

## **Trial Protocol Supplement**

Hooton TM, Vecchio M, Iroz A, et al. Increased daily water intake in premenopausal women with recurrent urinary tract infections: a randomized clinical trial. Published online October 1, 2018. *JAMA Intern Med*. doi:10.1001/jamainternmed.2018.4204

Clinical Study Protocol  
Clinical Study Protocol Amendment 1  
Clinical Study Protocol Amendment 2  
Clinical Study Protocol Amendment 3  
Statistical Analysis Plan

## CLINICAL STUDY PROTOCOL

**PRODUCTS:** WATER  
**PROTOCOL VERSION** 1 (FINAL)  
**STUDY CODE:** NU369

**INITIAL PROTOCOL  
DATE:** 19/11/2013

***The effect of increased water intake on the  
frequency of clinical recurrent urinary tract  
infections in pre-menopausal women:  
S-HYDRACYST***

**Principal Investigator:** MAYA DABCHEVA, MD  
MC "COMAC MEDICAL"  
13 ÜRIVICH STR., 3<sup>RD</sup> FLOOR  
1612 SOFIA, BULGARIA  
TEL.: +359 2 850 97 04 FAX: +359 2 850 60 96  
E-MAIL: MAYA.DABCHEVA@COMAC-MEDICAL.COM

**Sponsor:** DANONE RESEARCH  
ROUTE DEPARTEMENTALE 128  
91767 PALAISEAU CEDEX – FRANCE

**Sponsor Representative:** CHRISTINE M'RINI, MD, PHD  
LIFE SCIENCE DIRECTOR  
DANONE RESEARCH  
ROUTE DEPARTEMENTALE 128  
91767 PALAISEAU CEDEX – FRANCE  
TEL.: +33(0)1.69.35.70.00 FAX: +33(0)1.69.35.76.89  
E-MAIL: christine.mrini@danone.com

**Emergency Contact** MAYA DABCHEVA, MD  
TEL.: +359 2 850 97 04 FAX: +359 2 850 60 96

### **Ethics Statement**

This study will be conducted according to the protocol and in compliance with Good Clinical Practice (GCP), the ethical principles stated in the Declaration of Helsinki, and other local applicable regulatory requirements.

### **Confidentiality Statement**

The information provided in this document is the property of DANONE RESEARCH, and is shared with you and your staff in confidence. This information should not be disclosed to others without written authorization from DANONE RESEARCH, except to the extent necessary to ensure adequate conduct of the study.

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## 1. GENERAL INFORMATION

### 1.1 LIST OF PARTICIPANTS

**Principal Investigator:** MAYA DABCHEVA, MD  
MC "COMAC MEDICAL",  
13 ÜRIVICH STR., 3<sup>RD</sup> FLOOR  
1612 SOFIA, BULGARIA  
Tel.: +359 2 850 97 04 Fax: +359 2 850 60 96  
E-MAIL: MAYA.DABCHEVA@COMAC-MEDICAL.COM

The Appendix 2 provides the list of all participating investigational sites and Principal Investigators.

**Research Scientist/Engineer:** MARIACRISTINA VECCHIO  
DANONE RESEARCH  
ROUTE DEPARTEMENTALE 128  
91767 PALAISEAU CEDEX - FRANCE  
Tel. : +33 (0)1 69.35.68.47 Fax : +33(0)1.69.35.76.93

**Biostatistician:** PASCALINE PICARD [AIXIAL – PHARMA]  
DANONE RESEARCH  
ROUTE DEPARTEMENTALE 128  
91767 PALAISEAU CEDEX - FRANCE  
Tel. : +33 (0)7 78 41 10 44 Fax : +33 (1).69.35.76.89

**Sponsor's Medical Expert** CHRISTINE M'RINI, MD, PHD  
LIFE SCIENCE DIRECTOR  
DANONE RESEARCH  
ROUTE DEPARTEMENTALE 128  
91767 PALAISEAU CEDEX – FRANCE  
Tel.: +33(0)1.69.35.70.00 Fax: +33(0)1.69.35.76.89  
E-MAIL:christine.mrini@danone.com

**Emergency contact:** MAYA DABCHEVA, MD  
Tel.: +359 2 850 97 04 Fax: +359 2 850 60 96

**Clinical Study Manager:** LILIANA GALETESCU  
DANONE RESEARCH  
ROUTE DEPARTEMENTALE 128  
91767 PALAISEAU CEDEX - FRANCE  
Tel. : + 33 (1) 69 35 72 34 Fax : +33 (1).69.35.76.89

**Scientific Expert:** YAIR LOTAN  
CHIEF OF UROLOGIC ONCOLOGY  
UT SOUTHWESTERN MEDICAL CENTER AT DALLAS  
DEPARTMENT OF UROLOGY  
5323 HARRY HINES BLVD. J8.122  
DALLAS, TEXAS 75390-9110  
Tel. : 214-648-0389

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**Monitoring Clinical Research Organisation:** PHARM-OLAM INT.(UK) BULGARIA OFFICE  
PHARM-OLAM INT. (UK) BULGARIA OFFICE  
ADDRESS 55 PARCHEVICH STR.  
ADDRESS 1000 SOFIA, BULGARIA  
TEL: + 359 2 9888 704  
FAX : + 359 2 9888 701

**Central Laboratory:** MEDICAL DIAGNOSTIC LABORATORY RAMUS EOOD  
ADDRESS 2-4 ANGISTA STR.  
ADDRESS SOFIA 1527, BULGARIA  
Tel. + 359 2 9431196  
Fax: + 359 2 944 8206

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**1.2 PROTOCOL SIGNATURE PAGE - SPONSOR**

Protocol details	
Study title	<i>The effect of increased water intake on the frequency of clinical recurrent urinary tract infections in pre-menopausal women: S-HYDRACYST</i>

*Protocol approved by the Sponsor:*

I, the undersigned, have reviewed and approved this protocol, including the appendices.

SPONSOR	DATE	SIGNATURE
<b>Christine M'RINI, MD, PhD</b> LIFE SCIENCE DIRECTOR DANONE RESEARCH ROUTE DEPARTEMENTALE 128 91767 PALAISEAU CEDEX - FRANCE		

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**1.3 PROTOCOL SIGNATURE PAGE – PRINCIPAL INVESTIGATOR**

Protocol details	
Study title	<b><i>The effect of increased water intake on the frequency of clinical recurrent urinary tract infections in pre-menopausal women: S-HYDRACYST</i></b>

*Protocol approved by the Principal Investigator:*

I, the undersigned, have reviewed this protocol including the appendices and I am aware of my responsibility and I agree to the following:

To conduct the clinical study in compliance with the protocol as detailed in this document.

To apply ICH Good Clinical Practice the declaration of Helsinki and any other regulatory requirements.

To obtain protocol approval from an independent Ethics Committee and to comply with their requirements for ongoing review and reporting (if applicable).

To comply with procedures for data recording and reporting (with a particular focus on Safety reporting)

To permit monitoring, auditing and inspection by the sponsor and relevant regulatory agencies.

To retain study related documents according to regulatory requirements and as agreed with the sponsor.

PRINCIPAL INVESTIGATOR	DATE	SIGNATURE
<b>Maya DABCHEVA, MD</b>  MC "COMAC MEDICAL", 13 ÜRIVICH STR., 3 <sup>RD</sup> FLOOR 1612 SOFIA, BULGARIA		

*Three original copies of the clinical study protocol must be signed by the Principal Investigator and one original copy shall be filed by each of the parties (Sponsor and Principal Investigator) and one should be submitted to the Ethics Committee.*



**1.4 GLOSSARY**

ACR	Albumin Creatinine Ratio
AE	Adverse Event
AFU/EAU	Association Française d'Urologie (AFU)/European association of Urology
BMI	Body Mass Index
BP	Blood Pressure
CA	Competent Authority(ies)
CM	Concomitant Medication
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organisation
CUA	Cost utility analysis
CV	Curriculum vitae
DCF	Data Clarification Form
DBP	Diastolic Blood Pressure
EAU	European Association of Urology
EC	Ethics Committee
EDC	Electronic Data Capture
EPI	Epidemiology Collaboration
FAS	Full Analysis Set
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GP	General Practitioner
HR	Heart Rate
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ISF	Investigator Study File
IRB/IEC	Institutional Review Board/Independent Ethics Committee

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IWRS	Interactive Web Response Services/Systems
LDL	Low Density Lipoprotein
MCF	Mean Cumulative Function
OTC	Over The Counter
PI	Principal Investigator
PP	Per Protocol
QoL	Quality Of Life
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SD	Standard Deviation
SEM	Standard Error of Mean
SF	Screen Failure
SOP	Standard Operating Procedure
SS	Safety Set
SSAR	Suspected Serious Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
USG	Urine Specific Gravity
UTI	Urinary Tract Infection
V	Visit
VAS	Visual Analytical Scale
WHO	World Health Organisation

## 1.5 LIST OF DEFINITIONS

Active/Test product:	Product to be tested in the study.
Completed Subject:	Subject who has completed the study visits as required by the protocol. In the CRF the end of study status is available and it is : “Completed the study”.
Evaluation period:	This period extends from randomization (or allocation) up until the last visit where results are collected with the intent of evaluating any of the study criteria.
Eligible subject at visit X:	The subject is considered eligible when the Investigator certifies that he fulfills all inclusion criteria and does not meet any exclusion criteria for the current visit. This statement can be repeated as many times as required by the protocol. In the CRF the Investigator has answer YES to the question “Is the subject eligible at visit “X”?”
Follow up period:	This period, if applicable, follows the evaluation period in order to confirm the well-being of the subject or follow his recovering. Typically, minimal safety data are collected and no efficacy evaluation is performed.
Included (or enrolled):	The signature of the informed consent marks the inclusion of the subject in the study. This signature can be obtained solely after having fully briefed the subject about the study. At this step, a subject identification number is attributed to the subject. In the CRF the informed consent date(s) is/are available(s).
Principal Investigator:	Investigator responsible for the conduct of the clinical study at a study site. If a study is conducted by a team of individuals at a study site, the Principal Investigator is the responsible leader of the team and may be called the principal Investigator.
Pre-screening period:	Any actions performed by the site(s) in relation to the study recruitment that happens before the formal signature of the informed consent. No data is collected by the sponsor at this point.
Randomized (or allocated) subject:	Any subject who has been allocated to a study arm either by a randomization or by another method defined by the protocol. In the CRF the randomization number (or date) is available.
Screening period:	This period extends from the signature of the informed consent up until the randomization process.
Screen-failed subject:	The subject is considered a screen failure (SF) if: has been included but, did not complete the screening period successfully (which typically end at randomization). In the CRF an “end of study form” should be available with the reason for failure.

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Study product(s): Products to be used in the clinical study (Active/test products and control products).

Withdrawn (or Dropped out) Subject:

Any subject who has withdrawn from the study at any point after the screening period has ended but before having completed all the visits and assessments.

In the CRF the End of study status is available and it is different from: "Completed the study".

## 2. SYNOPSIS AND FLOW CHART

<b>Study title</b>	<i>The effect of increased water intake on the frequency of clinical recurrent urinary tract infections in pre-menopausal women: S-HYDRACYST</i>
<b>Study code</b>	<i>NU369 S-Hydracyst</i>
<b>Study sponsor</b>	<i>Christine M'Rini - DANONE RESEARCH, Palaiseau-France</i>
<b>Study principal investigator</b>	<i>Dr. Maya Dabcheva, MC "Comac Medical"</i>
<b>Study product description</b>	<p>The study is testing a medical recommendation, consisting in increasing the daily water intake in the tested group.</p> <p>For methodological reasons of homogeneity of access to water of the subjects, mineral commercialized water will be distributed to the subjects enrolled in the tested group, during the whole study duration.</p> <p>The mineral water is Evian® brand</p> <p>The intervention group will increase his fluid intake of 1.5L of Evian® water (on the top of their normal fluid intake)</p> <p>The control group will not change its water intake habits.</p>
<b>Study period</b>	<p><i>Intervention period: Nov 2013 –Apr 2015 (12 mo per subject)</i></p> <p><i>Start of study (first subject first visit): Nov 2013</i></p> <p><i>End date (clinical study report writing): Nov 2015</i></p>
<b>Study objectives</b>	<p><u>Study primary objective:</u></p> <p><i>To assess the effect of increased daily water intake on frequency of clinical recurrence of urinary tract infections (rUTI) among low drinking women suffering from recurrent community-acquired UTI</i></p> <p><u>Study secondary objectives:</u></p> <p><i>To evaluate the impact of increased daily water intake on use of antibiotics in recurrent UTI patients</i></p> <p><i>To evaluate the impact of increased daily water intake on the duration of urinary infection episodes</i></p> <p><i>To determine the changes in urinary hydration markers according to the changes in fluid intake habits</i></p> <p><i>To evaluate the impact of increased daily water intake on health costs in recurrent UTI patients</i></p> <p><i>To evaluate the impact of increased daily water intake on quality of life (QoL) in recurrent UTI patients</i></p> <p><u>Exploratory Objective:</u></p> <p><i>To evaluate the relationship between urinary hydration markers and number of UTI events</i></p>

	<i>To evaluate the relationship between urinary hydration markers and delay UTI events</i>
<b>Study Methodology</b>	<p>Prospective, mono-centric, open-label, randomised controlled trial in two parallel groups:</p> <ul style="list-style-type: none"> <li>❖ Control group not changing their fluid intake habits</li> <li>❖ Intervention group provided with low mineralised mineral water, fluid intake recommendations and regular <i>hydration coaching</i> support</li> </ul>
<b>Study population and sample size</b>	<p>The study population consists in pre-menopausal women diagnosed with rUTIs. and having a 'low drinker' profile. The total number of completed subjects is estimated at 84 subjects.</p> <p>In order to achieve 84 completed subjects, and considering a 40% drop out rate, we'll need to randomise 140 subjects</p> <p>Considering a 50% screen failure rate we might had to screen 280 subjects. In any case, the screening will be over until the 140 randomised subjects will be reached.</p>
<b>Subject recruitment</b>	This is a mono-centric study which will be performed in Bulgaria. 140 females will be randomized in this trial.
<b>Eligibility criteria</b>	<p><u>Eligibility criteria checked at V1</u></p> <p><u>Inclusion criteria:</u></p> <p>II01a. Women with at least 3 clinical recurrences* of (UTI in the last 12 months (at least one confirmed by bacteriological exam) and asymptomatic at V1 or II01b. Women with at least 3 clinical recurrences* of UTI in the last 12 months (at least one confirmed by bacteriological exam) and symptomatic at V1 or II01c. Women with at least 3 clinical recurrences* of UTI in the last 12 months (who did not performed any bacteriological exam in the last 12 months) and symptomatic at V1 II02 Age ≥ 18 years II03 Low-drinker (&lt; 1.5 L fluids per day) II04 Regular meal consumption (breakfast, lunch and dinner) II05 Access to Internet for information on hydration</p> <p>II06 Subject, upon briefing of the content of the present study, fully understanding and agreeing to its objective and having given written (dated and signed) informed consent to take part in the study. II07 Subject who is able to communicate well with the Investigator and to comply with the requirements of the entire study. II08 Literate subjects, able to fill in fluid questionnaires and QoL questionnaires II09 Women accepting to keep their lifestyle habits during the whole duration of the study II10 Women using any form of contraception II11 Subject covered by social security or covered by a similar system</p> <p><u>Exclusion criteria:</u> (checked at visit V1)</p> <p>IE01 Incapacity / non-willingness to consume 1.5 L of drinking water per day on top of their usual consumption</p>

	<p>IE02 Women with history of UTI complications (pyelonephritis or other) in the last 12 months</p> <p>IE03 Use of antibiotics or cranberries juice and extracts in the previous 2 weeks</p> <p>IE04 Chronic treatments with anti-coagulants therapy</p> <p>IE05 Chronic bladder inflammation (defined as permanent bladder bacterial infection)</p> <p>IE06 Chronic diarrhea or constipation treated with chronic use of laxative substances</p> <p>IE07 Interstitial cystitis</p> <p>IE08 Estrogen-dependent symptomatic vulvo-vaginitis</p> <p>IE09 Recent (&lt;1year) or active renal stone disease</p> <p>IE10 Urinary tract structural abnormalities</p> <p>IE11 Obesity or malnutrition (BMI &lt;18.5 Kg/m<sup>2</sup> and &gt;30 Kg/m<sup>2</sup>)</p> <p>IE12 Pregnant or lactating women</p> <p>IE13 Plans of any of the following 12 months after screening visit to become pregnant</p> <p>IE14 Menopausal and perimenopausal women</p> <p>IE15 Subjects general treated with drugs which can modify measurements performed in the study, in particular the assessment of the hydration status (diuretic intake, corticoids or treatment interfering with metabolism and nutrition behavior)</p> <p>IE16: Subjects with severe or uncontrolled organic disease, likely to interfere with the parameters of the study (e.g. neoplastic, cardiovascular, pulmonary and digestive disorders, unstabilized diabetes type I and II, untreated or uncontrolled clinically significant arterial blood hypertension) or mental disorders affecting eating and drinking behavior (i.e. primary polydipsia, bulimia nervosa, psychosis etc.)</p> <p>IE17: Women who have taken part in any other clinical study for the treatment of rUTI during the last 12 months</p> <p>IE18: Vulnerable subjects defined as individuals whose willingness to volunteer in the clinical trial may be unduly influenced by the expectation, whether justified or not, of benefits associated with participation, or of a retaliatory response from senior members of a hierarchy in case of refusal to participate. (Examples are members of a group with a hierarchical structure linked to the Investigator or to the Sponsor, such as medical, pharmacy, dental and nursing students, subordinate hospital and laboratory personnel, employees of the Investigator or of the Sponsor, members of the armed forces, and persons kept in detention).</p> <p>IE19: No legal capacity or limited legal capacity or unable to give an informed consent.</p> <p>IE20: Subjects unlikely to cooperate in the study, and/or poor compliance anticipated by the Investigator.</p> <p><i>* presence of bacteriuria in urine (<math>\geq 10^3</math> cfu/mL of a uropathogen in midstream urine culture) revealed by quantitative culture or microscopy in a sample taken from a patient or the typical symptoms of lower or upper urinary tract infection (defined as <math>\geq 3</math> urinary symptoms (difficult, painful, urgent and frequent urination, hematuria, supra-pubic pressure, increase in the number of white blood cells (&gt;10.000/ml) in the urine, with or without bacterial infection)).</i></p> <p><i>The presence of symptomatic bacteriuria can be established with a single urine sample</i></p> <p><i>Data source available at <a href="http://www.sign.ac.uk/pdf/sign88.pdf">http://www.sign.ac.uk/pdf/sign88.pdf</a></i></p> <p><u>Eligibility criteria checked at V2</u></p> <p><u>Randomization criteria</u></p> <p>RI01: Negative urine culture and asymptomatic state (i.e. silent phase) at V2 (randomization visit)</p> <p>RI02: Low-drinker confirmation (24 hours urinary volume &lt; 1.2 L per day)</p>
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	<p><u>Non-randomization criteria</u></p> <p>RE01: Chronic kidney disease (defined as decreased GFR (GFR&lt;60 ml/min/1.73m<sup>2</sup> calculated using EPI equation)</p> <p>RE02: Women suffering multiple antibiotic resistant bacterial strain<sup>§</sup></p> <p><sup>§</sup> Multiple antibiotic resistance is defined as resistance to 3 or more different antibiotics</p>
<p><b>Study product administration</b></p>	<p>Three bottles of natural mineral water (500 mL each) to be consumed daily, in addition to the subjects' usual fluid intake volume, for 12 months.</p> <ul style="list-style-type: none"> <li>- Bottles will be provided to subjects in the intervention group for free, along with the instruction to consume them at the beginning of every meal (breakfast, lunch and dinner) and fully drink them before the following meal. Coaching support will also be made available; this will be reinforced by individualised phone calls.</li> <li>- The study products will be provided by DANONE RESEARCH and will be supplied by a professional logistics company</li> </ul>



<p><b>Study description / Duration of subject participation</b></p>	<p>The total duration of the intervention phase will be 12 months per subject (between V2 and V4).</p> <p>Each subject will attend 4 visits:</p> <ol style="list-style-type: none"> <li>1. <i>Inclusion visit (V1)</i></li> <li>2. <i>Randomisation visit (V2; M0)</i></li> <li>3. <i>Visit 3 (V3; M6)</i></li> <li>4. <i>Visit 4 (V4; M12)</i></li> </ol> <p>The 3 days fluid diary will be administered every month (from M0 to M12). In order to standardize the data collection, women will be asked to complete the 3 day fluid questionnaire on Saturday-Sunday-Monday once a month.</p> <p>Quality of life questionnaire will be administered during three of the four visits (V2, V3 and V4).</p> <p>Laboratory exams will be performed at visit 2, 3 and 4. Fasting 8-12 hours will be required.</p> <p>Women will be asked to collect 24h urine and to complete a voiding diary outside the menstrual period.</p> <p>Symptoms of UTI will be recorded during all the visits and during the phone calls.</p> <p>Information about bladder emptying after sexual intercourses and active/inactive sexual life during the last month will be collected each month.</p> <p>Potential extra visits will be taken into account when/ if any acute episode during the course of the study. During the extra visits urine culture will be performed to confirm the presence of UTIs. If confirmed, the UTI episode will be treated following the normal clinical practice. Urine culture will be also performed at the end of the therapy to be sure that women are completely recovered. Start and end dates of each episode will be reported. Healthcare costs data related to UTI episodes will be collected during each relapse visit and during the visits 3 and 4 if needed.</p> <p>If women will report a UTI event during V2, V3 or V4, some of the urinary parameters collected during the visit (urine colour, urinary specific gravity) may be biased. For this reason they will be asked to come back once recovered to re-check urinary parameters.</p> <p>Physical examination will be performed during V2 and V4.</p> <p>Every month women will be contacted by phone by the investigators, to check the compliance with the intervention (if in the intervention group), adverse events, sexual activity during the past month, symptoms of UTI and, eventually, start and end date of episodes of UTI.</p>
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Inclusion visit: (V1)

Women willing to participate in the study will give their informed consent and be evaluated against inclusion and exclusion criteria. A pregnancy test will be performed.

Information about bladder emptying after sexual intercourses and active/inactive sexual life during the last month will be collected.

Urine culture to confirm the presence of UTI will be performed only in symptomatic women. They will be then sent home with a treatment against their acute episode (i.e. AFU/EAU guidelines for UTI management).

All participants will be asked to complete the 3 day fluid diary at home and to bring the questionnaires back to the site when they come back for the randomization visit.

Asymptomatic women at visit 1 will come back for the randomization visit after two weeks.

Symptomatic subjects showing positive urine culture (confirming the presence of UTI) will be sent home with a standardised treatment against their acute episode (i.e. AFU/EAU guidelines for UTI management) and they will be ask to come back for the randomization visit (V2) after 4 weeks.

Anthropometrical parameters (body weight, , waist circumference, height) will be collected.

Anamnestic data and vital signs will be collected.

Randomisation visit: (V2)

Subjects will be evaluated against randomisation and non-randomization criteria. Women will attend to the clinical site for confirmation of negative urine culture. A physical examination will be performed. Women will be randomly allocated into one of the two study arm. In order to evaluate their baseline hydration status, they will have been asked to collect and come along with a 24h urine sample beforehand. Urinary markers of hydration (urine volume, pH, osmolality, specific gravity, frequency of micturition, urine colour) will be monitored. Electrolytes in the urine sample (sodium, potassium, calcium, magnesium, chloride, oxalate, citrate) will be collected. Urine creatinine will be collected.

Information about bladder emptying after sexual intercourses and active/inactive sexual life during the last month will be collected.

A blood sample will be collected for determination of lipidic profile (HDL cholesterol, LDL cholesterol, triglyceride), glycosylated hemoglobin, serum creatinine, serum urea, copeptin. Estimated glomerular filtration rate (eGFR) will be calculated.

Vital signs and anthropometrical parameters (body weight, waist circumference) will be collected. Data on chronic medication (including all the anti-hypertensive agents) any other concomitant medications and AE will be collected.

The 3 day fluid diary (Saturday-Sunday-Monday) will be collected: patients will be asked to complete the questionnaire at home every month until the end of the study and to send them back to the investigating site by post.

In addition, patients in the intervention group will be asked to fill in a daily mineral water diary in order to help them to check the study product consumption. The daily diaries will be returned to the site each month by post together with the 3 day fluid intake diaries.

Patients will be asked to complete on site the Quality of Life questionnaire, AE, Concomitant medications.

Patients will receive instructions about study requirements accordingly.

Patients who will be randomized in the study group will be asked to consume 1,5L of Evian® brand mineral water which will be supplied to their home. This volume is on top of their normal water consumption

Evaluation visit: (V3)

Subjects will come along with a 24h urine sample to the clinical site. Urinary markers of hydration (urine volume, pH, osmolality, specific gravity, frequency of micturition, urine colour) will be monitored. Electrolytes in the urine sample (sodium, potassium)

Evaluation visit: (V3)

Subjects will come along with a 24h urine sample to the clinical site. Urinary markers of hydration (urine volume, pH, osmolality, specific gravity, frequency of micturition, urine colour) will be monitored. Electrolytes in the urine sample (sodium, potassium) will be collected. Urine creatinine will be collected. Vital signs and anthropometrical parameters (body weight, waist circumference) will be collected.

Information about bladder emptying after sexual intercourses and active/inactive sexual life during the last month will be collected.

. A blood sample will be collected for determination serum creatinine and copeptin. Estimated glomerular filtration rate (eGFR) will be calculated. 3 day fluid diary questionnaires will be completed every month until V4 and will be returned by post to the investigator site. Patients in the intervention group will continue to fill in a daily mineral water intake diary until V4 and will return them to the site by post, on monthly basis, together with the 3 day fluid intake diaries. Symptoms of UTI in the time between the visits will be recorded together with start and end date for each episode.

Patients will be asked to complete on site the Quality of Life questionnaire, AE, Concomitant medications.

Last visit: (V4)

Subjects will attend to the clinical site come along with a 24h urine sample. A physical examination will be performed. Urine culture to evaluate the presence of any infection will be performed. Urinary markers of hydration (urine volume, pH, osmolality, specific gravity, frequency of micturition, urine colour) will be monitored. Electrolytes in the urine sample (sodium, potassium, calcium, magnesium, chloride, oxalate, citrate) will be collected. Urine creatinine will be collected. Vital signs and anthropometrical parameters (body weight, waist circumference) will be collected along with quality of life, concomitant treatments, and AE.

Information about bladder emptying after sexual intercourses and active/inactive sexual life during the last month will be collected.

A blood sample will be collected for determination of lipidic profile (HDL cholesterol, LDL cholesterol, triglyceride), glycosylated hemoglobin, serum creatinine, serum urea, copeptin. Estimated glomerular filtration rate (eGFR) will be calculated. Data coming from the 3 day fluid intake questionnaires will be collected. Symptoms of UTI in the time between the visits will be recorded.

Relapse visits:

Subjects presenting with symptoms of UTI during the course of the study will be instructed not to stop the increased water intake (if in the intervention group) and to consult with the investigator for clinical examination; urine cultures will be systematically performed to confirm preliminary diagnosis. Data about duration of the infection (starting and ending date) will be registered. Information about bladder emptying after sexual intercourses and active/inactive sexual life during the last month will be collected. Healthcare costs data due to illness (for patients and/or conductors) will be collected. Concomitant treatments will be collected. Subjects will then be sent home with a standardised treatment against their acute episode (i.e. AFU/EAU guidelines for UTI management) irrespective of the study group they belong to. Urine culture will be also performed at the end of the therapy to be sure that women are completely recovered. Direct and indirect healthcare costs due to UTI event (for patients and conductors) will be collected.

Intake surveys & coaching support: throughout the duration of the study, subjects will be asked to complete a 3-day fluid questionnaire every month.

In order to reinforce their adherence to fluid intake recommendations, subjects allocated to the intervention group will be provided with bottled mineral water, will receive individualised e-mails/text messages/phone calls at regular intervals and will be provided with a coach. By contrast, subjects allocated to the control group won't receive any fluid intake recommendations nor any test product or coaching support.

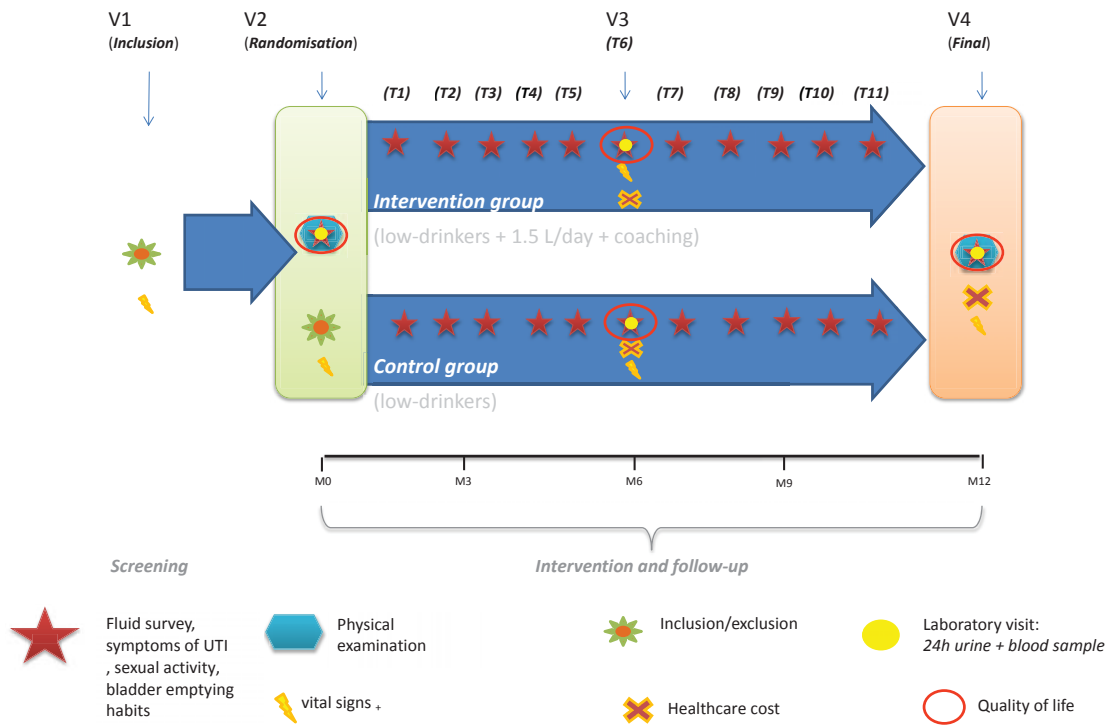
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		The protocol will follow ICHG6 guidelines on Good Clinical Practices and applicable Directives/local laws.
<b>Study schema</b>	<b>design</b>	<i>See below</i>

**Line of the data collection**

Procedures	Visits		Month 0		1st Month	2 <sup>nd</sup> Month	3rd Month	4th Month	5th month	6th month	Month 6	7th Month	8th Month	9th Month	10th Month	11th Month	12th Month	Month 12	Relapse visit
	Inclusion Visit (V1)	Home	Randomisation Visit (V2)	Home	Home	Home	Home	Home	Home	Visit 3 (V3)	Home	Home	Home	Home	Home	Home	Home	Completion (V4)	
Screening for acute UTI or previous episodes (at least 3 in the last 12 months)	X																		
Physical examination			X																X
Phone call to check compliance, AE and UTI events				X	X	X	X	X				X	X	X	X	X			
Medical & Surgical history	X																		
Informed consent	X																		
Inclusion / exclusion criteria	X																		
Randomisation/non randomisation criteria			X																
Demographic modules	X																		
Vital sign and anthropometrical parameters	X		X								X								X
Instructions on completion of 3 day fluid intake diary	X		X								X								
3 day fluid intake diary check			X								X								X
3 day diary fluid intake		X		X	X	X	X	X	X			X	X	X	X	X	X		
QoL questionnaire (SF-12 V2)			X								X								X
Urinary pregnancy test	X																		
Delivery of urine containers & instructions	X		X								X								
Urine 24h collection (+ voiding diary)		X*								X*							X*		
Collection of urine containers & Voiding diary			X								X								X
Blood sample			X								X								X
Symptoms of UTI	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Bacteriogram	X (if symptomatic)		X																X
Healthcare costs data																			X
Concomitant Treatments	X		X								X								X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Bladder emptying after sexual intercourse	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Active/inactive sexual life during the last month	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

\* 24h urine to be collected at home the day before the visit (V2, V3 and V4)



<p><b>Study Parameters</b></p>	<ul style="list-style-type: none"> <li>- <u>parameter 1</u>: total number of clinical recurrences of UTI during the Study period*</li> <li>- <u>parameter 2</u>: mean time elapsed between UTI episodes *</li> <li>- <u>parameter 3</u>: mean duration of UTI episodes</li> <li>- <u>parameter 4</u>: frequency of micturition during the 24h urine collection</li> <li>- <u>parameter 5</u>: 24h urine (volume, pH, osmolality, specific gravity, urine colour, sodium, potassium, calcium, magnesium, chloride, oxalate, citrate, creatinine)</li> <li>- <u>parameter 6</u>: blood sample (lipidic profile, glycosylated hemoglobin, serum creatinine, serum urea, copeptin)</li> <li>- <u>parameter 7</u>: vital signs (diastolic and systolic blood pressure, heart rate, body temperature)</li> <li>- <u>parameter 8</u>: quality of life (questionnaire SF12V2)</li> <li>- <u>parameter 9</u>: fluid intake (water, other types of fluids)</li> <li>- <u>parameter 10</u>: anthropometry (body weight, waist circumference, height, BMI)</li> <li>- <u>parameter 11</u>: pathology related costs</li> <li>- <u>parameter 12</u>: complications from UTIs (UTI related AE, SAE)</li> </ul> <p>*Based on the simultaneous presence of positive urine culture and UTIs symptoms</p>
<p><b>Study Endpoints</b></p>	<p><u>Principal:</u></p> <ul style="list-style-type: none"> <li>➤ Difference between groups in terms of number of UTI recurrence over 12 months of follow up</li> </ul> <p><u>Secondary:</u></p> <ul style="list-style-type: none"> <li>➤ Difference between groups in terms of duration of urinary infectious episodes</li> <li>➤ Average delay between each UTI over one year of study period</li> <li>➤ Change in urinary hydration markers following changes in water intake habits</li> <li>➤ Difference between groups in terms of change in QoL</li> <li>➤ Difference between groups in terms of antibiotic prescription/usage to treat UTI</li> <li>➤ Difference between groups in terms of health-costs associated with management of UTI recurrence(s)</li> <li>➤ Difference between groups in terms of cost utility analysis</li> </ul> <p><u>Exploratory:</u></p> <ul style="list-style-type: none"> <li>➤ Relationship between urinary hydration markers and number of UTI events</li> <li>➤ Relationship between urinary hydration markers and delay UTI events</li> </ul>
<p><b>Safety evaluation criteria</b></p>	<p>Blood pressure, heart rate, adverse events, serious adverse events</p>

<b>Statistical analysis</b>	<ul style="list-style-type: none"> <li>➤ The statistical methodology will be detailed in the statistical analysis plan written and finalized before the database lock.</li> <li>➤ Descriptive statistics will be given for each of the parameters (i.e. for continuous variables: group size, mean, standard deviation of the variable [SD], standard error of the mean [SEM], minimum, maximum, median, and possibly quartiles; for qualitative variables, group size and percentage).</li> <li>➤ For the main criterion, a nonparametric method called the mean cumulative function (MCF) will be used to analyse the multiple/repeated UTI events occurring. The null hypotheses is “There is no difference between groups on the average number of recurrent UTIs event experienced”. We will consider a Type I error rate (alpha) equal to 5%.</li> <li>➤ The analysis will be conducted with the statistical software package SAS 9.3.</li> </ul>
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### 3. INTRODUCTION

#### 3.1 SCIENTIFIC BACKGROUND INFORMATION

Urinary tract infection (UTI) is one of the most common clinical diagnoses in women. The lifetime risk for UTI in women is high (greater than 50%) and in the U.S. between 1988 and 1994 the overall lifetime prevalence of UTI was estimated to be 53,067/100,000 women.(1) The estimated global incidence of UTIs is at least 250 million cases per year.(2) UTIs are a source of significant cost and morbidity. Most UTIs are self-limiting but occasionally can be associated with significant complications such as pyelonephritis and sepsis. Composite data revealed that overall expenditures for the treatment of UTIs in women in the United States, excluding spending on outpatient prescriptions, were approximately 2.47 billion U.S. dollars in 2000.(1) According to the 1997 National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey, UTI accounted for nearly 7 million office visits and 1 million emergency department visits, resulting in 100,000 hospitalizations.(3) The exact frequency of UTIs is difficult to assess because they are not reportable diseases in the United States. This situation is further complicated by the fact that accurate diagnosis depends on both the presence of symptoms and a positive urine culture, although in most outpatient settings this diagnosis is made without the benefit of culture.

There are several higher risk populations for UTIs including infants, pregnant women, the elderly, patients with spinal cord injuries and/or catheters, patients with diabetes or multiple sclerosis, patients with acquired immunodeficiency disease syndrome/human immunodeficiency virus, and patients with underlying urologic abnormalities.(4) However, UTIs are very common in otherwise healthy women. It is estimated that at least a third of all women in the United States are diagnosed with a UTI before they are 24 years old.(5) There are both host and bacterial factors that contribute to UTIs.(6) In young women, sexual activity is associated with an increased risk for UTIs.(7) UTIs also have a propensity to recur. In otherwise healthy college women with a first UTI, the risk of a second episode within 6 months was 24%,(7) and in those with a history of one or more UTIs, the risk of a second within 1 year was 70%.(8) Even when UTI is not associated with long term consequences, the condition results in pain and suffering, and negatively impacts quality of life, albeit transiently.(9) Common symptoms in



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premenopausal women include frequent, urgent and painful urination and suprapubic pressure and hematuria may also be present.

There are multiple reasons to try to prevent UTIs in women. First and foremost is to reduce morbidity associated with the infections as well as reduce the cost of treatment. However, there are also important reasons to reduce the use of antibiotics in these patients.(10) First, there is increasing resistance of *Escherichia coli*, the primary causative agent of uncomplicated UTI, to a variety of antibiotics, including fluoroquinolones and extended-spectrum beta-lactamase (ESBL) resistance is increasingly observed among community acquired UTI.(11) Second, there can be significant impact of short courses of antibiotics on the gut and vaginal microbiota which can contribute to recurrence and antibiotic resistance.(12) Third, there are risks associated with antibiotic use such as allergic reactions and side effects of the drugs themselves. Finally there is a risk of vaginal candida infection, which occurs in up to 22 % of women treated for uncomplicated UTI.(13)

Moreover, due to the high prevalence and incidence, UTI has enormous economic implications.

As for other pathologies, costs related to UTI episodes should be divided into direct and indirect ones.

Direct costs include the costs of outpatient doctor visits, antibiotics and specific antimicrobial agent prescription, along with hospitalization expenses. The indirect costs include all the “non-medical costs” related to the pathology, such as travel and sick days for the patients and for the caregivers.

Direct and indirect annual costs related to acute episode of UTI have been estimated to be around \$1.6 billion for the US female population (14), including approximately \$936 million for indirect costs and \$659 million for direct ones.

To date, no data about specific European population are available. In spite of available evidence suggesting a link between urinary hydrodynamics and frequency of UTI episodes, cost-savings that could potentially be derived from appropriate fluid intake among UTI patients remain to be established.

Several strategies have been proposed to try to reduce the risk of recurrent UTIs.

While the use of daily antibiotics or post-coital antibiotics is effective, the rise of resistance and risk associated with antibiotics has made these strategies less attractive. Different approaches have been proposed including use of functional foods, lactobacillus and vaccines.(10) The most studied functional food thus far has been cranberries and their extracts. A Cochrane review on studies including cranberries included a total of 24 studies (six cross-over studies, 11 parallel group studies with two arms; five with three arms, and two studies with a factorial design) with a total of 4473 participants.(14) The comparison/control arms were placebo, no treatment, water, methenamine hippurate, antibiotics, or lactobacillus. The meta-analyses found that compared with placebo, water or not treatment, cranberry products did not significantly reduce the occurrence of symptomatic UTI overall (RR 0.86, 95% CI 0.71 to 1.04) or for any the subgroups: women with recurrent UTIs (RR 0.74, 95% CI 0.42 to 1.31); older people (RR 0.75, 95% CI 0.39 to 1.44); pregnant women (RR 1.04, 95% CI 0.97 to 1.17); children with recurrent UTI (RR 0.48, 95% CI 0.19 to 1.22); cancer patients (RR 1.15 95% CI 0.75 to 1.77); or people with neuropathic bladder or spinal injury (RR 0.95, 95% CI: 0.75 to 1.20). Studies evaluating lactobacillus have shown some promise but await further validation.(15,16)

### **3.2 DESCRIPTION OF THE TEST PRODUCT**

The approach that we propose involves randomizing premenopausal women with recurrent UTIs to high versus normal water intake to determine whether this will reduce risk of UTIs. For this purpose we will provide women with three bottles of natural mineral commercialized water (500 mL each) to be consumed daily for 12 months. Bottles will be provided to subjects in the intervention group for free, along with the instruction to consume them at the beginning of every meal (breakfast, lunch and dinner) and fully drink them before the following meal.

### **3.3 SCIENTIFIC RATIONALE**

The study will include only women who are low-drinkers (< 1.5 L fluids per day; urinary volume < 1.2 L per day) since they are most likely to have a predisposition to UTIs due to infrequent voiding. The rationale for this approach is that drinking more fluid will increase voiding frequency and voiding is the main defence of the bladder to reduce the number of bacteria in the bladder and avoid UTIs. As such, increasing fluid intake will increase the frequency and volume of voiding and potentially reduce risk of recurrent UTIs. Support for this hypothesis is multifold. A non-randomised, multivariate analysis comparing 791 women teachers who deliberately restricted their fluid intake (25% voided only once during working hours, or not at all) with women able to drink without restrictions found that women in the former group were at significantly higher risk of UTI than were women in the latter group (RR 2.21; 95% CI 1.45-3.38) after controlling for parity, voiding infrequently at work, and urge incontinence.(17) A study of 1613 women compared frequency of voiding based on age of women and impact on UTIs.(18) The study found that women aged 20-25 had a higher rate of this low voiding frequency than women 30-35 or 40-45 years and in all 3 groups, women who voided 3 times or less per day had significantly more urinary infections than those with 4 or more voidings per day, (p < 0.01). While results are inconsistent, several studies found that higher post-void residual volumes increases risk of UTI in women.(19-21) This supports the notion that efficacy of voiding and emptying the bladder can reduce risk of UTIs. One additional impact of fluid intake may involve reducing urine acidity. Low fluid intake is associated with an increase in urine osmolality and acidity which can predispose to bacterial adhesion to the bladder epithelium. A study in premenopausal women who had been treated for at least two idiopathic UTIs in the previous 6 months found that self-monitoring urine osmolality using a handheld 'traffic light' probe was associated with a significant shift towards urine of lower osmolality and a significant reduction in incidence of UTIs compared with the period in which the probe was not used.(22) Of the 17 patients who completed both 4-month periods, 14 felt that the probe had helped them to prevent infection.

#### *3.3.1 Rationale for the study purpose*

The above evidence and the heavy burden of recurrent UTIs on society demonstrate a significant need for strategies to prevent UTIs. The planned study will determine the efficacy of increased water intake in decreasing the risk of UTIs in women who are low volume drinkers and who suffer from recurrent UTIs compared to a control group.

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### *3.3.2 Rationale for the study population*

UTI is the most common infection in humans. It is highly prevalent in both men and women but its frequency is about 50 times higher in adult women of all age groups. This may be because the urethra is shorter in women than in men, making it easier for bacteria to ascend into the bladder and, once there, proliferate.

More than half of all women (50–60%) encounter with at least one UTI at some stage during their lives (23). Symptomatic infection of the bladder (lower UTI) has been estimated to occur in up to 30% of women during their life (24).

The estimated global incidence of UTI in women based on self-report of physician diagnosis is 11% per year resulting in at least 250 million cases per year (25). This incidence is higher (17.5%) during ages 18–24 and decreases to 9% for women 50 and over (5).

Approximately 5% of women with an initial UTI have multiple episodes within a year and a high recurrence rate, ranging between 25 to 30%, has been shown to affect the total female population (26). Observational studies have shown 6-month risk of recurrence ranging from 17 to 24% among otherwise healthy pre-menopausal women (5, 27). It is not surprising that one of the major risk factor for UTI among women is having a history of UTI.

Given the high burden, recurrent premenopausal women will be the target population of our study.

### *3.3.3 Rationale for dose(s) selected and/or dose(s) regimen*

We want women in the intervention group, to achieve a mean daily urine osmolality equivalent to that of plasma (approximately 285 mosm/kg). This osmolality goal is selected because it defines the transition from dilute to concentrated urine and is a safe end point under most any circumstance. To maintain such a low urine osmolality, we assume that women in the intervention group should increase their water intake of 1.5 L/day.

Moreover, to maintain a urinary volume of 1.5L we need to assure a total fluid intake of more than 1.3L/day (29). 1.5L/day will be our target knowing that the mean water intake in European women is less than 1L/day.

This dose hopes to gain an appreciation for how compliant patients would be in following a water prescription without inconveniencing them.

### *3.3.4 Rationale for the study design*

Given the necessity to establish a causality relation between increased water intake and UTI episodes, we will perform a monocentre, prospective, randomized, Open-label, controlled study. This model is often used where the double blind design is not applicable. It utilizes a strict randomization procedure: patients are allocated to different treatment regimens following the allocation concealment rules. In this way, the Investigator won't be able to subvert randomisation and select which patients get which treatment. The follow-up and treatment of patients is conducted openly in a way that adheres to accepted clinical principles and medical practice.

## **3.4 POTENTIAL RISKS AND BENEFITS**

We expect increased fluid intake to be efficient in reducing the risk of clinical recurrences of UTI in pre-menopausal women. Decreased incidence of this pathology will reduce the comorbidity associated with UTI events as well as the related healthcare costs.

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No risk linked to increased water intake has ever been shown in this population. More over the protocol recommends to split the water consumption over the day and consume 0,5L at the beginning of every meal (breakfast, lunch, dinner) and fully drink them before the following meal.

However, in case of ingestion of more than 1L of water in a short time interval (less than 10 min), the subject may have possible temporary stomach discomfort and an increased urge to use the toilet.

Blood samples will be drawn during the study visits. The risks related to this procedure are very weak and they are the same as for any type of blood drawing procedure in current medical practice:

- Pain during the sampling: it is especially connected to the speed of execution of the movement. This risk is minimized in this study as the staff performing the sampling is competent medical staff.
- Local secondary infection: this risk is theoretical because the used material is single-use, and the sampling is performed after local asepsis.
- Localized haematoma: this risk can be limited by the realization of an effective manual compression in the minutes following the sampling.

## 4. STUDY OBJECTIVES

### 4.1 PRIMARY STUDY OBJECTIVE

The primary objective of this study is to assess the effect of increased water intake on frequency of clinical recurrence of urinary tract infection among low drinking women suffering from recurrent community-acquired UTI.

### 4.2 SECONDARY STUDY OBJECTIVE(S)

To evaluate the impact of increased daily water intake on use of antibiotics in recurrent UTI patients  
To evaluate the impact of increased daily water intake on the duration of urinary infection episodes  
To determine the changes in urinary hydration markers according to the changes in fluid intake habits  
To evaluate the impact of increased daily water intake on health costs in recurrent UTI patients  
To evaluate the impact of increased daily water intake on quality of life (QoL) in recurrent UTI patients.

## 5. STUDY DESIGN

### 5.1 STUDY METHODOLOGY

This is an open label, prospective, single site, randomised, controlled trial, in two parallel groups with a 1:1 ratio allocation.

The randomization process will be applied using a centralised Interactive Web Response System (IWRS) that will allocate subject into one of the two groups in respect with the randomisation list generated at the beginning of the study.

### 5.2 STUDY EVALUATION CRITERIA

#### 5.2.1 Primary product effect evaluation endpoint

The primary criterion assessed during this study is the difference between groups in term of frequency of recurrence of Urinary Tract Infection events, defining by the number of events, using start and end dates of each one over a one year study period from randomisation visit (V2;M0) to end of study visit (V4;M12).

The start date of UTI event will be considered as the date of first symptoms, and the end date will be considered as the sampling date of urine culture performed at the end of the episode-therapy that provided negative urine culture.

#### 5.2.2 Secondary product effect evaluation criteria

The secondary criteria assessed during this study are as follows:

- Difference between groups in terms of duration of urinary infectious episodes
- Average delay between each UTI over one year follow up
- Change in urinary hydration markers following changes in water intake habits

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- Difference between groups in terms of change in QoL
- Difference between groups in terms of antibiotic prescription/usage to treat UTI
- Difference between groups in terms of health-costs associated with management of UTI recurrence(s)

As quality of life (QoL) is an important aspect of the intervention, the health economic evaluation will be considered through a cost utility analysis (CUA). Those health cost study will be conducted using two different perspectives. The perspective is the point of view from which the costs and benefits are recorded and assessed. Considering the research question, the two perspectives below will be considered:

- Social insurance
- Patients' perspective by determining patient out-of- pocket costs during the study intervention.

As several perspectives are included in the analysis, the results will be presented separately for each study perspective.

### *5.2.3 Exploratory product effect evaluation criteria*

The exploratory criteria assessed during this study are as follows:

- Relationship between urinary hydration markers and number of UTI events
- Relationship between urinary hydration markers and delay UTI events

### *5.2.4 Product safety evaluation criteria*

The safety criteria assessed during this study are as follows:

- Blood pressure
- Heart rate
- Body temperature
- Weight
- Urine sodium
- Urine potassium
- Physical Examination at V2 and V4
- Adverse events
- Serious adverse events

## **5.3 STUDY GLOBAL DESCRIPTION**

The subjects will be divided into 2 balanced groups of 70 subjects each. Subjects allocated to study test group (intervention group) will be asked to consume daily three bottles of natural mineral water (500 mL each) in addition to their usual fluid intake consumption, for 12 months. The subjects are advised to consume them at the beginning of every meal (breakfast, lunch and dinner) and fully drink them before the following meal. The subjects allocated to control group will be asked not to change the low fluid intake assessed at baseline.

The study will include 2 parts:

1. The screening period (from visit V1 to V2). During this period, subjects will be screened for inclusion and exclusion criteria.

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Symptomatic subjects who will fulfil all the inclusion and exclusion criteria, will be sent home with a treatment against their acute episode (i.e. AFU/EAU guidelines for UTI management) and will be ask to come back after one month for randomisation visit.

Asymptomatic subjects who will fulfil all the inclusion and exclusion criteria, will be asked to come back after two weeks for randomisation visit.

2. The study intervention period (12 months duration), from visits V2 to V4. During this period, the subjects will attend 2 evaluation visits (V3 at six months, V4 at twelve months).

Each subject will thus attend 4 visits in total at the clinical unit: Inclusion visit (V1), randomisation visit (V2), evaluation visit (V3) at month 6 and the end of study visit (V4) at month 12.

Each subject will undergo 3 blood sample collections, 3 24h urine collections and 2 urine cultures. Each appearance of UTI symptoms will lead to 2 additional urine cultures (one at the beginning of the episode and one after the treatment to confirm the recovery) over the study conduct.

For the blood samples, it is necessary for the subjects to be in a fasted state for 8-12 hours before the visit.

#### **5.4 STUDY DESIGN SCHEMA**

Refer to section 2.

## 5.5 DURATION OF THE STUDY PER SUBJECT

The total duration of the study is approximately thirteen (13) months for each subject, including an inclusion period of two-four weeks (2 to 4 weeks), a study intervention period of fifty-two weeks (twelve months).

# 6. SUBJECTS

## 6.1 SUBJECT RECRUITMENT AND SCREENING

The subjects included in the study will be women with recurrent episodes of UTI aged from 18 to premenopausal age and having a “low drinker profile”. Subjects with clinical manifestations of menopause such as women aged >45 y.o; with irregularities of the menstrual cycle during the last 6 months as well as menopausal women will be excluded.

The subject inclusion will be stopped as soon as 140 subjects are randomized.

At this time, all subjects already included (subjects who are within the screening V1 to V2) will be randomized and will complete all the study procedures and visits according to the protocol until their last study visit (V4).

Subjects potentially able to participate to this study will be pre-selected by physicians working in liberal practice (GPs, urologists, gynaecologists) from their patients' database. The pre-screened subjects will be referred to the Investigating Centre to be recruited in the clinical study. If necessary, in order to reinforce recruitment rate, the Investigating Centre may also use advertising and conduct a pre-screening of patients at the investigating site following their standard process.

The subject must have personally dated and signed their Informed Consent Form (ICF) before undergoing any medical or biological procedures required by the clinical study.

## 6.2 SUBJECT ELIGIBILITY CRITERIA

A subject is considered eligible when the Investigator certifies that the subject fulfils all inclusion criteria and does not meet any exclusion criteria for the current visit. This statement can potentially be repeated at different subjects' visits before subject's randomization as required by the study design.

### 6.2.1 Inclusion criteria (checked at the inclusion visit - V1)

In order to be eligible to participate in this study, a subject must meet all of the following criteria at the Visit 1:

Inclusion criteria:

II01a. Women with at least 3 clinical recurrences\* of UTI in the last 12 months (at least one confirmed by bacteriological exam) and asymptomatic at V1

or

II01b. Women with at least 3 clinical recurrences\* of UTI in the last 12 months (at least one confirmed by bacteriological exam) and symptomatic at V1

or

II01c. Women with at least 3 clinical recurrences\* of UTI in the last 12 months (who did not performed any bacteriological exam in the last 12 months) and symptomatic at V1

II02 Age  $\geq$  18 years

II03 Low-drinker (< 1.5 L fluids per day)



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- II04 Regular meal consumption (breakfast, lunch and dinner)
- II05 Access to Internet for information on hydration
- II06 Subject, upon briefing of the content of the present study, fully understanding and agreeing to its objective and having given written (dated and signed) informed consent to take part in the study.
- II07 Subject who is able to communicate well with the Investigator and to comply with the requirements of the entire study.
- II08 Literate subjects, able to fill in fluid questionnaires and QoL questionnaires
- II09 Women accepting to keep their lifestyle habits during the whole duration of the study
- II10 Women using any form of contraception
- II11 Subject covered by social security or covered by a similar system

Exclusion criteria: (checked at visit V1)

- IE01 Incapacity / non-willingness to consume 1.5 L of drinking water per day on top of their usual consumption
- IE02 Women with history of UTI complications (pyelonephritis or other) in the last 12 months
- IE03 Use of antibiotics or cranberries juice and extracts in the previous 2 weeks
- IE04 Chronic treatments with anti-coagulants therapy
- IE05 Chronic bladder inflammation (defined as permanent bladder bacterial infection)
- IE06 Chronic diarrhea or constipation treated with chronic use of laxative substances
- IE07 Interstitial cystitis
- IE08 Estrogen-dependent symptomatic vulvo-vaginitis
- IE09 Recent (<1year) or active renal stone disease
- IE10 Urinary tract structural abnormalities
- IE11 Obesity or malnutrition (BMI <18.5 Kg/m<sup>2</sup> and >30 Kg/m<sup>2</sup>)
- IE12 Pregnant or lactating women
- IE13 Plans of any of the following 12 months after screening visit to become pregnant
- IE14 Menopausal and perimenopausal women
- IE15 Subjects general treated with drugs which can modify measurements performed in the study, in particular the assessment of the hydration status (diuretic intake, corticoids or treatment interfering with metabolism and nutrition behavior)
- IE16: Subjects with severe or uncontrolled organic disease, likely to interfere with the parameters of the study (e.g. neoplastic, cardiovascular, pulmonary and digestive disorders, unstabilized diabetes type I and II, untreated or uncontrolled clinically significant arterial blood hypertension) or mental disorders affecting eating and drinking behavior (i.e. primary polydipsia, bulimia nervosa, psychosis etc.)
- IE17: Women who have taken part in any other clinical study for the treatment of rUTI during the last 12 months
- IE18: Vulnerable subjects defined as individuals whose willingness to volunteer in the clinical trial may be unduly influenced by the expectation, whether justified or not, of benefits associated with participation, or of a retaliatory response from senior members of a hierarchy in case of refusal to participate. (Examples are members of a group with a hierarchical structure linked to the Investigator or to the Sponsor, such as medical, pharmacy, dental and nursing students, subordinate hospital and laboratory personnel, employees of the Investigator or of the Sponsor, members of the armed forces, and persons kept in detention)
- IE19: No legal capacity or limited legal capacity or unable to give an informed consent
- IE20: Subjects unlikely to cooperate in the study, and/or poor compliance anticipated by the Investigator

*\* presence of bacteriuria in urine ( $\geq 10^3$  cfu/mL of a uropathogen in midstream urine culture) revealed by quantitative culture or microscopy in a sample taken from a patient or the typical symptoms of lower or upper urinary tract infection (defined as  $\geq 3$  urinary symptoms (difficult, painful, urgent and frequent urination, hematuria, supra-pubic pressure, increase in the number of white blood cells ( $>10.000/ml$ ) in the urine, with or without bacterial infection)).*

*The presence of symptomatic bacteriuria can be established with a single urine sample*

*Data source available at <http://www.sign.ac.uk/pdf/sign88.pdf>*

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*6.2.3 Randomisation criteria (checked at the randomisation visit – V2)*

Subjects may only be randomised in this study after meeting the inclusion criteria and presenting none of the exclusion criteria stipulated in paragraphs 6.2.1 and 6.2.2 above.

In addition, the subject must meet the following randomisation criteria checked during the randomisation visit V2:

- RI01: Negative urine culture and asymptomatic state (i.e. silent phase) at V2 (randomization visit)
- RI02: Low-drinker confirmation (24 hours urinary volume < 1.2 L per day)

*6.2.4 Non -Randomisation criteria (checked at the randomisation visit – V2)*

A potential subject who meets any of the following criteria at V2 will be excluded from participation in this study:

- RE01: Chronic kidney disease (defined as decreased GFR (GFR<60 ml/min/1.73m<sup>2</sup> calculated using EPI equation)
- RE02: Women suffering multiple antibiotic resistant bacterial strain<sup>§</sup>

<sup>§</sup> Multiple antibiotic resistance is defined as resistance to 3 or more different antibiotics.

**6.3 SUBJECT IDENTIFICATION**

*6.3.1 Subject Identification Number*

At V1, after the subject and the Investigator have personally dated and signed the Informed Consent Form, a Case Report Form (e-CRF) is created and a subject identification number is allocated manually to her in chronological order of inclusion. This number will be the main identification code for a subject for the duration of the study in order to protect the subject's identity and will appear throughout the eCRF for that particular subject. This number should also be documented on other relevant paper-based study documents where required (i.e. Quality of Life, Subject Diaries, source data in the Subject Medical File).

The Subject Identification number consists of 6 digits, which is based on:

- A 3 digit "Country number (will be 100)
- A 3 digit "Site" number (will be "001").
- A 3 digit "Subject" number. The first subject included at site will receive the number "001". Subject numbering will then be sequentially increased by one for each new included subject (example 002, 003 etc.).

**Example:** Subject Identification number "100-001010", refers to Site "001" and Subject "010".

*6.3.2 Randomisation Process*

Refer to section 10.1

**6.4 SUBJECT DISCONTINUATION**

*6.4.1 Criteria for subject discontinuation*

The Investigator should discontinue subject's participation in the study prematurely in the following situations:

- In the case where a subject has decided to resign from further participation in the study (withdrawal of consent) or
- In the case where further participation is a health risk for the subject, at the Investigator's discretion or

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- In the case of subject's pregnancy or
- In the case where a severe non-compliance to protocol or a protocol violation has been identified for the subject or
- In the case where the subject is lost to follow-up or
- In the case whereby the study is prematurely terminated or suspended by the sponsor.

*6.4.2 Replacement conditions*

Subjects withdrawing prematurely from the study after randomisation will not be replaced.

*6.4.3 Procedures in case of subject discontinuation*

In the event of premature withdrawal, the Investigator must notify the study monitor as soon as possible, and should make all efforts to contact the subject and if the subject agrees, ensure that all evaluations scheduled for the End of Study Visit (V4) are completed and reported in the eCRF.

Alternative follow-up of the discontinued subject is to be arranged by the Investigator if necessary.

For subjects who discontinue the study due to the occurrence of adverse events potentially related to the study product or to the study procedures, follow-up will take place (clinical or biological examinations) until the adverse event has abated, or until a stable situation has been reached (cf section 12), with findings being recorded in the CRF.

For subjects who discontinue the study due to pregnancy, follow-up will take place until birth or early termination of pregnancy. In addition to documenting this premature termination in the CRF, the Investigator should make every effort to collect information about the pregnancy and the infant and report to the Sponsor with a dated and signed Pregnancy Reporting Note as specified in the section 12.6.

**6.5 INSTRUCTIONS AND RESTRICTIONS DURING THE STUDY**

*6.5.1 Study product(s) consumption instructions*

The Investigator will provide the subject with the instructions on the study product consumption at the randomisation visit (V2).

Study intervention group:

The subject allocated in the study product intervention group will begin consumption of the study product the morning of the following day of the randomisation visit (V2) and will stop consumption the evening of the day before the last visit.

Throughout the entire fifty-two -week consumption period (365 days) of the study, the subject allocated in the intervention group will daily consume three (3) bottles of the test product (500 ml of commercialized Natural mineralized Evian water), one bottle of mineral water at the beginning of every meal (breakfast, lunch and dinner) and fully drink them before the following meal (About 500ml around the main meals).

At the same time, the subjects will be receiving coaching messages related to the fluid intake recommendations by e-mail, phone calls or directly from the Investigator during the study visits.

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The subjects will receive regularly and free of charge the bottles of mineral water at home and will enter their daily consumption into a paper diary (The Subject Compliance Diary).

The subject will report every day her consumption in the subject compliance diary, specifying the number of bottles consumed during the day and the quantity of water drunk for each bottle (total bottle, ¼ of bottle, ½ of bottle).

The water consumption will be checked at each visit by the Investigator (telephone visits and site visits) and a summary of the water consumption will be entered into the eCRF. The investigating site will also collect the paper subject compliance diaries in order to be able to check the information in case of inconsistencies.

Control group:

The subject allocated in the control group will remain stable in their fluid consumption. Their compliance will be assessed every month, using the 3 days fluid intake questionnaires.

They will not receive any water bottle nor any coaching support.

*6.5.2 Dietary instructions*

During the entire duration of the study (i.e. from V2 to V4), subjects will not have any dietary restriction with the exception of cranberries juice and extracts. All women included in the study will be asked not to consume these products from 2 weeks before the inclusion visit to the end of the study period.

The subjects will also be asked to avoid making any significant changes to their usual diet during the study period (i.e. no changes to the usual amount of fibre, no commencement of a weight loss diet etc).

The dietary restrictions stipulated in this study do not represent a risk for the subjects. There is no risk of deficiency in the nutrient related to this dietary restriction.

*6.5.3 Non-authorized medicinal products*

During all the study, all medicinal products are authorised excepting treatments listed in the exclusion criteria (anti-coagulants, corticoids, diuretics, laxatives or treatments interfering with metabolism and nutrition behaviour, cranberry juice or extracts)

Included subjects will be asked not to take any antibiotic therapy in the 2 weeks before the inclusion visit (V1), to avoid any inclusion bias due to the presence of a UTI masked by the use of antibiotics.

In case of any non-authorized medicinal products consumption or any non-authorized medical treatments, the subject will be asked to complete her subject diary with the name, the dose and the intake dates of the medicinal products or medical treatment. The subjects will be advised to contact the Investigator or the study nurse for recommendations.

## **7. STUDY PROCEDURES**

### **7.1 EVALUATIONS AND PROCEDURES PER VISIT**

Evaluations and procedures for the study visits are described below. See also the study flow chart in the Section 2.

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### 7.1.1 Inclusion Visit (Visit 1, Week –2 to –4)

This visit will take place within from –2 to –4 weeks prior to randomization visit (Visit 2).

At the beginning of the visit, the Investigator should fully inform the subject of all pertinent aspects of the clinical study including the written information and the approval by the IRB/IEC. The subject must be given the opportunity to ask questions and have them answered by the Investigator.

Prior to subject's participation in this clinical study (including screening phase), the subject must personally sign and date the written informed consent form. The subject cannot undergo any medical or biological procedures required by the clinical study before having personally dated and signed the Informed Consent Form. The Investigator must also sign and date the Informed Consent Form.

After the subject and the Investigator have personally dated and signed the Informed Consent Form, a subject identification number is allocated to her and a Case Report Form (CRF) is created.

The eligibility of the subject needs to be checked at the beginning of the visit and the tasks performed during the Visit 1 are the following (please refer to the flow chart in the section 2):

- Obtaining date and signature of subject on written informed consent form,
- Recording subject's demographic data (date of birth, gender),
- Reviewing the past medical and surgical history,
- Reviewing past and current medications history,
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,
- Requesting a urinary pregnancy test,
- Recording vital signs: systolic/diastolic blood pressure and heart rate, body temperature,
- Recording anthropometrical parameters; body weight and height, waist circumference,
- Checking all the inclusion and exclusion criteria for subject's eligibility validation,
- Checking symptoms of UTI
- Performing urine culture for symptomatic subjects and assign a therapy in case of acute episode,
- Providing instructions for subject's diary completion (including documentation of adverse events, instruction on completion of the 3 day fluid intake diary),
- Providing urine collection kits and instructions for 24h urine collection and transport logistics,
- Providing instruction on the voiding diary to fulfil during the 24h urine collection,
- Scheduling study visits for subjects who are eligible and available for the duration of the study.

The results of the pregnancy test must be negative and available prior to randomization of the subject.

If symptomatic subjects show positive urine culture (confirming the presence of UTI) they will be sent home with a standardised treatment against their acute episode (i.e. AFU/EAU guidelines for UTI management) and they will be ask to come back for the randomization visit (V2) after 4 weeks.

Asymptomatic women at V1 will be asked to come back for the randomization visit (V2) after two weeks.

If the Visit 1 eligibility criteria are respected, and symptomatic subjects show positive urine culture (confirming the presence of UTI) they will be delivered with:

- A treatment against their acute episode (i.e. AFU/EAU guidelines for UTI management),
- A urine collecting kit in order to collect one 24h urine samples during the day before the following visit (V2; randomization visit). These 24h urine samples will be returned to the site at the next visit (Visit 2),

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- A voiding diary, to collect frequency of voiding during the 24h urine collection.
- A 3 day fluid intake diary

These subjects will be asked to come back for the randomization visit (V2) after 4 weeks.

If the Visit 1 eligibility criteria are respected, and subjects are not symptomatic, they will be delivered with:

- A urine collecting kit in order to collect one 24h urine samples during the day before the following visit (V2; randomization visit). These 24h urine samples will be returned to the site at the next visit (Visit 2),
- A voiding diary, to collect frequency of voiding during the 24h urine collection.
- A 3 day fluid intake diary

These subjects will be asked to come back for the randomization visit (V2) after 2 weeks.

The subject will receive instructions on urine collection procedure.

Furthermore, the subject will be instructed to fill in the 3 days fluid intake questionnaire, during 3 consecutive days, on Saturday-Sunday-Monday, between Visit 1 and Visit 2 in order to:

- Verify the inclusion criteria II03: Low-drinker (< 1.5 L fluids per day)
- Evaluate the consumption of water and the total fluid intake before randomization

They will be also asked to document any adverse and serious adverse event.

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents are accurate and complete (refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit.

### 7.1.2 Randomisation Visit (Visit 2, Month 0)

The randomisation visit will take place at Day 0 (2 to 4 weeks after the inclusion visit).

At the randomisation visit, the subjects will bring back the following:

- 24h Urine samples
- Voiding diary
- 3 day fluid intake diary

The eligibility of the subject needs to be checked at the beginning of the visit and the tasks performed during the randomisation visit are the following (please refer to the flow chart in the section 2):

- Conducting a physical examination,
- Recording vital signs: systolic/diastolic blood pressure and heart rate, body temperature,
- Recording anthropometrical parameters; body weight , waist circumference,
- Checking all the randomization and non-randomization criteria,
- Reviewing V1-V2 subject's 3 day fluid intake questionnaire,
- Checking and recording of adverse events and of concomitant medications,
- Collecting of blood samples (after 8-12 hours of fasting),
- Collecting of 24h urine container (urine has been collected during the 24h before the visit),
- Collecting the voiding diary,
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,

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- Checking symptoms of UTI,
- Performing urine culture for all subjects to assess the absence of any acute UTI episode,
- Providing instructions for subject's diary completion (including documentation of adverse events, instruction on completion of the 3 day fluid intake diary and compliance diary),
- 
- Providing urine collection kits and instructions for 24h urine collection and transport logistics,
- Providing instructions for completing the QoL questionnaire (SF-12 V2),
- Providing instructions for study product consumption,
- Reminding instructions for urine collection and transport logistics (refer to section 7.4.2.6 for instructions to be provided),
- Providing instruction on the voiding diary to fulfil during the 24h urine collection,
- Delivery of V2-V3 subject's diary (only for the intervention group),
- Delivery of V2-V3 3 day fluid intake diary
- Delivery of 24h urine collection kits.

If the subject meets the randomisation criteria, and none of the non-randomisation criteria is confirmed, then the subject will be randomised into one of the 2 groups. Following the assignment of a randomisation number, the subject will be provided with:

- The recommendation to consume 1,5L/day (3 bottles of mineral water) starting from the morning after V2 to the day of the V4, in addition to their usual fluid intake consumption (only for the intervention group),
- A V2-V3 compliance diary for collecting self-reported subject data on compliance with the study product,
- A V2-V3 3 day fluid intake diary
- A urine collecting kit in order to collect one 24h urine samples during the day before the following visit (V3). These 24h urine samples will be returned to the site at the next visit (Visit 3),
- A voiding diary, to collect frequency of voiding during the 24h urine collection,
- The SF-12 V2 QoL questionnaire, to complete in situ but not in front of the Investigator,
- The Investigator will check the SF-12v2 questionnaire completion.

Two days after V2, after receiving lab reports of for eGFR and urine culture, the Investigator will assess eligibility with respect to the randomisation and non-randomisation criteria and will randomize the subject, if eligible.

If the subject is in the intervention group, the Investigator will inform the logistics company in order to provide the subject with the study products. The Investigator will inform the subject that she will receive the study products, she has to be compliant to the intervention (1,5L study product/day in addition to her normal water consumption) and she has to monitor the study product consumption in the paper compliance diary in addition to the 3 day fluid intake diary

If the subject is in the control group, the Investigator will call the subject and inform her that she has to continue her life habits and she doesn't need the paper compliance diary. She will have to continue filling in the 3 day fluid intake diary

Between V2 and V3, the subject in the intervention group has to send to the Investigator site by post the paper compliance diary corresponding to the previous 30 days of study product consumption and the 3day fluid intake diary The subject in the control group will have to return by post every month the 3 day fluid intake diary.

At the next visit (V3), the subject will be asked to bring back to the site: the last section of the V2-V3 paper subject compliance diary (for the intervention group), the last 3 day fluid intake diary, the 24h urine samples and the voiding diary.

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The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents need to be accurate and complete (refer to the section 9). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit.

*7.1.3 Evaluation Visit– Visit 3 (Month 6, +/- 10 days)*

This visit will take place 6 months (+/- 10 days) after the randomization visit (Visit 2).

At this evaluation visit, the subjects in both groups will bring back the following:

- 24h Urine samples
- Voiding diary
- The 3 day fluid intake diary

In addition, the subjects in the intervention group will bring the V2-V3 compliance diary for collecting self-reported subject data on compliance with the study product.

The tasks performed during the evaluation visit are the following (please refer to the flow chart in the section 3):

- Recording vital signs: systolic/diastolic blood pressure and heart rate, body temperature,
- Recording anthropometrical parameters: body weight, waist circumference,
- Reviewing V2-V3 subject's 3 day fluid intake diary,
- Checking and recording of adverse events and of concomitant medications,
- Collecting of blood samples (after 8-12 hours of fasting),
- Collecting of 24h urine container (urine has been collected during the 24h before the visit),
- Collecting the voiding diary,
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,
- Checking symptoms of UTI,
- Performing urine culture for all subjects presenting symptoms of UTI,
- Reminding instructions for subject's diary completion (including documentation of adverse events, instruction on completion of the 3 day fluid intake diary and compliance diary),
- Providing urine collection kits and instructions for 24h urine collection and transport logistics,
- Reminding instructions for completing the QoL questionnaire (SF-12 V2),
- Reminding instructions for study product consumption,
- Reminding instructions for urine collection and transport logistics (refer to section 7.4.2 for instructions to be provided),
- Providing instruction on the voiding diary to fulfil during the 24h urine collection,
- V3-V4 subject's compliance diary,
- V3-V4 3 day fluid intake diary
- Delivery of 24h urine collection kits.

At the end of the evaluation visit, the subject will be provided with:

- A V3-V4 compliance diary for collecting self-reported subject data on compliance with the study product if in the intervention group
- A V3-V4 3 day fluid intake diary
- A urine collecting kit in order to collect one 24h urine samples during the day before the following visit (V3). These 24h urine samples will be returned to the site at the next visit (Visit 3), for both groups
- A voiding diary, to collect frequency of voiding during the 24h urine collection, for both groups
- The SF-12 V2 QoL questionnaire, to complete in situ but not in front of the Investigator for both groups
- The Investigator will check the SF-12v2 questionnaire completion

Between V3 and V4, the subject in the intervention group has to send to the Investigator site by post the compliance diary corresponding to the previous 30 days of study product consumption and the 3 day fluid intake diary. The subject in the control group will return by post on monthly basis, the 3 day fluid intake diary



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At the next visit (V4), the subject will be asked to bring back to the site: the last part of the V3-V4 compliance diary (for the intervention group only), the 3 day fluid intake diary, the 24h urine samples and the voiding diary.

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents need to be accurate and complete (refer to the section 9). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit.

*7.1.4 Phone calls (Month 1, +/- 7 days, Month 2, +/- 7 days, Month 3, +/- 7 days, Month 4, +/- 7 days, Month 5, +/- 7 days, Month 7, +/- 7 days, Month 8, +/- 7 days, Month 9, +/- 7 days, Month 10, +/- 7 days, Month 11, +/- 7 days)*

Every month women will be contacted by phone by the principal Investigator, to check the compliance with the intervention (if in the intervention group), adverse events, symptoms of UTI and, eventually, start and end date of episodes of UTI.

Study personnel will call the subject at the following time schedule:

- Phone call 1 (Month 1, +/- 7 days), between Visit 2 and Visit 3,
- Phone call 2 (Month 2, +/- 7 days), between Visit 2 and Visit 3,
- Phone call 3 (Month 3, +/- 7 days), between Visit 2 and Visit 3,
- Phone call 4 (Month 4, +/- 7 days), between Visit 2 and Visit 3,
- Phone call 5 (Month 5, +/- 7 days), between Visit 2 and Visit 3,
- Phone call 6 (Month 7, +/- 7 days), between Visit 3 and Visit 4,
- Phone call 7 (Month 8, +/- 7 days), between Visit 3 and Visit 4,
- Phone call 8 (Month 9, +/- 7 days), between Visit 3 and Visit 4,
- Phone call 9 (Month 10, +/- 7 days), between Visit 3 and Visit 4,
- Phone call 10 (Month 11, +/- 7 days), between Visit 3 and Visit 4,

The objective of each phone call will be:

- Assessing the compliance with the intervention (if in the intervention group); for the 1<sup>st</sup> phone call, the investigator will ask the date of the 1<sup>st</sup> product consumption
- Assessing the level of fluid intake with the intervention based on subject's interview (if in the intervention group),
- Assessing the occurrence of adverse events based on subject's interview,
- Assessing whether any concomitant medications have been taken or modified,
- Assessing the presence of symptoms of UTI,
- Eventually assess the starting and end date of a UTI episode,
- Checking sexual life status and emptying bladder habits after sexual intercourses.

Each phone call must be recorded in the subject's source documentation (refer to the section 8). The subject's / e-CRF should be completed from the source documents and as soon as possible after the phone call.

*7.1.5 End of Study Visit – Visit 4 (Month 12, +/- 10 days)*

This visit will take place 12 months (+/- 10 days) after the randomization visit (Visit 2).

At this evaluation visit, the subjects will bring back the following:

- 24h Urine samples
- Voiding diary
- V3-V4 compliance diary for collecting self-reported subject data on compliance with the study product.

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- The 3 day fluid intake diary

The tasks performed during the evaluation visit are the following (please refer to the flow chart in the section 3):

- Conducting a physical examination,
- Recording vital signs: systolic/diastolic blood pressure and heart rate, body temperature,
- Recording anthropometrical parameters: body weight, waist circumference,
- Reviewing V3-V4 subject's 3 day fluid intake diary,
- Checking and recording of adverse events and of concomitant medications,
- Collecting of blood samples (after 8-12 hours of fasting),
- Collecting of 24h urine container (urine has been collected during the 24h before the visit),
- Collecting the voiding diary,
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,
- Checking symptoms of UTI,
- Performing urine culture for all subjects,
- Reminding instructions for completing the QoL questionnaire (SF-12 V2),
- Checking for QoL questionnaire completion.

At the end of the evaluation visit, the subject will be provided with:

- The SF-12 V2 QoL questionnaire, to complete in situ but not in front of the Investigator
- The Investigator will check the SF-12v2 questionnaire completion

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents need to be accurate and complete (refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit.

#### *7.1.6 Relapse visit*

This visit will take place during the intervention period, in case of UTI event.

The tasks performed during the evaluation visit are the following (please refer to the flow chart in the section 3):

- Performing urine culture for all subjects to confirm preliminary diagnosis
- Subjects will then be sent home with a standardised treatment against their acute episode (i.e. AFU/EAU guidelines for UTI management) irrespective of the study group they belong to
- Performing urine culture at the end of the therapy to be sure that women are completely recovered
- Reporting start and end dates of event
- Collecting data relative to healthcare costs due to illness at the end of event
- Collecting concomitant treatment

#### *7.1.7 Follow-up Visit*

Not applicable

#### *7.1.8 Early Subject's Discontinuation.*

In the event of early subject's discontinuation from the study (as specified in section 7.4), the Investigator should make every effort to entirely conduct the End of Study Visit evaluations and procedures (refer to section 6.4.3).

For all subjects who were randomised in the study, the reason(s) for early termination of the subject prior to completion of the study must be stated in the subject's source documentation and reported in the e-CRF.

The site(s) will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents need to be accurate and complete (refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit.

End of study form must be filled for all subjects that have signed the Informed Consent with available information. For screen failures this should minimally include: informed consent date, demographics, inclusion criteria, exclusion criteria and end of study form.

## **7.2 SUBJECT VISIT WINDOW**

For each subject's visit, allowable windows are defined and stated in the previous section (7.1) and in the table of the study procedures and stages as outlined in the section 2.

## **7.3 STUDY FLOW-CHART**

The table of the study evaluations, procedures and stages is outlined in the section 2.

## **7.4 STUDY ASSESSMENTS**

### *7.4.1 Clinical Assessments*

#### *7.4.1.1 Medical and Surgical History*

The Investigator will interview the subject with respect to her history of concomitant diseases, surgery. The history of renal diseases and treatments will be verified in depth and specific questions on the symptomatology, duration and management of the UTIs will be addressed. All information will be recorded in source data and reported into the e-CRF.

#### *7.4.1.2 Medications and Nutritional Supplements History*

The Investigator will check if the subjects consume the following products forbidden by the protocol:

- Cranberry juice during the last 2 weeks
- Antibiotics during the last 2 weeks
- Chronic anti-coagulant therapy
- Diuretics
- Corticoid treatment
- Chronic laxative treatment

In addition to this all concomitant medication taken within 2 weeks will be documented in the source documentation.

#### *7.4.1.3 Physical Examination*

The physical examination includes an assessment of general appearance and a review of systems (gastrointestinal, cardiovascular, OtoRhinoLaryngological (ORL), Neurological, Dermatological, Musculoskeletal, Urological/Nephrological,)

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**7.4.1.4 Vital Signs**

The following vital signs will be measured:

- Blood pressure (BP) (systolic and diastolic [mmHg]),
- Heart rate (HR) (beats per minute [bmp]),
- Axillary body temperature.

The measurement of blood pressure is performed after resting for at least 5 minutes, in sitting position.

**7.4.1.5 Demographics**

Demographics include date of birth and gender.

**7.4.1.6 Anthropometry (Body weight, waist circumference, Height)**

- Body weight is measured to the nearest 0.1 kg using a calibrated weighing scale without outerwear and shoes
- Body height is recorded to the nearest 1 cm
- Body weight and height are used to calculate the Body Mass Index (BMI) as followed:  
BMI = weight / (height)<sup>2</sup> where weight is in kilogram and height in meter.

**7.4.1.7 Other Clinical Assessment**

Not applicable.

**7.4.2 Laboratory Assessments**

Normal ranges and laboratory/technical procedures for clinical laboratory parameters are made available by the laboratory(ies) before start of the study in the Laboratory Manual.

**7.4.2.1 Clinical Laboratory Assessments**

Laboratory measurements will include the following parameters assessed per visit:

Parameters	V1	V2-1day	V2 rando (M0)	V3 (M6)	V4 (M12)	Extra visits
<b>Blood parameters</b>						
Lipidic profile**			X		X	
glycosylated hemoglobin			X		X	
serum creatinine			X	X	X	
estimated glomerular filtration rate (eGFR)			X	X	X	
Urea			X		X	
copeptin			X	X	X	
<b>24h urine parameters</b>						
Sodium		X		X	X	
Potassium		X		X	X	
Calcium		X			X	
Magnesium		X			X	
Chloride		X			X	
Oxalate		X			X	
Citrate		X			X	
Urine Specific Gravity		X		X	X	

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Osmolality		X		X	X	
Urine color		X		X	X	
pH		X		X	X	
Creatinine		X		X	X	
Volume		X		X	X	
Number of micturitions		X		X	X	
<b>Spot urine parameter</b>						
Urine Pregnancy Test	X					
Urine culture	X*		X		X	X

\* If symptomatic

\*\* Lipidic profile: HDL, LDL, cholesterol, tryglicerides

*7.4.2.2 Pregnancy Test*

Women of childbearing potential must have a negative pregnancy test. A urine pregnancy test will be used and results must be available prior to administration of study product. The Investigator is responsible for ensuring that the subject's pregnancy status is recorded accurately in the source documents. The Investigator will record the results of the pregnancy test in the subject's e-CRF.

*7.4.2.3 Special Assays or Procedures*

Not applicable

*7.4.2.4 Instructions for Specimen Preparation, Handling, and Storage*

*7.4.2.5 Blood Sample Collection and Handling*

Blood sampling will not exceed 20 mL per visit and will be taken after a period of fasting of 8-12 hours at visits V2, V3 and V4. This volume includes the volume required for back up samples in case of retest analysis. The blood sampling will be done at the Investigator site.

If fasting condition is not confirmed, subject has to come back for re sampling during a new visit scheduled within a maximum of 2 days after the initial scheduled visit in fasted state.

Normal ranges for all blood parameters are made available by the laboratory before start of the study in the Laboratory manual.

All blood samples will be processed, stored and transported under the best conditions in order to ensure the samples stability.

*7.4.2.6 Urine Sample Collection and Handling*

The 24h urines will be collected in a specific container during 24h and stored by the subject at about +4°C to ensure the stability of parameters analysed. In order to ease sample collection and homogenise the number of sample and time collection of each subject, urines are collected as follows: the first urine of the day is excluded. The collection includes all urinations in the following 24h including the first morning urine of the day after.

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If at least one micturition is not collected in the 24h urine collection, subject will be asked to do a new 24h urine collection and to come back for a new visit scheduled the following day after initial visit.

As some 24h urine parameters are impacted by UTI, if an extra-visit for UTI takes place at V3 or V4, a new 24h urine sampling has to be performed by patient once the urine culture is negative.

The subjects will bring back their urine collection to investigating site the morning of V2, V3 and of V4.

The urine samples will be processed, stored and transported under the optimal conditions in order to ensure the samples stability.

Urinary concentrations and excretions of sodium, potassium, calcium, magnesium, chloride, oxalate, citrate, creatinine, will be evaluated on 24h urine sample.

Urine volume, osmolality and pH

24h urine collection will be weighted and analyzed for their USG, osmolality and pH. The weight of the 24h urine collection and the USG will be performed in order to assess the urinary volume.

Urine colour

24h urine collected will be evaluated according to the Urine Color Chart provided to the investigating site. Urine Color Chart was originally published in Lawrence Armstrong's book titled Performing in Extreme environments. The scientific validation of this Urine Color Chart may be found in Armstrong, 1994 and Armstrong, 1998.

This assessment will be done in order to assess the change in urinary hydration markers following changes in water intake habits.

Urine culture and MIC determination

The collection will be performed from the 1st morning urine in midstream urine by clean catch method. Urine culture is performed to confirm the presence of UTI and determine the bacterial origin of the UTI. For the bacteria identified, a Minimum Inhibitory Concentration test (MIC test) will be performed in order to determine the bacteria resistance or susceptibility to antibiotics. The list of antibiotics is chosen on the result of Gram coloration (i.e.: Gram +/-).

In case of positive urine culture, an antibiotic treatment will be administered according to Investigator prescription. A new urine culture will be performed after the treatment in order to verify if urine culture is negative.

*7.4.2.7 Instructions for Specimen Shipments*

Frequency of shipment, labelling requirement and any special instructions will be defined before study start and specified in Laboratory Manual.

*7.4.2.8 Back up samples*

The remaining back up blood and urine samples will be kept by DANONE RESEARCH at a central laboratory facility, selected and contracted by DANONE RESEARCH These remaining back up blood and urine samples may

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be used for future researches such as the performance of additional analyses and/or the development of new analytical methodologies. In this case, this will be done under the confidentiality rules. In any case, these samples will not be used for human genetic analysis.

The subject will be informed, via the ICF, that the unused blood and urine samples will be frozen and stored for a maximum of 15 years, in order to make these samples available for repeated measurements as a substitution of analysis mistakes, or in case of development of new methodologies of analysis.

#### **7.4.3** *Other Assessments*

Not applicable.

##### **7.4.3.1** *Subject's Compliance with Study Product Intake*

The subject's compliance for study product intake will be assessed by the Investigator throughout the study using the following information:

- Subject's self-reported data of product intake into the paper diary,
- Subject's self-reported data on total fluid intake into the diary (3 consecutive days before each visit),
- The urine volume, osmolality and color measured at V2, V3 and V4.

For the product consumption control, the subject will record in a paper diary the volume of study product consumed (Evian® water) on a daily basis by specifying the number of bottles and the volume of water consumed from each bottle.

The subject will be asked to return her paper diary to the site each month by post. At each Telephone visit, the Investigator will review the subject's paper diary.

At the same time the Investigator site will receive the acknowledgment of receipt for the delivery of study product to the subject.

##### **7.4.3.2** *Dietary <Restrictions/Recommendations> and non-authorized products.*

Women included in the study will be asked not to consume any cranberries juice and extracts as well as any other nutritional complement which can impact the outcome of the study in the 2 weeks prior to the inclusion visit and for the all duration of the study.

## **8. SOURCE DATA AND SOURCE DOCUMENTS**

### **8.1 SOURCE DATA DEFINITION**

According to ICH GCP (E6), source data is all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

### **8.2 SOURCE DOCUMENTS DEFINITION**

According to ICH GCP (E6), source documents are original documents, data, and records (e.g., medical / hospital records, clinical and office charts, laboratory notes, memoranda, general practitioner letter, questionnaire used for

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diagnosis, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, X-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial).

### **8.3 SOURCE DATA MANAGEMENT**

For the following items, the data can be entered directly in the CRF/e-CRF, which will be considered as the source document:

- None

### **8.4 RIGHT OF ACCESS TO SOURCE DATA/DOCUMENTS**

The Investigator/institution should permit study-related monitoring, audits, IRB/IEC review and regulatory inspection(s), providing direct access to source data/documents.

In accordance with the Law, the subjects who wish may have access to their personal data. They must address their request to the Investigator in writing.

## **9. STUDY PRODUCT**

### **9.1 STUDY PRODUCT(S) DESCRIPTION AND COMPOSITION**

#### *9.1.1 Study product(s) description*

The study product is a low mineralised natural mineral water. It is manufactured according to the DANONE's quality policy in the factory of Evian® (France).

The study products are intended for oral use only and within the standard consumption patterns for physiological needs.

#### *9.1.2 Study product(s) composition*

Study product analyses during the study  
Not applicable.

<b>Name</b>	<b>Natural mineral water</b>
<b>Product Brands</b>	<b>Evian®</b>
<b>Type of Source Water</b>	<b>Cachat source (Evian, France)</b>
<b>Type of Packaging</b>	0.5L bottles made of polyethylene terephthalate [PET] represent the test unit
<b>pH, (pH units)</b>	<b>7.2</b>
<b>Dry Residue 180°C, mg/l</b>	<b>309</b>
<b>Silica, mg/l</b>	<b>15</b>
<b>Sodium, mg/l</b>	<b>6,5</b>
<b>Potassium, mg/l</b>	<b>1</b>
<b>Calcium, mg/l</b>	<b>80</b>
<b>Magnesium, mg/l</b>	<b>26</b>



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<b>Chlorides, mg/l</b>	<b>6,8</b>
<b>Bicarbonates, mg/l</b>	<b>360</b>
<b>Sulphates, mg/l</b>	<b>12,6</b>
<b>Nitrates, mg/l</b>	<b>3,7</b>

## **9.2 STUDY PRODUCT(S) LABELLING AND PACKAGING**

Boxes and bottles are labelled in accordance with applicable laws and regulation. Primary labels (on water bottles) are printed in French by default. Secondary labels (on carton boxes) will be written in local language (Bulgarian).

### **(a) Primary packaging (water bottle)**

The products are packaged in alimentary plastic bottles of 500mL. The primary labels affixed to these bottles contain the following information:

- Expiry date : DD/MM/YYYY
- Batch number : ZZZZ
- Content : 500 ml
- Storage temperature or conditions

### **Secondary packaging (carton box):**

The water bottles are gathered in batches of 24 units with the following information:

- Expiry date (DD/MM/YYYY)
- Study code / Name and address of Sponsor and Investigator
- Batch code allowing identification of the contents
- Weight
- Storage temperature or conditions
- Sentence: 'to be used for clinical study only'

NB: Labels are printed in Bulgarian.

## **9.3 SHIPMENT, STORAGE, DISPENSING, ACCOUNTABILITY AND DESTRUCTION**

### **9.3.1 *Shipment of study product(s)***

Study products are commercial products of the DANONE group and provided by DANONE RESEARCH.

The water bottles distributed to the subjects are manufactured, stored and delivered in accordance with the current sanitary regulations.

Study products are delivered in boxes and are identified as follows:

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- Name and address of sender
- Name and address of recipient
- Study code
- Batch number
- Type and Identification of products
- Storage conditions

Study products are accompanied by:

- An acknowledgement of receipt to be signed and returned by the consignee (subject) upon receipt of products and will be delivered to the investigating site by the logistics company.

*9.3.2 Storage of study product(s)*

The Investigator site ensures that the study products are stored in a temperature controlled, secure and closed area in accordance with the indications stated on the packaging for the products to be used.

The investigating site will ensure that the products are distributed to the subjects' homes in a timely manner and appropriate conditions. The subjects will be informed about water bottles storage conditions. The water bottles delivered should be used only for the study purposes.

*9.3.3 Delivery/Dispensing of study product(s)*

An external shipping company will deliver the water bottles at each subject enrolled in the study and randomised in the intervention study group. The Sponsor and the Investigator must ensure that water bottles are received under good condition by the subjects.

The subjects will sign an acknowledgment of receipt and will report any product quality issue to the delivery company. The subjects will dispose the empty bottles together with the household waste.

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**9.3.4 Accountability of study product(s)**

Delivery records from the transport company will be used to ensure the quantity of study product delivered to each study participant. The acknowledgement of receipt will be sent to the Investigator by the carrier.

A check between the acknowledgement of receipt and the recommended quantity of product delivery at subject home will be done by the CRA at each monitoring visit.

