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Randomised multi-centre effectiveness trial of rapid syndromic testing by panel assay in children presenting to European emergency departments with acute respiratory infections – trial protocol for the ADEQUATE Paediatric trial

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1 **ABSTRACT**

2 **Introduction:** Syndromic panel assays, i.e. using one test to simultaneously target multiple pathogens with

3 overlapping signs and symptoms., have been integrated into routine paediatric care over the past decade,

4 mainly for more severely ill and hospitalised patients. Their wider availability and short turnaround times

5 open the possibility to apply them to non-hospitalised patients as well. In this context, it is important to

6 trial how clinicians make use of pathogen detection data and if their early availability influences

7 management decisions, particularly antibiotic use and hospitalisation.

8 **Methods and analysis:** ADEQUATE is an individually-randomised, controlled, open-label effectiveness trial

9 comparing the impact of a respiratory pathogen panel assay used as a rapid syndromic test in addition to

10 the standard of care versus standard of care alone. The trial will 1:1 randomise 520 participants under the

11 age of 18 at 9 paediatric emergency departments in 6 European countries. Inclusion criteria for the trial

12 consist of two sets, with the first describing respiratory tract infections in paediatric patients and the

13 second describing the situation of potential management uncertainty in which test results may immediately

14 affect management decisions. Enrolment started in July 2021 and is expected to be complete in early 2024.

15 To investigate differences between the two arms for each endpoint separately, a two-sample t-test of the

16 log transformed mean time (in hours) on antibiotic treatment or alive out of hospital comparing those on

17 the standard of care arm (control) and the intervention arm will be performed, assuming a pooled variance

18 estimate.

19 **Ethics and dissemination:** The trial protocol and materials were approved by research ethics committees in

20 all participating countries. The respiratory pathogen panel assay is CE marked and FDA cleared for

21 diagnostic use. Participants and caregivers provide informed consent prior to study procedures

22 commencing. The trial results will be published in peer-reviewed journals and at national and international

23 conferences. Key messages will also be disseminated via press and social media where appropriate.

24 **Trial registration number:** NCT04781530

25

26

Strengths and limitations of this study (max 5 bullet points):

-   By design of the eligibility criteria, in this trial application of the test is targeted to a patient population where decisions are pending and test results may impact initial management decisions.

-   The trial's setting spans European countries with some difference in available resources and the results will therefore likely be generalisable to other high-income country settings.

-   Employing a pragmatic design, the protocol does not provide guidance on interpretation of test results and the results may therefore be sensitive to changing perceptions about current incidence of pathogens.

35 **INTRODUCTION**

36 Community-acquired acute respiratory infections (ARI) are the most frequent reason for unscheduled

37 healthcare visits and at the same time, the most frequent cause of inappropriate antibiotic use.[1, 2] While

38 most ARI cause mild symptoms and are self-limiting, lower respiratory tract infections, including

39 pneumonia, globally cause more than half a million deaths in <5 year old children per year.[3] Especially

40 since the wide roll-out of conjugate vaccines, most of these infections in children do not require treatment

41 with antibiotics. Antibiotic consumption is a driver of development of antimicrobial resistance (AMR) and

42 where use of antibiotics in the individual is not warranted, the ecological and economic cost of

43 antimicrobial resistance per antibiotic consumed is considerable.[4-6]

44 Determining which pathogen is the likely cause of an infectious episode is one common approach for

45 clinicians to decide on the probability of antibiotic treatment being beneficial in a patient. In paediatric

46 routine care, pathogen testing is usually limited to upper respiratory tract (URT) samples.[7] A wide range

47 of common respiratory pathogens that may cause more severe disease are frequently present in the URT of

48 asymptomatic children as well, thereby making it more difficult to determine the causative pathogen of an

49 episode. While for some viral pathogens, especially RSV, influenza virus, parainfluenza virus and human

50 metapneumovirus, there is a high probability that their detection explains the cause of an episode of

51 severe, for others, including *Streptococcus pneumoniae* and human rhinovirus, the association is much

52 weaker.[8] Uncertainty of aetiology may increase the probability of antibiotic prescriptions.[9]

53 Children hospitalised for ARI stay in hospital for a median of 2 to 3 days and resolution of symptoms takes

54 much longer.[3, 10] Interventions reducing hospital stays have a high potential to reduce psycho-social

55 costs for families and economic costs for the health system.

56 Syndromic panel assays, i.e. using one test to simultaneously target multiple pathogens with overlapping

57 signs and symptoms , have been integrated into routine paediatric care over the past decade, mainly for

58 more severely ill and hospitalised patients. Their wider availability and short turnaround times open the

59 possibility to apply them to non-hospitalised patients as well. In this context, it is important to trial how

60 clinicians make use of pathogen detection data and if their early availability influences management

61 decisions.

62 VALUE-Dx is the first Innovative Medicines Initiative project initiated by six in vitro diagnostic companies

63 who joined forces with 20 non-industry partners to combat AMR and improve patient outcomes. The

64 multidisciplinary consortium involves clinicians, microbiologists, health economists, social scientists, and

65 industry. The trial described here is a part of this VALUE-Dx project. It aims to determine if the integration

66 of a rapid syndromic test at an early point in time in the management workflow in paediatric emergency

67 departments can influence the decisions to treat a patient with antibiotics or to hospitalise them.

68 **METHODS AND ANALYSIS**

69 ADEQUATE is an individually randomised, controlled, open-label effectiveness trial comparing the impact of

70 a respiratory pathogen panel assay used as a rapid syndromic test in addition to the standard of care versus

71 standard of care alone on antibiotic use and hospitalisations in paediatric patients with ARI presenting to

72 EDs. The trial is part of workpackage 4 of the VALUE-Dx consortium.

Trial setting

74 The trial enrols participants at 9 paediatric EDs in 6 European countries (Germany, Greece, Italy, Spain,

75 Switzerland and the United Kingdom). Enrolment started in July 2021 and is expected to be complete in

76 early 2024.

Trial population

78 Inclusion criteria for the trial consist of two sets, with the first describing respiratory tract infections in

79 paediatric patients and the second describing the situation of potential management uncertainty in which

80 test results may immediately affect management decisions. Few exclusion criteria were introduced to

81 increase generalisability of the trial results. The full eligibility criteria are listed in table 1.

Inclusion criteria (all must be fulfilled)
1. ARI presentation Children of any age presenting to the ED with an acute illness (present for 14 days or less) with temperature ≥38.0°C measured at presentation or parental report of fever within the previous 72 hours AND at least two of the below: • Cough • Abnormal sounds on chest auscultation (crackles, reduced breath sounds, bronchial breathing, wheezing) • Clinical signs of dyspnea (chest indrawing, nasal flaring, grunting) • Signs of respiratory dysfunction: tachypnoea for age or decreased oxygen saturation (<92% in room air) • Signs of reduced general state: poor feeding, vomiting or lethargy/drowsiness
2. Management uncertainty At time of screening • Patient has undergone first assessment by managing clinical team (doctor or nurse, incl. triage) • Hospitalisation is not yet determined, i.e., neither by clinical presentation definitely requiring hospitalisation (e.g., per local guideline) nor by fixed decision of managing clinical team; admission to a short-stay unit or surveillance unit is not considered a hospitalisation for this trial • Antibiotic treatment or hospitalisation is being considered • The rapid syndromic diagnostic test result can be awaited up to 4 hours before the decision to discharge the patient or to initiate antibiotic treatment is made
Exclusion criteria (none may be fulfilled)
1. Development of ARTI more than 48 hours after hospital admission (hospital acquired);

2. Patients with a severe underlying medical condition dictating management decisions including hospitalisation and/or antibiotic treatment (e.g., cystic fibrosis, immunosuppression);

3. Hospitalisation for at least 24 hours within the last 14 days (healthcare-associated);

4. Confirmed pregnancy or breastfeeding;

5. Any clinically significant abnormality identified at the time of screening that in the judgment of the Investigator would preclude safe completion of the study or constrain endpoints assessment such as major systemic diseases or patients with short life expectancy;

6. Inability to obtain informed consent;

7. Alternative noninfectious diagnosis that explains clinical symptoms.

82   Table 1: Eligibility criteria

83   **Screening, recruitment and consent**

84   During working hours of study staff, patients in the emergency department or short-stay unit are screened

85   for eligibility by study staff. In most instances, screening takes place as soon as possible after initial triage.