The Investigator will check the reported quantity of water consumed by the subject in case of compliance deviation, he will check the acknowledgement of receipt for the deliver quantity.

**9.3.5 Destruction of study product(s)**

The Evian® empty water bottles will be disposed by the subjects together with their household waste.

The Investigator must instruct the subject that in case of detected or suspected study product abnormalities, the subject must not consume the related study products and return them to the investigational site.

**10. RANDOMISATION AND UNBLINDING**

**10.1 RANDOMISATION (AND STRATIFICATION IF APPLICABLE)**

After having checked all eligibility criteria at the randomization visit, the subject will be allocated to the study arm through the randomization system (IWRS), without any stratification factor.

**10.2 UNBLINDING PROCEDURE**

Not applicable, open label study.

**11. PRODUCT EFFECT PARAMETERS AND PRODUCT SAFETY PARAMETERS**

**11.1 DESCRIPTION OF PRODUCT EFFECT PARAMETERS**

The study intervention effect parameters are assessed by clinical parameters and laboratory parameters analysed in blood and urine.

<b>Product Effect parameters</b>	<b>Visit 1</b>	<b>Visit 2</b>	<b>Visit 3</b>	<b>Visit 4</b>	<b>Relapse visits</b>
Symptoms of UTI	X	X	X	X	X
Urine culture (bacteriogram if urine culture positive)	X ONLY IF SYMPTOMS	X		X	X
Frequency of micturitions during 24h urine collection		X	X	X	
Urine hydration markers (volume, USG, pH, osmolality,		X	X	X	

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urine colour)					
Fluid intake (daily volume)		X	X	X	
QoL questionnaire (SF-12V2)		X	X	X	
Antibiotic prescription data	X	X	X	X	X
Healthcare cost data					X

**11.2 DESCRIPTION OF PRODUCT SAFETY PARAMETERS**

The goal of the study intervention is to achieve a mean daily urine osmolality equivalent to that of plasma (approximately 285 mosm/kg). This osmolality goal is selected because it defines the transition from dilute to concentrated urine and is a safe end point under most any circumstance.

The safety parameters assessed are the urine osmolality and the urine concentration of sodium and potassium.

<b>Product Safety parameters</b>	<b>Visit 1</b>	<b>Visit 2</b>	<b>Visit 3</b>	<b>Visit 4</b>
Urine osmolality		X	X	X
Urine sodium		X	X	X
Urine potassium		X	X	X

## **12. SAFETY REPORTING**

### **12.1 DEFINITIONS (INSPIRED FROM ICH E2A GUIDELINES)**

#### *12.1.1 Adverse Event (AE)*

An Adverse Event (AE) is any untoward medical occurrence in a subject/patient or clinical study subject administered an investigational product but does not necessarily have a causal relationship with this investigational product.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the investigational product, whether or not related to the investigational product or to the protocol- mandated procedures (e.g., invasive procedures such as biopsies).

This includes events:

- Not present before the study but occurring after the signature of the Informed Consent;
- Pre-existing events that have worsened during the course of the study (increase in frequency or severity or change in nature during the study).
- The cystitis events are expected events, and will be considered as study parameters. Therefore, the related symptoms, signs and biological parameters will not be declared as AEs but will be collected in the CRF and on the lab reports and study analytical database.
- The typical symptoms of lower or upper urinary tract infection (defined as  $\geq 3$  urinary symptoms) are: difficult, painful, urgent and frequent urination, hematuria, supra-pubic pressure, increase in the number of white blood cells ( $>10\ 000/\text{ml}$  in the urine), with or without positive urinary bacteriological culture.

#### *12.1.2 Unexpected Adverse Event*

An unexpected adverse event is by its nature or severity not consistent with applicable product information contained in the relevant source document(s) (e.g. Protocol, Investigator's brochure).

The product distributed during the study is natural mineral water with a long commercial history and without any report of safety problem.

#### *12.1.3 Serious Adverse Event (SAE)*

A Serious Adverse Event (SAE) is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (at the time of the event)
- requires hospitalisation of a subject or prolongation of existing inpatients' hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital abnormality/birth defect

#### *12.1.4 Suspected Unexpected Serious Adverse Reaction (SUSAR)*

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is both:

- an SAE that is judged to be at least possibly related to the investigational product by either Investigator or sponsor,
- and by its frequency, nature or severity is unexpected (not listed in the Investigator's Brochure and in the study protocol).

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### 12.1.5 Emergent Adverse Event (EAE)

An adverse event will be considered as emergent if it began the day or after the first product consumption, or if it worsened the day or after the first product consumption.

## 12.2 (S)AE RECORDING

All untoward medical events occurring after subject's signature of the Informed Consent Form are recorded as (S)AEs except symptoms and biological parameters related to the cystitis episodes which are considered under the study parameters.

The typical symptoms of lower or upper urinary tract infection (defined as  $\geq 3$  urinary symptoms) are: difficult, painful, urgent and frequent urination, hematuria, supra-pubic pressure, increase in the number of white blood cells ( $>10\ 000/\text{ml}$  in the urine), with or without positive urinary bacteriological culture.

For each UTI event the following information will be collected into the CRF (and analytical database):

- start date (date of 1<sup>st</sup> symptoms)
- symptoms and signs
- urine bacteriological exam and antibiogram
- treatment prescribed
- end date of the episode (date of sampling of the last negative urine culture exam)

Complications of UTI (i.e. pyelonephritis) and symptoms, signs or abnormal values of biological parameters related to any other pathology, will be reported as S(AE).

Details of any (S)AE reported spontaneously by the subjects or observed by the Investigator or medical staff must be recorded in the (S)AE forms provided in the CRF during the course of the study. The Investigator must report the following information on the (S)AE form: nature of event (diagnosis or major symptoms/signs), start and end dates, severity, product-relatedness, action(s) taken regarding the (S) AE, action taken regarding the study product, and participant outcome.

SAEs must additionally be recorded on the SAE Report Form provided by DANONE RESEARCH. The Sponsor may request additional information from the site to evaluate the SAE.

When a subject undergoes a medical intervention or hospitalisation in absence of an adverse event (such as treatment of pre-existing condition or hospitalisation for elective surgery or diagnosis), this intervention/hospitalisation must be reported on the AE page and not as SAE. These procedures will be handled like AEs, and timelines for reporting are the same as for reporting AEs. Complications or prolongations of hospitalisation that result from procedures must be reported as SAEs, according to the applicable reporting timelines and procedures.

The severity of the (S)AE will be determined in the following manner:

- Mild: No interference with the subject's daily activities - transient or mild discomfort; no medical intervention/therapy required.
- Moderate: Moderate interference with the subject's daily activities but still acceptable - mild to moderate limitation in activity; some assistance may be needed; and/or minimal medical intervention/therapy required.

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- **Severe:** Major interference with the subject's daily activities and unacceptable - marked limitation in activity; some assistance usually required; and/or significant medical intervention/therapy/hospitalisation required.

The relationship of the (S)AE to the study product is assessed as being:

Not related	The AE follows no reasonable temporal relationship with the investigational product or does not follow a known response pattern to the investigational product and can be explained by the known characteristics of the subject/patient's clinical state, by underlying disease or other administrated products (e.g. nutritional products, OTCs, drugs).
Unlikely related	The AE has a time to investigational product intake that makes a relationship improbable (but not impossible) and concomitant administrated products (e.g. nutritional products, OTCs, drugs) or underlying disease provide plausible explanations.
Possibly related	The AE has reasonable time relationship to investigational product intake but could also be explained by an underlying disease or other administrated products (e.g. nutritional products, OTCs, drugs); information on drug withdrawal may be lacking or unclear
Probably related	The AE has a reasonable time relationship to investigational product intake, it is unlikely to be attributed to disease or other drugs and response to withdrawal of the investigational product is clinically reasonable; rechallenge information is not necessary
Definitely	The AE has plausible time relationship to investigational product intake and cannot be explained by underlying disease or other administrated products (e.g. nutritional products, OTCs or drugs); response to withdrawal of the investigational product is clinically plausible; rechallenge is satisfactory if necessary

### **12.3 SAE REPORTING BY THE INVESTIGATOR**

As soon as the Investigator becomes aware of a SAE and not later than 24 hours (1 working day), he/she completes the SAE Reporting Form and sends it together with other supporting documents (e.g. copy of pages of the case report form for medical history, on-going events and concomitant medications, etc.) via fax or e-mail to DANONE RESEARCH with copy to the monitoring CRO.

The Investigator ensures that any supportive documents submitted have been anonymised adequately.

### **12.4 SAE REVIEW AND REPORTING BY THE SPONSOR**

DANONE RESEARCH must review all reported SAE and independently assess the relationship of the SAE with the study product.

If the Investigator and the Sponsor both assess the relationship of the SAE with the study product as "Not related" or "Unlikely related", the SAE is considered as a regular SAE with no required expedited reporting to Ethics Committees.

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If one of them determines that the SAE is “Possibly”, “Probably” or “Definitely” related to the investigational product, and that this event has not been described in the Investigator’s Brochures or in the Clinical Study protocol, the SAE is considered as a SUSAR requiring expedited reporting to Ethics Committees. Should the assessments of the Sponsor and of the Investigator differ with regard to the relationship to the study product, then both will be reported.

DANONE RESEARCH must ensure that all SAEs and SUSARs are reported to the accredited Ethics Committee(s) that have approved the protocol (and Competent Authorities, if applicable) as follow:

- SAEs are reported annually as line listings according to the requirements of the Ethics Committee(s),
- SUSARs are reported within 7 days (fatal and life threatening) or 15 days (other events) after the first report. Reporting timelines include week-ends and public holidays.

The Sponsor will reply to all requests for further information concerning such events from the Ethics Committee (and/or Competent Authorities, if applicable).

### **12.5 FOLLOW-UP OF SAEs**

The Principal Investigator or his/her authorized representative will monitor and follow all SAEs until SAEs have abated, or until a stable situation has been reached and a satisfactory resolution is obtained.

Depending on the event, follow-up may require additional tests or medical procedures as indicated, and/ or referral to the general physician or a medical specialist or other health care provider.

Any clinical or biological examinations deemed necessary by the Principal Investigator will continue to be performed until a return to normal. The Principal Investigator will provide the Sponsor with follow-up SAE Reporting Form and with all the examination results and concomitant medications.

### **12.6 PREGNANCIES REPORTING BY THE INVESTIGATOR**

Although the study products are considered as safe for pregnant women, pregnancy may require specific diet, medication or procedure likely to interfere with the study product and/or outcomes.

For this reason subjects with a confirmed pregnancy have to be withdrawn from the study and followed up until birth or early termination of pregnancy. This premature termination of the protocol is documented in the CRF.

The Investigator should make every effort to collect information about the pregnancy and the infant and report it within a dated and signed Pregnancy Reporting Note including at least:

- Date of last menstruation
- Expected date of delivery
- Method of contraception used and if used according to instructions
- Medical history with information on familial disorders or risk factors that may influence the outcome of the pregnancy,
- Obstetric history with details on previous pregnancy (including termination or stillbirth)
- Medication taken prior and during pregnancy (should be available in the concomitant medication section of the CRF)
- Special tests and procedures performed during pregnancy if the case (e.g. amniocentesis, ultrasound etc.)
- Pregnancy associated events (if SAE during pregnancy)
- Pregnancy outcome (abortion, delivery)



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- Child outcome.

#### **12.7 INDIVIDUAL CODE-BREAK PROCEDURE**

Not applicable.

#### **12.8 NEW RELEVANT SAFETY INFORMATION**

Not applicable.

### **13. STATISTICS**

#### **13.1 SAMPLE SIZE CALCULATION**

By definition, the recurrent UTI event data consist of the inter event time of repetition of the same or different types for each subject. The measurements across subjects are considered to be statistically independent, but the times between UTI events for a specific subject are not necessarily independent. The mean cumulative function (MCF) contains the information of interest in the analysis of recurrent data.

Assume  $M(t)$  is the mean cumulative number of UTI events up to time  $t$ . (29)

$M(t) = E\{N(t)\}$ , where  $N(t)$  is a random variable for the number of events that have occurred up to time  $t$ . (30)

The main assumption of this approach is that the hazard or risk ratio is proportional over time, reason why a robust sandwich variance estimated is used to account for dependence of recurrent events on the same subject.

Assuming that all subjects had the same study period examination (365 days), and considering an interval of risk (inter event times) following an Uniform distribution, data have been simulated based on a mean number of events equal to 3 for control group and an expected intervention effect of 20% less events in the intervention group. One hundred samples have been replicated upon the simulated data. (31)

Considering the simulated data based on our hypotheses, a mean number of events during a 365 days period in control group equal to 3, the estimate sample size that would provide a power of 80% to detect a product effect of 20% less events occurred in the interventional study group for a bilateral test,  $\alpha=0.05$  will be equal to 42 subjects per group, i.e. 84 overall completed subjects (ratio 1:1).

As we consider a 40% drop out rate, the screening process will last until we will reach 140 subjects randomised.

#### **13.2 PLANNED STATISTICAL METHODS**

A Statistical Analysis Plan will be drawn up before data review meeting, reviewed by the Investigator and statisticians during the Data Review Meeting and signed by the sponsor before the database lock.

Statistical analyses will be performed by a specialized CRO, selected, contracted and followed under the responsibility of DANONE RESEARCH/located in Palaiseau (France) and using appropriate statistical software SAS® V9 or later using the files included in the database locked.

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### 13.2.1 Descriptive statistics

The distribution of study parameters will be summarised by group and overall depending on the type of variable for all criteria with a description of number of subjects and missing data.

The descriptive statistics for each criterion will be presented as follows:

- Continuous data: for each visit per group and overall: number of non missing observations, number of missing observations, mean, median, standard deviation (SD), minimum and maximum. (Standard error of the mean [SEM], Confident interval (CI) and quartiles optionally provided if mentioned in the Statistical Analysis Plan).
- For nominal data: for each visit per group and overall: number of missing observations, number and frequency of observations in each class.

For qualitative ordinal parameters

- Table of frequency (and/or mean and/or median), if necessary per parameter.

### 13.2.2 Statistical Hypotheses

In the present study the null hypothesis “There is no difference between groups on the average number of recurrent UTIs events experienced” versus the alternative hypothesis “the effect of intervention is different to the effect of the control” will be tested.

□ The test will be based on a nonparametric method called the mean cumulative function (MCF), that will be used to analyse the multiple/repeated UTI events occurring.

H<sub>0</sub>: Effect of fluid intake = Effect control

H<sub>1</sub>: Effect of fluid intake ≠ Effect control

Statistical tests will be conducted two-sided with a significance level of 5%. All confidence intervals will be presented two-sided with a confidence level of 95%. A resultant probability value of p<0.05 will be judged as being of statistical significance. The interaction factors (if any) will be considered as significant if the p value is < 0.10.

The MCF by group, estimated by a non-parametric estimator:

$$\widehat{MCF}(t) = \sum_{\{j|t_j \leq t\}} \frac{e_j}{n_{j-1}}$$

where e<sub>j</sub> is the number of events at time t<sub>j</sub>, n<sub>j-1</sub> is the number of subjects at risk just beyond time t<sub>j-1</sub>, and j the observed event times.

### 13.2.3 Interim Analysis

Not applicable.

### 13.2.4 Distribution and normality assessment

As a first step, the frequency distribution of the efficacy parameters will be analysed, including assessment of stem-and-leaf displays, boxplots and histograms per group and overall.

### 13.2.5 Statistical criteria to stop the research

Not applicable

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#### *13.2.6 Methods for dealing with missing, unused or non-valid data*

The way of handling missing, unused or non-valid data will be discussed at the Data Review Meeting and detailed in the Statistical Analysis Plan.

#### *13.2.7 Management of modifications made to the initial strategy of the analysis plan*

All the modifications of the statistical methodology will be detailed and justified in the Statistical Analysis Plan and described in the Clinical Study Report.

### **13.3 DISPOSITION OF SUBJECTS AND POPULATIONS DEFINITION**

#### *13.3.1 Disposition of subjects*

Disposition of subjects will be described as follows:

- Number of subjects included/enrolled.
- Number of subjects eligible at V1.
- Number of subjects screen failed at V2 (reasons of screen failure will be described).
- Number of subjects randomised per group and overall.
- Number of subjects premature withdrawals / drop out (reasons of premature withdrawals / will be described per group and overall).
- Number of subjects completed per group and overall.

#### *13.3.2 Deviations and populations definition*

A definition of minor and major protocol deviations will be detailed in a document attached to the Statistical Analysis Plan. The Data Review Meeting will allow a global review of the study data and then the deviations status to subjects in order to determine analysed populations before the final data base lock.

The populations will be defined as follows:

- The “Global Population”: all subjects included in the study and eligible at the end of Visit 1.
- The “Full Analysis Set” population (FAS): all subjects included in the study and randomised.
- The “Per Protocol” population (PP): all subjects included in the FAS population presenting no major protocol deviation.
- The “Safety Set” population (SS): all subjects included in the FAS population.

The analysis of baseline characteristics will be performed on the FAS population.

The analysis of main product effect criteria will be done on the FAS population. If the difference in number of subjects is few between the FAS and PP populations (difference lower than 10%), the analyses of the product effect criteria will be only done on the FAS population, except for the main criterion, for which the analysis will be performed on both populations.

The analysis of safety criteria will be done on the Safety Set (SS) population.

### **13.4 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS**

A descriptive analysis of subjects at baseline, corresponding to the data collected at inclusion and randomisation visits, will be performed by group and overall.

Following parameters will be described:

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- Demography, vital signs, anthropometrical parameters (body weight, waist circumference, height, BMI).
- Medical and surgical history
- Physical examination
- Quality of Life (SF12v2)
- Fluid intake consumption
- Contraception habits/method

**13.5 STUDY CONDUCT PARAMETERS**

*13.5.1 Compliance*

Subjects record the daily intake of study product in a diary on a daily basis (quantity of 3 daily bottles consumed per day). Parameters concerning compliance will be described by group and overall:

- Study product compliance
- Consumption of forbidden dietary products and treatments

*13.5.2 Quality of Life*

A quality of life questionnaire (SF-12v2) will be filled in by the subject at the randomisation visit (V2), at six-months (V3) and at the last visit (V4) in order to explore the quality of life of the subjects.

The short form 12 (SF-12v2) is a validated and frequently used questionnaire for the assessment of quality of life. It was developed from the more extensive short form 36 (SF-36) [<http://www.sf-36.org/tools/sf12.shtml>]. It includes 12 questions from the SF-36: 2 questions concerning physical functioning; 2 questions on role limitations because of physical health problems; 1 question on body pain; 1 question on general health perceptions; 1 question on vitality (energy/fatigue); 1 question on social functioning; 2 questions on role limitations because of emotional problems; and 2 questions on general mental health, psychological distress and psychological well-being. This will be a self-assessment questionnaire. However, if the subject is frail and requires help with filling in the form then the site staff will help.

A preference-based utility index, called the SF-6D, is also available to help understand economic benefit and will be assess in order to perform a cost utility analysis.

- 12-Items
- 8- Dimensions: Physical functioning, Role Physical, Bodily pain, Vitality, General health, Mental health, Social functioning and role emotional
- 2 composite score: Mental health and physical health
- Utility index based on SF-6Dimensions

**13.6 EVALUATION CRITERIA**

*13.6.1 Main evaluation criterion*

The main evaluation criterion is the number of recurrent UTI event experienced over a one year study period between intervention and control groups, evaluated by the difference between groups in the mean cumulative function. The primary criterion will be considered as statistically significant if confidence interval observed on the MCF difference between groups excludes zero, which suggests a statistical test and p value associated <0.05.

*13.6.2 Secondary evaluation criterion*

- Difference between groups in terms of duration of urinary infectious episodes
- Average delay between each UTI over one year study period

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- Change in urinary hydration markers following changes in water intake habits
- Difference between groups in terms of change in QoL
- Difference between groups in terms of antibiotic prescription/usage to treat UTI
- Difference between groups in terms of health-costs associated with management of UTI recurrence(s)
- Difference between groups in terms of cost utility analysis

The methodology to address the potential multiplicity issues will be detailed in the Statistical Analysis Plan.

*13.6.3 Exploratory criteria*

- Association measure between urinary hydration markers and number of UTI events
- Association measure between urinary hydration markers and delay UTI events

*13.6.4 Safety criteria*

*13.6.4.1 Extent of exposure / Study duration*

Descriptive summaries will be performed on study duration and extent of exposure by group and overall.

*13.6.4.2 AE / SAE*

The analysis of Adverse Events will be performed to evaluate the number of subjects with at least one adverse event and the number of adverse events by study group.

The adverse events will be presented by "body system" and "Preferred term" according to the MEDDRA coding system.

All adverse events will be summarised, in individual data listings, by subject with all details concerning the adverse event, the study product, the emergence, the intensity and the relationship to study product.

The Serious Adverse Events will be summarised, in individual data listings, by subject with all details concerning the adverse event, the study product, the emergence, the intensity and the relationship to study product.

*13.6.4.3 Concomitant Medications'*

The concomitant medications will be presented by ATC class (ATC1 and ATC3) according to the WHO-Drug coding system.

All concomitant medications will be summarised, in individual data listings, by subject with all details concerning the concomitant medications and the study product.

*13.6.4.4 Laboratory measurements*

Descriptive summaries will be performed for the following parameters by study group and overall:

Blood sample

- lipidic profile: HDL cholesterol, LDL cholesterol, triglyceride (mmol/L)
- glycosylated haemoglobin (%),
- serum creatinine ( $\mu\text{mol/L}$ ),
- serum urea (mmol/L),
- copeptin ( $\mu\text{g/L}$ )
- Estimated glomerular filtration rate (eGFR) will be calculated

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Urinary markers of hydration

- urine volume (L)
- pH
- osmolality (mOsm/kg)
- specific gravity
- frequency of micturition
- urine colour

Other urinary parameters

- Electrolytes: sodium, potassium, calcium, magnesium, chloride (mmol/L), oxalate ( $\mu\text{mol}/24\text{h}$ ), and citrate (mmol/L))
- Urine creatinine (mmol/L)

Information about bladder emptying after sexual intercourses and sexual activity during the last month will be also summarised by study group and overall.

#### 13.6.4.4 *Vital signs, anthropometrical and other parameters*

Descriptive summaries will be performed for the following parameters by study group and overall:

- Systolic and diastolic blood pressure (mmHg)
- Heart rate (bpm)
- Body temperature ( $^{\circ}\text{C}$ )
- Body weight (kg)
- Waist circumference (cm)
- Height (m)
- Body Mass Index (BMI) ( $\text{kg}/\text{m}^2$ )

## **14. STUDY REPORT AND PUBLICATION**

All information stemming from the study will be considered confidential and should not be divulged without the prior agreement of the sponsor (DANONE RESEARCH).

Following analysis of the study data, a final study report will be prepared in UK English, format ICH E3, according to the standard model of DANONE, describing the conditions under which the study was performed as well as the results. This report will be prepared and signed by the Sponsor's representative. The signature of the coordinating Investigator (for multicenter study) or of the Principal Investigator (for monocenter study) will be requested if relevant.

The identity of study subjects will under no circumstances be communicated to the sponsor.

Publication may be done only if before recruitment of the first subject, the sponsor has registered the study in a publicly accessible clinical trial database.

No publication will be done based upon interim analysis or result of one center of a multicentric study except if a written agreement has been signed by the sponsor.

## **15. STUDY MONITORING AND AUDITS**

Study monitoring is the act of overseeing the progress of a clinical study and of ensuring that:

- i) The rights and the well-being of human subjects are protected.
- ii) The reported trial data are accurate, complete, and verifiable from source documents.
- iii) The conduct of the clinical study is in accordance with the approved protocol/amendment(s), Good Clinical Practice (GCP, ICH E6), and the applicable local regulatory requirement(s).

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Monitoring includes on-site visits to assure that the investigation is conducted according to the approved protocol/amendment(s) and in order to comply with applicable regulations and deadlines. On-site monitoring includes, at least, the review of Informed consents, safety reporting, CRFs and supports the site management regarding protocol conduct and compliance or deviation(s).

The monitoring includes the review of forms for completeness, clarity, and consistency with source documents available for each subject and the management of the essential documents (Investigator Study File).

The Investigator and the investigating site staff must permit and be available for study-related monitoring visits, audits, review by the ethics committee and regulatory inspections, and allow direct access to source data and source documents provided that subject confidentiality is protected. In case of an audit appointed by DANONE RESEARCH, the Investigator will receive written notification in advance.

Study monitoring, under the responsibility of DANONE RESEARCH will be performed by a qualified staff of a company contracted by DANONE RESEARCH at various stages/on regular basis of the study (frequency of visits is specified in monitoring plan). Throughout the duration of the study period, the CRFs will be completed and signed by the Investigator, and he/she will be controlled individually by a Clinical Research Associate (CRA) designated by DANONE RESEARCH in order to verify data quality and compliance with study protocol and Good Clinical Practice (GCP, ICH E6).

Upon closure of the study, the company contracted by DANONE RESEARCH will perform study closeout.

## **16. ETHICAL CONSIDERATIONS**

### **16.1 BASIC PRINCIPLES AND REGULATIONS**

The Investigator must ensure that this study is conducted in full compliance with the principles of the 'World Medical Association Declaration of Helsinki' (64th WMA General Assembly, Seoul, October 2013) (Appendix I), ICH guidelines for Good Clinical Practice as appropriate for nutritional products, and local legislation of the country in which the research is conducted, whichever affords the greater protection to the participants.

### **16.2 ETHICS COMMITTEE**

This protocol and any accompanying material provided to the subjects, such as information and informed consent sheets, are submitted to the applicable Ethics Committee (IRB/IEC) by <the sponsor or the Investigator or the company contracted by DANONE RESEARCH> according to local legislation. Approval from the Ethics Committee must be obtained before starting the study, and should be documented in a letter to the sponsor specifying the date on which the Ethics Committee met and granted the approval, the composition of the Ethics Committee and their qualifications, and version and date of all submitted documents. If applicable, the documents will also be submitted to the Competent Authority in accordance with the local regulatory and legal requirements.

This study will be undertaken after approval from the appropriate Ethics Committee(s) (IRB/IEC) and of Health Authorities (if applicable).

During the study course, change(s) in any aspect of the study, such as modification(s) of the protocol, written ICF and any other written information to be provided to subjects should be submitted to the Ethics Committee (IRB/IEC)

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and Competent Authorities. All updates of the Investigator brochure will be supplied to Ethics Committee (IRB/IEC) as well.

If required, depending on local legislation, the Investigator must submit an annual progress report to the Ethics Committee which gave the favourable opinion. Annual progress reports should be submitted thereafter until the end of the study. DANONE RESEARCH could assist in the preparation/submission process.

Subject recruitment will start only after reception of a favourable opinion from the Ethics Committee and Health Authority (if applicable).

### **16.3 RECRUITMENT AND INFORMED CONSENT FORM**

The requirements of the research, the objectives of the research, the detailed research protocol and the risks and constraints of the research associated with this study must be explained to each subject both orally and in writing (subject information sheet) by the Investigator, or a person designated by the Investigator, before the start of the study.

The subject must personally initial each page of the Information to the subject and sign and date a written informed consent form (ICF) to take part in this study before the study starts (before the screening period, if applicable). This form must also be initialled and signed by the Investigator or a person designated by the Investigator. Two copies of the ICF are dated and signed: one is given to the subject and one is retained on site in the Investigator Site File.

Subjects may withdraw from the study at any time without having to provide justification. The confidentiality of medical data must be upheld.

Any substantial changes in the ICF and written information should receive the IRB/IEC's approval/favourable opinion before any use.

### **16.4 CONFIDENTIALITY OF STUDY DATA**

All information collected during the study is to be considered confidential and must not be disclosed without prior written agreement by the sponsor. The identity of study subjects must under no circumstances be communicated to the sponsor or to any official bodies.

### **16.5 COMPENSATION OF SUBJECTS**

[The subjects will be compensated for their participation in clinical studies. Compensation amount and the method and timing of disbursement are consistent with applicable regulatory requirement(s) of Bulgaria.

Each subject will receive an indemnity of 586 Bulgarian Leva for participating in this study if she has completed all study visits or in case of discontinuation due to medical reasons related to the protocol. In any other case of withdrawal, the subject will receive an indemnity for participation on a pro rata basis.

The reimbursement will be as follows:

- Inclusion visit (V1) = 30 Bulgarian Leva
- Randomization visit (V2)= 166 Bulgarian Leva.
- Evaluation visit (V3) = 185 Bulgarian Leva



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- Evaluation visit (V4)= 205 Bulgarian Leva.

#### **16.6 PROTOCOL AMENDMENTS**

Any protocol modification should be the object of an amendment, which will be dated and signed by the same parties than those invested in the initial protocol signature.

The amendment will be submitted to the appropriate Ethics Committee (IRB/IEC) and/or the Competent Authorities (if applicable), either for approval or for information, depending on the nature and the importance of the changes to the study conditions.

#### **16.7 STUDY SUSPENSION**

If the study or part of the study is prematurely terminated or suspended, the Investigators, the appropriate Ethics Committee(s) (IRB/IEC) and Competent Authorities should promptly be informed as specified by the applicable regulatory requirement(s).

#### **16.8 PROTOCOL DEVIATIONS**

No deviations are tolerated systematically. Any deviation from the approved protocol related to study inclusion or exclusion criteria, conduct of the trial, subject's management or subject's assessment should be documented and explained. DANONE RESEARCH will be informed of all protocol deviations, and these will be discussed before implementation according to prospective protocol deviation process or later at the data review meeting (blind review meeting), in order to define their status (minor – major).

#### **16.9 INSURANCE**

DANONE RESEARCH has contracted an insurance policy with an established insurance company in accordance with current regulatory requirements in Bulgaria to cover potential damage to subjects through injury or death caused by the study product and/or study procedures.

The insurance applies to the damage that becomes apparent during the study. DANONE RESEARCH also has liability insurance in accordance with the applicable legislation.

## **17. DATA COLLECTION, PROCESSING, AND MANAGEMENT**

#### **17.1 CASE REPORT FORM (CRF)**

An electronic CRF (eCRF) will be created by the Sponsor or its delegate in English. The final version will be approved by the Sponsor. The relevant study data defined in this Protocol will be collected and entered by the Investigator into the provided eCRF. A CRF Completion Guideline will be provided to the Investigator to facilitate CRF completion as well as provide answers for Frequently Asked Questions (FAQs)

Quality of life questionnaire will be printed on duplicate carbon copy paper by the Sponsor and provided to the Investigator:

- White (original) – Sponsor Copy (sent to the Biometry Contract Research Organization (CRO) for data processing).

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- Colour – To be kept at the study site by the Investigator

Each subject will be identified with a unique subject identification number (refer to the section 7.3). All study documents related to a subject must be identified with this subject identifier

All relevant CRF data will be single-entered by personnel at the Investigative site in the validated eCRF tool put in place by the sponsor. The Investigator must ensure that these data are complete and accurately represent the study subject information by electronically signing the appropriate forms in the eCRF.

QoL questionnaires and subject diaries will be in paper and single-entered by the DM CRO in the validated eCRF tool put in place by the sponsor. These questionnaires will be in paper and in Bulgarian but entered in English in the eCRF.

All external data will be provided to the DM CRO for integration to the database

The sponsor will ensure all study site personnel are appropriately trained on the use of the eCRF (i.e. data entry, queries, sign-off etc.). This training is MANDATORY for all site personnel (who are expected to use the system) and must be completed before access is granted to the system. Each user will be provided with a unique access (user name and password) to the eCRF.

***The combination of the user name and password to access the eCRF are the legally binding equivalent of a traditional handwritten signature, and as such, carries all of the same rights and responsibilities.*** User accounts are not interchangeable, and **must only be used by the person authorized to use that account.**

## **17.2 DATA PROCESSING**

Edit checks, to ensure the quality and consistency of the data, will be defined in a Data Validation Plan (DVP). These edit checks will be programmed and validated in the eCRF. During the data entry process, these checks will automatically be triggered (and become apparent to the user) in case of incoherent data.

## **17.3 DATA AUDIT TRAIL**

Any addition, modification, or deletion of subject data made after the initial entry (i.e. response to DCF or updated information) will be automatically tracked in the e-CRF's audit trail in compliance to ICH GCP and US FDA 21 CFR part 11.

## **17.4 DATA MANAGEMENT**

Data Management (DM) tasks for this study will be performed by a delegated DM CRO. The verification and validation of DM tasks will be performed by a Data Manager internal at the Sponsor. All DM tasks will be defined in a Data Management Plan (DMP) based on the current Sponsor and CRO Standard Operating Procedures (SOPs).

## **17.5 EXTERNAL DATA**

Data Transfer specifications for external data (i.e. central laboratory data) will be defined in a Data Transfer Agreement (DTA). All electronic external data transfers will be reconciliated with the main study data and validated by the DM CRO to ensure accuracy and consistency.

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### **17.6 DATABASE LOCK**

After all planned subject visits have been completed, entered into the study database, and considered clean, a Data Review Meeting (DRM) will be held by the Sponsor to discuss and review all study data. The main objective of this DRM will be to review tables, listings and summaries on study data, to review safety listings, to identify protocol deviations and to define subject analysis populations. Members of the Sponsor's Study Core Team, the designated CRO's as well as the Principal Investigator(s) may participate in this meeting.

Once any final issues resulting from the DRM have been resolved, the study database, including all external data will be locked. This will ensure that no further modifications to the study data are possible. The statistical analysis will be performed on this locked database.

Following the database lock, the sponsor will provide each Investigative site with a final, unmodifiable PDF copy of the completed CRFs, Questionnaires and Subject Diaries (including the audit trail ) of all subject's included at that site. This constitutes an exact representation of the information that was entered in the eCRF during the study. This will be provided on removable media (i.e. CD, DVD etc).

## **18. DOCUMENTATION AND ARCHIVING**

DANONE RESEARCH provides the Principal Investigator (s) with an Investigator Site File (ISF). Each Principal Investigator (s) is responsible to keep this ISF updated and available for review by the study monitor (CRA).

All documents pertaining to the conduct of the study must be kept by the Investigator for a period of 15 years.

Study documents should not be destroyed without prior written agreement between DANONE RESEARCH and the Investigator. Should the Investigator wish to assign study documents to another party, or move them to another location, DANONE RESEARCH must be notified first.

### **18.1 SPONSOR**

The following documents are to be archived by the sponsor for a minimum of 25 years after the completion of the study, in a room specifically designated for this purpose, access to which is controlled by the person responsible for archiving:

- Final version of the study protocol,
- Any forms containing protocol amendments,
- **FOR Paper questionnaire:** Original pages (White copy),
- **FOR eCRF:** The media (i.e. CD, DVD etc.) that contains the final PDF copy of the subject CRFs, Questionnaires, Subject Diaries (including audit trail ) that was extracted from the eCRF following the database lock,
- Ethics Committee approval forms,
- All correspondence between the sponsor and the Investigator,
- Curriculum vitae (CV) of Principal Investigator and other Investigators
- Acknowledgements of receipt and study product accountability forms as well as all other documents generated during the study.

## **18.2 INVESTIGATOR**

All documents concerning this study must be kept by the Investigator, including but not limited to:

- Subject's medical files including source documents
- Original (dated and signed) of the ICFs and the subject's identification code list
- Screening and enrolment Log
- **FOR Paper questionnaire:** Copy of the CRF (yellow copy)
- **FOR eCRF:** The media (i.e. CD, DVD etc.) provided by the sponsor that contains the final PDF copy of the subject CRFs, Questionnaires, Subject Diaries (including audit trail )
- Copy of accountability forms for products administered
- Copy of ethics committee approval and correspondence with the sponsor
- Signed Protocol(s), signed amendment(s)
- Advertisement (if any)
- Financial Contract(s)
- Insurance certificate
- EC correspondence including Approval Opinion and composition
- CV of Investigator and sub Investigator(s) and delegation task list
- Normal Values and technical procedures
- Instruction for handling products, shipping records, products accountability
- Decoding procedure
- Serious Adverse Events report forms

All correspondence between the sponsor and the Investigator,

The Investigator agrees to provide direct access to source documents during monitoring visits.

## **19. OWNERSHIP OF RESULTS**

All information and results issued from the study remain the property of the sponsor.

The study results may be published or presented by the Investigator or by experts responsible for analysis, in collaboration with the sponsor and with the prior written approval of the latter. The sponsor may use the results of the study for publications or communications with the written agreement of the Investigator or experts responsible for analysis if the latter are cited.

## **20. RESPONSIBILITIES**

### **20.1 SPONSOR**

- a. Manage the submission of the study protocol to the ethics committee//health authorities before the start of the study. The Sponsor should obtain the approval from all the competent authorities and from the EC.
- b. Before the start of the study, the sponsor must provide the Investigator with all documents required by the protocol and/or to provide information on the study products. In particular, the Sponsor must provide the Investigator with a document certifying that the study products are fit for human consumption.
- c. The Sponsor must take out a specific insurance policy for the study as required by current legislation of the region in which the study is being conducted.
- d. The Sponsor must provide the Investigator with insurance certification. The Sponsor may decide to terminate the study at any time. At the end of the study, the Sponsor must archive the study documents for the legally required duration and at least for 25 years.
- e. The Sponsor must carry out all procedures required by the relevant EC/health authorities including initial/amendment submission and Safety reporting.
- f. The Sponsor must set up data Quality Control at each stage of data handling.
- g. The Sponsor finances expenses related to the study
- h. The sponsor must monitor the study and ensure the correct adherence to protocol requirements and regulatory requirement are maintained (including the data protection and confidentiality)
- i. The Sponsor must record the study in the appropriate Governmental database

**20.2 INVESTIGATOR**

- a. Provides oral and written information for subjects and selects subjects in accordance with protocol inclusion and non-inclusion criteria.
- b. Keeps all study related information confidential
- c. Manages the of study products including randomisation, storage, dispensation, destruction, site accountability.
- d. Before the study, provides the following documents to the sponsor:
  - CV of coordinating Investigator and co-Investigators,
  - CV of the entire remaining investigating site staff involved in the study.
- e. Performs the clinical study within the scheduled dates.
- f. Establishes a written delegation task list, and allocate sufficient time and resources to properly conduct the study
- g. Reports of SAEs.
- h. Cooperates with Clinical Research Assistants at the periodic monitoring visits, or in case of audits and/or inspections
- i. Completes and corrects the case report forms, DCF.
- j. Give input in the clinical report for the study, if relevant.
- k. Maintain essential documentation
- l. Archives data for a minimum of 15 years after the date of the final report.

**20.3 MONITORING CLINICAL RESEARCH ORGANISATION (CRO)**

- a. Provides the required study materials in good time
- b. Performs Study start-up visit
- c. Verifies and updates the Trial Master File (TMF) and Investigator Study File.
- d. Performs the Monitoring during the study conduct (including at least the verification of the signed Informed consent, the validation of the data recorded in the CRF including Safety data from source documents, the verification of the study product's storage conditions .)
- e. Performs the End-of-study visit
- f. Communicate relevant information to the Sponsor
- g. Verifies the Data collection (accurate , complete and verifiable)

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## APPENDICES

# APPENDIX I

## DECLARATION OF HELSINKI

## **WORLD MEDICAL ASSOCIATION**

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:  
29th WMA General Assembly, Tokyo, Japan, October 1975  
35th WMA General Assembly, Venice, Italy, October 1983  
41st WMA General Assembly, Hong Kong, September 1989  
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996  
52nd WMA General Assembly, Edinburgh, Scotland, October 2000  
53rd WMA General Assembly, Washington, DC, USA, October 2002 (Note of Clarification added)  
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)  
59th WMA General Assembly, Seoul, Republic of Korea, October 2008  
64th WMA General Assembly, Fortaleza, Brazil, October 2013

### **PREAMBLE**

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

### **GENERAL PRINCIPLES**

3. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

### **RISKS, BURDENS AND BENEFITS**

16. In medical practice and in medical research, most interventions involve risks and burdens.

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Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

#### **VULNERABLE GROUPS AND INDIVIDUALS**

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

#### **SCIENTIFIC REQUIREMENTS AND RESEARCH PROTOCOLS**

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

#### **RESEARCH ETHICS COMMITTEES**

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

#### **PRIVACY AND CONFIDENTIALITY**

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

#### **INFORMED CONSENT**

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

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All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

### **USE OF PLACEBO**

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

### **POST-TRIAL PROVISIONS**

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

### **RESEARCH REGISTRATION AND PUBLICATION AND DISSEMINATION OF RESULTS**

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

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#### **UNPROVEN INTERVENTIONS IN CLINICAL PRACTICE**

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

#### **ARTICLE INFORMATION**

**Corresponding Author:** World Medical Association, 13, ch. du Levant, CIB - Bâtiment A, 01210 Ferney-Voltaire, France; [wma@wma.net](mailto:wma@wma.net).

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**Online-Only Content:** Audio podcast is available at [www.jama.com](http://www.jama.com).

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## **APPENDIX II**

### **List of Participating Investigational Sites and Principal Investigators**

**APPENDIX III**

**SERIOUS ADVERSE EVENT**

**TRANSMISSION FORM**

 <b>DANONE RESEARCH</b> CENTRE DANIEL CARASSO	<b>Serious Adverse Event Report Form</b>	<b>CLINICAL STUDIES PLATFORM</b> Dairy & Waters  <b>CONFIDENTIAL</b>
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## Serious Adverse Event Report Form

This form must be faxed to the Sponsor within 24h

<b>Sponsor:</b> DANONE RESEARCH	<b>Fax :</b>	<b>Tel :</b>
	<b>Clinical Study Manager (Name):</b>	
<b>Principal Investigator (Name):</b>		
<b>Study code : NU</b>	<b>Expeditor (Name):</b>	<b>Country :</b>
<b>Site Number:</b>		
<b>Transmission date :</b>       /       /             (dd/mm/yyyy)		
Number of pages (incl. this page)	_____ <b>Pages</b>	
Attached copies of completed e-CRF pages:	<input type="checkbox"/> Adverse events <input type="checkbox"/> Medical History and pre-existing conditions <input type="checkbox"/> Medications	




 <b>DANONE RESEARCH</b> CENTRE DANIEL CARASSO	<b>Serious Adverse Event Report Form</b>	<b>CLINICAL STUDIES PLATFORM</b> Dairy & Waters  <b>CONFIDENTIAL</b>
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This SAE report is :  Initial report  
 Follow-up report (Includes only new details)

Is the SAE aggravation of a pre-existing AE recorded in the e-CRF: <input type="checkbox"/> Yes <input type="checkbox"/> No				
If yes, please put the number of AE:  _ _				
<b>Subject Details</b>				
Subject Number:  _ _ _ / _ _ _ / _ _ _		Gender: <input type="checkbox"/> M <input type="checkbox"/> F		
Subject Initials:  _ _ _ / _		Date of birth:  _ _ / _ _ / _ _ _ _ _  (dd/mm/yyyy)		
Height:  _ ,  _ _  m Weight :  _ _ _ ,  _ _ kg				
<b>Description of Serious Adverse Event</b>				
Nature of SAE (diagnosis or major symptom and/or sign):				
Description of SAE:				
Severity of SAE: <input type="checkbox"/> Mild <input type="checkbox"/> Moderate <input type="checkbox"/> Severe				
Event start date:  _ _ / _ _ / _ _ _ _ _  (dd/mm/yyyy)		Event end date:  _ _ / _ _ / _ _ _ _ _  (dd/mm/yyyy)		
Time :  _ _  :  _ _  (Hours:Minutes)		Time :  _ _  :  _ _  (Hours:Minutes)		
<b>Category of SAE</b> (*as applicable to study protocol)				
<input type="checkbox"/> Death		<input type="checkbox"/> Persistent or significant disability or incapacity		
<input type="checkbox"/> Life threatening situation		<input type="checkbox"/> Congenital anomaly or birth defect		
<input type="checkbox"/> Hospitalization or Prolongation of existing Hospitalization				
<b>Study Product</b>				
<b>According to protocol, the dosage is:</b>		Dose:  _ _  Unit (Pot/Bottle): _____ Frequency: Daily Route: Oral		
<b>Information of study production distribution</b>	Study Product Number(s)	Last Product Batch Number:	Product Expiry date (dd/mm/yyyy)	Date of Last Product Distribution (dd/mm/yyyy)
	_ _ _ _ _	_ _ _ _ _	_ _ / _ _ / _ _ _ _ _	_ _ / _ _ / _ _ _ _ _
<b>Information of study product intake</b>			Date of last product intake (dd/mm/yyyy)	Date of last product intake before event (dd/mm/yyyy)
			_ _ / _ _ / _ _ _ _ _	_ _ / _ _ / _ _ _ _ _


	<b>Serious Adverse Event Report Form</b>	<b>CLINICAL STUDIES PLATFORM</b> Dairy & Waters  <b>CONFIDENTIAL</b>
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<b>Has the dosage been respected before the event?</b>	<input type="checkbox"/> Yes	<input type="checkbox"/> No If No, specify:	Dose:  _ _  Frequency: Daily	Unit (Pot/Bottle): _____
<b>Action taken with respect to the study product:</b>	<input type="checkbox"/> Dose Not Changed <span style="float:right;"><input type="checkbox"/> Not applicable</span> <input type="checkbox"/> Product Interrupted Stop date:  _ _ / _ _ / _ _ _ _  Did reaction disappear after stopping product? <input type="checkbox"/> Yes <input type="checkbox"/> No Reintroduction date:  _ _ / _ _ / _ _ _ _  Did reaction reappear after reintroduction? <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Product withdrawn => if yes please enter the stop date  _ _ / _ _ / _ _ _ _  (dd/mm/yyyy)			
<b>Has the code been broken?</b>	<input type="checkbox"/> Yes	<input type="checkbox"/> No	<input type="checkbox"/> Not Applicable	

Action taken with respect to the subject (check all that apply)	Outcome of SAE for the subject	Relationship to	
		Study Product	Study Procedure
<input type="checkbox"/> None <input type="checkbox"/> Medication <input type="checkbox"/> Hospitalization <input type="checkbox"/> Study discontinuation <input type="checkbox"/> Study withdrawal <input type="checkbox"/> Other, specify: _____ _____ _____	<input type="checkbox"/> Not Recovered <input type="checkbox"/> Recovering <input type="checkbox"/> Recovered with sequelae, being: ..... <input type="checkbox"/> Recovered without sequelae <input type="checkbox"/> Fatal /Death <input type="checkbox"/> Unknown	<input type="checkbox"/> Not related <input type="checkbox"/> Unlikely related <input type="checkbox"/> Possibly related <input type="checkbox"/> Probably related <input type="checkbox"/> Definitely related	<input type="checkbox"/> Not related <input type="checkbox"/> Unlikely related <input type="checkbox"/> Possibly related <input type="checkbox"/> Probably related <input type="checkbox"/> Definitely related

Relevant medical and surgical history / or copy of e-CRF pages (e-CRF page XXX) related to medical and surgical history				
No.	Disease or Surgery	Start date (dd/mm/yyyy)	End Date (dd/mm/yyyy)	Current medication /nutritional supplement?*
1		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
2		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No

	<b>Serious Adverse Event Report Form</b>	<b>CLINICAL STUDIES PLATFORM</b> Dairy & Waters  <b>CONFIDENTIAL</b>
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3		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
4		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
5		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
6		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
7		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
8		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
9		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
10		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
11		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
12		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
13		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
14		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
15		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No

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## **CLINICAL STUDY PROTOCOL**

**PRODUCTS:** WATER

**PROTOCOL VERSION** 2 (AMENDMENT 1)

**STUDY CODE:** NU369

**AMENDED PROTOCOL**

**DATE:** 22/04/2014

# ***The effect of increased water intake on the frequency of clinical recurrent urinary tract infections in pre-menopausal women: S-HYDRACYST***

**Principal Investigator:**

**MAYA DABCHEVA, MD**  
MC "COMAC MEDICAL"  
13 URVICH STR., 3<sup>RD</sup> FLOOR  
1612 SOFIA – BULGARIA  
TEL.: +359 2 850 97 04 FAX: +359 2 850 60 96  
E-MAIL: MAYA.DABCHEVA@COMAC-MEDICAL.COM

**Sponsor:**

DANONE RESEARCH  
ROUTE DEPARTEMENTALE 128  
91767 PALAISEAU CEDEX – FRANCE

**Sponsor Representative:**

**CHRISTINE M'RINI, MD, PHD**  
LIFE SCIENCE DIRECTOR  
DANONE RESEARCH  
ROUTE DÉPARTEMENTALE 128  
91767 PALAISEAU CEDEX – FRANCE  
TEL.: +33(0)1.69.35.70.00 FAX: +33(0)1.69.35.76.89  
E-MAIL: christine.mrini@danone.com

**Emergency Contact**

**MAYA DABCHEVA, MD**  
TEL.: +359 2 850 97 04 FAX: +359 2 850 60 96

### **Ethics Statement**

This study will be conducted according to the protocol and in compliance with Good Clinical Practice (GCP), the ethical principles stated in the Declaration of Helsinki, and other local applicable regulatory requirements.

### **Confidentiality Statement**

The information provided in this document is the property of DANONE RESEARCH, and is shared with you and your staff in confidence. This information should not be disclosed to others without written authorization from DANONE RESEARCH, except to the extent necessary to ensure adequate conduct of the study.



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## 1. GENERAL INFORMATION

### 1.1 LIST OF PARTICIPANTS

**Principal Investigator:** MAYA DABCHEVA, MD  
MC "Comac Medical",  
13 URVICH STR., 3<sup>RD</sup> FLOOR  
1612 SOFIA - BULGARIA  
Tel. : +359 2 850 97 04 Fax: +359 2 850 60 96  
E-MAIL: MAYA.DABCHEVA@COMAC-MEDICAL.COM

The Appendix 2 provides the list of all participating investigational sites and Principal Investigators.

**Research Scientist/Engineer:** MARIACRISTINA VECCHIO  
DANONE RESEARCH  
ROUTE DEPARTEMENTALE 128  
91767 PALAISEAU CEDEX - FRANCE  
Tel. : +33 (0)1 69.35.68.47 Fax : +33(0)1.69.35.76.93

**Biostatistician:** PASCALINE PICARD [AIXIAL – PHARMA]  
DANONE RESEARCH  
ROUTE DEPARTEMENTALE 128  
91767 PALAISEAU CEDEX - FRANCE  
Tel. : +33 (0)7 78 41 10 44 Fax : +33 (1).69.35.76.89

**Sponsor's Medical Expert** CHRISTINE M'RINI, MD, PHD  
LIFE SCIENCE DIRECTOR  
DANONE RESEARCH  
ROUTE DEPARTEMENTALE 128  
91767 PALAISEAU CEDEX – FRANCE  
Tel. : +33(0)1.69.35.70.00 Fax: +33(0)1.69.35.76.89  
E-MAIL:christine.mrini@danone.com

**Emergency contact:** MAYA DABCHEVA, MD  
Tel. : +359 2 850 97 04 Fax: +359 2 850 60 96

**Clinical Study Manager:** LILIANA GALETESCU  
DANONE RESEARCH  
ROUTE DEPARTEMENTALE 128  
91767 PALAISEAU CEDEX - FRANCE  
Tel. : + 33 (1) 69 35 72 34 Fax : +33 (1).69.35.76.89

**Scientific Expert:** YAIR LOTAN  
CHIEF OF UROLOGIC ONCOLOGY  
UT SOUTHWESTERN MEDICAL CENTER AT DALLAS  
DEPARTMENT OF UROLOGY  
5323 HARRY HINES BLVD. J8.122  
DALLAS, TEXAS 75390-9110  
Tel. : 214-648-0389

**Monitoring Clinical Research Organisation:** PHARM-OLAM INT.(UK) BULGARIA OFFICE  
PHARM-OLAM INT. (UK) BULGARIA OFFICE

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55 PARCHEVICH STR.  
1000 SOFIA - BULGARIA  
Tel. : + 359 2 9888 704  
Fax : + 359 2 9888 701

**Central Laboratory:**

MEDICAL DIAGNOSTIC LABORATORY RAMUS EOOD  
2-4 ANGISTA STR.  
SOFIA 1527 BULGARIA  
Tel. : + 359 2 9431196  
Fax: + 359 2 944 8206

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**1.2 PROTOCOL SIGNATURE PAGE - SPONSOR**

Protocol details	
Study title	<i>The effect of increased water intake on the frequency of clinical recurrent urinary tract infections in pre-menopausal women:S-HYDRACYST</i>

*Protocol approved by the Sponsor:*

I, the undersigned, have reviewed and approved this protocol, including the appendices.

SPONSOR	DATE	SIGNATURE
<b>Christine M'RINI, MD, PhD</b> LIFE SCIENCE DIRECTOR DANONE RESEARCH ROUTE DEPARTEMENTALE 128 91767 PALAISEAU CEDEX - FRANCE		

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**1.3 PROTOCOL SIGNATURE PAGE – PRINCIPAL INVESTIGATOR**

Protocol details	
Study title	<b><i>The effect of increased water intake on the frequency of clinical recurrent urinary tract infections in pre-menopausal women: S-HYDRACYST</i></b>

*Protocol approved by the Principal Investigator:*

I, the undersigned, have reviewed this protocol including the appendices and I am aware of my responsibility and I agree to the following:

To conduct the clinical study in compliance with the protocol as detailed in this document.

To apply ICH Good Clinical Practice the declaration of Helsinki and any other regulatory requirements.

To obtain protocol approval from an independent Ethics Committee and to comply with their requirements for ongoing review and reporting (if applicable).

To comply with procedures for data recording and reporting (with a particular focus on Safety reporting)

To permit monitoring, auditing and inspection by the sponsor and relevant regulatory agencies.

To retain study related documents according to regulatory requirements and as agreed with the sponsor.

PRINCIPAL INVESTIGATOR	DATE	SIGNATURE
<b>Maya DABCHEVA, MD</b>  MC "COMAC MEDICAL", 13 URVICH STR., 3 <sup>RD</sup> FLOOR 1612 SOFIA, BULGARIA		

*Three original copies of the clinical study protocol must be signed by the Principal Investigator and one original copy shall be filed by each of the parties (Sponsor and Principal Investigator) and one should be submitted to the Ethics Committee.*

**1.4 GLOSSARY**

ACR	Albumin Creatinine Ratio
AE	Adverse Event
AFU/EAU	Association Française d'Urologie (AFU)/European association of Urology
BMI	Body Mass Index
BP	Blood Pressure
CA	Competent Authority (ies)
CM	Concomitant Medication
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organisation
CUA	Cost utility analysis
CV	Curriculum vitae
DCF	Data Clarification Form
DBP	Diastolic Blood Pressure
EAU	European Association of Urology
EC	Ethics Committee
EDC	Electronic Data Capture
EPI	Epidemiology Collaboration
FAS	Full Analysis Set
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GP	General Practitioner
HR	Heart Rate
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ISF	Investigator Study File
IRB/IEC	Institutional Review Board/Independent Ethics Committee

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IWRS	Interactive Web Response Services/Systems
LDL	Low Density Lipoprotein
MCF	Mean Cumulative Function
OTC	Over The Counter
PI	Principal Investigator
PP	Per Protocol
QoL	Quality Of Life
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
SD	Standard Deviation
SEM	Standard Error of Mean
SF	Screen Failure
SOP	Standard Operating Procedure
SS	Safety Set
SSAR	Suspected Serious Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
USG	Urine Specific Gravity
UTI	Urinary Tract Infection
V	Visit
VAS	Visual Analytical Scale
WHO	World Health Organisation



## **1.5 LIST OF DEFINITIONS**

Active/Test product:	Product to be tested in the study.
Completed Subject:	Subject who has completed the study visits as required by the protocol. In the CRF the end of study status is available and it is: "Completed the study".
Evaluation period:	This period extends from randomization (or allocation) up until the last visit where results are collected with the intent of evaluating any of the study criteria.
Eligible subject at visit X:	The subject is considered eligible when the Investigator certifies that he fulfills all inclusion criteria and does not meet any exclusion criteria for the current visit. This statement can be repeated as many times as required by the protocol. In the CRF the Investigator has answer YES to the question "Is the subject eligible at visit "X"?"
Follow up period:	This period, if applicable, follows the evaluation period in order to confirm the well-being of the subject or follow his recovering. Typically, minimal safety data are collected and no efficacy evaluation is performed.
Included:	The signature of the informed consent marks the inclusion of the subject in the study. This signature can be obtained solely after having fully briefed the subject about the study. At this step, a subject identification number is attributed to the subject. In the CRF the informed consent date(s) is/are available(s).
Principal Investigator:	Investigator responsible for the conduct of the clinical study at a study site. If a study is conducted by a team of individuals at a study site, the Principal Investigator is the responsible leader of the team and may be called the principal Investigator.
Pre-screening period:	Any actions performed by the site(s) in relation to the study recruitment that happens before the formal signature of the informed consent. No data is collected by the sponsor at this point.
Randomized (or allocated) subject:	Any subject who has been allocated to a study arm either by a randomization or by another method defined by the protocol. In the CRF the randomization date is available.
Screening period:	This period extends from the signature of the informed consent up until the randomization process.
Screen-failed subject:	The subject is considered a screen failure (SF) if: has been included but, did not complete the screening period successfully (which typically end at randomization). In the CRF an "end of study form" should be available with the reason for failure.

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Study product(s): Products to be used in the clinical study (Active/test products and control products).

Withdrawn (or Dropped out) Subject:

Any subject who has withdrawn from the study at any point after the screening period has ended but before having completed all the visits and assessments.

In the CRF the End of study status is available and it is different from: "Completed the study".

## 2. SYNOPSIS AND FLOW CHART

<b>Study title</b>	<i>The effect of increased water intake on the frequency of clinical recurrent urinary tract infections in pre-menopausal women: S-HYDRACYST</i>
<b>Study code</b>	<i>NU369 S-Hydracyst</i>
<b>Study sponsor</b>	<i>Christine M'Rini - DANONE RESEARCH, Palaiseau-France</i>
<b>Study principal investigator</b>	<i>Dr. Maya Dabcheva, MC "Comac Medical"</i>
<b>Study product description</b>	<p>The study is testing a medical recommendation, consisting in increasing the daily water intake in the tested group.</p> <p>For methodological reasons of homogeneity of access to water of the subjects, mineral commercialized water will be distributed to the subjects included in the tested group, during the whole study duration.</p> <p>The mineral water is Evian® brand</p> <p>The intervention group will increase his fluid intake of 1.5L of Evian® water (on the top of their normal fluid intake)</p> <p>The control group will not change its water intake habits.</p>
<b>Study period</b>	<p><i>Intervention period: Dec 2013 – Oct 2015 (12 mo per subject)</i></p> <p><i>Start of study (first subject first visit): Dec 2013</i></p> <p><i>End date (clinical study report writing): May 2016</i></p>
<b>Study objectives</b>	<p><u>Study primary objective:</u></p> <p><i>To assess the effect of increased daily water intake on frequency of clinical recurrence of urinary tract infections (rUTI) among low drinking women suffering from recurrent community-acquired UTI</i></p> <p><u>Study secondary objectives:</u></p> <p><i>To evaluate the impact of increased daily water intake on use of antibiotics in recurrent UTI patients</i></p> <p><i>To evaluate the impact of increased daily water intake on the duration of urinary infection episodes</i></p> <p><i>To determine the changes in urinary hydration markers according to the changes in fluid intake habits</i></p> <p><i>To evaluate the impact of increased daily water intake on health costs in recurrent UTI patients</i></p> <p><i>To evaluate the impact of increased daily water intake on quality of life (QoL) in recurrent UTI patients</i></p> <p><u>Exploratory Objective:</u></p> <p><i>To evaluate the relationship between urinary hydration markers and number of UTI events</i></p>

	<i>To evaluate the relationship between urinary hydration markers and delay UTI events</i>
<b>Study Methodology</b>	Prospective, mono-centric, open-label, randomised controlled trial in two parallel groups: <ul style="list-style-type: none"> <li>❖ Control group not changing their fluid intake habits</li> <li>❖ Intervention group provided with low mineralised mineral water, fluid intake recommendations and regular <i>hydration coaching</i> support</li> </ul>
<b>Study population and sample size</b>	The study population consists in pre-menopausal women diagnosed with rUTIs. and having a 'low drinker' profile. The total number of completed subjects is estimated at 84 subjects.  In order to achieve 84 completed subjects, and considering a 40% drop out rate, we'll need to randomise 140 subjects.  Considering a 50% screen failure rate we might had to screen 280 subjects. In any case, the screening will be over until the 140 randomised subjects will be reached.
<b>Subject recruitment</b>	This is a mono-centric study which will be performed in Bulgaria. 140 females will be randomized in this trial.
<b>Eligibility criteria</b>	<p><u>Eligibility criteria checked at V1</u></p> <p><u>Inclusion criteria:</u></p> <p>II01a. Women with at least 3 clinical recurrences* of (UTI in the last 12 months (at least one confirmed by bacteriological exam) and asymptomatic at V1 or II01b. Women with at least 3 clinical recurrences* of UTI in the last 12 months (at least one confirmed by bacteriological exam) and symptomatic at V1 or II01c. Women with at least 3 clinical recurrences* of UTI in the last 12 months (who did not performed any bacteriological exam in the last 12 months) and symptomatic at V1 II02 Age ≥ 18 years II03 Low-drinker (&lt; 1.5 L fluids per day) II04 Regular meal consumption (breakfast, lunch and dinner) II05 Access to Internet for information on hydration  II06 Subject, upon briefing of the content of the present study, fully understanding and agreeing to its objective and having given written (dated and signed) informed consent to take part in the study II07 Subject who is able to communicate well with the Investigator and to comply with the requirements of the entire study II08 Literate subjects, able to fill in fluid questionnaires and QoL questionnaires II09 Women accepting to keep their lifestyle habits during the whole duration of the study II10 Women using any form of contraception II11 Subject covered by social security or covered by a similar system</p> <p><u>Exclusion criteria:</u> (checked at visit V1)</p> <p>IE01 Incapacity / non-willingness to consume 1.5 L of drinking water per day on top of their usual consumption IE02 Women with history of UTI complications (pyelonephritis or other) in the last 12 months IE03 Use of antibiotics or cranberries juice and extracts in the previous 2 weeks IE04 Chronic treatments with anti-coagulants therapy IE05 Chronic bladder inflammation (defined as permanent bladder bacterial infection)</p>

	<p>IE06 Chronic diarrhea or constipation treated with chronic use of laxative substances</p> <p>IE07 Interstitial cystitis</p> <p>IE08 Estrogen-dependent symptomatic vulvo-vaginitis</p> <p>IE09 Recent (&lt;1year) or active renal stone disease</p> <p>IE10 Urinary tract structural abnormalities</p> <p>IE11 Obesity or malnutrition (BMI &lt;18.5 Kg/m<sup>2</sup> and &gt;30 Kg/m<sup>2</sup>)</p> <p>IE12 Pregnant or lactating women</p> <p>IE13 Plans of any of the following 12 months after screening visit to become pregnant</p> <p>IE14 Menopausal and perimenopausal women.</p> <p>IE15 Subjects general treated with drugs which can modify measurements performed in the study, in particular the assessment of the hydration status (diuretic intake, corticoids or treatment interfering with metabolism and nutrition behavior)</p> <p>IE16: Subjects with severe or uncontrolled organic disease, likely to interfere with the parameters of the study (e.g. neoplastic, cardiovascular, pulmonary and digestive disorders, unstabilized diabetes type I and II, untreated or uncontrolled clinically significant arterial blood hypertension) or mental disorders affecting eating and drinking behavior (i.e. primary polydipsia, bulimia nervosa, psychosis etc.)</p> <p>IE17: Women who have taken part in any other clinical study for the treatment of rUTI during the last 12 months</p> <p>IE18: Vulnerable subjects defined as individuals whose willingness to volunteer in the clinical trial may be unduly influenced by the expectation, whether justified or not, of benefits associated with participation, or of a retaliatory response from senior members of a hierarchy in case of refusal to participate. (Examples are members of a group with a hierarchical structure linked to the Investigator or to the Sponsor, such as medical, pharmacy, dental and nursing students, subordinate hospital and laboratory personnel, employees of the Investigator or of the Sponsor, members of the armed forces, and persons kept in detention).</p> <p>IE19: No legal capacity or limited legal capacity or unable to give an informed consent.</p> <p>IE20: Subjects unlikely to cooperate in the study, and/or poor compliance anticipated by the Investigator.</p> <p><i>* presence of bacteriuria in urine (<math>\geq 10^3</math> cfu/mL of a uropathogen in midstream urine culture) revealed by quantitative culture or microscopy in a sample taken from a patient or the typical symptoms of lower or upper urinary tract infection (defined as <math>\geq 3</math> urinary symptoms (difficult, painful, urgent and frequent urination, hematuria, supra-pubic pressure, increase in the number of white blood cells (<math>&gt;10.000/ml</math>) in the urine, with or without bacterial infection)).</i></p> <p><i>The presence of symptomatic bacteriuria can be established with a single urine sample</i></p> <p><i>Data source available at <a href="http://www.sign.ac.uk/pdf/sign88.pdf">http://www.sign.ac.uk/pdf/sign88.pdf</a></i></p> <p><u>Eligibility criteria checked at V2</u></p> <p>Pregnancy test will be repeated for confirmation of the V1 eligibility criterion and to confirm perimenopausal status FSH blood test may be performed.</p> <p><u>Randomization criteria</u></p> <p>RI01: Negative urine culture and asymptomatic state (i.e. silent phase) at V2 (randomization visit)</p> <p>RI02: Low-drinker confirmation (24 hours urinary volume &lt; 1.2 L per day)</p> <p><u>Non-randomization criteria</u></p> <p>RE01: Chronic kidney disease (defined as decreased GFR (GFR&lt;60 ml/min/1.73m<sup>2</sup> calculated using EPI equation)</p> <p>RE02: Women suffering multiple antibiotic resistant bacterial strain<sup>s</sup></p>
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	§ Multiple antibiotic resistance is defined as resistance to 3 or more different antibiotics
<b>Study product administration</b>	<p>Three bottles of natural mineral water (500 mL each) to be consumed daily, in addition to the subjects' usual fluid intake volume, for 12 months.</p> <ul style="list-style-type: none"> <li>- Bottles will be provided to subjects in the intervention group for free. It is suggested to consume them at the beginning of every meal (breakfast, lunch and dinner) and fully drink them before the following meal. Coaching support will also be made available; this will be reinforced by individualised phone calls.</li> <li>- The study products will be provided by DANONE RESEARCH and will be supplied by a professional logistics company</li> </ul>
<b>Study description / Duration of subject participation</b>	<p>The total duration of the intervention phase will be 12 months per subject (between V2 and V4).</p> <p>Each subject will attend 4 visits:</p> <ol style="list-style-type: none"> <li>1. <i>Inclusion visit (V1)</i></li> <li>2. <i>Randomisation visit (V2; M0)</i></li> <li>3. <i>Visit 3 (V3; M6)</i></li> <li>4. <i>Visit 4 (V4; M12)</i></li> </ol> <p>The 3 days fluid diary will be administered every month (from M0 to M12). In order to standardize the data collection, women will be asked to complete the 3 days fluid intake diary on Saturday-Sunday-Monday once a month.</p> <p>Quality of life questionnaire will be administered during three of the four visits (V2, V3 and V4).</p> <p>Laboratory exams will be performed at visit 2, 3 and 4. Fasting 8-12 hours will be required.</p> <p>Women will be asked to collect 24h urine and to complete a voiding diary outside the menstrual period.</p> <p>Symptoms of UTI will be recorded during all the visits and during the phone calls.</p> <p>Information about bladder emptying after sexual intercourses and active/inactive sexual life during the last month will be collected each month.</p> <p>Potential extra visits will be taken into account when/ if any acute episode during the course of the study. During the extra visits urine culture will be performed to confirm the presence of UTIs. If confirmed, the UTI episode will be treated following the normal clinical practice. Urine culture will be also performed at the end of the therapy to be sure that women are completely recovered. Start and end dates of each episode will be reported. Healthcare costs data related to UTI episodes will be collected during each extra (relapse) visit and during the visits 3 and 4 if needed.</p> <p>If women will report a UTI event during V2, V3 or V4, some of the urinary parameters collected during the visit (urine colour, urinary specific gravity) may be biased. For this reason they will be asked to come back once recovered to re-check urinary parameters.</p> <p>Physical examination will be performed during V2 and V4.</p> <p>Every month women will be contacted by phone by the investigators, to check the compliance with the intervention (if in the intervention group), adverse events, sexual activity during the past month, symptoms of UTI and, eventually, start and end date of episodes of UTI.</p>

	<p><u>Inclusion visit:</u> (V1)</p> <p>Women willing to participate in the study will give their informed consent and be evaluated against inclusion and exclusion criteria. A pregnancy test will be performed.</p> <p>Information about bladder emptying after sexual intercourses and active/inactive sexual life during the last month will be collected.</p> <p>Urine culture to confirm the presence of UTI will be performed only in symptomatic women. They will be then sent home with a treatment against their acute episode (i.e. AFU/EAU guidelines for UTI management).</p> <p>All participants will be asked to complete the 3 days fluid intake diary at home and to bring the questionnaires back to the site when they come back for the randomization visit.</p> <p>Asymptomatic women at visit 1 will come back for the randomization visit after a minimum of 4 days and up to 2 months at the latest.</p> <p>Symptomatic subjects showing positive urine culture (confirming the presence of UTI) will be sent home with a standardised treatment against their acute episode (i.e. AFU/EAU guidelines for UTI management) and they will be asked to come back for the randomization visit (V2) after about 4 weeks after V1 and up to 2 months at the latest.</p> <p>Anthropometrical parameters (body weight, waist circumference, height) will be collected.</p> <p>Anamnestic data and vital signs will be collected.</p> <p><u>Randomisation visit:</u> (V2)</p> <p>Subjects will be evaluated against randomisation and non-randomization criteria. Women will attend to the clinical site for confirmation of negative urine culture. A physical examination will be performed. Women will be randomly allocated into one of the two study arms. In order to evaluate their baseline hydration status, they will have been asked to collect and come along with a 24h urine sample beforehand. Urinary markers of hydration (urine volume, pH, osmolality, specific gravity, frequency of micturition, urine colour) will be monitored. Electrolytes in the urine sample (sodium, potassium, calcium, magnesium, chloride, oxalate, citrate) will be collected. Urine creatinine will be collected.</p> <p>Information about bladder emptying after sexual intercourses and active/inactive sexual life during the last month will be collected.</p> <p>A blood sample will be collected for determination of lipidic profile (HDL cholesterol, LDL cholesterol, triglyceride), glycosylated hemoglobin, serum creatinine, serum urea, copeptin. Estimated glomerular filtration rate (eGFR) will be calculated. Pregnancy test will be repeated and FSH may be performed to confirm perimenopausal status.</p> <p>Vital signs and anthropometrical parameters (body weight, waist circumference) will be collected. Data on chronic medication (including all the anti-hypertensive agents) any other concomitant medications and AE will be collected.</p> <p>The 3 days fluid intake diary (Saturday-Sunday-Monday) will be collected: patients will be asked to complete the questionnaire at home every month until the end of the study and to send them back to the investigating site by courier</p> <p>In addition, patients in the intervention group will be asked to fill in a daily mineral water diary in order to help them to check the study product consumption. These daily diaries are called 'Subject Compliance Diaries' and will be returned to the site each month by courier together with the 3 days fluid intake diaries.</p> <p>Patients will be asked to complete on site the Quality of Life questionnaire, AE, Concomitant medications.</p> <p>Patients will be provided with urine colour chart for regular self-check of their hydration status and verbal instructions how to use them in case they will be allocated in the intervention group.</p>
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Patients will receive instructions about study requirements accordingly.

Patients who will be randomized in the study group will be asked to consume 1,5L of Evian® brand mineral water which will be supplied to their home. This volume is on top of their normal water consumption.

Evaluation visit: (V3)

Subjects will come along with a 24h urine sample to the clinical site. Urinary markers of hydration (urine volume, pH, osmolality, specific gravity, frequency of micturition, urine colour) will be monitored. Electrolytes in the urine sample (sodium, potassium) will be collected. Urine creatinine will be collected. Vital signs and anthropometrical parameters (body weight, waist circumference) will be collected.

Information about bladder emptying after sexual intercourses and active/inactive sexual life during the last month will be collected.

A blood sample will be collected for determination serum creatinine and copeptin. Estimated glomerular filtration rate (eGFR) will be calculated. 3 days fluid diary will be completed every month until V4 and will be returned by courriert to the investigator site. Patients in the intervention group will continue to fill in a daily mineral water intake diary until V4 and will return them to the site by courier on monthly basis, together with the 3 days fluid intake diary. Symptoms of UTI in the time between the visits will be recorded together with start and end date for each episode.

Patients will be asked to complete on site the Quality of Life questionnaire, AE, Concomitant medications.

Last visit: (V4)

Subjects will attend to the clinical site come along with a 24h urine sample. A physical examination will be performed. Urine culture to evaluate the presence of any infection will be performed. Urinary markers of hydration (urine volume, pH, osmolality, specific gravity, frequency of micturition, urine colour) will be monitored. Electrolytes in the urine sample (sodium, potassium, calcium, magnesium, chloride, oxalate, citrate) will be collected. Urine creatinine will be collected. Vital signs and anthropometrical parameters (body weight, waist circumference) will be collected along with quality of life, concomitant treatments, and AE.

Information about bladder emptying after sexual intercourses and active/inactive sexual life during the last month will be collected.

A blood sample will be collected for determination of lipidic profile (HDL cholesterol, LDL cholesterol, triglyceride), glycosylated hemoglobin, serum creatinine, serum urea, copeptin. Estimated glomerular filtration rate (eGFR) will be calculated. Data coming from the 3 days fluid intake diary will be collected. Symptoms of UTI in the time between the visits will be recorded.

Relapse visits:

Subjects presenting with symptoms of UTI during the course of the study will be instructed not to stop the increased water intake (if in the intervention group) and to consult with the investigator for clinical examination; urine cultures will be systematically performed to confirm preliminary diagnosis. Data about duration of the infection (starting and ending date) will be registered. Information about bladder emptying after sexual intercourses and active/inactive sexual life during the last month will be collected. Healthcare costs data due to illness (for patients and/or conductors) will be collected. Concomitant treatments will be collected. Subjects will then be sent home with a standardised treatment against their acute episode (i.e. AFU/EAU guidelines for UTI management) irrespective of the study group they belong to. Urine culture will be also performed at the end of the therapy to be sure that women are completely recovered. Direct and indirect healthcare costs due to UTI event (for patients and conductors) will be collected.

Intake surveys & coaching support: throughout the duration of the study, subjects will be asked to complete a 3 days fluid intake diary every month.



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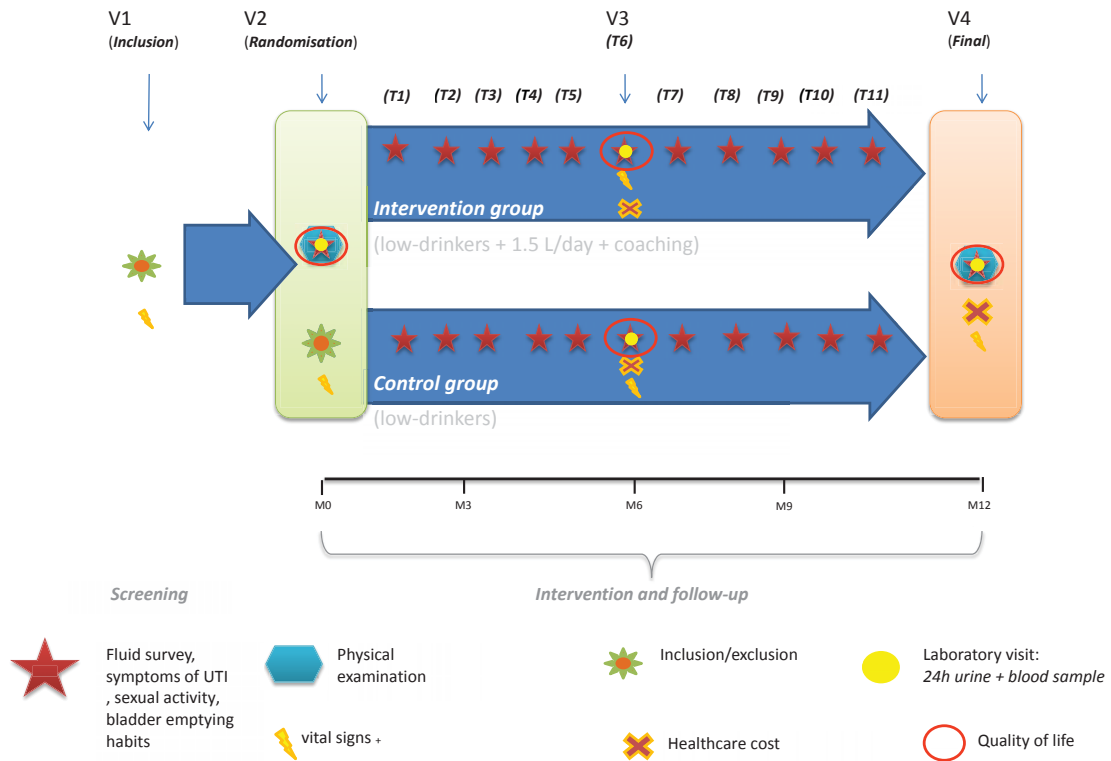
	<p>In order to reinforce their adherence to fluid intake recommendations, subjects allocated to the intervention group will be provided with bottled mineral water, will receive individualised phone calls at regular intervals and will be provided with coaching materials. By contrast, subjects allocated to the control group won't receive any fluid intake recommendations nor any test product or coaching support.</p> <p>Subjects will also be asked to complete a daily mineral water intake diary in order to assess the compliance to the study product intake (Subject Compliance Diary).</p> <p>The protocol will follow ICHG6 guidelines on Good Clinical Practices and applicable Directives/local laws.</p>
<p><b>Study design schema</b></p>	<p><i>See below</i></p>

Line of the data collection

Procedures	Visits		Month 0	1st Month	2 <sup>nd</sup> Month	3rd Month	4th Month	5th month	6th month	Month 6	7th Month	8th Month	9th Month	10th Month	11th Month	12th Month	Month 12	Relapse visit
	Inclusion Visit (V1)	Home	Randomisati on Visit (V2)	Home	Home	Home	Home	Home	Home	Visit 3 (V3)	Home	Home	Home	Home	Home	Home	Completion (V4)	
Screening for acute UTI or previous episodes (at least 3 in the last 12 months)	X																	
Physical examination			X															X
Phone call to check compliance, AE and UTI events				X	X	X	X	X			X	X	X	X	X			
Medical & Surgical history	X																	
Informed consent	X																	
Inclusion / exclusion criteria	X																	
Randomisation/non randomisation criteria			X															
Demographic modules	X																	
Vital sign and anthropometrical parameters	X		X							X								X
Instructions on completion of 3 days fluid intake diary	X		X							X								
3 days fluid intake diary check			X							X								X
3 days fluid intake diary completion		X		X	X	X	X	X	X		X	X	X	X	X	X		
Subject Compliance Diary (instructions for the test group)			x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Subject Compliance Diary completion (for the test group)				x	x	x	x	x	x		x	x	x	x	x	x		
Subject Compliance Diary check (for the test group)				x	x	x	x	x		x	x	x	x	x	x			x
QoL questionnaire (SF-12 V2)			X							X								X
Urine colour chart and instructions delivery (for the test group)			X															
Urinary pregnancy test	X		X															
Delivery of urine containers & Instructions	X		X							X								
Urine 24h collection (+ voiding diary)		X*							X*							X*		
Collection of urine containers & Voiding diary			X							X								X
Blood sample			X							X								X
Symptoms of UTI	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Bacteriogram	X (if symptomatic)		X															X
Healthcare costs data																		X

Concomitant Treatments	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Bladder emptying after sexual intercourses	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Active/inactive sexual life during the last month	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

\* 24h urine to be collected at home the day before the visit (V2, V3 and V4)



<p><b>Study Parameters</b></p>	<ul style="list-style-type: none"> <li>- <u>parameter 1</u>: total number of clinical recurrences of UTI during the Study period*</li> <li>- <u>parameter 2</u>: mean time elapsed between UTI episodes *</li> <li>- <u>parameter 3</u>: mean duration of UTI episodes</li> <li>- <u>parameter 4</u>: frequency of micturition during the 24h urine collection</li> <li>- <u>parameter 5</u>: 24h urine (volume, pH, osmolality, specific gravity, urine colour, sodium, potassium, calcium, magnesium, chloride, oxalate, citrate, creatinine)</li> <li>- <u>parameter 6</u>: blood sample (lipidic profile, glycosylated hemoglobin, serum creatinine, serum urea, copeptin)</li> <li>- <u>parameter 7</u>: vital signs (diastolic and systolic blood pressure, heart rate, body temperature)</li> <li>- <u>parameter 8</u>: quality of life (questionnaire SF12V2)</li> <li>- <u>parameter 9</u>: fluid intake (water, other types of fluids)</li> <li>- <u>parameter 10</u>: anthropometry (body weight, waist circumference, height, BMI)</li> <li>- <u>parameter 11</u>: pathology related costs</li> <li>- <u>parameter 12</u>: complications from UTIs (UTI related AE, SAE)</li> </ul> <p><i>*Based on the simultaneous presence of positive urine culture and UTIs symptoms</i></p>
<p><b>Study Endpoints</b></p>	<p><u>Principal:</u></p> <ul style="list-style-type: none"> <li>➤ Difference between groups in terms of number of UTI recurrence over 12 months of follow up</li> </ul> <p><u>Secondary:</u></p> <ul style="list-style-type: none"> <li>➤ Difference between groups in terms of duration of urinary infectious episodes</li> <li>➤ Average delay between each UTI over one year of study period</li> <li>➤ Change in urinary hydration markers following changes in water intake habits</li> <li>➤ Difference between groups in terms of change in QoL</li> <li>➤ Difference between groups in terms of antibiotic prescription/usage to treat UTI</li> <li>➤ Difference between groups in terms of health-costs associated with management of UTI recurrence(s)</li> <li>➤ Difference between groups in terms of cost utility analysis</li> </ul> <p><u>Exploratory:</u></p> <ul style="list-style-type: none"> <li>➤ Relationship between urinary hydration markers and number of UTI events</li> <li>➤ Relationship between urinary hydration markers and delay UTI events</li> </ul>
<p><b>Safety evaluation criteria</b></p>	<p>Blood pressure, heart rate, adverse events, serious adverse events</p>

<b>Statistical analysis</b>	<ul style="list-style-type: none"> <li>➤ The statistical methodology will be detailed in the statistical analysis plan written and finalized before the database lock.</li> <li>➤ Descriptive statistics will be given for each of the parameters (i.e. for continuous variables: group size, mean, standard deviation of the variable [SD], standard error of the mean [SEM], minimum, maximum, median, and possibly quartiles; for qualitative variables, group size and percentage).</li> <li>➤ For the main criterion, a nonparametric method called the mean cumulative function (MCF) will be used to analyse the multiple/repeated UTI events occurring. The null hypotheses is “There is no difference between groups on the average number of recurrent UTIs event experienced”. We will consider a Type I error rate (alpha) equal to 5%.</li> <li>➤ The analysis will be conducted with the statistical software package SAS 9.3.</li> </ul>
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### **3. INTRODUCTION**

#### **3.1 SCIENTIFIC BACKGROUND INFORMATION**

Urinary tract infection (UTI) is one of the most common clinical diagnoses in women. The lifetime risk for UTI in women is high (greater than 50%) and in the U.S. between 1988 and 1994 the overall lifetime prevalence of UTI was estimated to be 53,067/100,000 women.(1) The estimated global incidence of UTIs is at least 250 million cases per year.(2) UTIs are a source of significant cost and morbidity. Most UTIs are self-limiting but occasionally can be associated with significant complications such as pyelonephritis and sepsis. Composite data revealed that overall expenditures for the treatment of UTIs in women in the United States, excluding spending on outpatient prescriptions, were approximately 2.47 billion U.S. dollars in 2000.(1) According to the 1997 National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey, UTI accounted for nearly 7 million office visits and 1 million emergency department visits, resulting in 100,000 hospitalizations.(3) The exact frequency of UTIs is difficult to assess because they are not reportable diseases in the United States. This situation is further complicated by the fact that accurate diagnosis depends on both the presence of symptoms and a positive urine culture, although in most outpatient settings this diagnosis is made without the benefit of culture.

There are several higher risk populations for UTIs including infants, pregnant women, the elderly, patients with spinal cord injuries and/or catheters, patients with diabetes or multiple sclerosis, patients with acquired immunodeficiency disease syndrome/human immunodeficiency virus, and patients with underlying urologic abnormalities.(4) However, UTIs are very common in otherwise healthy women. It is estimated that at least a third of all women in the United States are diagnosed with a UTI before they are 24 years old.(5) There are both host and bacterial factors that contribute to UTIs.(6) In young women, sexual activity is associated with an increased risk for UTIs.(7) UTIs also have a propensity to recur. In otherwise healthy college women with a first UTI, the risk of a second episode within 6 months was 24%,(7) and in those with a history of one or more UTIs, the risk of a second within 1 year was 70%.(8) Even when UTI is not associated with long term consequences, the condition results in pain and suffering, and negatively impacts quality of life, albeit transiently.(9) Common symptoms in

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premenopausal women include frequent, urgent and painful urination and suprapubic pressure and hematuria may also be present.

There are multiple reasons to try to prevent UTIs in women. First and foremost is to reduce morbidity associated with the infections as well as reduce the cost of treatment. However, there are also important reasons to reduce the use of antibiotics in these patients.(10) First, there is increasing resistance of *Escherichia coli*, the primary causative agent of uncomplicated UTI, to a variety of antibiotics, including fluoroquinolones and extended-spectrum beta-lactamase (ESBL) resistance is increasingly observed among community acquired UTI.(11) Second, there can be significant impact of short courses of antibiotics on the gut and vaginal microbiota which can contribute to recurrence and antibiotic resistance.(12) Third, there are risks associated with antibiotic use such as allergic reactions and side effects of the drugs themselves. Finally there is a risk of vaginal candida infection, which occurs in up to 22 % of women treated for uncomplicated UTI.(13)

Moreover, due to the high prevalence and incidence, UTI has enormous economic implications.

As for other pathologies, costs related to UTI episodes should be divided into direct and indirect ones.

Direct costs include the costs of outpatient doctor visits, antibiotics and specific antimicrobial agent prescription, along with hospitalization expenses. The indirect costs include all the “non-medical costs” related to the pathology, such as travel and sick days for the patients and for the caregivers.

Direct and indirect annual costs related to acute episode of UTI have been estimated to be around \$1.6 billion for the US female population (14), including approximately \$936 million for indirect costs and \$659 million for direct ones.

To date, no data about specific European population are available. In spite of available evidence suggesting a link between urinary hydrodynamics and frequency of UTI episodes, cost-savings that could potentially be derived from appropriate fluid intake among UTI patients remain to be established.

Several strategies have been proposed to try to reduce the risk of recurrent UTIs.

While the use of daily antibiotics or post-coital antibiotics is effective, the rise of resistance and risk associated with antibiotics has made these strategies less attractive. Different approaches have been proposed including use of functional foods, lactobacillus and vaccines.(10) The most studied functional food thus far has been cranberries and their extracts. A Cochrane review on studies including cranberries included a total of 24 studies (six cross-over studies, 11 parallel group studies with two arms; five with three arms, and two studies with a factorial design) with a total of 4473 participants.(14) The comparison/control arms were placebo, no treatment, water, methenamine hippurate, antibiotics, or lactobacillus. The meta-analyses found that compared with placebo, water or not treatment, cranberry products did not significantly reduce the occurrence of symptomatic UTI overall (RR 0.86, 95% CI 0.71 to 1.04) or for any the subgroups: women with recurrent UTIs (RR 0.74, 95% CI 0.42 to 1.31); older people (RR 0.75, 95% CI 0.39 to 1.44); pregnant women (RR 1.04, 95% CI 0.97 to 1.17); children with recurrent UTI (RR 0.48, 95% CI 0.19 to 1.22); cancer patients (RR 1.15 95% CI 0.75 to 1.77); or people with neuropathic bladder or spinal injury (RR 0.95, 95% CI: 0.75 to 1.20). Studies evaluating lactobacillus have shown some promise but await further validation.(15,16)

### **3.2 DESCRIPTION OF THE TEST PRODUCT**

The approach that we propose involves randomizing premenopausal women with recurrent UTIs to high versus normal water intake to determine whether this will reduce risk of UTIs. For this purpose we will provide women with three bottles of natural mineral commercialized water (500 mL each) to be consumed daily for 12 months. Bottles will be provided to subjects in the intervention group for free, along with the suggestion to consume them at the beginning of every meal (breakfast, lunch and dinner) and fully drink them before the following meal.

### **3.3 SCIENTIFIC RATIONALE**

The study will include only women who are low-drinkers (< 1.5 L fluids per day; urinary volume < 1.2 L per day) since they are most likely to have a predisposition to UTIs due to infrequent voiding. The rationale for this approach is that drinking more fluid will increase voiding frequency and voiding is the main defence of the bladder to reduce the number of bacteria in the bladder and avoid UTIs. As such, increasing fluid intake will increase the frequency and volume of voiding and potentially reduce risk of recurrent UTIs. Support for this hypothesis is multifold. A non-randomised, multivariate analysis comparing 791 women teachers who deliberately restricted their fluid intake (25% voided only once during working hours, or not at all) with women able to drink without restrictions found that women in the former group were at significantly higher risk of UTI than were women in the latter group (RR 2.21; 95% CI 1.45-3.38) after controlling for parity, voiding infrequently at work, and urge incontinence.(17) A study of 1613 women compared frequency of voiding based on age of women and impact on UTIs.(18) The study found that women aged 20-25 had a higher rate of this low voiding frequency than women 30-35 or 40-45 years and in all 3 groups, women who voided 3 times or less per day had significantly more urinary infections than those with 4 or more voidings per day, (p < 0.01). While results are inconsistent, several studies found that higher post-void residual volumes increases risk of UTI in women.(19-21) This supports the notion that efficacy of voiding and emptying the bladder can reduce risk of UTIs. One additional impact of fluid intake may involve reducing urine acidity. Low fluid intake is associated with an increase in urine osmolality and acidity which can predispose to bacterial adhesion to the bladder epithelium. A study in premenopausal women who had been treated for at least two idiopathic UTIs in the previous 6 months found that self-monitoring urine osmolality using a handheld 'traffic light' probe was associated with a significant shift towards urine of lower osmolality and a significant reduction in incidence of UTIs compared with the period in which the probe was not used.(22) Of the 17 patients who completed both 4-month periods, 14 felt that the probe had helped them to prevent infection.

#### *3.3.1 Rationale for the study purpose*

The above evidence and the heavy burden of recurrent UTIs on society demonstrate a significant need for strategies to prevent UTIs. The planned study will determine the efficacy of increased water intake in decreasing the risk of UTIs in women who are low volume drinkers and who suffer from recurrent UTIs compared to a control group.

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### *3.3.2 Rationale for the study population*

UTI is the most common infection in humans. It is highly prevalent in both men and women but its frequency is about 50 times higher in adult women of all age groups. This may be because the urethra is shorter in women than in men, making it easier for bacteria to ascend into the bladder and, once there, proliferate.

More than half of all women (50–60%) encounter with at least one UTI at some stage during their lives (23). Symptomatic infection of the bladder (lower UTI) has been estimated to occur in up to 30% of women during their life (24).

The estimated global incidence of UTI in women based on self-report of physician diagnosis is 11% per year resulting in at least 250 million cases per year (25). This incidence is higher (17.5%) during ages 18–24 and decreases to 9% for women 50 and over (5).

Approximately 5% of women with an initial UTI have multiple episodes within a year and a high recurrence rate, ranging between 25 to 30%, has been shown to affect the total female population (26). Observational studies have shown 6-month risk of recurrence ranging from 17 to 24% among otherwise healthy pre-menopausal women (5, 27). It is not surprising that one of the major risk factor for UTI among women is having a history of UTI.

Given the high burden, recurrent premenopausal women will be the target population of our study.

### *3.3.3 Rationale for dose(s) selected and/or dose(s) regimen*

We want women in the intervention group, to achieve a mean daily urine osmolality equivalent to that of plasma (approximately 285 mosm/kg). This osmolality goal is selected because it defines the transition from dilute to concentrated urine and is a safe end point under most any circumstance. To maintain such a low urine osmolality, we assume that women in the intervention group should increase their water intake of 1.5 L/day.

Moreover, to maintain a urinary volume of 1.5L we need to assure a total fluid intake of more than 1.3L/day (29). 1.5L/day will be our target knowing that the mean water intake in European women is less than 1L/day.

This dose hopes to gain an appreciation for how compliant patients would be in following a water prescription without inconveniencing them.

### *3.3.4 Rationale for the study design*

Given the necessity to establish a causality relation between increased water intake and UTI episodes, we will perform a monocentre, prospective, randomized, Open-label, controlled study. This model is often used where the double blind design is not applicable. It utilizes a strict randomization procedure: patients are allocated to different treatment regimens following the allocation concealment rules. In this way, the Investigator won't be able to subvert randomisation and select which patients get which treatment. The follow-up and treatment of patients is conducted openly in a way that adheres to accepted clinical principles and medical practice.



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### **3.4 POTENTIAL RISKS AND BENEFITS**

We expect increased fluid intake to be efficient in reducing the risk of clinical recurrences of UTI in pre-menopausal women. Decreased incidence of this pathology will reduce the comorbidity associated with UTI events as well as the related healthcare costs.

No risk linked to increased water intake has ever been shown in this population. More over the protocol suggests to split the water consumption over the day and consume 0,5L at the beginning of every meal (breakfast, lunch, dinner) and fully drink them before the following meal.

However, in case of ingestion of more than 1L of water in a short time interval (less than 10 min), the subject may have possible temporary stomach discomfort and an increased urge to use the toilet.

Blood samples will be drawn during the study visits. The risks related to this procedure are very weak and they are the same as for any type of blood drawing procedure in current medical practice:

- Pain during the sampling: it is especially connected to the speed of execution of the movement. This risk is minimized in this study as the staff performing the sampling is competent medical staff.
- Local secondary infection: this risk is theoretical because the used material is single-use, and the sampling is performed after local asepsis.
- Localized haematoma: this risk can be limited by the realization of an effective manual compression in the minutes following the sampling.

## 4. STUDY OBJECTIVES

### 4.1 PRIMARY STUDY OBJECTIVE

The primary objective of this study is to assess the effect of increased water intake on frequency of clinical recurrence of urinary tract infection among low drinking women suffering from recurrent community-acquired UTI.

### 4.2 SECONDARY STUDY OBJECTIVE(S)

To evaluate the impact of increased daily water intake on use of antibiotics in recurrent UTI patients

To evaluate the impact of increased daily water intake on the duration of urinary infection episodes

To determine the changes in urinary hydration markers according to the changes in fluid intake habits

To evaluate the impact of increased daily water intake on health costs in recurrent UTI patients

To evaluate the impact of increased daily water intake on quality of life (QoL) in recurrent UTI patients.

## 5. STUDY DESIGN

### 5.1 STUDY METHODOLOGY

This is an open label, prospective, single site, randomised, controlled trial, in two parallel groups with a 1:1 ratio allocation.

The randomization process will be applied using a centralised Interactive Web Response System (IWRS) that will allocate subject into one of the two groups in respect with the randomisation list generated at the beginning of the study.

### 5.2 STUDY EVALUATION CRITERIA

#### 5.2.1 Primary product effect evaluation endpoint

The primary criterion assessed during this study is the difference between groups in term of frequency of recurrence of Urinary Tract Infection events, defining by the number of events, using start and end dates of each one over a one year study period from randomisation visit (V2;M0) to end of study visit (V4;M12).

The start date of UTI event will be considered as the date of first symptoms, and the end date will be considered as the sampling date of urine culture performed at the end of the episode-therapy that provided negative urine culture.

#### 5.2.2 Secondary product effect evaluation criteria

The secondary criteria assessed during this study are as follows:

- Difference between groups in terms of duration of urinary infectious episodes
- Average delay between each UTI over one year follow up
- Change in urinary hydration markers following changes in water intake habits

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- Difference between groups in terms of change in QoL
- Difference between groups in terms of antibiotic prescription/usage to treat UTI
- Difference between groups in terms of health-costs associated with management of UTI recurrence(s)

As quality of life (QoL) is an important aspect of the intervention, the health economic evaluation will be considered through a cost utility analysis (CUA). Those health cost study will be conducted using two different perspectives. The perspective is the point of view from which the costs and benefits are recorded and assessed. Considering the research question, the two perspectives below will be considered:

- Social insurance
- Patients' perspective by determining patient out-of- pocket costs during the study intervention.

As several perspectives are included in the analysis, the results will be presented separately for each study perspective.

*5.2.3 Exploratory product effect evaluation criteria*

The exploratory criteria assessed during this study are as follows:

- Relationship between urinary hydration markers and number of UTI events
- Relationship between urinary hydration markers and delay UTI events

*5.2.4 Product safety evaluation criteria*

The safety criteria assessed during this study are as follows:

- Blood pressure
- Heart rate
- Body temperature
- Weight
- Urine sodium
- Urine potassium
- Physical Examination at V2 and V4
- Adverse events
- Serious adverse events

**5.3 STUDY GLOBAL DESCRIPTION**

The subjects will be divided into 2 balanced groups of 70 subjects each. Subjects allocated to study test group (intervention group) will be asked to consume daily three bottles of natural mineral water (500 mL each) in addition to their usual fluid intake consumption, for 12 months. It is suggested to consume them at the beginning of every meal (breakfast, lunch and dinner) and fully drink them before the following meal. The subjects allocated to control group will be asked not to change the low fluid intake assessed at baseline.

The study will include 2 parts:

1. The screening period (from visit V1 to V2). During this period, subjects will be screened for inclusion and exclusion criteria.

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Symptomatic subjects who will fulfil all the inclusion and exclusion criteria and showing positive culture, will be sent home with a treatment against their acute episode (i.e. AFU/EAU guidelines for UTI management) and will be asked to come back about 4 weeks after V1 and up to 2 months at the latest for the randomization visit; it is up to the Investigator's decision and the time needed for these subjects to be treated/recovered so as to receive a negative bacteriogram..

Asymptomatic subjects who will fulfil all the inclusion and exclusion criteria, will be asked to come back after a minimum of 4 days and up to 2 months at the latest for randomisation visit.

2. The study intervention period (12 months duration), from visits V2 to V4. During this period, the subjects will attend 2 evaluation visits (V3 at six months, V4 at twelve months).

Each subject will thus attend 4 visits in total at the clinical unit: Inclusion visit (V1), randomisation visit (V2), evaluation visit (V3) at month 6 and the end of study visit (V4) at month 12. In case of UTI episodes, relapse visits are required.

Each subject will undergo 3 blood sample collections, 3 24h urine collections and 2 urine cultures. Each appearance of UTI symptoms will lead to 2 additional urine cultures (one at the beginning of the episode and one after the treatment to confirm the recovery) over the study conduct.

For the blood samples, it is necessary for the subjects to be in a fasted state for 8-12 hours before the visit.

#### **5.4 STUDY DESIGN SCHEMA**

Refer to section 2.

#### **5.5 DURATION OF THE STUDY PER SUBJECT**

The total duration of the study is approximately fourteen (14) months for each subject, including an inclusion period of up to 2 months, a study intervention period of fifty-two weeks (twelve months).

## 6. SUBJECTS

### 6.1 SUBJECT RECRUITMENT AND SCREENING

The subjects included in the study will be women with recurrent episodes of UTI aged from 18 to premenopausal age and having a “low drinker profile”. Subjects with clinical manifestations of menopause such as women aged about 45 y.o; with irregularities of the menstrual cycle during the last 6 months as well as menopausal women will be excluded. In case of menopausal symptoms, a FSH blood test may be performed.

The subject inclusion will be stopped as soon as 140 subjects are randomized.

At this time, all subjects already included (subjects who are within the screening V1 to V2) will be randomized and will complete all the study procedures and visits according to the protocol until their last study visit (V4).

Subjects potentially able to participate to this study will be pre-selected by physicians working in liberal practice (GPs, urologists, gynaecologists) from their patients' database. The pre-screened subjects will be referred to the Investigating Centre to be recruited in the clinical study. If necessary, in order to reinforce recruitment rate, the Investigating Centre may also use advertising and conduct a pre-screening of patients at the investigating site following their standard process.

Taking into account that the study is particularly long, the study team should discuss the subject's willingness to comply with all study related procedures stressing the length and rigorous requirements prior to the subject enrolling.

The subject must have personally dated and signed their Informed Consent Form (ICF) before undergoing any medical or biological procedures required by the clinical study.

### 6.2 SUBJECT ELIGIBILITY CRITERIA

A subject is considered eligible when the Investigator certifies that the subject fulfils all inclusion criteria and does not meet any exclusion criteria for the current visit. This statement can potentially be repeated at different subjects' visits before subject's randomization as required by the study design.

#### 6.2.1 Inclusion criteria (checked at the inclusion visit - V1)

In order to be eligible to participate in this study, a subject must meet all of the following criteria at the Visit 1:

Inclusion criteria:

II01a. Women with at least 3 clinical recurrences\* of UTI in the last 12 months (at least one confirmed by bacteriological exam) and asymptomatic at V1

or

II01b. Women with at least 3 clinical recurrences\* of UTI in the last 12 months (at least one confirmed by bacteriological exam) and symptomatic at V1

or

II01c. Women with at least 3 clinical recurrences\* of UTI in the last 12 months (who did not performed any bacteriological exam in the last 12 months) and symptomatic at V1

II02 Age  $\geq$  18 years

II03 Low-drinker (< 1.5 L fluids per day)

II04 Regular meal consumption (breakfast, lunch and dinner)

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- II05 Access to Internet for information on hydration
- II06 Subject, upon briefing of the content of the present study, fully understanding and agreeing to its objective and having given written (dated and signed) informed consent to take part in the study.
- II07 Subject who is able to communicate well with the Investigator and to comply with the requirements of the entire study.
- II08 Literate subjects, able to fill in fluid questionnaires and QoL questionnaires
- II09 Women accepting to keep their lifestyle habits during the whole duration of the study
- II10 Women using any form of contraception
- II11 Subject covered by social security or covered by a similar system

Exclusion criteria: (checked at visit V1)

- IE01 Incapacity / non-willingness to consume 1.5 L of drinking water per day on top of their usual consumption
- IE02 Women with history of UTI complications (pyelonephritis or other) in the last 12 months
- IE03 Use of antibiotics or cranberries juice and extracts in the previous 2 weeks
- IE04 Chronic treatments with anti-coagulants therapy
- IE05 Chronic bladder inflammation (defined as permanent bladder bacterial infection)
- IE06 Chronic diarrhea or constipation treated with chronic use of laxative substances
- IE07 Interstitial cystitis
- IE08 Estrogen-dependent symptomatic vulvo-vaginitis
- IE09 Recent (<1year) or active renal stone disease
- IE10 Urinary tract structural abnormalities
- IE11 Obesity or malnutrition (BMI <18.5 Kg/m<sup>2</sup> and >30 Kg/m<sup>2</sup>)
- IE12 Pregnant or lactating women
- IE13 Plans of any of the following 12 months after screening visit to become pregnant
- IE14 Menopausal and perimenopausal women
- IE15 Subjects general treated with drugs which can modify measurements performed in the study, in particular the assessment of the hydration status (diuretic intake, corticoids or treatment interfering with metabolism and nutrition behavior)
- IE16: Subjects with severe or uncontrolled organic disease, likely to interfere with the parameters of the study (e.g. neoplastic, cardiovascular, pulmonary and digestive disorders, unstabilized diabetes type I and II, untreated or uncontrolled clinically significant arterial blood hypertension) or mental disorders affecting eating and drinking behavior (i.e. primary polydipsia, bulimia nervosa, psychosis etc.)
- IE17: Women who have taken part in any other clinical study for the treatment of rUTI during the last 12 months
- IE18: Vulnerable subjects defined as individuals whose willingness to volunteer in the clinical trial may be unduly influenced by the expectation, whether justified or not, of benefits associated with participation, or of a retaliatory response from senior members of a hierarchy in case of refusal to participate. (Examples are members of a group with a hierarchical structure linked to the Investigator or to the Sponsor, such as medical, pharmacy, dental and nursing students, subordinate hospital and laboratory personnel, employees of the Investigator or of the Sponsor, members of the armed forces, and persons kept in detention)
- IE19: No legal capacity or limited legal capacity or unable to give an informed consent
- IE20: Subjects unlikely to cooperate in the study, and/or poor compliance anticipated by the Investigator

*\* presence of bacteriuria in urine ( $\geq 10^3$  cfu/mL of a uropathogen in midstream urine culture) revealed by quantitative culture or microscopy in a sample taken from a patient or the typical symptoms of lower or upper urinary tract infection (defined as  $\geq 3$  urinary symptoms (difficult, painful, urgent and frequent urination, hematuria, supra-pubic pressure, increase in the number of white blood cells ( $>10.000/ml$ ) in the urine, with or without bacterial infection)).*

*The presence of symptomatic bacteriuria can be established with a single urine sample*

*Data source available at <http://www.sign.ac.uk/pdf/sign88.pdf>*

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### 6.2.3 *Randomisation criteria (checked at the randomisation visit – V2)*

Subjects may only be randomised in this study after meeting the inclusion criteria and presenting none of the exclusion criteria stipulated in paragraphs 6.2.1 and 6.2.2 above. A pregnancy test will be repeated in order to confirm the inclusion criterion and FSH may be performed to confirm perimenopausal status.

In addition, the subject must meet the following randomisation criteria checked during the randomisation visit V2:

RI01: Negative urine culture and asymptomatic state (i.e. silent phase) at V2 (randomization visit)

RI02: Low-drinker confirmation (24 hours urinary volume < 1.2 L per day)

### 6.2.4 *Non -Randomisation criteria (checked at the randomisation visit – V2)*

A potential subject who meets any of the following criteria at V2 will be excluded from participation in this study:

RE01: Chronic kidney disease (defined as decreased GFR (GFR<60 ml/min/1.73m<sup>2</sup> calculated using EPI equation)

RE02: Women suffering multiple antibiotic resistant bacterial strain<sup>§</sup>

<sup>§</sup> Multiple antibiotic resistance is defined as resistance to 3 or more different antibiotics.

## **6.3 SUBJECT IDENTIFICATION**

### 6.3.1 *Subject Identification Number*

At V1, after the subject and the Investigator have personally dated and signed the Informed Consent Form, a Case Report Form (e-CRF) is created and a Subject Identification number is allocated manually to her in chronological order of inclusion. This number will be the main identification code for a subject for the duration of the study in order to protect the subject's identity and will appear throughout the eCRF for that particular subject. This number should also be reported on the IWRS (if the subject is randomized) and on other relevant paper-based study documents where required (i.e. Quality of Life, Subject Diaries, source data in the Subject Medical File).

The Subject Identification number consists of 6 digits, which is based on:

- A 3 digit "Country number (will be 100)
- A 3 digit "Site" number (will be "001").
- A 3 digit "Subject" number. The first subject included at site will receive the number "001". Subject numbering will then be sequentially increased by one for each new included subject (example 002, 003 etc.).

**Example:** Subject Identification number "100-001010", refers to Site "001" and Subject "010".

### 6.3.2 *Randomisation Process*

Refer to section 10.1

## **6.4 SUBJECT DISCONTINUATION**

### 6.4.1 *Criteria for subject discontinuation*

The Investigator should discontinue subject's participation in the study prematurely in the following situations:

- In the case where a subject has decided to resign from further participation in the study (withdrawal of consent) or
- In the case where further participation is a health risk for the subject, at the Investigator's discretion or

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- In the case of subject's pregnancy or
- In the case where a severe non-compliance to protocol or a protocol violation has been identified for the subject that may lead to protocol deviations described under *section 16.8. Such as may include:*.
- Non adherence to the targeted population defined as pre-menopausal low drinker women with recurrent urinary tract infections
- Recurrence of systematic non respect of investigator's instructions with respect to: scheduled visit urine samples collection, diaries' completion and return, fluid intake regimen. Taking into account that this non-adherence can impact the accuracy, the completeness and the legibility of the data reported. If such event arrives, the final decision of subject discontinuation must be taken by the sponsor and investigator.
- In the case where the subject is lost to follow-up or
- In the case whereby the study is prematurely terminated or suspended by the sponsor.

*6.4.2 Replacement conditions*

Subjects withdrawing prematurely from the study after randomisation will not be replaced and cannot be re-included at a later date.

*6.4.3 Procedures in case of subject discontinuation*

In the event of premature withdrawal, the Investigator must notify the study monitor as soon as possible, and should make all efforts to contact the subject and if the subject agrees, ensure that all evaluations scheduled for the End of Study Visit (V4) are completed and reported in the eCRF.

Alternative follow-up of the discontinued subject is to be arranged by the Investigator if necessary.

For subjects who discontinue the study due to the occurrence of adverse events potentially related to the study product or to the study procedures, follow-up will take place (clinical or biological examinations) until the adverse event has abated, or until a stable situation has been reached (crf section 12), with findings being recorded in the CRF.

For subjects who discontinue the study due to pregnancy, follow-up will take place until birth or early termination of pregnancy. In addition to documenting this premature termination in the CRF, the Investigator should make every effort to collect information about the pregnancy and the infant and report to the Sponsor with a dated and signed Pregnancy Reporting Note as specified in the section 12.6.

**6.5 INSTRUCTIONS AND RESTRICTIONS DURING THE STUDY**

*6.5.1 Study product(s) consumption instructions*

The Investigator will provide the subject with the instructions on the study product consumption at the randomisation visit (V2).

Study intervention group:



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The subject allocated in the study product intervention group will begin consumption of the study product the morning of the following day after the receipt of the study products following the randomisation call after visit (V2) and will stop consumption the evening of the day before the last visit.

Throughout the entire fifty-two-week consumption period (around 365 days) of the study, the subject allocated in the intervention group will daily consume three bottles (500 mL each) of the test product (i.e. 1,5 L of commercialized Natural mineralized Evian water in total). It is suggested to start consumption at the beginning of every meal (breakfast, lunch and dinner) and fully drink them before the following meal (about 500ml around the main meals).

At the same time, the subjects will be receiving coaching messages related to the fluid intake recommendations together with the study products.

The subjects will receive regularly and free of charge the bottles of mineral water at home and will enter their daily consumption into a paper diary (The Subject Compliance Diary).

The subject will report every day her consumption in the Subject Compliance Diary, specifying the number of bottles consumed during the day and the quantity of water drunk for each bottle (total bottle, ¼ of bottle, ½ of bottle).

The water consumption will be checked at each visit by the Investigator (telephone visits and site visits) and a summary of the water consumption will be entered into the eCRF. The investigating site will also collect the subject compliance diaries in order to be able to check the information in case of inconsistencies.

Control group:

The subject allocated in the control group will remain stable in their fluid consumption. Their compliance will be assessed every month, using the 3 days fluid intake diary.

They will not receive any water bottle nor any coaching support.

*6.5.2 Dietary instructions*

During the entire duration of the study (i.e. from V2 to V4), subjects will not have any dietary restriction with the exception of cranberries juice and extracts. All women included in the study will be asked not to consume these products from 2 weeks before the inclusion visit to the end of the study period.

The subjects will also be asked to avoid making any significant changes to their usual diet during the study period (i.e. no changes to the usual amount of fibre, no commencement of a weight loss diet etc).

The dietary restrictions stipulated in this study do not represent a risk for the subjects. There is no risk of deficiency in the nutrient related to this dietary restriction.

*6.5.3 Non-authorized medicinal products*

During all the study, all medicinal products are authorised excepting treatments listed in the exclusion criteria (anti-coagulants, corticoids, diuretics, laxatives or treatments interfering with metabolism and nutrition behaviour, cranberry juice or extracts)

Included subjects will be asked not to take any antibiotic therapy in the 2 weeks before the inclusion visit (V1), to avoid any inclusion bias due to the presence of a UTI masked by the use of antibiotics.

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In case of any non-authorised medicinal products consumption or any non-authorised medical treatments, the subject will be asked to complete her 3 days fluid intake diary with the name, the dose and the intake dates of the medicinal products or medical treatment. The subjects will be advised to contact the Investigator or the study nurse for recommendations.

## **7. STUDY PROCEDURES**

### **7.1 EVALUATIONS AND PROCEDURES PER VISIT**

Evaluations and procedures for the study visits are described below. See also the study flow chart in the Section 2.

#### *7.1.1 Inclusion Visit (Visit 1, –4 Days to –2 Months)*

This visit will take place within from –4 days to –2 months prior to randomization visit (Visit 2).

At the beginning of the visit, the Investigator should fully inform the subject of all pertinent aspects of the clinical study including the written information and the approval by the IRB/IEC. The subject must be given the opportunity to ask questions and have them answered by the Investigator.

Prior to subject's participation in this clinical study (including screening phase), the subject must personally sign and date the written informed consent form. The subject cannot undergo any medical or biological procedures required by the clinical study before having personally dated and signed the Informed Consent Form. The Investigator must also sign and date the Informed Consent Form.

After the subject and the Investigator have personally dated and signed the Informed Consent Form, a Subject Identification number is allocated to her and a Case Report Form (CRF) is created.

The eligibility of the subject needs to be checked at the beginning of the visit and the tasks performed during the Visit 1 are the following (please refer to the flow chart in the section 2):

- Obtaining date, signature of subject on written informed consent form,
- Recording subject's demographic data (date of birth, gender),
- Reviewing the past medical and surgical history,
- Reviewing past and current medications history,
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,
- Requesting a urinary pregnancy test,
- Recording vital signs: systolic/diastolic blood pressure and heart rate, body temperature,
- Recording anthropometrical parameters; body weight and height, waist circumference,
- Checking all the inclusion and exclusion criteria for subject's eligibility validation,
- Recommending FSH test in case of perimenopausal symptoms (to be performed at Visit 2)
- Checking symptoms of UTI,
- Performing urine culture for symptomatic subjects and assign a therapy in case of acute episode,
- Providing instructions for subject's diary completion (including documentation of adverse events, instruction on completion of the 3 days fluid intake diary),
- Providing urine collection kits and instructions for 24h urine collection, storage and transport logistics,
- Providing instructions on the clean catch method and sterile containers for urine collection intended for performing of urine culture,

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- Providing instruction on the voiding diary to fulfil during the 24h urine collection,
- Scheduling study visits for subjects who are eligible and available for the duration of the study.

The results of the pregnancy test must be negative and available prior to randomization of the subject.

If symptomatic subjects show positive urine culture (confirming the presence of UTI) they will be sent home with a standardised treatment against their acute episode (i.e. AFU/EAU guidelines for UTI management) and they will be asked to come back for the randomization visit (V2) after about weeks up to 2 months at the latest. It is up to the investigator to decide the appropriate moment (e.g. negative urine culture after the medical treatment completion) Asymptomatic women at V1 will be asked to come back for the randomization visit (V2) after a minimum of 4 days (the time needed to complete the 3 days fluid intake diary; this interval should include a week-end as the diary must be completed during Saturday, Sunday Monday) and up to 2 months..

If the Visit 1 eligibility criteria are respected, and symptomatic subjects show positive urine culture (confirming the presence of UTI) they will be delivered with:

- A treatment against their acute episode (i.e. AFU/EAU guidelines for UTI management),
- A urine collecting kit in order to collect one 24h urine samples during the day before the following visit (V2; randomization visit). These 24h urine samples will be returned to the site at the next visit (Visit 2),
- A voiding diary, to collect frequency of voiding during the 24h urine collection,
- A 3 days fluid intake diary.

If the Visit 1 eligibility criteria are respected, and subjects are not symptomatic, they will be delivered with:

- A urine collecting kit in order to collect one 24h urine samples during the day before the following visit (V2; randomization visit). These 24h urine samples will be returned to the site at the next visit (Visit 2),
- A voiding diary, to collect frequency of voiding during the 24h urine collection,
- A 3 days fluid intake diary.

In all cases, the subject will receive instructions on urine collection procedure.

Furthermore, the subject will be instructed to fill in the 3 days fluid intake diary, during 3 consecutive days, on Saturday-Sunday-Monday, between Visit 1 and Visit 2 in order to:

- Verify the inclusion criteria II03: Low-drinker (< 1.5 L fluids per day)
- Evaluate the consumption of water and the total fluid intake before randomization

They will be also asked to document any adverse and serious adverse event following the instructions provided.

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents are accurate and complete (refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit.

### *7.1.2 Randomisation Visit (Visit 2, Month 0)*

The randomisation visit will take place at Day 0 (min 4 days after the inclusion visit).

At the randomisation visit, the subjects will bring back the following:

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- 24h Urine samples
- Sterile urine sample
- Voiding diary
- 3 days fluid intake diary

The eligibility of the subject needs to be checked at the beginning of the visit and the tasks performed during the randomisation visit are the following (please refer to the flow chart in the section 2):

- Conducting a physical examination,
- Recording vital signs: systolic/diastolic blood pressure and heart rate, body temperature,
- Recording anthropometrical parameters; body weight , waist circumference,
- Repeating pregnancy test
- Checking all the randomization and non-randomization criteria,
- Reviewing V1-V2 subject's 3 days fluid intake diary,
- Checking and recording of adverse events and of concomitant medications,
- Collecting of blood samples (after 8-12 hours of fasting),
- Collecting of 24h urine container (urine has been collected during the 24h before the visit) as well as the sterile urine sample
- Collecting the voiding diary,
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,
- Checking symptoms of UTI,
- Performing urine culture for all subjects to assess the absence of any acute UTI episode,
- Providing instructions for subject's diaries completion (including documentation of adverse events, instruction on completion of the 3 days fluid intake diary and Subject Compliance Diary),
- Providing urine collection kit and instructions for 24h urine collection during the day before the following visit (V3) and for transport logistics (refer to section 7.4.2.6 for instructions),
- Providing instructions on the clean catch method and sterile containers for urine collection intended for performing of urine culture (refer to section 7.4.2.6 for instructions to be provided),
- Providing instructions for completing the QoL questionnaire (SF-12 V2) in situ but not in front of the Investigator. QoL questionnaire should be completed in the absence of UTI event (the subject is asymptomatic and has negative urine culture) The Investigator will check the SF-12v2 questionnaire completion.
- Providing instruction on the voiding diary to fulfil during the 24h urine collection,
- Delivery of urine colour chart for regular self-check of patient's hydration status and verbal instructions how to use them (only for the intervention group).
- Delivery of V2-V3 Subject Compliance Diary for collecting self-reported subject data on compliance with the study product (only for the intervention group),
- Delivery of V2-V3 3 days fluid intake diary,

After receiving lab reports for eGFR and urine culture results, the Investigator will assess eligibility with respect to the randomisation and non-randomisation criteria. Concerning the 24H urine, it may be repeated once in case of incoherent data as regards the 24h urine creatinine level. If considered eligible, the subject will be randomized into one of the 2 groups. The investigator will perform a randomization call in order to instruct the subject depending on her group allocation.

If the subject is in the intervention group, the Investigator will inform the logistics company in order to provide the subject with the study products. The Investigator will inform the subject that she will receive the study products and provide her with the recommendation to consume 1,5L/day (3 bottles of mineral water) starting from the morning after reception of study products following randomization to the day of the V4, in addition to their usual fluid intake consumption. She has to monitor the study product consumption in the Subject Compliance Diary in addition to the 3 days fluid intake diary.

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If the subject is in the control group, the Investigator will call the subject and inform her that she has to continue her life habits and she doesn't need the Subject Compliance Diary. She will have to continue filling in the 3 days fluid intake diary.

Between V2 and V3, the subject in the intervention group has to send to the Investigator site by courier the Subject Compliance Diary corresponding to the previous 30 days of study product consumption and the 3 days fluid intake diary. The subject in the control group will have to return by courier every month the 3 days fluid intake diary.

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents need to be accurate and complete (refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit.

*7.1.3 Evaluation Visit– Visit 3 (Month 6, +/- 10 days)*

This visit will take place 6 months (+/- 10 days) after the randomization visit (Visit 2).

At this evaluation visit, the subjects in both groups will bring back the following:

- 24h Urine samples
- the sterile urine sample
- Voiding diary
- The 3 days fluid intake diary

In addition, the subjects in the intervention group will bring the V2-V3 Subject Compliance Diary for collecting self-reported subject data on compliance with the study product.

The tasks performed during the evaluation visit are the following (please refer to the flow chart in the section 2):

- Recording vital signs: systolic/diastolic blood pressure and heart rate, body temperature,
- Recording anthropometrical parameters: body weight, waist circumference,
- Reviewing V2-V3 subject's 3 days fluid intake diary and Subject Compliance Diary (if relevant),
- Checking and recording of adverse events and of concomitant medications,
- Collecting of blood samples (after 8-12 hours of fasting),
- Collecting of 24h urine container (urine has been collected during the 24h before the visit),
- Collecting the voiding diary,
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,
- Checking symptoms of UTI,
- Performing urine culture for all subjects presenting symptoms of UTI,
- Reminding instructions for subject's diary completion (including documentation of adverse events, instruction on completion of the 3 days fluid intake diary and Subject Compliance Diary),
- Providing urine collection kit and instructions for 24h urine collection during the day before the following visit (V4) and for transport logistics (refer to section 7.4.2.6 for instructions),
- Reminding instructions for completing the QoL questionnaire (SF-12 V2) that will be performed in situ but not in front of the Investigator. QoL questionnaire should be completed in the absence of UTI event (the subject is asymptomatic and has negative urine culture) The Investigator will check the SF-12v2 questionnaire completion
- Reminding instructions for study product consumption (only for the intervention group),
- Delivery of a voiding diary and providing instruction to collect frequency of voiding during the 24h urine collection, for both groups,
- Delivery of V3-V4 Subject Compliance Diary for collecting self-reported subject data on compliance with the study product if in the intervention group,
- Delivery of V3-V4 3 days fluid intake diary,
-

Between V3 and V4, the subject in the intervention group has to send to the Investigator site by courier the Subject Compliance Diary corresponding to the previous 30 days of study product consumption and the 3 days fluid intake diary. The subject in the control group will return by courier on monthly basis, the 3 days fluid intake diary

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents need to be accurate and complete (refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit.

*7.1.4 Phone calls (Month 1, +/- 7 days, Month 2, +/- 7 days, Month 3, +/- 7 days, Month 4, +/- 7 days, Month 5, +/- 7 days, Month 7, +/- 7 days, Month 8, +/- 7 days, Month 9, +/- 7 days, Month 10, +/- 7 days, Month 11, +/- 7 days)*

Every month women will be contacted by phone by the principal Investigator, to check the compliance with the intervention (if in the intervention group), adverse events, symptoms of UTI and, eventually, start and end date of episodes of UTI.

Study personnel will call the subject at the following time schedule:

- Phone call 1 (Month 1, +/- 7 days), between Visit 2 and Visit 3,
- Phone call 2 ( Month 2, +/- 7 days), between Visit 2 and Visit 3,
- Phone call 3 (Month 3, +/- 7 days), between Visit 2 and Visit 3,
- Phone call 4 (Month 4, +/- 7 days), between Visit 2 and Visit 3,
- Phone call 5 (Month 5, +/- 7 days), between Visit 2 and Visit 3,
- Phone call 6 (Month 7, +/- 7 days), between Visit 3 and Visit 4,
- Phone call 7 (Month 8, +/- 7 days), between Visit 3 and Visit 4,
- Phone call 8 (Month 9, +/- 7 days), between Visit 3 and Visit 4,
- Phone call 9 (Month 10, +/- 7 days), between Visit 3 and Visit 4,
- Phone call 10 (Month 11, +/- 7 days), between Visit 3 and Visit 4,

The objective of each phone call will be:

- Assessing the compliance with the intervention (if in the intervention group), for the 1<sup>st</sup> phone call, the investigator will ask the date of the 1<sup>st</sup> product consumption
- Assessing the level of fluid intake with the intervention based on subject's interview (if in the intervention group),
- Asking about regular self-checks of subject's hydration status using the urine colour chart (only for the intervention group)
- Assessing the occurrence of adverse events based on subject's interview,
- Assessing whether any concomitant medications have been taken or modified,
- Assessing the presence of symptoms of UTI,
- Eventually assess the starting and end date of a UTI episode,
- Checking sexual life status and emptying bladder habits after sexual intercourses.

Each phone call must be recorded in the subject's source documentation (refer to the section 8). The subject's / e-CRF should be completed from the source documents and as soon as possible after the phone call.

*7.1.5 End of Study Visit – Visit 4 (Month 12, +/- 10 days)*

This visit will take place 12 months (+/- 10 days) after the randomization visit (Visit 2).

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At this evaluation visit, the subjects will bring back the following:

- 24h Urine samples
- the sterile urine sample
- Voiding diary
- V3-V4 Subject Compliance Diary if in the test group
- The 3 days fluid intake diary

The tasks performed during the evaluation visit are the following (please refer to the flow chart in the section 2):

- Conducting a physical examination,
- Recording vital signs: systolic/diastolic blood pressure and heart rate, body temperature,
- Recording anthropometrical parameters: body weight, waist circumference,
- Reviewing V3-V4 subject's 3 days fluid intake diary,
- Checking and recording of adverse events and of concomitant medications,
- Collecting of blood samples (after 8-12 hours of fasting),
- Collecting of 24h urine container (urine has been collected during the 24h before the visit),
- Collecting the voiding diary,
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,
- Checking symptoms of UTI,
- Performing urine culture for all subjects,
- Reminding instructions for completing the QoL questionnaire (SF-12 V2) that will be performed in situ but not in front of the Investigator. QoL questionnaire should be completed in the absence of UTI event (the subject is asymptomatic and has negative urine culture) The Investigator will check the SF-12v2 questionnaire completion

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents need to be accurate and complete (refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit.

#### *7.1.6 Relapse visit*

This visit will take place during the intervention period, in case of UTI event.

The tasks performed during the evaluation visit are the following (please refer to the flow chart in the section 2):

- Performing urine culture for all subjects to confirm preliminary diagnosis
- Subjects will then be sent home with a standardised treatment against their acute episode (i.e. AFU/EAU guidelines for UTI management) irrespective of the study group they belong to
- Performing urine culture at the end of the therapy to be sure that women are completely recovered
- Reporting start and end dates of event
- Collecting data relative to healthcare costs due to illness at the end of event
- Collecting concomitant treatment

#### *7.1.7 Follow-up Visit*

Not applicable

#### *7.1.8 Early Subject's Discontinuation.*

In the event of early subject's discontinuation from the study (as specified in section 6.4), the Investigator should make every effort to entirely conduct the End of Study Visit evaluations and procedures (refer to section 6.4.3).

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For all subjects who were randomised in the study, the reason(s) for early termination of the subject prior to completion of the study must be stated in the subject's source documentation and reported in the e-CRF.

The site(s) will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents need to be accurate and complete (refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit.

End of study form must be filled for all subjects that have signed the Informed Consent with available information. For screen failures this should minimally include: informed consent date, demographics, inclusion criteria, exclusion criteria and end of study form.

## **7.2 SUBJECT VISIT WINDOW**

For each subject's visit, allowable windows are defined and stated in the previous section (7.1) and in the table of the study procedures and stages as outlined in the section 2.

## **7.3 STUDY FLOW-CHART**

The table of the study evaluations, procedures and stages is outlined in the section 2.

## **7.4 STUDY ASSESSMENTS**

### *7.4.1 Clinical Assessments*

#### *7.4.1.1 Medical and Surgical History*

The Investigator will interview the subject with respect to her history of concomitant diseases, surgery. The history of renal diseases and treatments will be verified in depth and specific questions on the symptomatology, duration and management of the UTIs will be addressed. All information will be recorded in source data and reported into the e-CRF.

#### *7.4.1.2 Medications and Nutritional Supplements History*

The Investigator will check if the subjects consume the following products forbidden by the protocol:

- Cranberry juice during the last 2 weeks
- Antibiotics during the last 2 weeks
- Chronic anti-coagulant therapy
- Diuretics
- Corticoid treatment
- Chronic laxative treatment

In addition to this all concomitant medication taken within 2 weeks will be documented in the source documentation.



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#### *7.4.1.3 Physical Examination*

The physical examination includes an assessment of general appearance and a review of systems (gastrointestinal, cardiovascular, OtoRhinoLaryngological (ORL), Neurological, Dermatological, Musculoskeletal, Urological/Nephrological,)

#### *7.4.1.4 Vital Signs*

The following vital signs will be measured:

- Blood pressure (BP) (systolic and diastolic [mmHg]),
- Heart rate (HR) (beats per minute [bpm]),
- Axillary body temperature.

The measurement of blood pressure is performed after resting for at least 5 minutes, in sitting position.

#### *7.4.1.5 Demographics*

Demographics include date of birth and gender.

#### *7.4.1.6 Anthropometry (Body weight, waist circumference, Height)*

- Body weight is measured to the nearest 0.1 kg using a calibrated weighing scale without outerwear and shoes
- Body height is recorded to the nearest 1 cm
- Body weight and height are used to calculate the Body Mass Index (BMI) as followed:  
 $BMI = \text{weight} / (\text{height})^2$  where weight is in kilogram and height in meter.

#### *7.4.1.7 Other Clinical Assessment*

Not applicable.

#### *7.4.2 Laboratory Assessments*

Normal ranges and laboratory/technical procedures for clinical laboratory parameters are made available by the laboratory(ies) before start of the study in the Laboratory Manual.

*7.4.2.1 Clinical Laboratory Assessments*

Laboratory measurements will include the following parameters assessed per visit:

Parameters	V1	V2-1day	V2 rando (M0)	V3 (M6)	V4 (M12)	Extra visits
<b>Blood parameters</b>						
Lipidic profile**			X		X	
glycosylated hemoglobin			X		X	
serum creatinine			X	X	X	
estimated glomerular filtration rate (eGFR)			X	X	X	
Urea			X		X	
copeptin			X	X	X	
FSH***		X				
<b>24h urine parameters</b>						
Sodium		X		X	X	
Potassium		X		X	X	
Calcium		X			X	
Magnesium		X			X	
Chloride		X			X	
Oxalate		X			X	
Citrate		X			X	
Urine Specific Gravity		X		X	X	
Osmolality		X		X	X	
Urine color		X		X	X	
pH		X		X	X	
Creatinine		X		X	X	
Volume		X		X	X	
Number of micturitions		X		X	X	
<b>Spot urine parameter</b>						
Urine Pregnancy Test	X	X				
Urine culture	X*		X		X	X

\* If symptomatic

\*\* Lipidic profile: HDL, LDL, cholesterol, tryglicerides

\*\*\* if perimenopausal symptoms

*7.4.2.2 Pregnancy Test*

Women of childbearing potential must have a negative pregnancy test at both V1 and V2. A urine pregnancy test will be used and results must be available prior to administration of study product. The Investigator is responsible for ensuring that the subject's pregnancy status is recorded accurately in the source documents. The Investigator will record the results of the pregnancy tests in the subject's e-CRF.

*7.4.2.3 Special Assays or Procedures*

Not applicable

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*7.4.2.4 Instructions for Specimen Preparation, Handling, and Storage*

Not applicable

*7.4.2.5 Blood Sample Collection and Handling*

Blood sampling will not exceed 20 mL per visit and will be taken after a period of fasting of 8-12 hours at visits V2, V3 and V4. This volume includes the volume required for back up samples in case of retest analysis. The blood sampling will be done at the Investigator site.

If fasting condition is not confirmed, subject has to come back for re sampling during a new visit scheduled within a maximum of 2 days after the initial scheduled visit in fasted state.

Normal ranges for all blood parameters are made available by the laboratory before start of the study in the Laboratory Manual.

All blood samples will be processed, stored and transported under the best conditions in order to ensure the samples stability.

*7.4.2.6 Urine Sample Collection and Handling*

The 24h urines will be collected in a specific container during 24h and stored by the subject at about +4°C to ensure the stability of parameters analysed. In order to ease sample collection and homogenise the number of sample and time collection of each subject, urines are collected as follows: the first urine of the day is excluded. The collection includes all urinations in the following 24h including the first morning urine of the day after.

If at least one micturition is not collected in the 24h urine collection, subject will be asked to do a new 24h urine collection and to come back for a new visit scheduled the following day after initial visit.

As some 24h urine parameters are impacted by UTI, if an extra-visit for UTI takes place at V3 or V4, a new 24h urine sampling has to be performed by patient once the urine culture is negative.

The subjects will bring back their urine collection to investigating site the morning of V2, V3 and of V4.

The urine samples will be processed, stored and transported under the optimal conditions in order to ensure the samples stability.

Urinary concentrations and excretions of sodium, potassium, calcium, magnesium, chloride, oxalate, citrate, creatinine, will be evaluated on 24h urine sample.

Urine volume, osmolality and pH

24h urine collection will be weighted and analyzed for their USG, osmolality and pH. The weight of the 24h urine collection will be divided by the USG in order to calculate the urinary volume.

#### Urine colour

24h urine collected will be evaluated according to the Urine Color Chart provided to the investigating site. Urine Color Chart was originally published in Lawrence Armstrong's book titled Performing in Extreme environments. The scientific validation of this Urine Color Chart may be found in Armstrong, 1994 and Armstrong, 1998.

This assessment will be done in order to assess the change in urinary hydration markers following changes in water intake habits.

#### Urine culture and MIC determination

The collection will be performed from the 1st morning urine in midstream urine by clean catch method. Urine culture is performed to confirm the presence of UTI and determine the bacterial origin of the UTI. For the bacteria identified, a Minimum Inhibitory Concentration test (MIC test) will be performed in order to determine the bacteria resistance or susceptibility to antibiotics. The list of antibiotics is chosen on the result of Gram coloration (i.e.: Gram +/-).

In case of positive urine culture, an antibiotic treatment will be administered according to Investigator prescription. A new urine culture will be performed after the treatment in order to verify if urine culture is negative.

#### *7.4.2.7 Instructions for Specimen Shipments*

Frequency of shipment, labelling requirement and any special instructions will be defined before study start and specified in Laboratory Manual.

#### *7.4.2.8 Back up samples*

The remaining back up blood and urine samples will be kept during the study by the investigator site, following instructions described in the study site guidelines and up to the signature of the study report when DANONE RESEARCH will transfer them at a central laboratory facility, selected and contracted by DANONE RESEARCH. These remaining back up blood and urine samples may be used for future researches such as the performance of additional analyses and/or the development new analytical methodologies. In this case, this will be done under the confidentiality rules. In any case, these samples will not be used for human genetic analysis.

The subject will be informed, via the ICF, that the unused blood and urine samples will be frozen and stored for a maximum of 15 years, in order to make these samples available for repeated measurements as a substitution of analysis mistakes, or in case of development of new methodologies of analyse.

#### *7.4.3 Other Assessments*

Not applicable.

#### *7.4.3.1 Subject's Compliance with Study Product Intake*

The subject's compliance for study product intake will be assessed by the Investigator throughout the study using the following information:

- Subject's self-reported data of product intake into the Subject Compliance Diary,

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- Subject's self-reported data on total fluid intake into the 3 days fluid intake diary (3 consecutive days before each visit),
- The urine volume, osmolality and colour measured at V2, V3 and V4.

For the product consumption control, the subject will record in a Subject Compliance Diary the volume of study product consumed (Evian® water) on a daily basis by specifying the number of bottles and the volume of water consumed from each bottle.

The subject will be asked to return her Subject Compliance Diary to the site each month by courier. At each Telephone visit, the Investigator will review the subject's Subject Compliance Diary.

At the same time the Investigator site will receive the acknowledgment of receipt for the delivery of study product to the subject.

#### *7.4.3.2 Dietary <Restrictions/Recommendations> and non-authorized products.*

Women included in the study will be asked not to consume any cranberries juice and extracts as well as any other nutritional complement which can impact the outcome of the study in the 2 weeks prior to the inclusion visit and for the all duration of the study.

## **8. SOURCE DATA AND SOURCE DOCUMENTS**

### **8.1 SOURCE DATA DEFINITION**

According to ICH GCP (E6), source data is all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

### **8.2 SOURCE DOCUMENTS DEFINITION**

According to ICH GCP (E6), source documents are original documents, data, and records (e.g., medical / hospital records, clinical and office charts, laboratory notes, memoranda, general practitioner letter, questionnaire used for diagnosis, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, X-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial).

### **8.3 SOURCE DATA MANAGEMENT**

All information recorded in the CRF should be traceable and documented by source documents in the subject's medical records available at the study centre.

If the subject is not followed by the investigational site, the investigator will not be exempted of creating and/or maintaining a complete and accurate subject's file by ICH/GCP, guidelines of the medical profession and local laws and guidelines. The subject's file includes all source documents for the CRF completion and verification. A special attention will be stated for source documents on the inclusion/exclusion criteria, in order to ensure the targeted

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population. If relevant, the PI will solicit the subject's General Practitioner (GP) to ensure the accuracy, completeness and up to date information on past/current medical/medication history, or any other information required by the study.

#### **8.4 RIGHT OF ACCESS TO SOURCE DATA/DOCUMENTS**

The Investigator/institution should permit study-related monitoring, audits, IRB/IEC review and regulatory inspection(s), providing direct access to source data/documents.

In accordance with the Law, the subjects who wish may have access to their personal data. They must address their request to the Investigator in writing.

## **9. STUDY PRODUCT**

### **9.1 STUDY PRODUCT(S) DESCRIPTION AND COMPOSITION**

#### *9.1.1 Study product(s) description*

The study product is a low mineralised natural mineral water. It is manufactured according to the DANONE's quality policy in the factory of Evian® (France).

The study products are intended for oral use only and within the standard consumption patterns for physiological needs.

#### *9.1.2 Study product(s) composition*

<b>Name</b>	<b>Natural mineral water</b>
<b>Product Brands</b>	<b>Evian®</b>
<b>Type of Source Water</b>	<b>Cachat source (Evian, France)</b>
<b>Type of Packaging</b>	0.5L bottles made of polyethylene terephthalate [PET] represent the test unit
<b>pH, (pH units)</b>	<b>7.2</b>
<b>Dry Residue 180°C, mg/l</b>	<b>309</b>
<b>Silica, mg/l</b>	<b>15</b>
<b>Sodium, mg/l</b>	<b>6,5</b>
<b>Potassium, mg/l</b>	<b>1</b>
<b>Calcium, mg/l</b>	<b>80</b>
<b>Magnesium, mg/l</b>	<b>26</b>
<b>Chlorides, mg/l</b>	<b>6,8</b>
<b>Bicarbonates, mg/l</b>	<b>360</b>
<b>Sulphates, mg/l</b>	<b>12,6</b>
<b>Nitrates, mg/l</b>	<b>3,7</b>

#### *9.1.3 Study product analyses during the study*

Not applicable.

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## **9.2 STUDY PRODUCT(S) LABELLING AND PACKAGING**

Boxes and bottles are labelled in accordance with applicable laws and regulation. Primary labels (on water bottles) are printed in French by default. Secondary labels (on carton boxes) will be written in local language (Bulgarian).

### (a) Primary packaging (water bottle)

The products are packaged in alimentary plastic bottles of 500mL. The primary labels affixed to these bottles contain the following information:

- Expiry date : DD/MM/YYYY
- Batch number : ZZZZ
- Content :500 ml
- Storage temperature or conditions

### Secondary packaging (carton box):

The water bottles are gathered in batches of 24 units with the following information:

- Expiry date (DD/MM/YYYY)
- Study code / Name and address of Sponsor and Investigator
- Batch code allowing identification of the contents
- Weight
- Storage temperature or conditions
- Sentence: 'to be used for clinical study only'

NB: Labels are printed in Bulgarian.

## **9.3 SHIPMENT, STORAGE, DISPENSING, ACCOUNTABILITY AND DESTRUCTION**

### *9.3.1 Shipment of study product(s)*

Study products are commercial products of the DANONE group and provided by DANONE RESEARCH.

The water bottles distributed to the subjects are manufactured, stored and delivered in accordance with the current sanitary regulations.

Study products are delivered in boxes and are identified as follows:

- Name and address of sender
- Name and address of recipient
- Study code
- Batch number
- Type and Identification of products
- Storage conditions

Study products are accompanied by:

- An acknowledgement of receipt to be signed and returned by the consignee (subject) upon receipt of products and will be delivered to the investigating site by the logistics company

**9.3.2 Storage of study product(s)**  
The Investigator site ensures that the study products are stored in a temperature controlled, secure and closed area in accordance with the indications stated on the packaging for the products to be used.

The investigating site will ensure that the products are distributed to the subjects' homes in a timely manner and appropriate conditions. The subjects will be informed about water bottles storage conditions. The water bottles delivered should be used only for the study purposes.

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*9.3.3 Delivery/Dispensing of study product(s)*

An external shipping company will deliver the water bottles at each subject included in the study and randomised in the intervention study group. The Sponsor and the Investigator must ensure that water bottles are received under good condition by the subjects.

The subjects will sign an acknowledgment of receipt and will report any product quality issue to the delivery company. Details are provided in the study site guidelines. The subjects will dispose the empty bottles together with the household waste.

*9.3.4 Accountability of study product(s)*

Delivery records from the transport company will be used to ensure the quantity of study product delivered to each study participant. The acknowledgement of receipt will be sent to the Investigator by the carrier.

The Investigator will check the reported quantity of water consumed by the subject in case of compliance deviation, he will check the acknowledgement of receipt for the deliver quantity.

*9.3.5 Destruction of study product(s)*

The Evian® empty water bottles will be disposed by the subjects together with their household waste.

The Investigator must instruct the subject that in case of detected or suspected study product abnormalities, the subject must not consume the related study products and return them to the investigational site.



## 10. RANDOMISATION AND UNBLINDING

### 10.1 RANDOMISATION (AND STRATIFICATION IF APPLICABLE)

After having checked all eligibility criteria at the randomization visit, the subject will be allocated to the study arm through the randomization system (IWRS), without any stratification factor.

### 10.2 UNBLINDING PROCEDURE

Not applicable, open label study.

## 11. PRODUCT EFFECT PARAMETERS AND PRODUCT SAFETY PARAMETERS

### 11.1 DESCRIPTION OF PRODUCT EFFECT PARAMETERS

The study intervention effect parameters are assessed by clinical parameters and laboratory parameters analysed in blood and urine.

Product Effect parameters	Visit 1	Visit 2	Visit 3	Visit 4	Relapse visits
Symptoms of UTI	X	X	X	X	X
Urine culture (bacteriogram if urine culture positive)	X ONLY IF SYMPTOMS	X		X	X
Frequency of micturitions during 24h urine collection		X	X	X	
Urine hydration markers (volume, USG, pH, osmolality, urine colour)		X	X	X	
Fluid intake (daily volume)		X	X	X	
QoL questionnaire (SF-12V2)		X	X	X	
Antibiotic prescription data	X	X	X	X	X
Healthcare cost data					X

**11.2 DESCRIPTION OF PRODUCT SAFETY PARAMETERS**

The goal of the study intervention is to achieve a mean daily urine osmolality equivalent to that of plasma (approximately 285 mosm/kg). This osmolality goal is selected because it defines the transition from dilute to concentrated urine and is a safe end point under most any circumstance.

The safety parameters assessed are the urine osmolality and the urine concentration of sodium and potassium.

<b>Product Safety parameters</b>	<b>Visit 1</b>	<b>Visit 2</b>	<b>Visit 3</b>	<b>Visit 4</b>
Urine osmolality		X	X	X
Urine sodium		X	X	X
Urine potassium		X	X	X

## 12. SAFETY REPORTING

### 12.1 DEFINITIONS (INSPIRED FROM ICH E2A GUIDELINES)

#### 12.1.1 Adverse Event (AE)

An Adverse Event (AE) is any untoward medical occurrence in a subject/patient or clinical study subject administered an investigational product but does not necessarily have a causal relationship with this investigational product.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the investigational product, whether or not related to the investigational product or to the protocol- mandated procedures (e.g., invasive procedures such as biopsies).

This includes events:

- Not present before the study but occurring after the signature of the Informed Consent;
- Pre-existing events that have worsened during the course of the study (increase in frequency or severity or change in nature during the study).
- The cystitis events are expected events, and will be considered as study parameters. Therefore, the related symptoms, signs and biological parameters will not be declared as AEs but will be collected in the CRF and on the lab reports and study analytical database.
- The typical symptoms of lower or upper urinary tract infection (defined as  $\geq 3$  urinary symptoms) are: difficult, painful, urgent and frequent urination, hematuria, supra-pubic pressure, increase in the number of white blood cells ( $>10\ 000/\text{ml}$  in the urine), with or without positive urinary bacteriological culture.

#### 12.1.2 Unexpected Adverse Event

An unexpected adverse event is by its nature or severity not consistent with applicable product information contained in the relevant source document(s) (e.g. Protocol,).

The product distributed during the study is natural mineral water with a long commercial history and without any report of safety problem.

#### 12.1.3 Serious Adverse Event (SAE)

A Serious Adverse Event (SAE) is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (at the time of the event)
- requires hospitalisation of a subject or prolongation of existing inpatients' hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital abnormality/birth defect

#### 12.1.4 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is both:

- an SAE that is judged to be at least possibly related to the investigational product by either Investigator or sponsor,
- and by its frequency, nature or severity is unexpected (not listed in the and in the study protocol).

#### 12.1.5 Emergent Adverse Event (EAE)

An adverse event will be considered as emergent if it began the day or after the first product consumption, or if it worsened the day or after the first product consumption.

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## **12.2 (S)AE RECORDING**

All untoward medical events occurring after subject's signature of the Informed Consent Form are recorded as (S)AEs.

The symptoms and biological parameters related to the UTI events are considered under the study parameters. The typical symptoms of lower or upper urinary tract infection (defined as  $\geq 3$  urinary symptoms) are: difficult, painful, urgent and frequent urination, hematuria, supra-pubic pressure, increase in the number of white blood cells ( $>10\ 000/\text{ml}$  in the urine), with or without positive urinary bacteriological culture.

For each UTI event the following information will be collected into the eCRF (and analytical database):

- start date (date of 1<sup>st</sup> symptoms)
- symptoms and signs
- urine bacteriological exam and antibiogram
- treatment prescribed
- end date of the episode (date of sampling of the last negative urine culture exam)

For the UTI events occurring after the signature of the ICF and before the randomization of the subject the symptoms should be noted in the (S) AE section and the corresponding treatment into the concomitant treatment section of the eCRF.

The UTI events before signature of the ICF should be recorded in the section 'Significant Medical and Surgical History' of the eCRF.

For the UTI events occurring after randomization the symptoms and the related cost should be recorded into the relapse visit section and the related treatment in the UTI related Concomitant Medication section of the eCRF.

Complications of UTI (i.e. pyelonephritis) and symptoms, signs or abnormal values of biological parameters related to any other pathology, will be reported as S(AE).

Details of any (S)AE reported spontaneously by the subjects or observed by the Investigator or medical staff must be recorded in the (S)AE forms provided in the CRF during the course of the study. The Investigator must report the following information on the (S)AE form: nature of event (diagnosis or major symptoms/signs), start and end dates, severity, product-relatedness, action(s) taken regarding the (S) AE, action taken regarding the study product, and participant outcome.

SAEs must additionally be recorded on the SAE Report Form provided by DANONE RESEARCH. The Sponsor may request additional information from the site to evaluate the SAE.

When a subject undergoes a medical intervention or hospitalisation in absence of an adverse event (such as treatment of pre-existing condition or hospitalisation for elective surgery or diagnosis), this intervention/hospitalisation must be reported on the AE page and not as SAE. These procedures will be handled like AEs, and timelines for reporting are the same as for reporting AEs. Complications or prolongations of hospitalisation that result from procedures must be reported as SAEs, according to the applicable reporting timelines and procedures.

The severity of the (S)AE will be determined in the following manner:

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- Mild: No interference with the subject's daily activities - transient or mild discomfort; no medical intervention/therapy required.
- Moderate: Moderate interference with the subject's daily activities but still acceptable - mild to moderate limitation in activity; some assistance may be needed; and/or minimal medical intervention/therapy required.
- Severe: Major interference with the subject's daily activities and unacceptable - marked limitation in activity; some assistance usually required; and/or significant medical intervention/therapy/hospitalisation required.

The relationship of the (S)AE to the study product is assessed as being:

Not related	The AE follows no reasonable temporal relationship with the investigational product or does not follow a known response pattern to the investigational product and can be explained by the known characteristics of the subject/patient's clinical state, by underlying disease or other administrated products (e.g. nutritional products, OTCs, drugs).
Unlikely related	The AE has a time to investigational product intake that makes a relationship improbable (but not impossible) and concomitant administrated products (e.g. nutritional products, OTCs, drugs) or underlying disease provide plausible explanations.
Possibly related	The AE has reasonable time relationship to investigational product intake but could also be explained by an underlying disease or other administrated products (e.g. nutritional products, OTCs, drugs); information on drug withdrawal may be lacking or unclear
Probably related	The AE has a reasonable time relationship to investigational product intake, it is unlikely to be attributed to disease or other drugs and response to withdrawal of the investigational product is clinically reasonable; rechallenge information is not necessary
Definitely	The AE has plausible time relationship to investigational product intake and cannot be explained by underlying disease or other administrated products (e.g. nutritional products, OTCs or drugs); response to withdrawal of the investigational product is clinically plausible; rechallenge is satisfactory if necessary

### **12.3 SAE REPORTING BY THE INVESTIGATOR**

As soon as the Investigator becomes aware of a SAE and not later than 24 hours (1 working day), he/she completes the SAE Reporting Form and sends it together with other supporting documents (e.g. copy of pages of the case report form for medical history, on-going events and concomitant medications, etc.) via fax or e-mail to DANONE RESEARCH with copy to the monitoring CRO.

The Investigator ensures that any supportive documents submitted have been anonymised adequately.

### **12.4 SAE REVIEW AND REPORTING BY THE SPONSOR**

DANONE RESEARCH must review all reported SAE and independently assess the relationship of the SAE with the study product.

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If the Investigator and the Sponsor both assess the relationship of the SAE with the study product as “Not related “ or “Unlikely related”, the SAE is considered as a regular SAE with no required expedited reporting to Ethics Committees.

If one of them determines that the SAE is “Possibly”, “Probably” or “Definitely” related to the investigational product, and that this event has not been described in the or in the Clinical Study protocol, the SAE is considered as a SUSAR requiring expedited reporting to Ethics Committees. Should the assessments of the Sponsor and of the Investigator differ with regard to the relationship to the study product, then both will be reported.

DANONE RESEARCH must ensure that all SAEs and SUSARs are reported to the accredited Ethics Committee(s) that have approved the protocol (and Competent Authorities, if applicable) as follow:

- SAEs are reported annually as line listings according to the requirements of the Ethics Committee(s),
- SUSARs are reported within 7 days (fatal and life threatening) or 15 days (other events) after the first report. Reporting timelines include week-ends and public holidays.

The Sponsor will reply to all requests for further information concerning such events from the Ethics Committee (and/or Competent Authorities, if applicable).

#### **12.5 FOLLOW-UP OF SAEs**

The Principal Investigator or his/her authorized representative will monitor and follow all SAEs until SAEs have abated, or until a stable situation has been reached and a satisfactory resolution is obtained.

Depending on the event, follow-up may require additional tests or medical procedures as indicated, and/ or referral to the general physician or a medical specialist or other health care provider.

Any clinical or biological examinations deemed necessary by the Principal Investigator will continue to be performed until a return to normal. The Principal Investigator will provide the Sponsor with follow-up SAE Reporting Form and with all the examination results and concomitant medications.

#### **12.6 PREGNANCIES REPORTING BY THE INVESTIGATOR**

Although the study products are considered as safe for pregnant women, pregnancy may require specific diet, medication or procedure likely to interfere with the study product and/or outcomes.

For this reason subjects with a confirmed pregnancy have to be withdrawn from the study and followed up until birth or early termination of pregnancy. This premature termination of the protocol is documented in the CRF.

The Investigator should make every effort to collect information about the pregnancy and the infant and report it within a dated and signed Pregnancy Reporting Note including at least:

- Date of last menstruation
- Expected date of delivery
- Method of contraception used and if used according to instructions
- Medical history with information on familial disorders or risk factors that may influence the outcome of the pregnancy,

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- Obstetric history with details on previous pregnancy (including termination or stillbirth)
- Medication taken prior and during pregnancy (should be available in the concomitant medication section of the CRF)
- Special tests and procedures performed during pregnancy if the case (e.g. amniocentesis, ultrasound etc.)
- Pregnancy associated events (if SAE during pregnancy)
- Pregnancy outcome (abortion, delivery)
- Child outcome.

**12.7 INDIVIDUAL CODE-BREAK PROCEDURE**

Not applicable.

**12.8 NEW RELEVANT SAFETY INFORMATION**

Not applicable.

## **13. STATISTICS**

**13.1 SAMPLE SIZE CALCULATION**

By definition, the recurrent UTI event data consist of the inter event time of repetition of the same or different types for each subject. The measurements across subjects are considered to be statistically independent, but the times between UTI events for a specific subject are not necessarily independent. The mean cumulative function (MCF) contains the information of interest in the analysis of recurrent data.

Assume  $M(t)$  is the mean cumulative number of UTI events up to time  $t$ . (29)

$M(t) = E\{N(t)\}$ , where  $N(t)$  is a random variable for the number of events that have occurred up to time  $t$ . (30)

The main assumption of this approach is that the hazard or risk ratio is proportional over time, reason why a robust sandwich variance estimated is used to account for dependence of recurrent events on the same subject.

Assuming that all subjects had the same study period examination (around 365 days), and considering an interval of risk (inter event times) following an Uniform distribution, data have been simulated based on a mean number of events equal to 3 for control group and an expected intervention effect of 20% less events in the intervention group. One hundred samples have been replicated upon the simulated data. (31)

Considering the simulated data based on our hypotheses, a mean number of events during a 365 days period in control group equal to 3, the estimate sample size that would provide a power of 80% to detect a product effect of 20% less events occurred in the interventional study group for a bilateral test,  $\alpha=0.05$  will be equal to 42 subjects per group, i.e. 84 overall completed subjects (ratio 1:1).

As we consider a 40% drop out rate, the screening process will last until we will reach 140 subjects randomised.

**13.2 PLANNED STATISTICAL METHODS**

A Statistical Analysis Plan will be drawn up before data review meeting, reviewed by the Investigator and statisticians during the Data Review Meeting and signed by the sponsor before the database lock.

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Statistical analyses will be performed by a specialized CRO, selected, contracted and followed under the responsibility of DANONE RESEARCH/located in Palaiseau (France) and using appropriate statistical software SAS® V9 or later using the files included in the database locked.

*13.2.1 Descriptive statistics*

The distribution of study parameters will be summarised by group and overall depending on the type of variable for all criteria with a description of number of subjects and missing data.

The descriptive statistics for each criterion will be presented as follows:

- Continuous data: for each visit per group and overall: number of non missing observations, number of missing observations, mean, median, standard deviation (SD), minimum and maximum. (Standard error of the mean [SEM], Confident interval (CI) and quartiles optionally provided if mentioned in the Statistical Analysis Plan).
- For nominal data: for each visit per group and overall: number of missing observations, number and frequency of observations in each class.

For qualitative ordinal parameters

- Table of frequency (and/or mean and/or median), if necessary per parameter.

*13.2.2 Statistical Hypotheses*

In the present study the null hypothesis “There is no difference between groups on the average number of recurrent UTIs events experienced” versus the alternative hypothesis “the effect of intervention is different to the effect of the control” will be tested.

□ The test will be based on a nonparametric method called the mean cumulative function (MCF), that will be used to analyse the multiple/repeated UTI events occurring.

H<sub>0</sub>: Effect of fluid intake = Effect control

H<sub>1</sub>: Effect of fluid intake ≠ Effect control

Statistical tests will be conducted two-sided with a significance level of 5%. All confidence intervals will be presented two-sided with a confidence level of 95%. A resultant probability value of p<0.05 will be judged as being of statistical significance. The interaction factors (if any) will be considered as significant if the p value is < 0.10.

The MCF by group, estimated by a non-parametric estimator:

$$\widehat{MCF}(t) = \sum_{\{j|t_j \leq t\}} \frac{e_j}{n_{j-1}}$$

where e<sub>j</sub> is the number of events at time t<sub>j</sub>, n<sub>j-1</sub> is the number of subjects at risk just beyond time t<sub>j-1</sub>, and j the observed event times.

*13.2.3 Interim Analysis*

Not applicable.

*13.2.4 Distribution and normality assessment*

As a first step, the frequency distribution of the efficacy parameters will be analysed, including assessment of stem-and-leaf displays, boxplots and histograms per group and overall.



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*13.2.5 Statistical criteria to stop the research*

Not applicable

*13.2.6 Methods for dealing with missing, unused or non-valid data*

The way of handling missing, unused or non-valid data will be discussed at the Data Review Meeting and detailed in the Statistical Analysis Plan.

*13.2.7 Management of modifications made to the initial strategy of the analysis plan*

All the modifications of the statistical methodology will be detailed and justified in the Statistical Analysis Plan and described in the Clinical Study Report.

**13.3 DISPOSITION OF SUBJECTS AND POPULATIONS DEFINITION**

*13.3.1 Disposition of subjects*

Disposition of subjects will be described as follows:

- Number of subjects included.
- Number of subjects eligible at V1.
- Number of subjects screen failed at V2 (reasons of screen failure will be described).
- Number of subjects randomised per group and overall.
- Number of subjects premature withdrawals / drop out (reasons of premature withdrawals / will be described per group and overall).
- Number of subjects completed per group and overall.

*13.3.2 Deviations and populations definition*

A definition of minor and major protocol deviations will be detailed in a document attached to the Statistical Analysis Plan. The Data Review Meeting will allow a global review of the study data and then the deviations status to subjects in order to determine analysed populations before the final data base lock.

The populations will be defined as follows:

- The “Global Population”: all subjects included in the study and eligible at the end of Visit 1.
- The “Full Analysis Set” population (FAS): all subjects included in the study and randomised.
- The “Per Protocol” population (PP): all subjects included in the FAS population presenting no major protocol deviation.
- The “Safety Set” population (SS): all subjects included in the FAS population.

The analysis of baseline characteristics will be performed on the FAS population.

The analysis of main product effect criteria will be done on the FAS population. If the difference in number of subjects is few between the FAS and PP populations (difference lower than 10%), the analyses of the product effect criteria will be only done on the FAS population, except for the main criterion, for which the analysis will be performed on both populations.

The analysis of safety criteria will be done on the Safety Set (SS) population.

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### **13.4 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS**

A descriptive analysis of subjects at baseline, corresponding to the data collected at inclusion and randomisation visits, will be performed by group and overall.

Following parameters will be described:

- Demography, vital signs, anthropometrical parameters (body weight, waist circumference, height, BMI).
- Medical and surgical history
- Physical examination
- Quality of Life (SF12v2)
- Fluid intake consumption
- Contraception habits/method

### **13.5 STUDY CONDUCT PARAMETERS**

#### *13.5.1 Compliance*

Subjects record the daily intake of study product in a diary on a daily basis (quantity of 3 daily bottles consumed per day). Parameters concerning compliance will be described by group and overall:

- Study product compliance
- Consumption of forbidden dietary products and treatments

#### *13.5.2 Quality of Life*

A quality of life questionnaire (SF-12v2) will be filled in by the subject at the randomisation visit (V2), at six-months (V3) and at the last visit (V4) in order to explore the quality of life of the subjects.

The short form 12 (SF-12v2) is a validated and frequently used questionnaire for the assessment of quality of life. It was developed from the more extensive short form 36 (SF-36) [<http://www.sf-36.org/tools/sf12.shtml>]. It includes 12 questions from the SF-36: 2 questions concerning physical functioning; 2 questions on role limitations because of physical health problems; 1 question on body pain; 1 question on general health perceptions; 1 question on vitality (energy/fatigue); 1 question on social functioning; 2 questions on role limitations because of emotional problems; and 2 questions on general mental health, psychological distress and psychological well-being. This will be a self-assessment questionnaire. However, if the subject is frail and requires help with filling in the form then the site staff will help.

A preference-based utility index, called the SF-6D, is also available to help understand economic benefit and will be assess in order to perform a cost utility analysis.

- 12-Items
- 8- Dimensions: Physical functioning, Role Physical, Bodily pain, Vitality, General health, Mental health, Social functioning and role emotional
- 2 composite score: Mental health and physical health
- Utility index based on SF-6Dimensions

### **13.6 EVALUATION CRITERIA**

#### *13.6.1 Main evaluation criterion*

The main evaluation criterion is the number of recurrent UTI event experienced over a one year study period between intervention and control groups, evaluated by the difference between groups in the mean cumulative

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function. The primary criterion will be considered as statistically significant if confidence interval observed on the MCF difference between groups excludes zero, which suggests a statistical test and p value associated <0.05.

*13.6.2 Secondary evaluation criterion*

- Difference between groups in terms of duration of urinary infectious episodes
- Average delay between each UTI over one year study period
- Change in urinary hydration markers following changes in water intake habits
- Difference between groups in terms of change in QoL
- Difference between groups in terms of antibiotic prescription/usage to treat UTI
- Difference between groups in terms of health-costs associated with management of UTI recurrence(s)
- Difference between groups in terms of cost utility analysis

The methodology to address the potential multiplicity issues will be detailed in the Statistical Analysis Plan.

*13.6.3 Exploratory criteria*

- Association measure between urinary hydration markers and number of UTI events
- Association measure between urinary hydration markers and delay UTI events

*13.6.4 Safety criteria*

*13.6.4.1 Extent of exposure / Study duration*

Descriptive summaries will be performed on study duration and extent of exposure by group and overall.

*13.6.4.2 AE / SAE*

The analysis of Adverse Events will be performed to evaluate the number of subjects with at least one adverse event and the number of adverse events by study group.

The adverse events will be presented by "body system" and "Preferred term" according the MEDDRA coding system.

All adverse events will be summarised, in individual data listings, by subject with all details concerning the adverse event, the study product, the emergence, the intensity and the relationship to study product.

The Serious Adverse Events will be summarised, in individual data listings, by subject with all details concerning the adverse event, the study product, the emergence, the intensity and the relationship to study product.

*13.6.4.3 Concomitant Medications'*

The concomitant medications will be presented by ATC class (ATC1 and ATC3) according to the WHO-Drug coding system.

All concomitant medications will be summarised, in individual data listings, by subject with all details concerning the concomitant medications and the study product.

*13.6.4.4 Laboratory measurements*

Descriptive summaries will be performed for the following parameters by study group and overall:

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**Blood sample**

- lipidic profile: HDL cholesterol, LDL cholesterol, triglyceride (mmol/L)
- glycosylated haemoglobin (%),
- serum creatinine ( $\mu\text{mol/L}$ ),
- serum urea (mmol/L),
- copeptin ( $\mu\text{g/L}$ )
- Estimated glomerular filtration rate (eGFR) will be calculated

**Urinary markers of hydration**

- urine volume (L)
- pH
- osmolality (mOsm/kg)
- specific gravity
- frequency of micturition
- urine colour

**Other urinary parameters**

- Electrolytes: sodium, potassium, calcium, magnesium, chloride (mmol/L), oxalate ( $\mu\text{mol/24h}$ ), and citrate (mmol/L))
- Urine creatinine (mmol/L)

Information about bladder emptying after sexual intercourses and sexual activity during the last month will be also summarised by study group and overall.

**13.6.4.4**      *Vital signs, anthropometrical and other parameters*

Descriptive summaries will be performed for the following parameters by study group and overall:

- Systolic and diastolic blood pressure (mmHg)
- Heart rate (bpm)
- Body temperature ( $^{\circ}\text{C}$ )
- Body weight (kg)
- Waist circumference (cm)
- Height (m)
- Body Mass Index (BMI) ( $\text{kg/m}^2$ )

## **14.      STUDY REPORT AND PUBLICATION**

All information stemming from the study will be considered confidential and should not be divulged without the prior agreement of the sponsor (DANONE RESEARCH).

Following analysis of the study data, a final study report will be prepared in UK English, format ICH E3, according to the standard model of DANONE, describing the conditions under which the study was performed as well as the results. This report will be prepared and signed by the Sponsor's representative. The signature of the coordinating Investigator (for multicenter study) or of the Principal Investigator (for monocenter study) will be requested if relevant.

The identity of study subjects will under no circumstances be communicated to the sponsor.

Publication may be done only if before recruitment of the first subject, the sponsor has registered the study in a publicly accessible clinical trial database.

No publication will be done based upon interim analysis or result of one center of a multicentric study except if a written agreement has been signed by the sponsor.

## **15. STUDY MONITORING AND AUDITS**

Study monitoring is the act of overseeing the progress of a clinical study and of ensuring that:

- i) The rights and the well-being of human subjects are protected.
- ii) The reported trial data are accurate, complete, and verifiable from source documents.
- iii) The conduct of the clinical study is in accordance with the approved protocol/amendment(s), Good Clinical Practice (GCP, ICH E6), and the applicable local regulatory requirement(s).

Monitoring includes on-site visits to assure that the investigation is conducted according to the approved protocol/amendment(s) and in order to comply with applicable regulations and deadlines. On-site monitoring includes, at least, the review of Informed consents, safety reporting, CRFs and supports the site management regarding protocol conduct and compliance or deviation(s).

The monitoring includes the review of forms for completeness, clarity, and consistency with source documents available for each subject and the management of the essential documents (Investigator Study File).

The Investigator and the investigating site staff must permit and be available for study-related monitoring visits, audits, review by the ethics committee and regulatory inspections, and allow direct access to source data and source documents provided that subject confidentiality is protected. In case of an audit appointed by DANONE RESEARCH, the Investigator will receive written notification in advance.

Study monitoring, under the responsibility of DANONE RESEARCH will be performed by a qualified staff of a company contracted by DANONE RESEARCH at various stages/on regular basis of the study (frequency of visits is specified in monitoring plan). Throughout the duration of the study period, the CRFs will be completed and signed by the Investigator, and he/she will be controlled individually by a Clinical Research Associate (CRA) designated by DANONE RESEARCH in order to verify data quality and compliance with study protocol and Good Clinical Practice (GCP, ICH E6).

Upon closure of the study, the company contracted by DANONE RESEARCH will perform study closeout.

## **16. ETHICAL CONSIDERATIONS**

### **16.1 BASIC PRINCIPLES AND REGULATIONS**

The Investigator must ensure that this study is conducted in full compliance with the principles of the 'World Medical Association Declaration of Helsinki' (64th WMA General Assembly, Fortaleza, October 2013) (Appendix I), ICH guidelines for Good Clinical Practice as appropriate for nutritional products, and local legislation of the country in which the research is conducted, whichever affords the greater protection to the participants.

### **16.2 ETHICS COMMITTEE**

This protocol and any accompanying material provided to the subjects, such as information and informed consent sheets, are submitted to the applicable Ethics Committee (IRB/IEC) by <the sponsor or the Investigator or the company contracted by DANONE RESEARCH> according to local legislation. Approval from the Ethics Committee must be obtained before starting the study, and should be documented in a letter to the sponsor specifying the date on which the Ethics Committee met and granted the approval, the composition of the Ethics Committee and their

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qualifications, and version and date of all submitted documents. If applicable, the documents will also be submitted to the Competent Authority in accordance with the local regulatory and legal requirements.

This study will be undertaken after approval from the appropriate Ethics Committee(s) (IRB/IEC) and of Health Authorities (if applicable).

During the study course, change(s) in any aspect of the study, such as modification(s) of the protocol, written ICF and any other written information to be provided to subjects should be submitted to the Ethics Committee (IRB/IEC) and Competent Authorities.

If required, depending on local legislation, the Investigator must submit an annual progress report to the Ethics Committee which gave the favourable opinion. Annual progress reports should be submitted thereafter until the end of the study. DANONE RESEARCH could assist in the preparation/submission process.

Subject recruitment will start only after reception of a favourable opinion from the Ethics Committee and Health Authority (if applicable).

### **16.3 RECRUITMENT AND INFORMED CONSENT FORM**

The requirements of the research, the objectives of the research, the detailed research protocol and the risks and constraints of the research associated with this study must be explained to each subject both orally and in writing (subject information sheet) by the Investigator, or a person designated by the Investigator, before the start of the study.

The subject must personally initial each page of the Information to the subject and sign and date a written informed consent form (ICF) to take part in this study before the study starts (before the screening period, if applicable). This form must also be initialled and signed by the Investigator or a person designated by the Investigator. Two copies of the ICF are dated and signed: one is given to the subject and one is retained on site in the patent's files.

Subjects may withdraw from the study at any time without having to provide justification. The confidentiality of medical data must be upheld.

Any substantial changes in the ICF and written information should receive the IRB/IEC's approval/favourable opinion before any use.

### **16.4 CONFIDENTIALITY OF STUDY DATA**

All information collected during the study is to be considered confidential and must not be disclosed without prior written agreement by the sponsor. The identity of study subjects must under no circumstances be communicated to the sponsor or to any official bodies.

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### **16.5 COMPENSATION OF SUBJECTS**

The subjects will be compensated for their participation in this clinical study on a pro rata basis. Compensation amount and the method and timing of disbursement are consistent with applicable regulatory requirement(s) of Bulgaria.

Each subject will receive an indemnity of 557 Bulgarian Leva for participating in this study if she has completed all study visits or in case of discontinuation due to medical reasons related to the protocol. In any other case of withdrawal, the subject will receive an indemnity for participation on a pro rata basis.

The reimbursement will be as follows:

- Inclusion visit (V1) = 28 Bulgarian Leva
- Randomization visit (V2)= 158 Bulgarian Leva.
- Evaluation visit (V3) = 176 Bulgarian Leva
- Evaluation visit (V4)= 195 Bulgarian Leva.

### **16.6 PROTOCOL AMENDMENTS**

Any protocol modification should be the object of an amendment, which will be dated and signed by the same parties than those invested in the initial protocol signature.

The amendment will be submitted to the appropriate Ethics Committee (IRB/IEC) and/or the Competent Authorities (if applicable), either for approval or for information, depending on the nature and the importance of the changes to the study conditions.

### **16.7 STUDY SUSPENSION**

If the study or part of the study is prematurely terminated or suspended, the Investigators, the appropriate Ethics Committee(s) (IRB/IEC) and Competent Authorities should promptly be informed as specified by the applicable regulatory requirement(s).

### **16.8 PROTOCOL DEVIATIONS**

No deviations are tolerated systematically. Any deviation from the approved protocol related to study inclusion or exclusion criteria, conduct of the trial, subject's management or subject's assessment should be documented and explained

The main categories of deviations are based at least on the following:

- Informed consent process and documentation
- Enrolment: Assessment of the 'low drinker profile' (based on the coherence between fluid intake volume, complete 24H urine volume, urine osmolality and urine creatinine)
- Randomisation: Inclusion criteria/Non-Inclusion Criteria based on documented medical history of diseases) and/or Randomisation not controlled
- Biological sample collection (e.g 24H urine volume collection)
- Product compliance and product exposure duration (based on subject diaries and questionnaire)
- Time respect between visits
- Follow-up of dietary and forbidden treatment/medication
- Safety issues (e.g lack of declaration of UTI symptoms)

And any other procedure described in the study protocol and site guidelines.

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DANONE RESEARCH will be informed of all protocol deviations, and these will be discussed before implementation according to prospective protocol deviation process or later at the data review meeting (blind review meeting), in order to define their status (minor – major).

#### **16.9 INSURANCE**

DANONE RESEARCH has contracted an insurance policy with an established insurance company in accordance with current regulatory requirements in Bulgaria to cover potential damage to subjects through injury or death caused by the study product and/or study procedures.

The insurance applies to the damage that becomes apparent during the study. DANONE RESEARCH also has liability insurance in accordance with the applicable legislation.

## **17. DATA COLLECTION, PROCESSING, AND MANAGEMENT**

### **17.1 CASE REPORT FORM (CRF)**

An electronic CRF (eCRF) will be created by the Sponsor or its delegate in English. The final version will be approved by the Sponsor. The relevant study data defined in this Protocol will be collected and entered by the Investigator into the provided eCRF. A CRF Completion Guideline will be provided to the Investigator to facilitate CRF completion as well as provide answers for Frequently Asked Questions (FAQs)

Quality of life questionnaire will be provided by the Sponsor to the Investigator:

- Original form should stay at the investigator's site and a photocopy should be sent to the Biometry Contract Research Organization (CRO) for data processing).

Each subject will be identified with a unique Subject Identification number (refer to the section 7.3). All study documents related to a subject must be identified with this subject identifier

All relevant CRF data will be single-entered by personnel at the Investigative site in the validated eCRF tool put in place by the sponsor. The Investigator must ensure that these data are complete and accurately represent the study subject information by electronically signing the appropriate forms in the eCRF.

QoL questionnaires and subject diaries will be in paper and single-entered by the DM CRO in the validated eCRF tool put in place by the sponsor. These questionnaires will be in paper and in Bulgarian but entered in English in the eCRF.

All external data will be provided to the DM CRO for integration to the database

The sponsor will ensure all study site personnel are appropriately trained on the use of the eCRF (i.e. data entry, queries, sign-off etc.). This training is MANDATORY for all site personnel (who are expected to use the system) and must be completed before access is granted to the system. Each user will be provided with a unique access (user name and password) to the eCRF.



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The combination of the user name and password to access the eCRF are the legally binding equivalent of a traditional handwritten signature, and as such, carries all of the same rights and responsibilities. User accounts are not interchangeable, and must only be used by the person authorized to use that account.

### **17.2 DATA PROCESSING**

Edit checks, to ensure the quality and consistency of the data, will be defined in a Data Validation Plan (DVP). These edit checks will be programmed and validated in the eCRF. During the data entry process, these checks will automatically be triggered (and become apparent to the user) in case of incoherent data.

### **17.3 DATA AUDIT TRAIL**

Any addition, modification, or deletion of subject data made after the initial entry (i.e. response to DCF or updated information) will be automatically tracked in the e-CRF's audit trail in compliance to ICH GCP and US FDA 21 CFR part 11.

### **17.4 DATA MANAGEMENT**

Data Management (DM) tasks for this study will be performed by a delegated DM CRO. The verification and validation of DM tasks will be performed by a Data Manager internal at the Sponsor. All DM tasks will be defined in a Data Management Plan (DMP) based on the current Sponsor and CRO Standard Operating Procedures (SOPs).

### **17.5 EXTERNAL DATA**

Data Transfer specifications for external data (i.e. central laboratory data) will be defined in a Data Transfer Agreement (DTA). All electronic external data transfers will be reconciliated with the main study data and validated by the DM CRO to ensure accuracy and consistency.

### **17.6 DATABASE LOCK**

After all planned subject visits have been completed, entered into the study database, and considered clean, a Data Review Meeting (DRM) will be held by the Sponsor to discuss and review all study data. The main objective of this DRM will be to review tables, listings and summaries on study data, to review safety listings, to identify protocol deviations and to define subject analysis populations. Members of the Sponsor's Study Core Team, the designated CRO's as well as the Principal Investigator(s) may participate in this meeting.

Once any final issues resulting from the DRM have been resolved, the study database, including all external data will be locked. This will ensure that no further modifications to the study data are possible. The statistical analysis will be performed on this locked database.

Following the database lock, the sponsor will provide each Investigative site with a final, unmodifiable PDF copy of the completed CRFs, Questionnaires and Subject Diaries (including the audit trail ) of all subject's included at that site. This constitutes an exact representation of the information that was entered in the eCRF during the study. This will be provided on removable media (i.e. CD, DVD etc).

## **18. DOCUMENTATION AND ARCHIVING**

DANONE RESEARCH provides the Principal Investigator (s) with an Investigator Site File (ISF). Each Principal Investigator (s) is responsible to keep this ISF updated and available for review by the study monitor (CRA).

All documents pertaining to the conduct of the study must be kept by the Investigator for a period of 15 years.

Study documents should not be destroyed without prior written agreement between DANONE RESEARCH and the Investigator. Should the Investigator wish to assign study documents to another party, or move them to another location, DANONE RESEARCH must be notified first.

### **18.1 SPONSOR**

The following documents are to be archived by the sponsor for a minimum of 25 years after the completion of the study, in a room specifically designated for this purpose, access to which is controlled by the person responsible for archiving:

- Final version of the study protocol,
- Any forms containing protocol amendments,
- **FOR QoL questionnaire:** Original pages
- **FOR eCRF:** The media (i.e. CD, DVD etc.) that contains the final PDF copy of the subject CRFs, Questionnaires, Subject Diaries (including audit trail ) that was extracted from the eCRF following the database lock,
- Ethics Committee approval forms,
- All correspondence between the sponsor and the Investigator,
- Curriculum vitae (CV) of Principal Investigator and other Investigators
- Acknowledgements of receipt and study product accountability forms as well as all other documents generated during the study.

### **18.2 INVESTIGATOR**

All documents concerning this study must be kept by the Investigator, including but not limited to:

- Subject's medical files including source documents
- Original (dated and signed) of the ICFs and the subject's identification code list
- Screening and enrolment Log
- **FOR QoL questionnaire:** Copy
- **FOR eCRF:** The media (i.e. CD, DVD etc.) provided by the sponsor that contains the final PDF copy of the subject CRFs, Questionnaires, Subject Diaries (including audit trail )
- Copy of accountability forms for products administered
- Copy of ethics committee approval and correspondence with the sponsor
- Signed Protocol(s), signed amendment(s)
- Advertisement (if any)
- Financial Contract(s)
- Insurance certificate
- EC correspondence including Approval Opinion and composition
- CV of Investigator and sub Investigator(s) and delegation task list
- Normal Values and technical procedures
- Instruction for handling products, shipping records, products accountability
- Decoding procedure
- Serious Adverse Events report forms

All correspondence between the sponsor and the Investigator,

The Investigator agrees to provide direct access to source documents during monitoring visits.

## **19. OWNERSHIP OF RESULTS**

All information and results issued from the study remain the property of the sponsor.

The study results may be published or presented by the Investigator or by experts responsible for analysis, in collaboration with the sponsor and with the prior written approval of the latter. The sponsor may use the results of the study for publications or communications with the written agreement of the Investigator or experts responsible for analysis if the latter are cited.

## **20. RESPONSIBILITIES**

### **20.1 SPONSOR**

- a. Manage the submission of the study protocol to the ethics committee//health authorities before the start of the study. The Sponsor should obtain the approval from all the competent authorities and from the EC.
- b. Before the start of the study, the sponsor must provide the Investigator with all documents required by the protocol and/or to provide information on the study products. In particular, the Sponsor must provide the Investigator with a document certifying that the study products are fit for human consumption.
- c. The Sponsor must take out a specific insurance policy for the study as required by current legislation of the region in which the study is being conducted.
- d. The Sponsor must provide the Investigator with insurance certification. The Sponsor may decide to terminate the study at any time. At the end of the study, the Sponsor must archive the study documents for the legally required duration and at least for 25 years.
- e. The Sponsor must carry out all procedures required by the relevant EC/health authorities including initial/amendment submission and Safety reporting.
- f. The Sponsor must set up data Quality Control at each stage of data handling.
- g. The Sponsor finances expenses related to the study
- h. The sponsor must monitor the study and ensure the correct adherence to protocol requirements and regulatory requirements are maintained (including the data protection and confidentiality)
- i. The Sponsor must record the study in the appropriate Governmental database

### **20.2 INVESTIGATOR**

- a. Provides oral and written information for subjects and selects subjects in accordance with protocol inclusion and non-inclusion criteria.
- b. Keeps all study related information confidential
- c. Manages the of study products including randomisation, storage, dispensation, destruction, site accountability.
- d. Before the study, provides the following documents to the sponsor:
  - CV of coordinating Investigator and co-Investigators,
  - CV of the entire remaining investigating site staff involved in the study.
- e. Performs the clinical study within the scheduled dates.
- f. Establishes a written delegation task list, and allocate sufficient time and resources to properly conduct the study
- g. Reports of SAEs.
- h. Cooperates with Clinical Research Assistants at the periodic monitoring visits, or in case of audits and/or inspections
- i. Completes and corrects the case report forms, DCF.
- j. Give input in the clinical report for the study, if relevant.
- k. Maintain essential documentation
- l. Archives data for a minimum of 15 years after the date of the final report.

### **20.3 MONITORING CLINICAL RESEARCH ORGANISATION (CRO)**

- a. Provides the required study materials in good time

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- b. Performs Study start-up visit
- c. Verifies and updates the Trial Master File (TMF) and Investigator Study File.
- d. Performs the Monitoring during the study conduct (including at least the verification of the signed Informed consent, the validation of the data recorded in the CRF including Safety data from source documents, the verification of the study product's storage conditions .)
- e. Performs the End-of-study visit
- f. Communicate relevant information to the Sponsor
- g. Verifies the Data collection (accurate , complete and verifiable)

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## **22. APPENDICES**

# **APPENDIX I**

## **DECLARATION OF HELSINKI**



## **WORLD MEDICAL ASSOCIATION**

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:  
29th WMA General Assembly, Tokyo, Japan, October 1975  
35th WMA General Assembly, Venice, Italy, October 1983  
41st WMA General Assembly, Hong Kong, September 1989  
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996  
52nd WMA General Assembly, Edinburgh, Scotland, October 2000  
53rd WMA General Assembly, Washington, DC, USA, October 2002 (Note of Clarification added)  
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)  
59th WMA General Assembly, Seoul, Republic of Korea, October 2008  
64th WMA General Assembly, Fortaleza, Brazil, October 2013

### **PREAMBLE**

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

### **GENERAL PRINCIPLES**

3. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

### **RISKS, BURDENS AND BENEFITS**

16. In medical practice and in medical research, most interventions involve risks and burdens.

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Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

#### **VULNERABLE GROUPS AND INDIVIDUALS**

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

#### **SCIENTIFIC REQUIREMENTS AND RESEARCH PROTOCOLS**

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

#### **RESEARCH ETHICS COMMITTEES**

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

#### **PRIVACY AND CONFIDENTIALITY**

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

#### **INFORMED CONSENT**

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be included in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

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All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

### **USE OF PLACEBO**

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

### **POST-TRIAL PROVISIONS**

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

### **RESEARCH REGISTRATION AND PUBLICATION AND DISSEMINATION OF RESULTS**

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

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#### **UNPROVEN INTERVENTIONS IN CLINICAL PRACTICE**

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

#### **ARTICLE INFORMATION**

**Corresponding Author:** World Medical Association, 13, ch. du Levant, CIB - Bâtiment A, 01210 Ferney-Voltaire, France; [wma@wma.net](mailto:wma@wma.net).

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**Online-Only Content:** Audio podcast is available at [www.jama.com](http://www.jama.com).