86   Informed consent is sought from all patients meeting the eligibility criteria at the time of screening. The

87   health status of patients might rapidly deteriorate between screening and randomisation. Therefore, all

88   eligibility criteria are be re-evaluated and confirmed prior to the decision to randomise the patient.

89   Screening failures are defined as patients who were found eligible per screening but have either not given

90   informed consent, or have deteriorated between screening and randomisation, and therefore no longer

91   fulfil eligibility criteria. Screening failures are recorded anonymously on a screening log detailing the reason

92   for screening failure and are not randomised. No diagnostic procedures are performed for the purpose of

93   checking eligibility criteria specifically, i.e., any procedures indicated for the standard of care patient

94   management will be performed but none will be added to check eligibility criteria.

95   **Randomisation and blinding**

96   Participants are randomised with equal probability into two allocation groups: (a) the control group,

97   receiving the current standard of care at the respective trial site, which may include rapid diagnostic testing

98   for specific pathogens or syndromic testing with results reported after a longer time than four hours, or (b)

99   the intervention group, receiving the standard of care plus immediately a nasopharyngeal swab tested with

100 the BIOFIRE® Respiratory Panel 2.1*plus* (RP2.1*plus*). The intervention is a multiplexed nucleic acid test for

101 the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic

102 acids in nasopharyngeal swabs obtained from patients suspected of respiratory tract infections. The assay is

103 licensed in CE marked and FDA cleared, for the use intended in this trial. The pathogens included in the

104 assay are adenovirus, coronaviruses (229 E, HKU1, NL63, OC43, SARS-CoV-2), human metapneumovirus,

105 human rhinovirus/enterovirus, influenza A, including subtypes H1, H1-2009, and H3, influenza B, middle

106 east respiratory syndrome coronavirus (MERS-CoV), parainfluenza virus (1, 2, 3, 4), respiratory syncytial

107 virus, *Bordetella parapertussis*, *Bordetella pertussis*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*.

108 After all eligibility criteria have been verified and informed consent has been obtained, randomisation is

109 performed using the built-in randomisation module of the eCRF system. Allocation is concealed until the

110 moment of randomisation. To this end, block randomisation is used with variable blocks of size 2, 4 and 6.

111 Randomisation is stratified by centre. In the intervention group, a URT swab is obtained by trained trial or

112 clinical staff and submitted to the panel assay test with as little delay as possible. After the decision to

113 randomise the subject is made, subjects will not be excluded from the trial. Due to the nature of the

114 intervention, blinding is not possible. If the allocated intervention is not applied for any reason, this will be

115 recorded and follow-up for the participant will be completed.

116 **Outcome measures and assessments**

117 The co-primary study endpoints are:

118 1. Days alive out of hospital within 14 days after study enrolment

119 2. Days on Therapy (DOT) with antibiotics within 14 days after study enrolment

120 The secondary endpoints are listed in table 2.

Non-inferiority safety endpoint:

- For initially hospitalised patients: i) any readmission, ii) ICU admission => 24 hours after

  hospitalisation, or iii) death, within 30 days after study enrolment

- For initially non-admitted patients: any admission or death within 30 days after study enrolment.

Direct costs and indirect costs within 30 days after enrolment.

Change in quality of life as determined by EQ-5D-5L (or suitable alternative for age), days away from

usual childcare routine or school and healthcare utilisation on day 1, 14, and 30 after enrolment.

Proportion of participants with an identified respiratory pathogen in both study groups on randomisation day samples.

Proportion of participants on non-first-line anti-infective regimens (as defined by local guidelines)

Time to de-escalation and time to stop of anti-infective therapy

Proportion of hospitalised participants with detection of cephalosporin-, carbapenem- or chinolone-resistant Enterobacteriaceae on any standard of care samples >7 days after randomisation

Hours in individual or cohort isolation in hospitalised participants

121   Table 2: Secondary endpoints

122   Primary endpoints were adapted after a decision to terminate the recruitment of adult patients on a

123   partner protocol on 3rd May 2022. Prior to this adaptation, the non-inferiority safety endpoint was

124   considered a third co-primary endpoint. Because mortality in the study population in high-resource settings

125   is extremely low, and secondary admission rates among children initially managed in the community as well

126   as re-admission and secondary ICU admission rates among primarily admitted children are likely to be in

127   the range of below 5%, this endpoint was judged to unlikely be relevant or appropriate for the paediatric

128   population. Additionally, secondary admissions will still provide safety information on the first co-primary

129   endpoint. Based on this, the safety endpoint is considered a key secondary endpoint.