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## **APPENDIX II**

### **List of Participating Investigational Sites and Principal Investigators**

**APPENDIX III**

**SERIOUS ADVERSE EVENT**

**TRANSMISSION FORM**

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## Serious Adverse Event Report Form

This form must be faxed to the Sponsor within 24h

<b>Sponsor:</b> <b>DANONE RESEARCH</b>	<b>Fax :</b>	<b>Tel :</b>	
	<b>Clinical Study Manager (Name):</b>		
<b>Principal Investigator (Name):</b>			
<b>Study code : NU</b>    _ _ _	<b>Expeditor (Name):</b>	<b>Country :</b>  _ _ _	<b>Site Number:</b>  _ _ _
<b>Transmission date :</b>  _ _ / _ _ / _ _ _ _  (dd/mm/yyyy)			
Number of pages (incl. this page)	_____ <b>Pages</b>		
Attached copies of completed e-CRF pages:	<input type="checkbox"/> Adverse events <input type="checkbox"/> Medical History and pre-existing conditions <input type="checkbox"/> Medications		

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This SAE report is :  Initial report  
 Follow-up report (Includes only new details)

Is the SAE aggravation of a pre-existing AE recorded in the e-CRF: <input type="checkbox"/> Yes <input type="checkbox"/> No				
If yes, please put the number of AE:  _ _				
<b>Subject Details</b>				
Subject Number:  _ _ _ / _ _ _ / _ _ _		Gender: <input type="checkbox"/> M <input type="checkbox"/> F		
Subject Initials:  _ _ _ / _		Date of birth:  _ _ / _ _ / _ _ _ _  (dd/mm/yyyy)		
Height:  _ ,  _ _  m Weight :  _ _ _ ,  _ kg				
<b>Description of Serious Adverse Event</b>				
Nature of SAE (diagnosis or major symptom and/or sign):				
Description of SAE:				
Severity of SAE: <input type="checkbox"/> Mild <input type="checkbox"/> Moderate <input type="checkbox"/> Severe				
Event start date:  _ _ / _ _ / _ _ _ _  (dd/mm/yyyy)		Event end date:  _ _ / _ _ / _ _ _ _  (dd/mm/yyyy)		
Time :  _ _  :  _ _  (Hours:Minutes)		Time :  _ _  :  _ _  (Hours:Minutes)		
<b>Category of SAE</b> (*as applicable to study protocol)				
<input type="checkbox"/> Death		<input type="checkbox"/> Persistent or significant disability or incapacity		
<input type="checkbox"/> Life threatening situation		<input type="checkbox"/> Congenital anomaly or birth defect		
<input type="checkbox"/> Hospitalization or Prolongation of existing Hospitalization				
<b>Study Product</b>				
<b>According to protocol, the dosage is:</b>		Dose:  _ _  Unit (Pot/Bottle): _____ Frequency: Daily Route: Oral		
<b>Information of study production distribution</b>	Study Product Number(s)	Last Product Batch Number:	Product Expiry date (dd/mm/yyyy)	Date of Last Product Distribution (dd/mm/yyyy)
	_ _ _ _ _ _ _	_ _ _ _ _ _ _	_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _
<b>Information of study product intake</b>			Date of last product intake (dd/mm/yyyy)	Date of last product intake before event (dd/mm/yyyy)
			_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _
<b>Has the dosage been respected before the event?</b>	<input type="checkbox"/> Yes	<input type="checkbox"/> No	Dose:  _ _  Unit (Pot/Bottle): _____	
	If No, specify:		Frequency: Daily	



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<b>Action taken with respect to the study product:</b>	<input type="checkbox"/> Dose Not Changed <span style="float: right;"><input type="checkbox"/> Not applicable</span>		
	<input type="checkbox"/> Product Interrupted Stop date:  __ _ / __ _ / _ _ _ _  Did reaction disappear after stopping product? <input type="checkbox"/> Yes <input type="checkbox"/> No Reintroduction date:  __ _ / __ _ / _ _ _ _  Did reaction reappear after reintroduction? <input type="checkbox"/> Yes <input type="checkbox"/> No  <input type="checkbox"/> Product withdrawn => if yes please enter the stop date  __ _ / __ _ / _ _ _ _  (dd/mm/yyyy)		
<b>Has the code been broken?</b>	<input type="checkbox"/> Yes	<input type="checkbox"/> No	<input type="checkbox"/> Not Applicable

Action taken with respect to the subject (check all that apply)	Outcome of SAE for the subject	Relationship to	
		Study Product	Study Procedure
<input type="checkbox"/> None <input type="checkbox"/> Medication <input type="checkbox"/> Hospitalization <input type="checkbox"/> Study discontinuation <input type="checkbox"/> Study withdrawal <input type="checkbox"/> Other, specify: _____ _____ _____ _____	<input type="checkbox"/> Not Recovered <input type="checkbox"/> Recovering <input type="checkbox"/> Recovered with sequelae, being: ..... <input type="checkbox"/> Recovered without sequelae <input type="checkbox"/> Fatal /Death <input type="checkbox"/> Unknown	<input type="checkbox"/> Not related <input type="checkbox"/> Unlikely related <input type="checkbox"/> Possibly related <input type="checkbox"/> Probably related <input type="checkbox"/> Definitely related	<input type="checkbox"/> Not related <input type="checkbox"/> Unlikely related <input type="checkbox"/> Possibly related <input type="checkbox"/> Probably related <input type="checkbox"/> Definitely related

Relevant medical and surgical history / or copy of e-CRF pages (e-CRF page XXX) related to medical and surgical history				
No.	Disease or Surgery	Start date (dd/mm/yyyy)	End Date (dd/mm/yyyy)	Current medication /nutritional supplement?*
1		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
2		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
3		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
4		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
5		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No

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6		_  /  _  /  _  _  _	_  /  _  /  _  _  _   Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
7		_  /  _  /  _  _  _	_  /  _  /  _  _  _   Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
8		_  /  _  /  _  _  _	_  /  _  /  _  _  _   Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
9		_  /  _  /  _  _  _	_  /  _  /  _  _  _   Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
10		_  /  _  /  _  _  _	_  /  _  /  _  _  _   Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
11		_  /  _  /  _  _  _	_  /  _  /  _  _  _   Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
12		_  /  _  /  _  _  _	_  /  _  /  _  _  _   Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
13		_  /  _  /  _  _  _	_  /  _  /  _  _  _   Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
14		_  /  _  /  _  _  _	_  /  _  /  _  _  _   Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
15		_  /  _  /  _  _  _	_  /  _  /  _  _  _   Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No

## CLINICAL STUDY PROTOCOL

**PRODUCTS:** WATER

**PROTOCOL VERSION** 3 (AMENDMENT 2)

**STUDY CODE:** NU369

**AMENDED PROTOCOL**

**DATE:** 30/01/2015

***The effect of increased water intake on the  
frequency of clinical recurrent urinary tract  
infections in pre-menopausal women:  
S-HYDRACYST***

**Principal Investigator:**

**MAYA DABCHEVA, MD**  
MC "COMAC MEDICAL"  
13 URVICH STR., 3<sup>RD</sup> FLOOR  
1612 SOFIA – BULGARIA  
TEL.: +359 2 850 97 04 FAX: +359 2 850 60 96  
E-MAIL: MAYA.DABCHEVA@COMAC-MEDICAL.COM

**Sponsor:**

DANONE RESEARCH  
ROUTE DEPARTEMENTALE 128  
91767 PALAISEAU CEDEX – FRANCE

**Sponsor Representative:**

**PETER SUENAERT, MD, PHD**  
DIRECTOR CLINICAL AFFAIRS AND  
HUMAN TRANSLATIONAL RESEARCH  
DANONE RESEARCH  
ROUTE DEPARTEMENTALE 128  
91767 PALAISEAU CEDEX – FRANCE  
TEL.: +33(0)6 10 99 42 18 FAX: +33(0)1.69.35.76.89  
E-MAIL: PETER.SUENAERT@DANONE.COM

**Emergency Contact**

**MAYA DABCHEVA, MD**  
TEL.: +359 2 850 97 04 FAX: +359 2 850 60 96

**Ethics Statement**

This study will be conducted according to the protocol and in compliance with Good Clinical Practice (GCP), the ethical principles stated in the Declaration of Helsinki, and other local applicable regulatory requirements.

**Confidentiality Statement**

The information provided in this document is the property of DANONE RESEARCH, and is shared with you and your staff in confidence. This information should not be disclosed to others without written authorization from DANONE RESEARCH, except to the extent necessary to ensure adequate conduct of the study.

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## 1. GENERAL INFORMATION

### 1.1 LIST OF PARTICIPANTS

**Principal Investigator:** **MAYA DABCHEVA, MD**  
MC "Comac Medical",  
13 URVICH STR., 3<sup>RD</sup> FLOOR  
1612 SOFIA - BULGARIA  
Tel.: +359 2 850 97 04 Fax: +359 2 850 60 96  
E-MAIL: MAYA.DABCHEVA@COMAC-MEDICAL.COM

**Research Scientist/Engineer:** **MARIACRISTINA VECCHIO**  
DANONE RESEARCH  
ROUTE DEPARTEMENTALE 128  
91767 PALAISEAU CEDEX - FRANCE  
Tel.: +33 (0)1 69 35 68 47 Fax: +33(0)1.69.35.76.93

**Biostatistician:** **QUENTIN DORNIC** [Aixial on behalf of DANONE RESEARCH]  
DANONE RESEARCH  
ROUTE DEPARTEMENTALE 128  
91767 PALAISEAU CEDEX - FRANCE  
Tel.: +33 (0)7 78 41 70 38 Fax: +33 (1).69.35.76.89

**Sponsor's Medical Reviewer:** **LILIANA GALESTESCU, MD**  
*[to be contacted for safety reporting]*  
DANONE RESEARCH  
ROUTE DEPARTEMENTALE 128  
91767 PALAISEAU CEDEX – FRANCE  
Tel. : +33(0)1 69 35 72 34 Fax: +33(0)1.69.35.76.89

**Emergency contact:** **MAYA DABCHEVA, MD**  
Tel. : +359 2 850 97 04 Fax: +359 2 850 60 96

**Clinical Study Manager:** **LAURENCE DE LA HOSSERAYE** [Hays Pharma on behalf of  
DANONE RESEARCH]  
DANONE RESEARCH  
ROUTE DÉPARTEMENTALE 128  
91767 PALAISEAU CEDEX - FRANCE  
Tel.: + 33 (0)6 13 61 27 02 Fax: +33 (1).69.35.76.89

**Scientific Expert:** **YAIR LOTAN**  
CHIEF OF UROLOGIC ONCOLOGY  
UT SOUTHWESTERN MEDICAL CENTER AT DALLAS  
DEPARTMENT OF UROLOGY  
5323 HARRY HINES BLVD. J8.122  
DALLAS, TEXAS 75390-9110  
Tel.: 214-648-0389

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**Monitoring Clinical Research Organisation:** PHARM-OLAM INT.(UK) BULGARIA OFFICE  
PHARM-OLAM INT. (UK) BULGARIA OFFICE  
55 PARCHEVICH STR.  
1000 SOFIA - BULGARIA  
Tel.: + 359 2 9888 704  
Fax: + 359 2 9888 701

**Central Laboratory:** MEDICAL DIAGNOSTIC LABORATORY RAMUS EOOD  
2-4 ANGISTA STR.  
SOFIA 1527 - BULGARIA  
Tel.: + 359 2 9431196  
Fax: + 359 2 944 8206

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**1.2 PROTOCOL SIGNATURE PAGE - SPONSOR**

Protocol details	
Study title	<i>The effect of increased water intake on the frequency of clinical recurrent urinary tract infections in pre-menopausal women: S-HYDRACYST</i>

*Protocol approved by the Sponsor:*

I, the undersigned, have reviewed and approved this protocol, including the appendices.

SPONSOR	DATE	SIGNATURE
<b>PETER SUENAERT, MD, PhD</b> DIRECTOR CLINICAL AFFAIRS AND HUMAN TRANSLATIONAL RESEARCH  DANONE RESEARCH ROUTE DEPARTEMENTALE 128 91767 PALAISEAU CEDEX - FRANCE		



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**1.3 PROTOCOL SIGNATURE PAGE – PRINCIPAL INVESTIGATOR**

Protocol details	
Study title	<b><i>The effect of increased water intake on the frequency of clinical recurrent urinary tract infections in pre-menopausal women: S-HYDRACYST</i></b>

*Protocol approved by the Principal Investigator:*

I, the undersigned, have reviewed this protocol including the appendices and I am aware of my responsibility and I agree to the following:

To conduct the clinical study in compliance with the protocol as detailed in this document.

To apply ICH Good Clinical Practice the declaration of Helsinki and any other regulatory requirements.

To obtain protocol approval from an independent Ethics Committee and to comply with their requirements for ongoing review and reporting (if applicable).

To comply with procedures for data recording and reporting (with a particular focus on Safety reporting)

To permit monitoring, auditing and inspection by the sponsor and relevant regulatory agencies.

To retain study related documents according to regulatory requirements and as agreed with the sponsor.

PRINCIPAL INVESTIGATOR	DATE	SIGNATURE
<b>MAYA DABCHEVA, MD</b> MC "COMAC MEDICAL", 13 URVICH STR., 3 <sup>RD</sup> FLOOR 1612 SOFIA - BULGARIA		

*Three original copies of the clinical study protocol must be signed by the Principal Investigator and one original copy shall be filed by each of the parties (Sponsor and Principal Investigator) and one should be submitted to the Ethics Committee.*

**1.4 GLOSSARY**

ACR	Albumin Creatinine Ratio
AE	Adverse Event
AFU/EAU	Association Française d'Urologie (AFU)/European Association of Urology
BMI	Body Mass Index
BP	Blood Pressure
CA	Competent Authority (ies)
CI	Confidence Interval
CM	Concomitant Medication
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organisation
CUA	Cost Utility Analysis
CV	Curriculum Vitae
DCF	Data Clarification Form
DBP	Diastolic Blood Pressure
EAU	European Association of Urology
EC	Ethics Committee
EDC	Electronic Data Capture
e-CRF	Electronic Case Report Form
EPI	Epidemiology Collaboration
FAS	Full Analysis Set
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GP	General Practitioner
HR	Heart Rate
ICF	Informed Consent Form

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ICH	International Conference on Harmonisation
ISF	Investigator Study File
IRB/IEC	Institutional Review Board/Independent Ethics Committee
IWRS	Interactive Web Response Services/Systems
LDL	Low Density Lipoprotein
MCF	Mean Cumulative Function
MSU	Midstream Specimen of Urine
OTC	Over The Counter
PI	Principal Investigator
PP	Per Protocol
QoL	Quality Of Life
RR	Relative Risk
rUTI	Recurrent Urinary Tract Infection
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SD	Standard Deviation
SEM	Standard Error of Mean
SF	Screen Failure
SOP	Standard Operating Procedure
SS	Safety Set
SSAR	Suspected Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
UCC	Urine Color Chart
USG	Urine Specific Gravity
UTI	Urinary Tract Infection

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V	Visit
VAS	Visual Analytical Scale
WHO	World Health Organisation

## 1.5 LIST OF DEFINITIONS

Active/Test product:	Product to be tested in the study.
Completed Subject:	Subject who has completed the study visits as required by the protocol. In the e-CRF the end of study status is available and it is: "Completed the study".
Evaluation period:	This period extends from randomisation (or allocation) up until the last visit where results are collected with the intent of evaluating any of the study criteria.
Eligible subject at visit 1 and visit 2:	The subject is considered eligible when the Investigator certifies that she fulfills all inclusion criteria and does not meet any exclusion criteria for the current visit. This statement can be repeated as many times as required by the protocol. In the e-CRF the Investigator has answered YES to the question "Is the subject eligible at visit 1 and visit 2?"
Included subject:	The signature of the informed consent marks the inclusion of the subject in the study. This signature can be obtained solely after having fully informed the subject about the study. At this step, a subject identification number is attributed to the subject. In the e-CRF the informed consent date is available.
Low drinker profile:	A low drinker profile is defined as follows: <ul style="list-style-type: none"><li>- total self-reported fluid intake &lt; 1,5 l/day</li><li>- and total 24 hours urinary volume &lt; 1,2 L</li><li>- and urine osmolality <math>\geq</math> 500 mOsmol/kg</li><li>- and urine creatinine within the laboratory normal ranges</li></ul>
Menopausal subject:	Subject without menstrual period for 12 months following the final menstrual period in the absence of pregnancy or other biological causes.
Multiple antibiotic resistance:	Multiple antibiotic resistance is defined as resistance to 3 or more different antibiotic groups.
Perimenopausal subject:	Subject with irregular menstrual cycles (on average 4 years before the final menstrual period) and hormone fluctuation often accompanied by hot flushes, sleep disturbances, mood symptoms and vaginal dryness [1].
Pre-menopausal subject:	Subject with normal menstrual cycles with monthly period, unless she is pregnant.

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Principal Investigator:	Investigator responsible for the conduct of the clinical study at a study site. If a study is conducted by a team of individuals at a study site, the Principal Investigator is the responsible leader of the team and may be called the principal Investigator.
Pre-screening period:	Any actions performed by the site(s) in relation to the study recruitment that happens before the formal signature of the informed consent. No data is collected by the sponsor at this point.
Positive urine culture:	Positive urine culture is defined as $> 10^3$ CFU/mL in the midstream specimen of urine (MSU) [31].
Randomised (or allocated) subject:	Any subject who has been allocated to a study arm either by a randomisation or by another method defined by the protocol. The randomisation date is available in the e-CRF.
Screening period:	This period extends from the signature of the informed consent up until the randomisation call.
Screen-failed subject:	The subject is considered a screen failure (SF) if she has signed the informed consent but did not complete the screening period successfully and not been randomised). In the e-CRF an “end of study form” should be available with the reason for failure.
Study product(s):	Products to be used in the clinical study (test products).
Symptoms of UTI:	Dysuria (i.e. painful urination, usually described by the patient as burning, stinging or itching) and / or urgency and / or increased frequency of urination and / or suprapubic pain (i.e. pain occurring from above the pubis) [31].
UTI:	Bacterial infection of the urinary.
UTI event:	Diagnosed by at least 1 symptom of urinary tract infection and positive urine culture.
Withdrawn (or Dropped out) Subject:	Any subject who has withdrawn from the study at any point after the randomisation but before having completed all the visits and assessments. In the e-CRF the end of study status is available and it is different from: “Completed the study”.

## 2. SYNOPSIS AND FLOW CHART

<b>Study title</b>	<i>The effect of increased water intake on the frequency of clinical recurrent urinary tract infections in pre-menopausal women: S-HYDRACYST</i>
<b>Study code</b>	<i>NU369 S-Hydracyst</i>
<b>Study sponsor</b>	<i>Peter SUENAERT - DANONE RESEARCH, Palaiseau - France</i>
<b>Study principal investigator</b>	<i>Dr. Maya DABCHEVA, MC "COMAC MEDICAL"</i>
<b>Study product description</b>	<p>The study is testing a medical recommendation, consisting in increasing the daily water intake in the intervention group.</p> <p>For methodological reasons of homogeneity of access to water of the subjects, mineral commercialised water will be distributed to the subjects included in the intervention group, during the whole study duration.</p> <p>The mineral water is Evian® brand.</p> <p>The intervention group will increase its fluid intake of 1,5L of Evian® water (on the top of their normal fluid intake).</p> <p>The control group will not change its water intake habits.</p>
<b>Study period</b>	<p>Intervention period: Dec 2013 – Jan 2017 (around 12 months per subject)</p> <p>Study start date: (first subject first visit): Dec 2013</p> <p>Study end date (clinical study report writing): Q3 2017</p>
<b>Study objectives</b>	<p><u>Study primary objective:</u></p> <p>To assess the effect of increased daily water intake on the frequency of clinical recurrent urinary tract infections (rUTIs) among low drinking pre-menopausal women suffering from recurrent community-acquired UTI over 12 consecutive months of study product consumption.</p> <p><u>Study secondary objectives:</u></p> <p>To evaluate the impact of increased daily water intake on the use of antibiotics in recurrent UTI patients over 12 consecutive months of study product consumption.</p> <p>To evaluate the impact of increased daily water intake on the mean time elapsed between UTI episodes over 12 consecutive months of study product consumption.</p> <p>To evaluate the impact of increased daily water intake on urinary hydration markers over 6 and 12 consecutive months of study product consumption.</p> <p>To evaluate the impact of increased daily water intake on health costs in recurrent UTI patients over 12 consecutive months of study product consumption.</p> <p>To evaluate the impact of increase of daily water intake on the cost utility analysis during the study intervention using two different perspectives: National Health insurance and subject's perspective (subject's own out-of-pocket costs).</p> <p>To evaluate the impact of increased daily water intake on quality of life (QoL) in recurrent UTI patients over 6 and 12 consecutive months of study product</p>

	<p>consumption.</p> <p><u>Exploratory Objectives:</u></p> <p>To evaluate the relationship between urinary hydration markers and number of UTI events over 12 consecutive months of study intervention from D0.</p> <p>To evaluate the relationship between urinary hydration markers and delay UTI events over 12 consecutive months of study intervention from D0.</p> <p>To evaluate the impact of increased daily water intake on crystallisation risk index (Tiselius) over 12 consecutive months of study intervention from D0.</p> <p>To evaluate the number of UTI events confirmed by positive urine culture and number of subjects presenting at least one UTI event over 12 consecutive months of study intervention from D0 (by group).</p> <p>To evaluate the number of UTI events not confirmed by positive urine culture and number of subjects presenting at least one UTI event over 12 consecutive months of study intervention from D0 (by group).</p> <p>To evaluate the number of UTI events confirmed or not by positive urine culture and number of subjects presenting at least one UTI event over 12 consecutive months of the study intervention from D0 (by group).</p>
<b>Study Methodology</b>	<p>Prospective, single-site, open-label, randomised controlled trial in two parallel groups:</p> <ul style="list-style-type: none"> <li>❖ Control group not changing their fluid intake habits</li> <li>❖ Intervention group provided with low mineralised mineral water, fluid intake recommendations and regular hydration coaching support</li> </ul> <p>Subject will be allocated to the study arm through the randomisation system (IWRS) without any stratification factor.</p>
<b>Study population and sample size</b>	<p>The study population consists in pre-menopausal women diagnosed with rUTIs and having a 'low drinker' profile. The total number of completed subjects is estimated at 84 subjects.</p> <p>In order to achieve 84 completed subjects, and considering a 40% drop out rate, 140 subjects will be randomised.</p> <p>Considering a 50% screen failure rate, around 280 screened subjects will be needed in order to get 140 randomised subjects.</p>
<b>Subject recruitment</b>	<p>This is a single-site study which will be performed in Bulgaria. 140 women will be randomised in this trial.</p>
<b>Eligibility criteria</b>	<p><u>Eligibility criteria checked at V1</u></p> <p><u>Inclusion criteria:</u></p> <p>II01a: Women with at least 3 clinical recurrences of symptomatic UTI in the last 12 months (at least one UTI must be confirmed by a positive documented bacteriological exam) and asymptomatic at V1</p> <p>or</p> <p>II01b: Women with at least 3 clinical recurrences of symptomatic UTI in the last 12 months (at least one UTI must be confirmed by a positive documented bacteriological exam) and symptomatic at V1</p> <p>or</p> <p>II01c: Women with at least 2 clinical recurrences of symptomatic UTI in the last 12 months (who did not perform any bacteriological exam during the last 12 months), symptomatic at V1 and with positive documented bacteriological exam between V1 and V2</p>



	<p>II02: Age ≥ 18 years</p> <p>II03: Fluid intake &lt; 1,5 L per day based on patient's declaration</p> <p>II04: Regular meal consumption (breakfast, lunch and dinner)</p> <p>II05: Access to Internet for information on hydration</p> <p>II06: Subject, upon briefing of the content of the present study, fully understanding and agreeing to its objective and having given written (dated and signed) informed consent to take part in the study</p> <p>II07: Subject who is able to communicate well with the Investigator and to comply with the requirements of the entire study</p> <p>II08: Literate subjects, able to fill in fluid diaries and QoL questionnaire</p> <p>II09: Women accepting to keep their lifestyle habits during the whole duration of the study</p> <p>II10: Women using any form of contraception</p> <p>II11: Subject covered by the National Health Insurance system</p> <p><u>Exclusion criteria:</u></p> <p>IE01: Incapacity / non-willingness to consume 1,5 L of drinking water per day on top of their usual consumption</p> <p>IE02: Women with history of UTI complications (pyelonephritis or other) in the last 12 months</p> <p>IE03: Use of antibiotics or cranberries juice and/or extracts in the previous 2 weeks</p> <p>IE04: Chronic treatments with anti-coagulants therapy</p> <p>IE05: Chronic bladder inflammation (defined as permanent bladder bacterial infection)</p> <p>IE06: Chronic diarrhea or constipation treated with chronic use of laxative substances</p> <p>IE07: Interstitial cystitis</p> <p>IE08: Estrogen-dependent symptomatic vulvo-vaginitis</p> <p>IE09: Recent (&lt;1year) or active renal stone disease</p> <p>IE10: Urinary tract structural abnormalities</p> <p>IE11: Obesity or malnutrition (BMI &lt;18,5 Kg/m<sup>2</sup> and &gt;30 Kg/m<sup>2</sup>)</p> <p>IE12: Pregnant or lactating women</p> <p>IE13: Women planning to become pregnant during the study</p> <p>IE14: Menopausal and peri-menopausal women</p> <p>IE15: On-going or planned therapy during the study which can modify the study measurements, in particular the assessment of the hydration status (diuretic intake, corticoids or drug treatment interfering with nutrition behaviour)</p> <p>IE16: Subjects with severe or uncontrolled organic disease, likely to interfere with the parameters of the study (e.g. neoplastic, cardiovascular, pulmonary and digestive disorders, uncontrolled diabetes type I and II, untreated or uncontrolled clinically significant arterial blood hypertension) or mental disorders affecting eating and drinking behaviour (i.e. primary polydipsia, bulimia nervosa, psychosis etc.)</p> <p>IE17: Women who have taken part in any other clinical study for the treatment of rUTI during the last 12 months</p> <p>IE18: Vulnerable subjects defined as individuals whose willingness to volunteer in the clinical trial may be unduly influenced by the expectation, whether justified or not, of benefits associated with participation, or of a retaliatory response from senior members of a hierarchy in case of refusal to participate. (Examples are members of a group with a hierarchical structure linked to the Investigator or to the Sponsor, such as medical, pharmacy, dental and nursing students, subordinate hospital and laboratory personnel, employees of the Investigator or of the Sponsor, members of the armed forces, and persons kept in detention).</p> <p>IE19: No legal capacity or limited legal capacity or unable to give an informed consent.</p>
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	<p>IE20: Subjects unlikely to cooperate in the study, and/or poor compliance anticipated by the Investigator.</p> <p>IE 21: Women who have taken part in this study and/or enrolled in another clinical study during the last month and/or currently participating in another clinical study.</p> <p><b><u>Randomisation criteria checked at the pre-randomisation visit (V2) and before subject's randomisation:</u></b></p> <p>RI01: Negative urine culture and asymptomatic state (i.e. silent phase) at V2 (pre-randomisation visit)</p> <p>RI02: Low-drinker confirmation defined as:</p> <ul style="list-style-type: none"> <li>- total self-reported fluid intake &lt; 1,5 L/day</li> <li>- and 24 hours urinary volume &lt; 1,2 L per day</li> <li>- and osmolality ≥ 500 mOsmol/kg</li> <li>- and urine creatinine within the laboratory normal ranges</li> </ul> <p>The following <u>non-randomisation criterion</u> should not be met:</p> <p>RE01: Chronic kidney disease (defined as decreased eGFR (eGFR&lt;60 ml/min/1,73m<sup>2</sup> calculated using EPI equation)</p> <p>RE02: Women suffering multiple antibiotic resistant bacterial strain*</p> <p>IE12: Pregnant or lactating women</p> <p>IE14: Menopausal and peri-menopausal women**</p> <p><i>* Multiple antibiotic resistance is defined as resistance to 3 or more different antibiotic groups</i></p> <p><i>** Based on V2 FSH assessment as per PI's discretion</i></p>
<p><b>Study product administration</b></p>	<p>For the subject in the intervention group, three (3) bottles of natural mineral water (500 mL each) to be consumed daily, in addition to the subjects' usual fluid intake volume, for the whole study duration (i.e. until successful completion of the End of Study visit).</p> <ul style="list-style-type: none"> <li>- Bottles will be provided to subjects in the intervention group for free, it is suggested to consume them at the beginning of every meal (breakfast, lunch and dinner) and fully drink them before the following meal. Coaching support will also be made available; this will be reinforced by individualised phone calls to motivate compliance.</li> <li>- The study products will be provided by DANONE RESEARCH and will be supplied by a professional logistics company.</li> </ul>

<p><b>Study description / Duration of subject participation</b></p>	<p><b><u>Informed consent:</u></b></p> <p>All subjects must sign and personally date the approved informed consent form before any specific study procedure is performed.</p> <p>The total duration of the intervention phase will be around 12 months per subject [between D0 (randomisation call) and V4 (end of study visit)].</p> <p>Each subject will attend at least 4 visits:</p> <p><u>During the Screening period</u> [from V1 to D0 (randomisation call)]</p> <ol style="list-style-type: none"> <li>1. Inclusion visit (V1)</li> <li>2. Pre-randomisation visit (V2). V2 can be rescheduled or repeated in case of UTI event discovered at V2. PI or another authorised study team member will randomise the subject via the IWRS. Then the PI or another authorised study team perform a randomisation call (D0) and inform the subject on her eligibility. D0 has to be performed no later than 8 weeks from V1.</li> </ol> <p><u>During the evaluation period</u> [from D0 (randomisation call) to V4 (end of study visit)]</p> <ol style="list-style-type: none"> <li>3. Evaluation visit (V3)</li> <li>4. End of study visit (V4). V4 can be rescheduled or repeated in case of UTI event discovered at V4.</li> </ol> <p>Additionally, for each UTI event or in case of symptom(s) of UTI, a <b>relapse</b> visit will be done.</p> <p>Furthermore, during the evaluation period, the PI or another authorised study team member will call the subject every month (except at month 6 and 12).</p> <p>All visits (i.e. V3, V4) after D0 (randomisation call) may be conducted within <math>\pm 10</math> days of the theoretical visit date except in case in UTI symptom(s) as mentioned in section 7.1.4. and 7.1.6.</p> <p>All phone calls after the D0 (randomisation call) may be conducted within <math>\pm 7</math> days of the theoretical call date.</p> <p><b><u>Dietary instructions and non-authorized products during the study:</u></b></p> <p>Starting from V1, subjects will be motivated to maintain their usual diet and fluid intake habits and not to consume cranberries juice and/or extracts.</p>
	<p><b><u>Inclusion visit (V1): (Refer to section 7.1.1)</u></b></p> <p>At V1 UTI events in the past 12 months need to be verified. The status of all UTI events must be reported on the referral bulletin provided and signed by the medical doctor referring the patient and / or on the subject's medical records.</p> <p>At V1, subjects meeting all inclusion criteria and none of the exclusion criteria will be included after signature of the approved informed consent form. They will enter into a maximum of 8-weeks screening period.</p> <p><b>1/ Symptomatic subjects will perform the V1. A urine culture will be performed:</b></p> <p><b>1a/ If positive urine culture:</b></p> <p>The subject will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a <b>control</b> urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered and that results are not biased by the antibiotic treatment taken by the subjects.</p>

	<p>Depending on <b>control</b> urine culture results:</p> <ul style="list-style-type: none"> <li>• if <b>negative</b> control urine culture, the subject will come back to site to perform the pre-randomisation visit (V2) as soon as the subject is completely recovered and up to a maximum of 7 weeks from V1,</li> <li>• if <b>positive</b> control urine culture, the subject will be treated till a negative control urine culture is obtained and will come back to the site to perform the pre-randomisation visit (V2) as soon as the subject is completely recovered and up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.</li> </ul> <p>Any UTI symptom(s) with or without a positive urine culture, occurring between V1 and V2 will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.</p> <p><b>1b/ If negative urine culture:</b></p> <p>The subject may be treated or not with a standard of care treatment at the PI's discretion:</p> <ul style="list-style-type: none"> <li>○ Subject <i>receiving</i> a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to site to perform the pre-randomisation visit (V2) up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.</li> <li>○ Subject <i>not receiving</i> any treatment and declaring complete recover from previous symptoms will come back to the site to perform the pre-randomisation visit (V2) as soon as the symptoms are completely disappeared (subject is completely recovered) and up to a maximum of 7 weeks from V1.</li> <li>○ Subject <i>not receiving</i> any treatment but with persisting symptoms will re-perform a urine culture. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standard of care treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the pre-randomisation visit (V2) up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.</li> </ul> <p>Any UTI symptom(s) with or without a positive urine culture, occurring between V1 and V2 will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.</p> <p><b>2/ Asymptomatic subjects at V1</b> will come back to the site to perform the pre-randomisation Visit (V2) after a minimum of 4 days (i.e. the minimum time needed to complete the 3-days fluid intake diary) and up to 7 weeks from V1.</p> <p>Data on past medical and surgical history, past and current medications, sexual life, urine pregnancy test, demographic, vital signs and anthropometrical parameters (body weight and height, waist circumference), UTI symptom(s) will be collected.</p>
	<p><b><u>Over the Screening period [after V1 up to D0 (Randomisation call)]</u></b></p> <p>All subjects will have to complete the <b>3-days fluid intake diary</b> and the <b>Voiding diary</b>. Both diaries, a sterile urine sample (a sample from the first morning micturition on the V2 day) and the 24-hours urine sample outside of menstruation period (collection to be started during the day before V2 and ended with the first morning urine of the day after) will be brought back to the site at pre-randomisation visit. (V2).</p>

	<p>All subjects must be instructed to contact the PI in case of any symptom(s) of UTI. In this case, they will be treated or / not with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management) according to PI's decision, and, the V2, will be rescheduled once the subjects are recovered and / or UTI symptom(s) have disappeared (i.e. a control urine culture will be done at least 48h after the end of the therapy and /or disappearance of all UTI symptoms and within 1 week)</p>
	<p><b><u>Pre-randomisation visit (V2): (Refer to section 7.1.2)</u></b></p> <p>1/ If a subject coming to <b>V2 presents UTI symptom(s), whatever the urine culture results</b>, she won't be able to perform the visit, only urine culture is analysed. V2 will be rescheduled.</p> <p><b>1a/ Symptomatic subjects at V2 with a positive urine culture:</b></p> <p>The subject will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a <b>control</b> urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that the subject is completely recovered.</p> <p>Depending on <b>control</b> urine culture results:</p> <ul style="list-style-type: none"> <li>• If <b>negative</b> control urine culture, the subject will come back to the site to perform the pre-randomisation visit (V2) as soon as the subject is completely recovered and up to a maximum of 7 weeks from V1.</li> <li>• If <b>positive</b> control urine culture, the subject will be treated till a negative control urine culture is obtained and will come back to site perform the pre-randomisation visit (V2) as soon as the subject is completely recovered and up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.</li> </ul> <p>Any UTI symptom(s) with or without a positive urine culture, occurring between V2 and D0 (randomisation call) will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.</p> <p><b>1b/ Symptomatic subjects at V2 with a negative urine culture:</b></p> <p>The subject may be treated or not with a standard of care treatment at the PI's discretion:</p> <ul style="list-style-type: none"> <li>○ Subject <i>receiving</i> a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the pre-randomisation visit (V2) up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.</li> <li>○ Subject <i>not receiving</i> any treatment and declaring complete recover from previous symptoms will come back to site to perform the pre-randomisation visit (V2) as soon as the symptoms are completely disappeared (subject is completely recovered) and up to a maximum of 7 weeks from V1.</li> <li>○ Subject <i>not receiving</i> any treatment but with persisting symptoms will re-perform a urine culture. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standard of care treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the pre-randomisation visit (V2) up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.</li> </ul> <p>Any UTI symptom(s) with or without a positive urine culture, occurring between V2 and randomisation call (D0) will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.</p>

	<p><b>2/Asymptomatic subject will perform V2.</b></p> <p><b>2a/ Asymptomatic subjects at V2 with a positive urine culture:</b></p> <p>The subject will be treated for the UTI event with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a <b>control</b> urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered:</p> <p>If a negative <b>control</b> urine culture is obtained:</p> <ul style="list-style-type: none"> <li>• ≤ 28 days, after V2 (i.e. negative control urine culture obtained at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered), a new QoL questionnaire will be completed by the subject after UTI is cured.</li> <li>• &gt; 28 days, after V2, the V2 should be repeated.</li> </ul> <p>Any UTI symptom(s), with or without a positive urine culture, occurring between V2 and randomisation call (D0) will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.</p> <p><b>2b/ Asymptomatic subjects at V2 with a negative urine culture</b> and fulfilling all randomisation and none of non-randomisation criteria will be randomised by the PI no later than 1 week after V2.</p> <p>Diaries data and any changes in the usual diet and fluid intake habits will be checked (including non-authorized product consumption).</p> <p>Urinary samples will be analysed. Data on sexual life, urine pregnancy test and FSH (to confirm the perimenopausal status if needed), vital signs and anthropometrical parameters (body weight, waist circumference), UTI symptom(s), AE and concomitant medications will be collected.</p> <p>Quality of Life questionnaire will be completed by the subject.</p> <p>A blood sample will be collected for determination of lipidic profile (Total Cholesterol, HDL cholesterol, LDL cholesterol, triglycerides), glycosylated hemoglobin, serum creatinine, serum urea, copeptin. Estimated glomerular filtration rate (eGFR) will be calculated. Patients will receive instructions about study requirements accordingly.</p>
	<p><b><u>Randomisation call (D0): (Refer to section 7.1.3)</u></b></p> <p>Randomisation will be performed once the investigator receives the results of urine analysed confirming the randomisation and non-randomisation criteria.</p> <p>Subject randomisation will be done through the IWRS. Women will be randomly allocated into one of the two study arms.</p> <p>Subjects who will be allocated to the intervention study group will be asked to consume 1,5L of Evian® brand mineral water which will be regularly supplied to their home or office. This volume is on top of their normal water consumption.</p> <p>PI or another documented authorized study team member will contact the subject to inform her about her randomisation group and to provide the appropriate instructions as soon as possible.</p>

	<p><b><u>Over the 12-months evaluation period</u></b></p> <p>All subjects will complete every month till the end of the study, a <b>3-days fluid intake diary</b> (Saturday-Sunday-Monday or Friday-Saturday-Sunday) at home and will have to send it back to the investigational site by courier or to hand it in personally if preferred every month.</p> <p>In addition, subjects in the intervention group will fill in a <b>Subject Compliance Diary</b> which will help them to check the study product consumption. Subjects will send them back to the site each month by courier or to hand it in personally if preferred together with the 3-days fluid intake diary.</p> <p><b>Healthcare costs data</b> related to UTI episodes will be completed during each relapse visit.</p> <p>Subjects will be called every month, except at months 6 (V3) and 12 (V4), by the PI or another documented authorized study team member to check the study compliance (including fluid intake and study product consumption if any), adverse event(s), symptom(s) of UTI and / or UTI event(s), concomitant treatment(s).</p>
	<p><b><u>Evaluation visit (V3): (Refer to section 7.1.4)</u></b></p> <p>Subjects in fasting condition will come along with a 24h urine sample and appropriate pages of the diaries to the clinical site.</p> <p><b>If a subject coming to V3 presents UTI symptom(s), she won't be able to perform the visit. V3 will be rescheduled</b> once the subject is recovered and UTI symptoms disappeared.</p> <p><b>Given the presence of symptoms, a urine culture will be performed and a relapse visit will be done (refer to section 7.1.7).</b></p> <p><b>1a/ Symptomatic subjects at V3 with a positive urine culture:</b></p> <p>The subject will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a <b>control</b> urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered.</p> <p>Depending on the <b>control</b> urine culture results:</p> <ul style="list-style-type: none"> <li>• If <b>negative</b> control urine culture, the subject will come back to site to perform the evaluation visit (V3) as soon as the subject is completely recovered.</li> <li>• If <b>positive</b> control urine culture, the subject will be treated till a negative control urine culture is obtained and will come back to the site to perform the evaluation visit (V3) as soon as the subject is completely recovered.</li> </ul> <p>Any UTI symptom(s), with or without a positive urine culture at V3 will be reported in a relapse visit.</p>

	<p><b>1b/ Symptomatic subjects at V3 with a negative urine culture:</b></p> <p>The subject may be treated or not with a standard of care treatment at the PI's discretion:</p> <ul style="list-style-type: none"> <li>○ Subject <i>receiving</i> a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained. They will come back to site to perform the evaluation visit (V3) as soon as the subject is completely recovered.</li> <li>○ Subject <i>not receiving</i> any treatment, and declaring complete recover from previous symptoms, will come back to the site to perform the evaluation visit (V3) as soon as the symptoms are completely disappeared (subject is completely recovered).</li> <li>○ Subject <i>not receiving</i> any treatment but with persisting symptoms will <b>re-perform a urine culture</b>. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standard of care treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the evaluation visit (V3).</li> </ul> <p>Any UTI symptom(s), with or without a positive urine culture before the V3 will be reported in a relapse visit.</p> <p><b>2/Asymptomatic subject will perform V3.</b></p> <p>Urinary samples will be analysed (urine culture is not performed).</p> <p>Data on sexual life, vital signs and anthropometrical parameters (body weight, waist circumference), UTI symptom(s), AE and concomitant medications will be collected.</p> <p>Quality of Life questionnaire will be completed by the subject.</p> <p>A blood sample will be collected for determination of serum creatinine and copeptin. Estimated glomerular filtration rate (eGFR) will be calculated.</p> <p>Patients will be asked to complete on site the Quality of Life questionnaire.</p>
	<p><b><u>End of study visit (V4): (Refer to Section 7.1.6)</u></b></p> <p>Subjects in fasting condition will come along with a 24h urine sample, sterile urine sample and the appropriate pages of the diaries.</p> <p>1/ If a subject coming to <b>V4 presents UTI symptom(s), whatever the urine culture results</b>, she won't be able to perform the visit, only urine culture is analysed. <b>V4 will be rescheduled</b> once the subject is recovered.</p>



**1a/ Symptomatic subjects at V4 with a positive urine culture:**

The subject will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management) and will be advised not to stop water intake (if she is in the intervention group) till the end of study visit completion. After the completion of UTI treatment, a **control** urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that the subject is completely recovered.

Depending on **control** urine culture results:

- If **negative** control urine culture, the subject will come back to the site to perform the end of study visit (V4) as soon as the subject is completely recovered.
- If **positive** control urine culture, the subject will be treated till a negative control urine culture is obtained and will come back to the site to perform the end of study visit (V4) as soon as the subject is completely recovered.

Any UTI symptom(s), confirmed or not with a positive urine culture before the V4 will be reported in a **relapse** visit.

Once the negative control urine culture is obtained, the end of study form in the e-CRF will be completed.

**1b/ Symptomatic subjects at V4 with a negative urine culture:**

The subject will be advised not to stop water intake (if she is in the intervention group) till the end of study visit completion.

The subject may be treated or not with a standard of care treatment at the PI's discretion:

- Subject *receiving* a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained. They will come back to site to perform the end of study visit (V4) as soon as they are completely recovered.
- Subject *not receiving* any treatment and declaring complete recover from previous symptoms will come back to site to perform the end of study visit (V4) as soon as the symptoms are completely disappeared (subject is completely recovered).
- Subject *not receiving* any treatment but with persisting symptoms will re-perform a urine culture. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standard of care treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the end of study visit (V4).

Any UTI symptom(s), confirmed or not with a positive urine culture before the V4 will be reported in a relapse visit.

Once the negative control urine culture is obtained, the end of study form in the e-CRF will be completed

	<p><b>2/Asymptomatic subject will perform V4.</b></p> <p><b>2a/ Asymptomatic subjects at V4 with a positive urine culture:</b></p> <p>The subject will be treated for the UTI event with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a <b>control</b> urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered and symptoms disappeared.</p> <p>Depending on <b>control</b> urine culture results, if a negative control urine culture is obtained:</p> <ul style="list-style-type: none"> <li>• ≤ 28 days after V4 (i.e. negative control urine culture obtained at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered), a new QoL questionnaire will be completed by the subject after UTI is cured</li> <li>• &gt; 28 days after V4, the V4 should be repeated.</li> </ul> <p>All asymptomatic events with a positive urine culture, occurring at V4 will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.</p> <p>Once the negative control urine culture is obtained, the end of study form in the e-CRF will be completed.</p> <p><b>2b/ For asymptomatic subjects at V4 with a negative urine culture,</b> the end of study form in the e-CRF will be completed.</p> <p>Diary data and any change in the usual diet and fluid intake habits will be checked (including non-authorized product consumption).</p> <p>An urine culture will be performed to evaluate the presence of any infection at the end of study.</p> <p>Urinary samples will be analysed. Data on physical examination, sexual life, vital signs and anthropometrical parameters (body weight, waist circumference), UTI symptom(s), AE and concomitant medications will be collected.</p> <p>Quality of Life questionnaire will be completed by the subject.</p> <p>A blood sample will be collected for determination of lipidic profile (Total Cholesterol, HDL cholesterol, LDL cholesterol, triglycerides), glycosylated hemoglobin, serum creatinine, serum urea, copeptin. Estimated glomerular filtration rate (eGFR) will be calculated.</p>
	<p><b><u>Relapse visits:</u> (Refer to Section 7.1.7)</b></p> <p>Subjects presenting any symptoms of UTI during the course of the study will be instructed not to stop the increased water intake (if in the intervention group) and to come back to the investigational site for clinical examination.</p> <p>UTI events will be diagnosed.</p> <p>Only in case of a positive urine culture, healthcare costs due to illness will be collected. Concomitant treatments will be collected.</p>

	<p><b><u>Intake surveys &amp; coaching support:</u></b></p> <p>In order to reinforce their adherence to fluid intake recommendations, subjects allocated to the intervention group will be provided with bottled mineral water, and will receive individualised phone calls at regular intervals and will be provided with coaching materials. By contrast, subjects allocated to the control group won't receive any fluid intake recommendations nor any test product or coaching support.</p> <p>Subjects allocated to the intervention group will also be asked to complete a Subject Compliance Diary in order to assess the compliance to the study product intake.</p> <p>The protocol will follow ICHG6 guidelines on Good Clinical Practices and applicable Directives/local laws.</p>
<p><b>Study design schema</b></p>	<p><i>See on the next page "Study Flow-Chart"</i></p>

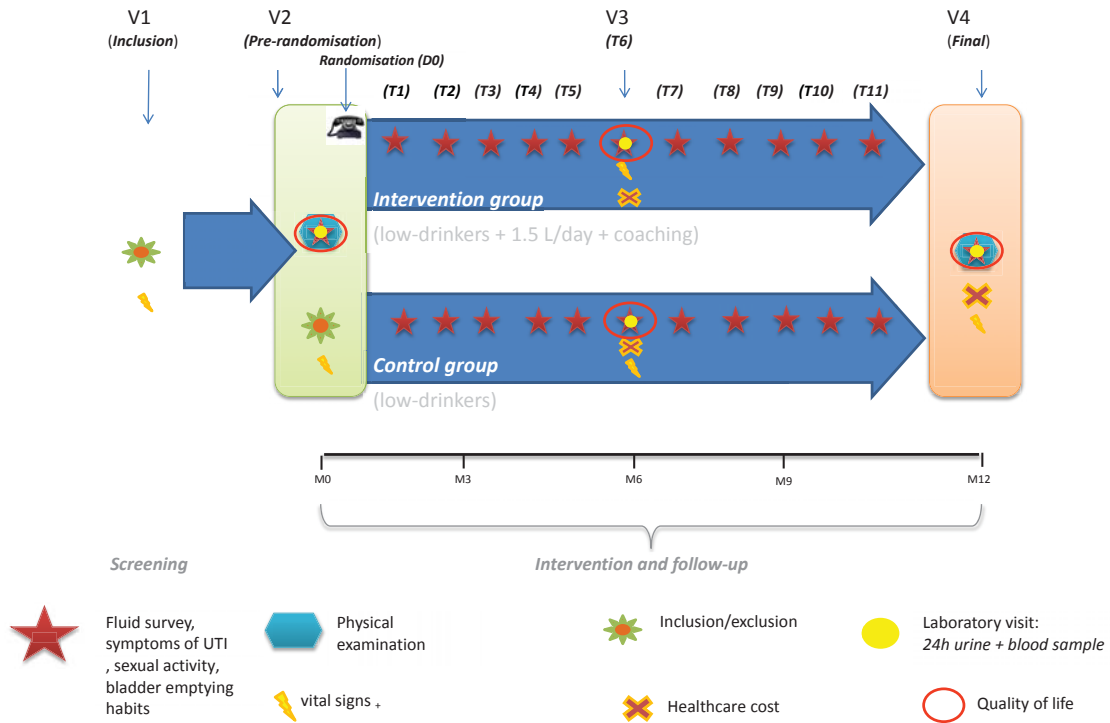
**STUDY FLOW-CHART**

Visits Procedures	MONTH 0				1st Month ± 7 days	2 <sup>nd</sup> Month ± 7 days	3rd Month ± 7 days	4th Month ± 7 days	5th month ± 7 days	6th month ± 7 days	Month 6 ± 10 days	7th Month ± 7 days	8th Month ± 7 days	9th Month ± 7 days	10th Month ± 7 days	11th Month ± 7 days	12th Month	Month 12± 10 days	Relapse visit
	Inclusion Visit (V1)	Home	Pre- randomisation visit (V2)	Randomisation call Day 0	Call	Call	Call	Call	Call	Home	Visit 3 (V3)	Call	Call	Call	Call	Call	Home	Completion (V4)	
Screening for acute UTI or previous episodes (at least 3 in the last 12 months)	X																		
Inclusion / exclusion criteria	X		X																
Randomisation/non randomisation criteria			X	X															
Informed consent form signature	X																		
Study group allocation				X															
Demographic data (date of birth, gender)	X																		
Urine pregnancy test	X		X																
FSH test if applicable			X																
Physical examination			X															X	
Phone call to check compliance, AE and UTI events					X	X	X	X	X			X	X	X	X	X			
Medical & Surgical history	X																		
Vital sign and anthropometrical parameters	X		X								X							X	
Instructions for Subject's diary completion and return for the 3-days fluid intake	X		X	X	X	X	X	X	X		X	X	X	X	X	X		X	X
Subject's diary completion for 3-days fluid intake by the subject		X			X	X	X	X	X			X	X	X	X	X	X		
Check of 3-days fluid intake diary			X		X	X	X	X			X	X	X	X	X	X		X	
Subject Compliance Diary (instructions for the test group)			X		x	x	x	x	x		X	x	x	x	x	x			
Subject Compliance Diary completion (for the test group)					x	x	x	x	x	X		x	x	x	x	x	X		
Subject Compliance with the study product check (for the test group)					x	x	x	x	x		X	x	x	x	x	x		x	
QoL questionnaire (SF-12 V2)			X								X							X	
Delivery of urine kits & Instructions	X		X								X								x
Urine 24h collection + Voiding diary completed by the subject			X*							X*							X*		
Delivery of UCC			X																
Collection of urine containers & Voiding diary			X								X							X	
Blood samples			X								X							X	
Symptoms of UTI	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Urine culture	X** (only if symptomatic)		X**								X** (only if symptomatic)							X**	X**
Healthcare costs data																			X

Visits Procedures	MONTH 0			1st Month ± 7 days	2 <sup>nd</sup> Month ± 7 days	3rd Month ± 7 days	4th Month ± 7 days	5th month ± 7 days	6th month ± 7 days	Month 6 ± 10 days	7th Month ± 7 days	8th Month ± 7 days	9th Month ± 7 days	10th Month ± 7 days	11th Month ± 7 days	12th Month	Month 12± 10 days		
	Inclusion Visit (V1)	Home	Pre- randomisation visit (V2)	Randomisation call Day 0	Call	Call	Call	Call	Call	Home	Visit 3 (V3)	Call	Call	Call	Call	Call	Home	Completion (V4)	Relapse visit
Bladder emptying after sexual intercourses	X		X		X	X	X	X	X		X	X	X	X	X	X		X	X
Active/inactive sexual life during the last month	X		X		X	X	X	X	X		X	X	X	X	X	X		X	X
Past and Concomitant Treatments	X		X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X

\* 24h urine to be collected at home the day before the visit (V2, V3 and V4)

\*\* In case of UTI infection a control urine culture should be performed at least 48H after the end of treatment and within 1 week to confirm that subjects are completely recovered



<p><b>Study Parameters</b></p>	<ul style="list-style-type: none"> <li>- <u>parameter 1</u>: total number of clinical recurrences of UTI*</li> <li>- <u>parameter 2</u>: mean time elapsed between UTI* episodes</li> <li>- <u>parameter 3</u>: total days of antibiotics therapy related to UTI episodes</li> <li>- <u>parameter 4</u>: frequency of micturition during the 24h urine collection</li> <li>- <u>parameter 5</u>: 24h urine parameters (volume, pH, osmolality, specific gravity, urine colour, sodium, potassium, calcium, magnesium, chloride, oxalate, citrate, creatinine)</li> <li>- <u>parameter 6</u>: eGFR</li> <li>- <u>parameter 7</u>: Tiselius Crystallisation Risk Index</li> <li>- <u>parameter 8</u>: blood sample (lipidic profile, glycosylated hemoglobin, serum creatinine, serum urea, copeptin)</li> <li>- <u>parameter 9</u>: vital signs (diastolic and systolic blood pressure, heart rate, axillary body temperature)</li> <li>- <u>parameter 10</u>: quality of life (questionnaire SF12V2)</li> <li>- <u>parameter 11</u>: fluid intake (water, other types of fluids)</li> <li>- <u>parameter 12</u>: anthropometry (body weight, waist circumference, height, BMI)</li> <li>- <u>parameter 13</u>: pathology related costs</li> <li>- <u>parameter 14</u>: complications from UTIs* (UTI related AE, and/or SAE)</li> </ul> <p>*Based on the simultaneous presence of positive urine culture and UTIs symptom(s)</p>
<p><b>Study Endpoints</b></p>	<p><u>Primary endpoint:</u></p> <ul style="list-style-type: none"> <li>➤ Difference between groups in terms of number of UTI recurrence over 12 months of study intervention from randomisation call (D0).</li> </ul> <p><u>Secondary endpoints:</u></p> <ul style="list-style-type: none"> <li>➤ Difference between groups in terms of antibiotic prescription/usage to treat UTI event over 12 months of study intervention from D0,</li> <li>➤ Difference between groups on average delay between each UTI event over 12 months of study intervention from D0,</li> <li>➤ Difference between groups on urinary hydration markers over 6 and 12 consecutive months of study intervention from D0,</li> <li>➤ Difference between groups in terms of health-costs associated with management of UTI recurrence(s) over 12 months of study intervention from D0,</li> <li>➤ Difference between groups in terms of cost utility analysis over 12 months of study intervention from D0,</li> <li>➤ Difference between groups in terms of change in QoL over 6 and 12 consecutive months of study intervention from D0.</li> </ul>

	<p><u>Exploratory endpoints:</u></p> <ul style="list-style-type: none"> <li>➤ Relationship between urinary hydration markers and number of UTI events over 12 consecutive months of study intervention from D0,</li> <li>➤ Relationship between urinary hydration markers and delay between UTI events over 12 consecutive months of study intervention from D0,</li> <li>➤ Impact of increased daily water intake on crystallisation risk index (Tiselius) over 12 consecutive months of study intervention from D0,</li> <li>➤ Number of events and number of subjects with at least one UTI event confirmed by positive urine culture over 12 consecutive months of study intervention (by group) from D0</li> <li>➤ Number of events and number of subjects with at least one UTI event not confirmed by positive urine culture over 12 consecutive months of study intervention (by group) from D0,</li> <li>➤ Number of events and number of subjects with at least one UTI event confirmed or not by positive urine culture during the intervention period (by group) from D0.</li> </ul>
<p><b>Safety evaluation criteria</b></p>	<p>Blood sample (Total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, glycosylated haemoglobin, serum creatinine, serum urea, and eGFR), blood pressure, heart rate, axillary body temperature, weight, urine sodium, urine potassium, physical examination at V2 and V4, adverse events, serious adverse events</p>
<p><b>Statistical analysis</b></p>	<ul style="list-style-type: none"> <li>➤ The statistical methodology will be detailed in the statistical analysis plan (SAP) written and finalized before the database lock.</li> <li>➤ Descriptive statistics will be given for each of the parameters (i.e. for continuous variables: group size, mean, standard deviation of the variable [SD], standard error of the mean [SEM], minimum, maximum, median, and possibly quartiles; for qualitative variables, group size and percentage).</li> <li>➤ For the main criterion, a nonparametric method called the mean cumulative function (MCF) will be used to analyse the multiple/repeated UTI events occurring. The null hypothesis is “There is no difference between groups on the average number of recurrent UTIs event experienced”. We will consider a Type I error rate (alpha) equal to 5%.</li> <li>➤ The analysis will be conducted with the statistical software package SAS 9.3.</li> </ul>



### **3. INTRODUCTION**

#### **3.1 SCIENTIFIC BACKGROUND INFORMATION**

Urinary tract infection (UTI) is one of the most common clinical diagnoses in women. The lifetime risk for UTI in women is high (greater than 50%) and in the U.S. between 1988 and 1994 the overall lifetime prevalence of UTI was estimated to be 53,067/100,000 women [2]. The estimated global incidence of UTIs is at least 250 million cases per year [3]. UTIs are a source of significant cost and morbidity. Most UTIs are self-limiting but occasionally can be associated with significant complications such as pyelonephritis and sepsis. Composite data revealed that overall expenditures for the treatment of UTIs in women in the United States, excluding spending on outpatient prescriptions, were approximately 2.47 billion U.S. dollars in 2000 [2]. According to the 1997 National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey, UTI accounted for nearly 7 million office visits and 1 million emergency department visits, resulting in 100,000 hospitalisations [4]. The exact frequency of UTIs is difficult to assess because they are not reportable diseases in the United States. This situation is further complicated by the fact that accurate diagnosis depends on both the presence of symptoms and a positive urine culture, although in most outpatient settings this diagnosis is made without the benefit of culture.

There are several higher risk populations for UTIs including infants, pregnant women, the elderly, patients with spinal cord injuries and/or catheters, patients with diabetes or multiple sclerosis, patients with acquired immunodeficiency disease syndrome/human immunodeficiency virus, and patients with underlying urologic abnormalities [5]. However, UTIs are very common in otherwise healthy women. It is estimated that at least a third of all women in the United States are diagnosed with a UTI before they are 24 years old [6]. There are both host and bacterial factors that contribute to UTIs [7,8]. In young women, sexual activity is associated with an increased risk for UTIs [7,8]. UTIs also have a propensity to recur. In otherwise healthy college women with a first UTI, the risk of a second episode within 6 months was 24% [8], and in those with a history of one or more UTIs, the risk of a second within 1 year was 70% [9]. Even when UTI is not associated with long term consequences, the condition results in pain and suffering, and negatively impacts quality of life, albeit transiently [10]. Common symptoms in pre-menopausal women include frequent, urgent and painful urination and suprapubic pressure and hematuria may also be present.

There are multiple reasons to try to prevent UTIs in women. First and foremost is to reduce morbidity associated with the infections as well as reduce the cost of treatment. However, there are also important reasons to reduce the use of antibiotics in these patients [11]. First, there is increasing resistance of *Escherichia coli*, the primary causative agent of uncomplicated UTI, to a variety of antibiotics, including fluoroquinolones and extended-spectrum beta-lactamase (ESBL) resistance is increasingly observed among community acquired UTI [12].) Second, there can be significant impact of short courses of antibiotics on the gut and vaginal microbiota which can contribute to recurrence and antibiotic resistance [13]. Third, there are risks associated with antibiotic use such as allergic reactions and side effects of the drugs themselves. Finally there is a risk of vaginal candida infection, which occurs in up to 22 % of women treated for uncomplicated UTI [14].

Moreover, due to the high prevalence and incidence, UTI has enormous economic implications.

As for other pathologies, costs related to UTI episodes should be divided into direct and indirect ones.

Direct costs include the costs of outpatient doctor visits, antibiotics and specific antimicrobial agent prescription, along with hospitalisation expenses. The indirect costs include all the “non-medical costs” related to the pathology, such as travel and sick days for the patients and for the caregivers.

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Direct and indirect annual costs related to acute episode of UTI have been estimated to be around \$1.6 billion for the US female population [5,6], including approximately \$936 million for indirect costs and \$659 million for direct ones.

To date, no data about specific European population are available. In spite of available evidence suggesting a link between urinary hydrodynamics and frequency of UTI episodes, cost-savings that could potentially be derived from appropriate fluid intake among UTI patients remain to be established.

Several strategies have been proposed to try to reduce the risk of recurrent UTIs.

While the use of daily antibiotics or post-coital antibiotics is effective, the rise of resistance and risk associated with antibiotics has made these strategies less attractive. Different approaches have been proposed including use of functional foods, lactobacillus and vaccines [11]. The most studied functional food thus far has been cranberries and their extracts. A Cochrane review on studies including cranberries included a total of 24 studies (six cross-over studies, 11 parallel group studies with two arms; five with three arms, and two studies with a factorial design) with a total of 4473 participants [15]. The comparison/control arms were placebo, no treatment, water, methenamine hippurate, antibiotics, or lactobacillus. The meta-analyses found that compared with placebo, water or not treatment, cranberry products did not significantly reduce the occurrence of symptomatic UTI overall (RR 0.86, 95% CI 0.71 to 1.04) or for any the subgroups: women with recurrent UTIs (RR 0.74, 95% CI 0.42 to 1.31); older people (RR 0.75, 95% CI 0.39 to 1.44); pregnant women (RR 1.04, 95% CI 0.97 to 1.17); children with recurrent UTI (RR 0.48, 95% CI 0.19 to 1.22); cancer patients (RR 1.15 95% CI 0.75 to 1.77); or people with neuropathic bladder or spinal injury (RR 0.95, 95% CI: 0.75 to 1.20). Studies evaluating lactobacillus have shown some promise but await further validation [16,17].

### 3.2 DESCRIPTION OF THE TEST PRODUCT

The approach that we propose involves randomising low-drinking pre-menopausal women with recurrent UTIs to high versus usual water intake habits to determine whether this will reduce risk of UTIs. For this purpose, we will provide women with three (3) bottles of natural mineral commercialised water (500 mL each) to be consumed daily for approximately twelve (12) months. Bottles will be provided to subjects in the intervention group for free, along with the suggestion to consume them at the beginning of every meal (breakfast, lunch and dinner) and fully drink them before the following meal.

### 3.3 SCIENTIFIC RATIONALE

The study will include only women who are low-drinkers (< 1.5 L fluids per day; urinary volume < 1.2 L per day) since they are most likely to have a predisposition to UTIs due to infrequent voiding. The rationale for this approach is that drinking more fluid will increase voiding frequency and voiding is the main defence of the bladder to reduce the number of bacteria in the bladder and avoid UTIs. As such, increasing fluid intake will increase the frequency and volume of voiding and potentially reduce risk of recurrent UTIs. Support for this hypothesis is multifold. A non-randomised, multivariate analysis comparing 791 women teachers who deliberately restricted their fluid intake (25% voided only once during working hours, or not at all) with women able to drink without restrictions found that women in the former group were at significantly higher risk of UTI than were women in the latter group (RR 2.21; 95% CI 1.45-3.38) after controlling for parity, voiding infrequently

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at work, and urge incontinence [18]. A study of 1613 women compared frequency of voiding based on age of women and impact on UTIs [19]. The study found that women aged 20-25 had a higher rate of this low voiding frequency than women 30-35 or 40-45 years and in all 3 groups, women who voided 3 times or less per day had significantly more urinary infections than those with 4 or more voidings per day, ( $p < 0.01$ ). While results are inconsistent, several studies found that higher post-void residual volumes increases risk of UTI in women [20-22]. This supports the notion that efficacy of voiding and emptying the bladder can reduce risk of UTIs. One additional impact of fluid intake may involve reducing urine acidity. Low fluid intake is associated with an increase in urine osmolality and acidity which can predispose to bacterial adhesion to the bladder epithelium. A study in pre-menopausal women who had been treated for at least 2 idiopathic UTIs in the previous 6 months found that self-monitoring urine osmolality using a handheld 'traffic light' probe was associated with a significant shift towards urine of lower osmolality and a significant reduction in incidence of UTIs compared with the period in which the probe was not used [23]. Of the 17 patients who completed both 4-month periods, 14 felt that the probe had helped them to prevent infection.

### 3.3.1 *Rationale for the study purpose*

The above evidence and the heavy burden of recurrent UTIs on society demonstrate a significant need for strategies to prevent UTIs. The planned study will determine the efficacy of increased water intake in decreasing the risk of UTIs in women who are low volume drinkers and who suffer from recurrent UTIs compared to a control group.

### 3.3.2 *Rationale for the study population*

UTI is the most common infection in humans. It is highly prevalent in both men and women but its frequency is about 50 times higher in adult women of all age groups. This may be because the urethra is shorter in women than in men, making it easier for bacteria to ascend into the bladder and, once there, proliferate.

More than half of all women (50–60%) encounter with at least one UTI at some stage during their lives. [24] Symptomatic infection of the bladder (lower UTI) has been estimated to occur in up to 30% of women during their life [25].

The estimated global incidence of UTI in women based on self-report of physician diagnosis is 11% per year resulting in at least 250 million cases per year [26]. This incidence is higher (17.5%) during ages 18–24 and decreases to 9% for women 50 and over [6].

Approximately 5% of women with an initial UTI have multiple episodes within a year and a high recurrence rate, ranging between 25 to 30%, has been shown to affect the total female population [27]. Observational studies have shown 6-month risk of recurrence ranging from 17 to 24% among otherwise healthy pre-menopausal women [6, 28]. It is not surprising that one of the major risk factor for UTI among women is having a history of UTI.

Given the high burden, recurrent pre-menopausal women will be the target population of our study.

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### 3.3.3 *Rationale for dose(s) selected and/or dose(s) regimen*

We want women in the intervention group, to achieve a mean daily urine osmolality equivalent to that of plasma (approximately 285 mOsm/kg). This osmolality goal is selected because it defines the transition from concentrated to dilute urine and is a safe end point under most circumstances. To maintain such a low urine osmolality, we assume that women in the intervention group should increase their water intake of 1.5 L/day.

Moreover, to maintain a urinary volume of 1.5L we need to assure a total fluid intake of more than 1.3L/day [29]. 1.5L/day will be our target knowing that the mean water intake in European women is less than 1L/day.

### 3.3.4 *Rationale for the study design*

Given the necessity to establish a causality relation between increased water intake and UTI episodes, we will perform a single-site, prospective, randomised, open-label, controlled study. This model is often used where the double-blind design is not applicable. A strict randomisation procedure is used: patients are allocated to different group regimens following the allocation concealment rules. In this way, the Investigator won't be able to subvert randomisation and select which patients get intervention or control allocation. The follow-up and intervention phase is conducted openly in a way that adheres to accepted clinical principles and medical practice.

## **3.4 POTENTIAL RISKS AND BENEFITS**

We expect increased fluid intake to be efficient in reducing the risk of clinical recurrences of UTI in women (except peri-menopausal and menopausal women. Decreased incidence of this pathology will reduce the comorbidity associated with UTI events as well as the related healthcare costs.

No risk linked to increased water intake has ever been shown in this population. Moreover the protocol suggests to split the water consumption over the day and consume 0,5L at the beginning of every meal (breakfast, lunch, dinner) and fully drink them before the following meal.

However, in case of ingestion of more than 1L of water in a short time interval (less than 10 min), the subject may have possible temporary stomach discomfort and an increased urge to use the toilet.

Blood samples will be drawn during the study visits. The risks related to this procedure are very weak and they are the same as for any type of blood drawing procedure in current medical practice:

- Pain during the sampling: it is especially connected to the speed of execution of the movement. This risk is minimised in this study as the staff performing the sampling is competent medical staff.
- Local secondary infection: this risk is theoretical because the used material is single-use, and the sampling is performed after local asepsis.
- Localized haematoma: this risk can be limited by the realisation of an effective manual compression in the minutes following the sampling.

## **4. STUDY OBJECTIVES**

### **4.1 PRIMARY STUDY OBJECTIVE**

The primary objective of this study is to assess the effect of increased daily water intake on the frequency of clinical recurrent urinary tract infections (rUTIs) among low drinking pre-menopausal women suffering from recurrent community-acquired UTI over 12 consecutive months of study product consumption.

### **4.2 SECONDARY STUDY OBJECTIVE(S)**

- To evaluate the impact of increased daily water intake on the use of antibiotics in recurrent UTI patients over 12 consecutive months of study product consumption
- To evaluate the impact of increased daily water intake on the mean time elapsed between UTI episodes over 12 consecutive months of study product consumption
- To evaluate the impact of increased daily water intake on urinary hydration markers over 6 and 12 consecutive months of study product consumption
- To evaluate the impact of increased daily water intake on health costs in recurrent UTI patients over 12 consecutive months of study product consumption
- To evaluate the impact of increase of daily water intake on the cost utility analysis during the study intervention using two different perspectives: National Health insurance and subject's perspective (subject's out-of-pocket costs)
- To evaluate the impact of increased daily water intake on quality of life (QoL) in recurrent UTI patients over 6 and 12 consecutive months of study product consumption

### **4.3 EXPLORATORY STUDY OBJECTIVE(S)**

- To evaluate the relationship between urinary hydration markers and number of UTI events over 12 consecutive months of study intervention from D0.
- To evaluate the relationship between urinary hydration markers and delay UTI events over 12 consecutive months of study intervention from D0.
- To evaluate the impact of increased daily water intake on crystallisation risk index (Tiselius) over 12 consecutive months of study intervention from D0.
- To evaluate the number of UTI events confirmed by positive urine culture and number of subjects presenting at least one UTI event over 12 consecutive months of study intervention from D0 (by group).
- To evaluate the number of UTI events not confirmed by positive urine culture and number of subjects presenting at least one UTI event over 12 consecutive months of study intervention from D0 (by group).
- To evaluate the number of UTI events confirmed or not by positive urine culture and number of subjects presenting at least one UTI event over 12 consecutive months of study intervention from D0 (by group).

## **5. STUDY DESIGN**

### **5.1 STUDY METHODOLOGY**

This is an open label, prospective, single site, randomised, controlled trial, in two parallel groups with a 1:1 ratio allocation.

The randomisation process will be applied using a centralised Interactive Web Response System (IWRS) that will allocate subject into one of the two groups in respect with the randomisation list generated informatically at the beginning of the study.

### **5.2 STUDY EVALUATION CRITERIA**

#### *5.2.1 Primary endpoint*

The primary endpoint assessed during this study is the difference between groups in terms of number of UTI recurrence over about 12 months of follow-up of study intervention from randomisation call (D0).

Only uncomplicated symptomatic UTI events will be considered for the primary endpoint analysis.

Uncomplicated UTI events are defined as follow:

- At least one symptom of UTI (i.e.: dysuria (i.e. painful urination, usually described by the patient as burning, stinging or itching) and / or urgency and / or frequency and / or suprapubic pain (i.e. pain occurring from above the pubis) [30]

And

- Positive urine culture (i.e.  $\geq 10^3$  CFU/mL in the midstream specimen of urine (MSU) [30].

The start date of UTI event will be the date of first symptoms, and the end date will be the sampling date of urine culture performed at the end of the episode-therapy that provides negative urine culture.

#### *5.2.2 Secondary endpoints*

The secondary endpoints assessed during this study are as follows:

- Difference between groups in terms of antibiotic prescription/usage to treat UTI event over 12 months of study intervention from D0
- Difference between groups on average delay between each UTI event over 12 months of study intervention from D0
- Difference between groups on urinary hydration markers over 6 and 12 consecutive months of study intervention from D0
- Difference between groups in terms of health-costs associated with management of UTI recurrence(s) over 12 months of study intervention from D0
- Difference between groups in terms of cost utility analysis over 12 months of study intervention from D0
- Difference between groups in terms of change in QoL over 6 and 12 consecutive months of study intervention from D0

As quality of life (QoL) is an important aspect of the intervention, the health economic evaluation will be considered through a cost utility analysis (CUA). Those health cost study will be conducted using two different

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perspectives. The perspective is the point of view from which the costs and benefits are recorded and assessed. Considering the research question, the two perspectives below will be considered:

- National Health insurance
- Patients' perspective by determining patient own out-of- pocket costs during the study intervention.

As several perspectives are included in the analysis, the results will be presented separately for each study perspective.

### 5.2.3 *Exploratory endpoints*

The exploratory endpoints assessed during this study are as follows:

- Relationship between urinary hydration markers and number of UTI events over 12 consecutive months of study intervention from D0
- Relationship between urinary hydration markers and delay of UTI events over 12 consecutive months of study intervention from D0
- Impact of increased daily water intake on crystallisation risk index (Tiselius) over 12 consecutive months of study intervention from D0
- Number of UTI events confirmed by positive urine culture and number of subjects with at least one UTI event over 12 consecutive months of study intervention from D0 (by group)
- Number of UTI events not confirmed by positive urine culture and number of subjects with at least one UTI event over 12 consecutive months of study intervention from D0 (by group)
- Number of UTI events confirmed or not by positive urine culture and number of subjects with at least one UTI event over 12 consecutive months of study intervention from D0 (by group)

### 5.2.4 *Product safety evaluation criteria*

The safety criteria assessed during this study are as follows:

- Blood sample (Total Cholesterol, HDL Cholesterol, LDL Cholesterol, Triglycerides, Glycosylated haemoglobin, serum creatinine, serum urea, and eGFR),
- Blood pressure (systolic and diastolic)
- Heart rate
- Axillary Body temperature
- Weight
- Urine sodium
- Urine potassium
- Physical Examination at V2 and V4
- Adverse events
- Serious adverse events

## **5.3 STUDY GLOBAL DESCRIPTION**

The subjects will be divided into 2 balanced groups of 70 randomised subjects each. Subjects allocated to study test group (intervention group) will be asked to consume daily three (3) bottles of natural mineral water (500 mL each) in addition to their usual fluid intake consumption, for approximately 12 months. It is suggested to

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consume them at the beginning of every meal (breakfast, lunch and dinner) and fully drink them before the following meal. The subjects allocated to control group will be asked not to change the low fluid intake assessed at baseline (V1).

The study will include 2 parts:

1. The **screening period**, from V1 to D0 (randomisation call).

During this period, subjects will be screened for inclusion and exclusion criteria and checked for randomisation/non-randomisation criteria.

2. The **study intervention period** (approximately 12 months duration), from D0 (randomisation call) to V4 (end of study visit).

During this period, subjects will be checked for randomisation/non-randomisation criteria and will attend 1 evaluation visit (V3) at six months and an end of study visit (V4) at twelve months from D0 (randomisation call). Subjects will be called every month by the PI or another documented authorized study team member to check the study compliance (including fluid intake and study product consumption if any), adverse event(s), symptoms of UTI and / or UTI event(s), concomitant medication(s) and sexual life habits.

In case of symptom(s) of UTI, a relapse visit will be performed.

Each subject will undergo :

- at least 2 urine pregnancy tests (V1 and V2),
- at least 3 blood sample collections (V2, V3 and V4),
- at least 3 urine 24-hours collections (collection to be started the day before V2, V3 and V4),
- And at least 2 urine cultures (V2 and V4).

Each appearance of UTI symptoms will lead to:

- 1 urine culture at the beginning of the episode,
- 1 urine culture, after the standard of care treatment to confirm the recovery, if the first one was positive.

For the blood samples, it is necessary for the subjects to be in a fasted state for 8-12 hours before the visit.

#### **5.4 STUDY DESIGN SCHEMA**

Refer to section 2.

#### **5.5 DURATION OF THE STUDY PER SUBJECT**

The total duration of the study is approximately fourteen (14) months for each subject, including a screening period of up to eight (8) weeks, and a study intervention period around twelve (12) months.



## **6. SUBJECTS**

### **6.1 SUBJECT RECRUITMENT AND SCREENING**

The subjects included in the study will be pre-menopausal women with recurrent symptomatic episodes of UTI aged not below 18 and having a “low drinker profile” defined as:

- total self-reported fluid intake < 1,5 L/day;
- and total 24 hours urinary volume < 1,2 L per day
- and urine osmolality  $\geq$  500 mOsmol/kg
- and urine creatinine within the laboratory normal ranges

Subjects with clinical manifestations of menopause as menopausal women will be excluded. In case of menopausal symptoms, a FSH blood test must be performed at V2.

The subject inclusion will be stopped as soon as 140 subjects are randomised.

At this time, all eligible subjects already included (subjects who are within the screening period (from V1 to V2)) will be randomised and are expected to complete all the study procedures and visits according to the protocol until their end of study visit (V4).

Subjects potentially able to participate to this study will be pre-selected by physicians working in liberal practice (GPs, urologists, gynaecologists) from their patients' database. The pre-screened subjects will be referred to the Investigating Centre with a referral bulletin mentioning the status of all UTI events as well as with the last positive results of urine cultures performed within the last 12 months, except for the symptomatic subjects with 2 UTI events at V1 (i.e. correspond to criteria II01.C for which the documentation of positive urine culture must be the one performed between V1 and V2).

If necessary, in order to reinforce recruitment rate, the Investigating Centre may also use advertising, only after EC approval, and conduct a **pre-screening** of patients at the investigating site following their standard process.

Taking into account that the study is particularly long, the study team should discuss the subject's willingness to comply with all study related procedures stressing the length and rigorous requirements prior to the subject enrolling.

The subject must have personally dated and signed their Patient Information and Informed Consent Form (ICF) before undergoing any study procedures.

### **6.2 SUBJECT ELIGIBILITY CRITERIA**

A subject is considered eligible when the Investigator certifies that the subject fulfils all inclusion criteria and does not meet any exclusion criteria for the current visit. This statement can potentially be repeated at different subjects' visits before subject's randomisation as required by the study design.

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**6.2.1 Inclusion criteria (checked at the inclusion visit - V1)**

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

II01a: Women with at least 3 clinical recurrences\* of symptomatic UTI in the last 12 months (at least one UTI must be confirmed by a positive documented bacteriological exam) and asymptomatic at V1

**or**

II01b: Women with at least 3 clinical recurrences\* of symptomatic UTI in the last 12 months (at least one UTI must be confirmed by a positive documented bacteriological exam) and symptomatic at V1

**or**

II01c: Women with at least 2 clinical recurrences\* of symptomatic UTI in the last 12 months (who did not perform any bacteriological exam during the last 12 months), symptomatic at V1 and with positive documented bacteriological exam between V1 and V2

II02: Age  $\geq$  18 years

II03: Fluid intake  $<$  1.5 L per day based on patient's declaration

II04: Regular meal consumption (breakfast, lunch and dinner)

II05: Access to Internet for information on hydration

II06: Subject, upon briefing of the content of the present study, fully understanding and agreeing to its objective and having given written (dated and signed) informed consent to take part in the study

II07: Subject who is able to communicate well with the Investigator and to comply with the requirements of the entire study.

II08: Literate subjects, able to fill in fluid diaries and QoL (Quality of life) questionnaire

II09: Women accepting to keep their lifestyle habits during the whole duration of the study

II10: Women using any form of contraception

II11: Subject covered by the National Health insurance system

**6.2.2 Exclusion criteria (checked at the inclusion visit - V1)**

A potential subject who meets any of the following criteria will be excluded from participation in this study:

IE01: Incapacity / non-willingness to consume 1.5 L of drinking water per day on top of their usual consumption

IE02: Women with history of UTI complications (pyelonephritis or other) in the last 12 months

IE03: Use of antibiotics or cranberries juice and / or extracts in the previous 2 weeks

IE04: Chronic treatments with anti-coagulants therapy

IE05: Chronic bladder inflammation (defined as permanent bladder bacterial infection)

IE06: Chronic diarrhea or constipation treated with chronic use of laxative substances

IE07: Interstitial cystitis

IE08: Estrogen-dependent symptomatic vulvo-vaginitis

IE09: Recent ( $<$ 1year) or active renal stone disease

IE10: Urinary tract structural abnormalities

IE11: Obesity or malnutrition (BMI  $<$ 18.5 Kg/m<sup>2</sup> and  $>$ 30 Kg/m<sup>2</sup>)

IE12: Pregnant or lactating women

IE13: Women planning to become pregnant during the study

IE14: Menopausal and peri-menopausal women

IE15: On-going or planned therapy during the study which can modify the study measurements, in particular the assessment of the hydration status (diuretic intake, corticoids or drug treatment interfering with nutrition behaviour)

IE16: Subjects with severe or uncontrolled organic disease, likely to interfere with the parameters of the study (e.g. neoplastic, cardiovascular, pulmonary and digestive disorders, unbalanced diabetes type I and II, untreated or uncontrolled clinically significant arterial blood hypertension) or mental disorders affecting eating and drinking behaviour (i.e. primary polydipsia, bulimia nervosa, psychosis, etc.)

IE17: Women who have taken part in any other clinical study for the treatment of rUTI during the last 12 months

IE18: Vulnerable subjects defined as individuals whose willingness to volunteer in the clinical trial may be unduly influenced by the expectation, whether justified or not, of benefits associated with participation, or of a retaliatory response from senior members of a hierarchy in case of refusal to participate.

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(Examples are members of a group with a hierarchical structure linked to the Investigator or to the Sponsor, such as medical, pharmacy, dental and nursing students, subordinate hospital and laboratory personnel, employees of the Investigator or of the Sponsor, members of the armed forces, and persons kept in detention)

IE19: No legal capacity or limited legal capacity or unable to give an informed consent

IE20: Subjects unlikely to cooperate in the study, and/or poor compliance anticipated by the Investigator

IE21: Women who have taken part in this study and/or enrolled in another clinical study during the last month and/or any other clinical study during the last month

*6.2.3 Randomisation criteria [checked at the pre-randomisation visit (V2) and before the subject's randomisation]*

RI01: Negative urine culture and asymptomatic state (i.e. silent phase) at V2 (pre-randomisation visit)

RI02: Low-drinker confirmation defined as:

- total self-reported fluid intake < 1,5 L/day,
- and 24 hours urinary volume < 1.2 L per day,
- and osmolality > 500 mOsmol/kg,
- and urine creatinine within the laboratory normal ranges.

*6.2.4 Non -Randomisation criteria [checked at the pre-randomisation visit (V2)]*

Subjects may only be randomised in this study after meeting the inclusion criteria and presenting none of the non-inclusion criteria stipulated in paragraphs 6.2.1, 6.2.2 and 6.2.3 above.

A potential subject who meets any of the following non-randomisation criteria at V2 and before the D0 (randomisation call) will be excluded from participation in this study:

RE01: Chronic kidney disease (defined as decreased GFR ( $GFR < 60 \text{ ml/min/1.73m}^2$  calculated using EPI equation)

RE02: Women suffering multiple antibiotic resistant bacterial strain\*

IE12: Pregnant or lactating women

IE14: Menopausal and peri-menopausal women\*\*

\* Multiple antibiotic resistance is defined as resistance to 3 or more different antibiotics

\*\* Based on V2 FSH assessment as per PI's discretion

**6.3 SUBJECT IDENTIFICATION**

*6.3.1 Subject Identification Number*

At V1, after the subject and the Investigator have personally dated and signed the Informed Consent Form, a Case Report Form (e-CRF) is created and a Subject Identification number is allocated manually to her in chronological order of inclusion. This number will be the main identification code for a subject for the duration of the study in order to protect the subject's identity and will appear throughout the e-CRF for that particular subject. This number should also be reported on the IWRS (if the subject is randomised) and on other relevant paper-based study documents where required (i.e. Quality of Life, Subject Diaries, source data in the Subject Medical File).

The Subject Identification number consists of 11 characters, which is based on:

- A 3 digit "Country number (will be "100")
- A 3 digit "Site" number (will be "001")

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- A 3 digit “Subject” number.

The first subject included at site will receive the number “001”. Subject numbering will then be sequentially increased by one for each new included subject (example 002, 003 etc.).

Each of these 3 elements is separated by a score “-”.

Example: Subject Identification number “100-001-010”, refers to Site “001” and Subject “010”.

### 6.3.2 *Randomisation Process*

Refer to section 10.1.

## **6.4 SUBJECT DISCONTINUATION**

### 6.4.1 *Criteria for subject discontinuation*

The Investigator should discontinue subject’s participation in the study prematurely at any time during the study in the following situations:

- In the case where a subject has decided to resign from further participation in the study (withdrawal of consent) or
- In the case where further participation is a health risk for the subject, at the Investigator’s discretion or
- In the case of subject’s pregnancy or
- In the case where a severe non-compliance to protocol or a protocol violation has been identified for the subject that may lead to protocol deviations described under *section 16.8. Such as may include:*
- Non adherence to the targeted population defined as pre-menopausal low drinker women with recurrent urinary tract infections
- Recurrence of systematic non respect of investigator’s instructions with respect to: scheduled visit urine samples collection, diaries’ completion and return, fluid intake regimen. Taking into account that this non-adherence can impact the accuracy, the completeness and the legibility of the data reported. If such event arrives, the final decision of subject discontinuation must be taken by the sponsor and the investigator.
- In the case where the subject is lost to follow-up or
- In the case whereby the study is prematurely terminated or suspended by the sponsor.

### 6.4.2 *Replacement conditions*

**Subjects withdrawing prematurely from the study at any time will not be replaced and cannot be re-included at a later date.**

### 6.4.3 *Procedures in case of subject discontinuation*

In case of premature withdrawal, the Investigator must notify the study monitor as soon as possible, and should make all efforts to contact the subject and if the subject agrees, ensure that all evaluations scheduled for the End of Study Visit (V4) are completed and reported in the e-CRF.

Alternative follow-up of the discontinued subject is to be arranged by the Investigator if necessary.

For subjects who discontinue the study due to the occurrence of adverse events potentially related to the study product or to the study procedures, follow-up will take place (clinical or biological examinations) until the adverse

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event has abated, or until a stable situation has been reached (please refer to the section 12), with findings being recorded in the e-CRF.

For subjects who discontinue the study due to pregnancy, follow-up will take place until birth or early termination of pregnancy. In addition to documenting this premature termination in the e-CRF, the Investigator should make every effort to collect information about the pregnancy and the infant and report to the Sponsor with a dated and signed Pregnancy Reporting Note as specified in the section 12.6.

## **6.5 INSTRUCTIONS AND RESTRICTIONS DURING THE STUDY**

### *6.5.1 Study product(s) consumption instructions*

The Investigator will provide the subject with the instructions on the study product consumption at the pre-randomisation visit (V2).

#### **Study intervention group:**

The subject allocated in the study product intervention group will begin consumption of the study product the morning of the following day after the receipt of the study products and will stop consumption the day of end of study visit (V4).

Throughout the entire consumption period (around 12 months) of the study, the subject allocated in the intervention group will daily consume three (3) bottles (500 mL each) of the test product (i.e. 1,5 L of commercialised natural mineralised Evian water in total).

It is suggested to start consumption at the beginning of every meal (breakfast, lunch and dinner) and fully drink them before the following meal (about 500ml around the main meals).

At the same time, the subjects will be receiving coaching messages (in particular 4 newsletters in total) related to the fluid intake recommendations and study products.

The subjects will receive regularly, and free of charge, the bottles of mineral water personally or to a delegated person, at home or office.

The subject will report every day her consumption in the Subject Compliance Diary (please refer to the section 6.5.5), specifying the number of bottles consumed during the day and the quantity of water drunk for each bottle (total bottle, ¼ of bottle, ½ of bottle).

The water consumption will be checked at each visit (i.e. telephone call and site visits) by the PI or another documented authorised study team member and a summary of the water consumption will be entered into the e-CRF. The investigating site will also collect the Subject Compliance Diaries every month in order to be able to check the information in case of inconsistencies.

Subjects will record every month their fluid intakes using the 3-days fluid intake diary (please refer to the section 6.5.4).

#### **Study control group:**

The subject allocated in the control group will remain stable in her fluid consumption. Her compliance will be assessed every month, using the 3-days fluid intake diary (please refer to the section 6.5.4).

The subject will not receive any bottle of water nor any coaching support.

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#### *6.5.2 Dietary instructions*

During the entire duration of the study (i.e. from V2 to V4), subjects will not have any dietary restriction with the exception of cranberries juice and/or extracts. All women included in the study will be asked not to consume these products from 2 weeks before the inclusion visit to the end of the study period.

The subjects will also be asked to avoid making any significant changes to their usual diet during the study period (i.e. no changes to the usual amount of fibre, not to begin a weight loss diet, etc...).

The dietary restrictions stipulated in this study do not represent a risk for the subjects. There is no risk of deficiency in the nutrient related to this dietary restriction.

#### *6.5.3 Non-authorized medicinal products and nutritional supplements*

During the entire duration of the study (i.e. from V1 to V4), all medicinal products are authorised excepting treatments listed in the exclusion criteria (anti-coagulants, corticoids, diuretics, laxatives or treatments interfering with nutrition behaviour, cranberry juice or extracts).

Included subjects will be asked not to take any antibiotic therapy in the 2 weeks before the inclusion visit (V1), to avoid any inclusion bias due to the presence of a UTI masked by the use of antibiotics.

In case of any non-authorized medicinal products consumption or any non-authorized medical treatments, the subject will be asked to complete her 3 days fluid intake diary with the name, the dose and the intake dates of the medicinal products or medical treatment. The subjects will be advised to contact the Investigator or the study nurse for recommendations.

#### *6.5.4 3-days fluid intake diary*

Before the planned visits (i.e. phone call and visit), the subject will be instructed to fill in the 3-days fluid intake diary throughout the entire study duration in order to document the fluid intake during 3 days (Saturday-Sunday-Monday or Friday-Saturday-Sunday).

#### *6.5.5 Subject Compliance Diary*

The subject randomised in the intervention group will be instructed to daily fill in the Subject Compliance diary to report her study product consumption.

#### *6.5.6 Voiding diary*

During the 24 hours collection, the subject will be instructed to fill in the Voiding diary outside of menstruation period before V2, V3 and V4 (and in case of 24H collection repetition) to report all micturitions collected during 24 hours.

## **7. STUDY PROCEDURES**

### **7.1 EVALUATIONS AND PROCEDURES PER VISIT**

Evaluations and procedures for the study visits are described below. Please refer also to the Study flow-chart in the Section 2.

24 h urine collection nor any visits can be performed in case of menstrual period.

#### *7.1.1 Inclusion Visit (V1)*

This visit will take place no more than 7 weeks before the pre- randomisation visit (V2).

At the beginning of V1, the Investigator should fully inform the subject of all aspects of the clinical study including the written information and the approval by the IRB/IEC. The subject must be given the opportunity to ask questions and have them answered by the Investigator.

Prior to subject's participation in this clinical study, the subject must personally sign and date the approved informed consent form. The subject cannot undergo any study procedures before having personally dated and signed the approved Informed Consent Form. The Investigator must also sign and date the Informed Consent Form.

After the subject and the Investigator have personally dated and signed the approved Informed Consent Form, a Subject Identification number is allocated to the subject an electronic Case Report Form (e-CRF) is created.

The eligibility of the subject has to be checked at the beginning of the visit and the study procedures at V1 are the following (please refer to the Study flow-chart in the section 2):

- Obtaining date (incl. time), signature of subject on approved informed consent form,
- Recording subject's demographic data (date of birth, gender),
- Checking all the inclusion and exclusion criteria for subject's eligibility validation (please refer to the section 6.2),
- Reviewing the past medical and surgical history,
- Reviewing the past and current medications,
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,
- Performing a urinary pregnancy test,
- Recording vital signs: systolic/diastolic blood pressure (please refer to the section 7.4.1.4 for instructions) and heart rate, axillary body temperature,
- Recording anthropometrical parameters; body weight and height, waist circumference,
- Checking for (peri)menopausal symptoms. If there are such, a FSH test is to be performed at V2,
- Checking symptoms of UTI
- Checking and recording of adverse events and of concomitant medications,
- **Performing urine culture for symptomatic subjects only** and assign or not a therapy (i.e. AFU/EAU guidelines for UTI management) according to PI's decision.

**Symptomatic subjects will perform V1 (please refer to the Appendix IV / Management of symptomatic/Asymptomatic subjects per visits). A urine culture will be performed:**

**1a/If positive urine culture:**

The subject will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a **control** urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered and that results are not biased by the antibiotic treatment taken by the subjects.

Depending on **control** urine culture results:

- If **negative** control urine culture, the subject will come back to site to perform the pre-randomisation visit (V2) as soon as the subject is completely recovered and up to a maximum of 7 weeks from V1
- If **positive** control urine culture, the subject will be treated till a negative control urine culture is obtained and will come back to site to perform the pre-randomisation visit (V2) as soon as the subject is completely recovered and up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.

Any UTI symptom(s) with or without a positive urine culture, occurring between V1 and V2 will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.

**1b/ If negative urine culture:**

The subject may be treated or not with a standard of care treatment at the PI's discretion:

- Subject **receiving** a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained. They will come back to the site to perform the pre-randomisation visit (V2) up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.
- Subject **not receiving** any treatment and declaring complete recover from previous symptoms will come back to site to perform the pre-randomisation visit (V2) as soon as the symptoms are completely disappeared (subject is completely recovered) and up to a maximum of 7 weeks from V1.
- Subject **not receiving** any treatment but with persisting symptoms will **re-perform** a urine culture. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standard of care treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the pre-randomisation visit (V2) up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.

Any UTI symptom(s) with or without a positive urine culture, occurring between V1 and V2 will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.

- Scheduling study visits (i.e. phone calls and visits) for subjects who are eligible and available for the duration of the study.

**If the eligibility criteria are met, the subject is included in the study.**

Furthermore, the subject will be provided with instructions for:

- Coming back to the site in case of any UTI symptom(s)
- Diet and non-authorized products (please refer to the sections 6.5.2 and 6.5.3)
- Documenting any adverse and serious adverse event.
- Completing the 3-days fluid intake diary provided and return at the next visit (V2) (please refer to the section 6.5.4)
- Completing the Voiding diary provided and returning at the next visit (V2) (please refer to the section 6.5.6)
- Collecting, storing and transportation of urine samples. Subject will be provided with the kits for urine sterile sample and 24h urine collection (please refer to the section 7.4.2.6)



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**Asymptomatic subjects at V1 (please refer to the Appendix IV / Management of symptomatic/Asymptomatic subjects per visits)** will come back to the site to perform the pre-randomisation Visit (V2) after a minimum of 4 days (i.e. the minimum time needed to complete the 3-days fluid intake diary) and up to 7 weeks from V1.

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents are accurate and complete (please refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit and no later than 5 working days to the date of the visit. In case of unavailable data, the e-CRF should be completed within 48 hours after the missing data's receipt.

#### 7.1.2 Pre-randomisation Visit (V2)(from + 4 days to + 7 weeks after V1)

The pre-randomisation visit (V2) will take place at the minimum of 4 days after V1 and at the maximum of 7 weeks after V1.

At the pre-randomisation visit (V2), the subjects will bring back the following:

- 24hours urine samples
- Sterile urine sample
- Voiding diary
- 3 days fluid intake diary

1/ If a subject coming to **V2 presents UTI symptom(s), whatever the urine culture results**, she won't be able to perform the visit, only urine culture is analysed. **V2 will be rescheduled** once the subject is recovered. **(please refer to the Appendix IV / Management of symptomatic/Asymptomatic subjects per visits)**

#### **1a/ Symptomatic subjects at V2 with a positive urine culture:**

The subject will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a **control** urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that the subject is completely recovered.

Depending on **control** urine culture results:

- If **negative** control urine culture, the subject will come back to the site to perform the pre-randomisation visit (V2) as soon as the subject is completely recovered and up to a maximum of 7 weeks from V1
- If **positive** control urine culture, the subject will be treated till a negative control urine culture is obtained and will come back to site to perform the pre-randomisation visit (V2) as soon as the subject is completely recovered and up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.

Any UTI symptom(s) with or without a positive urine culture, occurring between V2 and D0 (randomisation call) will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.

#### **1b/ Symptomatic subjects at V2 with a negative urine culture:**

The subject may be treated or not with a standard of care treatment at the PI's discretion:

- Subject **receiving** a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the pre-randomisation visit (V2) up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.
- Subject **not receiving** any treatment and declaring complete recover from previous symptoms will come back to the site to perform the pre-randomisation visit (V2) as soon as the symptoms are completely disappeared (subject is completely recovered) and up to a maximum of 7 weeks from V1.

- Subject **not receiving** any treatment but with persisting symptoms will **re-perform** a urine culture. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standardised treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the pre-randomisation visit (V2) up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.

Any UTI symptom(s) with or without a positive urine culture, occurring between V2 and D0 (randomisation call) will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.

**2/ Asymptomatic subject will perform V2 (please refer to the Appendix IV / Management of asymptomatic/Asymptomatic subjects per visits).**

The eligibility of the subject has to be checked at the beginning of the visit and the study procedures during the pre-randomisation visit are the following (please refer to the Study flow-chart in the section 2):

- Checking that 24 hours urine samples have been collected as well as the sterile urine sample,
- Conducting a physical examination,
- Recording vital signs: systolic/diastolic blood pressure (please refer to the section 7.4.1.4 for instructions) and heart rate, axillary body temperature,
- Recording anthropometrical parameters; body weight , waist circumference,
- Repeating pregnancy test
- Checking all the randomisation and non-randomisation criteria (please refer to the section 6.2),
- Collecting and reviewing V1-V2 subject diary for 3-days fluid intake,
- Checking and recording of adverse events and of concomitant medications,
- Checking non-authorized product compliance,
- Collecting of blood samples (after 8-12 hours of fasting), including the request for FSH test if needed in case of perimenopausal symptoms (please refer to the section 7.4.2.1),
- Collecting and checking of the Voiding diary,
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,
- Checking symptoms of UTI,
- **Performing urine culture for all subjects** to assess the absence of any acute UTI episode,
- Reminding the instructions for diet and non-authorized products (please refer to the section 6.5.2),
- Reminding instruction for coming back to the site in case of any UTI symptom(s),
- Providing instructions for completing the QoL questionnaire (SF-12 V2) in situ but not in front of the Investigator. The Investigator will check the SF-12 V2 questionnaire completion,
- Providing the 3-days fluid intake diary and Subject's Compliance Diary pages (only for the patients in the intervention group) covering the period between V2 and V3 (inclusive pre-printed envelopes for sending via courier) and repeating the instructions for their completion,
- Providing instructions for collecting, storing and transportation of urine samples. The subject will be provided with kits for urine sterile sample (in case of UTI symptoms between V2 and V3) for the 24h urine collection (please refer to the section 7.4.2.6),
- Providing a Voiding diary in order to collect frequency of voiding during the 24h urine collection before V3,
- Providing UCC and instructions to use.

**2a/ Asymptomatic subjects at V2 with a positive urine culture**, the subject will be treated for the UTI event with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a **control** urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered.

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If a negative **control** urine culture is obtained:

- ≤ 28 days, after V2 (i.e. negative control urine culture obtained at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered), a new QoL questionnaire will be completed by the subject after UTI is cured.
- > 28 days, after V2, the V2 should be repeated.

Any UTI symptom(s), with or without a positive urine culture, occurring between V2 and D0 (randomisation call) will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.

**2b/ Asymptomatic subjects at V2 with a negative urine culture (please refer to the Appendix IV / Management of symptomatic/Asymptomatic subjects per visits)** and fulfilling all randomisation and none of the non-randomisation criteria will be randomised by the PI or another documented authorized study team member no later than 1 week after V2.

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents are accurate and complete (please refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit and no later than 5 days to the date of the visit. In case of unavailable data, the e-CRF should be completed within 48 hours after the missing data's receipt.

#### *7.1.3 Randomisation call (D0)(maximum 8 weeks after V1)*

The investigator will verify randomisation and non-randomisation criteria after having received the relevant lab results. If considered eligible, the subject will be randomised into one of the 2 groups through the IWRS.

The investigator or another documented authorized study team member will perform a randomisation call the same day of subject randomisation through the IWRS, in order to instruct the subject on the study procedures to follow, depending on her group allocation.

**If the subject is in the intervention group**, the Investigator will arrange the logistics company to be informed for the requirement to deliver to the subject the study products. The subject will be informed that she will receive the study products and she will be provided with the recommendation to consume 1,5 L/day (3 bottles of mineral water) starting from the morning after reception of study products until the completion of the study, in addition to their usual fluid intake consumption. She has to report the study product consumption in the Subject Compliance Diary in addition to the 3-days fluid intake diary.

**If the subject is in the control group**, the Investigator or another documented authorized study team member will call the subject and inform her that she has to continue her life habits and she doesn't need to complete the Subject Compliance Diary. She will have to continue to complete monthly the 3-days fluid intake diary.

#### **Between D0 (randomisation call) and V3 (evaluation visit):**

The subject in the **intervention group** has to send back to the Investigational site the Subject Compliance Diary, corresponding to the previous month of study product consumption, and the 3-days fluid intake diary by courier or personally if preferred.

The subject in the **control group** will have to return the 3-days fluid intake diary every month to the site by courier or personally if preferred.

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents are accurate and complete (please refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit and no later than 5 days to the date of the visit. In case of unavailable data, the e-CRF should be completed within 48 hours after the missing data's receipt.

*7.1.4 Evaluation Visit– Visit 3 (Month 6 after the D0 (randomisation call) within +/- 10 days allowable time window or + 28 days in case of UTI event)*

Visit 3 will take place 6 months (+/- 10 days or + 28 days in case of UTI event) after the randomisation call (D0).

At V3, the subjects in both groups will bring back to the site the following:

- 24h Urine samples
- Voiding diary
- 3 days fluid intake diary
- Subject Compliance Diary for subject as appropriate.

The study procedures performed during the evaluation visit are the following (please refer to the Study flow-chart in the section 2):

- Checking symptoms of UTI

**Asymptomatic subject will perform V3 (please refer to the Appendix IV / Management of symptomatic/Asymptomatic subjects per visits).**

**If a subject coming to V3 presents UTI symptom(s), she won't be able to perform the visit. Given the presence of symptoms, a relapse visit will be done (please refer to the section 7.1.7). V3 will be rescheduled once the subject is recovered (please refer to the Appendix IV / Management of symptomatic/Asymptomatic subjects per visits).**

**1a/ Symptomatic subjects at V3 with a positive urine culture:**

The subject will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a **control** urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered.

Depending on the **control** urine culture results:

- If **negative** control urine culture, the subject will come back to the site to perform the evaluation visit (V3) as soon as the subject is completely recovered.
- If **positive** control urine culture, the subject will be treated till a negative control urine culture is obtained and will come back to the site to perform the evaluation visit (V3) as soon as the subject is completely recovered.

Any UTI symptom(s), with or without a positive urine culture, verified just before the V3 will be reported in a relapse visit.

**1b/ Symptomatic subjects at V3 with a negative urine culture:**

The subject may be treated or not with a standard of care treatment at the PI's discretion:

- Subject **receiving** a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the evaluation visit (V3) as soon as the patient is completely recovered.
- Subject **not receiving** any treatment and declaring complete recover from previous symptoms will come back to site to perform the evaluation visit (V3) as soon as the symptoms are completely disappeared (subject is completely recovered).

- Subject **not receiving** any treatment but with persisting symptoms will **re-perform** a urine culture. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standard of care treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the evaluation visit (V3).

Any UTI symptom(s), with or without a positive urine culture, verified just before the V3 will be reported in a relapse visit.

The study procedures performed during the evaluation visit are the following (please refer to the Study flow-chart in the section 2):

- Recording vital signs: systolic/diastolic blood pressure (please refer to the section 7.4.1.4. for instructions) and heart rate, axillary body temperature,
- Recording anthropometrical parameters: body weight, waist circumference,
- Collecting and reviewing V2-V3 subject's diaries 3 days fluid intake and Subject Compliance Diary (if applicable),
- Checking and recording adverse events and concomitant medications,
- Checking non-authorized product compliance,
- Collecting of blood samples (after 8-12 hours of fasting) (please refer to the section 7.4.2.1),
- Checking that 24h urine sample has been collected,
- Collecting and checking the voiding diary,
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,
- Providing instructions for completing the QoL questionnaire (SF-12 V2) in situ but not in front of the Investigator. The Investigator will check the SF-12v2 questionnaire completion,

At the end of the evaluation visit (V3), the subject will be:

- Reminded with instructions for diet and non-authorized products (please refer to the section 6.5.2),
  - Reminded with instructions for:
    - 3-days fluid intake diary completion (including documentation of adverse events),
    - Subject Compliance Diary
    - Voiding diary as appropriate.
- Appropriate pages of the diaries will be provided to the subject. (please refer to the section 6.5.4, 6.5.5 and 6.5.6)
- Provided with instructions for urine collections, storages and transportations. Subject will be provided with the kits for urine sterile sample and 24h urine collection (please refer to the section 7.4.2.6 for instructions),
  - Reminded with instructions for coming back to the site in case of any UTI symptom(s),
  - Reminded with instructions for study product consumption (only for the intervention group).

#### **Between V3 and V4:**

- The subjects in the **intervention group** have to send back to the Investigator's site the Subject Compliance Diary, corresponding to the previous month of study product consumption, and the 3 days fluid intake diary by courier or personally if preferred,
- The subjects in the **control group** will send back to the Investigator's site the 3 days fluid intake diary by courier or personally if preferred on monthly basis.

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents are accurate and complete (please refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit and no later than

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5 days to the date of the visit. In case of unavailable data, the e-CRF should be completed within 48 hours after the missing data's receipt.

*7.1.5 Phone calls (From Month 1 to Month 5 and from Month 7 to Month 11, all phone calls will be performed within the respective number of months after the randomisation call (D0) within +/- 7 days allowable time window)*

Every month, except at Month 6 (V3) and month 12 (V4), the subjects will be contacted by phone by the principal Investigator or another documented authorised study team member.

PI or study personnel will call the subject at the following time schedule:

- Phone call 1 (Month 1, +/- 7 days), between Randomisation call (D0) and V3
- Phone call 2 (Month 2, +/- 7 days), between Randomisation call (D0) and V3
- Phone call 3 (Month 3, +/- 7 days), between Randomisation call (D0) and V3
- Phone call 4 (Month 4, +/- 7 days), between Randomisation call (D0) and V3,
- Phone call 5 (Month 5, +/- 7 days), between Randomisation call (D0) and V3
- Phone call 6 (Month 7, +/- 7 days), between V3 and V4
- Phone call 7 (Month 8, +/- 7 days), between V3 and V4,
- Phone call 8 (Month 9, +/- 7 days), between V3 and V4,
- Phone call 9 (Month 10, +/- 7 days), between V3 and V4
- Phone call 10 (Month 11, +/- 7 days), between V3 and V4

The objective of each phone call will be:

- Reminding the instructions for diet and and non-authorized products (please refer to the section 6.5.2 and 6.5.3),
- Ensuring that 3-days fluid intake diary, Subject compliance diary (as appropriate) are regularly completed and sent back to the site (please refer to the section 6.5.4),
- Assessing the compliance with the study product consumption as applicable. During the 1<sup>st</sup> phone call, the Investigator will collect the date of the 1<sup>st</sup> product consumption,
- Assessing the level of fluid intake based on subject's interview,
- Assessing the occurrence of adverse events based on subject's interview,
- Assessing whether any concomitant medications have been taken or modified,
- Assessing the presence of symptom(s) of UTI at the time of the phone call as well as in the period between the last contact to the subject and the current phone call,
- Assessing the start date and the end date of a UTI episode if any. In this case, the PI must remind to the subject that in case of any UTI symptom(s) a relapse visit must be done,
- Checking sexual life status and emptying bladder habits after sexual intercourses,
- Inquiring for diagnosed pregnancy or any doubts for such after the last visit (including phone call).
- For the 5<sup>th</sup> and 11<sup>th</sup> month follow-up calls; PI will remind the instructions for urine collections, storages and transports for urine sterile sample (as applicable for the visit) and 24h urine collection (please refer to the section 7.4.2.6 for instructions) as well as the instructions to complete the voiding diary.

The site will ensure that the phone call procedures and evaluations are properly documented in the subject's medical file and that the source documents are accurate and complete (please refer to the section 8). The subject's / e-CRF should be completed from the source documents and as soon as possible after the phone call and no later than 5 days to the date of the phone call. In case of unavailable data, the e-CRF should be completed within 48 hours after the missing data's receipt.

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*7.1.6 End of Study Visit – Visit 4 (Month 12 after the D0 (randomisation call) within +/- 10 days allowable time window or + 28 days in case of UTI event)*

The End of study visit (V4) will take place 12 months (+/- 10 days or +28 days in case of UTI event)) after the randomisation call (D0).

At V4, the subjects (in fasting condition) will bring back to the site the following:

- 24h Urine samples
- Sterile urine sample
- Voiding diary
- V3-V4 Subject Compliance Diary as appropriate
- 3 days fluid intake diary

1/ If a subject coming to V4 **presents UTI symptom(s), whatever the urine culture results**, she won't be able to perform the visit, only urine culture is analysed. **V4 will be rescheduled** as soon as the subject is recovered **(please refer to the Appendix IV / Management of symptomatic/Asymptomatic subjects per visits)**.

**1a/ Symptomatic subjects at V4 with a positive urine culture:**

The subject will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a control urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered.

Depending on **control** urine culture results:

- If a **negative** control urine culture, the subject will come back to the site to perform the end of study visit (V4) as soon as the subject is completely recovered.
- If a **positive** control urine culture, the subject will be treated till a negative control urine culture is obtained and will come back to site to perform the end of study visit (V4) as soon as the subject is completely recovered.

Any UTI symptom(s), confirmed or not with a positive urine culture just before the V4 will be reported in a relapse visit.

Once the negative control urine culture is obtained, the end of study form in the e-CRF will be completed

**1b/ Symptomatic subjects at V4 with a negative urine culture:**

The subject may be treated or not with a standard of care treatment at the PI's discretion:

- Subject **receiving** a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained. They will come back to site to perform the end of study visit (V4) as soon as they are completely recovered.
- Subject **not receiving** any treatment and declaring complete recover from previous symptoms will come back to site to perform the end of study visit (V4) as soon as the symptoms are completely disappeared (subject is completely recovered).
- Subject **not receiving** any treatment but with persisting symptoms will re-perform a urine culture. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standardised treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to site to perform the end of study visit (V4).

Any UTI symptom(s) confirmed or not with a positive urine culture just before the V4 will be reported in a relapse visit.

Once the negative control urine culture is obtained, the end of study form in the e-CRF will be completed

**2/Asymptomatic subject will perform V4 (please refer to the Appendix IV / Management of symptomatic/Asymptomatic subjects per visits).**

The study procedures performed during the End of study visit (V4) are the following (please refer to the Study flow-chart in the section 2):

- Conducting a physical examination,
- Recording vital signs: systolic/diastolic blood pressure (please refer to the section 7.4.1.4 for instructions) and heart rate, axillary body temperature,

- Recording anthropometrical parameters: body weight, waist circumference,
- Reviewing V3-V4 3-days fluid intake diary, Voiding diary and Subject's compliance diary as appropriate,
- Checking and recording adverse events and concomitant medications,
- Collecting of blood samples (after 8-12 hours of fasting), (please refer to the section 7.4.2.1),
- Checking that 24h urine samples and sterile urine sample have been collected,
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,
- Checking symptom(s) of UTI,
- **Performing urine culture for all subjects,**
- Reminding instructions for completing the QoL questionnaire (SF-12 V2) that will be performed in situ but not in front of the Investigator. QoL questionnaire should be completed in the absence of UTI event (the subject is asymptomatic and has negative urine culture) The Investigator will check the SF-12v2 questionnaire completion

**2a/ Asymptomatic subjects at V4 with a positive urine culture:**

The subject will be treated for the UTI event with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management)., After the completion of UTI treatment, a **control** urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered.

Depending on **control** urine culture results:

- If a **negative** control urine culture is obtained  $\leq 28$  days after V4 (i.e. negative control urine culture obtained at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered), a new QoL questionnaire will be completed by the subject after UTI is cured,
- If a **negative** control urine culture is obtained  $> 28$  days after V4, the V4 should be repeated

All asymptomatic events with a positive urine culture, occurring at V4 will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF

Once the negative control urine culture is obtained, the end of study form in the e-CRF will be completed.

**2b/ For asymptomatic subjects at V4 with a negative urine culture,** the end of study form in the e-CRF will be completed. Date of end of study will be the date of lab reports of last negative urine culture.

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents are accurate and complete (please refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit and no later than 5 days to the date of the visit. In case of unavailable data, the e-CRF should be completed within 48 hours after the missing data's receipt.

**7.1.7 Relapse visit**

Subjects presenting with symptoms of UTI during the course of the study (from D0 to V4), will be instructed not to stop the increased water intake (if in the intervention group) and to come back to the investigational site as soon as possible for clinical examination.

**UTI events will be diagnosed as at least 1 symptom of urinary tract infection and positive urine culture.**

Any UTI symptom(s), with or without a positive urine culture, occurring during the study will be reported in a relapse visit.

Urine cultures will be always performed to confirm presence of infection. Data about duration of the infection (starting and ending date) will be registered.



The tasks performed during the relapse visit are the following (please refer to the Study flow-chart in the section 2):

- Performing urine culture to confirm preliminary diagnosis
- Reporting start date of event (defined as the date of the appearance of the first symptom(s))
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,
- Checking and recording adverse events and concomitant medications

**1a/ Subjects with a positive urine culture**, will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management).

**1b/ Subjects with a negative urine culture**, may be treated/or not with a standard of care treatment at the PI's discretion:

- o Subject **receiving** a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained.
- o Subject **not receiving** any treatment but with persisting symptoms will **re-perform** a urine culture. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standard of care treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained.

At the end of UTI event treatment or disappearance of symptom(s), the following study procedures will be performed:

- Performing urine culture at least 48h after the end of the therapy or disappearance of symptom(s) of the UTI event and within 1 week to be sure that women are completely recovered
- Reporting end date of event (defined as the date of sampling of the last negative urine culture exam confirming the remission of patients after treatment)
- Collecting UTI related treatment if any (to be reported in the "UTI related Concomitant Medication" section of the e-CRF)
- Completion of health care cost questionnaire related to the UTI event at the end of event ONLY in the case of symptomatic event confirmed by positive urine culture
- Checking and recording adverse events and concomitant medications since the previous contact with the subject.

The visit date will be the date of sampling of the (last) negative urine culture

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents are accurate and complete (please refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit and no later than 5 days to the date of the visit. In case of unavailable data, the e-CRF should be completed within 48 hours after the missing data's receipt.

#### *7.1.8 Follow-up Visit*

Not applicable

#### *7.1.9 Early Subject's Discontinuation.*

In the event of early subject's discontinuation from the study (as specified in section 6.4), the Investigator should make every effort to entirely conduct the End of Study Visit evaluations and procedures (please refer to the section 6.4.3).

For all subjects who were randomised in the study, the reason(s) for early termination of the subject prior to completion of the study must be stated in the subject's source documentation and reported in the e-CRF.

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The site(s) will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents are accurate and complete (please refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit and no later than 5 days to the date of the visit. In case of unavailable data, the e-CRF should be completed within 48 hours after the missing data's receipt.

End of study form must be filled for all subjects that have signed the Informed Consent.

For screen failures this should minimally include: informed consent date, demographics, inclusion criteria, exclusion criteria and end of study form.

## **7.2 SUBJECT VISIT WINDOW**

For each subject's visit, allowable windows are defined and stated in the previous section (7.1) and in the table of the study procedures and stages as outlined in the section 2.

## **7.3 STUDY FLOW-CHART**

The table of the study evaluations, procedures and stages is outlined in the section 2.

## **7.4 STUDY ASSESSMENTS**

### *7.4.1 Clinical Assessments*

#### *7.4.1.1 Medical and Surgical History*

The Investigator will interview the subject with respect to her history of concomitant diseases, surgery. The history of renal diseases and treatments will be verified in depth and specific questions on the symptomatology, duration and management of the UTIs will be addressed. All information will be recorded in source data and reported into the e-CRF.

#### *7.4.1.2 Medications and Nutritional Supplements History*

The Investigator will check if the subjects consume the following products forbidden by the protocol:

- Cranberry juice and/or extracts during the last 2 weeks
- Antibiotics during the last 2 weeks
- Chronic anti-coagulant therapy
- Diuretics
- Corticoid treatment
- Chronic laxative treatment

In addition to this all concomitant medication taken within 2 weeks will be documented in the source documentation.

#### *7.4.1.3 Physical Examination*

The physical examination includes an assessment of general appearance and a review of systems (gastrointestinal, cardiovascular, OtoRhinoLaryngological (ORL), Neurological, Dermatological, Musculoskeletal, Urological/Nephrological,)

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**7.4.1.4 Vital Signs**

The following vital signs will be measured:

- Blood pressure (BP) (systolic and diastolic [mmHg]),
- Heart rate (HR) (beats per minute [bmp]),
- Axillary body temperature.

The measurement of blood pressure is performed after resting for at least 5 minutes, in sitting position.

**7.4.1.5 Demographics**

Demographics include date of birth and gender.

**7.4.1.6 Anthropometry (Body weight, waist circumference, Height)**

- Body weight is measured to the nearest 0.1 kg using a calibrated weighing scale without outerwear and shoes
- Body height is recorded to the nearest 1 cm
- Body weight and height are used to calculate the Body Mass Index (BMI) as followed:  
BMI = weight / (height)<sup>2</sup> where weight is in kilogram and height in meter.

**7.4.1.7 Other Clinical Assessment**

Not applicable.

**7.4.2 Laboratory Assessments**

Normal ranges and laboratory/technical procedures for clinical laboratory parameters are made available by the laboratory(ies) before start of the study in the Laboratory Manual.

**7.4.2.1 Clinical Laboratory Assessments**

Laboratory measurements will include the following parameters assessed per visit:

Parameters	V1	V2 Pre- randomisation	V3 (M6)	V4 (M12)	Relapse visits
<b>Blood parameters</b>					
Lipidic profile**		X		X	
Glycosylated hemoglobin		X		X	
Serum creatinine		X	X	X	
Estimated glomerular filtration rate (eGFR)		X	X	X	
Serum Urea		X		X	
Copeptin		X	X	X	
FSH***		X			
<b>24h urine parameters</b>					
Sodium		X	X	X	
Potassium		X	X	X	
Calcium		X		X	
Magnesium		X		X	
Chloride		X		X	
Oxalate		X		X	
Citrate		X		X	

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Urine Specific Gravity		X	X	X	
Osmolality		X	X	X	
Urine color		X	X	X	
pH		X	X	X	
Urine Creatinine		X	X	X	
Volume		X	X	X	
Number of micturitions		X	X	X	
<b>Spot urine parameter</b>					
Urine Pregnancy Test	X	X			
Urine culture****	X*	X	X*	X	X

\* If symptomatic

\*\* Lipidic profile: HDL, LDL, total cholesterol, tryglicerides

\*\*\* if peri-menopausal symptoms

\*\*\*\* In case of UTI infection a control urine culture should be performed at least 48h and within 1 week after the end of treatment

*7.4.2.2 Pregnancy Test*

Women of childbearing potential must have a negative pregnancy test both at V1 and V2. A urine pregnancy test will be used and results must be available prior to the subject randomisation. The Investigator is responsible for ensuring that the subject's pregnancy status is recorded accurately in the source documents. The Investigator will record the results of the pregnancy tests in the source documents and in the subject's e-CRF.

*7.4.2.3 Special Assays or Procedures*

Not applicable

*7.4.2.4 Instructions for Specimen Preparation, Handling, and Storage*

Not applicable

*7.4.2.5 Blood Sample Collection and Handling*

Blood sampling will not exceed 20 mL per visit and will be taken after a period of fasting of 8-12 hours at visits V2, V3 and V4. This volume includes the volume required for back up samples in case of retest analysis. The blood sampling will be done at the Investigational site.

The clinical examinations and the blood and urine collections related to the study visits may be repeated in case of UTI events.

If fasting condition is not confirmed, the subject has to come back to the investigational site for re-sampling during a new visit scheduled within a maximum of 2 days after the initial scheduled visit in fasted state.

All blood samples will be processed, stored and transported under the conditions described in the laboratory specification in order to ensure the samples stability.

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#### *7.4.2.6 Urine Sample Collection and Handling*

The 24h urines will be collected outside of menstruation period in a specific container during 24h and stored by the subject at about +4°C to ensure the stability of parameters analysed. In order to ease sample collection and homogenise the number of sample and time collection of each subject, urines are collected as follows: the first urine of the day is excluded. The collection includes all urinations in the following 24h including the first morning urine of the day after.

**If at least one micturition is not collected in the 24h urine collection, subject will be asked to perform a new 24h urine collection (and complete a new Voiding diary accordingly) and to come back for a new visit scheduled the earliest possible day (and no later than 10 days) after the initial visit.**

The subjects will bring back their urine collection to the investigational site the morning of V2, V3, V4 or on the day of re-test as applicable.

The urine samples will be processed, stored and transported under the optimal conditions stipulated in the Protocol as well as in the laboratory specification in order to ensure the samples stability.

Urinary concentrations and excretions of sodium, potassium, calcium, magnesium, chloride, oxalate, citrate, creatinine, will be evaluated on 24h urine sample as per requirement for the concerned study visit

#### Urine volume, osmolality and pH

24h urine collection will be weighted and analysed for their Urine Specific Gravity (USG), osmolality and pH. The weight of the 24h urine collection will be divided by the USG in order to calculate the urinary volume.

#### Urine colour

24h urine collected will be evaluated according to the Urine Color Chart (UCC) provided to the investigating site. Urine Color Chart was originally published in Lawrence Armstrong's book titled Performing in Extreme environments. The scientific validation of this UCC may be found in the literature [31,32]

This assessment will be done in order to assess the change in urinary hydration markers following changes in water intake habits.

#### Urine culture and MIC determination

The collection will be performed from the 1st morning urine in midstream urine by clean catch method. Urine culture is performed to confirm the presence of UTI and determine the bacterial origin of the UTI. For the bacteria identified, a Minimum Inhibitory Concentration test (MIC test) will be performed in order to determine the bacteria resistance or susceptibility to antibiotics. The list of antibiotics is chosen on the result of Gram coloration (i.e.: Gram +/-).

In case of positive urine culture, an antibiotic treatment will be administered according to Investigator prescription. A new urine culture will be performed after the treatment in order to verify if urine culture is negative. This urine culture will be performed at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered and that results are not biased by the antibiotic treatment taken by the subjects.

#### Tiselius Crystallisation Risk Index

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Subjects exhibiting rather highly concentrated urine due to their dietary habits and low fluid intake may be seen as potential future stone-formers in which a higher water intake may reduce the risk of crystallization and finally the risk of a lithiasis event.

Several indexes of crystallization risk have been proposed to evaluate the risk of crystallization in urine. Most of them have been developed and proposed by Tiselius who is well known for his Tiselius Crystallisation Risk Index (Tiselius, 1991):

$$AP(\text{CaOx}) \text{ index EQ} = 1.9 \times \text{Ca}^{0.84} \times \text{Ox} \times \text{Mg}^{-0.12} \times \text{Cit}^{-0.22} \times \text{V}^{-1.03}$$

Where Ca (calcium), Ox (oxalate), Mg (magnesium), Cit (citrate) are urinary excretions expressed in millimoles excreted during the period, and V represent the urine volume in litres corresponding to the sample considered from 24 hours urine collection.

#### 7.4.2.7 Instructions for Specimen Shipments

Frequency of shipment, labelling requirement and any special instructions will be defined before study start and specified in Laboratory Manual.

#### 7.4.2.8 Back up samples

The remaining back up blood and urine samples will be kept during the study by the investigator site, following instructions described in the laboratory specification as well as in the laboratory manual at the site and up to the signature of the study report when DANONE RESEARCH will transfer them at a central laboratory facility, selected and contracted by DANONE RESEARCH. These remaining back up blood and urine samples may be used for future researches such as the performance of additional analyses and/or the development new analytical methodologies. In this case, this will be done under the confidentiality rules. In any case, these samples will not be used for human genetic analysis.

The subject will be informed, via the ICF, that the unused blood and urine samples will be frozen and stored for a maximum of 15 years, in order to make these samples available for repeated measurements as a substitution of analysis mistakes, or in case of development of new methodologies of analyse.

#### 7.4.3. Other Assessments

##### 7.4.3.1. Subject's Compliance with Study Product Intake

The subject's compliance for study product intake will be assessed by the Investigator throughout the study using the following information:

- Subject's self-reported data of product intake into the Subject Compliance Diary (please refer to the section 6.5.5),
- Subject's self-reported data on total fluid intake into the Subject diary for 3-days fluid intake (data collected on 3 consecutive days including the 2 days of the week-end and one working day (Saturday-Sunday-Monday or Friday-Saturday-Sunday) (please refer to the section 6.5.4),
- The urine volume, osmolality and colour measured at V2, V3 and V4.

For the **intervention group**, the subjects will record in a Subject Compliance Diary the volume of study product consumed (Evian® water) on a daily basis by specifying the number of bottles and the volume of water consumed from each bottle (total bottle, ¼ of bottle, ½ of bottle).

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During the monthly phone calls, the subject will be reminded to return her Subject Compliance Diary to the site each month by courier or personally if preferred. Before each Telephone call, the Investigator will review the subject's Subject Compliance Diary.

At the same time, the Investigational site will receive the acknowledgment of receipt for the delivery of study product to the subject.

*7.4.3.2. Recommendations and non-authorised products*

Women included in the study will be asked not to consume any cranberries juice and/or extracts as well as any other nutritional complement (such as infusion(s) / product(s) with diuretic action(s)...) which can impact the outcome of the study in the 2 weeks prior to the inclusion visit and for the all duration of the study.

## **8. SOURCE DATA AND SOURCE DOCUMENTS**

### **8.1 SOURCE DATA DEFINITION**

According to ICH GCP (E6), source data is all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

### **8.2 SOURCE DOCUMENTS DEFINITION**

According to ICH GCP (E6), source documents are original documents, data, and records (e.g., medical / hospital records, clinical and office charts, laboratory notes, memoranda, general practitioner letter, questionnaire used for diagnosis, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, X-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial).

### **8.3 SOURCE DATA MANAGEMENT**

All information recorded in the CRF should be traceable and documented by source documents in the subject's medical records available at the study centre.

If the subject is not followed by the investigational site, the investigator will not be exempted of creating and/or maintaining a complete and accurate subject's file by ICH/GCP, guidelines of the medical profession and local laws and guidelines. The subject's file includes all source documents for the e-CRF completion and verification. A special attention will be stated for source documents on the inclusion/exclusion criteria and randomisation/ non randomisation criteria, in order to ensure the targeted population. If relevant, the PI or another documented authorised study team member will solicit the subject's General Practitioner (GP) to ensure the accuracy, completeness and up to date information on past/current medical/medication history, or any other information required by the study.

The e-CRF must be completed within 5 working days to the date of the visit.

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#### **8.4 RIGHT OF ACCESS TO SOURCE DATA/DOCUMENTS**

The Investigator/institution should permit study-related monitoring, audits, IRB/IEC review and regulatory inspection(s), providing direct access to source data/documents.

In accordance with the Law, the subjects who wish may have access to their personal data. They must address their request to the Investigator in writing.

## **9. STUDY PRODUCT**

### **9.1 STUDY PRODUCT(S) DESCRIPTION AND COMPOSITION**

#### *9.1.1 Study product(s) description*

The study product is a low mineralised natural mineral water. It is manufactured according to the DANONE's quality policy in the factory of Evian® (France).

The study products are intended for oral use only and within the standard consumption patterns for physiological needs.

#### *9.1.2 Study product(s) composition*

<b>Name</b>	<b>Natural mineral water</b>
<b>Product Brands</b>	<b>Evian®</b>
<b>Type of Source Water</b>	<b>Cachat source (Evian, France)</b>
<b>Type of Packaging</b>	0.5L bottles made of polyethylene terephthalate [PET] represent the test unit
<b>pH, (pH units)</b>	<b>7.2</b>
<b>Dry Residue 180°C, mg/l</b>	<b>309</b>
<b>Silica, mg/l</b>	<b>15</b>
<b>Sodium, mg/l</b>	<b>6,5</b>
<b>Potassium, mg/l</b>	<b>1</b>
<b>Calcium, mg/l</b>	<b>80</b>
<b>Magnesium, mg/l</b>	<b>26</b>
<b>Chlorides, mg/l</b>	<b>6,8</b>
<b>Bicarbonates, mg/l</b>	<b>360</b>
<b>Sulphates, mg/l</b>	<b>12,6</b>
<b>Nitrates, mg/l</b>	<b>3,7</b>

#### *9.1.3 Study product analyses during the study*

Not applicable.

### **9.2 STUDY PRODUCT(S) LABELLING AND PACKAGING**

Boxes and bottles are labelled in accordance with applicable laws and regulation. Primary labels (on water bottles) are printed in French by default. Secondary labels (on carton boxes) will be written in local language (Bulgarian).



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(a) Primary packaging (water bottle)

The products are packaged in alimentary plastic bottles of 500mL. The primary labels affixed to these bottles contain the following information:

- Expiry date : DD/MM/YYYY
- Batch number : ZZZZ
- Content : 500 ml
- Storage temperature or conditions

Secondary packaging (carton box):

The water bottles are gathered in batches of 24 units with the following information:

- Expiry date (DD/MM/YYYY)
- Study code / Name and address of Sponsor and Investigator
- Batch code allowing identification of the contents
- Weight
- Storage temperature or conditions
- Sentence: 'to be used for clinical study only'

NB: Labels are printed in Bulgarian.

### **9.3 SHIPMENT, STORAGE, DISPENSING, ACCOUNTABILITY AND DESTRUCTION**

#### *9.3.1 Shipment of study product(s)*

Study products are commercial products of the DANONE group and provided by DANONE RESEARCH.

The water bottles distributed to the subjects are manufactured, stored and delivered in accordance with the current sanitary regulations.

Study products are delivered in boxes and are identified as follows:

- Name and address of sender
- Name and address of recipient
- Study code
- Batch number
- Type and Identification of products
- Storage conditions

Study products are accompanied by:

- An acknowledgement of receipt to be signed and returned by the consignee (the subject herself or another person delegated by the subject) upon receipt of products and will be delivered to the investigating site by the logistics company.

#### *9.3.2 Storage of study product(s)*

The Investigator site ensures that the study products are stored in a temperature controlled, secure and closed area in accordance with the indications stated on the packaging for the products to be used.

The investigating site will ensure that the products are distributed personally or to a delegated person to the subjects' home/or office in a timely manner and appropriate conditions. The subjects will be informed about water bottles storage conditions. The water bottles delivered should be used only for the study purposes.

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### 9.3.3 *Delivery/Dispensing of study product(s)*

An external shipping company will deliver the water bottles at each subject included in the study and randomised in the intervention study group. The Sponsor and the Investigator must ensure that water bottles are received under good condition by the subjects.

The subjects (or the preliminary indicated designee) will sign an acknowledgment of receipt and will report any product quality issue to the delivery company.

The subject may delegate the receipt of the study products to another person (e.g. a family member) which has to be documented in the source documents. A confirmation from the subject *a posteriori* stating that she has received the products has to be collected in the source documents (e.g. during the monthly phone calls).

If the subject has planned to travel abroad and she will not be able to carry the water bottles, the investigator should encourage the subject to continue drinking water (any type of drinking water) at the recommended volume (1,5L/day on top of their usual consumption).

The subjects will dispose the empty bottles together with the household waste.

### 9.3.4 *Accountability of study product(s)*

Delivery records from the transport company will be used to ensure the quantity of study product delivered to each study participant. The acknowledgement of receipt will be sent to the Investigator by the carrier.

The Investigator will check the reported quantity of water consumed by the subject in case of compliance deviation, he will check the acknowledgement of receipt for the deliver quantity.

### 9.3.5 *Destruction of study product(s)*

The Evian® empty water bottles will be disposed by the subjects together with their household waste.

At the end of the study, the subject will keep any left bottle(s).

The Investigator must instruct the subject that in case of detected or suspected study product abnormalities, the subject must not consume the related study products and return them to the investigational site.

## **10. RANDOMISATION AND UNBLINDING**

### **10.1 RANDOMISATION (AND STRATIFICATION IF APPLICABLE)**

After having checked all eligibility criteria at pre-randomisation visit (V2), the subject will be allocated to the study arm through the randomisation system (IWRS), without any stratification factor.

The randomisation process will be applied using a centralised Interactive Web Response System (IWRS) that will allocate subject into one of the two groups in respect with the randomisation list generated informatically at the beginning of the study.

### **10.2 UNBLINDING PROCEDURE**

Not applicable (open label study).

## 11. PRODUCT EFFECT PARAMETERS AND PRODUCT SAFETY PARAMETERS

### 11.1 DESCRIPTION OF PRODUCT EFFECT PARAMETERS

The study intervention effect parameters are assessed by clinical parameters and laboratory parameters analysed in blood and urine.

Product Effect parameters	Visit 1	Visit 2	Visit 3	Visit 4	Relapse visits
Symptoms of UTI	X	X	X	X	X
Urine culture (bacteriogram if urine culture positive)	X* Only if symptoms	X *	X* Only if symptoms	X *	X *
Frequency of micturitions during 24h urine collection		X	X	X	
Urine hydration markers (volume, USG, pH, osmolality, urine colour)		X	X	X	
Fluid intake (daily volume)		X	X	X	
QoL questionnaire (SF-12V2)		X	X	X	
Antibiotic prescription data	X	X	X	X	X
Healthcare cost data					X

\* in case of UTI confirmation, a **control** urine culture will be systematically performed at least 48h after the end of the treatment and within 1 week

### 11.2 DESCRIPTION OF PRODUCT SAFETY PARAMETERS

The study safety parameters are assessed by clinical parameters and laboratory parameters analysed in blood and urine.

Product Safety parameters	Visit 1	Visit 2	Visit 3	Visit 4
Urine sodium		X	X	X
Urine potassium		X	X	X
Total cholesterol		X		X
HDL cholesterol		X		X
LDL cholesterol		X		X
Triglycerides		X		X

Glycosylated haemoglobin		X		X
Serum creatinine		X	X	X
Serum urea		X		X
eGFR		X	X	X
Blood pressure		X	X	X
Heart rate		X	X	X
Axillary body temperature		X	X	X
Physical examination		X		X
AE/SAE	X	X	X	X

## 12. SAFETY REPORTING

### 12.1 DEFINITIONS (INSPIRED FROM ICH E2A GUIDELINES)

#### 12.1.1 Adverse Event (AE)

An Adverse Event (AE) is any untoward medical occurrence in a subject/patient or clinical study subject administered an investigational product but does not necessarily have a causal relationship with this investigational product.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the investigational product, whether or not related to the investigational product or to the protocol- mandated procedures (e.g., invasive procedures such as biopsies).

This includes events:

- Not present before the study but occurring after the signature of the Informed Consent,
- Pre-existing events that have worsened during the course of the study (increase in frequency or severity or change in nature during the study),
- All asymptomatic UTI detected at V4 (please refer to the Appendix II),
- All symptomatic and asymptomatic UTI detected between V1 and randomisation call (D0) (please refer to the Appendix II),
- All worsening UTI (as per protocol definition) collected in relapse visit.

For this study, the cystitis events are expected events, and will be considered as study parameters. Therefore, the related symptoms, signs and biological parameters will not be declared as AEs (with exception described into the protocol and above) but will be collected in the e-CRF.

#### 12.1.2 Unexpected Adverse Event

An unexpected adverse event is by its nature or severity not consistent with applicable product information contained in the relevant source document(s) (e.g. Protocol, Inform Consent Form).

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The product distributed during the study is natural mineral water with a long commercial history and without any report of safety problem.

#### 12.1.3 *Serious Adverse Event (SAE)*

A Serious Adverse Event (SAE) is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (at the time of the event)
- requires hospitalisation of a subject or prolongation of existing inpatients' hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital abnormality/birth defect
- is an important medical event.

**Complications of UTI (i.e. pyelonephritis) and symptoms, signs or abnormal values of biological parameters related to any other pathology, will be reported as S(AE).**

#### 12.1.4 *Suspected Unexpected Serious Adverse Reaction (SUSAR)*

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is both:

- an SAE that is judged to be at least possibly related to the investigational product by either Investigator or sponsor,
- and by its frequency, nature or severity is unexpected (not listed in the study protocol).

#### 12.1.5 *Emergent Adverse Event (EAE)*

An adverse event will be considered as emergent if it began the day or after the first product consumption, or if it worsened the day or after the first product consumption.

### **12.2 (S)AE RECORDING**

Details of any (S)AE reported spontaneously by the subjects or observed by the Investigator or medical staff must be recorded in the (S)AE forms provided in the e-CRF during the course of the study. The Investigator must report the following information on the (S)AE form: nature of event (diagnosis or major symptoms/signs), start and end dates, severity, product-relatedness, action(s) taken regarding the (S) AE, action taken regarding the study product, and subject outcome.

SAEs must additionally be recorded on the SAE Report Form provided by DANONE RESEARCH. The Sponsor may request additional information from the site to evaluate the SAE.

When a subject undergoes a medical intervention or hospitalisation in absence of an adverse event (such as treatment of pre-existing condition or hospitalisation for elective surgery or diagnosis), this intervention/hospitalisation must be reported on the AE page and not as SAE. These procedures will be handled like AEs, and timelines for reporting are the same as for reporting AEs. Complications or prolongations of hospitalisation that result from procedures must be reported as SAEs, according to the applicable reporting timelines and procedures.

The severity of the (S)AE will be determined in the following manner:

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- Mild: No interference with the subject's daily activities - transient or mild discomfort; no medical intervention/therapy required.
- Moderate: Moderate interference with the subject's daily activities but still acceptable - mild to moderate limitation in activity; some assistance may be needed; and/or minimal medical intervention/therapy required.
- Severe: Major interference with the subject's daily activities and unacceptable - marked limitation in activity; some assistance usually required; and/or significant medical intervention/therapy/hospitalisation required.

The relationship of the (S)AE to the study product is assessed as being:

Not related	The AE follows no reasonable temporal relationship with the investigational product or does not follow a known response pattern to the investigational product and can be explained by the known characteristics of the subject/patient's clinical state, by underlying disease or other administrated products (e.g. nutritional products, OTCs, drugs).
Unlikely related	The AE has a time to investigational product intake that makes a relationship improbable (but not impossible) and concomitant administrated products (e.g. nutritional products, OTCs, drugs) or underlying disease provide plausible explanations.
Possibly related	The AE has reasonable time relationship to investigational product intake but could also be explained by an underlying disease or other administrated products (e.g. nutritional products, OTCs, drugs); information on drug withdrawal may be lacking or unclear
Probably related	The AE has a reasonable time relationship to investigational product intake, it is unlikely to be attributed to disease or other drugs and response to withdrawal of the investigational product is clinically reasonable; rechallenge information is not necessary
Definitely	The AE has plausible time relationship to investigational product intake and cannot be explained by underlying disease or other administrated products (e.g. nutritional products, OTCs or drugs); response to withdrawal of the investigational product is clinically plausible; rechallenge is satisfactory if necessary

**12.3 SAE REPORTING BY THE INVESTIGATOR**

As soon as the Investigator becomes aware of a SAE and not later than 24 hours (1 working day), or within 3 calendar days of the event if this period includes a week-end or public holiday, he/she completes the SAE Reporting Form and sends it together with other supporting documents (e.g. copy of pages of the case report form for medical history, on-going events and concomitant medications, etc.) via fax or e-mail to DANONE RESEARCH with copy to the monitoring CRO.

The Investigator ensures that any supportive documents submitted have been anonymised adequately. The Investigator also ensures that e-CRF pages related to the SAE are duly completed (e.g. medical history, on-going events and concomitant medications, etc).

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#### **12.4 SAE REVIEW AND REPORTING BY THE SPONSOR**

DANONE RESEARCH must review all reported SAE and independently assess the relationship of the SAE with the study product.

If the Investigator and the Sponsor both assess the relationship of the SAE with the study product as “Not related “ or “Unlikely related”, the SAE is considered as a regular SAE with no required expedited reporting to Ethics Committees.

If one of them determines that the SAE is “Possibly”, “Probably” or “Definitely” related to the investigational product, and that this event has not been described in the or in the Clinical Study protocol, the SAE is considered as a SUSAR requiring expedited reporting to Ethics Committees. Should the assessments of the Sponsor and of the Investigator differ with regard to the relationship to the study product, then both will be reported.

DANONE RESEARCH must ensure that all SAEs and SUSARs are reported to the accredited Ethics Committee(s) that have approved the protocol (and Competent Authorities, if applicable) as follow:

- SAEs are reported annually as line listings according to the requirements of the Ethics Committee(s),
- SUSARs are reported within 7 days (fatal and life threatening) or 15 days (other events) after the first report. Reporting timelines include week-ends and public holidays.

The Sponsor will reply to all requests for further information concerning such events from the Ethics Committee (and/or Competent Authorities, if applicable).

#### **12.5 FOLLOW-UP OF SAEs**

The Principal Investigator or his/her authorised representative will monitor and follow all SAEs until SAEs have abated, or until a stable situation has been reached and a satisfactory resolution is obtained.

Depending on the event, follow-up may require additional tests or medical procedures as indicated, and/ or referral to the general physician or a medical specialist or other health care provider.

Any clinical or biological examinations deemed necessary by the Principal Investigator will continue to be performed until a return to normal. The Principal Investigator will provide the Sponsor with follow-up SAE Reporting Form and with all the examination results and concomitant medications, as soon as the Investigator becomes aware of new information and not later than 24 hours (1 working day) or within 3 calendar days of the new information if this period includes a week-end or public holiday.

#### **12.6 PREGNANCIES REPORTING BY THE INVESTIGATOR**

Although the study products are considered as safe for pregnant women, pregnancy may require specific diet, medication or procedure likely to interfere with the study product and/or outcomes.

For this reason subjects with a confirmed pregnancy have to be withdrawn from the study and followed up until birth or early termination of pregnancy. This premature termination of the protocol is documented in the e-CRF.

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The Investigator should make every effort to collect information about the pregnancy and the infant and report it within a dated and signed Pregnancy Reporting Note including at least:

- Date of last menstruation
- Expected date of delivery
- Method of contraception used and if used according to instructions
- Medical history with information on familial disorders or risk factors that may influence the outcome of the pregnancy,
- Obstetric history with details on previous pregnancy (including termination or stillbirth)
- Medication taken prior and during pregnancy (should be available in the concomitant medication section of the e-CRF)
- Special tests and procedures performed during pregnancy if the case (e.g. amniocentesis, ultrasound etc.)
- Pregnancy associated events (if SAE during pregnancy)
- Pregnancy outcome (abortion, delivery)
- Child outcome.

**12.7 INDIVIDUAL CODE-BREAK PROCEDURE**

Not applicable.

**12.8 NEW RELEVANT SAFETY INFORMATION**

Not applicable.

## **13. STATISTICS**

### **13.1 SAMPLE SIZE CALCULATION**

By definition, the recurrent UTI event data consist of the inter event time of repetition of the same or different types for each subject. The measurements across subjects are considered to be statistically independent, but the times between UTI events for a specific subject are not necessarily independent. The mean cumulative function (MCF) contains the information of interest in the analysis of recurrent data.

Assume  $M(t)$  is the mean cumulative number of UTI events up to time  $t$  [33].

$M(t) = E\{N(t)\}$ , where  $N(t)$  is a random variable for the number of events that have occurred up to time  $t$  [34]

The main assumption of this approach is that the hazard or risk ratio is proportional over time, reason why a robust sandwich variance estimated is used to account for dependence of recurrent events on the same subject.

Assuming that all subjects had the same study period examination (approximately 12 months), and considering an interval of risk (inter event times) following an Uniform distribution, data have been simulated based on a mean number of events equal to 3 for control group and an expected intervention effect of 20% less events in the intervention group. One hundred samples have been replicated upon the simulated data [35].

Considering the simulated data based on our hypotheses, a mean number of events during a 12 months period in control group equal to 3, the estimate sample size that would provide a power of 80% to detect a product effect of 20% less events occurred in the interventional study group for a bilateral test,  $\alpha=0.05$  will be equal to 42 subjects per group, i.e. 84 overall completed subjects (ratio 1:1).



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In order to achieve 84 completed subjects, and considering a 40% drop out rate, the screening process will last until we will reach 140 subjects randomised.

Considering a 50% screen failure rate, around 280 screened subjects will be needed in order to get 140 randomised subjects.

### **13.2 PLANNED STATISTICAL METHODS**

A Statistical Analysis Plan (SAP) will be drawn up before data review meeting, reviewed by the Investigator and statisticians during the Data Review Meeting and signed by the sponsor before the database lock.

Statistical analyses will be performed by a specialized CRO, selected, contracted and followed under the responsibility of DANONE RESEARCH/located in Palaiseau (France) and using appropriate statistical software SAS® V9 or later using the files included in the database locked.

#### *13.2.1 Descriptive statistics*

The distribution of study parameters will be summarised by group and overall depending on the type of variable for all criteria with a description of number of subjects and missing data.

The descriptive statistics for each criterion will be presented as follows:

- Continuous data: for each visit per group and overall: number of non-missing observations, number of missing observations, mean, median, standard deviation (SD), minimum and maximum. (Standard error of the mean [SEM], Confident interval (CI) and quartiles optionally provided if mentioned in the SAP).
- For nominal data: for each visit per group and overall: number of missing observations, number and frequency of observations in each class.

For qualitative ordinal parameters

- Table of frequency (and/or mean and/or median), if necessary per parameter.

#### *13.2.2 Statistical Hypotheses*

In the present study the null hypothesis “There is no difference between groups on the average number of recurrent UTIs events experienced” versus the alternative hypothesis “the effect of intervention is different to the effect of the control” will be tested.

The test will be based on a non-parametric method called the mean cumulative function (MCF), that will be used to analyse the multiple/repeated UTI events occurring.

$H_0$ : Effect of fluid intake = Effect control

$H_1$ : Effect of fluid intake  $\neq$  Effect control

Statistical tests will be conducted two-sided with a significance level of 5%. All confidence intervals will be presented two-sided with a confidence level of 95%. A resultant probability value of  $p < 0.05$  will be judged as being of statistical significance. The interaction factors (if any) will be considered as significant if the p value is  $< 0.10$ .

The MCF by group, estimated by a non-parametric estimator:

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$$\widehat{MCF}(t) = \sum_{\{j|t_j \leq t\}} \frac{e_j}{n_{j-1}}$$

where  $e_j$  is the number of events at time  $t_j$ ,  $n_{j-1}$  is the number of subjects at risk just beyond time  $t_{j-1}$ , and  $j$  the observed event times.

### 13.2.3 *Interim Analysis*

Not applicable.

### 13.2.4 *Distribution and normality assessment*

As a first step, the frequency distribution of the efficacy parameters will be analysed, including assessment of stem-and-leaf displays, boxplots and histograms per group and overall.

### 13.2.5 *Statistical criteria to stop the research*

Not applicable

### 13.2.6 *Methods for dealing with missing, unused or non-valid data*

The way of handling missing, unused or non-valid data will be discussed at the Data Review Meeting and detailed in the SAP.

### 13.2.7 *Management of modifications made to the initial strategy of the analysis plan*

All the modifications of the statistical methodology will be detailed and justified in the SAP and described in the Clinical Study Report.

## 13.3 DISPOSITION OF SUBJECTS AND POPULATIONS DEFINITION

### 13.3.1 *Disposition of subjects*

Disposition of subjects will be described as follows:

- Number of subjects included.
- Number of subjects eligible at V1.
- Number of subjects screen failed at V2 (reasons of screen failure will be described).
- Number of subjects randomised per group and overall.
- Number of subjects premature withdrawals / drop out (reasons of premature withdrawals / will be described per group and overall).
- Number of subjects completed per group and overall.

### 13.3.2 *Deviations and populations definition*

A definition of minor and major protocol deviations will be detailed in a document attached to the SAP. The Data Review Meeting will allow a global review of the study data and then the deviations status of subjects in order to determine analysed populations before the final data base lock.

The populations will be defined as follows:

- The “Global Population”: all subjects included in the study and eligible at the end of Visit 1.

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- The “Full Analysis Set” population (FAS): all subjects included in the study and randomised.
- The “Per Protocol” population (PP): all subjects included in the FAS population presenting no major protocol deviation.
- The “Safety Set” population (SS): all subjects included in the FAS population.

The analysis of baseline characteristics will be performed on the FAS population.

The analysis of main product effect criteria will be done on the FAS population. If the difference in number of subjects is few between the FAS and PP populations (difference lower than 10%), the analyses of the product effect criteria will be only done on the FAS population, except for the main criterion, for which the analysis will be performed on both populations.

The analysis of safety criteria will be done on the Safety Set (SS) population.

#### **13.4 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS**

A descriptive analysis of subjects at baseline, corresponding to the data collected at inclusion and pre-randomisation visits, will be performed by group and overall.

Following parameters will be described:

- Demography, vital signs, anthropometrical parameters (body weight, waist circumference, height, BMI).
- Medical and surgical history
- Physical examination
- Quality of Life (SF12v2)
- Fluid intake consumption
- Contraception habits/method

#### **13.5 STUDY CONDUCT PARAMETERS**

##### *13.5.1 Compliance*

Subjects record the daily intake of study product in a diary on a daily basis (quantity of 3 daily bottles consumed per day). Parameters concerning compliance will be described by group and overall:

- Study product compliance
- Consumption of forbidden dietary products and treatments

##### *13.5.2 Quality of Life*

A quality of life questionnaire (**SF-12v2**) will be filled in by the subject at:

- the pre-randomisation visit (V2),
- at 6-months (V3),
- at the last visit (V4),

in order to explore the quality of life of the subjects.

The short form 12 (SF-12v2) is a validated and frequently used questionnaire for the assessment of quality of life. It was developed from the more extensive short form 36 (SF-36) [<http://www.sf-36.org/tools/sf12.shtml>]. It includes 12 questions from the SF-36: 2 questions concerning physical functioning; 2 questions on role limitations because of physical health problems; 1 question on body pain; 1 question on general health perceptions; 1 question on vitality (energy/fatigue); 1 question on social functioning; 2 questions on role limitations because of emotional problems; and 2 questions on general mental health, psychological distress and psychological well-being. This will be a self-

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assessment questionnaire. However, if the subject is frail and requires help with filling in the form then the site staff will help.

A preference-based utility index, **called the SF-6D**, is also available to help understand economic benefit and will be assessed in order to perform a cost utility analysis.

- 12-Items
- 8- Dimensions: Physical functioning, Role Physical, Bodily pain, Vitality, General health, Mental health, Social functioning and role emotional
- 2 composite score: Mental health and physical health
- Utility index based on SF-6Dimensions

### 13.6 EVALUATION CRITERIA

#### 13.6.1 *Main evaluation criterion*

The main evaluation criterion is the number of recurrent UTI event experienced over a one (1) year study period between intervention and control groups, evaluated by the difference between groups in the mean cumulative function. The primary criterion will be considered as statistically significant if confidence interval observed on the MCF difference between groups excludes zero, which suggests a statistical test and p value associated <0.05.

#### 13.6.2 *Secondary evaluation criterion*

- Difference between groups in terms of antibiotic prescription/usage to treat UTI event over 12 months of study intervention from D0
- Difference between groups on average delay between each UTI event over 12 months of study intervention from D0
- Difference between groups on urinary hydration markers over 6 and 12 consecutive months of study intervention from D0
- Difference between groups in terms of health-costs associated with management of UTI recurrence(s) over 12 months of study intervention from D0
- Difference between groups in terms of cost utility analysis over 12 months of study intervention from D0
- Difference between groups in terms of change in QoL over 6 and 12 consecutive months of study intervention from D0.

The methodology to address the potential multiplicity issues will be detailed in the SAP.

#### 13.6.3 *Exploratory criteria*

- Relationship between urinary hydration markers and number of UTI events over 12 consecutive months of study intervention from D0
- Relationship between urinary hydration markers and delay UTI events over 12 consecutive months of study intervention from D0
- Impact of increased daily water intake on crystallisation risk index (Tiselius) over 12 consecutive months of study intervention from D0
- Number of UTI events confirmed by positive urine culture and number of subjects presenting at least one UTI event during the intervention period from D0 (by group)
- Number of UTI events not confirmed by positive urine culture and number of subjects presenting at least one UTI event during the intervention period from D0 (by group)
- Number of UTI events confirmed or not by positive urine culture and number of subjects presenting at least one UTI event during the intervention period from D0 (by group).

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*13.6.4 Safety criteria*

*13.6.4.1 Extent of exposure / Study duration*

Descriptive summaries will be performed on study duration and extent of exposure by group and overall.

*13.6.4.2 AE / SAE*

The analysis of Adverse Events will be performed to evaluate the number of subjects with at least one adverse event and the number of adverse events by study group.

The adverse events will be presented by "body system" and "Preferred term" according to the MEDDRA coding system.

All adverse events will be summarised, in individual data listings, by subject with all details concerning the adverse event, the study product, the emergence, the intensity and the relationship to study product.

The Serious Adverse Events will be summarised, in individual data listings, by subject with all details concerning the adverse event, the study product, the emergence, the intensity and the relationship to study product.

*13.6.4.3 Concomitant Medications*

The concomitant medications will be presented by ATC class (ATC1 and ATC3) according to the WHO-Drug coding system.

All concomitant medications will be summarised, in individual data listings, by subject with all details concerning the concomitant medications and the study product.

*13.6.4.4 Laboratory measurements*

Descriptive summaries will be performed for the following parameters by study group and overall:

Blood sample:

- lipidic profile: HDL cholesterol, LDL cholesterol, Total Cholesterol, triglycerides (mmol/L)
- glycosylated haemoglobin (%)
- serum creatinine ( $\mu\text{mol/L}$ ),
- serum urea (mmol/L),
- copeptin ( $\mu\text{g/L}$ )
- estimated glomerular filtration rate (eGFR) will be calculated.

Urinary markers of hydration:

- urine volume (L)
- pH
- osmolality (mOsm/kg)
- specific gravity
- frequency of micturition
- urine colour

Other urinary parameters:

- Electrolytes: sodium, potassium, calcium, magnesium, chloride (mmol/L), oxalate ( $\mu\text{mol/24h}$ ), and citrate (mmol/L)
- Urine creatinine (mmol/L)
- Crystallisation risk index (Tiselius).

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Information about bladder emptying after sexual intercourses and sexual activity during the last month will be also summarised by study group and overall.

#### *13.6.4.4 Vital signs, anthropometrical and other parameters*

Descriptive summaries will be performed for the following parameters by study group and overall:

- Systolic and diastolic blood pressure (mmHg)
- Heart rate (bpm)
- Axillary Body temperature (°C)
- Body weight (kg)
- Waist circumference (cm)
- Height (m)
- Body Mass Index (BMI) (kg/m<sup>2</sup>)

## **14. STUDY REPORT AND PUBLICATION**

All information stemming from the study will be considered confidential and should not be divulged without the prior agreement of the sponsor (DANONE RESEARCH).

Following analysis of the study data, a final study report will be prepared in UK English, format ICH E3, according to the standard model of DANONE, describing the conditions under which the study was performed as well as the results. This report will be prepared and signed by the Sponsor's representative. The signature of the Principal Investigator will be requested if relevant.

The identity of study subjects will under no circumstances be communicated to the sponsor.

Publication may be done only if before recruitment of the first subject, the sponsor has registered the study in a publicly accessible clinical trial database.

No publication will be done based upon interim analysis or result of one site of a study except if a written agreement has been signed by the sponsor.

## **15. STUDY MONITORING AND AUDITS**

Study monitoring is the act of overseeing the progress of a clinical study and of ensuring that:

- i) The rights and the well-being of human subjects are protected.
- ii) The reported trial data are accurate, complete, and verifiable from source documents.
- iii) The conduct of the clinical study is in accordance with the approved protocol/amendment(s), Good Clinical Practice (GCP, ICH E6), and the applicable local regulatory requirement(s).

Monitoring includes on-site visits to assure that the study is conducted according to the approved protocol/amendment(s) and in order to comply with applicable regulations and deadlines. On-site monitoring includes, at least, the review of Informed consents, safety reporting, e-CRFs and supports to the site management regarding protocol conduct and compliance or deviation(s).

The monitoring includes the review of forms for completeness, clarity, and consistency with source documents available for each subject and the management of the essential documents (Investigator Study File).

The Investigator and the investigating site staff must permit and be available for study-related monitoring visits, audits, review by the ethics committee and regulatory inspections, and allow direct access to source data and

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source documents provided that subject confidentiality is protected. In case of an audit appointed by DANONE RESEARCH, the Investigator will receive written notification in advance.

Study monitoring, under the responsibility of DANONE RESEARCH will be performed by a qualified staff of a company contracted by DANONE RESEARCH at various stages/on regular basis of the study (frequency of visits is specified in the Monitoring plan). Throughout the duration of the study period, the e-CRFs will be completed and signed by the Investigator, and he/she will be controlled individually by a Clinical Research Associate (CRA) designated by DANONE RESEARCH in order to verify data quality and compliance with study protocol and Good Clinical Practice (GCP, ICH E6).

Upon closure of the study, the company contracted by DANONE RESEARCH will perform study closeout.

## **16. ETHICAL CONSIDERATIONS**

### **16.1 BASIC PRINCIPLES AND REGULATIONS**

The Investigator must ensure that this study is conducted in full compliance with the principles of the ‘World Medical Association Declaration of Helsinki’ (64th WMA General Assembly, Fortaleza, Brazil, October 2013) (Appendix I), ICH guidelines for Good Clinical Practice as appropriate for nutritional products, and local legislation of the country in which the research is conducted, whichever affords the greater protection to the participants.

### **16.2 ETHICS COMMITTEE**

This protocol and any accompanying material provided to the subjects, such as information and informed consent sheets, are submitted to the applicable Ethics Committee (IRB/IEC) by the Investigator according to local legislation. Approval from the Ethics Committee must be obtained before starting the study, and should be documented in a letter to the sponsor specifying the date on which the Ethics Committee met and granted the approval, the composition of the Ethics Committee and their qualifications, and version and date of all submitted documents. If applicable, the documents will also be submitted to the Competent Authority in accordance with the local regulatory and legal requirements.

This study will be undertaken after approval from the appropriate Ethics Committee(s) (IRB/IEC).

During the study course, change(s) in any aspect of the study, such as modification(s) of the protocol, written ICF and any other written information to be provided to subjects should be submitted to the Ethics Committee (IRB/IEC).

If required, depending on local legislation, the Investigator must submit an annual progress report to the Ethics Committee which gave the favourable opinion. Annual progress reports should be submitted thereafter until the end of the study. DANONE RESEARCH could assist in the preparation/submission process.

Subject recruitment will start only after reception of a favourable opinion from the Ethics Committee.

### **16.3 RECRUITMENT AND INFORMED CONSENT FORM**

The requirements of the research, the objectives of the research, the detailed research protocol and the risks and constraints of the research associated with this study must be explained to each subject both orally and in writing (subject information sheet) by the Investigator, or a person designated by the Investigator, before the start of the study.

The subject must personally initial each page of the Information to the subject and sign and date a written informed consent form (ICF) to take part in this study before the study starts (before the screening period, if applicable). This form must also be initialled and signed by the Investigator or a Physician designated by the Investigator. Two copies of the ICF are dated and signed: one is given to the subject and one is retained on site in the subject's files.

Subjects may withdraw from the study at any time without having to provide justification. The confidentiality of medical data must be upheld.

Any substantial changes in the ICF and written information should receive the IRB/IEC's approval/favourable opinion before any use.

### **16.4 CONFIDENTIALITY OF STUDY DATA**

All information collected during the study is to be considered confidential and must not be disclosed without prior written agreement by the sponsor. The identity of study subjects must under no circumstances be communicated to the sponsor or to any official bodies.

### **16.5 COMPENSATION OF SUBJECTS**

The subjects will be compensated for their participation in this clinical study on a pro rata basis. Compensation amount and the method and timing of disbursement are consistent with applicable regulatory requirement(s) of Bulgaria.

Each subject will receive an indemnity of 557 Bulgarian Leva for participating in this study if she has completed all study visits or in case of discontinuation due to medical reasons related to the protocol. In any other case of withdrawal, the subject will receive an indemnity for participation on a pro rata basis.

The reimbursement will be as follows:

- Inclusion visit (V1) = 28 Bulgarian Leva
- Pre-randomisation visit (V2)= 158 Bulgarian Leva
- Evaluation visit (V3) = 176 Bulgarian Leva
- Evaluation visit (V4)= 195 Bulgarian Leva

### **16.6 PROTOCOL AMENDMENTS**

Any protocol modification should be the object of an amendment, which will be dated and signed by the same parties than those invested in the initial protocol signature.

The amendment will be submitted to the appropriate Ethics Committee (IRB/IEC), either for approval or for information, depending on the nature and the importance of the changes to the study conditions.



### **16.7 STUDY SUSPENSION**

If the study or part of the study is prematurely terminated or suspended, the Investigators, the appropriate Ethics Committee(s) (IRB/IEC) should promptly be informed as specified by the applicable regulatory requirement(s).

### **16.8 PROTOCOL DEVIATIONS**

No deviations are tolerated without sponsor approval. Any deviation from the approved protocol related to study eligibility criteria, conduct of the trial, subject's management or subject's assessment should be documented and explained

The main categories of deviations are based at least on the following:

- Informed consent process and documentation,
- Enrolment: Assessment of the 'low drinker profile' (based on the coherence between fluid intake volume, complete 24H urinary volume, urine osmolality and urine creatinine),
- Randomisation: Eligibility criteria based on documented medical history of diseases,
- Biological sample collection (i.e. blood samples and/or urine samples and voiding diary),
- Product compliance and product exposure duration (based on subject compliance dairies, 3-days flude intake diary and questionnaire),
- Time respect between visits,
- Follow-up of dietary and forbidden treatment/medication,
- Safety issues (e.g lack of declaration of UTI symptoms),
- And any other procedure described in the study protocol.

DANONE RESEARCH will be informed of all protocol deviations, and these will be discussed before implementation according to prospective protocol deviation process or later at the data review meeting (blind review meeting), in order to define their status (minor – major).

### **16.9 INSURANCE**

DANONE RESEARCH has contracted an insurance policy with an established insurance company in accordance with current regulatory requirements in Bulgaria to cover potential damage to subjects through injury or death caused by the study product and/or study procedures.

The insurance applies to the damage that becomes apparent during the study. DANONE RESEARCH also has liability insurance in accordance with the applicable legislation.

## **17. DATA COLLECTION, PROCESSING, AND MANAGEMENT**

### **17.1 CASE REPORT FORM (CRF)**

An electronic CRF (e-CRF) will be created by the Sponsor or its delegate in English. The final version will be approved by the Sponsor. The relevant study data defined in this Protocol will be collected and entered by the Investigator or the responsible team members into the provided e-CRF. An e-CRF Completion Guideline will be provided to the Investigator to facilitate e-CRF completion as well as provide answers for Frequently Asked Questions (FAQs)

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Quality of life questionnaire will be provided by the Sponsor to the Investigator:

- Original form should stay at the investigator's site and a copy should be sent to the Biometry Contract Research Organisation (CRO) for data processing.

Each subject will be identified with a unique Subject Identification number (please refer to the section 7.3). All study documents related to a subject must be identified with this subject identifier.

All relevant e-CRF data will be single-entered by personnel at the Investigational site in the validated e-CRF tool put in place by the sponsor. The Investigator must ensure that these data are complete and accurately represent the study subject information by electronically signing the appropriate forms in the e-CRF.

QoL questionnaires and subject diaries will be in paper and single-entered by the CRO Data-Management in the validated e-CRF tool put in place by the sponsor. These questionnaires will be in paper and in Bulgarian but entered in English in the e-CRF by the PI.

All external data will be provided to the CRO Data-Management for integration to the database.

The sponsor will ensure all study site personnel are appropriately trained on the use of the e-CRF (i.e. data entry, queries, sign-off etc.). This training is MANDATORY for all site personnel (who are expected to use the system) and must be completed before access is granted to the system. Each user will be provided with a unique access (user name and password) to the e-CRF.

The combination of the user name and password to access to the e-CRF are the legally binding equivalent of a traditional handwritten signature, and as such, carries all of the same rights and responsibilities. User accounts are not interchangeable, and must only be used by the person authorized to use that account.

## **17.2 DATA PROCESSING**

Edit checks, to ensure the quality and consistency of the data, will be defined in a Data Validation Plan (DVP). These edit checks will be programmed and validated in the e-CRF. During the data entry process, these checks will automatically be triggered (and become apparent to the user) in case of incoherent data.

## **17.3 DATA AUDIT TRAIL**

Any addition, modification, or deletion of subject data made after the initial entry (i.e. response to Data Clarification Forms (DCFs) or updated information) will be automatically tracked in the e-CRF's audit trail in compliance to ICH GCP and US FDA 21 CFR part 11.

## **17.4 DATA MANAGEMENT**

Data Management (DM) tasks for this study will be performed by a delegated Data-Management CRO. The verification and validation of Data-Management tasks will be performed by a Data Manager internal at the Sponsor. All Data-Management tasks will be defined in a Data Management Plan (DMP) based on the current Sponsor and CRO Standard Operating Procedures (SOPs).

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### **17.5 EXTERNAL DATA**

Data Transfer specifications for external data (i.e. central laboratory data) will be defined in a Data Transfer Agreement (DTA). All electronic external data transfers will be reconciliated with the main study data and validated by the CRO Data-Management to ensure accuracy and consistency.

### **17.6 DATABASE LOCK**

After all planned subject visits have been completed, entered into the study database, and considered clean, a Data Review Meeting (DRM) will be held by the Sponsor to discuss and review all study data. The main objective of this Data Review Meeting (DRM) will be to review tables, listings and summaries on study data, to review safety listings, to identify protocol deviations and to define subject analysis populations. Members of the Sponsor's Study Core Team, the designated CRO's as well as the Principal Investigator(s) may participate in this meeting.

Once any final issues resulting from the Data Review Meeting (DRM) have been resolved, the study database, including all external data will be locked. This will ensure that no further modifications to the study data are possible. The statistical analysis will be performed on this locked database.

Following the database lock, the sponsor will provide each Investigational site with a final, unmodifiable PDF copy of the completed e-CRFs, Questionnaires and Subject Diaries (including the audit trail) of all subject's included at that site. This constitutes an exact representation of the information that was entered in the e-CRF during the study. This will be provided on removable media (i.e. CD, DVD etc).

## **18. DOCUMENTATION AND ARCHIVING**

DANONE RESEARCH provides the Principal Investigator (s) with an Investigator Site File (ISF). Each Principal Investigator (s) is responsible to keep this ISF updated and available for review by the study monitor (CRA).

All documents pertaining to the conduct of the study must be kept by the Investigator for a period of 15 years.

Study documents should not be destroyed without prior written agreement between DANONE RESEARCH and the Investigator. Should the Investigator wish to assign study documents to another party, or move them to another location, DANONE RESEARCH must be notified first.

### **18.1 SPONSOR**

The following documents are to be archived by the sponsor for a minimum of 25 years after the completion of the study, in a room specifically designated for this purpose, access to which is controlled by the person responsible for archiving:

- Final version of the study protocol,
- Any forms containing protocol amendments,
- **FOR QoL questionnaire:** Original pages
- **FOR e-CRF:** The media (i.e. CD, DVD etc.) that contains the final PDF copy of the subject e-CRFs, Questionnaires, Subject Diaries (including audit trail) that was extracted from the e-CRF following the database lock,
- Ethics Committee approval forms,

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- All correspondence between the sponsor and the Investigator,
- Curriculum vitae (CV) of Principal Investigator and other Investigators,
- Acknowledgements of receipt – as well as all other documents generated during the study.

## **18.2 INVESTIGATOR**

All documents concerning this study must be kept by the Investigator, including but not limited to:

- Subject's medical files including source documents
- Original (dated and signed) of the ICFs and the subject's identification code list
- Screening and enrolment Log
- **FOR QoL questionnaire:** Copy
- **FOR e-CRF:** The media (i.e. CD, DVD etc.) provided by the sponsor that contains the final PDF copy of the subject e-CRFs, Questionnaires, Subject Diaries (including audit trail)
- Copy of accountability forms for products administered
- Copy of ethics committee approval(s) and correspondence with the sponsor
- Signed Protocol(s), signed amendment(s)
- Advertisement (if any)
- Financial Contract(s)
- Insurance certificate
- EC correspondence including Approval Opinion and composition
- CV of Investigator and sub Investigator(s) and delegation task list
- Normal Values and technical procedures
- Instruction for handling products, shipping records, products accountability
- Decoding procedure
- Serious Adverse Events report forms

All correspondence between the sponsor and the Investigator,

The Investigator agrees to provide direct access to source documents during monitoring visits.

## **19. OWNERSHIP OF RESULTS**

All information and results issued from the study remain the property of the sponsor.

The study results may be published or presented by the Investigator or by experts responsible for analysis, in collaboration with the sponsor and with the prior written approval of the latter. The sponsor may use the results of the study for publications or communications with the written agreement of the Investigator or experts responsible for analysis if the latter are cited.

## **20. RESPONSIBILITIES**

### **20.1 SPONSOR**

- a. Manage the submission of the study protocol to the ethics committee before the start of the study. The Sponsor should obtain the approval from all the competent authorities and from the EC.
- b. Before the start of the study, the sponsor must provide the Investigator with all documents required by the protocol and/or to provide information on the study products. In particular, the Sponsor must provide the Investigator with a document certifying that the study products are fit for human consumption.
- c. The Sponsor must take out a specific insurance policy for the study as required by current legislation of the region in which the study is being conducted.
- d. The Sponsor must provide the Investigator with insurance certification. The Sponsor may decide to terminate the study at any time. At the end of the study, the Sponsor must archive the study documents for the legally required duration and at least for 25 years.

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- e. The Sponsor must carry out all procedures required by the relevant EC including initial/amendment submission and Safety reporting.
- f. The Sponsor must set up data Quality Control at each stage of data handling.
- g. The Sponsor finances expenses related to the study
- h. The sponsor must monitor the study and ensure the correct adherence to protocol requirements and regulatory requirement are maintained (including the data protection and confidentiality)
- i. The Sponsor must record the study in the appropriate Governmental database

**20.2 INVESTIGATOR**

- a. Provides oral and written information for subjects and selects subjects in accordance with protocol inclusion and non-inclusion criteria.
- b. Keeps all study related information confidential
- c. Manages the study products including randomisation, storage, dispensation, destruction, site accountability based on the data provided in the compliance diaries and on the acknowledgements of receipt
- d. Before the study, provides the following documents to the sponsor:
  - CV of Principal Investigator and co-Investigators,
  - CV of the entire remaining investigating site staff involved in the study.
- e. Performs the clinical study within the scheduled dates.
- f. Establishes a written delegation task list, and allocate sufficient time and resources to properly conduct the study
- g. Reports of SAEs.
- h. Cooperates with Clinical Research Associates at the monitoring visits, or in case of audits and/or inspections
- i. Completes and corrects the e-CRFs, Data clarification Forms.
- j. Give input in the clinical report for the study, if relevant.
- k. Maintain essential documentation
- l. Archives data for a minimum of 15 years after the date of the final report.

**20.3 MONITORING CLINICAL RESEARCH ORGANISATION (CRO)**

- a. Provides the required study materials in good time
- b. Performs Study start-up visit
- c. Verifies and updates the Trial Master File (TMF) and Investigator Study File SMF)
- d. Performs the Monitoring during the study conduct (including at least the verification of the signed Informed consent, the validation of the data recorded in the e-CRF including Safety data from source documents
- e. Performs the End-of-study visit
- f. Communicate relevant information to the Sponsor
- g. Verifies the Data collection (accurate, complete and verifiable)

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## **22. APPENDICES**

# **APPENDIX I**

## **DECLARATION OF HELSINKI**

## **WORLD MEDICAL ASSOCIATION**

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:  
29th WMA General Assembly, Tokyo, Japan, October 1975  
35th WMA General Assembly, Venice, Italy, October 1983  
41st WMA General Assembly, Hong Kong, September 1989  
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996  
52nd WMA General Assembly, Edinburgh, Scotland, October 2000  
53rd WMA General Assembly, Washington, DC, USA, October 2002 (Note of Clarification added)  
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)  
59th WMA General Assembly, Seoul, Republic of Korea, October 2008  
64th WMA General Assembly, Fortaleza, Brazil, October 2013

### **PREAMBLE**

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

### **GENERAL PRINCIPLES**

3. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

### **RISKS, BURDENS AND BENEFITS**

16. In medical practice and in medical research, most interventions involve risks and burdens.

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Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

#### **VULNERABLE GROUPS AND INDIVIDUALS**

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

#### **SCIENTIFIC REQUIREMENTS AND RESEARCH PROTOCOLS**

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

#### **RESEARCH ETHICS COMMITTEES**

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

#### **PRIVACY AND CONFIDENTIALITY**

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

#### **INFORMED CONSENT**

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be included in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

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All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

### **USE OF PLACEBO**

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

### **POST-TRIAL PROVISIONS**

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

### **RESEARCH REGISTRATION AND PUBLICATION AND DISSEMINATION OF RESULTS**

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

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### UNPROVEN INTERVENTIONS IN CLINICAL PRACTICE

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

### ARTICLE INFORMATION

**Corresponding Author:** World Medical Association, 13, ch. du Levant, CIB - Bâtiment A, 01210 Ferney-Voltaire, France; [wma@wma.net](mailto:wma@wma.net).

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**APPENDIX II**

**SERIOUS ADVERSE EVENT**

**TRANSMISSION FORM**

Life Science Clinical Studies & Biometrics – Dairy & Waters

## Serious Adverse Event Report Form

This form must be faxed to the Sponsor within 24h

<b>Sponsor:</b> <b>DANONE RESEARCH</b>	<b>Fax :</b>	<b>Tel :</b>	
	<b>Clinical Study Manager (Name):</b>		
<b>Principal Investigator (Name):</b>			
<b>Study code : NU</b>    _ _ _	<b>Expeditor (Name):</b>	<b>Country :</b>  _ _ _	<b>Site Number:</b>  _ _ _
<b>Transmission date :</b>  _ _ / _ _ / _ _ _ _  (dd/mm/yyyy)			
Number of pages (incl. this page)	_____ <b>Pages</b>		
Attached copies of completed e-CRF pages:	<input type="checkbox"/> Adverse events <input type="checkbox"/> Medical History and pre-existing conditions <input type="checkbox"/> Medications		

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This SAE report is :  Initial report  
 Follow-up report (Includes only new details)

Is the SAE aggravation of a pre-existing AE recorded in the e-CRF:  Yes  No

If yes, please put the number of AE: |\_|\_|

**Subject Details**

Subject Number: |\_|\_|\_|/|\_|\_|\_|/|\_|\_|\_|

Gender:  M  F

Subject Initials: |\_|\_|\_|/|\_|

Date of birth: |\_|\_|/|\_|\_|/|\_|\_|\_|\_| (dd/mm/yyyy)

Height: |\_|, |\_|\_| m Weight : |\_|\_|\_|, |\_|kg

**Description of Serious Adverse Event**

Nature of SAE (diagnosis or major symptom and/or sign):

Description of SAE:

Severity of SAE:  Mild  Moderate  Severe

Event start date: |\_|\_|/|\_|\_|/|\_|\_|\_|\_| (dd/mm/yyyy)

Event end date: |\_|\_|/|\_|\_|/|\_|\_|\_|\_| (dd/mm/yyyy)

Time : |\_|\_| : |\_|\_| (Hours:Minutes)

Time : |\_|\_| : |\_|\_| (Hours:Minutes)

**Category of SAE (\*as applicable to study protocol)**

- Death  Persistent or significant disability or incapacity  
 Life threatening situation  Congenital anomaly or birth defect  
 Hospitalization or Prolongation of existing Hospitalization

**Study Product**

**According to protocol, the dosage is:**

Dose: |\_|\_| Unit (Pot/Bottle): \_\_\_\_\_ Frequency: Daily Route: Oral

**Information of study production distribution**

Study Product Number(s)	Last Product Batch Number:	Product Expiry date (dd/mm/yyyy)	Date of Last Product Distribution (dd/mm/yyyy)
_ _ _ _ _	_ _ _ _ _	_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _

**Information of study product intake**

Date of last product intake (dd/mm/yyyy)	Date of last product intake before event (dd/mm/yyyy)
_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _

**Has the dosage been respected before the event?**

Yes  No  
If No, specify: Dose: |\_|\_| Unit (Pot/Bottle): \_\_\_\_\_  
Frequency: Daily



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<b>Action taken with respect to the study product:</b>	<input type="checkbox"/> Dose Not Changed <span style="float: right;"><input type="checkbox"/> Not applicable</span>		
	<input type="checkbox"/> Product Interrupted Stop date:  _ _ / _ _ / _ _ _ _  Did reaction disappear after stopping product? <input type="checkbox"/> Yes <input type="checkbox"/> No Reintroduction date:  _ _ / _ _ / _ _ _ _  Did reaction reappear after reintroduction? <input type="checkbox"/> Yes <input type="checkbox"/> No  <input type="checkbox"/> Product withdrawn => if yes please enter the stop date  _ _ / _ _ / _ _ _ _  (dd/mm/yyyy)		
<b>Has the code been broken?</b>	<input type="checkbox"/> Yes	<input type="checkbox"/> No	<input type="checkbox"/> Not Applicable

Action taken with respect to the subject (check all that apply)	Outcome of SAE for the subject	Relationship to	
		Study Product	Study Procedure
<input type="checkbox"/> None <input type="checkbox"/> Medication <input type="checkbox"/> Hospitalization <input type="checkbox"/> Study discontinuation <input type="checkbox"/> Study withdrawal <input type="checkbox"/> Other, specify: _____ _____ _____ _____	<input type="checkbox"/> Not Recovered <input type="checkbox"/> Recovering <input type="checkbox"/> Recovered with sequelae, being: ..... <input type="checkbox"/> Recovered without sequelae <input type="checkbox"/> Fatal /Death <input type="checkbox"/> Unknown	<input type="checkbox"/> Not related <input type="checkbox"/> Unlikely related <input type="checkbox"/> Possibly related <input type="checkbox"/> Probably related <input type="checkbox"/> Definitely related	<input type="checkbox"/> Not related <input type="checkbox"/> Unlikely related <input type="checkbox"/> Possibly related <input type="checkbox"/> Probably related <input type="checkbox"/> Definitely related

Relevant medical and surgical history / or copy of e-CRF pages (e-CRF page XXX) related to medical and surgical history				
No.	Disease or Surgery	Start date (dd/mm/yyyy)	End Date (dd/mm/yyyy)	Current medication /nutritional supplement?*
1		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
2		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
3		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
4		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
5		_ _ / _ _ / _ _ _ _	_ _ / _ _ / _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No

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6		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
7		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
8		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
9		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
10		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
11		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
12		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
13		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
14		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
15		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No

## **APPENDIX III**

# **MANAGEMENT OF ANY UTI SYMPTOM(S) AND POSITIVE URINE CULTURE DURING THE STUDY**

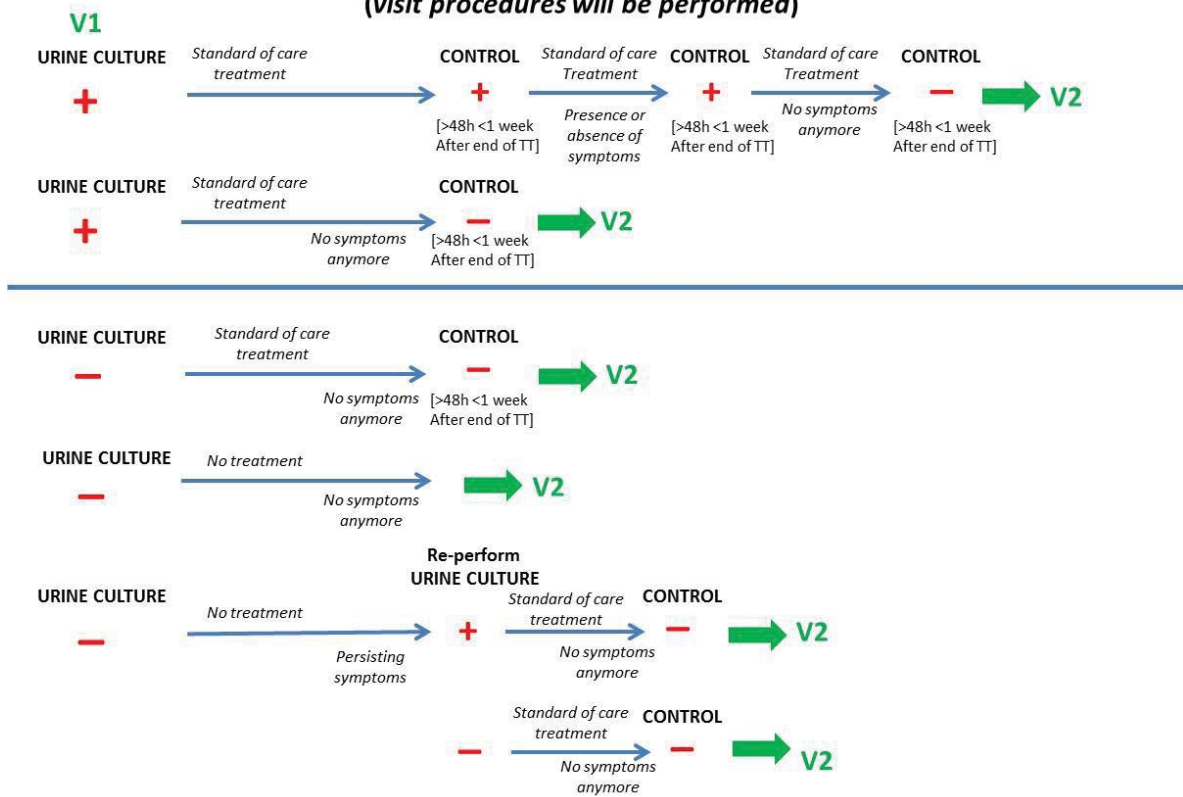
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Subject				
Visit	Symptomatic & B +	Symptomatic & B -	Asymptomatic & B +	Asymptomatic & B -
V1 Urine culture only on symptomatic subjects	Complete an Adverse Event form and Concomitant medication page		NA	NA
V2 Urine culture for all subjects	Complete an Adverse Event form and Concomitant medication page			-
Randomization – D0	NA	NA	NA	NA
V3 Urine culture only on symptomatic subjects	Complete Relapse visit and UTI related Concomitant Treatment		NA	NA
V4 Urine culture for all subjects	Complete Relapse visit and UTI related Concomitant Treatment		Complete an Adverse Event form and Concomitant medication page	Complete EoS form with date of last urine culture

## **APPENDIX IV**

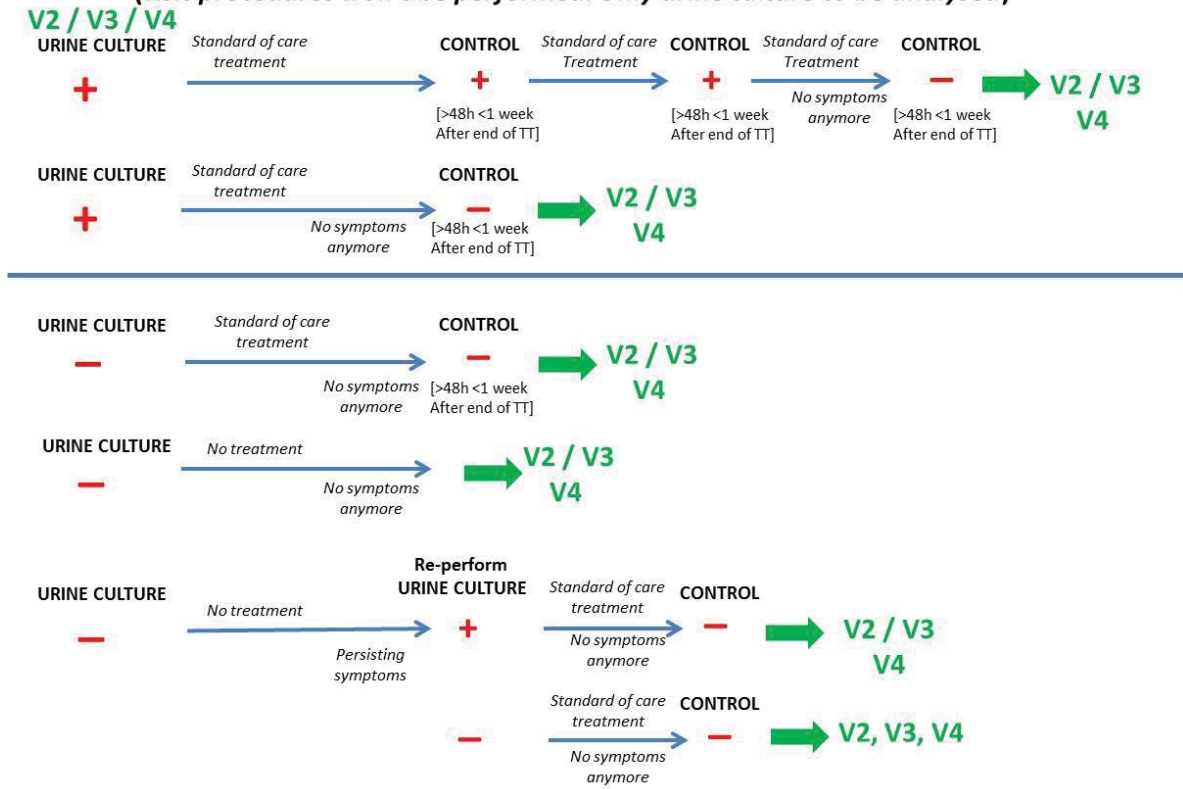
# **MANAGEMENT OF SYMPTOMATIC/ASYMPTOMATIC SUBJECTS PER VISITS**

**Management of V1 for SYMPTOMATIC subjects**  
**(visit procedures will be performed)**



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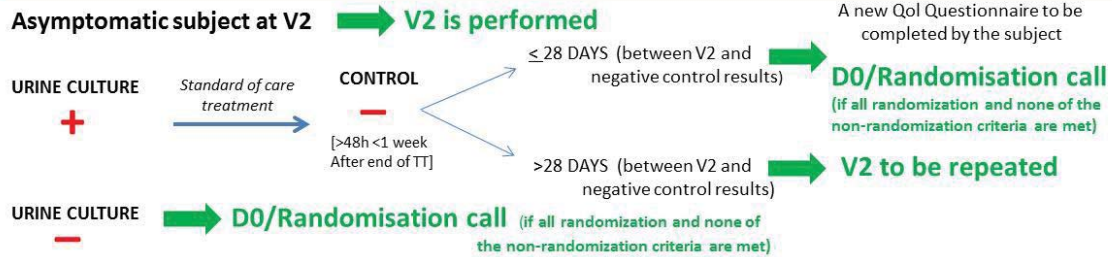
**Management of V2 / V3 / V4 for SYMPTOMATIC subjects**  
*(visit procedures won't be performed. Only urine culture to be analysed)*



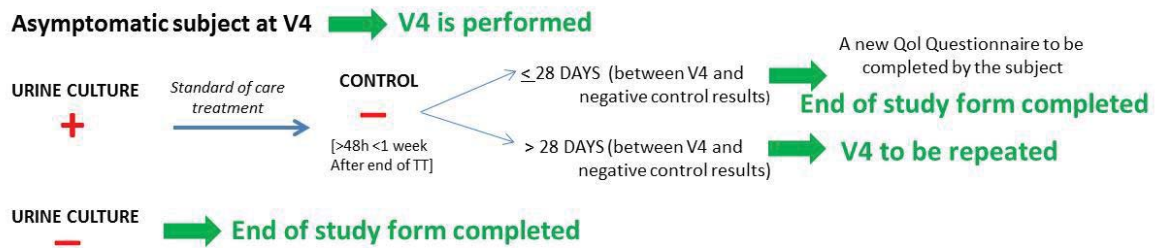
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**Management of V1 / V2 / V3 / V4 for ASYMPTOMATIC subjects**  
*(visit will be performed)*

Asymptomatic subject at V1 → V2 is performed



Asymptomatic subject at V3 → V3 is performed





## CLINICAL STUDY PROTOCOL

**PRODUCTS:** WATER

**PROTOCOL VERSION** 4 (AMENDMENT 3)

**STUDY CODE:** NU369

**AMENDED PROTOCOL**

**DATE:** 29/02/2016

***The effect of increased water intake on the  
frequency of clinical recurrent urinary tract  
infections in pre-menopausal women:  
S-HYDRACYST***

**Principal Investigator:**

**MAYA DABCHEVA, MD**  
MC "COMAC MEDICAL"  
13 URVICH STR., 3<sup>RD</sup> FLOOR  
1612 SOFIA – BULGARIA  
TEL.: +359 2 850 97 04      FAX: +359 2 850 60 96  
E-MAIL: MAYA.DABCHEVA@COMAC-MEDICAL.COM

**Sponsor:**

DANONE RESEARCH  
ROUTE DEPARTEMENTALE 128  
91767 PALAISEAU CEDEX – FRANCE

**Sponsor Representative:**

**CHRISTINE M'RINI, MD, PHD**  
LIFE SCIENCE DIRECTOR  
DANONE RESEARCH  
ROUTE DÉPARTEMENTALE 128  
91767 PALAISEAU CEDEX – FRANCE  
TEL.: +33(0)6 18 78 56 17      FAX: +33(0)1 69 35 70 42  
E-MAIL: CHRISTINE.MRINI@DANONE.COM

**Emergency Contact:**

**CHRISTINE M'RINI, MD, PHD**  
TEL.: +33(0)6 18 78 56 17      FAX: +33(0)1 69 35 70 42

**Ethics Statement**

This study will be conducted according to the protocol and in compliance with Good Clinical Practice (GCP), the ethical principles stated in the Declaration of Helsinki, and other local applicable regulatory requirements.