130   Participants are followed up until 30 days after randomisation. Standard of care clinical and microbiological

131   data are collected. The participant dataset summarises the illness episode and outcome, microbiological

132   testing, antimicrobial use, use of healthcare facilities including hospitalisations and return to normal

133   activity, childcare arrangements and quality of life. Data is entered into case report forms in a GCP-

134   compliant database held at the Julius Center, UMC Utrecht. Follow-up information including data for health

135   economic analysis is collected on day 14 (visit window: day 12 – 16) and on day 30 (visit window: day 28 –

136   32) after randomisation. Parents or participants themselves (where age-appropriate) are contacted by

137   study staff for the follow-up visits, usually via telephone but in case of hospitalisation or hospital

138   attendance during the visit window face-to-face visits are acceptable. Quality of life is measured by EQ-5D,

139   using age-appropriate versions including proxy versions that are emailed to families. In case of failure to

140   successfully contact families at the end of trial participation, the participant's general practitioner is

141   contacted to complete information on the primary endpoints.

Sample size and power

In particular, a reduction of one day in antibiotic treatment or increase of one day in days alive out of

hospital appear to be relevant for a clinically relevant reduction in antibiotic prescribing and a reduction in

hospital costs, respectively. In children, the co-primary superiority endpoints are likely to be dominated by

the DOT with antibiotics, as ambulatory exposure to antibiotics is likely to be common in the absence of

hospital admission, whereas many admitted children would be expected to be treated with antibiotics as

well.

The sample size estimation was performed for this endpoint. From a recent publication on variations in

antibiotic prescribing in febrile children presenting to European EDs, the standard deviation for days on

antibiotic treatment was estimated as 3.7 days. Based on this, recruitment of 170 children per arm (total of

340 children) will be sufficient to detect a difference of one day in this endpoint (power 80%, alpha 0.05).

To account for uncertainty about the variability in both co-primary endpoints in the paediatric study

population, we adopt a highly conservative approach aiming to recruit 252 evaluable children per arm

(total of 504 children), resulting in adequate power to detect a difference in one day in both endpoints

(table 3).

SD	Delta	Alpha	Beta	Correction	Sample size per arm
2.5	1	0.025	0.2	1	99
3.0	1	0.025	0.2	1	142
3.5	1	0.025	0.2	1	193
3.7	1	0.025	0.2	1	215
4.0	1	0.025	0.2	1	252
4.5	1	0.025	0.2	1	318
5.0	1	0.025	0.2	1	393

Table 3: Sample sizes for Days on antibiotic treatment (paediatric) using different assumptions

Analysis plan

The analysis will be performed by the trial statistician using the R language and environment for statistical

computing (version 3.6 or higher). Reporting will follow the CONSORT guidelines.

161 Both co-primary endpoints will be tested separately, and superiority is confirmed if either one or both are

162 superior in terms of the primary analysis.

163 To investigate differences between the two arms for each endpoint separately, a two-sample t-test of the

164 log transformed mean time (in hours) on antibiotic treatment or alive out of hospital comparing those on

165 the standard of care arm (control) and the intervention arm will be performed, assuming a pooled variance

166 estimate.

167 An adjusted linear mixed effects model will be fitted with log transformed days on antibiotic treatment or

168 days alive out of hospital as dependent variable, and an indicator variable for the randomised arm, age

169 groups (<5y, 5-17y) and comorbidities (stratified according to modified Charlson comorbidity index: 0, 1,

170 +1) as independent variables. Further independent variables will be considered in post hoc analyses. The

171 model will include a random intercept for each country (and potentially, emergency department in country

172 if cluster sizes allow). Zero-inflated or similar models will be considered if data are heavily skewed.