**Confidentiality Statement**

The information provided in this document is the property of DANONE RESEARCH, and is shared with you and your staff in confidence. This information should not be disclosed to others without written authorization from DANONE RESEARCH, except to the extent necessary to ensure adequate conduct of the study.

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## 1. GENERAL INFORMATION

### 1.1 LIST OF PARTICIPANTS

<b>Principal Investigator:</b>	<b>MAYA DABCHEVA, MD</b> MC "Comac Medical", 13 URVICH STR., 3 <sup>RD</sup> FLOOR 1612 SOFIA - BULGARIA Tel.: +359 2 850 97 04      Fax: +359 2 850 60 96 E-MAIL: MAYA.DABCHEVA@COMAC-MEDICAL.COM
<b>Research Scientist/Engineer:</b>	<b>MARIACRISTINA VECCHIO</b> DANONE RESEARCH ROUTE DEPARTEMENTALE 128 91767 PALAISEAU CEDEX - FRANCE Tel.: +33 (0)1 69 35 68 47      Fax: +33(0)1 69 35 76 93
<b>Biostatistician:</b>	<b>QUENTIN DORNIC</b> DANONE RESEARCH ROUTE DEPARTEMENTALE 128 91767 PALAISEAU CEDEX - FRANCE Tel.: 33(0)6 13 33 01 00.      Fax: +33 (1) 69 35 70 42
<b>Sponsor's Medical Reviewer:</b> <i>[to be contacted for safety reporting]</i>	<b>LILIANA GALETESCU, MD</b> DANONE RESEARCH ROUTE DEPARTEMENTALE 128 91767 PALAISEAU CEDEX – FRANCE Tel. : +33(0)1 69 35 72 34      Fax: +33(0)1 69 35 70 42
<b>Emergency contact:</b>	<b>CHRISTINE M'RINI, MD, PHD</b> TEL.: +33(0)6 18 78 56 17      FAX: +33(0)1 69 35 70 42
<b>Clinical Study Manager:</b>	<b>LAURENCE DE LA HOSSERAYE</b> [HAYS PHARMA on behalf of DANONE RESEARCH] DANONE RESEARCH ROUTE DEPARTEMENTALE 128 91767 PALAISEAU CEDEX - FRANCE Tel.: + 33 (0)6 13 61 27 02      Fax: +33 (1) 69 35 70 42
<b>Scientific Expert:</b>	<b>YAIR LOTAN</b> CHIEF OF UROLOGIC ONCOLOGY UT SOUTHWESTERN MEDICAL CENTER AT DALLAS DEPARTMENT OF UROLOGY 5323 HARRY HINES BLVD. J8.122 DALLAS, TEXAS 75390-9110 Tel.: 214-648-0389

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**Monitoring Clinical Research Organisation:** PHARM-OLAM INT.(UK) BULGARIA OFFICE  
PHARM-OLAM INT. (UK) BULGARIA OFFICE  
55 PARCHEVICH STR.  
1000 SOFIA - BULGARIA  
Tel.: + 359 2 9888 704  
Fax: + 359 2 9888 701

**Central Laboratory:** MEDICAL DIAGNOSTIC LABORATORY RAMUS EOOD  
2-4 ANGISTA STR.  
SOFIA 1527 - BULGARIA  
Tel.: + 359 2 9431196  
Fax: + 359 2 944 8206

**1.2 PROTOCOL SIGNATURE PAGE - SPONSOR**

Protocol details	
Study title	<i>The effect of increased water intake on the frequency of clinical recurrent urinary tract infections in pre-menopausal women:S-HYDRACYST</i>

*Protocol approved by the Sponsor:*

I, the undersigned, have reviewed and approved this protocol, including the appendices.

SPONSOR	DATE	SIGNATURE
<b>CHRISTINE M'RINI, MD, PhD</b>  DIRECTOR LIFE SCIENCE DANONE RESEARCH ROUTE DEPARTEMENTALE 128 91767 PALAISEAU CEDEX – FRANCE		

**1.3 PROTOCOL SIGNATURE PAGE – PRINCIPAL INVESTIGATOR**

Protocol details	
Study title	<b><i>The effect of increased water intake on the frequency of clinical recurrent urinary tract infections in pre-menopausal women: S-HYDRACYST</i></b>

*Protocol approved by the Principal Investigator:*

I, the undersigned, have reviewed this protocol including the appendices and I am aware of my responsibility and I agree to the following:

To conduct the clinical study in compliance with the protocol as detailed in this document.

To apply ICH Good Clinical Practice the declaration of Helsinki and any other regulatory requirements.

To obtain protocol approval from an independent Ethics Committee and to comply with their requirements for ongoing review and reporting (if applicable).

To comply with procedures for data recording and reporting (with a particular focus on Safety reporting)

To permit monitoring, auditing and inspection by the sponsor and relevant regulatory agencies.

To retain study related documents according to regulatory requirements and as agreed with the sponsor.

PRINCIPAL INVESTIGATOR	DATE	SIGNATURE
<b>MAYA DABCHEVA, MD</b> MC "COMAC MEDICAL", 13 URVICH STR., 3 <sup>RD</sup> FLOOR 1612 SOFIA - BULGARIA		

*Three original copies of the clinical study protocol must be signed by the Principal Investigator and one original copy shall be filed by each of the parties (Sponsor and Principal Investigator) and one should be submitted to the Ethics Committee.*

**1.4 GLOSSARY**

ACR	Albumin Creatinine Ratio
AE	Adverse Event
AFU/EAU	Association Française d'Urologie (AFU)/ European association of Urology
BMI	Body Mass Index
BP	Blood Pressure
CA	Competent Authority (ies)
CI	Confidence Interval
CM	Concomitant Medication
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organisation
CUA	Cost Utility Analysis
CV	Curriculum Vitae
DCF	Data Clarification Form
DBP	Diastolic Blood Pressure
EAU	European Association of Urology
EC	Ethics Committee
EDC	Electronic Data Capture
e-CRF	Electronic Case Report Form
EPI	Epidemiology Collaboration
FAS	Full Analysis Set
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GP	General Practitioner
HR	Heart Rate
ICF	Informed Consent Form



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ICH	International Conference on Harmonisation
ISF	Investigator Study File
IRB/IEC	Institutional Review Board/Independent Ethics Committee
IWRS	Interactive Web Response Services/Systems
LDL	Low Density Lipoprotein
MCF	Mean Cumulative Function
MSU	Midstream Specimen of Urine
OTC	Over The Counter
PI	Principal Investigator
PP	Per Protocol
QoL	Quality Of Life
RR	Relative Risk
rUTI	Recurrent Urinary Tract Infection
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SD	Standard Deviation
SEM	Standard Error of Mean
SF	Screen Failure
SOP	Standard Operating Procedure
SS	Safety Set
SSAR	Suspected Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
UCC	Urine Color Chart
USG	Urine Specific Gravity
UTI	Urinary Tract Infection

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V	Visit
VAS	Visual Analytical Scale
WHO	World Health Organisation

## 1.5 LIST OF DEFINITIONS

Active/Test product:	Product to be tested in the study.
Completed Subject:	Subject who has completed the study visits as required by the protocol. In the e-CRF the end of study status is available and it is: "Completed the study".
Evaluation period:	This period extends from randomisation (or allocation) up until the last visit where results are collected with the intent of evaluating any of the study criteria.
Eligible subject at visit 1 and visit 2:	The subject is considered eligible when the Investigator certifies that she fulfills all inclusion criteria and does not meet any exclusion criteria for the current visit. This statement can be repeated as many times as required by the protocol. In the e-CRF the Investigator has answered YES to the question "Is the subject eligible at visit 1 and visit 2?"
Included subject:	The signature of the informed consent marks the inclusion of the subject in the study. This signature can be obtained solely after having fully informed the subject about the study. At this step, a subject identification number is attributed to the subject. In the e-CRF the informed consent date is available.
Low drinker profile:	A low drinker profile is defined as follows: <ul style="list-style-type: none"><li>- total self-reported fluid intake &lt; 1,5 l/day</li><li>- and total 24 hours urinary volume &lt; 1,2 L</li><li>- and urine osmolality <math>\geq</math> 500 mOsmol/kg</li><li>- and urine creatinine within the laboratory normal ranges</li></ul>
Menopausal subject:	Subject without menstrual period for 12 months following the final menstrual period in the absence of pregnancy or other biological causes.
Multiple antibiotic resistance:	Multiple antibiotic resistance is defined as resistance to 3 or more different antibiotic groups.
Perimenopausal subject:	Subject with irregular menstrual cycles (on average 4 years before the final menstrual period) and hormone fluctuation often accompanied by hot flushes, sleep disturbances, mood symptoms and vaginal dryness [1].
Pre-menopausal subject:	Subject with normal menstrual cycles with monthly period, unless she is pregnant.

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Principal Investigator:	Investigator responsible for the conduct of the clinical study at a study site. If a study is conducted by a team of individuals at a study site, the Principal Investigator is the responsible leader of the team and may be called the principal Investigator.
Pre-screening period:	Any actions performed by the site(s) in relation to the study recruitment that happens before the formal signature of the informed consent. No data is collected by the sponsor at this point.
Positive urine culture:	Positive urine culture is defined as $> 10^3$ CFU/mL in the midstream specimen of urine (MSU) [31].
Randomised (or allocated) subject:	Any subject who has been allocated to a study arm either by a randomisation or by another method defined by the protocol. The randomisation date is available in the e-CRF.
Screening period:	This period extends from the signature of the informed consent up until the randomisation call.
Screen-failed subject:	The subject is considered a screen failure (SF) if she has signed the informed consent but did not complete the screening period successfully and not been randomised). In the e-CRF an “end of study form” should be available with the reason for failure.
Study product(s):	Products to be used in the clinical study (test products).
Symptoms of UTI:	Dysuria (i.e. painful urination, usually described by the patient as burning, stinging or itching) and / or urgency and / or increased frequency of urination and / or suprapubic pain (i.e. pain occurring from above the pubis) [31].
UTI:	Bacterial infection of the urinary.
UTI event:	Diagnosed by at least 1 symptom of urinary tract infection and positive urine culture.
Withdrawn (or Dropped out) Subject:	Any subject who has withdrawn from the study at any point after the randomisation but before having completed all the visits and assessments. In the e-CRF the end of study status is available and it is different from: “Completed the study”.

## 2. SYNOPSIS AND FLOW CHART

<b>Study title</b>	<i>The effect of increased water intake on the frequency of clinical recurrent urinary tract infections in pre-menopausal women: S-HYDRACYST</i>
<b>Study code</b>	<i>NU369 S-Hydracyst</i>
<b>Study sponsor</b>	<i>CHRISTINE M'RINI - DANONE RESEARCH, Palaiseau - France</i>
<b>Study principal investigator</b>	<i>Dr. Maya DABCHEVA, MC "COMAC MEDICAL"</i>
<b>Study product description</b>	<p>The study is testing a medical recommendation, consisting in increasing the daily water intake in the intervention group.</p> <p>For methodological reasons of homogeneity of access to water of the subjects, mineral commercialised water will be distributed to the subjects included in the intervention group, during the whole study duration.</p> <p>The mineral water is Evian® brand.</p> <p>The intervention group will increase its fluid intake of 1,5L of Evian® water (on the top of their normal fluid intake).</p> <p>The control group will not change its water intake habits.</p>
<b>Study period</b>	<p>Intervention period: Dec 2013 – Sep 2016 (around 12 months per subject)</p> <p>Study start date: (first subject first visit): Dec 2013</p> <p>Study end date (clinical study report writing): Q3 2017</p>
<b>Study objectives</b>	<p><u>Study primary objective:</u></p> <p>To assess the effect of increased daily water intake on the frequency of clinical recurrent urinary tract infections (rUTIs) among low drinking pre-menopausal women suffering from recurrent community-acquired UTI over 12 consecutive months of study product consumption.</p> <p><u>Study secondary objectives:</u></p> <p>To evaluate the impact of increased daily water intake on the use of antibiotics in recurrent UTI patients over 12 consecutive months of study product consumption.</p> <p>To evaluate the impact of increased daily water intake on the mean time elapsed between UTI episodes over 12 consecutive months of study product consumption.</p> <p>To evaluate the impact of increased daily water intake on urinary hydration markers over 6 and 12 consecutive months of study product consumption.</p> <p>To evaluate the impact of increased daily water intake on health costs in recurrent UTI patients over 12 consecutive months of study product consumption.</p> <p>To evaluate the impact of increase of daily water intake on the cost utility analysis during the study intervention using two different perspectives: National Health insurance and subject's perspective (subject's own out-of-pocket costs).</p> <p>To evaluate the impact of increased daily water intake on quality of life (QoL) in recurrent UTI patients over 6 and 12 consecutive months of study product</p>

	<p>consumption.</p> <p><u>Exploratory Objectives:</u></p> <p>To evaluate the relationship between urinary hydration markers and number of UTI events over 12 consecutive months of study intervention from D0.</p> <p>To evaluate the relationship between urinary hydration markers and delay UTI events over 12 consecutive months of study intervention from D0.</p> <p>To evaluate the impact of increased daily water intake on crystallisation risk index (Tiselius) over 12 consecutive months of study intervention from D0.</p> <p>To evaluate the number of UTI events confirmed by positive urine culture and number of subjects presenting at least one UTI event over 12 consecutive months of study intervention from D0 (by group).</p> <p>To evaluate the number of UTI events not confirmed by positive urine culture and number of subjects presenting at least one UTI event over 12 consecutive months of study intervention from D0 (by group).</p> <p>To evaluate the number of UTI events confirmed or not by positive urine culture and number of subjects presenting at least one UTI event over 12 consecutive months of the study intervention from D0 (by group).</p>
<b>Study Methodology</b>	<p>Prospective, single-site, open-label, randomised controlled trial in two parallel groups:</p> <ul style="list-style-type: none"> <li>❖ Control group not changing their fluid intake habits</li> <li>❖ Intervention group provided with low mineralised mineral water, fluid intake recommendations and regular hydration coaching support</li> </ul> <p>Subject will be allocated to the study arm through the randomisation system (IWRS) without any stratification factor.</p>
<b>Study population and sample size</b>	<p>The study population consists in pre-menopausal women diagnosed with rUTIs and having a 'low drinker' profile. The total number of completed subjects is estimated at 84 subjects.</p> <p>In order to achieve 84 completed subjects, and considering a 40% drop out rate, 140 subjects will be randomised.</p> <p>Considering a 50% screen failure rate, around 280 screened subjects will be needed in order to get 140 randomised subjects.</p>
<b>Subject recruitment</b>	<p>This is a single-site study which will be performed in Bulgaria. 140 women will be randomised in this trial.</p>
<b>Eligibility criteria</b>	<p><u>Eligibility criteria checked at V1</u></p> <p><u>Inclusion criteria:</u></p> <p>II01a: Women with at least 3 clinical recurrences of symptomatic UTI in the last 12 months (at least one UTI must be confirmed by a positive documented bacteriological exam) and asymptomatic at V1</p> <p>or</p> <p>II01b: Women with at least 3 clinical recurrences of symptomatic UTI in the last 12 months (at least one UTI must be confirmed by a positive documented bacteriological exam) and symptomatic at V1</p> <p>or</p> <p>II01c: Women with at least 2 clinical recurrences of symptomatic UTI in the last 12 months (who did not perform any bacteriological exam during the last 12 months), symptomatic at V1 and with positive documented bacteriological exam between V1 and V2</p>

	<p>II02: Age <math>\geq</math> 18 years</p> <p>II03: Fluid intake &lt; 1,5 L per day based on patient's declaration</p> <p>II04: Regular meal consumption (breakfast, lunch and dinner)</p> <p>II05: Access to Internet for information on hydration</p> <p>II06: Subject, upon briefing of the content of the present study, fully understanding and agreeing to its objective and having given written (dated and signed) informed consent to take part in the study</p> <p>II07: Subject who is able to communicate well with the Investigator and to comply with the requirements of the entire study</p> <p>II08: Literate subjects, able to fill in fluid diaries and QoL questionnaire</p> <p>II09: Women accepting to keep their lifestyle habits during the whole duration of the study</p> <p>II10: Women using any form of contraception</p> <p>II11: Subject covered by the National Health Insurance system</p> <p><u>Exclusion criteria:</u></p> <p>IE01: Incapacity / non-willingness to consume 1,5 L of drinking water per day on top of their usual consumption</p> <p>IE02: Women with history of UTI complications (pyelonephritis or other) in the last 12 months</p> <p>IE03: Use of antibiotics or cranberries juice and/or extracts in the previous 2 weeks</p> <p>IE04: Chronic treatments with anti-coagulants therapy</p> <p>IE05: Chronic bladder inflammation (defined as permanent bladder bacterial infection)</p> <p>IE06: Chronic diarrhea or constipation treated with chronic use of laxative substances</p> <p>IE07: Interstitial cystitis</p> <p>IE08: Estrogen-dependent symptomatic vulvo-vaginitis</p> <p>IE09: Recent (&lt;1year) or active renal stone disease</p> <p>IE10: Urinary tract structural abnormalities</p> <p>IE11: Obesity or malnutrition (BMI &lt;18,5 Kg/m<sup>2</sup> and &gt;30 Kg/m<sup>2</sup>)</p> <p>IE12: Pregnant or lactating women</p> <p>IE13: Women planning to become pregnant during the study</p> <p>IE14: Menopausal and peri-menopausal women</p> <p>IE15: On-going or planned therapy during the study which can modify the study measurements, in particular the assessment of the hydration status (diuretic intake, corticoids or drug treatment interfering with nutrition behaviour)</p> <p>IE16: Subjects with severe or uncontrolled organic disease, likely to interfere with the parameters of the study (e.g. neoplastic, cardiovascular, pulmonary and digestive disorders, uncontrolled diabetes type I and II, untreated or uncontrolled clinically significant arterial blood hypertension) or mental disorders affecting eating and drinking behaviour (i.e. primary polydipsia, bulimia nervosa, psychosis etc.)</p> <p>IE17: Women who have taken part in any other clinical study for the treatment of rUTI during the last 12 months</p> <p>IE18: Vulnerable subjects defined as individuals whose willingness to volunteer in the clinical trial may be unduly influenced by the expectation, whether justified or not, of benefits associated with participation, or of a retaliatory response from senior members of a hierarchy in case of refusal to participate. (Examples are members of a group with a hierarchical structure linked to the Investigator or to the Sponsor, such as medical, pharmacy, dental and nursing students, subordinate hospital and laboratory personnel, employees of the Investigator or of the Sponsor, members of the armed forces, and persons kept in detention).</p> <p>IE19: No legal capacity or limited legal capacity or unable to give an informed consent.</p>
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	<p>IE20: Subjects unlikely to cooperate in the study, and/or poor compliance anticipated by the Investigator.</p> <p>IE 21: Women who have taken part in this study and/or enrolled in another clinical study during the last month and/or currently participating in another clinical study.</p> <p><b><u>Randomisation criteria checked at the pre-randomisation visit (V2) and before subject's randomisation:</u></b></p> <p>RI01: Negative urine culture and asymptomatic state (i.e. silent phase) at V2 (pre-randomisation visit)</p> <p>RI02: Low-drinker confirmation defined as:</p> <ul style="list-style-type: none"> <li>- total self-reported fluid intake &lt; 1,5 L/day</li> <li>- and 24 hours urinary volume &lt; 1,2 L per day</li> <li>- and osmolality ≥ 500 mOsmol/kg</li> <li>- and urine creatinine within the laboratory normal ranges</li> </ul> <p>The following <u>non-randomisation criterion</u> should not be met:</p> <p>RE01: Chronic kidney disease (defined as decreased eGFR (eGFR&lt;60 ml/min/1,73m<sup>2</sup> calculated using EPI equation)</p> <p>RE02: Women suffering multiple antibiotic resistant bacterial strain*</p> <p>IE12: Pregnant or lactating women</p> <p>IE14: Menopausal and peri-menopausal women**</p> <p><i>* Multiple antibiotic resistance is defined as resistance to 3 or more different antibiotic groups</i></p> <p><i>** Based on V2 FSH assessment as per PI's discretion</i></p>
<p><b>Study product administration</b></p>	<p>For the subject in the intervention group, three (3) bottles of natural mineral water (500 mL each) to be consumed daily, in addition to the subjects' usual fluid intake volume, for the whole study duration (i.e. until successful completion of the End of Study visit).</p> <ul style="list-style-type: none"> <li>- Bottles will be provided to subjects in the intervention group for free, it is suggested to consume them at the beginning of every meal (breakfast, lunch and dinner) and fully drink them before the following meal. Coaching support will also be made available; this will be reinforced by individualised phone calls to motivate compliance.</li> <li>- The study products will be provided by DANONE RESEARCH and will be supplied by a professional logistics company.</li> </ul>



<p><b>Study description / Duration of subject participation</b></p>	<p><b><u>Informed consent:</u></b></p> <p>All subjects must sign and personally date the approved informed consent form before any specific study procedure is performed.</p> <p>The total duration of the intervention phase will be around 12 months per subject [between D0 (randomisation call) and V4 (end of study visit)].</p> <p>Each subject will attend at least 4 visits:</p> <p><u>During the Screening period</u> [from V1 to D0 (randomisation call)]</p> <ol style="list-style-type: none"> <li>1. Inclusion visit (V1)</li> <li>2. Pre-randomisation visit (V2). V2 can be rescheduled or repeated in case of UTI event discovered at V2. PI or another authorised study team member will randomise the subject via the IWRS. Then the PI or another authorised study team perform a randomisation call (D0) and inform the subject on her eligibility. D0 has to be performed no later than 8 weeks from V1.</li> </ol> <p><u>During the evaluation period</u> [from D0 (randomisation call) to V4 (end of study visit)]</p> <ol style="list-style-type: none"> <li>3. Evaluation visit (V3)</li> <li>4. End of study visit (V4). V4 can be rescheduled or repeated in case of UTI event discovered at V4.</li> </ol> <p>Additionally, for each UTI event or in case of symptom(s) of UTI, a <b>relapse</b> visit will be done.</p> <p>Furthermore, during the evaluation period, the PI or another authorised study team member will call the subject every month (except at month 6 and 12).</p> <p>All visits (i.e. V3, V4) after D0 (randomisation call) may be conducted within <math>\pm</math> 10 days of the theoretical visit date except in case in UTI symptom(s) as mentioned in section 7.1.4. and 7.1.6.</p> <p>All phone calls after the D0 (randomisation call) may be conducted within <math>\pm</math> 7 days of the theoretical call date.</p> <p><b><u>Dietary instructions and non-authorised products during the study:</u></b> Starting from V1, subjects will be motivated to maintain their usual diet and fluid intake habits and not to consume cranberries juice and/or extracts.</p>
	<p><b><u>Inclusion visit (V1): (Refer to section 7.1.1)</u></b></p> <p>At V1 UTI events in the past 12 months need to be verified. The status of all UTI events must be reported on the referral bulletin provided and signed by the medical doctor referring the patient and / or on the subject's medical records.</p> <p>At V1, subjects meeting all inclusion criteria and none of the exclusion criteria will be included after signature of the approved informed consent form. They will enter into a maximum of 8-weeks screening period.</p> <p><b>1/ Symptomatic subjects will perform the V1. A urine culture will be performed:</b></p> <p><b>1a/ If positive urine culture:</b></p> <p>The subject will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a <b>control</b> urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered and that results are not biased by the antibiotic treatment taken by the subjects.</p>

	<p>Depending on <b>control</b> urine culture results:</p> <ul style="list-style-type: none"> <li>• if <b>negative</b> control urine culture, the subject will come back to site to perform the pre-randomisation visit (V2) as soon as the subject is completely recovered and up to a maximum of 7 weeks from V1,</li> <li>• if <b>positive</b> control urine culture, the subject will be treated till a negative control urine culture is obtained and will come back to the site to perform the pre-randomisation visit (V2) as soon as the subject is completely recovered and up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.</li> </ul> <p>Any UTI symptom(s) with or without a positive urine culture, occurring between V1 and V2 will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.</p> <p><b>1b/ If negative urine culture:</b></p> <p>The subject may be treated or not with a standard of care treatment at the PI's discretion:</p> <ul style="list-style-type: none"> <li>○ Subject <i>receiving</i> a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to site to perform the pre-randomisation visit (V2) up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.</li> <li>○ Subject <i>not receiving</i> any treatment and declaring complete recover from previous symptoms will come back to the site to perform the pre-randomisation visit (V2) as soon as the symptoms are completely disappeared (subject is completely recovered) and up to a maximum of 7 weeks from V1.</li> <li>○ Subject <i>not receiving</i> any treatment but with persisting symptoms will re-perform a urine culture. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standard of care treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the pre-randomisation visit (V2) up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.</li> </ul> <p>Any UTI symptom(s) with or without a positive urine culture, occurring between V1 and V2 will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.</p> <p><b>2/ Asymptomatic subjects at V1</b> will come back to the site to perform the pre-randomisation Visit (V2) after a minimum of 4 days (i.e. the minimum time needed to complete the 3-days fluid intake diary) and up to 7 weeks from V1.</p> <p>Data on past medical and surgical history, past and current medications, sexual life, urine pregnancy test, demographic, vital signs and anthropometrical parameters (body weight and height, waist circumference), UTI symptom(s) will be collected.</p>
	<p><b><u>Over the Screening period [after V1 up to D0 (Randomisation call)]</u></b></p> <p>All subjects will have to complete the <b>3-days fluid intake diary</b> and the <b>Voiding diary</b>. Both diaries, a sterile urine sample (a sample from the first morning micturition on the V2 day) and the 24-hours urine sample outside of menstruation period (collection to be started during the day before V2 and ended with the first morning urine of the day after) will be brought back to the site at pre-randomisation visit. (V2).</p>

	<p>All subjects must be instructed to contact the PI in case of any symptom(s) of UTI. In this case, they will be treated or / not with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management) according to PI's decision, and, the V2, will be rescheduled once the subjects are recovered and / or UTI symptom(s) have disappeared (i.e. a control urine culture will be done at least 48h after the end of the therapy and /or disappearance of all UTI symptoms and within 1 week)</p>
	<p><b><u>Pre-randomisation visit (V2): (Refer to section 7.1.2)</u></b></p> <p>1/ If a subject coming to <b>V2 presents UTI symptom(s), whatever the urine culture results</b>, she won't be able to perform the visit, only urine culture is analysed. V2 will be rescheduled.</p> <p><b>1a/ Symptomatic subjects at V2 with a positive urine culture:</b></p> <p>The subject will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a <b>control</b> urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that the subject is completely recovered.</p> <p>Depending on <b>control</b> urine culture results:</p> <ul style="list-style-type: none"> <li>• If <b>negative</b> control urine culture, the subject will come back to the site to perform the pre-randomisation visit (V2) as soon as the subject is completely recovered and up to a maximum of 7 weeks from V1.</li> <li>• If <b>positive</b> control urine culture, the subject will be treated till a negative control urine culture is obtained and will come back to site perform the pre-randomisation visit (V2) as soon as the subject is completely recovered and up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.</li> </ul> <p>Any UTI symptom(s) with or without a positive urine culture, occurring between V2 and D0 (randomisation call) will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.</p> <p><b>1b/ Symptomatic subjects at V2 with a negative urine culture:</b></p> <p>The subject may be treated or not with a standard of care treatment at the PI's discretion:</p> <ul style="list-style-type: none"> <li>○ Subject <i>receiving</i> a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the pre-randomisation visit (V2) up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.</li> <li>○ Subject <i>not receiving</i> any treatment and declaring complete recover from previous symptoms will come back to site to perform the pre-randomisation visit (V2) as soon as the symptoms are completely disappeared (subject is completely recovered) and up to a maximum of 7 weeks from V1.</li> <li>○ Subject <i>not receiving</i> any treatment but with persisting symptoms will re-perform a urine culture. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standard of care treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the pre-randomisation visit (V2) up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.</li> </ul> <p>Any UTI symptom(s) with or without a positive urine culture, occurring between V2 and randomisation call (D0) will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.</p>

	<p><b>2/Asymptomatic subject will perform V2.</b></p> <p><b>2a/ Asymptomatic subjects at V2 with a positive urine culture:</b></p> <p>The subject will be treated for the UTI event with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a <b>control</b> urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered:</p> <p>If a negative <b>control</b> urine culture is obtained:</p> <ul style="list-style-type: none"> <li>• ≤ 28 days, after V2 (i.e. negative control urine culture obtained at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered), a new QoL questionnaire will be completed by the subject after UTI is cured.</li> <li>• &gt; 28 days, after V2, the V2 should be repeated.</li> </ul> <p>Any UTI symptom(s), with or without a positive urine culture, occurring between V2 and randomisation call (D0) will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.</p> <p><b>2b/ Asymptomatic subjects at V2 with a negative urine culture</b> and fulfilling all randomisation and none of non-randomisation criteria will be randomised by the PI no later than 1 week after V2.</p> <p>Diaries data and any changes in the usual diet and fluid intake habits will be checked (including non-authorized product consumption).</p> <p>Urinary samples will be analysed. Data on sexual life, urine pregnancy test and FSH (to confirm the perimenopausal status if needed), vital signs and anthropometrical parameters (body weight, waist circumference), UTI symptom(s), AE and concomitant medications will be collected.</p> <p>Quality of Life questionnaire will be completed by the subject.</p> <p>A blood sample will be collected for determination of lipidic profile (Total Cholesterol, HDL cholesterol, LDL cholesterol, triglycerides), glycosylated hemoglobin, serum creatinine, serum urea, copeptin. Estimated glomerular filtration rate (eGFR) will be calculated. Patients will receive instructions about study requirements accordingly.</p>
	<p><b><u>Randomisation call (D0): (Refer to section 7.1.3)</u></b></p> <p>Randomisation will be performed once the investigator receives the results of urine analysed confirming the randomisation and non-randomisation criteria.</p> <p>Subject randomisation will be done through the IWRS. Women will be randomly allocated into one of the two study arms.</p> <p>Subjects who will be allocated to the intervention study group will be asked to consume 1,5L of Evian® brand mineral water which will be regularly supplied to their home or office. This volume is on top of their normal water consumption.</p> <p>PI or another documented authorized study team member will contact the subject to inform her about her randomisation group and to provide the appropriate instructions as soon as possible.</p>

	<p><b><u>Over the 12-months evaluation period</u></b></p> <p>All subjects will complete every month till the end of the study, a <b>3-days fluid intake diary</b> (Saturday-Sunday-Monday or Friday-Saturday-Sunday) at home and will have to send it back to the investigational site by courier or to hand it in personally if preferred every month.</p> <p>In addition, subjects in the intervention group will fill in a <b>Subject Compliance Diary</b> which will help them to check the study product consumption. Subjects will send them back to the site each month by courier or to hand it in personally if preferred together with the 3-days fluid intake diary.</p> <p><b>Healthcare costs data</b> related to UTI episodes will be completed during each relapse visit.</p> <p>Subjects will be called every month, except at months 6 (V3) and 12 (V4), by the PI or another documented authorized study team member to check the study compliance (including fluid intake and study product consumption if any), adverse event(s), symptom(s) of UTI and / or UTI event(s), concomitant treatment(s).</p>
	<p><b><u>Evaluation visit (V3): (Refer to section 7.1.4)</u></b></p> <p>Subjects in fasting condition will come along with a 24h urine sample and appropriate pages of the diaries to the clinical site.</p> <p><b>If a subject coming to V3 presents UTI symptom(s), she won't be able to perform the visit. V3 will be rescheduled</b> once the subject is recovered and UTI symptoms disappeared.</p> <p><b>Given the presence of symptoms, a urine culture will be performed and a relapse visit will be done (refer to section 7.1.7).</b></p> <p><b>1a/ Symptomatic subjects at V3 with a positive urine culture:</b></p> <p>The subject will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a <b>control</b> urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered.</p> <p>Depending on the <b>control</b> urine culture results:</p> <ul style="list-style-type: none"> <li>• If <b>negative</b> control urine culture, the subject will come back to site to perform the evaluation visit (V3) as soon as the subject is completely recovered.</li> <li>• If <b>positive</b> control urine culture, the subject will be treated till a negative control urine culture is obtained and will come back to the site to perform the evaluation visit (V3) as soon as the subject is completely recovered.</li> </ul> <p>Any UTI symptom(s), with or without a positive urine culture at V3 will be reported in a relapse visit.</p>

	<p><b>1b/ Symptomatic subjects at V3 with a negative urine culture:</b></p> <p>The subject may be treated or not with a standard of care treatment at the PI's discretion:</p> <ul style="list-style-type: none"> <li>○ Subject <i>receiving</i> a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained. They will come back to site to perform the evaluation visit (V3) as soon as the subject is completely recovered.</li> <li>○ Subject <i>not receiving</i> any treatment, and declaring complete recover from previous symptoms, will come back to the site to perform the evaluation visit (V3) as soon as the symptoms are completely disappeared (subject is completely recovered).</li> <li>○ Subject <i>not receiving</i> any treatment but with persisting symptoms will <b>re-perform a urine culture</b>. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standard of care treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the evaluation visit (V3).</li> </ul> <p>Any UTI symptom(s), with or without a positive urine culture before the V3 will be reported in a relapse visit.</p> <p><b>2/Asymptomatic subject will perform V3.</b></p> <p>Urinary samples will be analysed (urine culture is not performed).</p> <p>Data on sexual life, vital signs and anthropometrical parameters (body weight, waist circumference), UTI symptom(s), AE and concomitant medications will be collected.</p> <p>Quality of Life questionnaire will be completed by the subject.</p> <p>A blood sample will be collected for determination of serum creatinine and copeptin. Estimated glomerular filtration rate (eGFR) will be calculated.</p> <p>Patients will be asked to complete on site the Quality of Life questionnaire.</p>
	<p><b><u>End of study visit (V4): (Refer to Section 7.1.6)</u></b></p> <p>Subjects in fasting condition will come along with a 24h urine sample, sterile urine sample and the appropriate pages of the diaries.</p> <p>1/ If a subject coming to <b>V4 presents UTI symptom(s), whatever the urine culture results</b>, she won't be able to perform the visit, only urine culture is analysed. <b>V4 will be rescheduled</b> once the subject is recovered.</p>

**1a/ Symptomatic subjects at V4 with a positive urine culture:**

The subject will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management) and will be advised not to stop water intake (if she is in the intervention group) till the end of study visit completion. After the completion of UTI treatment, a **control** urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that the subject is completely recovered.

Depending on **control** urine culture results:

- If **negative** control urine culture, the subject will come back to the site to perform the end of study visit (V4) as soon as the subject is completely recovered.
- If **positive** control urine culture, the subject will be treated till a negative control urine culture is obtained and will come back to the site to perform the end of study visit (V4) as soon as the subject is completely recovered.

Any UTI symptom(s), confirmed or not with a positive urine culture before the V4 will be reported in a **relapse** visit.

Once the negative control urine culture is obtained, the end of study form in the e-CRF will be completed.

**1b/ Symptomatic subjects at V4 with a negative urine culture:**

The subject will be advised not to stop water intake (if she is in the intervention group) till the end of study visit completion.

The subject may be treated or not with a standard of care treatment at the PI's discretion:

- Subject *receiving* a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained. They will come back to site to perform the end of study visit (V4) as soon as they are completely recovered.
- Subject *not receiving* any treatment and declaring complete recover from previous symptoms will come back to site to perform the end of study visit (V4) as soon as the symptoms are completely disappeared (subject is completely recovered).
- Subject *not receiving* any treatment but with persisting symptoms will re-perform a urine culture. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standard of care treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the end of study visit (V4).

Any UTI symptom(s), confirmed or not with a positive urine culture before the V4 will be reported in a relapse visit.

Once the negative control urine culture is obtained, the end of study form in the e-CRF will be completed

	<p><b>2/Asymptomatic subject will perform V4.</b></p> <p><b>2a/ Asymptomatic subjects at V4 with a positive urine culture:</b></p> <p>The subject will be treated for the UTI event with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a <b>control</b> urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered and symptoms disappeared.</p> <p>Depending on <b>control</b> urine culture results, if a negative control urine culture is obtained:</p> <ul style="list-style-type: none"> <li>• ≤ 28 days after V4 (i.e. negative control urine culture obtained at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered), a new QoL questionnaire will be completed by the subject after UTI is cured</li> <li>• &gt; 28 days after V4, the V4 should be repeated.</li> </ul> <p>All asymptomatic events with a positive urine culture, occurring at V4 will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.</p> <p>Once the negative control urine culture is obtained, the end of study form in the e-CRF will be completed.</p> <p><b>2b/ For asymptomatic subjects at V4 with a negative urine culture,</b> the end of study form in the e-CRF will be completed.</p> <p>Diary data and any change in the usual diet and fluid intake habits will be checked (including non-authorized product consumption).</p> <p>An urine culture will be performed to evaluate the presence of any infection at the end of study.</p> <p>Urinary samples will be analysed. Data on physical examination, sexual life, vital signs and anthropometrical parameters (body weight, waist circumference), UTI symptom(s), AE and concomitant medications will be collected.</p> <p>Quality of Life questionnaire will be completed by the subject.</p> <p>A blood sample will be collected for determination of lipidic profile (Total Cholesterol, HDL cholesterol, LDL cholesterol, triglycerides), glycosylated hemoglobin, serum creatinine, serum urea, copeptin. Estimated glomerular filtration rate (eGFR) will be calculated.</p>
	<p><b><u>Relapse visits: (Refer to Section 7.1.7)</u></b></p> <p>Subjects presenting any symptoms of UTI during the course of the study will be instructed not to stop the increased water intake (if in the intervention group) and to come back to the investigational site for clinical examination.</p> <p>UTI events will be diagnosed.</p> <p>Only in case of a positive urine culture, healthcare costs due to illness will be collected. Concomitant treatments will be collected.</p>



	<p><b><u>Intake surveys &amp; coaching support:</u></b></p> <p>In order to reinforce their adherence to fluid intake recommendations, subjects allocated to the intervention group will be provided with bottled mineral water, and will receive individualised phone calls at regular intervals and will be provided with coaching materials. By contrast, subjects allocated to the control group won't receive any fluid intake recommendations nor any test product or coaching support.</p> <p>Subjects allocated to the intervention group will also be asked to complete a Subject Compliance Diary in order to assess the compliance to the study product intake.</p> <p>The protocol will follow ICHE6 guidelines on Good Clinical Practices and applicable Directives/local laws.</p>
<p><b>Study design schema</b></p>	<p><i>See on the next page "Study Flow-Chart"</i></p>

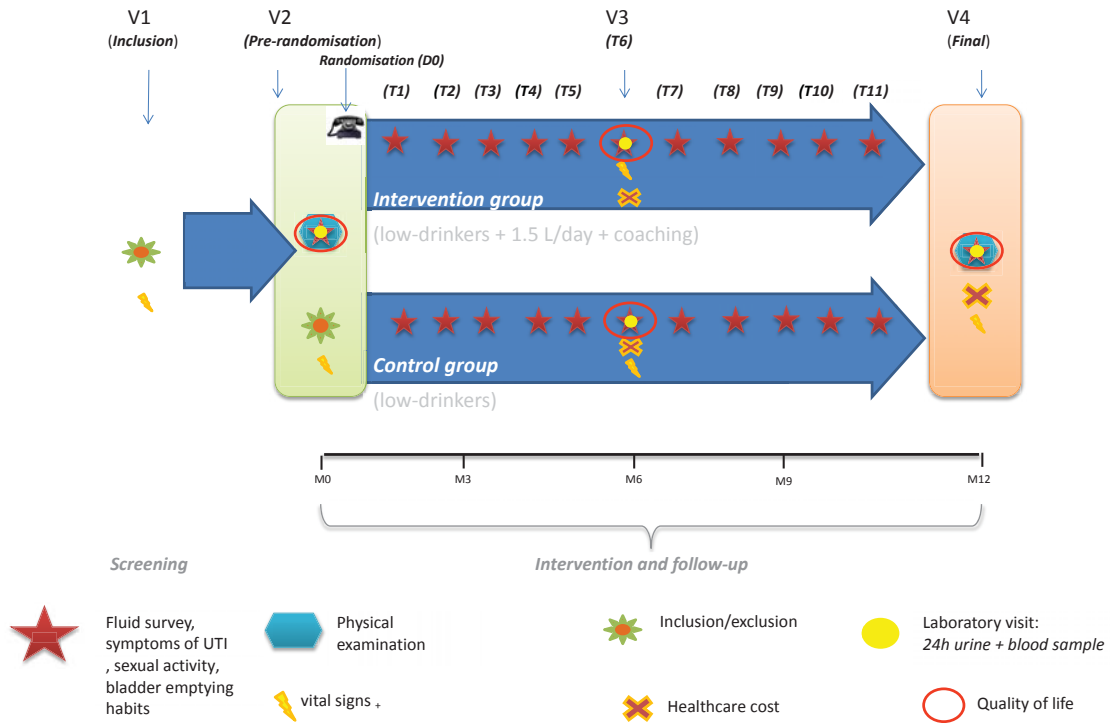
STUDY FLOW-CHART

Visits	Procedures	MONTH 0				1st Month ± 7 days	2 <sup>nd</sup> Month ± 7 days	3rd Month ± 7 days	4th Month ± 7 days	5th month ± 7 days	6th month ± 7 days	Month 6 ± 10 days	7th Month ± 7 days	8th Month ± 7 days	9th Month ± 7 days	10th Month ± 7 days	11th Month ± 7 days	12th Month	Month 12± 10 days	Relapse visit
		Inclusion Visit (V1)	Home	Pre- randomisation visit (V2)	Randomisation call Day 0	Call	Call	Call	Call	Call	Home	Visit 3 (V3)	Call	Call	Call	Call	Call	Home	Completion (V4)	
	Screening for acute UTI or previous episodes (at least 3 in the last 12 months)	X																		
	Inclusion / exclusion criteria	X		X																
	Randomisation/non randomisation criteria			X	X															
	Informed consent form signature	X																		
	Study group allocation				X															
	Demographic data (date of birth, gender)	X																		
	Urine pregnancy test	X		X																
	FSH test if applicable			X																
	Physical examination			X															X	
	Phone call to check compliance, AE and UTI events					X	X	X	X	X		X	X	X	X	X				
	Medical & Surgical history	X																		
	Vital sign and anthropometrical parameters	X		X							X								X	
	Instructions for Subject's diary completion and return for the 3-days fluid intake	X		X	X	X	X	X	X		X	X	X	X	X	X			X	X
	Subject's diary completion for 3-days fluid intake by the subject		X			X	X	X	X	X		X	X	X	X	X	X			
	Check of 3-days fluid intake diary		X			X	X	X	X	X	X	X	X	X	X	X			X	
	Subject Compliance Diary (instructions for the test group)		X			x	x	x	x	x	X	x	x	x	x	x				
	Subject Compliance Diary completion (for the test group)					x	x	x	x	x	X	x	x	x	x	x	X			
	Subject Compliance with the study product check (for the test group)					x	x	x	x	x	X	x	x	x	x	x			x	
	QoL questionnaire (SF-12 V2)		X								X								X	
	Delivery of urine kits & Instructions	X		X							X									x
	Urine 24h collection + Voiding diary completed by the subject		X*							X*							X*			
	Delivery of UCC		X																	
	Collection of urine containers & Voiding diary		X								X								X	
	Blood samples		X								X								X	
	Symptoms of UTI	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
	Urine culture	X** (only if symptomatic)		X**							X** (only if symptomatic)								X**	X**
	Healthcare costs data																			X

Visits Procedures	MONTH 0				1st Month ± 7 days	2 <sup>nd</sup> Month ± 7 days	3rd Month ± 7 days	4th Month ± 7 days	5th month ± 7 days	6th month ± 7 days	Month 6 ± 10 days	7th Month ± 7 days	8th Month ± 7 days	9th Month ± 7 days	10th Month ± 7 days	11th Month ± 7 days	12th Month	Month 12± 10 days	
	Inclusion Visit (V1)	Home	Pre-randomisation visit (V2)	Randomisation call Day 0	Call	Call	Call	Call	Call	Home	Visit 3 (V3)	Call	Call	Call	Call	Call	Home	Completion (V4)	Relapse visit
Bladder emptying after sexual intercourses	X		X		X	X	X	X	X		X	X	X	X	X	X		X	X
Active/inactive sexual life during the last month	X		X		X	X	X	X	X		X	X	X	X	X	X		X	X
Past and Concomitant Treatments	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

\* 24h urine to be collected at home the day before the visit (V2, V3 and V4)

\*\* In case of UTI infection a control urine culture should be performed at least 48H after the end of treatment and within 1 week to confirm that subjects are completely recovered



<p><b>Study Parameters</b></p>	<ul style="list-style-type: none"> <li>- <u>parameter 1</u>: total number of clinical recurrences of UTI*</li> <li>- <u>parameter 2</u>: mean time elapsed between UTI* episodes</li> <li>- <u>parameter 3</u>: total days of antibiotics therapy related to UTI episodes</li> <li>- <u>parameter 4</u>: frequency of micturition during the 24h urine collection</li> <li>- <u>parameter 5</u>: 24h urine parameters (volume, pH, osmolality, specific gravity, urine colour, sodium, potassium, calcium, magnesium, chloride, oxalate, citrate, creatinine)</li> <li>- <u>parameter 6</u>: eGFR</li> <li>- <u>parameter 7</u>: Tiselius Crystallisation Risk Index</li> <li>- <u>parameter 8</u>: blood sample (lipidic profile, glycosylated hemoglobin, serum creatinine, serum urea, copeptin)</li> <li>- <u>parameter 9</u>: vital signs (diastolic and systolic blood pressure, heart rate, axillary body temperature)</li> <li>- <u>parameter 10</u>: quality of life (questionnaire SF12V2)</li> <li>- <u>parameter 11</u>: fluid intake (water, other types of fluids)</li> <li>- <u>parameter 12</u>: anthropometry (body weight, waist circumference, height, BMI)</li> <li>- <u>parameter 13</u>: pathology related costs</li> <li>- <u>parameter 14</u>: complications from UTIs* (UTI related AE, and/or SAE)</li> </ul> <p>*Based on the simultaneous presence of positive urine culture and UTIs symptom(s)</p>
<p><b>Study Endpoints</b></p>	<p><u>Primary endpoint:</u></p> <ul style="list-style-type: none"> <li>➤ Difference between groups in terms of number of UTI recurrence over 12 months of study intervention from randomisation call (D0).</li> </ul> <p><u>Secondary endpoints:</u></p> <ul style="list-style-type: none"> <li>➤ Difference between groups in terms of antibiotic prescription/usage to treat UTI event over 12 months of study intervention from D0,</li> <li>➤ Difference between groups on average delay between each UTI event over 12 months of study intervention from D0,</li> <li>➤ Difference between groups on urinary hydration markers over 6 and 12 consecutive months of study intervention from D0,</li> <li>➤ Difference between groups in terms of health-costs associated with management of UTI recurrence(s) over 12 months of study intervention from D0,</li> <li>➤ Difference between groups in terms of cost utility analysis over 12 months of study intervention from D0,</li> <li>➤ Difference between groups in terms of change in QoL over 6 and 12 consecutive months of study intervention from D0.</li> </ul>

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	<p><u>Exploratory endpoints:</u></p> <ul style="list-style-type: none"> <li>➤ Relationship between urinary hydration markers and number of UTI events over 12 consecutive months of study intervention from D0,</li> <li>➤ Relationship between urinary hydration markers and delay between UTI events over 12 consecutive months of study intervention from D0,</li> <li>➤ Impact of increased daily water intake on crystallisation risk index (Tiselius) over 12 consecutive months of study intervention from D0,</li> <li>➤ Number of events and number of subjects with at least one UTI event confirmed by positive urine culture over 12 consecutive months of study intervention (by group) from D0</li> <li>➤ Number of events and number of subjects with at least one UTI event not confirmed by positive urine culture over 12 consecutive months of study intervention (by group) from D0,</li> <li>➤ Number of events and number of subjects with at least one UTI event confirmed or not by positive urine culture during the intervention period (by group) from D0.</li> </ul>
<p><b>Safety evaluation criteria</b></p>	<p>Blood sample (Total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, glycosylated haemoglobin, serum creatinine, serum urea, and eGFR), blood pressure, heart rate, axillary body temperature, weight, urine sodium, urine potassium, physical examination at V2 and V4, adverse events, serious adverse events</p>
<p><b>Statistical analysis</b></p>	<ul style="list-style-type: none"> <li>➤ The statistical methodology will be detailed in the statistical analysis plan (SAP) written and finalized before the database lock.</li> <li>➤ Descriptive statistics will be given for each of the parameters (i.e. for continuous variables: group size, mean, standard deviation of the variable [SD], standard error of the mean [SEM], minimum, maximum, median, and possibly quartiles; for qualitative variables, group size and percentage).</li> <li>➤ For the main criterion, a nonparametric method called the mean cumulative function (MCF) will be used to analyse the multiple/repeated UTI events occurring. The null hypothesis is “There is no difference between groups on the average number of recurrent UTIs event experienced”. We will consider a Type I error rate (alpha) equal to 5%.</li> <li>➤ The analysis will be conducted with the statistical software package SAS 9.3.</li> </ul>

### 3. INTRODUCTION

#### 3.1 SCIENTIFIC BACKGROUND INFORMATION

Urinary tract infection (UTI) is one of the most common clinical diagnoses in women. The lifetime risk for UTI in women is high (greater than 50%) and in the U.S. between 1988 and 1994 the overall lifetime prevalence of UTI was estimated to be 53,067/100,000 women [2]. The estimated global incidence of UTIs is at least 250 million cases per year [3]. UTIs are a source of significant cost and morbidity. Most UTIs are self-limiting but occasionally can be associated with significant complications such as pyelonephritis and sepsis. Composite data revealed that overall expenditures for the treatment of UTIs in women in the United States, excluding spending on outpatient prescriptions, were approximately 2.47 billion U.S. dollars in 2000 [2]. According to the 1997 National Ambulatory Medical Care Survey and National Hospital Ambulatory Medical Care Survey, UTI accounted for nearly 7 million office visits and 1 million emergency department visits, resulting in 100,000 hospitalisations [4]. The exact frequency of UTIs is difficult to assess because they are not reportable diseases in the United States. This situation is further complicated by the fact that accurate diagnosis depends on both the presence of symptoms and a positive urine culture, although in most outpatient settings this diagnosis is made without the benefit of culture.

There are several higher risk populations for UTIs including infants, pregnant women, the elderly, patients with spinal cord injuries and/or catheters, patients with diabetes or multiple sclerosis, patients with acquired immunodeficiency disease syndrome/human immunodeficiency virus, and patients with underlying urologic abnormalities [5]. However, UTIs are very common in otherwise healthy women. It is estimated that at least a third of all women in the United States are diagnosed with a UTI before they are 24 years old [6]. There are both host and bacterial factors that contribute to UTIs [7,8]. In young women, sexual activity is associated with an increased risk for UTIs [7,8]. UTIs also have a propensity to recur. In otherwise healthy college women with a first UTI, the risk of a second episode within 6 months was 24% [8], and in those with a history of one or more UTIs, the risk of a second within 1 year was 70% [9]. Even when UTI is not associated with long term consequences, the condition results in pain and suffering, and negatively impacts quality of life, albeit transiently [10]. Common symptoms in pre-menopausal women include frequent, urgent and painful urination and suprapubic pressure and hematuria may also be present.

There are multiple reasons to try to prevent UTIs in women. First and foremost is to reduce morbidity associated with the infections as well as reduce the cost of treatment. However, there are also important reasons to reduce the use of antibiotics in these patients [11]. First, there is increasing resistance of *Escherichia coli*, the primary causative agent of uncomplicated UTI, to a variety of antibiotics, including fluoroquinolones and extended-spectrum beta-lactamase (ESBL) resistance is increasingly observed among community acquired UTI [12].) Second, there can be significant impact of short courses of antibiotics on the gut and vaginal microbiota which can contribute to recurrence and antibiotic resistance [13]. Third, there are risks associated with antibiotic use such as allergic reactions and side effects of the drugs themselves. Finally there is a risk of vaginal candida infection, which occurs in up to 22 % of women treated for uncomplicated UTI [14].

Moreover, due to the high prevalence and incidence, UTI has enormous economic implications.

As for other pathologies, costs related to UTI episodes should be divided into direct and indirect ones.

Direct costs include the costs of outpatient doctor visits, antibiotics and specific antimicrobial agent prescription, along with hospitalisation expenses. The indirect costs include all the “non-medical costs” related to the pathology, such as travel and sick days for the patients and for the caregivers.

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Direct and indirect annual costs related to acute episode of UTI have been estimated to be around \$1.6 billion for the US female population [5,6], including approximately \$936 million for indirect costs and \$659 million for direct ones.

To date, no data about specific European population are available. In spite of available evidence suggesting a link between urinary hydrodynamics and frequency of UTI episodes, cost-savings that could potentially be derived from appropriate fluid intake among UTI patients remain to be established.

Several strategies have been proposed to try to reduce the risk of recurrent UTIs.

While the use of daily antibiotics or post-coital antibiotics is effective, the rise of resistance and risk associated with antibiotics has made these strategies less attractive. Different approaches have been proposed including use of functional foods, lactobacillus and vaccines [11]. The most studied functional food thus far has been cranberries and their extracts. A Cochrane review on studies including cranberries included a total of 24 studies (six cross-over studies, 11 parallel group studies with two arms; five with three arms, and two studies with a factorial design) with a total of 4473 participants [15]. The comparison/control arms were placebo, no treatment, water, methenamine hippurate, antibiotics, or lactobacillus. The meta-analyses found that compared with placebo, water or not treatment, cranberry products did not significantly reduce the occurrence of symptomatic UTI overall (RR 0.86, 95% CI 0.71 to 1.04) or for any the subgroups: women with recurrent UTIs (RR 0.74, 95% CI 0.42 to 1.31); older people (RR 0.75, 95% CI 0.39 to 1.44); pregnant women (RR 1.04, 95% CI 0.97 to 1.17); children with recurrent UTI (RR 0.48, 95% CI 0.19 to 1.22); cancer patients (RR 1.15 95% CI 0.75 to 1.77); or people with neuropathic bladder or spinal injury (RR 0.95, 95% CI: 0.75 to 1.20). Studies evaluating lactobacillus have shown some promise but await further validation [16,17].

### **3.2 DESCRIPTION OF THE TEST PRODUCT**

The approach that we propose involves randomising low-drinking pre-menopausal women with recurrent UTIs to high versus usual water intake habits to determine whether this will reduce risk of UTIs. For this purpose, we will provide women with three (3) bottles of natural mineral commercialised water (500 mL each) to be consumed daily for approximately twelve (12) months. Bottles will be provided to subjects in the intervention group for free, along with the suggestion to consume them at the beginning of every meal (breakfast, lunch and dinner) and fully drink them before the following meal.

### **3.3 SCIENTIFIC RATIONALE**

The study will include only women who are low-drinkers (< 1.5 L fluids per day; urinary volume < 1.2 L per day) since they are most likely to have a predisposition to UTIs due to infrequent voiding. The rationale for this approach is that drinking more fluid will increase voiding frequency and voiding is the main defence of the bladder to reduce the number of bacteria in the bladder and avoid UTIs. As such, increasing fluid intake will increase the frequency and volume of voiding and potentially reduce risk of recurrent UTIs. Support for this hypothesis is multifold. A non-randomised, multivariate analysis comparing 791 women teachers who deliberately restricted their fluid intake (25% voided only once during working hours, or not at all) with women able to drink without restrictions found that women in the former group were at significantly higher risk of UTI than were women in the latter group (RR 2.21; 95% CI 1.45-3.38) after controlling for parity, voiding infrequently



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at work, and urge incontinence [18]. A study of 1613 women compared frequency of voiding based on age of women and impact on UTIs [19]. The study found that women aged 20-25 had a higher rate of this low voiding frequency than women 30-35 or 40-45 years and in all 3 groups, women who voided 3 times or less per day had significantly more urinary infections than those with 4 or more voidings per day, ( $p < 0.01$ ). While results are inconsistent, several studies found that higher post-void residual volumes increases risk of UTI in women [20-22]. This supports the notion that efficacy of voiding and emptying the bladder can reduce risk of UTIs. One additional impact of fluid intake may involve reducing urine acidity. Low fluid intake is associated with an increase in urine osmolality and acidity which can predispose to bacterial adhesion to the bladder epithelium. A study in pre-menopausal women who had been treated for at least 2 idiopathic UTIs in the previous 6 months found that self-monitoring urine osmolality using a handheld 'traffic light' probe was associated with a significant shift towards urine of lower osmolality and a significant reduction in incidence of UTIs compared with the period in which the probe was not used [23]. Of the 17 patients who completed both 4-month periods, 14 felt that the probe had helped them to prevent infection.

### 3.3.1 *Rationale for the study purpose*

The above evidence and the heavy burden of recurrent UTIs on society demonstrate a significant need for strategies to prevent UTIs. The planned study will determine the efficacy of increased water intake in decreasing the risk of UTIs in women who are low volume drinkers and who suffer from recurrent UTIs compared to a control group.

### 3.3.2 *Rationale for the study population*

UTI is the most common infection in humans. It is highly prevalent in both men and women but its frequency is about 50 times higher in adult women of all age groups. This may be because the urethra is shorter in women than in men, making it easier for bacteria to ascend into the bladder and, once there, proliferate.

More than half of all women (50–60%) encounter with at least one UTI at some stage during their lives. [24] Symptomatic infection of the bladder (lower UTI) has been estimated to occur in up to 30% of women during their life [25].

The estimated global incidence of UTI in women based on self-report of physician diagnosis is 11% per year resulting in at least 250 million cases per year [26]. This incidence is higher (17.5%) during ages 18–24 and decreases to 9% for women 50 and over [6].

Approximately 5% of women with an initial UTI have multiple episodes within a year and a high recurrence rate, ranging between 25 to 30%, has been shown to affect the total female population [27]. Observational studies have shown 6-month risk of recurrence ranging from 17 to 24% among otherwise healthy pre-menopausal women [6, 28]. It is not surprising that one of the major risk factor for UTI among women is having a history of UTI.

Given the high burden, recurrent pre-menopausal women will be the target population of our study.

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3.3.3 *Rationale for dose(s) selected and/or dose(s) regimen*

We want women in the intervention group, to achieve a mean daily urine osmolality equivalent to that of plasma (approximately 285 mOsm/kg). This osmolality goal is selected because it defines the transition from concentrated to dilute urine and is a safe end point under most circumstances. To maintain such a low urine osmolality, we assume that women in the intervention group should increase their water intake of 1.5 L/day.

Moreover, to maintain a urinary volume of 1.5L we need to assure a total fluid intake of more than 1.3L/day [29]. 1.5L/day will be our target knowing that the mean water intake in European women is less than 1L/day.

3.3.4 *Rationale for the study design*

Given the necessity to establish a causality relation between increased water intake and UTI episodes, we will perform a single-site, prospective, randomised, open-label, controlled study. This model is often used where the double-blind design is not applicable. A strict randomisation procedure is used: patients are allocated to different group regimens following the allocation concealment rules. In this way, the Investigator won't be able to subvert randomisation and select which patients get intervention or control allocation. The follow-up and intervention phase is conducted openly in a way that adheres to accepted clinical principles and medical practice.

**3.4 POTENTIAL RISKS AND BENEFITS**

We expect increased fluid intake to be efficient in reducing the risk of clinical recurrences of UTI in women (except peri-menopausal and menopausal women. Decreased incidence of this pathology will reduce the comorbidity associated with UTI events as well as the related healthcare costs.

No risk linked to increased water intake has ever been shown in this population. Moreover the protocol suggests to split the water consumption over the day and consume 0,5L at the beginning of every meal (breakfast, lunch, dinner) and fully drink them before the following meal.

However, in case of ingestion of more than 1L of water in a short time interval (less than 10 min), the subject may have possible temporary stomach discomfort and an increased urge to use the toilet.

Blood samples will be drawn during the study visits. The risks related to this procedure are very weak and they are the same as for any type of blood drawing procedure in current medical practice:

- Pain during the sampling: it is especially connected to the speed of execution of the movement. This risk is minimised in this study as the staff performing the sampling is competent medical staff.
- Local secondary infection: this risk is theoretical because the used material is single-use, and the sampling is performed after local asepsis.
- Localized haematoma: this risk can be limited by the realisation of an effective manual compression in the minutes following the sampling.

## **4. STUDY OBJECTIVES**

### **4.1 PRIMARY STUDY OBJECTIVE**

The primary objective of this study is to assess the effect of increased daily water intake on the frequency of clinical recurrent urinary tract infections (rUTIs) among low drinking pre-menopausal women suffering from recurrent community-acquired UTI over 12 consecutive months of study product consumption.

### **4.2 SECONDARY STUDY OBJECTIVE(S)**

- To evaluate the impact of increased daily water intake on the use of antibiotics in recurrent UTI patients over 12 consecutive months of study product consumption
- To evaluate the impact of increased daily water intake on the mean time elapsed between UTI episodes over 12 consecutive months of study product consumption
- To evaluate the impact of increased daily water intake on urinary hydration markers over 6 and 12 consecutive months of study product consumption
- To evaluate the impact of increased daily water intake on health costs in recurrent UTI patients over 12 consecutive months of study product consumption
- To evaluate the impact of increase of daily water intake on the cost utility analysis during the study intervention using two different perspectives: National Health insurance and subject's perspective (subject's out-of-pocket costs)
- To evaluate the impact of increased daily water intake on quality of life (QoL) in recurrent UTI patients over 6 and 12 consecutive months of study product consumption

### **4.3 EXPLORATORY STUDY OBJECTIVE(S)**

- To evaluate the relationship between urinary hydration markers and number of UTI events over 12 consecutive months of study intervention from D0.
- To evaluate the relationship between urinary hydration markers and delay UTI events over 12 consecutive months of study intervention from D0.
- To evaluate the impact of increased daily water intake on crystallisation risk index (Tiselius) over 12 consecutive months of study intervention from D0.
- To evaluate the number of UTI events confirmed by positive urine culture and number of subjects presenting at least one UTI event over 12 consecutive months of study intervention from D0 (by group).
- To evaluate the number of UTI events not confirmed by positive urine culture and number of subjects presenting at least one UTI event over 12 consecutive months of study intervention from D0 (by group).
- To evaluate the number of UTI events confirmed or not by positive urine culture and number of subjects presenting at least one UTI event over 12 consecutive months of study intervention from D0 (by group).

## 5. STUDY DESIGN

### 5.1 STUDY METHODOLOGY

This is an open label, prospective, single site, randomised, controlled trial, in two parallel groups with a 1:1 ratio allocation.

The randomisation process will be applied using a centralised Interactive Web Response System (IWRS) that will allocate subject into one of the two groups in respect with the randomisation list generated informatically at the beginning of the study.

### 5.2 STUDY EVALUATION CRITERIA

#### 5.2.1 Primary endpoint

The primary endpoint assessed during this study is the difference between groups in terms of number of UTI recurrence over about 12 months of follow-up of study intervention from randomisation call (D0).

Only uncomplicated symptomatic UTI events will be considered for the primary endpoint analysis.

Uncomplicated UTI events are defined as follow:

- At least one symptom of UTI (i.e.: dysuria (i.e. painful urination, usually described by the patient as burning, stinging or itching) and / or urgency and / or frequency and / or suprapubic pain (i.e. pain occurring from above the pubis) [30]

And

- Positive urine culture (i.e.  $\geq 10^3$  CFU/mL in the midstream specimen of urine (MSU) [30].

The start date of UTI event will be the date of first symptoms, and the end date will be the sampling date of urine culture performed at the end of the episode-therapy that provides negative urine culture.

#### 5.2.2 Secondary endpoints

The secondary endpoints assessed during this study are as follows:

- Difference between groups in terms of antibiotic prescription/usage to treat UTI event over 12 months of study intervention from D0
- Difference between groups on average delay between each UTI event over 12 months of study intervention from D0
- Difference between groups on urinary hydration markers over 6 and 12 consecutive months of study intervention from D0
- Difference between groups in terms of health-costs associated with management of UTI recurrence(s) over 12 months of study intervention from D0
- Difference between groups in terms of cost utility analysis over 12 months of study intervention from D0
- Difference between groups in terms of change in QoL over 6 and 12 consecutive months of study intervention from D0

As quality of life (QoL) is an important aspect of the intervention, the health economic evaluation will be considered through a cost utility analysis (CUA). Those health cost study will be conducted using two different

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perspectives. The perspective is the point of view from which the costs and benefits are recorded and assessed.

Considering the research question, the two perspectives below will be considered:

- National Health insurance
- Patients' perspective by determining patient own out-of- pocket costs during the study intervention.

As several perspectives are included in the analysis, the results will be presented separately for each study perspective.

### 5.2.3 *Exploratory endpoints*

The exploratory endpoints assessed during this study are as follows:

- Relationship between urinary hydration markers and number of UTI events over 12 consecutive months of study intervention from D0
- Relationship between urinary hydration markers and delay of UTI events over 12 consecutive months of study intervention from D0
- Impact of increased daily water intake on crystallisation risk index (Tiselius) over 12 consecutive months of study intervention from D0
- Number of UTI events confirmed by positive urine culture and number of subjects with at least one UTI event over 12 consecutive months of study intervention from D0 (by group)
- Number of UTI events not confirmed by positive urine culture and number of subjects with at least one UTI event over 12 consecutive months of study intervention from D0 (by group)
- Number of UTI events confirmed or not by positive urine culture and number of subjects with at least one UTI event over 12 consecutive months of study intervention from D0 (by group)

### 5.2.4 *Product safety evaluation criteria*

The safety criteria assessed during this study are as follows:

- Blood sample (Total Cholesterol, HDL Cholesterol, LDL Cholesterol, Triglycerides, Glycosylated haemoglobin, serum creatinine, serum urea, and eGFR),
- Blood pressure (systolic and diastolic)
- Heart rate
- Axillary Body temperature
- Weight
- Urine sodium
- Urine potassium
- Physical Examination at V2 and V4
- Adverse events
- Serious adverse events

## **5.3 STUDY GLOBAL DESCRIPTION**

The subjects will be divided into 2 balanced groups of 70 randomised subjects each. Subjects allocated to study test group (intervention group) will be asked to consume daily three (3) bottles of natural mineral water (500 mL each) in addition to their usual fluid intake consumption, for approximately 12 months. It is suggested to

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consume them at the beginning of every meal (breakfast, lunch and dinner) and fully drink them before the following meal. The subjects allocated to control group will be asked not to change the low fluid intake assessed at baseline (V1).

The study will include 2 parts:

1. The **screening period**, from V1 to D0 (randomisation call).

During this period, subjects will be screened for inclusion and exclusion criteria and checked for randomisation/non-randomisation criteria.

2. The **study intervention period** (approximately 12 months duration), from D0 (randomisation call) to V4 (end of study visit).

During this period, subjects will be checked for randomisation/non-randomisation criteria and will attend 1 evaluation visit (V3) at six months and an end of study visit (V4) at twelve months from D0 (randomisation call). Subjects will be called every month by the PI or another documented authorized study team member to check the study compliance (including fluid intake and study product consumption if any), adverse event(s), symptoms of UTI and / or UTI event(s), concomitant medication(s) and sexual life habits.

In case of symptom(s) of UTI, a relapse visit will be performed.

Each subject will undergo:

- at least 2 urine pregnancy tests (V1 and V2),
- at least 3 blood sample collections (V2, V3 and V4),
- at least 3 urine 24-hours collections (collection to be started the day before V2, V3 and V4),
- And at least 2 urine cultures (V2 and V4).

Each appearance of UTI symptoms will lead to:

- 1 urine culture at the beginning of the episode,
- 1 urine culture, after the standard of care treatment to confirm the recovery, if the first one was positive.

For the blood samples, it is necessary for the subjects to be in a fasted state for 8-12 hours before the visit.

#### **5.4 STUDY DESIGN SCHEMA**

Refer to section 2.

#### **5.5 DURATION OF THE STUDY PER SUBJECT**

The total duration of the study is approximately fourteen (14) months for each subject, including a screening period of up to eight (8) weeks, and a study intervention period around twelve (12) months.

## 6. SUBJECTS

### 6.1 SUBJECT RECRUITMENT AND SCREENING

The subjects included in the study will be pre-menopausal women with recurrent symptomatic episodes of UTI aged not below 18 and having a “low drinker profile” defined as:

- total self-reported fluid intake < 1,5 L/day;
- and total 24 hours urinary volume < 1,2 L per day
- and urine osmolality  $\geq$  500 mOsmol/kg
- and urine creatinine within the laboratory normal ranges

Subjects with clinical manifestations of menopause as menopausal women will be excluded. In case of menopausal symptoms, a FSH blood test must be performed at V2.

The subject inclusion will be stopped as soon as 140 subjects are randomised.

At this time, all eligible subjects already included (subjects who are within the screening period (from V1 to V2)) will be randomised and are expected to complete all the study procedures and visits according to the protocol until their end of study visit (V4).

Subjects potentially able to participate to this study will be pre-selected by physicians working in liberal practice (GPs, urologists, gynaecologists) from their patients' database. The pre-screened subjects will be referred to the Investigating Centre with a referral bulletin mentioning the status of all UTI events as well as with the last positive results of urine cultures performed within the last 12 months, except for the symptomatic subjects with 2 UTI events at V1 (i.e. correspond to criteria II01.C for which the documentation of positive urine culture must be the one performed between V1 and V2).

If necessary, in order to reinforce recruitment rate, the Investigating Centre may also use advertising, only after EC approval, and conduct a **pre-screening** of patients at the investigating site following their standard process.

Taking into account that the study is particularly long, the study team should discuss the subject's willingness to comply with all study related procedures stressing the length and rigorous requirements prior to the subject enrolling.

The subject must have personally dated and signed their Patient Information and Informed Consent Form (ICF) before undergoing any study procedures.

### 6.2 SUBJECT ELIGIBILITY CRITERIA

A subject is considered eligible when the Investigator certifies that the subject fulfils all inclusion criteria and does not meet any exclusion criteria for the current visit. This statement can potentially be repeated at different subjects' visits before subject's randomisation as required by the study design.

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6.2.1 *Inclusion criteria (checked at the inclusion visit - V1)*

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

II01a: Women with at least 3 clinical recurrences\* of symptomatic UTI in the last 12 months (at least one UTI must be confirmed by a positive documented bacteriological exam) and asymptomatic at V1

**or**

II01b: Women with at least 3 clinical recurrences\* of symptomatic UTI in the last 12 months (at least one UTI must be confirmed by a positive documented bacteriological exam) and symptomatic at V1

**or**

II01c: Women with at least 2 clinical recurrences\* of symptomatic UTI in the last 12 months (who did not perform any bacteriological exam during the last 12 months), symptomatic at V1 and with positive documented bacteriological exam between V1 and V2

II02: Age  $\geq$  18 years

II03: Fluid intake < 1.5 L per day based on patient's declaration

II04: Regular meal consumption (breakfast, lunch and dinner)

II05: Access to Internet for information on hydration

II06: Subject, upon briefing of the content of the present study, fully understanding and agreeing to its objective and having given written (dated and signed) informed consent to take part in the study

II07: Subject who is able to communicate well with the Investigator and to comply with the requirements of the entire study.

II08: Literate subjects, able to fill in fluid diaries and QoL (Quality of life) questionnaire

II09: Women accepting to keep their lifestyle habits during the whole duration of the study

II10: Women using any form of contraception

II11: Subject covered by the National Health insurance system

6.2.2 *Exclusion criteria (checked at the inclusion visit - V1)*

A potential subject who meets any of the following criteria will be excluded from participation in this study:

IE01: Incapacity / non-willingness to consume 1.5 L of drinking water per day on top of their usual consumption

IE02: Women with history of UTI complications (pyelonephritis or other) in the last 12 months

IE03: Use of antibiotics or cranberries juice and / or extracts in the previous 2 weeks

IE04: Chronic treatments with anti-coagulants therapy

IE05: Chronic bladder inflammation (defined as permanent bladder bacterial infection)

IE06: Chronic diarrhea or constipation treated with chronic use of laxative substances

IE07: Interstitial cystitis

IE08: Estrogen-dependent symptomatic vulvo-vaginitis

IE09: Recent (<1year) or active renal stone disease

IE10: Urinary tract structural abnormalities

IE11: Obesity or malnutrition (BMI <18.5 Kg/m<sup>2</sup> and >30 Kg/m<sup>2</sup>)

IE12: Pregnant or lactating women

IE13: Women planning to become pregnant during the study

IE14: Menopausal and peri-menopausal women

IE15: On-going or planned therapy during the study which can modify the study measurements, in particular the assessment of the hydration status (diuretic intake, corticoids or drug treatment interfering with nutrition behaviour)

IE16: Subjects with severe or uncontrolled organic disease, likely to interfere with the parameters of the study (e.g. neoplastic, cardiovascular, pulmonary and digestive disorders, uncontrolled diabetes type I and II, untreated or uncontrolled clinically significant arterial blood hypertension) or mental disorders affecting eating and drinking behaviour (i.e. primary polydipsia, bulimia nervosa, psychosis, etc.)

IE17: Women who have taken part in any other clinical study for the treatment of rUTI during the last 12 months

IE18: Vulnerable subjects defined as individuals whose willingness to volunteer in the clinical trial may be unduly influenced by the expectation, whether justified or not, of benefits associated with participation, or of a retaliatory response from senior members of a hierarchy in case of refusal to participate.



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(Examples are members of a group with a hierarchical structure linked to the Investigator or to the Sponsor, such as medical, pharmacy, dental and nursing students, subordinate hospital and laboratory personnel, employees of the Investigator or of the Sponsor, members of the armed forces, and persons kept in detention)

IE19: No legal capacity or limited legal capacity or unable to give an informed consent

IE20: Subjects unlikely to cooperate in the study, and/or poor compliance anticipated by the Investigator

IE21: Women who have taken part in this study and/or enrolled in another clinical study during the last month and/or any other clinical study during the last month

*6.2.3 Randomisation criteria [checked at the pre-randomisation visit (V2) and before the subject's randomisation]*

RI01: Negative urine culture and asymptomatic state (i.e. silent phase) at V2 (pre-randomisation visit)

RI02: Low-drinker confirmation defined as:

- total self-reported fluid intake < 1,5 L/day,
- and 24 hours urinary volume < 1.2 L per day,
- and osmolality > 500 mOsmol/kg,
- and urine creatinine within the laboratory normal ranges.

*6.2.4 Non -Randomisation criteria [checked at the pre-randomisation visit (V2)]*

Subjects may only be randomised in this study after meeting the inclusion criteria and presenting none of the non-inclusion criteria stipulated in paragraphs 6.2.1, 6.2.2 and 6.2.3 above.

A potential subject who meets any of the following non-randomisation criteria at V2 and before the D0 (randomisation call) will be excluded from participation in this study:

RE01: Chronic kidney disease (defined as decreased GFR ( $GFR < 60 \text{ ml/min/1.73m}^2$  calculated using EPI equation)

RE02: Women suffering multiple antibiotic resistant bacterial strain\*

IE12: Pregnant or lactating women

IE14: Menopausal and peri-menopausal women\*\*

\* Multiple antibiotic resistance is defined as resistance to 3 or more different antibiotics

\*\* Based on V2 FSH assessment as per PI's discretion

**6.3 SUBJECT IDENTIFICATION**

*6.3.1 Subject Identification Number*

At V1, after the subject and the Investigator have personally dated and signed the Informed Consent Form, a Case Report Form (e-CRF) is created and a Subject Identification number is allocated manually to her in chronological order of inclusion. This number will be the main identification code for a subject for the duration of the study in order to protect the subject's identity and will appear throughout the e-CRF for that particular subject. This number should also be reported on the IWRS (if the subject is randomised) and on other relevant paper-based study documents where required (i.e. Quality of Life, Subject Diaries, source data in the Subject Medical File).

The Subject Identification number consists of 11 characters, which is based on:

- A 3 digit "Country number (will be "100")
- A 3 digit "Site" number (will be "001")

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- A 3 digit “Subject” number.

The first subject included at site will receive the number “001”. Subject numbering will then be sequentially increased by one for each new included subject (example 002, 003 etc.).

Each of these 3 elements is separated by a score “-”.

Example: Subject Identification number “100-001-010”, refers to Site “001” and Subject “010”.

#### 6.3.2 *Randomisation Process*

Refer to section 10.1.

### 6.4 SUBJECT DISCONTINUATION

#### 6.4.1 *Criteria for subject discontinuation*

The Investigator should discontinue subject’s participation in the study prematurely at any time during the study in the following situations:

- In the case where a subject has decided to resign from further participation in the study (withdrawal of consent) or
- In the case where further participation is a health risk for the subject, at the Investigator’s discretion or
- In the case of subject’s pregnancy or
- In the case where a severe non-compliance to protocol or a protocol violation has been identified for the subject that may lead to protocol deviations described under *section 16.8. Such as may include:*
- Non adherence to the targeted population defined as pre-menopausal low drinker women with recurrent urinary tract infections
- Recurrence of systematic non respect of investigator’s instructions with respect to: scheduled visit urine samples collection, diaries’ completion and return, fluid intake regimen. Taking into account that this non-adherence can impact the accuracy, the completeness and the legibility of the data reported. If such event arrives, the final decision of subject discontinuation must be taken by the sponsor and the investigator.
- In the case where the subject is lost to follow-up or
- In the case whereby the study is prematurely terminated or suspended by the sponsor.

#### 6.4.2 *Replacement conditions*

**Subjects withdrawing prematurely from the study at any time will not be replaced and cannot be re-included at a later date.**

#### 6.4.3 *Procedures in case of subject discontinuation*

In case of premature withdrawal, the Investigator must notify the study monitor as soon as possible, and should make all efforts to contact the subject and if the subject agrees, ensure that all evaluations scheduled for the End of Study Visit (V4) are completed and reported in the e-CRF.

Alternative follow-up of the discontinued subject is to be arranged by the Investigator if necessary.

For subjects who discontinue the study due to the occurrence of adverse events potentially related to the study product or to the study procedures, follow-up will take place (clinical or biological examinations) until the adverse

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event has abated, or until a stable situation has been reached (please refer to the section 12), with findings being recorded in the e-CRF.

For subjects who discontinue the study due to pregnancy, follow-up will take place until birth or early termination of pregnancy. In addition to documenting this premature termination in the e-CRF, the Investigator should make every effort to collect information about the pregnancy and the infant and report to the Sponsor with a dated and signed Pregnancy Reporting Form as specified in the section 12.6.

## 6.5 INSTRUCTIONS AND RESTRICTIONS DURING THE STUDY

### 6.5.1 Study product(s) consumption instructions

The Investigator will provide the subject with the instructions on the study product consumption at the pre-randomisation visit (V2).

#### **Study intervention group:**

The subject allocated in the study product intervention group will begin consumption of the study product the morning of the following day after the receipt of the study products and will stop consumption the day of end of study visit (V4).

Throughout the entire consumption period (around 12 months) of the study, the subject allocated in the intervention group will daily consume three (3) bottles (500 mL each) of the test product (i.e. 1,5 L of commercialised natural mineralised Evian water in total).

It is suggested to start consumption at the beginning of every meal (breakfast, lunch and dinner) and fully drink them before the following meal (about 500ml around the main meals).

At the same time, the subjects will be receiving coaching messages (in particular 4 newsletters in total) related to the fluid intake recommendations and study products.

The subjects will receive regularly, and free of charge, the bottles of mineral water personally or to a delegated person, at home or office.

The subject will report every day her consumption in the Subject Compliance Diary (please refer to the section 6.5.5), specifying the number of bottles consumed during the day and the quantity of water drunk for each bottle (total bottle, ¼ of bottle, ½ of bottle).

The water consumption will be checked at each visit (i.e. telephone call and site visits) by the PI or another documented authorised study team member and a summary of the water consumption will be entered into the e-CRF. The investigating site will also collect the Subject Compliance Diaries every month in order to be able to check the information in case of inconsistencies.

Subjects will record every month their fluid intakes using the 3-days fluid intake diary (please refer to the section 6.5.4).

#### **Study control group:**

The subject allocated in the control group will remain stable in her fluid consumption. Her compliance will be assessed every month, using the 3-days fluid intake diary (please refer to the section 6.5.4).

The subject will not receive any bottle of water nor any coaching support.

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**6.5.2** *Dietary instructions*

During the entire duration of the study (i.e. from V2 to V4), subjects will not have any dietary restriction with the exception of cranberries juice and/or extracts. All women included in the study will be asked not to consume these products from 2 weeks before the inclusion visit to the end of the study period.

The subjects will also be asked to avoid making any significant changes to their usual diet during the study period (i.e. no changes to the usual amount of fibre, not to begin a weight loss diet, etc...).

The dietary restrictions stipulated in this study do not represent a risk for the subjects. There is no risk of deficiency in the nutrient related to this dietary restriction.

**6.5.3** *Non-authorized medicinal products and nutritional supplements*

During the entire duration of the study (i.e. from V1 to V4), all medicinal products are authorised excepting treatments listed in the exclusion criteria (anti-coagulants, corticoids, diuretics, laxatives or treatments interfering with nutrition behaviour, cranberry juice or extracts).

Included subjects will be asked not to take any antibiotic therapy in the 2 weeks before the inclusion visit (V1), to avoid any inclusion bias due to the presence of a UTI masked by the use of antibiotics.

In case of any non-authorized medicinal products consumption or any non-authorized medical treatments, the subject will be asked to complete her 3 days fluid intake diary with the name, the dose and the intake dates of the medicinal products or medical treatment. The subjects will be advised to contact the Investigator or the study nurse for recommendations.

**6.5.4** *3-days fluid intake diary*

Before the planned visits (i.e. phone call and visit), the subject will be instructed to fill in the 3-days fluid intake diary throughout the entire study duration in order to document the fluid intake during 3 days (Saturday-Sunday-Monday or Friday-Saturday-Sunday).

**6.5.5** *Subject Compliance Diary*

The subject randomised in the intervention group will be instructed to daily fill in the Subject Compliance diary to report her study product consumption.

**6.5.6** *Voiding diary*

During the 24 hours collection, the subject will be instructed to fill in the Voiding diary outside of menstruation period before V2, V3 and V4 (and in case of 24H collection repetition) to report all micturitions collected during 24 hours.

## 7. STUDY PROCEDURES

### 7.1 EVALUATIONS AND PROCEDURES PER VISIT

Evaluations and procedures for the study visits are described below. Please refer also to the Study flow-chart in the Section 2.

24 h urine collection nor any visits can be performed in case of menstrual period.

#### 7.1.1 Inclusion Visit (V1)

This visit will take place no more than 7 weeks before the pre- randomisation visit (V2).

At the beginning of V1, the Investigator should fully inform the subject of all aspects of the clinical study including the written information and the approval by the IRB/IEC. The subject must be given the opportunity to ask questions and have them answered by the Investigator.

Prior to subject's participation in this clinical study, the subject must personally sign and date the approved informed consent form. The subject cannot undergo any study procedures before having personally dated and signed the approved Informed Consent Form. The Investigator must also sign and date the Informed Consent Form.

After the subject and the Investigator have personally dated and signed the approved Informed Consent Form, a Subject Identification number is allocated to the subject an electronic Case Report Form (e-CRF) is created.

The eligibility of the subject has to be checked at the beginning of the visit and the study procedures at V1 are the following (please refer to the Study flow-chart in the section 2):

- Obtaining date (incl. time), signature of subject on approved informed consent form,
- Recording subject's demographic data (date of birth, gender),
- Checking all the inclusion and exclusion criteria for subject's eligibility validation (please refer to the section 6.2),
- Reviewing the past medical and surgical history,
- Reviewing the past and current medications,
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,
- Performing a urinary pregnancy test,
- Recording vital signs: systolic/diastolic blood pressure (please refer to the section 7.4.1.4 for instructions) and heart rate, axillary body temperature,
- Recording anthropometrical parameters; body weight and height, waist circumference,
- Checking for (peri)menopausal symptoms. If there are such, a FSH test is to be performed at V2,
- Checking symptoms of UTI
- Checking and recording of adverse events and of concomitant medications,
- **Performing urine culture for symptomatic subjects only** and assign or not a therapy (i.e. AFU/EAU guidelines for UTI management) according to PI's decision.

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**Symptomatic subjects will perform V1 (please refer to the Appendix IV / Management of symptomatic/Asymptomatic subjects per visits). A urine culture will be performed:**

**1a/If positive urine culture:**

The subject will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a **control** urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered and that results are not biased by the antibiotic treatment taken by the subjects.

Depending on **control** urine culture results:

- If **negative** control urine culture, the subject will come back to site to perform the pre-randomisation visit (V2) as soon as the subject is completely recovered and up to a maximum of 7 weeks from V1
- If **positive** control urine culture, the subject will be treated till a negative control urine culture is obtained and will come back to site to perform the pre-randomisation visit (V2) as soon as the subject is completely recovered and up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.

Any UTI symptom(s) with or without a positive urine culture, occurring between V1 and V2 will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.

**1b/ If negative urine culture:**

The subject may be treated or not with a standard of care treatment at the PI's discretion:

- Subject **receiving** a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained. They will come back to the site to perform the pre-randomisation visit (V2) up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.
- Subject **not receiving** any treatment and declaring complete recover from previous symptoms will come back to site to perform the pre-randomisation visit (V2) as soon as the symptoms are completely disappeared (subject is completely recovered) and up to a maximum of 7 weeks from V1.
- Subject **not receiving** any treatment but with persisting symptoms will **re-perform** a urine culture. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standard of care treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the pre-randomisation visit (V2) up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.

Any UTI symptom(s) with or without a positive urine culture, occurring between V1 and V2 will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.

- Scheduling study visits (i.e. phone calls and visits) for subjects who are eligible and available for the duration of the study.

**If the eligibility criteria are met, the subject is included in the study.**

Furthermore, the subject will be provided with instructions for:

- Coming back to the site in case of any UTI symptom(s)
- Diet and non-authorized products (please refer to the sections 6.5.2 and 6.5.3)
- Documenting any adverse and serious adverse event.
- Completing the 3-days fluid intake diary provided and return at the next visit (V2) (please refer to the section 6.5.4)
- Completing the Voiding diary provided and returning at the next visit (V2) (please refer to the section 6.5.6)
- Collecting, storing and transportation of urine samples. Subject will be provided with the kits for urine sterile sample and 24h urine collection (please refer to the section 7.4.2.6)

**Asymptomatic subjects at V1 (please refer to the Appendix IV / Management of symptomatic/Asymptomatic subjects per visits)** will come back to the site to perform the pre-randomisation Visit (V2) after a minimum of 4 days (i.e. the minimum time needed to complete the 3-days fluid intake diary) and up to 7 weeks from V1.

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents are accurate and complete (please refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit and no later than 5 working days to the date of the visit. In case of unavailable data, the e-CRF should be completed within 48 hours after the missing data's receipt.

#### 7.1.2 Pre-randomisation Visit (V2)(from + 4 days to + 7 weeks after V1)

The pre-randomisation visit (V2) will take place at the minimum of 4 days after V1 and at the maximum of 7 weeks after V1.

At the pre-randomisation visit (V2), the subjects will bring back the following:

- 24hours urine samples
- Sterile urine sample
- Voiding diary
- 3 days fluid intake diary

1/ If a subject coming to **V2 presents UTI symptom(s), whatever the urine culture results**, she won't be able to perform the visit, only urine culture is analysed. **V2 will be rescheduled** once the subject is recovered. **(please refer to the Appendix IV / Management of symptomatic/Asymptomatic subjects per visits)**

#### 1a/ Symptomatic subjects at V2 with a positive urine culture:

The subject will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a **control** urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that the subject is completely recovered.

Depending on **control** urine culture results:

- If **negative** control urine culture, the subject will come back to the site to perform the pre-randomisation visit (V2) as soon as the subject is completely recovered and up to a maximum of 7 weeks from V1
- If **positive** control urine culture, the subject will be treated till a negative control urine culture is obtained and will come back to site to perform the pre-randomisation visit (V2) as soon as the subject is completely recovered and up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.