173 We especially anticipate days alive out of hospital data to be heavily right skewed in the full analysis set,

174 and therefore suitable transformations or modelling approaches will be considered as appropriate.

175 Subgroup analyses of the primary endpoints will include

- 176 • by age groups (<5, >5)

- 177 • by admission at baseline (yes/no)

- 178 • by receipt of antibiotics at baseline (yes/no)

- 179 • for those on antibiotic therapy at baseline, we will dichotomise days on treatment into two groups

180 (0="1-5 days", 1=">5 days"), and fit a (mixed effects) logistic model with this grouping as

181 dependent variable, adjusting as above.

- 182 • by country

- 183 • by emergency department (if the number of patients allows).

184 A detailed analysis plan for all secondary objectives will be finalised before the trial's data base closure and

185 will be under version control at the Paediatric Research Centre, University of Basel Children's Hospital.

186 **Sub-study and biobanking**

187 The sub-study will have its own analysis plan which will be finalised before the respective database is

188 locked.

189 The aim of the microbiology study, located at the University of Antwerp, is to use suitable methods,

190 including metagenomic sequencing, to characterise changes in microbiological colonisation and

191 antimicrobial resistance patterns dependent on treatment with antibiotics. In a subset of study sites and

192 participants (up to 150 participants), additional oropharyngeal samples are obtained from participants. One

193 sample is obtained on the day of randomisation and one sample on day 30 (visit window: day 28 – 32) after

194 randomisation. Specific procedures for collection and processing are provided to sites. After receiving

195 specific instructions, the day 30 swab can be obtained at home and sent to the local study site via mail.

196 Inclusion in the microbiology study will require separate informed consent.

197 Biological samples obtained for the study (including leftovers from the specimens obtained for the

198 intervention and for the microbiology study) are be stored at all sites and shipped to the University of

199 Antwerp for inclusion in a biobank, subject to the condition that separate informed consent for biobanking

200 is given.

201 Participation in the main study does not depend on consent for the microbiology study or for biobanking.

202 **Monitoring**

203 Representatives of the trial management team and a designated study monitor conducted a remote site

204 initiation visit at each study site to verify qualifications of the local investigators and inform the local teams

205 of responsibilities and the procedures for ensuring adequate and correct documentation and use of the

206 electronic data capture system as well as providing training on implementing all trial activities.

207 Sites are requested to enter data in the eCRF within 5 working days following each subject's visit. The

208 monitor ensures that data is entered in a timely manner. When queries regarding the data entered in the

209 eCRF are raised, the site is expected to resolve them within 10 working days.

210 The monitor visits a site at least once during the course of the study, when at least 3 subjects are

211 randomised and completed data collection in the eCRF up to at least Day 30. Depending on the subject

212 enrollment rate and any site-specific issues, the total number of on-site monitoring visits may be increased.

213   The visits include Source Data Verification (SDV) for selected variables: 100% SDV is performed on all

214   Informed Consent Form (ICF) versions and consent process in the source; a total of 10% of subjects (always

215   including the first 3 randomised subjects, thereafter randomly selected) have SDV performed on the

216   primary and secondary endpoint CRFs. 100% (S)AEs, (S)ADEs and DD that are reported in accordance with

217   the study protocol, including potential unreported events for these subjects reviewed.

218   In accordance with ICH GCP guidelines,[11] audits may be performed by the ethics committees and

219   competent authorities during the course of the study.

220

ETHICS AND DISSEMINATION

Ethical and regulatory compliance

Prior to study conduct, protocol, proposed patient information, consent form and other study-specific

documents were approved by all local ethics committees, with the first approval received in Switzerland

(Ethikkommission Nordwest- und Zentralschweiz (2021-00713)). The current protocol version is 4.0. The

trial is sponsored by the Penta Foundation, Corso Stati Uniti, 4, 35127 Padova, Italy.

Before commencement of the trial, a risk classification following the ISO 201916 standard and ICH-GCP E6

guidelines was carried out. The risk classification of the ADEQUATE Study is defined as negligible, because

participation in the intervention group has no significant additional risks compared to the standard of care.

This study is registered on https://clinicaltrials.gov (NCT04781530).