Any UTI symptom(s) with or without a positive urine culture, occurring between V2 and D0 (randomisation call) will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.

#### 1b/ Symptomatic subjects at V2 with a negative urine culture:

The subject may be treated or not with a standard of care treatment at the PI's discretion:

- Subject **receiving** a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the pre-randomisation visit (V2) up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.
- Subject **not receiving** any treatment and declaring complete recover from previous symptoms will come back to the site to perform the pre-randomisation visit (V2) as soon as the symptoms are completely disappeared (subject is completely recovered) and up to a maximum of 7 weeks from V1.

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- Subject **not receiving** any treatment but with persisting symptoms will **re-perform** a urine culture. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standardised treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the pre-randomisation visit (V2) up to a maximum of 7 weeks from V1 and only if the subject is not multiple antibiotic resistant.

Any UTI symptom(s) with or without a positive urine culture, occurring between V2 and D0 (randomisation call) will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.

**2/ Asymptomatic subject will perform V2 (please refer to the Appendix IV / Management of symptomatic/Asymptomatic subjects per visits).**

The eligibility of the subject has to be checked at the beginning of the visit and the study procedures during the pre-randomisation visit are the following (please refer to the Study flow-chart in the section 2):

- Checking that 24 hours urine samples have been collected as well as the sterile urine sample,
- Conducting a physical examination,
- Recording vital signs: systolic/diastolic blood pressure (please refer to the section 7.4.1.4 for instructions) and heart rate, axillary body temperature,
- Recording anthropometrical parameters; body weight , waist circumference,
- Repeating pregnancy test
- Checking all the randomisation and non-randomisation criteria (please refer to the section 6.2),
- Collecting and reviewing V1-V2 subject diary for 3-days fluid intake,
- Checking and recording of adverse events and of concomitant medications,
- Checking non-authorized product compliance,
- Collecting of blood samples (after 8-12 hours of fasting), including the request for FSH test if needed in case of perimenopausal symptoms (please refer to the section 7.4.2.1),
- Collecting and checking of the Voiding diary,
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,
- Checking symptoms of UTI,
- **Performing urine culture for all subjects** to assess the absence of any acute UTI episode,
- Reminding the instructions for diet and non-authorized products (please refer to the section 6.5.2),
- Reminding instruction for coming back to the site in case of any UTI symptom(s),
- Providing instructions for completing the QoL questionnaire (SF-12 V2) in situ but not in front of the Investigator. The Investigator will check the SF-12 V2 questionnaire completion,
- Providing the 3-days fluid intake diary and Subject's Compliance Diary pages (only for the patients in the intervention group) covering the period between V2 and V3 (inclusive pre-printed envelopes for sending via courier) and repeating the instructions for their completion,
- Providing instructions for collecting, storing and transportation of urine samples. The subject will be provided with kits for urine sterile sample (in case of UTI symptoms between V2 and V3) for the 24h urine collection (please refer to the section 7.4.2.6),
- Providing a Voiding diary in order to collect frequency of voiding during the 24h urine collection before V3,
- Providing UCC and instructions to use.

**2a/ Asymptomatic subjects at V2 with a positive urine culture**, the subject will be treated for the UTI event with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a **control** urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered.



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If a negative **control** urine culture is obtained:

- ≤ 28 days, after V2 (i.e. negative control urine culture obtained at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered), a new QoL questionnaire will be completed by the subject after UTI is cured.
- > 28 days, after V2, the V2 should be repeated.

Any UTI symptom(s), with or without a positive urine culture, occurring between V2 and D0 (randomisation call) will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF.

**2b/ Asymptomatic subjects at V2 with a negative urine culture (please refer to the Appendix IV / Management of symptomatic/Asymptomatic subjects per visits)** and fulfilling all randomisation and none of the non-randomisation criteria will be randomised by the PI or another documented authorized study team member no later than 1 week after V2.

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents are accurate and complete (please refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit and no later than 5 days to the date of the visit. In case of unavailable data, the e-CRF should be completed within 48 hours after the missing data's receipt.

#### *7.1.3 Randomisation call (D0)(maximum 8 weeks after V1)*

The investigator will verify randomisation and non-randomisation criteria after having received the relevant lab results. If considered eligible, the subject will be randomised into one of the 2 groups through the IWRS.

The investigator or another documented authorized study team member will perform a randomisation call the same day of subject randomisation through the IWRS, in order to instruct the subject on the study procedures to follow, depending on her group allocation.

**If the subject is in the intervention group**, the Investigator will arrange the logistics company to be informed for the requirement to deliver to the subject the study products. The subject will be informed that she will receive the study products and she will be provided with the recommendation to consume 1,5 L/day (3 bottles of mineral water) starting from the morning after reception of study products until the completion of the study, in addition to their usual fluid intake consumption. She has to report the study product consumption in the Subject Compliance Diary in addition to the 3-days fluid intake diary.

**If the subject is in the control group**, the Investigator or another documented authorized study team member will call the subject and inform her that she has to continue her life habits and she doesn't need to complete the Subject Compliance Diary. She will have to continue to complete monthly the 3-days fluid intake diary.

#### **Between D0 (randomisation call) and V3 (evaluation visit):**

The subject in the **intervention group** has to send back to the Investigational site the Subject Compliance Diary, corresponding to the previous month of study product consumption, and the 3-days fluid intake diary by courier or personally if preferred.

The subject in the **control group** will have to return the 3-days fluid intake diary every month to the site by courier or personally if preferred.

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents are accurate and complete (please refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit and no later than 5 days to the date of the visit. In case of unavailable data, the e-CRF should be completed within 48 hours after the missing data's receipt.

*7.1.4 Evaluation Visit– Visit 3 (Month 6 after the D0 (randomisation call) within +/- 10 days allowable time window or + 28 days in case of UTI event)*

Visit 3 will take place 6 months (+/- 10 days or + 28 days in case of UTI event) after the randomisation call (D0).

At V3, the subjects in both groups will bring back to the site the following:

- 24h Urine samples
- Voiding diary
- 3 days fluid intake diary
- Subject Compliance Diary for subject as appropriate.

The study procedures performed during the evaluation visit are the following (please refer to the Study flow-chart in the section 2):

- Checking symptoms of UTI

**Asymptomatic subject will perform V3 (please refer to the Appendix IV / Management of symptomatic/Asymptomatic subjects per visits).**

**If a subject coming to V3 presents UTI symptom(s), she won't be able to perform the visit. Given the presence of symptoms, a relapse visit will be done (please refer to the section 7.1.7). V3 will be rescheduled once the subject is recovered (please refer to the Appendix IV / Management of symptomatic/Asymptomatic subjects per visits).**

**1a/ Symptomatic subjects at V3 with a positive urine culture:**

The subject will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a **control** urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered.

Depending on the **control** urine culture results:

- If **negative** control urine culture, the subject will come back to the site to perform the evaluation visit (V3) as soon as the subject is completely recovered.
- If **positive** control urine culture, the subject will be treated till a negative control urine culture is obtained and will come back to the site to perform the evaluation visit (V3) as soon as the subject is completely recovered.

Any UTI symptom(s), with or without a positive urine culture, verified just before the V3 will be reported in a relapse visit.

**1b/ Symptomatic subjects at V3 with a negative urine culture:**

The subject may be treated or not with a standard of care treatment at the PI's discretion:

- Subject **receiving** a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the evaluation visit (V3) as soon as the patient is completely recovered.
- Subject **not receiving** any treatment and declaring complete recover from previous symptoms will come back to site to perform the evaluation visit (V3) as soon as the symptoms are completely disappeared (subject is completely recovered).

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- Subject **not receiving** any treatment but with persisting symptoms will **re-perform** a urine culture. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standard of care treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to the site to perform the evaluation visit (V3).

Any UTI symptom(s), with or without a positive urine culture, verified just before the V3 will be reported in a relapse visit.

The study procedures performed during the evaluation visit are the following (please refer to the Study flow-chart in the section 2):

- Recording vital signs: systolic/diastolic blood pressure (please refer to the section 7.4.1.4. for instructions) and heart rate, axillary body temperature,
- Recording anthropometrical parameters: body weight, waist circumference,
- Collecting and reviewing V2-V3 subject's diaries 3 days fluid intake and Subject Compliance Diary (if applicable),
- Checking and recording adverse events and concomitant medications,
- Checking non-authorized product compliance,
- Collecting of blood samples (after 8-12 hours of fasting) (please refer to the section 7.4.2.1),
- Checking that 24h urine sample has been collected,
- Collecting and checking the voiding diary,
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,
- Providing instructions for completing the QoL questionnaire (SF-12 V2) in situ but not in front of the Investigator. The Investigator will check the SF-12v2 questionnaire completion,

At the end of the evaluation visit (V3), the subject will be:

- Reminded with instructions for diet and non-authorized products (please refer to the section 6.5.2),
- Reminded with instructions for:
  - 3-days fluid intake diary completion (including documentation of adverse events),
  - Subject Compliance Diary
  - Voiding diary as appropriate.Appropriate pages of the diaries will be provided to the subject. (please refer to the section 6.5.4, 6.5.5 and 6.5.6)
- Provided with instructions for urine collections, storages and transportations. Subject will be provided with the kits for urine sterile sample and 24h urine collection (please refer to the section 7.4.2.6 for instructions),
- Reminded with instructions for coming back to the site in case of any UTI symptom(s),
- Reminded with instructions for study product consumption (only for the intervention group).

**Between V3 and V4:**

- The subjects in the **intervention group** have to send back to the Investigator's site the Subject Compliance Diary, corresponding to the previous month of study product consumption, and the 3 days fluid intake diary by courier or personally if preferred,
- The subjects in the **control group** will send back to the Investigator's site the 3 days fluid intake diary by courier or personally if preferred on monthly basis.

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents are accurate and complete (please refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit and no later than

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5 days to the date of the visit. In case of unavailable data, the e-CRF should be completed within 48 hours after the missing data's receipt.

*7.1.5 Phone calls (From Month 1 to Month 5 and from Month 7 to Month 11, all phone calls will be performed within the respective number of months after the randomisation call (D0) within +/- 7 days allowable time window)*

Every month, except at Month 6 (V3) and month 12 (V4), the subjects will be contacted by phone by the principal Investigator or another documented authorised study team member.

PI or study personnel will call the subject at the following time schedule:

- Phone call 1 (Month 1, +/- 7 days), between Randomisation call (D0) and V3
- Phone call 2 ( Month 2, +/- 7 days), between Randomisation call (D0) and V3
- Phone call 3 (Month 3, +/- 7 days), between Randomisation call (D0) and V3
- Phone call 4 (Month 4, +/- 7 days), between Randomisation call (D0) and V3,
- Phone call 5 (Month 5, +/- 7 days), between Randomisation call (D0) and V3
- Phone call 6 (Month 7, +/- 7 days), between V3 and V4
- Phone call 7 (Month 8, +/- 7 days), between V3 and V4,
- Phone call 8 (Month 9, +/- 7 days), between V3 and V4,
- Phone call 9 (Month 10, +/- 7 days), between V3 and V4
- Phone call 10 (Month 11, +/- 7 days), between V3 and V4

The objective of each phone call will be:

- Reminding the instructions for diet and and non-authorized products (please refer to the section 6.5.2 and 6.5.3),
- Ensuring that 3-days fluid intake diary, Subject compliance diary (as appropriate) are regularly completed and sent back to the site (please refer to the section 6.5.4),
- Assessing the compliance with the study product consumption as applicable. During the 1<sup>st</sup> phone call, the Investigator will collect the date of the 1<sup>st</sup> product consumption,
- Assessing the level of fluid intake based on subject's interview,
- Assessing the occurrence of adverse events based on subject's interview,
- Assessing whether any concomitant medications have been taken or modified,
- Assessing the presence of symptom(s) of UTI at the time of the phone call as well as in the period between the last contact to the subject and the current phone call,
- Assessing the start date and the end date of a UTI episode if any. In this case, the PI must remind to the subject that in case of any UTI symptom(s) a relapse visit must be done,
- Checking sexual life status and emptying bladder habits after sexual intercourses,
- Inquiring for diagnosed pregnancy or any doubts for such after the last visit (including phone call).
- For the 5<sup>th</sup> and 11<sup>th</sup> month follow-up calls; PI will remind the instructions for urine collections, storages and transports for urine sterile sample (as applicable for the visit) and 24h urine collection (please refer to the section 7.4.2.6 for instructions) as well as the instructions to complete the voiding diary.

The site will ensure that the phone call procedures and evaluations are properly documented in the subject's medical file and that the source documents are accurate and complete (please refer to the section 8). The subject's / e-CRF should be completed from the source documents and as soon as possible after the phone call and no later than 5 days to the date of the phone call. In case of unavailable data, the e-CRF should be completed within 48 hours after the missing data's receipt.

*7.1.6 End of Study Visit – Visit 4 (Month 12 after the D0 (randomisation call) within +/- 10 days allowable time window or + 28 days in case of UTI event)*

The End of study visit (V4) will take place 12 months (+/- 10 days or +28 days in case of UTI event) after the randomisation call (D0).

At V4, the subjects (in fasting condition) will bring back to the site the following:

- 24h Urine samples
- Sterile urine sample
- Voiding diary
- V3-V4 Subject Compliance Diary as appropriate
- 3 days fluid intake diary

**1/** If a subject coming to V4 **presents UTI symptom(s), whatever the urine culture results**, she won't be able to perform the visit, only urine culture is analysed. **V4 will be rescheduled** as soon as the subject is recovered (**please refer to the Appendix IV / Management of symptomatic/Asymptomatic subjects per visits**).

**1a/ Symptomatic subjects at V4 with a positive urine culture:**

The subject will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management). After the completion of UTI treatment, a control urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered.

Depending on **control** urine culture results:

- If a **negative** control urine culture, the subject will come back to the site to perform the end of study visit (V4) as soon as the subject is completely recovered.
- If a **positive** control urine culture, the subject will be treated till a negative control urine culture is obtained and will come back to site to perform the end of study visit (V4) as soon as the subject is completely recovered.

Any UTI symptom(s), confirmed or not with a positive urine culture just before the V4 will be reported in a relapse visit.

Once the negative control urine culture is obtained, the end of study form in the e-CRF will be completed

**1b/ Symptomatic subjects at V4 with a negative urine culture:**

The subject may be treated or not with a standard of care treatment at the PI's discretion:

- Subject **receiving** a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained. They will come back to site to perform the end of study visit (V4) as soon as they are completely recovered.
- Subject **not receiving** any treatment and declaring complete recover from previous symptoms will come back to site to perform the end of study visit (V4) as soon as the symptoms are completely disappeared (subject is completely recovered).
- Subject **not receiving** any treatment but with persisting symptoms will re-perform a urine culture. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standardised treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained. The subject will come back to site to perform the end of study visit (V4).

Any UTI symptom(s) confirmed or not with a positive urine culture just before the V4 will be reported in a relapse visit.

Once the negative control urine culture is obtained, the end of study form in the e-CRF will be completed

**2/Asymptomatic subject will perform V4 (please refer to the Appendix IV / Management of symptomatic/Asymptomatic subjects per visits).**

The study procedures performed during the End of study visit (V4) are the following (please refer to the Study flow-chart in the section 2):

- Conducting a physical examination,
- Recording vital signs: systolic/diastolic blood pressure (please refer to the section 7.4.1.4 for instructions) and heart rate, axillary body temperature,
  
- Recording anthropometrical parameters: body weight, waist circumference,
- Reviewing V3-V4 3-days fluid intake diary, Voiding diary and Subject's compliance diary as appropriate,
- Checking and recording adverse events and concomitant medications,
- Collecting of blood samples (after 8-12 hours of fasting), (please refer to the section 7.4.2.1),
- Checking that 24h urine samples and sterile urine sample have been collected,
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,
- Checking symptom(s) of UTI,
- **Performing urine culture for all subjects,**
- Reminding instructions for completing the QoL questionnaire (SF-12 V2) that will be performed in situ but not in front of the Investigator. QoL questionnaire should be completed in the absence of UTI event (the subject is asymptomatic and has negative urine culture) The Investigator will check the SF-12v2 questionnaire completion

**2a/ Asymptomatic subjects at V4 with a positive urine culture:**

The subject will be treated for the UTI event with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management)., After the completion of UTI treatment, a **control** urine culture will be done at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered.

Depending on **control** urine culture results:

- If a **negative** control urine culture is obtained  $\leq$  28 days after V4 (i.e. negative control urine culture obtained at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered), a new QoL questionnaire will be completed by the subject after UTI is cured,
- If a **negative** control urine culture is obtained  $>$  28 days after V4, the V4 should be repeated

All asymptomatic events with a positive urine culture, occurring at V4 will be considered and collected as AE/ SAE. Concomitant medications will be reported in the e-CRF

Once the negative control urine culture is obtained, the end of study form in the e-CRF will be completed.

**2b/ For asymptomatic subjects at V4 with a negative urine culture,** the end of study form in the e-CRF will be completed. Date of end of study will be the date of lab reports of last negative urine culture.

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents are accurate and complete (please refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit and no later than 5 days to the date of the visit. In case of unavailable data, the e-CRF should be completed within 48 hours after the missing data's receipt.

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7.1.7 *Relapse visit*

Subjects presenting with symptoms of UTI during the course of the study (from D0 to V4), will be instructed not to stop the increased water intake (if in the intervention group) and to come back to the investigational site as soon as possible for clinical examination.

**UTI events will be diagnosed as at least 1 symptom of urinary tract infection and positive urine culture.**

Any UTI symptom(s), with or without a positive urine culture, occurring during the study will be reported in a relapse visit.

Urine cultures will be always performed to confirm presence of infection. Data about duration of the infection (starting and ending date) will be registered.

The tasks performed during the relapse visit are the following (please refer to the Study flow-chart in the section 2):

- Performing urine culture to confirm preliminary diagnosis
- Reporting start date of event (defined as the date of the appearance of the first symptom(s))
- Checking for active/inactive sexual life during the past month,
- Checking for bladder emptying habits after sexual intercourses,
- Checking and recording adverse events and concomitant medications

**1a/ Subjects with a positive urine culture**, will be treated with a standard of care treatment (i.e. AFU/EAU guidelines for UTI management).

**1b/ Subjects with a negative urine culture**, may be treated/or not with a standard of care treatment at the PI's discretion:

- o Subject **receiving** a standard of care treatment will be treated till symptom(s) are completely disappeared and a negative control urine culture is obtained.
- o Subject **not receiving** any treatment but with persisting symptoms will **re-perform** a urine culture. Whatever the result of urine culture, given the persistence of symptoms, the subject will be treated with a standard of care treatment till symptom(s) are completely disappeared and a negative control urine culture is obtained.

At the end of UTI event treatment or disappearance of symptom(s), the following study procedures will be performed:

- Performing urine culture at least 48h after the end of the therapy or disappearance of symptom(s) of the UTI event and within 1 week to be sure that women are completely recovered
- Reporting end date of event (defined as the date of sampling of the last negative urine culture exam confirming the remission of patients after treatment)
- Collecting UTI related treatment if any (to be reported in the "UTI related Concomitant Medication" section of the e-CRF)
- Completion of health care cost questionnaire related to the UTI event at the end of event ONLY in the case of symptomatic event confirmed by positive urine culture
- Checking and recording adverse events and concomitant medications since the previous contact with the subject.

The visit date will be the date of sampling of the (last) negative urine culture

The site will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents are accurate and complete (please refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit and no later than 5 days to the date of the visit. In case of unavailable data, the e-CRF should be completed within 48 hours after the missing data's receipt.

#### 7.1.8 *Follow-up Visit*

Not applicable

#### 7.1.9 *Early Subject's Discontinuation.*

In the event of early subject's discontinuation from the study (as specified in section 6.4), the Investigator should make every effort to entirely conduct the End of Study Visit evaluations and procedures (please refer to the section 6.4.3).

For all subjects who were randomised in the study, the reason(s) for early termination of the subject prior to completion of the study must be stated in the subject's source documentation and reported in the e-CRF.

The site(s) will ensure that the visit procedures and evaluations are properly documented in the subject medical file and that the source documents are accurate and complete (please refer to the section 8). The subject's e-CRF should be completed from the source documents and as soon as possible after the subject's visit and no later than 5 days to the date of the visit. In case of unavailable data, the e-CRF should be completed within 48 hours after the missing data's receipt.

End of study form must be filled for all subjects that have signed the Informed Consent.

For screen failures this should minimally include: informed consent date, demographics, inclusion criteria, exclusion criteria and end of study form.

### **7.2 SUBJECT VISIT WINDOW**

For each subject's visit, allowable windows are defined and stated in the previous section (7.1) and in the table of the study procedures and stages as outlined in the section 2.

### **7.3 STUDY FLOW-CHART**

The table of the study evaluations, procedures and stages is outlined in the section 2.

### **7.4 STUDY ASSESSMENTS**

#### 7.4.1 *Clinical Assessments*

##### 7.4.1.1 *Medical and Surgical History*

The Investigator will interview the subject with respect to her history of concomitant diseases, surgery. The history of renal diseases and treatments will be verified in depth and specific questions on the symptomatology, duration and management of the UTIs will be addressed. All information will be recorded in source data and reported into the e-CRF.



#### 7.4.1.2 Medications and Nutritional Supplements History

The Investigator will check if the subjects consume the following products forbidden by the protocol:

- Cranberry juice and/or extracts during the last 2 weeks
- Antibiotics during the last 2 weeks
- Chronic anti-coagulant therapy
- Diuretics
- Corticoid treatment
- Chronic laxative treatment

In addition to this all concomitant medication taken within 2 weeks will be documented in the source documentation.

#### 7.4.1.3 Physical Examination

The physical examination includes an assessment of general appearance and a review of systems (gastrointestinal, cardiovascular, OtoRhinoLaryngological (ORL), Neurological, Dermatological, Musculoskeletal, Urological/Nephrological,)

#### 7.4.1.4 Vital Signs

The following vital signs will be measured:

- Blood pressure (BP) (systolic and diastolic [mmHg]),
- Heart rate (HR) (beats per minute [bpm]),
- Axillary body temperature.

The measurement of blood pressure is performed after resting for at least 5 minutes, in sitting position.

#### 7.4.1.5 Demographics

Demographics include date of birth and gender.

#### 7.4.1.6 Anthropometry (Body weight, waist circumference, Height)

- Body weight is measured to the nearest 0.1 kg using a calibrated weighing scale without outerwear and shoes
- Body height is recorded to the nearest 1 cm
- Body weight and height are used to calculate the Body Mass Index (BMI) as followed:  
$$\text{BMI} = \text{weight} / (\text{height})^2$$
 where weight is in kilogram and height in meter.

#### 7.4.1.7 Other Clinical Assessment

Not applicable.

#### 7.4.2 Laboratory Assessments

Normal ranges and laboratory/technical procedures for clinical laboratory parameters are made available by the laboratory(ies) before start of the study in the Laboratory Manual.

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7.4.2.1 Clinical Laboratory Assessments

Laboratory measurements will include the following parameters assessed per visit:

Parameters	V1	V2 Pre- randomisation	V3 (M6)	V4 (M12)	Relapse visits
<b>Blood parameters</b>					
Lipidic profile**		X		X	
Glycosylated hemoglobin		X		X	
Serum creatinine		X	X	X	
Estimated glomerular filtration rate (eGFR)		X	X	X	
Serum Urea		X		X	
Copeptin		X	X	X	
FSH***		X			
<b>24h urine parameters</b>					
Sodium		X	X	X	
Potassium		X	X	X	
Calcium		X		X	
Magnesium		X		X	
Chloride		X		X	
Oxalate		X		X	
Citrate		X		X	
Urine Specific Gravity		X	X	X	
Osmolality		X	X	X	
Urine color		X	X	X	
pH		X	X	X	
Urine Creatinine		X	X	X	
Volume		X	X	X	
Number of micturitions		X	X	X	
<b>Spot urine parameter</b>					
Urine Pregnancy Test	X	X			
Urine culture****	X*	X	X*	X	X

\* If symptomatic

\*\* Lipidic profile: HDL, LDL, total cholesterol, tryglicerides

\*\*\* if peri-menopausal symptoms

\*\*\*\* In case of UTI infection a control urine culture should be performed at least 48h and within 1 week after the end of treatment

7.4.2.2 Pregnancy Test

Women of childbearing potential must have a negative pregnancy test both at V1 and V2. A urine pregnancy test will be used and results must be available prior to the subject randomisation. The Investigator is responsible for ensuring that the subject's pregnancy status is recorded accurately in the source documents. The Investigator will record the results of the pregnancy tests in the source documents and in the subject's e-CRF.

7.4.2.3 Special Assays or Procedures

Not applicable

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*7.4.2.4 Instructions for Specimen Preparation, Handling, and Storage*

Not applicable

*7.4.2.5 Blood Sample Collection and Handling*

Blood sampling will not exceed 20 mL per visit and will be taken after a period of fasting of 8-12 hours at visits V2, V3 and V4. This volume includes the volume required for back up samples in case of retest analysis. The blood sampling will be done at the Investigational site.

The clinical examinations and the blood and urine collections related to the study visits may be repeated in case of UTI events.

If fasting condition is not confirmed, the subject has to come back to the investigational site for re-sampling during a new visit scheduled within a maximum of 2 days after the initial scheduled visit in fasted state.

All blood samples will be processed, stored and transported under the conditions described in the laboratory specification in order to ensure the samples stability.

*7.4.2.6 Urine Sample Collection and Handling*

The 24h urines will be collected outside of menstruation period in a specific container during 24h and stored by the subject at about +4°C to ensure the stability of parameters analysed. In order to ease sample collection and homogenise the number of sample and time collection of each subject, urines are collected as follows: the first urine of the day is excluded. The collection includes all urinations in the following 24h including the first morning urine of the day after.

**If at least one micturition is not collected in the 24h urine collection, subject will be asked to perform a new 24h urine collection (and complete a new Voiding diary accordingly) and to come back for a new visit scheduled the earliest possible day (and no later than 10 days) after the initial visit.**

The subjects will bring back their urine collection to the investigational site the morning of V2, V3, V4 or on the day of re-test as applicable.

The urine samples will be processed, stored and transported under the optimal conditions stipulated in the Protocol as well as in the laboratory specification in order to ensure the samples stability.

Urinary concentrations and excretions of sodium, potassium, calcium, magnesium, chloride, oxalate, citrate, creatinine, will be evaluated on 24h urine sample as per requirement for the concerned study visit

Urine volume, osmolality and pH

24h urine collection will be weighted and analysed for their Urine Specific Gravity (USG), osmolality and pH. The weight of the 24h urine collection will be divided by the USG in order to calculate the urinary volume.

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#### Urine colour

24h urine collected will be evaluated according to the Urine Color Chart (UCC) provided to the investigating site. Urine Color Chart was originally published in Lawrence Armstrong's book titled Performing in Extreme environments. The scientific validation of this UCC may be found in the literature [31,32]

This assessment will be done in order to assess the change in urinary hydration markers following changes in water intake habits.

#### Urine culture and MIC determination

The collection will be performed from the 1st morning urine in midstream urine by clean catch method. Urine culture is performed to confirm the presence of UTI and determine the bacterial origin of the UTI. For the bacteria identified, a Minimum Inhibitory Concentration test (MIC test) will be performed in order to determine the bacteria resistance or susceptibility to antibiotics. The list of antibiotics is chosen on the result of Gram coloration (i.e.: Gram +/-).

In case of positive urine culture, an antibiotic treatment will be administered according to Investigator prescription. A new urine culture will be performed after the treatment in order to verify if urine culture is negative. This urine culture will be performed at least 48h after the end of the therapy and within 1 week to be sure that women are completely recovered and that results are not biased by the antibiotic treatment taken by the subjects.

#### Tiselius Crystallisation Risk Index

Subjects exhibiting rather highly concentrated urine due to their dietary habits and low fluid intake may be seen as potential future stone-formers in which a higher water intake may reduce the risk of crystallization and finally the risk of a lithiasis event.

Several indexes of crystallization risk have been proposed to evaluate the risk of crystallization in urine. Most of them have been developed and proposed by Tiselius who is well known for his Tiselius Crystallisation Risk Index (Tiselius, 1991):

$$AP(\text{CaOx}) \text{ index EQ} = 1.9 \times \text{Ca}^{0.84} \times \text{Ox} \times \text{Mg}^{-0.12} \times \text{Cit}^{-0.22} \times \text{V}^{-1.03}$$

Where Ca (calcium), Ox (oxalate), Mg (magnesium), Cit (citrate) are urinary excretions expressed in millimoles excreted during the period, and V represent the urine volume in litres corresponding to the sample considered from 24 hours urine collection.

#### *7.4.2.7 Instructions for Specimen Shipments*

Frequency of shipment, labelling requirement and any special instructions will be defined before study start and specified in Laboratory Manual.

#### *7.4.2.8 Back up samples*

The remaining back up blood and urine samples will be kept during the study by the investigator site, following instructions described in the laboratory specification as well as in the laboratory manual at the site and up to the signature of the study report when DANONE RESEARCH will transfer them at a central laboratory facility, selected and contracted by DANONE RESEARCH. These remaining back up blood and urine samples may be used for

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future researches such as the performance of additional analyses and/or the development new analytical methodologies. In this case, this will be done under the confidentiality rules. In any case, these samples will not be used for human genetic analysis.

The subject will be informed, via the ICF, that the unused blood and urine samples will be frozen and stored for a maximum of 15 years, in order to make these samples available for repeated measurements as a substitution of analysis mistakes, or in case of development of new methodologies of analyse.

#### *7.4.3. Other Assessments*

##### *7.4.3.1. Subject's Compliance with Study Product Intake*

The subject's compliance for study product intake will be assessed by the Investigator throughout the study using the following information:

- Subject's self-reported data of product intake into the Subject Compliance Diary (please refer to the section 6.5.5),
- Subject's self-reported data on total fluid intake into the Subject diary for 3-days fluid intake (data collected on 3 consecutive days including the 2 days of the week-end and one working day (Saturday-Sunday-Monday or Friday-Saturday-Sunday) (please refer to the section 6.5.4),
- The urine volume, osmolality and colour measured at V2, V3 and V4.

For the **intervention group**, the subjects will record in a Subject Compliance Diary the volume of study product consumed (Evian® water) on a daily basis by specifying the number of bottles and the volume of water consumed from each bottle (total bottle, ¼ of bottle, ½ of bottle).

During the monthly phone calls, the subject will be reminded to return her Subject Compliance Diary to the site each month by courier or personally if preferred. Before each Telephone call, the Investigator will review the subject's Subject Compliance Diary.

At the same time, the Investigational site will receive the acknowledgment of receipt for the delivery of study product to the subject.

##### *7.4.3.2. Recommendations and non-authorized products*

Women included in the study will be asked not to consume any cranberries juice and/or extracts as well as any other nutritional complement (such as infusion(s) / product(s) with diuretic action(s)...) which can impact the outcome of the study in the 2 weeks prior to the inclusion visit and for the all duration of the study.

## **8. SOURCE DATA AND SOURCE DOCUMENTS**

### **8.1 SOURCE DATA DEFINITION**

According to ICH GCP (E6), source data is all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

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## **8.2 SOURCE DOCUMENTS DEFINITION**

According to ICH GCP (E6), source documents are original documents, data, and records (e.g., medical / hospital records, clinical and office charts, laboratory notes, memoranda, general practitioner letter, questionnaire used for diagnosis, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, X-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial).

## **8.3 SOURCE DATA MANAGEMENT**

All information recorded in the CRF should be traceable and documented by source documents in the subject's medical records available at the study centre.

If the subject is not followed by the investigational site, the investigator will not be exempted of creating and/or maintaining a complete and accurate subject's file by ICH/GCP, guidelines of the medical profession and local laws and guidelines. The subject's file includes all source documents for the e-CRF completion and verification. A special attention will be stated for source documents on the inclusion/exclusion criteria and randomisation/ non randomisation criteria, in order to ensure the targeted population. If relevant, the PI or another documented authorised study team member will solicit the subject's General Practitioner (GP) to ensure the accuracy, completeness and up to date information on past/current medical/medication history, or any other information required by the study.

The e-CRF must be completed within 5 working days to the date of the visit.

## **8.4 RIGHT OF ACCESS TO SOURCE DATA/DOCUMENTS**

The Investigator/institution should permit study-related monitoring, audits, IRB/IEC review and regulatory inspection(s), providing direct access to source data/documents.

In accordance with the Law, the subjects who wish may have access to their personal data. They must address their request to the Investigator in writing.

# **9. STUDY PRODUCT**

## **9.1 STUDY PRODUCT(S) DESCRIPTION AND COMPOSITION**

### *9.1.1 Study product(s) description*

The study product is a low mineralised natural mineral water. It is manufactured according to the DANONE's quality policy in the factory of Evian® (France).

The study products are intended for oral use only and within the standard consumption patterns for physiological needs.

9.1.2 Study product(s) composition

Name	Natural mineral water
Product Brands	Evian®
Type of Source Water	Cachat source (Evian, France)
Type of Packaging	0.5L bottles made of polyethylene terephthalate [PET] represent the test unit
pH, (pH units)	7.2
Dry Residue 180°C, mg/l	309
Silica, mg/l	15
Sodium, mg/l	6,5
Potassium, mg/l	1
Calcium, mg/l	80
Magnesium, mg/l	26
Chlorides, mg/l	6,8
Bicarbonates, mg/l	360
Sulphates, mg/l	12,6
Nitrates, mg/l	3,7

9.1.3

Study product analyses during the study

Not applicable.

**9.2 STUDY PRODUCT(S) LABELLING AND PACKAGING**

Boxes and bottles are labelled in accordance with applicable laws and regulation. Primary labels (on water bottles) are printed in French by default. Secondary labels (on carton boxes) will be written in local language (Bulgarian).

(a) Primary packaging (water bottle)

The products are packaged in alimentary plastic bottles of 50 cL. The primary labels affixed to these bottles contain the following information:

- Expiry date (DD/MM/YYYY)
- Batch number: ZZZZ
- Content: 50 cL
- Storage temperature or conditions: to be kept out of direct sunshine and stored in a clean and dry place at room temperature.

(b) Secondary packaging (carton box):

The water bottles are gathered in batches of 24 units with the following information:

- Expiry date (DD/MM/YYYY)
- Study code
- Name and address of Sponsor and Investigator
- Batch code allowing identification of the contents
- Weight
- Storage temperature or conditions
- Mineral composition (mg/L)
- Sentence: 'To be used for clinical study only'

NB: Labels affixed to the carton box are printed in Bulgarian.

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### **9.3 SHIPMENT, STORAGE, DISPENSING, ACCOUNTABILITY AND DESTRUCTION**

#### *9.3.1 Shipment of study product(s)*

Study products are commercial products of the DANONE group and provided by DANONE RESEARCH.

The water bottles distributed to the subjects are manufactured, stored and delivered in accordance with the current sanitary regulations.

Study products are delivered in boxes and are identified as follows:

- Name and address of sender
- Name and address of recipient
- Study code
- Batch number
- Type and Identification of products
- Storage conditions

Study products are accompanied by:

- An acknowledgement of receipt to be signed and returned by the consignee (the subject herself or another person delegated by the subject) upon receipt of products and will be delivered to the investigating site by the logistics company.

#### *9.3.2 Storage of study product(s)*

The Investigator site ensures that the study products are stored at room temperature in a secure and closed area in accordance with the indications stated on the packaging for the products to be used.

The investigating site will ensure that the products are distributed personally or to a delegated person to the subjects' home/or office in a timely manner and appropriate conditions. The subjects will be informed about water bottles storage conditions. The water bottles delivered should be used only for the study purposes.

#### *9.3.3 Delivery/Dispensing of study product(s)*

An external shipping company will deliver the water bottles at each subject included in the study and randomised in the intervention study group. The Sponsor and the Investigator must ensure that water bottles are received under good condition by the subjects.

The subjects (or the preliminary indicated designee) will sign an acknowledgment of receipt and will report any product quality issue to the delivery company.

The subject may delegate the receipt of the study products to another person (e.g. a family member) which has to be documented in the source documents. A confirmation from the subject *a posteriori* stating that she has received the products has to be collected in the source documents (e.g. during the monthly phone calls).

If the subject has planned to travel abroad and she will not be able to carry the water bottles, the investigator should encourage the subject to continue drinking water (any type of drinking water) at the recommended volume (1,5L/day on top of their usual consumption).

The subjects will dispose the empty bottles together with the household waste.

#### *9.3.4 Accountability of study product(s)*

Delivery records from the transport company will be used to ensure the quantity of study product delivered to each study participant. The acknowledgement of receipt will be sent to the Investigator by the carrier.



The Investigator will check the reported quantity of water consumed by the subject in case of compliance deviation, he will check the acknowledgement of receipt for the deliver quantity.

**9.3.5 Destruction of study product(s)**

The Evian® empty water bottles will be disposed by the subjects together with their household waste.

At the end of the study, the subject will keep any left bottle(s).

The Investigator must instruct the subject that in case of detected or suspected study product abnormalities, the subject must not consume the related study products and return them to the investigational site.

## **10. RANDOMISATION AND UNBLINDING**

### **10.1 RANDOMISATION (AND STRATIFICATION IF APPLICABLE)**

After having checked all eligibility criteria at pre-randomisation visit (V2), the subject will be allocated to the study arm through the randomisation system (IWRS), without any stratification factor.

The randomisation process will be applied using a centralised Interactive Web Response System (IWRS) that will allocate subject into one of the two groups in respect with the randomisation list generated informatically at the beginning of the study.

### **10.2 UNBLINDING PROCEDURE**

Not applicable (open label study).

## **11. PRODUCT EFFECT PARAMETERS AND PRODUCT SAFETY PARAMETERS**

### **11.1 DESCRIPTION OF PRODUCT EFFECT PARAMETERS**

The study intervention effect parameters are assessed by clinical parameters and laboratory parameters analysed in blood and urine.

<b>Product Effect parameters</b>	<b>Visit 1</b>	<b>Visit 2</b>	<b>Visit 3</b>	<b>Visit 4</b>	<b>Relapse visits</b>
Symptoms of UTI	X	X	X	X	X
Urine culture (bacteriogram if urine culture positive)	X* Only if symptoms	X *	X* Only if symptoms	X *	X *
Frequency of micturitions during 24h urine collection		X	X	X	
Urine hydration markers (volume, USG, pH, osmolality, urine colour)		X	X	X	

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Fluid intake (daily volume)		X	X	X	
QoL questionnaire (SF-12V2)		X	X	X	
Antibiotic prescription data	X	X	X	X	X
Healthcare cost data					X

\* in case of UTI confirmation, a **control** urine culture will be systematically performed at least 48h after the end of the treatment and within 1 week

**11.2 DESCRIPTION OF PRODUCT SAFETY PARAMETERS**

The study safety parameters are assessed by clinical parameters and laboratory parameters analysed in blood and urine.

<b>Product Safety parameters</b>	<b>Visit 1</b>	<b>Visit 2</b>	<b>Visit 3</b>	<b>Visit 4</b>
Urine sodium		X	X	X
Urine potassium		X	X	X
Total cholesterol		X		X
HDL cholesterol		X		X
LDL cholesterol		X		X
Triglycerides		X		X
Glycosylated haemoglobin		X		X
Serum creatinine		X	X	X
Serum urea		X		X
eGFR		X	X	X
Blood pressure		X	X	X
Heart rate		X	X	X
Axillary body temperature		X	X	X
Physical examination		X		X
AE/SAE	X	X	X	X

## **12. SAFETY REPORTING**

### **12.1 DEFINITIONS (INSPIRED FROM ICH E2A GUIDELINES)**

#### *12.1.1 Adverse Event (AE)*

An Adverse Event (AE) is any untoward medical occurrence in a subject/patient or clinical study subject administered an investigational product but does not necessarily have a causal relationship with this investigational product.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the investigational product, whether or not related to the investigational product or to the protocol- mandated procedures (e.g., invasive procedures such as biopsies).

This includes events:

- Not present before the study but occurring after the signature of the Informed Consent Form,
- Pre-existing events that have worsened during the course of the study (increase in frequency or severity or change in nature during the study),
- All asymptomatic UTI detected at V4 (please refer to the Appendix II),
- All symptomatic and asymptomatic UTI detected between V1 and randomisation call (D0) (please refer to the Appendix II),
- All worsening UTI (as per protocol definition) collected in relapse visit.

For this study, the cystitis events are expected events, and will be considered as study parameters. Therefore, the related symptoms, signs and biological parameters will not be declared as AEs (with exception described into the protocol and above) but will be collected in the e-CRF.

#### *12.1.2 Unexpected Adverse Event*

An unexpected adverse event is by its nature or severity not consistent with applicable product information contained in the relevant source document(s) (e.g. Protocol, Inform Consent Form).

The product distributed during the study is natural mineral water with a long commercial history and without any report of safety problem.

#### *12.1.3 Serious Adverse Event (SAE)*

A Serious Adverse Event (SAE) is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (at the time of the event)
- requires hospitalisation of a subject or prolongation of existing inpatients' hospitalisation
- results in persistent or significant disability or incapacity
- is a congenital abnormality/birth defect
- is an important medical event.

**Complications of UTI (i.e. pyelonephritis) and symptoms, signs or abnormal values of biological parameters related to any other pathology, will be reported as S(AE).**

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**12.1.4 Suspected Unexpected Serious Adverse Reaction (SUSAR)**

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is both:

- an SAE that is judged to be at least possibly related to the investigational product by either Investigator or sponsor,
- and by its frequency, nature or severity is unexpected (not listed in the study protocol).

**12.1.5 Emergent Adverse Event (EAE)**

An adverse event will be considered as emergent if it began the day or after the first product consumption, or if it worsened the day or after the first product consumption.

**12.2 (S)AE RECORDING**

Details of any (S)AE reported spontaneously by the subjects or observed by the Investigator or medical staff must be recorded in the (S)AE forms provided in the e-CRF during the course of the study. The Investigator must report the following information on the (S)AE form: nature of event (diagnosis or major symptoms/signs), start and end dates, severity, product-relatedness, action(s) taken regarding the (S) AE, action taken regarding the study product, and subject outcome.

SAEs must additionally be recorded on the SAE Report Form provided by DANONE RESEARCH (see Appendix II). The Sponsor may request additional information from the site to evaluate the SAE.

When a subject undergoes a medical intervention or hospitalisation in absence of an adverse event (such as treatment of pre-existing condition or hospitalisation for elective surgery or diagnosis), this intervention/hospitalisation must be reported on the AE page and not as SAE. These procedures will be handled like AEs, and timelines for reporting are the same as for reporting AEs. Complications or prolongations of hospitalisation that result from procedures must be reported as SAEs, according to the applicable reporting timelines and procedures.

The severity of the (S)AE will be determined in the following manner:

- Mild: No interference with the subject's daily activities - transient or mild discomfort; no medical intervention/therapy required.
- Moderate: Moderate interference with the subject's daily activities but still acceptable - mild to moderate limitation in activity; some assistance may be needed; and/or minimal medical intervention/therapy required.
- Severe: Major interference with the subject's daily activities and unacceptable - marked limitation in activity; some assistance usually required; and/or significant medical intervention/therapy/hospitalisation required.

The relationship of the (S)AE to the study product is assessed as being:

Not related	The AE follows no reasonable temporal relationship with the investigational product or does not follow a known response pattern to the investigational product and can be explained by the known characteristics of the subject/patient's clinical state, by underlying disease or other administrated products (e.g. nutritional products, OTCs, drugs).
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Unlikely related	The AE has a time to investigational product intake that makes a relationship improbable (but not impossible) and concomitant administered products (e.g. nutritional products, OTCs, drugs) or underlying disease provide plausible explanations.
Possibly related	The AE has reasonable time relationship to investigational product intake but could also be explained by an underlying disease or other administered products (e.g. nutritional products, OTCs, drugs); information on drug withdrawal may be lacking or unclear
Probably related	The AE has a reasonable time relationship to investigational product intake, it is unlikely to be attributed to disease or other drugs and response to withdrawal of the investigational product is clinically reasonable; rechallenge information is not necessary
Definitely	The AE has plausible time relationship to investigational product intake and cannot be explained by underlying disease or other administered products (e.g. nutritional products, OTCs or drugs); response to withdrawal of the investigational product is clinically plausible; rechallenge is satisfactory if necessary

**12.3 SAE REPORTING BY THE INVESTIGATOR**

As soon as the Investigator becomes aware of a SAE and not later than 24 hours (1 working day), or within 3 calendar days of the event if this period includes a week-end or public holiday, he/she completes the SAE Reporting Form (see Appendix II) and sends it together with other supporting documents (e.g. copy of pages of the case report form for medical history, on-going events and concomitant medications, etc.) via fax or e-mail to DANONE RESEARCH with copy to the monitoring CRO.

Follow-up information of the SAE should be provided by the investigator as soon as possible.

The Investigator ensures that any supportive documents submitted have been adequately anonymised and reported into the e-CRF session related to the SAE.

**12.4 SAE REVIEW AND REPORTING BY THE SPONSOR**

DANONE RESEARCH must review all reported SAE and independently assess the relationship of the SAE with the study product.

If the Investigator and the Sponsor both assess the relationship of the SAE with the study product as “Not related “ or “Unlikely related”, the SAE is considered as a regular SAE with no required expedited reporting to Ethics Committees.

If one of them determines that the SAE is “Possibly”, “Probably” or “Definitely” related to the investigational product, and that this event has not been described in the or in the Clinical Study protocol, the SAE is considered as a SUSAR requiring expedited reporting to Ethics Committees. Should the assessments of the Sponsor and of the Investigator differ with regard to the relationship to the study product, then both will be reported.

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DANONE RESEARCH must ensure that all SAEs and SUSARs are reported to the accredited Ethics Committee(s) that have approved the protocol (and Competent Authorities, if applicable) as follow:

- SAEs are reported annually as line listings according to the requirements of the Ethics Committee(s),
- SUSARs are reported within 7 days (fatal and life threatening) or 15 days (other events) after the first report. Reporting timelines include week-ends and public holidays.

The Sponsor will reply to all requests for further information concerning such events from the Ethics Committee (and/or Competent Authorities, if applicable).

#### **12.5 FOLLOW-UP OF SAEs**

The Principal Investigator or his/her authorised representative will monitor and follow all SAEs until SAEs have abated, or until a stable situation has been reached and a satisfactory resolution is obtained.

Depending on the event, follow-up may require additional tests or medical procedures as indicated, and/ or referral to the general physician or a medical specialist or other health care provider.

Any clinical or biological examinations deemed necessary by the Principal Investigator will continue to be performed until a return to normal. The Principal Investigator will provide the Sponsor with follow-up SAE Reporting Form and with all the examination results and concomitant medications, as soon as the Investigator becomes aware of new information and not later than 24 hours (1 working day) or within 3 calendar days of the new information if this period includes a week-end or public holiday.

#### **12.6 PREGNANCIES REPORTING BY THE INVESTIGATOR**

Although the study products are considered as safe for pregnant women, pregnancy may require specific diet, medication or procedure likely to interfere with the study product and/or outcomes.

For this reason subjects with a confirmed pregnancy have to be withdrawn from the study and followed up until birth or early termination of pregnancy.

The Investigator should make every effort to collect information about the pregnancy and the infant and report it within a dated and signed Pregnancy Reporting Form (see Appendix V).

#### **12.7 INDIVIDUAL CODE-BREAK PROCEDURE**

Not applicable.

#### **12.8 NEW RELEVANT SAFETY INFORMATION**

Not applicable.

## 13. STATISTICS

### 13.1 SAMPLE SIZE CALCULATION

By definition, the recurrent UTI event data consist of the inter event time of repetition of the same or different types for each subject. The measurements across subjects are considered to be statistically independent, but the times between UTI events for a specific subject are not necessarily independent. The mean cumulative function (MCF) contains the information of interest in the analysis of recurrent data.

Assume  $M(t)$  is the mean cumulative number of UTI events up to time  $t$  [33].

$M(t) = E\{N(t)\}$ , where  $N(t)$  is a random variable for the number of events that have occurred up to time  $t$  [34]

The main assumption of this approach is that the hazard or risk ratio is proportional over time, reason why a robust sandwich variance estimated is used to account for dependence of recurrent events on the same subject.

Assuming that all subjects had the same study period examination (approximately 12 months), and considering an interval of risk (inter event times) following an Uniform distribution, data have been simulated based on a mean number of events equal to 3 for control group and an expected intervention effect of 20% less events in the intervention group. One hundred samples have been replicated upon the simulated data [35].

Considering the simulated data based on our hypotheses, a mean number of events during a 12 months period in control group equal to 3, the estimate sample size that would provide a power of 80% to detect a product effect of 20% less events occurred in the interventional study group for a bilateral test,  $\alpha=0.05$  will be equal to 42 subjects per group, i.e. 84 overall completed subjects (ratio 1:1).

In order to achieve 84 completed subjects, and considering a 40% drop out rate, the screening process will last until we will reach 140 subjects randomised.

Considering a 50% screen failure rate, around 280 screened subjects will be needed in order to get 140 randomised subjects.

### 13.2 PLANNED STATISTICAL METHODS

A Statistical Analysis Plan (SAP) will be drawn up before data review meeting, reviewed by the Investigator and statisticians during the Data Review Meeting and signed by the sponsor before the database lock.

Statistical analyses will be performed by a specialized CRO, selected, contracted and followed under the responsibility of DANONE RESEARCH/located in Palaiseau (France) and using appropriate statistical software SAS® V9 or later using the files included in the database locked.

#### 13.2.1 Descriptive statistics

The distribution of study parameters will be summarised by group and overall depending on the type of variable for all criteria with a description of number of subjects and missing data.

The descriptive statistics for each criterion will be presented as follows:

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- Continuous data: for each visit per group and overall: number of non-missing observations, number of missing observations, mean, median, standard deviation (SD), minimum and maximum. (Standard error of the mean [SEM], Confident interval (CI) and quartiles optionally provided if mentioned in the SAP).
- For nominal data: for each visit per group and overall: number of missing observations, number and frequency of observations in each class.

For qualitative ordinal parameters

- Table of frequency (and/or mean and/or median), if necessary per parameter.

*13.2.2 Statistical Hypotheses*

In the present study the null hypothesis “There is no difference between groups on the average number of recurrent UTIs events experienced” versus the alternative hypothesis “the effect of intervention is different to the effect of the control” will be tested.

The test will be based on a non-parametric method called the mean cumulative function (MCF), that will be used to analyse the multiple/repeated UTI events occurring.

$H_0$ : Effect of fluid intake = Effect control

$H_1$ : Effect of fluid intake  $\neq$  Effect control

Statistical tests will be conducted two-sided with a significance level of 5%. All confidence intervals will be presented two-sided with a confidence level of 95%. A resultant probability value of  $p < 0.05$  will be judged as being of statistical significance. The interaction factors (if any) will be considered as significant if the p value is  $< 0.10$ .

The MCF by group, estimated by a non-parametric estimator:

$$\widehat{MCF}(t) = \sum_{\{j|t_j \leq t\}} \frac{e_j}{n_{j-1}}$$

where  $e_j$  is the number of events at time  $t_j$ ,  $n_{j-1}$  is the number of subjects at risk just beyond time  $t_{j-1}$ , and  $j$  the observed event times.

*13.2.3 Interim Analysis*

Not applicable.

*13.2.4 Distribution and normality assessment*

As a first step, the frequency distribution of the efficacy parameters will be analysed, including assessment of stem-and-leaf displays, boxplots and histograms per group and overall.

*13.2.5 Statistical criteria to stop the research*

Not applicable

*13.2.6 Methods for dealing with missing, unused or non-valid data*

The way of handling missing, unused or non-valid data will be discussed at the Data Review Meeting and detailed in the SAP.



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*13.2.7 Management of modifications made to the initial strategy of the analysis plan*

All the modifications of the statistical methodology will be detailed and justified in the SAP and described in the Clinical Study Report.

**13.3 DISPOSITION OF SUBJECTS AND POPULATIONS DEFINITION**

*13.3.1 Disposition of subjects*

Disposition of subjects will be described as follows:

- Number of subjects included.
- Number of subjects eligible at V1.
- Number of subjects screen failed at V2 (reasons of screen failure will be described).
- Number of subjects randomised per group and overall.
- Number of subjects premature withdrawals / drop out (reasons of premature withdrawals / will be described per group and overall).
- Number of subjects completed per group and overall.

*13.3.2 Deviations and populations definition*

A definition of minor and major protocol deviations will be detailed in a document attached to the SAP. The Data Review Meeting will allow a global review of the study data and then the deviations status of subjects in order to determine analysed populations before the final data base lock.

The populations will be defined as follows:

- The “Global Population”: all subjects included in the study and eligible at the end of Visit 1.
- The “Full Analysis Set” population (FAS): all subjects included in the study and randomised.
- The “Per Protocol” population (PP): all subjects included in the FAS population presenting no major protocol deviation.
- The “Safety Set” population (SS): all subjects included in the FAS population.

The analysis of baseline characteristics will be performed on the FAS population.

The analysis of main product effect criteria will be done on the FAS population. If the difference in number of subjects is few between the FAS and PP populations (difference lower than 10%), the analyses of the product effect criteria will be only done on the FAS population, except for the main criterion, for which the analysis will be performed on both populations.

The analysis of safety criteria will be done on the Safety Set (SS) population.

**13.4 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS**

A descriptive analysis of subjects at baseline, corresponding to the data collected at inclusion and pre-randomisation visits, will be performed by group and overall.

Following parameters will be described:

- Demography, vital signs, anthropometrical parameters (body weight, waist circumference, height, BMI).
- Medical and surgical history
- Physical examination
- Quality of Life (SF12v2)
- Fluid intake consumption
- Contraception habits/method

### 13.5 STUDY CONDUCT PARAMETERS

#### 13.5.1 Compliance

Subjects record the daily intake of study product in a diary on a daily basis (quantity of 3 daily bottles consumed per day). Parameters concerning compliance will be described by group and overall:

- Study product compliance
- Consumption of forbidden dietary products and treatments

#### 13.5.2 Quality of Life

A quality of life questionnaire (**SF-12v2**) will be filled in by the subject at:

- the pre-randomisation visit (V2),
- at 6-months (V3),
- at the last visit (V4),

in order to explore the quality of life of the subjects.

The short form 12 (SF-12v2) is a validated and frequently used questionnaire for the assessment of quality of life. It was developed from the more extensive short form 36 (SF-36) [<http://www.sf-36.org/tools/sf12.shtml>]. It includes 12 questions from the SF-36: 2 questions concerning physical functioning; 2 questions on role limitations because of physical health problems; 1 question on body pain; 1 question on general health perceptions; 1 question on vitality (energy/fatigue); 1 question on social functioning; 2 questions on role limitations because of emotional problems; and 2 questions on general mental health, psychological distress and psychological well-being. This will be a self-assessment questionnaire. However, if the subject is frail and requires help with filling in the form then the site staff will help.

A preference-based utility index, **called the SF-6D**, is also available to help understand economic benefit and will be assessed in order to perform a cost utility analysis.

- 12-Items
- 8- Dimensions: Physical functioning, Role Physical, Bodily pain, Vitality, General health, Mental health, Social functioning and role emotional
- 2 composite score: Mental health and physical health
- Utility index based on SF-6Dimensions

### 13.6 EVALUATION CRITERIA

#### 13.6.1 Main evaluation criterion

The main evaluation criterion is the number of recurrent UTI event experienced over a one (1) year study period between intervention and control groups, evaluated by the difference between groups in the mean cumulative function. The primary criterion will be considered as statistically significant if confidence interval observed on the MCF difference between groups excludes zero, which suggests a statistical test and p value associated <0.05.

#### 13.6.2 Secondary evaluation criterion

- Difference between groups in terms of antibiotic prescription/usage to treat UTI event over 12 months of study intervention from D0
- Difference between groups on average delay between each UTI event over 12 months of study intervention from D0

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- Difference between groups on urinary hydration markers over 6 and 12 consecutive months of study intervention from D0
- Difference between groups in terms of health-costs associated with management of UTI recurrence(s) over 12 months of study intervention from D0
- Difference between groups in terms of cost utility analysis over 12 months of study intervention from D0
- Difference between groups in terms of change in QoL over 6 and 12 consecutive months of study intervention from D0.

The methodology to address the potential multiplicity issues will be detailed in the SAP.

*13.6.3 Exploratory criteria*

- Relationship between urinary hydration markers and number of UTI events over 12 consecutive months of study intervention from D0
- Relationship between urinary hydration markers and delay UTI events over 12 consecutive months of study intervention from D0
- Impact of increased daily water intake on crystallisation risk index (Tiselius) over 12 consecutive months of study intervention from D0
- Number of UTI events confirmed by positive urine culture and number of subjects presenting at least one UTI event during the intervention period from D0 (by group)
- Number of UTI events not confirmed by positive urine culture and number of subjects presenting at least one UTI event during the intervention period from D0 (by group)
- Number of UTI events confirmed or not by positive urine culture and number of subjects presenting at least one UTI event during the intervention period from D0 (by group).

*13.6.4 Safety criteria*

*13.6.4.1 Extent of exposure / Study duration*

Descriptive summaries will be performed on study duration and extent of exposure by group and overall.

*13.6.4.2 AE / SAE*

The analysis of Adverse Events will be performed to evaluate the number of subjects with at least one adverse event and the number of adverse events by study group.

The adverse events will be presented by "body system" and "Preferred term" according to the MEDDRA coding system.

All adverse events will be summarised, in individual data listings, by subject with all details concerning the adverse event, the study product, the emergence, the intensity and the relationship to study product.

The Serious Adverse Events will be summarised, in individual data listings, by subject with all details concerning the adverse event, the study product, the emergence, the intensity and the relationship to study product.

*13.6.4.3 Concomitant Medications*

The concomitant medications will be presented by ATC class (ATC1 and ATC3) according to the WHO-Drug coding system.

All concomitant medications will be summarised, in individual data listings, by subject with all details concerning the concomitant medications and the study product.

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13.6.4.4 *Laboratory measurements*

Descriptive summaries will be performed for the following parameters by study group and overall:

Blood sample:

- lipidic profile: HDL cholesterol, LDL cholesterol, Total Cholesterol, triglycerides (mmol/L)
- glycosylated haemoglobin (%),
- serum creatinine ( $\mu\text{mol/L}$ ),
- serum urea (mmol/L),
- copeptin ( $\mu\text{g/L}$ )
- estimated glomerular filtration rate (eGFR) will be calculated.

Urinary markers of hydration:

- urine volume (L)
- pH
- osmolality (mOsm/kg)
- specific gravity
- frequency of micturition
- urine colour

Other urinary parameters:

- Electrolytes: sodium, potassium, calcium, magnesium, chloride (mmol/L), oxalate ( $\mu\text{mol/24h}$ ), and citrate (mmol/L)
- Urine creatinine (mmol/L)
- Crystallisation risk index (Tiselius).

Information about bladder emptying after sexual intercourses and sexual activity during the last month will be also summarised by study group and overall.

13.6.4.4 *Vital signs, anthropometrical and other parameters*

Descriptive summaries will be performed for the following parameters by study group and overall:

- Systolic and diastolic blood pressure (mmHg)
- Heart rate (bpm)
- Axillary Body temperature ( $^{\circ}\text{C}$ )
- Body weight (kg)
- Waist circumference (cm)
- Height (m)
- Body Mass Index (BMI) ( $\text{kg/m}^2$ )

## 14. STUDY REPORT AND PUBLICATION

All information stemming from the study will be considered confidential and should not be divulged without the prior agreement of the sponsor (DANONE RESEARCH).

Following analysis of the study data, a final study report will be prepared in UK English, format ICH E3, according to the standard model of DANONE, describing the conditions under which the study was performed as well as the results. This report will be prepared and signed by the Sponsor's representative. The signature of the Principal Investigator will be requested if relevant.

The identity of study subjects will under no circumstances be communicated to the sponsor.

Publication may be done only if before recruitment of the first subject, the sponsor has registered the study in a publicly accessible clinical trial database.

No publication will be done based upon interim analysis or result of one site of a study except if a written agreement has been signed by the sponsor.

## **15. STUDY MONITORING AND AUDITS**

Study monitoring is the act of overseeing the progress of a clinical study and of ensuring that:

- i) The rights and the well-being of human subjects are protected.
- ii) The reported trial data are accurate, complete, and verifiable from source documents.
- iii) The conduct of the clinical study is in accordance with the approved protocol/amendment(s), Good Clinical Practice (GCP, ICH E6), and the applicable local regulatory requirement(s).

Monitoring includes on-site visits to assure that the study is conducted according to the approved protocol/amendment(s) and in order to comply with applicable regulations and deadlines. On-site monitoring includes, at least, the review of Informed consents, safety reporting, e-CRFs and supports to the site management regarding protocol conduct and compliance or deviation(s).

The monitoring includes the review of forms for completeness, clarity, and consistency with source documents available for each subject and the management of the essential documents (Investigator Study File).

The Investigator and the investigating site staff must permit and be available for study-related monitoring visits, audits, review by the ethics committee and regulatory inspections, and allow direct access to source data and source documents provided that subject confidentiality is protected. In case of an audit appointed by DANONE RESEARCH, the Investigator will receive written notification in advance.

Study monitoring, under the responsibility of DANONE RESEARCH will be performed by a qualified staff of a company contracted by DANONE RESEARCH at various stages/on regular basis of the study (frequency of visits is specified in the Monitoring plan). Throughout the duration of the study period, the e-CRFs will be completed and signed by the Investigator, and he/she will be controlled individually by a Clinical Research Associate (CRA) designated by DANONE RESEARCH in order to verify data quality and compliance with study protocol and Good Clinical Practice (GCP, ICH E6).

Upon closure of the study, the company contracted by DANONE RESEARCH will perform study closeout.

## **16. ETHICAL CONSIDERATIONS**

### **16.1 BASIC PRINCIPLES AND REGULATIONS**

The Investigator must ensure that this study is conducted in full compliance with the principles of the 'World Medical Association Declaration of Helsinki' (64th WMA General Assembly, Fortaleza, Brazil, October 2013) (Appendix I), ICH guidelines for Good Clinical Practice as appropriate for nutritional products, and local legislation of the country in which the research is conducted, whichever affords the greater protection to the participants.

### **16.2 ETHICS COMMITTEE**

This protocol and any accompanying material provided to the subjects, such as information and informed consent sheets, are submitted to the applicable Ethics Committee (IRB/IEC) by the Investigator according to local legislation. Approval from the Ethics Committee must be obtained before starting the study, and should be documented in a letter to the sponsor specifying the date on which the Ethics Committee met and granted the

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approval, the composition of the Ethics Committee and their qualifications, and version and date of all submitted documents. If applicable, the documents will also be submitted to the Competent Authority in accordance with the local regulatory and legal requirements.

This study will be undertaken after approval from the appropriate Ethics Committee(s) (IRB/IEC).

During the study course, change(s) in any aspect of the study, such as modification(s) of the protocol, written ICF and any other written information to be provided to subjects should be submitted to the Ethics Committee (IRB/IEC).

If required, depending on local legislation, the Investigator must submit an annual progress report to the Ethics Committee which gave the favourable opinion. Annual progress reports should be submitted thereafter until the end of the study. DANONE RESEARCH could assist in the preparation/submission process.

Subject recruitment will start only after reception of a favourable opinion from the Ethics Committee.

### **16.3 RECRUITMENT AND INFORMED CONSENT FORM**

The requirements of the research, the objectives of the research, the detailed research protocol and the risks and constraints of the research associated with this study must be explained to each subject both orally and in writing (subject information sheet) by the Investigator, or a person designated by the Investigator, before the start of the study.

The subject must personally initial each page of the Information to the subject and sign and date a written informed consent form (ICF) to take part in this study before the study starts (before the screening period, if applicable). This form must also be initialled and signed by the Investigator or a Physician designated by the Investigator. Two copies of the ICF are dated and signed: one is given to the subject and one is retained on site in the subject's files.

Subjects may withdraw from the study at any time without having to provide justification. The confidentiality of medical data must be upheld.

Any substantial changes in the ICF and written information should receive the IRB/IEC's approval/favourable opinion before any use.

### **16.4 CONFIDENTIALITY OF STUDY DATA**

All information collected during the study is to be considered confidential and must not be disclosed without prior written agreement by the sponsor. The identity of study subjects must under no circumstances be communicated to the sponsor or to any official bodies.

### **16.5 COMPENSATION OF SUBJECTS**

The subjects will be compensated for their participation in this clinical study on a pro rata basis. Compensation amount and the method and timing of disbursement are consistent with applicable regulatory requirement(s) of Bulgaria.

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Each subject will receive an indemnity of 557 Bulgarian Leva for participating in this study if she has completed all study visits or in case of discontinuation due to medical reasons related to the protocol. In any other case of withdrawal, the subject will receive an indemnity for participation on a pro rata basis.

The reimbursement will be as follows:

- Inclusion visit (V1) = 28 Bulgarian Leva
- Pre-randomisation visit (V2)= 158 Bulgarian Leva
- Evaluation visit (V3) = 176 Bulgarian Leva
- Evaluation visit (V4)= 195 Bulgarian Leva

#### **16.6 PROTOCOL AMENDMENTS**

Any protocol modification should be the object of an amendment, which will be dated and signed by the same parties than those invested in the initial protocol signature.

The amendment will be submitted to the appropriate Ethics Committee (IRB/IEC), either for approval or for information, depending on the nature and the importance of the changes to the study conditions.

#### **16.7 STUDY SUSPENSION**

If the study or part of the study is prematurely terminated or suspended, the Investigators, the appropriate Ethics Committee(s) (IRB/IEC) should promptly be informed as specified by the applicable regulatory requirement(s).

#### **16.8 PROTOCOL DEVIATIONS**

No deviations are tolerated without sponsor approval. Any deviation from the approved protocol related to study eligibility criteria, conduct of the trial, subject's management or subject's assessment should be documented and explained

The main categories of deviations are based at least on the following:

- Informed consent process and documentation,
- Enrolment: Assessment of the 'low drinker profile' (based on the coherence between fluid intake volume, complete 24H urinary volume, urine osmolality and urine creatinine),
- Randomisation: Eligibility criteria based on documented medical history of diseases,
- Biological sample collection (i.e. blood samples and/or urine samples and voiding diary),
- Product compliance and product exposure duration (based on subject compliance dairies, 3-days fluide intake diary and questionnaire),
- Time respect between visits,
- Follow-up of dietary and forbidden treatment/medication,
- Safety issues (e.g lack of declaration of UTI symptoms),
- And any other procedure described in the study protocol.

DANONE RESEARCH will be informed of all protocol deviations, and these will be discussed before implementation according to prospective protocol deviation process or later at the data review meeting (blind review meeting), in order to define their status (minor – major).

#### **16.9 INSURANCE**

DANONE RESEARCH has contracted an insurance policy with an established insurance company in accordance with current regulatory requirements in Bulgaria to cover potential damage to subjects through injury or death caused by the study product and/or study procedures.

The insurance applies to the damage that becomes apparent during the study. DANONE RESEARCH also has liability insurance in accordance with the applicable legislation.

## **17. DATA COLLECTION, PROCESSING, AND MANAGEMENT**

### **17.1 CASE REPORT FORM (CRF)**

An electronic CRF (e-CRF) will be created by the Sponsor or its delegate in English. The final version will be approved by the Sponsor. The relevant study data defined in this Protocol will be collected and entered by the Investigator or the responsible team members into the provided e-CRF. An e-CRF Completion Guideline will be provided to the Investigator to facilitate e-CRF completion as well as provide answers for Frequently Asked Questions (FAQs)

Quality of life questionnaire will be provided by the Sponsor to the Investigator:

- Original form should stay at the investigator's site and a copy should be sent to the Biometry Contract Research Organisation (CRO) for data processing.

Each subject will be identified with a unique Subject Identification number (please refer to the section 7.3). All study documents related to a subject must be identified with this subject identifier.

All relevant e-CRF data will be single-entered by personnel at the Investigational site in the validated e-CRF tool put in place by the sponsor. The Investigator must ensure that these data are complete and accurately represent the study subject information by electronically signing the appropriate forms in the e-CRF.

QoL questionnaires and subject diaries will be in paper and single-entered by the CRO Data-Management in the validated e-CRF tool put in place by the sponsor. These questionnaires will be in paper and in Bulgarian but entered in English in the e-CRF by the PI.

All external data will be provided to the CRO Data-Management for integration to the database.

The sponsor will ensure all study site personnel are appropriately trained on the use of the e-CRF (i.e. data entry, queries, sign-off etc.). This training is MANDATORY for all site personnel (who are expected to use the system) and must be completed before access is granted to the system. Each user will be provided with a unique access (user name and password) to the e-CRF.

The combination of the user name and password to access to the e-CRF are the legally binding equivalent of a traditional handwritten signature, and as such, carries all of the same rights and responsibilities. User accounts are not interchangeable, and must only be used by the person authorized to use that account.

### **17.2 DATA PROCESSING**

Edit checks, to ensure the quality and consistency of the data, will be defined in a Data Validation Plan (DVP). These edit checks will be programmed and validated in the e-CRF. During the data entry process, these checks will automatically be triggered (and become apparent to the user) in case of incoherent data.



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### **17.3 DATA AUDIT TRAIL**

Any addition, modification, or deletion of subject data made after the initial entry (i.e. response to Data Clarification Forms (DCF) or updated information) will be automatically tracked in the e-CRF's audit trail in compliance to ICH GCP and US FDA 21 CFR part 11.

### **17.4 DATA MANAGEMENT**

Data Management (DM) tasks for this study will be performed by a delegated Data-Management CRO. The verification and validation of Data-Management tasks will be performed by a Data Manager internal at the Sponsor. All Data-Management tasks will be defined in a Data Management Plan (DMP) based on the current Sponsor and CRO Standard Operating Procedures (SOPs).

### **17.5 EXTERNAL DATA**

Data Transfer specifications for external data (i.e. central laboratory data) will be defined in a Data Transfer Agreement (DTA). All electronic external data transfers will be reconciliated with the main study data and validated by the CRO Data-Management to ensure accuracy and consistency.

### **17.6 DATABASE LOCK**

After all planned subject visits have been completed, entered into the study database, and considered clean, a Data Review Meeting (DRM) will be held by the Sponsor to discuss and review all study data. The main objective of this Data Review Meeting (DRM) will be to review tables, listings and summaries on study data, to review safety listings, to identify protocol deviations and to define subject analysis populations. Members of the Sponsor's Study Core Team, the designated CRO's as well as the Principal Investigator(s) may participate in this meeting.

Once any final issues resulting from the Data Review Meeting (DRM) have been resolved, the study database, including all external data will be locked. This will ensure that no further modifications to the study data are possible. The statistical analysis will be performed on this locked database.

Following the database lock, the sponsor will provide each Investigational site with a final, unmodifiable PDF copy of the completed e-CRFs, Questionnaires and Subject Diaries (including the audit trail) of all subject's included at that site. This constitutes an exact representation of the information that was entered in the e-CRF during the study. This will be provided on removable media (i.e. CD, DVD etc).

## **18. DOCUMENTATION AND ARCHIVING**

DANONE RESEARCH provides the Principal Investigator (s) with an Investigator Site File (ISF). Each Principal Investigator (s) is responsible to keep this ISF updated and available for review by the study monitor (CRA).

All documents pertaining to the conduct of the study must be kept by the Investigator for a period of 15 years.

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Study documents should not be destroyed without prior written agreement between DANONE RESEARCH and the Investigator. Should the Investigator wish to assign study documents to another party, or move them to another location, DANONE RESEARCH must be notified first.

### **18.1 SPONSOR**

The following documents are to be archived by the sponsor for a minimum of 25 years after the completion of the study, in a room specifically designated for this purpose, access to which is controlled by the person responsible for archiving:

- Final version of the study protocol,
- Any forms containing protocol amendments,
- **FOR QoL questionnaire:** Original pages
- **FOR e-CRF:** The media (i.e. CD, DVD etc.) that contains the final PDF copy of the subject e-CRFs, Questionnaires, Subject Diaries (including audit trail) that was extracted from the e-CRF following the database lock,
- Ethics Committee approval forms,
- All correspondence between the sponsor and the Investigator,
- Curriculum vitae (CV) of Principal Investigator and other Investigators,
- Acknowledgements of receipt – as well as all other documents generated during the study.

### **18.2 INVESTIGATOR**

All documents concerning this study must be kept by the Investigator, including but not limited to:

- Subject's medical files including source documents
- Original (dated and signed) of the ICFs and the subject's identification code list
- Screening and enrolment Log
- **FOR QoL questionnaire:** Copy
- **FOR e-CRF:** The media (i.e. CD, DVD etc.) provided by the sponsor that contains the final PDF copy of the subject e-CRFs, Questionnaires, Subject Diaries (including audit trail)
- Copy of accountability forms for products administered
- Copy of ethics committee approval(s) and correspondence with the sponsor
- Signed Protocol(s), signed amendment(s)
- Advertisement (if any)
- Financial Contract(s)
- Insurance certificate
- EC correspondence including Approval Opinion and composition
- CV of Investigator and sub Investigator(s) and delegation task list
- Normal Values and technical procedures
- Instruction for handling products, shipping records, products accountability
- Decoding procedure
- Serious Adverse Events report forms

All correspondence between the sponsor and the Investigator,

The Investigator agrees to provide direct access to source documents during monitoring visits.

## **19. OWNERSHIP OF RESULTS**

All information and results issued from the study remain the property of the sponsor.

The study results may be published or presented by the Investigator or by experts responsible for analysis, in collaboration with the sponsor and with the prior written approval of the latter. The sponsor may use the results of

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the study for publications or communications with the written agreement of the Investigator or experts responsible for analysis if the latter are cited.

## **20. RESPONSIBILITIES**

### **20.1 SPONSOR**

- a. Manage the submission of the study protocol to the ethics committee before the start of the study. The Sponsor should obtain the approval from all the competent authorities and from the EC.
- b. Before the start of the study, the sponsor must provide the Investigator with all documents required by the protocol and/or to provide information on the study products. In particular, the Sponsor must provide the Investigator with a document certifying that the study products are fit for human consumption.
- c. The Sponsor must take out a specific insurance policy for the study as required by current legislation of the region in which the study is being conducted.
- d. The Sponsor must provide the Investigator with insurance certification. The Sponsor may decide to terminate the study at any time. At the end of the study, the Sponsor must archive the study documents for the legally required duration and at least for 25 years.
- e. The Sponsor must carry out all procedures required by the relevant EC including initial/amendment submission and Safety reporting.
- f. The Sponsor must set up data Quality Control at each stage of data handling.
- g. The Sponsor finances expenses related to the study
- h. The sponsor must monitor the study and ensure the correct adherence to protocol requirements and regulatory requirement are maintained (including the data protection and confidentiality)
- i. The Sponsor must record the study in the appropriate Governmental database

### **20.2 INVESTIGATOR**

- a. Provides oral and written information for subjects and selects subjects in accordance with protocol inclusion and non-inclusion criteria.
- b. Keeps all study related information confidential
- c. Manages the study products including randomisation, storage, dispensation, destruction, site accountability based on the data provided in the compliance diaries and on the acknowledgements of receipt
- d. Before the study, provides the following documents to the sponsor:
  - CV of Principal Investigator and co-Investigators,
  - CV of the entire remaining investigating site staff involved in the study.
- e. Performs the clinical study within the scheduled dates.
- f. Establishes a written delegation task list, and allocate sufficient time and resources to properly conduct the study
- g. Reports of SAEs.
- h. Cooperates with Clinical Research Associates at the monitoring visits, or in case of audits and/or inspections
- i. Completes and corrects the e-CRFs, Data clarification Forms.
- j. Give input in the clinical report for the study, if relevant.
- k. Maintain essential documentation
- l. Archives data for a minimum of 15 years after the date of the final report.

### **20.3 MONITORING CLINICAL RESEARCH ORGANISATION (CRO)**

- a. Provides the required study materials in good time
- b. Performs Study start-up visit
- c. Verifies and updates the Trial Master File (TMF) and Investigator Study File SMF)
- d. Performs the Monitoring during the study conduct (including at least the verification of the signed Informed consent, the validation of the data recorded in the e-CRF including Safety data from source documents
- e. Performs the End-of-study visit
- f. Communicate relevant information to the Sponsor
- g. Verifies the Data collection (accurate, complete and verifiable)

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## **22. APPENDICES**

# **APPENDIX I**

## **DECLARATION OF HELSINKI**

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## **WORLD MEDICAL ASSOCIATION**

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:  
29th WMA General Assembly, Tokyo, Japan, October 1975  
35th WMA General Assembly, Venice, Italy, October 1983  
41st WMA General Assembly, Hong Kong, September 1989  
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996  
52nd WMA General Assembly, Edinburgh, Scotland, October 2000  
53rd WMA General Assembly, Washington, DC, USA, October 2002 (Note of Clarification added)  
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)  
59th WMA General Assembly, Seoul, Republic of Korea, October 2008  
64th WMA General Assembly, Fortaleza, Brazil, October 2013

### **PREAMBLE**

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

### **GENERAL PRINCIPLES**

3. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.



### **RISKS, BURDENS AND BENEFITS**

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

### **VULNERABLE GROUPS AND INDIVIDUALS**

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

### **SCIENTIFIC REQUIREMENTS AND RESEARCH PROTOCOLS**

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

### **RESEARCH ETHICS COMMITTEES**

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

### **PRIVACY AND CONFIDENTIALITY**

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

### **INFORMED CONSENT**

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be included in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal.

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Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

### **USE OF PLACEBO**

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

### **POST-TRIAL PROVISIONS**

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

### **RESEARCH REGISTRATION AND PUBLICATION AND DISSEMINATION OF RESULTS**

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations

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and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

#### **UNPROVEN INTERVENTIONS IN CLINICAL PRACTICE**

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

#### **ARTICLE INFORMATION**

**Corresponding Author:** World Medical Association, 13, ch. du Levant, CIB - Bâtiment A, 01210 Ferney-Voltaire, France; [wma@wma.net](mailto:wma@wma.net).

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**Online-Only Content:** Audio podcast is available at [www.jama.com](http://www.jama.com).

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**APPENDIX II**

**SERIOUS ADVERSE EVENT**

**REPORTING FORM**

## SERIOUS ADVERSE EVENT REPORT FORM

**This form must be faxed and mailed to the Sponsor within 24h**

<b>Sponsor:</b> <b>DANONE RESEARCH</b>	<b>Fax : 33 (0)1 69 35 70 42</b>  <b>Email : christine.mrini@danone.com</b> <b>          liliana.galetescu@danone.com</b> <b>          jean-francois.jeanne@danone.com</b>	
	<b>Clinical Study Manager (Name): Laurence de LA HOSSERAYE [from HAYS Pharma on behalf of DANONE Research]</b>  <b>Medical reviewer (Name): Liliana GALETESCU</b>	
<b>Principal Investigator (Name): Dr Maya DABCHEVA</b>		
<b>Study code : NU  3 6 9 </b>	<b>Expeditor (Name):</b>	<b>Country:  _ _ _ </b>
<b>Site Number:  0 0 1 </b>		
<b>Transmission date :  _ _ / _ _ / _ _ _ _  (dd/mm/yyyy)</b>		
<b>Number of pages (incl. this page)</b>	<b>_____ Pages</b>	
<b>Attached copies of completed CRF pages:</b>	<input type="checkbox"/> Adverse events <input type="checkbox"/> Medical History and pre-existing conditions <input type="checkbox"/> Medications	

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This SAE report is :  Initial report  
 Follow-up report (Includes only new details)

Is the SAE aggravation of a pre-existing AE recorded in the CRF: <input type="checkbox"/> Yes <input type="checkbox"/> No				
If yes, please put the number of AE:  _ _				
<b>Subject Details</b>				
Subject Number:  _ _ _ / _ _ _ / _ _ _		Gender: <input type="checkbox"/> M <input type="checkbox"/> F		
Subject Initials:  _ _ _ / _  (if required)		Age:  _ _		
		Height:  _ ,  _ _  m      Weight :  _ _ _ ,  _ _ kg		
<b>Description of Serious Adverse Event</b>				
Nature of SAE (diagnosis or major symptom and/or sign):				
Description of SAE:				
Severity of SAE: <input type="checkbox"/> Mild <input type="checkbox"/> Moderate <input type="checkbox"/> Severe				
Event start date:  _ _ / _ _ / _ _ _ _  (dd/mm/yyyy)		Event end date:  _ _ / _ _ / _ _ _ _  (dd/mm/yyyy)		
Time : _ _  :  _ _  (Hours:Minutes)		Time : _ _  :  _ _  (Hours:Minutes)		
<b>Category of SAE</b> (*as applicable to study protocol)				
<input type="checkbox"/> Death		<input type="checkbox"/> Persistent or significant disability or incapacity		
<input type="checkbox"/> Life threatening situation		<input type="checkbox"/> Congenital anomaly or birth defect		
<input type="checkbox"/> Hospitalization or Prolongation of existing Hospitalization		<input type="checkbox"/> Other Important medical event		
<b>Study Product</b>				
<b>According to protocol, the dosage is:</b>		Dose:  _ _       Unit (Pot/Bottle): _____      Frequency: Daily      Route: Oral		
<b>Information of study production distribution</b>	Study Product Number(s)	Last Product Batch Number:	Product Expiry date (dd/mm/yyyy)	Date of Last Product Distribution (dd/mm/yyyy)
	_ _ _ _ _ _ _	_ _ _ _ _ _ _	_ _ _ / _ _ _ / _ _ _ _	_ _ _ / _ _ _ / _ _ _ _
<b>Information of study product intake</b>			Date of last product intake (dd/mm/yyyy)	Date of last product intake before event (dd/mm/yyyy)
			_ _ _ / _ _ _ / _ _ _ _	_ _ _ / _ _ _ / _ _ _ _
<b>Has the dosage been respected before the event?</b>	<input type="checkbox"/> Yes	<input type="checkbox"/> No If No, specify:	Dose:  _ _       Unit (Pot/Bottle): _____ Frequency: Daily	

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<b>Action taken with respect to the study product:</b>	<input type="checkbox"/> Dose Not Changed <span style="float: right;"><input type="checkbox"/> Not applicable</span>		
	<input type="checkbox"/> Product Interrupted Stop date:  _ _ / _ _ / _ _ _ _ _ _  Did reaction disappear after stopping product? <input type="checkbox"/> Yes <input type="checkbox"/> No Reintroduction date:  _ _ / _ _ / _ _ _ _ _ _  Did reaction reappear after reintroduction? <input type="checkbox"/> Yes <input type="checkbox"/> No  <input type="checkbox"/> Product withdrawn => if yes please enter the stop date  _ _ / _ _ / _ _ _ _ _ _  (dd/mm/yyyy)		
<b>Has the code been broken?</b>	<input type="checkbox"/> Yes	<input type="checkbox"/> No	<input type="checkbox"/> Not Applicable

<b>Action taken with respect to the subject</b> (check all that apply)	<b>Outcome of SAE for the subject</b>	<b>Relationship to</b>	
		<b>Study Product</b>	<b>Study Procedure</b>
<input type="checkbox"/> None <input type="checkbox"/> Medication <input type="checkbox"/> Hospitalization <input type="checkbox"/> Study discontinuation <input type="checkbox"/> Study withdrawal <input type="checkbox"/> Other, specify: _____ _____ _____	<input type="checkbox"/> Not Recovered <input type="checkbox"/> Recovering <input type="checkbox"/> Recovered with sequelae, being: ..... <input type="checkbox"/> Recovered without sequelae <input type="checkbox"/> Fatal /Death <input type="checkbox"/> Unknown	<input type="checkbox"/> Not related <input type="checkbox"/> Unlikely related <input type="checkbox"/> Possibly related <input type="checkbox"/> Probably related <input type="checkbox"/> Definitely related	<input type="checkbox"/> Not related <input type="checkbox"/> Unlikely related <input type="checkbox"/> Possibly related <input type="checkbox"/> Probably related <input type="checkbox"/> Definitely related

<b>Relevant medical and surgical history / or copy of CRF pages (CRF page XXX) related to medical and surgical history</b>				
No.	Disease or Surgery	Start date (dd/mm/yyyy)	End Date (dd/mm/yyyy)	Current medication /nutritional supplement?*
1		_ _ / _ _ / _ _ _ _ _ _	_ _ / _ _ / _ _ _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
2		_ _ / _ _ / _ _ _ _ _ _	_ _ / _ _ / _ _ _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
3		_ _ / _ _ / _ _ _ _ _ _	_ _ / _ _ / _ _ _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
4		_ _ / _ _ / _ _ _ _ _ _	_ _ / _ _ / _ _ _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
5		_ _ / _ _ / _ _ _ _ _ _	_ _ / _ _ / _ _ _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
6		_ _ / _ _ / _ _ _ _ _ _	_ _ / _ _ / _ _ _ _ _ _  Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No

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7		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
8		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
9		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
10		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
11		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
12		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
13		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
14		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No
15		____/____/____	____/____/____ Or <input type="checkbox"/> Ongoing	<input type="checkbox"/> Yes <input type="checkbox"/> No



Prior and Concomitant Medication or Alimentary Supplement						<input type="checkbox"/> None	
#	Product name (Trade name)	Indication for use	Subcategory of treatment	Dose description (Dose, Unit, Frequency)	Route (1)	Start Date (dd/mm/yyyy) End Date (dd/mm/yyyy)	Ongoing at the end of the study (2)
1	..... ..... .....	..... ..... .....	<input type="checkbox"/> Prescription <input type="checkbox"/> Self-medication		/_/_/	Start  _ _ / _ _ / _ _ _ _ _  End  _ _ / _ _ / _ _ _ _ _	<input type="checkbox"/>
2	..... ..... .....	..... ..... .....	<input type="checkbox"/> Prescription <input type="checkbox"/> Self-medication		/_/_/	Start  _ _ / _ _ / _ _ _ _ _  End  _ _ / _ _ / _ _ _ _ _	<input type="checkbox"/>
3	..... ..... .....	..... ..... .....	<input type="checkbox"/> Prescription <input type="checkbox"/> Self-medication		/_/_/	Start  _ _ / _ _ / _ _ _ _ _  End  _ _ / _ _ / _ _ _ _ _	<input type="checkbox"/>
4	..... ..... .....	..... ..... .....	<input type="checkbox"/> Prescription <input type="checkbox"/> Self-medication		/_/_/	Start  _ _ / _ _ / _ _ _ _ _  End  _ _ / _ _ / _ _ _ _ _	<input type="checkbox"/>

(1) **Route:** AU = Auricular; IA = Intra-Articular; SC = Subcutaneous; ID = Intradermal; IM = Intramuscular; IV = Intravenous; LO = Local/Topical; NA = Nasal.

OP = Ophthalmic; PO = Oral; SL = Sub-Lingual; RE = Rectal; NK = Not Known; OT = Other.

(2) If the treatment is ongoing at the end of the study, tick off the box. If not, write the end date.