The study is carried out according to the protocol and with principles enunciated in the current version of

the Declaration of Helsinki,[12] the guidelines of Good Clinical Practice (GCP) issued by ICH,[11] in case of

medical device: the European Directive on medical devices 93/42/EEC and the ISO Norm 14155,[13].

Patient and Public Involvement

This protocol was written without patient involvement. Patients or guardians were not invited to comment

on the study design or to contribute to the writing or editing of this document for readability or accuracy.

Dissemination of results

The data from all centres will be analysed together and published as soon as possible in peer-reviewed

journals, as well as being presented at national or international conferences.

The results of this trial will be submitted for Open Access publication in high impact peer-reviewed journals

likely to be read by health professionals in the management of ARI in children in Europe. The work will be

presented at key medical conferences. To maximise the impact of the trial across Europe, its findings will be

245 disseminated more widely through abstracts for oral and poster presentations submitted to some of the

246 main relevant national and international conferences.

247 Findings will further be distributed through activities of the VALUE-Dx consortium's workpackage 6,

248 including press releases, the consortium website and educational activities and materials. The social media

249 presence of the organisations involved will also be used to highlight news about the trial.

250

TRIAL STATUS AND DISCUSSION

Currently, 348 children have been enrolled in the trial. Follow-up has been completed for x and x have

missed the 14d and 28d follow-up visit, but data on primary endpoints may still be completed following GP

enquiry. Recruitment accrual is at 67% of target.

A 2014 Cochrane Review found a trend towards reduced antibiotic use with use of rapid syndromic tests in

paediatric EDs.[14] Since then, two single-centre randomised controlled trials (RCT), one from Finland and

one from the US, found no effect of a similar test as used in our trial on antibiotic prescribing in EDs.[15, 16]

Both trials employed a similar strategy of approaching children at an early point in time and before clinical

assessment. Our trial differs in that children for whom (a) a fixed decision to admit them had already been

made, e.g. as part of a treatment guideline or local standard operating procedure, or (b) where it was

deemed obvious by clinicians that neither antibiotics nor hospitalisation were considered, are excluded

from the trial. Also, both trials did not investigate duration of antibiotics, thereby potentially missing an

effect on antibiotic use if results from the test would make clinicians more likely to stop antibiotics early.

Finally, both trials were designed to show a difference in antibiotic prescribing but did not complement this

with decisions to hospitalise patients. Thus, our trial adds to the previous literature

-    by employing the same protocol across a range of different settings,

-    by studying the intervention in a population in which clinicians express an initial degree of

     uncertainty about management,

-    by treating hospitalisation and its duration as equally important effects of a rapid syndromic test as

     treatment with antibiotics,

-    and by capturing delayed effects of the test on both

The trial's primary endpoint was adapted after the start of the trial. Although this is often considered

acceptable, it is still a decision that needs careful deliberation and explanation. The ADEQUATE trial was

initially designed as two partner trials in EDs, one in the adult and one in the paediatric population. The

primary outcomes were planned to be analysed together, thus a safety non-inferiority endpoint with high

relevance mainly for the adult population was introduced into the primary endpoints. Because of the low

risk of meeting this endpoint, demonstrating non-inferiority was dominating the sample size estimation for

the paediatric trial. Following the obligation to restrict the number of individuals in clinical trials to the

279   number necessary to generate robust findings, we decided to move the non-inferiority endpoint to the

280   secondary endpoints as soon as the adult trial was terminated due to changes in routine care making the

281   trial unfeasible.

282   Paediatric ARI is a common condition with diverse aetiology. A diagnostic intervention reducing length of

283   hospital stay and antibiotics has a high potential to (a) reduce strain on healthcare resources, (b) reduce

284   evolution of antimicrobial resistance and (c) improve children's and parents' well-being. The ADEQUATE

285   trial will provide conclusive evidence on the effectiveness of a rapid syndromic test for this purpose.

286

STATEMENTS

A. contributorship statement

Conceptualisation: Cristina Prat Aymerich, Malte Kohns Vasconcelos, Andrew Atkinson, Henri van Werkhoven, Surbhi Malhotra-Kumar, Herman Goossens, Marc Bonten, Julia A. Bielicki

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323 Bonten, Julia A. Bielicki

324 Writing – review & editing: all authors

325

B. competing interests

327 Benjamin Hommel, Marie Tessonneau, Sophie Vandepitte, Jean-Louis Tissier, Florence Allantaz and Philippe

328 Cleuziat are employees of bioMérieux, the manufacturer of the diagnostic tool under study in this trial.

329 They were involved in the administration of the trial, provided resources and monitored the trial progress.

330 They were not involved in the design or analysis of the trial. The other authors have no potential conflict of

331 interest to disclose.

332

C. funding

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338

D. data sharing statement

340 The article does not contain a report on analysed data.

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SPIRIT ✓

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title ✓	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration ✓	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version l225	3	Date and version identifier
Funding✓	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors contributor statement and acknowledgements
	5b	Name and contact information for the trial sponsor named ll225-6
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities contributor statement and acknowledgements
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) contributor statement and acknowledgements
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention ll36-61, 65-7, 255-7
	6b	Explanation for choice of comparators ll56-61
Objectives	7	Specific objectives or hypotheses ll69-72

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Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) ll69,96,145

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained ll73-6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) table 1, ll84-8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered ll100-12
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) n/a
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) n/a
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial ll96-9
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended respective section (l116ff)
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) ll130-41, we decided against a diagram because the structure of FU is very simple in this trial
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations respective section (l142ff)
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size n/a

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions ll108-11
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned ll108-11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions ll108-11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how ll113-4
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial n/a

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol ll130-41
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols ll139-41
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol ll133-4, 202ff
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol respective section (l158ff)
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) referred to SAP

| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) ==referred to SAP== |

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed ==acknowledgements==
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial ==n/a==
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct ==table 2 and ll216-7==
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor ==ll218-9==

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval ==respective section (l222ff)==
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) ==respective section (l222ff)==
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) ==respective section (l83ff)==
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable ==ll197-210==
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial ==ll133-4, 202ff==
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site ==funding and competing interests statements==

Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators <mark>data sharing statement</mark>
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation <mark>n/a</mark>
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions <mark>respective section (l239ff)</mark>
	31b	Authorship eligibility guidelines and any intended use of professional writers <mark>n/a</mark>
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code <mark>none available yet</mark>

Appendices

| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates <mark>supplement</mark> |
| Biological specimens | 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable <mark>ll186ff</mark> |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

Randomised multi-centre effectiveness trial of rapid syndromic testing by panel assay in children presenting to European emergency departments with acute respiratory infections – trial protocol for the ADEQUATE Paediatric trial

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Randomised multi-centre effectiveness trial of rapid syndromic testing by panel assay in children presenting to European emergency departments with acute respiratory infections – trial protocol for the ADEQUATE Paediatric trial

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1 **ABSTRACT**

2 **Introduction:** Syndromic panel assays, i.e. using one test to simultaneously target multiple pathogens with

3 overlapping signs and symptoms, have been integrated into routine paediatric care over the past decade,

4 mainly for more severely ill and hospitalised patients. Their wider availability and short turnaround times

5 open the possibility to apply them to non-hospitalised patients as well. In this context, it is important to

6 trial how clinicians make use of pathogen detection data and if their early availability influences

7 management decisions, particularly antibiotic use and hospitalisation.

8 **Methods and analysis:** ADEQUATE is an individually-randomised, controlled, open-label effectiveness trial

9 comparing the impact of a respiratory pathogen panel assay (BIOFIRE® Respiratory Panel 2.1*plus*) used as a

10 rapid syndromic test on nasopharyngeal swabs in addition to the standard of care versus standard of care

11 alone. The trial will 1:1 randomise 520 participants under the age of 18 at 9 paediatric emergency

12 departments in 6 European countries. Inclusion criteria for the trial consist of two sets, with the first

13 describing respiratory tract infections in paediatric patients and the second describing the situation of

14 potential management uncertainty in which test results may immediately affect management decisions.

15 Enrolment started in July 2021 and is expected to be complete in early 2024. We will perform a two-sample

16 t-test assuming a pooled variance estimate to compare the log transformed mean time on antibiotic

17 treatment (in hours) and number of days alive out of the hospital within 14 days after study enrolment

18 between the control and intervention arms.