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<p>Comments: .....</p> <p>.....</p> <p>.....</p> <p>.....</p>
---

Completed by:			
Name	Position	Signature	Date

**APPENDIX III**

**MANAGEMENT OF ANY UTI SYMPTOM(S)  
AND POSITIVE URINE CULTURE DURING  
THE STUDY**

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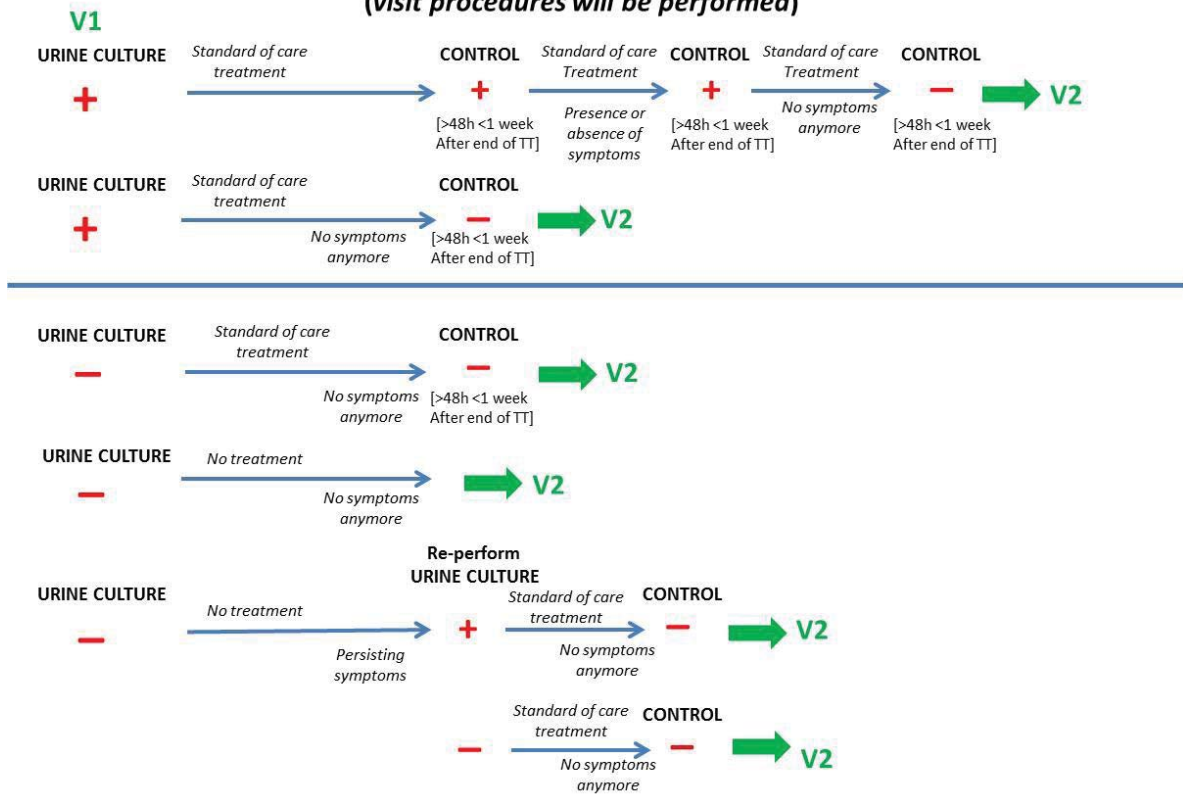
Subject					
Visit	Symptomatic & B +	Symptomatic & B -	Asymptomatic & B +	Asymptomatic & B -	
V1 Urine culture only on symptomatic subjects	Complete an Adverse Event form and Concomitant medication page		NA	NA	
V2 Urine culture for all subjects	Complete an Adverse Event form and Concomitant medication page			-	
Randomization – D0	NA	NA	NA	NA	
V3 Urine culture only on symptomatic subjects	Complete Relapse visit and UTI related Concomitant Treatment		NA	NA	
V4 Urine culture for all subjects	Complete Relapse visit and UTI related Concomitant Treatment		Complete an Adverse Event form and Concomitant medication page	Complete EoS form with date of last urine culture	

**APPENDIX IV**

**MANAGEMENT OF  
SYMPTOMATIC/ASYMPTOMATIC SUBJECTS  
PER VISITS**

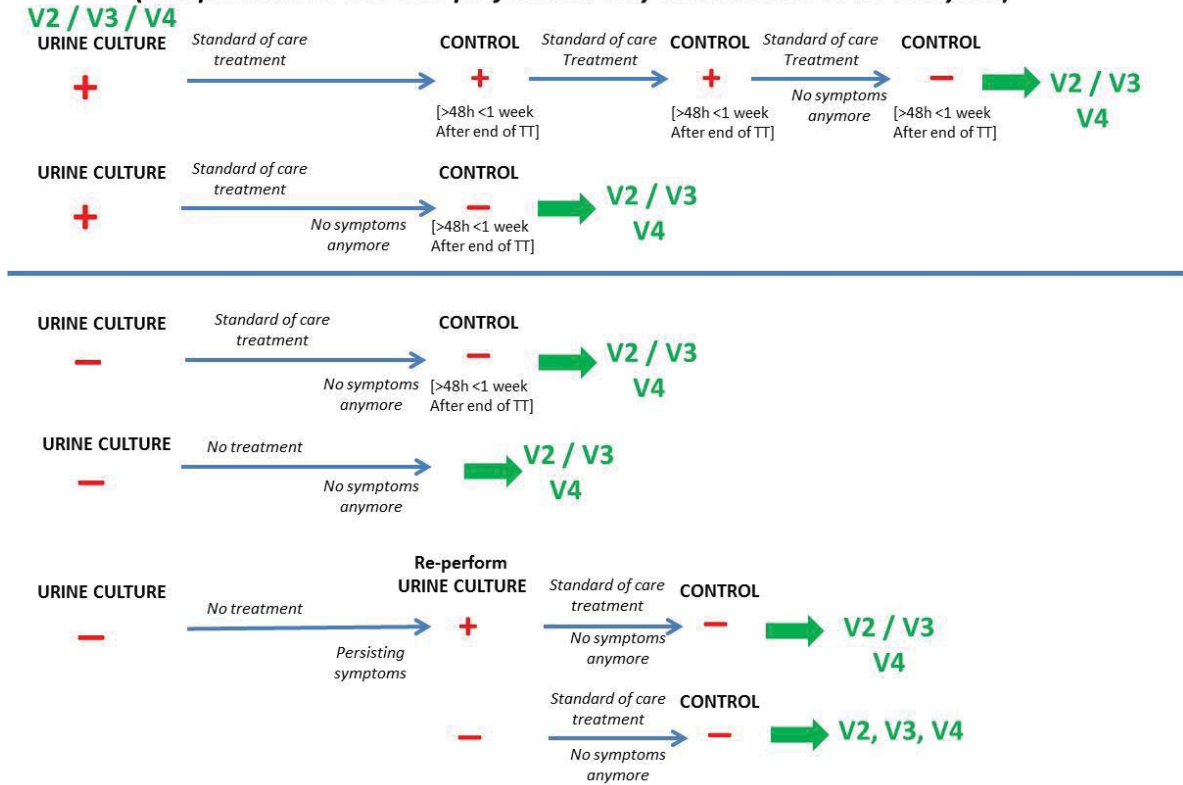
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**Management of V1 for SYMPTOMATIC subjects**  
*(visit procedures will be performed)*



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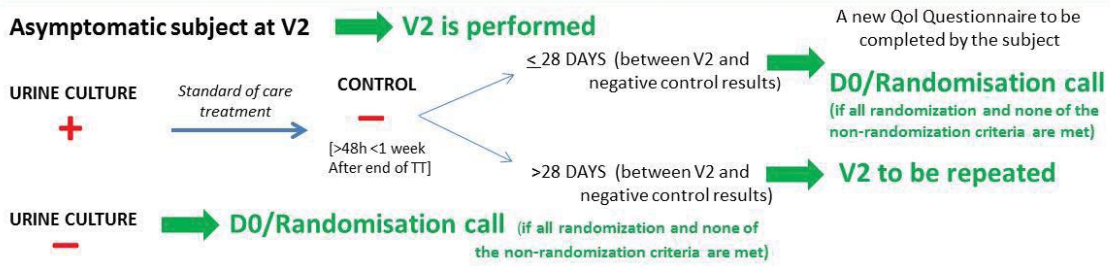
**Management of V2 / V3 /V4 for SYMPTOMATIC subjects**  
(visit procedures won't be performed. Only urine culture to be analysed)



**Management of V1 / V2 / V3 / V4 for ASYMPTOMATIC subjects  
(visit will be performed)**

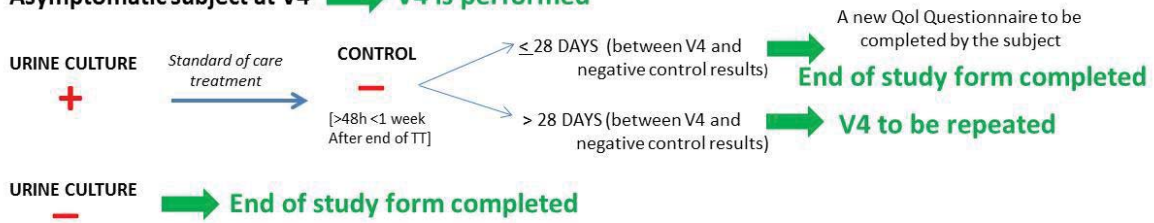
Asymptomatic subject at V1 → V2 is performed

Asymptomatic subject at V2 → V2 is performed



Asymptomatic subject at V3 → V3 is performed

Asymptomatic subject at V4 → V4 is performed





**APPENDIX V**

**PREGNANCY REPORTING FORM**

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**PREGNANCY REPORTING FORM**

STUDY NAME	SITE ID	SUBJECT ID
NU 369	Location : Site ID  0 0 1	_ _ _
Allocation arm (if applicable):		

*☞ This form must be mailed to the sponsor within 24h*

Sponsor: <b>DANONE RESEARCH</b>	Email: liliana.galetescu@danone.com	Tel: +33 (0)1 69 35 76 89
	jean-francois.jeanne@danone.com	Tel: +33 (0)6 25 13 86 96
Clinical Study Manager (Name): Laurence de LA HOSSERAYE (HAYS Pharma on behalf of DANONE RESEARCH)		
Medical reviewer (Name): Liliana GALETESCU		
Principal Investigator (Name): Dr Maya DABCHEVA		
Study code: NU  3 6 9	Expeditor (Name):	Site Number:  0 0 1
Transmission date :  _ _ / _ _ / _ _ _ _  (dd/mm/yyyy)		
Number of pages (incl. this page)	_____ Pages	
Attached copies of completed CRF pages:	<input type="checkbox"/> Adverse events <input type="checkbox"/> Medical History and pre-existing conditions <input type="checkbox"/> Medications	

**FIRST INFORMATIONS**

INFORMATIONS concerning the site :	
Completed by:	
Position :	
Signature :	
Date :	
PI Name :	
Phone :	
Mail :	



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STUDY NAME	SITE ID	SUBJECT ID
NU 369	Location : Site ID   0   0   1	□□□□
Allocation arm (if applicable):		

INFORMATIONS about the SUBJECT :	
Date of last menstruation :	□□   □□   □□   □□
Expected Date of delivery :	□□   □□   □□   □□
Method of contraception used:  Method used according to instructions:	
Medical history with information on familial disorders or risk factors that may influence the outcome of the pregnancy:	
Obstetric history with details on previous pregnancy (including termination or stillbirth):	

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Medication taken prior and during pregnancy:						
Medication or Alimentary Supplement						<input type="checkbox"/> None
#	Product name (Trade name)	Indication for use	Subcategory of treatment	Dose description (Dose, Unit, Frequency)	Route (1)	Start Date (dd/mm/yyyy) End Date (dd/mm/yyyy)
1	..... ..... .....	..... ..... .....	<input type="checkbox"/> Prescription <input type="checkbox"/> Self-medication		/ / /	Start  _ _ / _ _ / _ _ _ _  End  _ _ / _ _ / _ _ _ _
2	..... ..... .....	..... ..... .....	<input type="checkbox"/> Prescription <input type="checkbox"/> Self-medication		/ / /	Start  _ _ / _ _ / _ _ _ _  End  _ _ / _ _ / _ _ _ _
3	..... ..... .....	..... ..... .....	<input type="checkbox"/> Prescription <input type="checkbox"/> Self-medication		/ / /	Start  _ _ / _ _ / _ _ _ _  End  _ _ / _ _ / _ _ _ _
4	..... ..... .....	..... ..... .....	<input type="checkbox"/> Prescription <input type="checkbox"/> Self-medication		/ / /	Start  _ _ / _ _ / _ _ _ _  End  _ _ / _ _ / _ _ _ _
<p>(1) <b>Route:</b> AU = Auricular; IA = Intra-Articular; SC = Subcutaneous; ID = Intradermal; IM = Intramuscular; IV = Intravenous; LO = Local/Topical; NA = Nasal; OP = Ophthalmic; PO = Oral; SL = Sub-Lingual; RE = Rectal, NK = Not Known; OT = Other.</p>						
<p><b>Special tests and procedures performed during pregnancy if the case (e.g. amniocentesis, ultrasound etc.):</b></p>						
<p><b>Pregnancy associated events (if SAE during pregnancy):</b></p>						
<p><b>Pregnancy outcome (abortion, delivery):</b></p>						
<p><b>Child outcome:</b></p>						

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### NU369

The effect of increased water intake on the frequency of clinical recurrent urinary tract infections in pre-menopausal women:


### S-HYDRACYST

Version : 1.0

Date : 06 December 2016



Scientist:	Mariacristina Vecchio
Coordination of the study	Laurence de la Hossieraye
Statistician	Quentin Dornic
Head of Biometrics	Christophe Sauce
Data Manager	Simon Lebeau
CRO Statistician	Isabelle Josse
Sponsor Contact details	DANONE RESEARCH Route départementale 128 91767 Palaiseau Cedex – France
Principal Investigator:	Maya Dabcheva MC "Comac Medical" 13 Urvich Str. 3 <sup>rd</sup> floor 1612 Sofia - Bulgaria Tel.: +369 2 850 97 04 email: maya.dabcheva@comac-medical.com
Statistics CRO	Lincoln 4 Rue Danjou, Bâtiment B 92517 Boulogne-Billancourt Cedex Tel.: +331 46 99 36 65

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### Approval



DANONE RESEARCH	Name	Signature	Date
Scientist	Mariacristina Vecchio		
Statistician	Quentin Dornic		
Biometrics Director	Christophe Sauce		
CRO: Lincoln			
Statistician	Isabelle Josse		

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## 1 Version history


Version	Version Date	Changes*	Author
1.0	06/12/2016	Creation	Q. Dornic (Danone)

**\*Change:**

*A: Addition,*

*M: Modification,*

*D: Deletion*

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## 2 Abbreviations and definitions

AE	Adverse event
ATC	Anatomical Therapeutic Classification
CI	Confidence Interval
eCRF	Electronic Case Report Form
FAS	Full Analysis Set
ICH	International Conference of Harmonisation
INN	International Nonproprietary Names
LLT	Lowest Level Term
MCF	Mean Cumulative Function
NHIF	National Health Insurance Fund
NA	Not Applicable
PP	Per Protocol
PT	Preferred Term
QoL	Quality of Life
RS	Randomised Set
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SE	Standard Error
SEM	Standard error of the mean
SOC	System Organ Class
SS	Safety Set
USG	Urine Specific Gravity
UTI	Urinary Tract Infection

## 3 Introduction

This Statistical Analysis Plan (SAP) describes the different statistical analyses which will be provided on the study, in conformity with the version 4 amendment 3 of the Clinical Study Protocol, dated the 29/02/2016 (including the amendment N°1; 2; 3) and the version 4.0 of CRF (dated the 29/07/2016) and other forms, used for data management of the study.

The Clinical Study Report including statistical and clinical results and statistical appendices will be in English language, format ICHE3, according to the Life Science standard templates (CSP\_T030 to T034). For the documentation of statistical methods, the analyses and the results, this report will have to take into account all information defined in this SAP.

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## 4 Description of the study

### 4.1 Study objectives

#### 4.1.1 Primary study objective

To assess the effect of increased daily water intake on the frequency of clinical recurrent urinary tract infections (rUTIs) among low drinking pre-menopausal women suffering from recurrent community-acquired UTI over 12 consecutive months of study product consumption.

#### 4.1.2 Secondary study objective

To evaluate the impact of increased daily water intake on the use of antibiotics in rUTI subjects over 12 consecutive months of study product consumption.

To evaluate the impact of increased daily water intake on the mean time elapsed between UTI episodes over 12 consecutive months of study product consumption.

To evaluate the impact of increased daily water intake on urinary hydration markers over 6 and 12 consecutive months of study product consumption.

To evaluate the impact of increased daily water intake on health costs in rUTI subjects over 12 consecutive months of study product consumption.

To evaluate the impact of increase of daily water intake on the cost utility analysis during the study intervention using two different perspectives: National Health insurance and subject's perspective (subject's own out-of-pocket costs).

To evaluate the impact of increased daily water intake on quality of life (QoL) in rUTI subjects over 6 and 12 consecutive months of study product consumption.

#### 4.1.3 Exploratory study objective


To evaluate the relationship between urinary hydration markers and number of UTI events over 12 consecutive months of study intervention from D0.

To evaluate the relationship between urinary hydration markers and time to UTI events over 12 consecutive months of study intervention from D0.

To evaluate the impact of increased daily water intake on crystallisation risk index (Tiselius) over 12 consecutive months of study intervention from D0.

To evaluate the number of UTI events confirmed by positive urine culture and number of subjects presenting at least one UTI event over 12 consecutive months of study intervention from D0 (by group).

To evaluate the number of UTI events not confirmed by positive urine culture and number of subjects presenting at least one UTI event over 12 consecutive months of study intervention from D0 (by group).



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To evaluate the number of UTI events confirmed or not by positive urine culture and number of subjects presenting at least one UTI event over 12 consecutive months of the study intervention from D0 (by group).

## 4.2 Study design



This is an open label, prospective, single site, randomised, controlled trial, in two parallel groups with a 1:1 ratio allocation.

The randomisation process will be applied using a centralised Interactive Web Response System (IWRS) that will allocate subject into one of the two groups in respect with the randomisation list generated informatically at the beginning of the study.



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**STUDY FLOW-CHART**



Visits Procedures	MONTH 0				1st Month ± 7 days	2 <sup>nd</sup> Month ± 7 days	3rd Month ± 7 days	4th Month ± 7 days	5th month ± 7 days	6th month ± 7 days	Month 6 ± 10 days	7th Month ± 7 days	8th Month ± 7 days	9th Month ± 7 days	10th Month ± 7 days	11th Month ± 7 days	12th Month	Month 12± 10 days	Rela pse visit
	Inclusio n Visit (V1)	Ho me	Pre- randomis ation visit (V2)	Randomi sation call Day 0	Call	Call	Call	Call	Call	Ho me	Visit 3 (V3)	Call	Call	Call	Call	Call	Home	Completion (V4)	
Screening for acute UTI or previous episodes (at least 3 in the last 12 months)	X																		
Inclusion / exclusion criteria	X		X																
Randomisati on/non randomisati on criteria			X	X															
Informed consent form signature	X																		
Study group				X															

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Visits	Procedures	MONTH 0				1st Month ± 7 days	2 <sup>nd</sup> Month ± 7 days	3rd Month ± 7 days	4th Month ± 7 days	5th month ± 7 days	6th month ± 7 days	Month 6 ± 10 days	7th Month ± 7 days	8th Month ± 7 days	9th Month ± 7 days	10th Month ± 7 days	11th Month ± 7 days	12th Month	Month 12± 10 days	Relapse visit
		Inclusion Visit (V1)	Home	Pre- randomis- ation visit (V2)	Randomi- sation call Day 0	Call	Call	Call	Call	Call	Home	Visit 3 (V3)	Call	Call	Call	Call	Call	Home	Completion (V4)	
allocation																				
Demographic data (date of birth, gender)	X																			
Urine pregnancy test	X		X																	
FSH test if applicable			X																	
Physical examination			X																X	
Phone call to check compliance, AE and UTI events					X	X		X	X	X		X	X	X	X	X				
Medical & Surgical history	X																			
Vital sign and anthropomet	X		X								X								X	



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Visits	MONTH 0				1st Month ± 7 days	2 <sup>nd</sup> Month ± 7 days	3rd Month ± 7 days	4th Month ± 7 days	5th month ± 7 days	6th month ± 7 days	Month 6 ± 10 days	7th Month ± 7 days	8th Month ± 7 days	9th Month ± 7 days	10th Month ± 7 days	11th Month ± 7 days	12th Month	Month 12± 10 days	
	Inclusion Visit (V1)	Home	Pre-randomisation visit (V2)	Randomisation call Day 0	Call	Call	Call	Call	Call	Home	Visit 3 (V3)	Call	Call	Call	Call	Call	Home	Completion (V4)	Relapse visit
Procedural parameters																			
Instructions for Subject's diary completion and return for the 3-days fluid intake	X		X	X	X	X	X	X			X	X	X	X	X			X	X
Subject's diary completion for 3-days fluid intake by the subject		X			X	X	X	X	X			X	X	X	X	X	X		
Check of 3-days fluid intake diary			X		X	X	X	X		X	X	X	X	X	X			X	
Subject Compliance Diary (instructions)			X		x	x	x	x		X	x	x	x	x	x	x			



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Visits Procedures	MONTH 0				1st Month ± 7 days	2 <sup>nd</sup> Month ± 7 days	3rd Month ± 7 days	4th Month ± 7 days	5th month ± 7 days	6th month ± 7 days	Month 6 ± 10 days	7th Month ± 7 days	8th Month ± 7 days	9th Month ± 7 days	10th Month ± 7 days	11th Month ± 7 days	12th Month	Month 12± 10 days		
	Inclusion Visit (V1)	Home	Pre-randomisation visit (V2)	Randomisation call Day 0	Call	Call	Call	Call	Call	Home	Visit 3 (V3)	Call	Call	Call	Call	Call	Home	Completion (V4)	Relapse visit	
(for the test group)																				
Subject Compliance Diary completion (for the test group)					x	x	x	x	x	X		x	x	x	x	x	X			
Subject Compliance with the study product check (for the test group)					x	x	x	x	x		X	x	x	x	x	x		x		
QoL questionnaire (SF-12 V2)			X								X							X		
Delivery of urine kits & Instructions	X		X								X									x



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Visits	Inclusion Visit (V1)	MONTH 0				1st Month ± 7 days	2 <sup>nd</sup> Month ± 7 days	3rd Month ± 7 days	4th Month ± 7 days	5th month ± 7 days	6th month ± 7 days	Month 6 ± 10 days	7th Month ± 7 days	8th Month ± 7 days	9th Month ± 7 days	10th Month ± 7 days	11th Month ± 7 days	12th Month	Month 12± 10 days	Relapse visit
		Home	Pre-randomisation visit (V2)	Randomisation call Day 0	Call	Call	Call	Call	Call	Home	Visit 3 (V3)	Call	Call	Call	Call	Call	Home	Completion (V4)		
Urine 24h collection + Voiding diary completed by the subject		X*								X*							X*			
Delivery of UCC			X																	
Collection of urine containers & Voiding diary			X								X							X		
Blood samples			X								X							X		
Symptoms of UTI	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Urine culture	X** (only if symptomatic)		X**								X** (only if symptomatic)							X**	X**	
Healthcare costs data																			X	

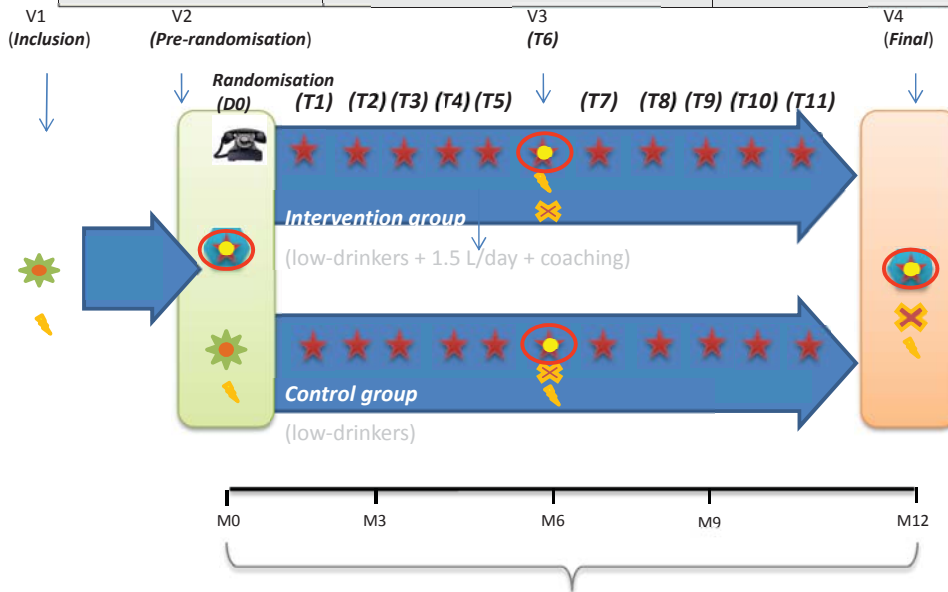
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Visits	Inclusion Visit (V1)	Home	MONTH 0				1st Month ± 7 days	2 <sup>nd</sup> Month ± 7 days	3rd Month ± 7 days	4th Month ± 7 days	5th month ± 7 days	6th month ± 7 days	Month 6 ± 10 days	7th Month ± 7 days	8th Month ± 7 days	9th Month ± 7 days	10th Month ± 7 days	11th Month ± 7 days	12th Month	Month 12± 10 days	Relapse visit
			Pre-randomisation visit (V2)	Randomisation call Day 0	Call	Call	Call	Call	Call	Home	Visit 3 (V3)	Call	Call	Call	Call	Call	Home	Completion (V4)			
Bladder emptying after sexual intercourses	X		X			X	X	X	X	X		X	X	X	X	X	X		X	X	
Active/Inactive sexual life during the last month	X		X			X	X	X	X	X		X	X	X	X	X	X		X	X	
Past and Concomitant Treatments	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Adverse events	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

\* 24h urine to be collected at home the day before the visit (V2, V3 and V4)

\*\* In case of UTI infection a control urine culture should be performed at least 48H after the end of treatment and within 1 week to confirm that subjects are completely recovered

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**Screening**

**Intervention and follow-up**

-  Fluid survey, symptoms of UTI
  -  Physical examination
  -  Inclusion/exclusion
  -  Laboratory visit: 24h urine + blood sample
-  vital signs
 
 Healthcare cost
 

 Quality of life

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### 4.3 Sample size

By definition, the rUTI event data consist of the inter event time of repetition of the same or different types for each subject. The measurements across subjects are considered to be statistically independent, but the times between UTI events for a specific subject are not necessarily independent. The mean cumulative function (MCF) contains the information of interest in the analysis of recurrent data.

Assume  $M(t)$  is the mean cumulative number of UTI events up to time  $t$   
 $M(t) = E\{N(t)\}$ , where  $N(t)$  is a random variable for the number of events that have occurred up to time  $t$



The main assumption of this approach is that the hazard or risk ratio is proportional over time, reason why a robust sandwich variance estimated is used to account for dependence of recurrent events on the same subject.

Assuming that all subjects had the same study period examination (approximately 12 months), and considering an interval of risk (inter event times) following an Uniform distribution, data have been simulated based on a mean number of events equal to 3 for control group and an expected intervention effect of 20% less events in the intervention group. One hundred samples have been replicated upon the simulated data.

Considering the simulated data based on our hypotheses, a mean number of events during a 12 months period in control group equal to 3, the estimate sample size that would provide a power of 80% to detect a product effect of 20% less events occurred in the interventional study group for a bilateral test,  $\alpha=0.05$  will be equal to 42 subjects per group, i.e. 84 overall completed subjects (ratio 1:1).

In order to achieve 84 completed subjects, and considering a 40% drop out rate, the screening process will last until we will reach 140 subjects randomised.

Considering a 50% screen failure rate, around 280 screened subjects will be needed in order to get 140 randomised subjects.

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#### 4.4 Changes in the conduct of the study compared to protocol

A site audit, performed in July 2014, outlined several major problems in the study (misunderstandings of certain aspects of the protocol). As a consequence, inclusions were stopped from 01/02/2014 to 01/06/2015. Training for the site was performed before the restart of the inclusions.

The impact of these findings will be evaluated by subgroup analyses on the different batches of subjects (see section 6.6).

#### 4.5 Unblinding of the study

Not applicable.

### 5 Analysis sets and products groups

#### 5.1 Analysis sets

The populations will be defined as follows:

- The “Global Population”: all subjects included in the study and eligible at the end of screening period (eligible at the end of V2).
- The “Full Analysis Set” (FAS): all subjects included in the study and randomised.
- The “Per Protocol” population (PP): all subjects included in the FAS presenting no major protocol deviation.
- The “Safety Set” (SS): all subjects included in the FAS.

The analysis of baseline characteristics will be performed on the FAS.

The analysis of primary criteria will be done on the FAS and PP population. If the difference in number of subjects is few between the FAS and PP population (difference lower than 10%), the analyses of secondary criteria will be only done on the FAS (see details in section 7).


The safety and tolerance analyses will be performed on the SS, see details respectively in section 8 and 9.

#### 5.2 Products groups

The study is testing a medical recommendation, consisting in increasing the daily water intake in the intervention group.

For methodological reasons of homogeneity of access to water of the subjects, mineral commercialised water will be distributed to the subjects included in the intervention group, during the whole study duration.

The mineral water is Evian® brand.

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The intervention group will increase its fluid intake of 1,5L of Evian® water (on the top of their normal fluid intake).

The control group will not change its water intake habits.

## 6 Statistical elements

### 6.1 Hypothesis and significance level

In the present study the null hypothesis “There is no difference between groups on the average number of recurrent UTIs events experienced” versus the alternative hypothesis “the effect of intervention on the average number of recurrent UTIs events experienced is different to the effect of the control” will be tested.

The test will be based on a non-parametric method (MCF) that will be used to analyse the multiple/repeated UTI events occurring.

H<sub>0</sub>: Effect of fluid intake = Effect control

H<sub>1</sub>: Effect of fluid intake ≠ Effect control

Statistical tests will be conducted two-sided with a significance level of 5%. All confidence intervals will be presented two-sided with a confidence level of 95%. A resultant probability value of p<0.05 will be judged as being of statistical significance. The interaction factors (if any) will be considered as significant if the p value is < 0.10.

The MCF by group, estimated by a non-parametric estimator:


$$\widehat{MCF}(t) = \sum_{\{j|t_j \leq t\}} \frac{e_j}{n_{j-1}}$$

where e<sub>j</sub> is the number of events at time t<sub>j</sub>, n<sub>j-1</sub> is the number of subjects at risk just beyond time t<sub>j-1</sub>, and j the observed event times.

### 6.2 Distribution and normality assessment

The underlying assumptions of normality of residuals for analysis covariance (ANCOVA) model will be checked (amongst others by using skewness and kurtosis statistics, histogram and QQ plot). A distribution will be considered as approximately normal if the values of Skewness and Kurtosis falls within the interval [-1.5, 1.5].

If the assumption of normality is not satisfied, box-cox transformation could be performed if relevant or nonparametric method (Wilcoxon test) will be used.

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For cox model, the proportional hazard assumption will be graphically checked by plotting the survival curves. For qualitative data, the log of the negative log of the survival curve will be plotted, and Harell test will be performed. For quantitative data, the deviance and martingales residuals will be plotted, and Harell test will be performed.

### 6.3 Handling of missing data

No imputation method will be use.

### 6.4 Handling multiple testing

For the primary criterion, no adjustment will be done.

Within each secondary criterion category (use of antibiotics, average time between each UTI, urinary hydration markers, health costs, Quality of Life), a Bonferroni correction will be applied to ensure a global  $\alpha=0.05$ . The corrections to apply are specified in section 7.7.

For the exploratory criteria, no adjustment will be done.

### 6.5 Outlier management


For analysis on the primary and secondary parameters only: in case the data review meeting and/or database explorations reveal outliers, as identified by independent experts and/or statistical and/or Medical Reviewer outlier identification, a sensitivity analysis could be performed excluding these outliers. Reasons for outlier classification and results of these sensitivity analyses will be described separately in the study report.

### 6.6 Subgroup analyses

Subjects will be divided into two subgroups, one containing all the subjects included before the 01<sup>st</sup> of June 2015 (subgroup: 1<sup>st</sup> batch), and the other containing all the subjects included since the 01<sup>st</sup> of June 2015 (subgroup: 2<sup>nd</sup> batch). Theses subgroups will be used in sensitivity analyses.

### 6.7 Interim analysis

Not applicable.

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## 6.8 Statistical computer software

The statistical analysis will be performed by CRO using SAS 9.2.

## 6.9 Reporting of results

Results will be presented in the clinical study report, provided by the CRO responsible of the analysis.

## 6.10 Format tables and graphs

### 6.10.1 Tables

For continuous data:

- For each visit per group and overall, raw data: number of non-missing observations, number of missing observations, mean, median, standard deviation (SD), minimum and maximum, quartile (Q1 and Q3), confidence interval [CI] at 95% of the mean. Standard error mean [SEM] could be added if relevant.

For nominal data:

- For each visit per group and overall, raw data: number of non-missing observations, number of missing observations, number and frequency of observations in each class.

For qualitative ordinal parameters

- For each visit per group and overall, raw data: number of non-missing observations, number of missing observations, number and frequency of observations in each class.

The unit of the parameters will be always specified in the titles

The p values will be given with only 4 decimals.


### 6.10.2 Graphs

For MCF analysis, the estimated MCF will be plotted by group on the same graph.

For survival analysis, the Kaplan Meier curves will be plotted by group on the same graph.

For repeated measures ANCOVA, the means  $\pm$  SD, by visit, will be plotted by group on the same graph.



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## 7 Statistical analyses

### 7.1 Status of subjects, protocol deviations and population definition

**Objective:** to describe the status of subjects, the protocol deviations and populations.

#### 7.1.1 Status of subjects

The number and percentage of subjects included (performed V1), eligible at V1, eligible at V2 and randomised will be provided overall and by study group and subgroup.

The number and percentage of subjects at each visit will be provided overall and by study group and subgroup.

#### 7.1.2 Protocol Deviations

The deviations and their status (minor or major) are defined in the document “S-HYDRACYST, DRP v2\_2.docx”.



The number and percentage of subjects having at least one protocol deviation, overall and by study group and subgroup, will be described on FAS.

The deviation frequency and number and percentage of subjects concerned by deviation type, overall and by study group and subgroup, will be described on FAS.

#### 7.1.3 Populations

Number and percentage of subjects in each population will be described, overall and by study group and subgroup.

A listing of subjects excluded from each population will be provided.

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## 7.2 Demography

**Objective:** description of demographic characteristics at baseline.

Descriptive summaries will be performed for the following parameters:

Variable name	Variable source <sup>(1)</sup>	Type <sup>(2)</sup>	Unit	Set
Age	R	C	years	FAS (all, subgroups)

(1) (R)aw or (D)erived data

(2) (C)ontinuous or (O)rdinal or (N)ominal


Derived data are defined in section 10.

## 7.3 Baseline characteristics

**Objective:** description of baseline characteristics in order to assess the clinical comparability of groups.

Descriptive summaries will be performed for the following parameters:

Variable name	Variable source <sup>(1)</sup>	Type <sup>(2)</sup>	Unit	Set
<b>Vital signs and Anthropometry</b>				
Height	R	C	cm	FAS (all, subgroups)
Weight	R	C	kg	FAS (all, subgroups)
Waist circumference	R	C	cm	FAS (all, subgroups)
Body Mass Index	D	C	kg/m <sup>2</sup>	FAS (all, subgroups)
Body temperature	R	C	°C	FAS (all, subgroups)
SBP	R	C	mmHg	FAS (all, subgroups)
DBP	R	C	mmHg	FAS (all, subgroups)
Clinical significance of blood pressure	R	N		FAS (all, subgroups)
Heart rate	R	C	beats/min	FAS (all, subgroups)
Clinical significance of heart rate	R	N		FAS (all, subgroups)
<b>Physical examination</b>				
Result (Normal / Abnormal)	R	N		FAS (all)
<b>Contraceptive Method</b>				
Effective contraception (Yes / No)	R	N		FAS (all)
If yes, method used	R	N		FAS (all)
<b>Sexual Life Habits</b>				

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Sexual status during the last month (Active/Not Active)	R	N		FAS (all, subgroups)
If active, emptying bladder during the last month (Yes / No)	R	N		FAS (all, subgroups)
<b>Urinary Pregnancy test</b>				
Result	R	N		FAS (all)
<b>UTI symptoms</b>				
Symptoms of UTI (Yes / No)	R	N		FAS (all, subgroups)
If yes, Type of symptoms	R	N		FAS (all, subgroups)
<b>Significant Medical and Surgical History</b>				
Disease or Surgery (description by system organ class and preferred term)	R	Text		FAS (all, subgroups)
<b>Urinary hydration markers</b>				
Weight	R	C	g	FAS (all, subgroups)
Urine volume	R	C	mL	FAS (all, subgroups)
pH	R	C		FAS (all, subgroups)
Osmolality	R	C	mOsm/kg	FAS (all, subgroups)
Urine Specific gravity	R	C		FAS (all, subgroups)
Micturition	R	C		FAS (all, subgroups)
Urine colour	R	C		FAS (all, subgroups)
Tiselius Crystallisation risk index	D	C		FAS (all, subgroups)
<b>Quality of Life (SF12V2)</b>				
Each of the 12 items	R	O		FAS (all, subgroups)
Each of the 8 dimensions	D	C		FAS (all, subgroups)
Mental health score	D	C		FAS (all, subgroups)
Physical health score	D	C		FAS (all, subgroups)
Utility index	D	C		FAS (all, subgroups)

(1) (R)aw or (D)erived data



(2) (C)ontinuous or (O)rdinal or (N)ominal

Derived data are defined in section 10.

## 7.4 Study conduct parameters

**Objective:** description of study duration and compliance.

Descriptive summaries will be performed for the following parameters:

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Variable name	Variable source <sup>(1)</sup>	Type <sup>(2)</sup>	Unit	Set
<b>Study duration (visit date)</b>				
Study duration	D	C	days	FAS (all, subgroups)
Study Intervention phase duration	D	C	days	FAS (all, subgroups)
<b>Compliance to product consumption (product intake date)</b>				
Study product exposure	D	C	days	FAS (all, subgroups)
Study product compliance (for intervention group only)	D	C	%	FAS (all, subgroups)
Fluid consumption over 3 days: Average quantity of fluid consumed per day	D	C	L	FAS (all, subgroups)

(1) (R)aw or (D)erived data

(2) (C)ontinuous or (O)rdinal or (N)ominal

Derived data are defined in section 10.

## 7.5 Concomitant medications

**Objective:** description of prior and concomitant medications taken on following periods:



- Treatments taken prior inclusion
- Treatments taken during the screening period
- Treatments taken during the intervention period

The concomitant medications and nutritional supplement will be presented by ATC class (Medication class and Preferred Term) according to the latest WHO-Drug coding system (Version Q1 2015).

Frequency ordering will be used for table's presentation.

They will be presented globally, and by relationship to UTI.

Variable name	Variable source <sup>(1)</sup>	Type <sup>(2)</sup>	Unit	Set
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Concomitant medications				
Prior medication and nutritional supplement	R	Text		SS (all, subgroups)
screening period medication and nutritional supplement	R	Text		
intervention period medication and nutritional supplement	R	Text		

(1) (R)aw or (D)erived data

(2) (C)ontinuous or (O)rdinal or (N)ominal

## 7.6 Analysis of primary criteria

**Objective:** To assess the effect of increased daily water intake on the frequency of clinical rUTIs.

### 7.6.1 Description of primary outcome parameters

Descriptive summaries will be performed for the following parameters:

Variable name	Variable source <sup>(1)</sup>	Type <sup>(2)</sup>	Unit	Set
number of confirmed UTIs events experienced over 12 months	D	C, N		FAS(all, subgroups), PP (all)

(1) (R)aw or (D)erived data

(2) (C)ontinuous or (O)rdinal or (N)ominal


Derived data are defined in section 10.

### 7.6.2 Analysis of primary outcome parameters

The main evaluation criterion is the number of confirmed UTI event experienced over a one (1) year study period between intervention and control groups, evaluated by the difference between groups in the mean cumulative function (poisson model). The p-value considered for statistical significance will be  $p=0.05$ .

As sensitivity analyses, the same analysis will be done first adjusted on subgroup and group\*subgroup interaction, and then using a negative binomial model (adjusted and unadjusted).

The same analysis will be done on the PP population (all subjects only).

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## 7.7 Analyses of secondary criteria



**Objective:** To evaluate the impact of increased daily water intake on:

- the use of antibiotics,
- the mean time elapsed between each UTI,
- urinary hydration markers,
- health costs,
- the cost utility,
- Quality of Life (QoL).

### 7.7.1 Description of secondary outcome parameters

Descriptive summaries will be performed for the following parameters:

Variable name	Variable source <sup>(1)</sup>	Type <sup>(2)</sup>	Unit	Set
<b>Antibiotic prescription/usage to treat UTI event over 12 months</b>				
Number of antibiotic prescription/usage related to UTIs	D	C		FAS (all, subgroups)
<b>Time between each UTI event over 12 months</b>				
Average time between each confirmed UTI event	D	C	days	FAS (all, subgroups)
<b>Urinary hydration markers over 6 and 12 months</b>				
Weight (raw data and change from baseline)	R	C	g	FAS (all, subgroups)
Urine volume (raw data and change from baseline)	R	C	mL	FAS (all, subgroups)
pH (raw data and change from baseline)	R	C		FAS (all, subgroups)
Osmolality (raw data and change from baseline)	R	C	mOsm/kg	FAS (all, subgroups)
Urine Specific gravity (raw data and change from baseline)	R	C		FAS (all, subgroups)
Micturition (raw data and change from baseline)	R	C		FAS (all, subgroups)

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Urine colour (raw data and change from baseline)	R	C		FAS (all, subgroups)
<b>QoL over 6 and 12 months</b>				
Each of the 12 items (raw data)	R	O		FAS (all, subgroups)
Each of the 8 dimensions (raw data)	D	C		FAS (all, subgroups)
Mental health score (raw data and change from baseline)	D	C		FAS (all, subgroups)
Physical health score (raw data and change from baseline)	D	C		FAS (all, subgroups)
Utility index (raw data and change from baseline)	D	C		FAS (all, subgroups)
<b>Health Cost over 12 months</b>				
Mean cost of a UTI for the subject (cost from the study data)	D	C	BGN	FAS (all, subgroups)
Mean cost of a UTI for the subject (cost from standard practice)	D	C	BGN	FAS (all, subgroups)
Mean cost of a UTI for the National Health Insurance Fund (NHIF) (cost from standard practice)	D	C	BGN	FAS (all, subgroups)


- (1) (R)aw or (D)erived data  
 (2) (C)ontinuous or (O)rdinal or (N)ominal

Derived data are defined in section 10.

### 7.7.2 Analysis of secondary outcome parameters

The secondary outcome parameters will be analysed using the following models.

The difference between groups in terms of antibiotic prescription/usage (number of prescription/usage of antibiotic) will be analysed using the MCF, with a poisson model (adjusted on age); a negative

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binomial model will be used as a sensitivity analysis. The p-value considered for statistical significance will be  $p=0.05$ .

The difference between groups in terms of average time between each UTI event will be analysed using a gamma model (adjusted on age). The p-value considered for statistical significance will be  $p=0.05$ .


The difference between groups in terms of urinary hydration markers (urine volume, pH, Osmolality, USG, Urine colour) will be analysed using repeated measures analysis of covariance for change from baseline (adjusted on age and baseline value as fixed effects). The p-value considered for statistical significance will be 0.05/5.

The difference between groups in terms of QoL (mental health score, physical health score, and utility index) will be analysed using repeated measures analysis of covariance on change from baseline (adjusted on age and baseline value as fixed effects). The p-value considered for statistical significance will be 0.05/3.

The difference between groups in terms of health cost associated with management of UTI recurrence (subject perspective, study data) and cost utility analysis (subject perspective and NHIF perspective, standard practice) will be analysed using analyse of covariance for each perspective (social insurance and subject perspectives) (adjusted on age as fixed effect). The p-value considered for statistical significance will be 0.05/3

As sensitivity analyses, the same analyses will be done without adjustment, and adjusted on subgroup and group\*subgroup interaction. If this interaction is statistically significant further investigations will be done,



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## 7.8 Analysis of exploratory criteria

**Objective:** to explore:

- the links between urinary hydration markers and UTIs (number and time between UTIs),
- the impact of increased daily water intake on crystallisation risk index
- UTIs (all, confirmed, suspected).

### 7.8.1 Description of exploratory outcome parameters

Descriptive summaries will be performed for the following parameters:

Variable name	Variable source <sup>(1)</sup>	Type <sup>(2)</sup>	Unit	Set
<b>Urinary markers of hydration</b>				
Tiselius Crystallisation risk index	D	C		FAS (all)
<b>Urinary Tract Infection</b>				
Time to first confirmed UTI event (for subject with at least 1 confirmed UTI event)	D	C		FAS (all)
<b>UTIs</b>				
number of subjects presenting at least one confirmed UTI event	D	N		FAS (all)
number of subjects presenting at least one suspected UTI event	D	N		FAS (all)
number of suspected UTI events	D	C, N		FAS (all)
number of subjects presenting at least one UTI event (confirmed or suspected)	D	N		FAS (all)
number of UTI events (confirmed or suspected)	D	C, N		FAS (all)


(1) (R)aw or (D)erived data

(2) (C)ontinuous or (O)rdinal or (N)ominal

Derived data are defined in section 10.

### 7.8.2 Analysis of exploratory outcome parameters

The exploratory parameters will be analysed using the following models.

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The relationship between each of the urinary hydration markers at baseline (urine volume, pH, Osmolality, USG, Urine colour) and the number of UTI events between groups will be analysed using a poisson regression model (adjusted on age).

The relationship between each of the urinary hydration markers at baseline (urine volume, pH, Osmolality, USG, Urine colour) and the average time between UTI events between groups will be analysed using analyse of covariance (adjusted on age as fixed effect).

The relationship between each of the urinary hydration markers at baseline (urine volume, pH, Osmolality, USG, Urine colour) and the time to first confirmed UTI event between groups will be analysed using Cox model (adjusted on age) and survival plots.

The impact of increased daily water intake on crystallisation risk index (Tiselius) will be evaluated using analysis of covariance (adjusted on age and baseline value as fixed effects). As sensitivity analysis, the same analysis will be done without adjustment.

The number of suspected UTI events and the number of UTI event (confirmed or suspected) will be analysed using the MCF, with a poisson and a negative binomial model.

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## 8 Analysis of safety

**Objective:** to check if the study product presents any safety issue.

### 8.1 Adverse events

The analysis of adverse events will be performed to evaluate the number of subjects with at least one adverse events and the number of adverse events by study group and subgroup.

The adverse events will be presented by "*Body System*" and "*Preferred term*" according the MedDRA coding system (Version 18.0).

The analysis of emergent adverse events will also be performed.

**Adverse Event:**

Number of subjects with at least one adverse event during the study, by primary system organ class, by preferred term and by total. Number of adverse events during the study, by primary system organ class and preferred term.

**Serious Adverse Event:**

Number of subjects with at least one serious adverse event during the study, by primary system organ class and preferred term.

Number of serious adverse events during the study, by primary system organ class and preferred term.

**Emergent Adverse Event:**


Number of subjects with at least one emergent adverse event during the study, by primary system organ class and preferred term.

Number of emergent adverse events during the study, by primary system organ class and preferred term.

**Serious Emergent Adverse Event:**

Number of subjects with at least one serious emergent adverse event during the study, by primary system organ class and preferred term.

Number of serious emergent adverse events during the study, by primary system organ class and preferred term.

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**Emergent Adverse Event, by intensity:**

Number of subjects with at least one emergent adverse event during the study, by primary system organ class and preferred term, according to the intensity

Number of emergent adverse events during the study, by primary system organ class and preferred term, according to the intensity

**Emergent Adverse Event, by outcome:**

Number of subjects with at least one emergent adverse event during the study, by primary system organ class and preferred term, according to the outcome.

Number of emergent adverse events during the study, by primary system organ class and preferred term, according to the outcome

**Emergent Adverse Event, by relationship to product:**

Number of subjects with at least one emergent adverse event during the study, by primary system organ class and preferred term, according to the relationship to product.

Number of emergent adverse events during the study, by primary system organ class and preferred term, according to the relationship to product

**Serious Emergent Adverse Event, by relationship to product:**

Number of subjects with at least one serious emergent adverse event during the study, by primary system organ class and preferred term, according to the relationship to product.

Number of serious emergent adverse events during the study, by primary system organ class and preferred term, according to the relationship to product


**Emergent Adverse Event, by action taken:**

Number of subjects with at least one emergent adverse event during the study, by primary system organ class and preferred term, according to the action taken.

Number of emergent adverse events during the study, by primary system organ class and preferred term, according to the action taken

All adverse events will be summarised, in individual data listings, by subject with all details concerning the adverse event, the study product, and the emergence.

The serious adverse events will be summarised, in individual data listings, by subject with all details concerning the adverse event, the study product, and the emergence.

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## 9 Analysis of the product tolerance

**Objective:** to check if the study product presents any tolerance issue.

### 9.1 Vital signs

Descriptive summaries will be performed for the following parameters:

Variable name	Variable source <sup>(1)</sup>	Type <sup>(2)</sup>	Unit	Set
Weight (raw data and change from baseline)	R	C	kg	SS (all)
Waist circumference (raw data and change from baseline)	R	C	cm	SS (all)
BMI (raw data and change from baseline)	D	C	kg/m <sup>2</sup>	SS (all)
Body temperature (raw data and change from baseline)	R	C	°C	SS (all)
SBP (raw data and change from baseline)	R	C	mmHg	SS (all)
DBP (raw data and change from baseline)	R	C	mmHg	SS (all)
Clinical significance of BP	R	N		SS (all)
HR (raw data and change from baseline)	R	C	bpm	SS (all)
Clinical significance of heart rate	R	N		SS (all)



(1) (R)aw or (D)erived data

(2) (C)ontinuous or (O)rdinal or (N)ominal

Derived data are defined in section 10.

### 9.2 Laboratory parameters

Descriptive summaries will be performed for the following parameters:

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Variable name	Variable source <sup>(1)</sup>	Type <sup>(2)</sup>	Unit	Set
<b>Blood sample – Biochemistry</b>				
HbA1c (raw data and change from baseline)	R	C	%	SS (all)
HDL cholesterol (raw data and change from baseline)	R	C	mmol/L	SS (all)
LDL cholesterol (raw data and change from baseline)	R	C	mmol/L	SS (all)
Total cholesterol (raw data and change from baseline)	R	C	mmol/L	SS (all)
Tryglicerides (raw data and change from baseline)	R	C	mmol/L	SS (all)
Serum creatinine (raw data and change from baseline)	R	C	μmol/L	SS (all)
Serum urea (raw data and change from baseline)	R	C	mmol/L	SS (all)
Copeptin (raw data and change from baseline)	R	C	pmol/L	SS (all)
Glomerular filtration rate (raw data and change from baseline)	R	C	mL/min/1.73m <sup>2</sup>	SS (all)
<b>Urinary parameters</b>				
Sodium (raw data and change from baseline)	R	C	mmol/day	SS (all)
Potassium (raw data and change from baseline)	R	C	mmol/day	SS (all)
Calcium (raw data and change from baseline)	R	C	mmol/day	SS (all)
Magnesium (raw data and change from baseline)	R	C	mmol/day	SS (all)
Chloride (raw data and change from baseline)	R	C	mmol/day	SS (all)
Oxalate (raw data and change from baseline)	R	C	μmol/day	SS (all)
Citrate (raw data and change from baseline)	R	C	mg/day	SS (all)
Urine creatinine (raw data and change from baseline)	R	C	mmol/L	SS (all)



(1) (R)aw or (D)erived data

(2) (C)ontinuous or (O)rdinal or (N)ominal

Derived data are defined in section 10.

### 9.3 Other tolerance criteria

Not applicable.

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## 10 Definitions and derivations

### 10.1.1 Compliance and duration

#### Baseline:

Value at V2 if available, else value at V1

For not completed subjects the data of visit V4 will be reassigned to the first missed scheduled visit (V3 if V3 is not performed, else V4), whatever their actual date

#### Change from baseline:

Value at Vx – value at baseline

#### Compliance with study product intake

If the subject is in the intervention group:

- Global compliance with quantity of study product intake  

$$= \frac{\text{Number of products consumed between V2 and V4}}{3 \times (\text{study product exposure})} \times 100$$

Subject has a “Good compliance”, if compliance is included in [80%; 120%]

Subject has a “Bad compliance”, if compliance is <80% or > 120%

#### Duration

- Study duration: end of study date – date of V1 + 1
- Intervention duration:  
 for control group: end of study date – date of randomisation + 1  
 for intervention group: end of study date – date of first intake + 1
- Study product exposure (days)  
 = Last intake date – First intake date + 1



#### Concomitant medication:

- Treatments during the screening period:  
 Start date ≥ date of V1 or  
 Start date < date of V1 and stop date ≥ date of V1
- Treatments during the intervention period  
 Start date ≥ first product intake (randomisation date for the control group) or  
 Start date < first product intake (randomisation date for the control group) and stop date ≥ first product intake (randomisation date for the control group)

### 10.1.2 Primary evaluation criteria

Suspected UTI event: symptoms of UTI without positive bacteriogram associated

Confirmed UTI event: symptoms of UTI with positive bacteriogram associated

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UTI event: any suspected or confirmed UTI event

For end of study visit, if symptoms are reported and the bacteriogram is positive, then it will be considered as a confirmed UTI event. If symptoms are reported and the bacteriogram is negative, then it will be considered as a suspected UTI event. The start date will be the date of end of study visit. The end date will be the end of last negative urine culture

End date of UTI:

If the end date is different than the date of last negative urine culture, then replace it with this date.

### 10.1.3 Secondary evaluation criteria

Number of antibiotic prescription/usage; Number of antibiotic prescribed+number of antibiotic taken as automedication

Antibiotic related to UTI: an antibiotic is considered related to an UTI if it begins between the start date and the end date of an UTI (confirmed or suspected).

Antibiotic, codes list:

WHODDE, Version: March 1, 2016

J ANTIINFECTIVES FOR SYSTEMIC USE

J01 ANTIBACTERIALS FOR SYSTEMIC USE

J01A TETRACYCLINES

J01B AMPHENICOLS

J01C BETA-LACTAM ANTIBACTERIALS, PENICILLINS

J01CA Penicillins with extended spectrum

J01CE Beta-lactamase sensitive penicillins

J01CF Beta-lactamase resistant penicillins

J01CG Beta-lactamase inhibitors

J01CR Combinations of penicillins, incl. beta-lactamase inhibitors

J01D OTHER BETA-LACTAM ANTIBACTERIALS

J01E SULFONAMIDES AND TRIMETHOPRIM

J01F MACROLIDES, LINCOSAMIDES AND STREPTOGRAMINS

J01FA Macrolides

J01FF Lincosamides


J01FG Streptogramins

J01W HERBAL ANTIBACTERIALS AND ANTIINFECTIVES FOR SYSTEMIC USE

J01WA Herbal antibacterials for systemic use

J01WB Herbal urinary antiseptics and antiinfectives



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J01X OTHER ANTIBACTERIALS

- J01XA Glycopeptide antibacterials
- J01XB Polymyxins
- J01XC Steroid antibacterials
- J01XD Imidazole derivatives
- J01XE Nitrofurans derivatives
- J01XX Other antibacterials

Fluid consumption over 3 days:

Sum of the 3 days fluid intake / number of days recorded

Time between UTI events:

For the first UTI event:

Start date of the UTI – randomisation date

For the others UTI events:

Start date of the UTI – end date of the previous UTI

Average time between UTI:

Sum of all the times between UTI / number of UTI

For censored data (subjects without UTI events): end of study date – date of randomisation

Cost of UTI:

The followings costs corresponds to the pricing done by a Bulgarian expert, Antoniya Dimova, MSC, PHD, associate professor at the Department of Health Economics and Management of Varna University of Medicine


Cost of UTI from study data (for each UTI):

-mean cost of a compulsory health insurance in Bulgaria (Q1)

Monthly expenses for SHI contributions for employed under labour contract individuals. Calculations is based on mean Mouny salary for the selected years, 8 % health insurance contribution (defined by law) split between employee and employer at ratio 40:60 (3.2 % of the contribution is paid by employees and 4.8% by employers. This ratio is also defined by law).

2014				2015			
employee		employer		employee		Employer	
BGN	euro	BGN	euro	BGN	euro	BGN	Euro
26,3	13,4	39,5	20,2	28,6	14,6	42,9	21,9

If Q1= Yes, then add 28.6 BGN

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-mean cost of a basic hospitalization (Q4)

NHIF expenses per day of hospitalization on clinical pathway related with UTI complications  
Consumer fee paid by patient for each day of hospitalization

BGN	euro
84	42,9
5,8	3,0

If Q1= Yes, then add 5.8 BGN \*, (number of days)

If Q1= No, then add (5.8+84) BGN \* (number of days)

-mean cost of additional payment such as (Q5):

Consumer fee

Huge variation between hospitals

“Luxury” conditions (private room, TV)

Mean price per day per patient based available information about prices-lists of hospitals in Sofia, Varna and Plovdiv

Choice of a physician

Huge variation between hospitals and services in a same hospital. The maximal price for choice of a physician is set at 500 BGN and for choice of a team - at 900 BGN by law

Choice of a team

BGN	Euro
60	30,7
500	255,6
900	460,2

If luxury conditions, then add 60 BGN

If choice of a physician or a team, then add 900 BGN

If informal payments, then add the value

-mean cost of complication of UTI such as(Q6)

Fever

Pyelonephritis

The NHIF price for hospital episode (clinical pathway); minimal hospital stay - 5 days

BGN	Euro
420	214,7

-mean cost of diagnostic procedures such as (Q7):

Urine culture

Urine microscopy (leuco'setc)

Haematology lab

Test strip

Echography


CT

CT with contrast

mean cost based on laboratories' prices lists

market prices for non-insured patients or for elective services; these prices are referred to patients who responded "No" to question 8

BGN	Euro
15	7,7
2,5	1,3
6,5	3,3
4	2,0
35	17,9
152	77,7
250	127,8

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	fee-for-service paid by the NHIF fixed for all out-patient labs	BGN	Euro
Urine culture		6	3,1
Urine microscopy (leuco's etc)		1,98	1,0
Haematology lab		1,98	1,0
Test strip	in case patients answered "Yes" to question 8	0,8	0,4
Echography		13,77	7,0
CT		76,94	39,3

If Q1= Yes, then add (cost in BGN for each procedure – cost for the NHIF in BGN for each procedure) \* number of times  
 If Q1= No, then add the cost in BGN for each procedure \* number of times  
 For the UT scan, the price consider will be the price of CT

- mean cost of a GP (Q9)


		BGN	Euro
-mean cost of a GP visit (Q9)	price per visit paid by non-insured person	20	10,2
-mean cost of a GP visit (Q9)	consumer fee for health insured person per visit	2,9	1,5
-mean cost of a GP visit (Q9)	consumer fee for pensioners per visit	1	0,5

If Q1= Yes, then add 2.9 BGN \* number of times  
 If Q1= No, then add 20 BGN \* number of times

-mean cost of other specialists (Q11, Q12, Q13)

		BGN	Euro
mean cost of any specialist first visit	price per visit for non-insured	30	15,3
mean cost of any specialist second visit	price per visit for uninsured patient	20	10,2
mean cost of any specialist first visit	consumer fee per visit for insured patient	2,9	1,5
mean cost of any specialist first visit	consumer fee per visit for insured pensioner	1	0,5
mean cost of any specialist first visit	fee-for-service paid by the NHIF for every first visit for insured person	19	9,7
mean cost of any specialist second visit	fee-for-service paid by the NHIF for secondary visit for insured person	9,5	4,9

If Q1= Yes, then add 2.9 BGN \* number of times  
 If Q1= No, then add 30 BGN + 20 BGN \* (number of times-1)

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-mean cost for transportation (Q14)

-mean cost par KM (fuel) and cost of a ticket bus (Q14)

fuel

taxi, price per km

bus ticket

2014		2015	
BGN	euro	BGN	Euro
2,11	1,1	2,09	1,1
		0,8	0,4
		1	0,5

If car or motorbike, then add 2.09 BGN \* number of Km \* 2

If bus, train or taxi, then add the fare paid

-mean cost par hour of a the following (Q17):

Home help

GP home visit

Nurse

4 hours

8 hour

12 hours

24 hours

manipulation sterile urine

	BGN	Euro
Home help		
GP home visit	30	15,3
Nurse 4 hours	25	12,8
Nurse 8 hour	35	17,9
Nurse 12 hours	48	24,5
Nurse 24 hours	70	35,8
manipulation sterile urine	15	7,7

If Nurse, and duration is in ]0;4] then add 25

If Nurse, and duration is in ]4;8] then add 35


If Nurse, and duration is in ]8;12] then add 48

If Nurse, and duration is in ]12;24] then add 70.

If Nurse and duration >24 hours, repeat the preceding scheme.

Cost of UTI from standard practice (for each UTI)

	Service/drug	SHI path		Free market
		cost for the patient	cost for the NHIF	cost for the patient
Scenario 1: uncomplicated infections of the lower urinary tract – cystitis and urethritis treated at primary medical care level (by GP)	GP visit	2,9	0	20
	Urine test strip	2	0,8	4
	Urine microscopy (leuco'setc)		1,98	2,5
	Antibiotics	22	0	22
	GP second visit	2,9	0	0
	<b>Total in BGN</b>	<b>29,8</b>	<b>2,78</b>	<b>48,5</b>

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Scenario 2: complicated infections of the lower urinary tract – cystitis and urethritis treated at primary and specialized out-patient medical care level	<b>Total in Euro</b>	<b>15,24</b>	<b>1,42</b>	<b>24,80</b>
	GP visit	2,9	0	0
	Urine microscopy (leuco'setc)	2	1,98	2,5
	Urine culture	2	6	15
	specialist first visit	2,9	19	30
	specialist second visit	2,9	9,5	20
	Haematology lab	2	1,98	6,5
	Echography	2,9	13,77	35
	Antibiotics	22	0	22
	Other drugs (sedatives, antipyretics and immunostimulants)	5,89	0	5,89
	Total in BGN	45,49	52,23	136,89
	<b>Total in Euro</b>	<b>23,26</b>	<b>26,70</b>	<b>69,99</b>
Scenario 3: infections of the upper urinary tract and acute pyelonephritis treated in a hospital	GP visit	2,9	0	0
	Urine microscopy (leuco'setc)	2	1,98	2,5
	Urine culture	2	6	15
	specialist visit	2,9	19	30
	Haematology lab	2	1,98	6,5
	Echography	2,9	13,77	35
	CT	2,9	76,94	250
	hospital stay 5 days	29	420	720
	Total in BGN	46,6	539,67	1059
	<b>Total in Euro</b>	<b>23,83</b>	<b>275,93</b>	<b>541,46</b>



If no complication, specialist visit, or hospitalisation, then cost of the UTI is 29.8 BGN from patient perspective, and 2.78 BGN from NHIF perspective.

If visit to a specialist without hospitalisation, then cost of the UTI is 45.49 BGN from patient perspective, and 52.23 BGN from NHIF perspective.

If hospitalisation then cost of the UTI is 46.6 BGN from patient perspective, and 539.67 BGN from NHIF perspective.

#### 10.1.4 Exploratory criteria

##### Tiselius crystallisation risk index

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$$AP(\text{CaOx}) \text{ index EQ} = 1.9 \times \text{Ca}^{0.84} \times \text{Ox} \times \text{Mg}^{-0.12} \times \text{Cit}^{-0.22} \times \text{V}^{-1.03}$$

Where Ca (calcium), Ox (oxalate), Mg (magnesium), Cit (citrate) are urinary excretions expressed in millimoles excreted during the period, and V represent the urine volume in litres corresponding to the sample considered from 24 hours urine collection.

For parameter expressed in mmol/day, the result will be multiplied by 1 to have the value for 1 day.

For parameter expressed in mg/day, the result will be divided by 192 to obtain the value in mmol/day, and multiplied by 1 to have the value for 1 day.

### 10.1.5 Safety criteria

#### Incomplete start date (for AE):

For incomplete start date, the following rules will be applied:

If year is missing, it will be completed with the year of randomisation.

If month is missing, it will be completed with January. If this completed date is before randomisation, the month will be completed with the month of randomisation.

If day is missing, it will be completed with 01. If this completed date is before randomisation, the day will be completed with the day of randomisation.

#### Incomplete end date (for AE, except for outcome not recovered):

For incomplete end date, the following rules will be applied:

If year is missing, it will be completed with the year of end of study.

If month is missing, it will be completed with December. If this completed date is after end of study visit, the month will be completed with the month of end of study visit.

If day is missing, it will be completed with last day of the month. If this completed date is after end of study visit, the day will be completed with the day of end of study visit.

#### Adverse event emergence:

For the intervention group, an event will be considered as emergent if it began (or when existing symptoms worsened) between the day of first product consumption and the day of last study product consumption +1 day.

For the control group, an event will be considered as emergent if it began (or when existing symptoms worsened) between the date of randomisation and the date of last visit.



### 10.1.6 Tolerance Criteria

BMI at visit X (kg/m<sup>2</sup>)

Weight at visit X (kg) / (Height at baseline (m))<sup>2</sup>

## 11 Changes in the SAP with respect to the protocol

Addition: a subgroup has been defined.


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Modification: the definition of the Global Population has been modified, due to an eligibility criteria (pregnancy test) checked at V2 instead of V1 during the first phase of inclusion.

Modification: a secondary criterion has been modified, due to a translation mistake; delay between UTI events has been replaced by average time between UTI events.

## 12 References

1. "Sample size estimation for trials of recurrent events" KUOLONG HU ET AL, 2008
2. "On reporting results from randomized controlled trials with recurrent events" LISA KURAMOTO, BORIS G SOBOLEV AND MEGHAN G DONALDSON, BMC MEDICAL RESEARCH METHODOLOGY 2008, 8:35 DOI:10.1186/1471-2288-8-35
3. PHARMASUG2011 – PAPER SP07 "Statistical analysis of adverse events in randomized clinical trials using SAS ", DONGSUN CAO, ICON CLINICAL RESEARCH ET AL;
4. SUGI 28 – PAPER 273-28 "Analysis of data from recurrent events", GORDON JOHNSTON AND YING SO, SAS INSTITUTE INC.

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## 13 Mock outputs

### 13.1 Status of subjects, protocol deviations and population definitions

**Table 13.1.1: Disposition of subjects – All /Subgroup**

		Intervention Group	Control group	Total
Included				xx
Eligible at V1	No			xx (xx.x%)
	Yes			xx (xx.x%)
If no, reason for non-eligibility at V1	Inclusion criteria xx :			xx (xx.x%)
	Inclusion criteria xx :			xx (xx.x%)
	Non-inclusion criteria xx :			xx (xx.x%)
Eligible at V2	No			xx (xx.x%)
	Yes			xx (xx.x%)
If no, reason for non-eligibility at V2	Non-eligible at V1			xx (xx.x%)
	Inclusion criteria xx:			xx (xx.x%)
	Inclusion criteria xx:			xx (xx.x%)
Randomised	No			xx (xx.x%)
	Yes	xx	xx	xx (xx.x%)
Completion or reason of withdrawal on randomised subjects	Completed as per study protocol	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
	Adverse event	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
	Screen failure	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
	Protocol deviation	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
	Sponsor Decision	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
	Investigator decision	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
	Withdrawal of consent	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
	Lost to follow up	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
	Other	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)





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**Table 13.1.2: Number and percentage of subjects having at least one protocol deviation - Full Analysis Set – All / Subgroup**

	Intervention Group (N=xx)	Control group (N=xx)	Total (N=xx)
At least one minor deviation	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
At least one major deviation	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
No protocol deviation	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)

**Table 13.1.3: Deviation frequency and number and percentage of subjects concerned by deviation type – Full Analysis Set – All / Subgroup**

	Intervention Group (N=xx)		Control group (N=xx)		Total (N=xx)	
	ND	N (%)	ND	N (%)	ND	N (%)
Protocol Deviation						
All	x	xx (xx.x%)	x	xx (xx.x%)	x	xx (xx.x%)
Minor	x	xx (xx.x%)	x	xx (xx.x%)	x	xx (xx.x%)
Dev n°x :	x	xx (xx.x%)	x	xx (xx.x%)	x	xx (xx.x%)
Dev n°x :	x	xx (xx.x%)	x	xx (xx.x%)	x	xx (xx.x%)
Major	x	xx (xx.x%)	x	xx (xx.x%)	x	xx (xx.x%)
Dev n°x :	x	xx (xx.x%)	x	xx (xx.x%)	x	xx (xx.x%)
ND : Number of deviations						

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**Table 13.1.4: Number and percentage of subjects in each population - All / Subgroup**

	Intervention Group	Control group	Total
Included Subjects			xx
Global Population	xx	xx	xx (xx.x%)
Randomised Subjects	xx	xx	xx (xx.x%)
FAS	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
PP Population	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)
Safety Set	xx (xx.x%)	xx (xx.x%)	xx (xx.x%)

Note: For global population and for randomised subjects, the percentages are calculated on included subjects. For FAS and Safety set, the percentages are calculated on randomised subjects. For PP Population, the percentages are calculated on FAS

**Listing 13.1.1: Subjects included in each analysis set.**



Group	Subgroup	Subject ID	Full Analysis Set	PP Population	Safety Set

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## 13.2 Demography

**Table 13.2.1: Demographic characteristics at baseline - Full Analysis set – All / Subgroup**

	Intervention Group (N=xx)	Control group (N=xx)	Total (N=xx)
n	xx	xx	xx
Mean (SD)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Quantitative parameter	[CIinf- CIsup]	[CIinf- CIsup]	[CIinf- CIsup]
CI 95%	xx.x	xx.x	xx.x
Median	xx.x ; xx.x	xx.x ; xx.x	xx.x ; xx.x
Q1 ; Q3	xx ; xx	xx ; xx	xx ; xx
Min ; Max	xx	xx	xx
Missing	xx	xx	xx
n	xx	xx	xx
Qualitative parameter	xx (xx.x)	xx (xx.x)	xx (xx.x)
Level 1	xx (xx.x)	xx (xx.x)	xx (xx.x)
...	xx (xx.x)	xx (xx.x)	xx (xx.x)
Level n	xx	xx	xx
Missing	xx	xx	xx

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### 13.3 Baseline characteristics

**Table 13.3.1: Baseline characteristics - Characteristic (eg. Questionnaire, smoking habits,...) - Full Analysis set – All / Subgroup**

	Intervention Group (N=xx)	Control group (N=xx)	Total (N=xx)
n	xx	xx	xx
Mean (SD)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
Quantitative parameter	[CIinf- CIsup]	[CIinf- CIsup]	[CIinf- CIsup]
CI 95%			
Median	xx.x	xx.x	xx.x
Q1 ; Q3	xx.x ; xx.x	xx.x ; xx.x	xx.x ; xx.x
Min ; Max	xx ; xx	xx ; xx	xx ; xx
Missing	xx	xx	xx
Qualitative parameter			
n	xx	xx	xx
Level 1	xx (xx.x)	xx (xx.x)	xx (xx.x)
...	xx (xx.x)	xx (xx.x)	xx (xx.x)
Level n	xx (xx.x)	xx (xx.x)	xx (xx.x)
Missing	xx	xx	xx

**Table 13.3.2: Baseline characteristics Medical and surgical characteristics – Description by system organ class and preferred term – Full Analysis set – All / Subgroup**

System organ class / Preferred term	Intervention Group (N=xx)	Control group (N=xx)	Total (N=xx)
All	xx (xx.x)	xx (xx.x)	xx (xx.x)
SOC - A	xx (xx.x)	xx (xx.x)	xx (xx.x)
PT - 1	xx (xx.x)	xx (xx.x)	xx (xx.x)
PT - 2	xx (xx.x)	xx (xx.x)	xx (xx.x)
SOC - B	xx (xx.x)	xx (xx.x)	xx (xx.x)
PT - 1	xx (xx.x)	xx (xx.x)	xx (xx.x)
SOC - ...	xx (xx.x)	xx (xx.x)	xx (xx.x)
PT - ...	xx (xx.x)	xx (xx.x)	xx (xx.x)

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### 13.4 Study conduct parameters

**Table 13.4.1: Study duration – Full Analysis set – All / Subgroup**

	Intervention Group (N=xx)	Control group (N=xx)	Total (N=xx)
n	xx	xx	xx
Mean (SD)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
SEM	xx.x	xx.x	xx.x
Quantitative parameter CI 95%	[CIinf- CIsup]	[CIinf- CIsup]	[CIinf- CIsup]
Median	xx.x	xx.x	xx.x
Q1 ; Q3	xx.x ; xx.x	xx.x ; xx.x	xx.x ; xx.x
Min ; Max	xx ; xx	xx ; xx	xx ; xx
Missing	xx	xx	xx

**Table 13.4.3: Compliance – Full Analysis set – All / Subgroup**



	Intervention Group (N=xx)	Control group (N=xx)	Total (N=xx)
n	xx	xx	xx
Mean (SD)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
SEM	xx.x	xx.x	xx.x
Quantitative parameter CI 95%	[CIinf- CIsup]	[CIinf- CIsup]	[CIinf- CIsup]
Median	xx.x	xx.x	xx.x
Q1 ; Q3	xx.x ; xx.x	xx.x ; xx.x	xx.x ; xx.x
Min ; Max	xx ; xx	xx ; xx	xx ; xx
Missing	xx	xx	xx
Qualitative parameter n	xx	xx	xx
Level 1	xx (xx.x)	xx (xx.x)	xx (xx.x)
...	xx (xx.x)	xx (xx.x)	xx (xx.x)
Level n	xx (xx.x)	xx (xx.x)	xx (xx.x)
Missing	xx	xx	xx

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### 13.5 Concomitant medications

**Table 13.5.1: Concomitant medications - Description by medication class, by preferred term and by relationship to UTI – Safety Set – All treatments/Treatments related to UTI/Treatments non related to UTI – All / Subgroup**


	Intervention Group (N=xx)	Control group (N=xx)	Total (N=xx)
ALL	xx (xx.x)	xx (xx.x)	xx (xx.x)
Medication class - A			
PT - A	xx (xx.x)	xx (xx.x)	xx (xx.x)
PT - B	xx (xx.x)	xx (xx.x)	xx (xx.x)
Medication class - B			
PT - A	xx (xx.x)	xx (xx.x)	xx (xx.x)
PT - B	xx (xx.x)	xx (xx.x)	xx (xx.x)

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## 13.6 Primary evaluation criteria

**Table 13.6.1: Primary efficacy criteria: Number of confirmed UTI event - Analysis Set – All/Subgroup**

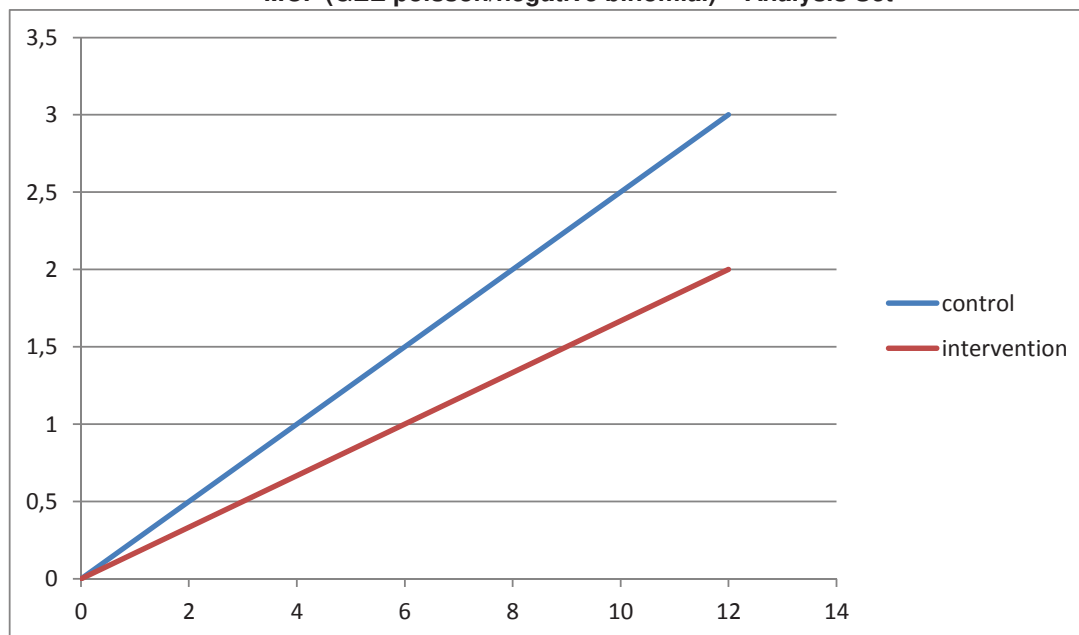
	Intervention Group (N=xx)	Control group (N=xx)	Total (N=xx)	
n	xx	xx	xx	
Mean (SD)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)	
CI 95%	[CIinf- CI sup]	[CIinf- CI sup]	[CIinf- CI sup]	
Quantitative parameter	Median	xx.x	xx.x	
	Q1 ; Q3	xx.x ; xx.x	xx.x ; xx.x	
	Min ; Max	xx ; xx	xx ; xx	
	Missing	xx	xx	xx
Qualitative parameter	n	xx	xx	
	Level 1	xx (xx.x)	xx (xx.x)	xx (xx.x)
	...	xx (xx.x)	xx (xx.x)	xx (xx.x)
	Level n	xx (xx.x)	xx (xx.x)	xx (xx.x)
	Missing	xx	xx	xx

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**Table 13.6.2: Primary efficacy criteria: Confirmed UTI events  
MCF (GEE poisson/negative binomial) – Analysis Set**

	Intervention Group (N=xx)	Control Group (N=xx)
<b>Number of subjects</b>	xx	xx
<b>Estimate</b>	xx.xx (xx.xx)	xx.xx (xx.xx)
<b>95%CI</b>	[xx.xx;xx.xx]	[xx.xx;xx.xx]
<b>Product effect ratio</b>		
<b>Estimate</b>	xx.xx (xx.xx)	
<b>95%CI</b>	[xx.xx;xx.xx]	
<b>p-value</b>	0.xxx	
<b>AIC</b>	xx.xx	
<b>Sensitivity: subgroup effect</b>		
<b>product effect: p-value</b>	0.xxx	
<b>Inclusion period: p-value</b>	0.xxx	
<b>interaction: p-value</b>	0.xxx	

**Graph 13.6.1: Primary efficacy criteria: Confirmed UTI events  
MCF (GEE poisson/negative binomial) – Analysis Set**







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### 13.7 Secondary evaluation criteria

**Table 13.7.1: Secondary efficacy criteria: xxxxx**  
**Descriptive statistics & change from baseline – Analysis Set– All / Subgroup (N=xxx)**


		Intervention Group (N=xx)	Control group (N=xx)	Total (N=xx)
	n	xx	xx	xx
	Missing	xx	xx	xx
	Mean (SD)	xx (xx.x)	xx (xx.x)	xx (xx.x)
<b>Vx</b>	CI 95%	[CIinf- CIsup]	[CIinf- CIsup]	[CIinf- CIsup]
	Median	xx.x	xx.x	xx.x
	Q1 ; Q3	xx.x ; xx.x	xx.x ; xx.x	xx.x ; xx.x
	Min ; Max	xx ; xx	xx ; xx	xx ; xx
	n	xx	xx	xx
	Missing	xx	xx	xx
	Mean (SD)	xx (xx.x)	xx (xx.x)	xx (xx.x)
<b>Quantitative parameter Vy</b>	CI 95%	[CIinf- CIsup]	[CIinf- CIsup]	[CIinf- CIsup]
	Median	xx.x	xx.x	xx.x
	Q1 ; Q3	xx.x ; xx.x	xx.x ; xx.x	xx.x ; xx.x
	Min ; Max	xx ; xx	xx ; xx	xx ; xx
	n	xx	xx	xx
	Missing	xx	xx	xx
	Mean (SD)	xx (xx.x)	xx (xx.x)	xx (xx.x)
<b>Change Vy-Vx</b>	CI 95%	[CIinf- CIsup]	[CIinf- CIsup]	[CIinf- CIsup]
	Median	xx.x	xx.x	xx.x
	Q1 ; Q3	xx.x ; xx.x	xx.x ; xx.x	xx.x ; xx.x
	Min ; Max	xx ; xx	xx ; xx	xx ; xx
	n	xx	xx	xx

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**Table 13.7.2: Secondary efficacy criteria: xxxxx**


Mixed ANCOVA linear model with ... as covariate & Non-parametric Analysis

	<b>Intervention Group (N=xx)</b>	<b>Control Group (N=xx)</b>
<b>Number of subjects</b>	xx	xx
<b>Adjusted mean (SE)</b>	xx.xx (xx.xx)	xx.xx (xx.xx)
<b>Adjusted Mean difference I- C (SE)</b>	xx.xx (xx.xx)	
<b>95%CI</b>	[xx.xx;xx.xx]	
<b>Pvalue</b>	0.xxx	
<b>Pvalue (Wilcoxon Rank-Sum test)</b>	0.xxx	
<b>Covariate analysis</b>		
<b>Covariable Effect</b>	0.xxx	
<b>Skewness</b>	x.xxx	
<b>Kurtosis</b>	x.xxx	
<b>Sensitivity: subgroup effect</b>		
<b>Group: p-value</b>	0.xxx	
<b>Inclusion period: p-value</b>	0.xxx	
<b>interaction: p-value</b>	0.xxx	

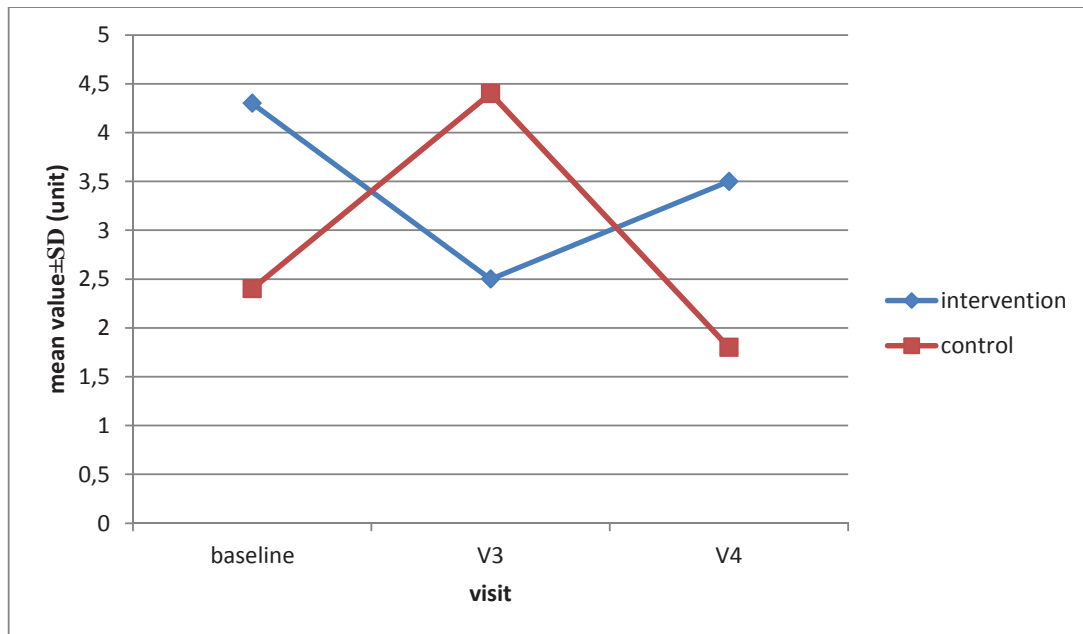
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Mixed ANCOVA linear model for repeated measures ( XX Matrix) & Non-parametric Analysis

	Intervention Group (N=xx)	Control Group (N=xx)
<b>Number of subjects</b>	xx	xx
<b>Product Effect at Visit 3</b>		
Adjusted mean (SE)	xx.xx (xx.xx)	xx.xx (xx.xx)
Adjusted Mean difference I- C (SE)	xx.xx (xx.xx)	
95%CI	[xx.xx;xx.xx]	
Pvalue	0.xxx	
Pvalue (Wilcoxon Rank-Sum test)	0.xxx	
<b>Product Effect at Visit 4</b>		
Adjusted mean (SE)	xx.xx (xx.xx)	xx.xx (xx.xx)
Adjusted Mean difference I- C (SE)	xx.xx (xx.xx)	
95%CI	[xx.xx;xx.xx]	
Pvalue	0.xxx	
Pvalue (Wilcoxon Rank-Sum test)	0.xxx	
<b>Covariate analysis</b>		
Covariable Effect	0.xxx	
Visit Effect	0.xxx	
Product*Visit Effect	0.xxx	
<b>Skewness</b>	x.xxx	
<b>Kurtosis</b>	x.xxx	
<b>Sensitivity: subgroup effect</b>		
Product Effect at Visit 3: p-value	0.xxx	
Product Effect at Visit 4: p-value	0.xxx	
Inclusion period: p-value	0.xxx	
interaction: p-value	0.xxx	

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**Graph 13.7.1: Parameter X: raw mean value and standard deviation over time – Analysis Set – All**



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Generalised linear model (gamma)



	Intervention Group (N=xx)	Control Group (N=xx)
<b>Number of subjects</b>	xx	xx
<b>Estimate</b>	xx.xx (xx.xx)	xx.xx (xx.xx)
<b>95%CI</b>	[xx.xx;xx.xx]	[xx.xx;xx.xx]
<b>Product effect ratio</b>		
<b>Estimate</b>	xx.xx (xx.xx)	
<b>95%CI</b>	[xx.xx;xx.xx]	
<b>p-value</b>	0.xxx	
<b>Covariate analysis</b>		
<b>Covariable Effect</b>	0.xxx	
<b>AIC</b>	xx.xx	
<b>Sensitivity: subgroup effect</b>		
<b>Group: p-value</b>	0.xxx	
<b>Inclusion period: p-value</b>	0.xxx	
<b>interaction: p-value</b>	0.xxx	

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## 13.8 Exploratory evaluation criteria

**Table 13.7.1: Exploratory efficacy criteria: xxxxx  
– Analysis Set – All / Subgroup**


	Intervention Group (N=xx)	Control group (N=xx)	Total (N=xx)	
Quantitative parameter	n	xx	xx	
	Mean (SD)	xx.x (xx.x)	xx.x (xx.x)	xx.x (xx.x)
	CI 95%	[CIinf- CIsup]	[CIinf- CIsup]	[CIinf- CIsup]
	Median	xx.x	xx.x	xx.x
	Q1 ; Q3	xx.x ; xx.x	xx.x ; xx.x	xx.x ; xx.x
	Min ; Max	xx ; xx	xx ; xx	xx ; xx
	Missing	xx	xx	xx
Qualitative parameter	n	xx	xx	
	Level 1	xx (xx.x)	xx (xx.x)	xx (xx.x)
	...	xx (xx.x)	xx (xx.x)	xx (xx.x)
	Level n	xx (xx.x)	xx (xx.x)	xx (xx.x)
	Missing	xx	xx	xx

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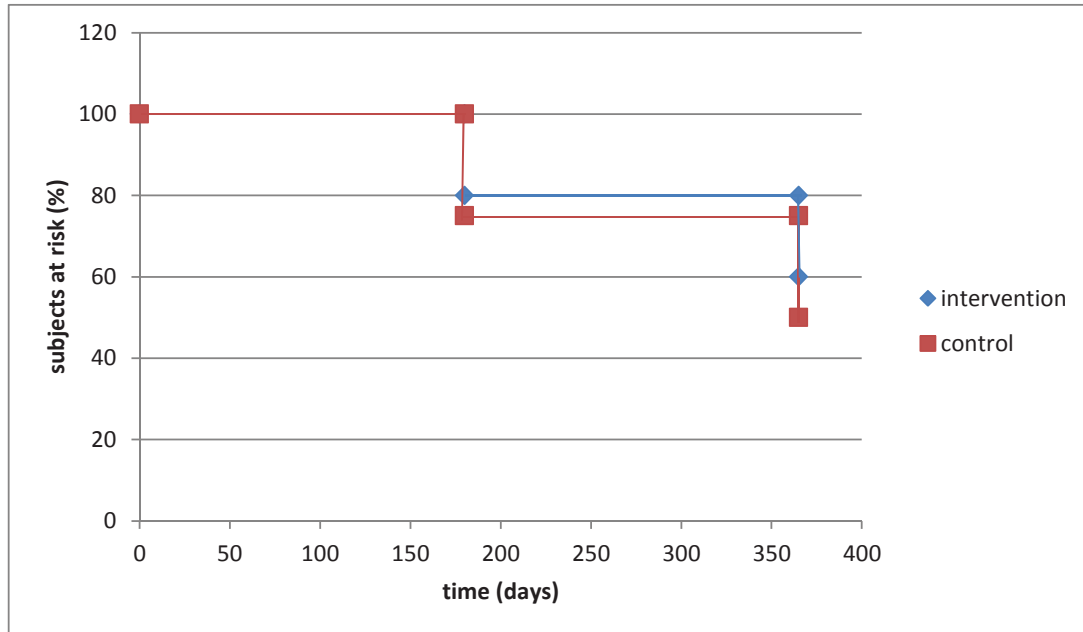
*For MCF (table only, no graph) and ANCOVA, refer to the preceding sections*

**Table 13.8.2: Exploratory criteria: xxxxx  
Cox model – Analysis Set – All / Subgroup**



	Intervention Group (N=xx)	Control Group (N=xx)
<b>Number of subjects</b>	xx	xx
<b>Number of events</b>	xx.xx (xx.xx)	xx.xx (xx.xx)
<b>Hazard ratio : group</b>		
<b>Estimate</b>	xx.xx (xx.xx)	
<b>95%CI</b>	[xx.xx;xx.xx]	
<b>p-value</b>	0.xxx	
<b>Hazard ratio : urinary marker</b>		
<b>Estimate</b>	xx.xx (xx.xx)	
<b>95%CI</b>	[xx.xx;xx.xx]	
<b>p-value</b>	0.xxx	
<b>Covariate analysis</b>		
<b>Covariable Effect</b>	0.xxx	

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**Graph 13.8.1: Exploratory criteria: xxxxx  
Kaplan-Meier curves – Analysis Set – All**






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## 13.9 Safety

**Table 13.9.1: Adverse events - Descriptive statistics - Subjects having at least one adverse event by Primary SOC and preferred term - Safety Set- All / Subgroup**

SOC / Preferred term	Intervention Group (N=xx)		Control group (N=xx)		Total (N=xx)	
	Event (1)	N (%) (2)	Event (1)	N (%) (2)	Event (1)	N (%) (2)
All	xxx	xx (xx.x)	xxx	xx (xx.x)	xxx	xx (xx.x)
SOC - A	xxx	xx (xx.x)	xxx	xx (xx.x)	xxx	xx (xx.x)
PT - 1	xxx	xx (xx.x)	xxx	xx (xx.x)	xxx	xx (xx.x)
PT - 2	xxx	xx (xx.x)	xxx	xx (xx.x)	xxx	xx (xx.x)
SOC - B	xxx	xx (xx.x)	xxx	xx (xx.x)	xxx	xx (xx.x)
PT - 1	xxx	xx (xx.x)	xxx	xx (xx.x)	xxx	xx (xx.x)
SOC - ...	xxx	xx (xx.x)	xxx	xx (xx.x)	xxx	xx (xx.x)
PT - ...	xxx	xx (xx.x)	xxx	xx (xx.x)	xxx	xx (xx.x)



(1) Number of events  
(2) Number and percentage of subjects

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### 13.10 Product tolerance



**Table 13.10.1: Vital signs at Vx – Safety Set – All / Subgroup**

		Intervention Group (N=xx)	Control group (N=xx)	Total (N=xx)
	n	xx	xx	xx
	Missing	xx	xx	xx
	Mean (SD)	xx (xx.x)	xx (xx.x)	xx (xx.x)
	<b>Vx</b>			
	CI 95%	[CIinf- CIsup]	[CIinf- CIsup]	[CIinf- CIsup]
	Median	xx.x	xx.x	xx.x
	Q1 ; Q3	xx.x ; xx.x	xx.x ; xx.x	xx.x ; xx.x
	Min ; Max	xx ; xx	xx ; xx	xx ; xx
	n	xx	xx	xx
	Missing	xx	xx	xx
	Mean (SD)	xx (xx.x)	xx (xx.x)	xx (xx.x)
<b>Quantitative parameter</b>	<b>Vy</b>			
	CI 95%	[CIinf- CIsup]	[CIinf- CIsup]	[CIinf- CIsup]
	Median	xx.x	xx.x	xx.x
	Q1 ; Q3	xx.x ; xx.x	xx.x ; xx.x	xx.x ; xx.x
	Min ; Max	xx ; xx	xx ; xx	xx ; xx
	n	xx	xx	xx
	Missing	xx	xx	xx
	Mean (SD)	xx (xx.x)	xx (xx.x)	xx (xx.x)
	<b>Change Vy-Vx</b>			
	CI 95%	[CIinf- CIsup]	[CIinf- CIsup]	[CIinf- CIsup]
	Median	xx.x	xx.x	xx.x
	Q1 ; Q3	xx.x ; xx.x	xx.x ; xx.x	xx.x ; xx.x
	Min ; Max	xx ; xx	xx ; xx	xx ; xx

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**Table 13.10.2: Descriptive statistics of <laboratory parameter> – Safety Set – All / Subgroup**

		Intervention Group (N=xx)	Control group (N=xx)	Total (N=xx)
	n	xx	xx	xx
	Missing	xx	xx	xx
	Mean (SD)	xx (xx.x)	xx (xx.x)	xx (xx.x)
	<b>Vx</b>			
	CI 95%	[CIinf- CIsup]	[CIinf- CIsup]	[CIinf- CIsup]
	Median	xx.x	xx.x	xx.x
	Q1 ; Q3	xx.x ; xx.x	xx.x ; xx.x	xx.x ; xx.x
	Min ; Max	xx ; xx	xx ; xx	xx ; xx
	n	xx	xx	xx
	Missing	xx	xx	xx
	Mean (SD)	xx (xx.x)	xx (xx.x)	xx (xx.x)
<b>Quantitative parameter</b>	<b>Vy</b>			
	CI 95%	[CIinf- CIsup]	[CIinf- CIsup]	[CIinf- CIsup]
	Median	xx.x	xx.x	xx.x
	Q1 ; Q3	xx.x ; xx.x	xx.x ; xx.x	xx.x ; xx.x
	Min ; Max	xx ; xx	xx ; xx	xx ; xx
	n	xx	xx	xx
	Missing	xx	xx	xx
	Mean (SD)	xx (xx.x)	xx (xx.x)	xx (xx.x)
	<b>Change Vy-Vx</b>			
	CI 95%	[CIinf- CIsup]	[CIinf- CIsup]	[CIinf- CIsup]
	Median	xx.x	xx.x	xx.x
	Q1 ; Q3	xx.x ; xx.x	xx.x ; xx.x	xx.x ; xx.x
	Min ; Max	xx ; xx	xx ; xx	xx ; xx

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## 14 SAS Syntax

MCF analysis:

```
proc genmod data =x ;
class group;
model var = group / dist = distance link=log scale=deviance type3 offset=duration;
estimate 'control' Intercept 1 group 0 1 / EXP;
estimate 'Intervention' Intercept 1 group 1 0 / EXP;
lsmeans group / DIFF CL;
estimate 'Product effect ratio' group 1 -1 / EXP;
run;
```

where


*Group*= group of the subject (intervention or control)  
*distance*= poison for the primary analysis and negln for the sensitivity analysis  
*duration*= log(intervention duration)

gamma model:

```
proc genmod data =x ;
class group;
model var = group / dist = gamma link=log scale=deviance type3 offset=duration;
estimate 'control' Intercept 1 group 0 1 / EXP;
estimate 'Intervention' Intercept 1 group 1 0 / EXP;
lsmeans group / DIFF CL;
estimate 'Product effect ratio' group 1 -1 / EXP;
run;
```

ANCOVA:

```
proc mixed data=x;
class group;
model var= baseline age group;
lsmeans group;
estimate "group Intervention vs Control" group -1 1;
run;
```

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repeated measures ANCOVA

```
proc mixed data=x;
class group visitnum;
model var= baseline age group visitnum group*visitnum;
lsmeans group group*visitnum;
estimates "group at visit3 group 1 -1 visitnum 0 0 1 0 group*visitnum 0 0 1 0 0 0 -1 0;
estimates "group at visit4 group 1 -1 visitnum 0 0 0 1 group*visitnum 0 0 0 1 0 0 0 -1;
repeated visitnum/subject=usubjid type=cov;
run;
```

where cov= UN, AR(1) or CS; The 3 method will be tested, and the one with the smallest AIC will be presented.

Cox survival analysis

```
proc phreg data=X plot=survival;
model time*censor(0)= age Umarker group/RL;
run;
```