19 **Ethics and dissemination:** The trial protocol and materials were approved by research ethics committees in

20 all participating countries. The respiratory pathogen panel assay is CE marked and FDA cleared for

21 diagnostic use. Participants and caregivers provide informed consent prior to study procedures

22 commencing. The trial results will be published in peer-reviewed journals and at national and international

23 conferences. Key messages will also be disseminated via press and social media where appropriate.

24 **Trial registration number:** NCT04781530

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27   **Strengths and limitations of this study (max 5 bullet points):**

28   -   The eligibility criteria in this trial are tailored to include a patient population where decisions are

29   pending and test results may impact initial management decisions.

30   -   The trial's setting spans European countries with some difference in available resources and the

31   results will therefore likely be generalisable to other high-income country settings.

32   -   The panel assay used in the trial is assessed as a test close to the point of care in the emergency

33   department and use of the test in other scenarios may result in different estimates for

34   effectiveness.

35   -   Due to the pragmatic design with minimised interference with routine procedures and clinician

36   judgement, results may lose applicability with major changes in the respective health system.

37   -

INTRODUCTION

Community-acquired acute respiratory infections (ARI) are the most frequent reason for unscheduled healthcare visits and at the same time, the most frequent cause of inappropriate antibiotic use.[1, 2] While most ARI cause mild symptoms and are self-limiting, lower respiratory tract infections, including pneumonia, globally cause more than half a million deaths in <5 year old children per year.[3] Especially since the wide roll-out of conjugate vaccines, most of these infections in children do not require treatment with antibiotics. Antibiotic consumption is a driver of development of antimicrobial resistance (AMR) and where use of antibiotics in the individual is not warranted, the ecological and economic cost of antimicrobial resistance per antibiotic consumed is considerable.[4-6]

Determining which pathogen is the likely cause of an infectious episode is one common approach for clinicians to decide on the probability of antibiotic treatment being beneficial in a patient. In paediatric routine care, pathogen testing is usually limited to upper respiratory tract (URT) samples.[7] A wide range of common respiratory pathogens that may cause more severe disease are frequently present in the URT of asymptomatic children as well, thereby making it more difficult to determine the causative pathogen of an episode.[8] While for some viral pathogens, especially RSV, influenza virus, parainfluenza virus and human metapneumovirus, there is a high probability that their detection explains the cause of an episode of severe ARI, for others, including *Streptococcus pneumoniae* and human rhinovirus, the association is much weaker.[9] Detection of a viral pathogen does not exclude a bacterial aetiology of an illness episode and uncertainty of aetiology may increase the probability of antibiotic prescriptions.[10]

Children hospitalised for ARI stay in hospital for a median of 2 to 3 days and resolution of symptoms takes much longer.[3, 11] Interventions reducing hospital stays have a high potential to reduce psycho-social costs for families and economic costs for the health system.

Syndromic panel assays, i.e. using one test to simultaneously target multiple pathogens with overlapping signs and symptoms, have been integrated into routine paediatric care including in emergency departments over the past decade, mainly for more severely ill and hospitalised patients. Their wider availability and short turnaround times open the possibility to apply them to non-hospitalised patients as

64 well. In this context, it is important to trial how clinicians make use of pathogen detection data and if their

65 early availability influences management decisions.

66 VALUE-Dx is the first Innovative Medicines Initiative project initiated by six in vitro diagnostic companies

67 who joined forces with 20 non-industry partners to combat AMR and improve patient outcomes. The

68 multidisciplinary consortium involves clinicians, microbiologists, health economists, social scientists, and

69 industry. The trial described here is a part of this VALUE-Dx project. It aims to determine if the integration

70 of a rapid syndromic test at an early point in time in the management workflow in paediatric emergency

71 departments can influence the decisions to treat a patient with antibiotics or to hospitalise them.

72 **METHODS AND ANALYSIS**

73 ADEQUATE is an individually randomised, controlled, open-label superiority effectiveness trial comparing

74 the impact of a respiratory pathogen panel assay used as a rapid syndromic test in addition to the standard

75 of care versus standard of care alone on antibiotic use and hospitalisations in paediatric patients with ARI

76 presenting to EDs. The trial is part of workpackage 4 of the VALUE-Dx consortium.

77 **Trial setting**

78 The trial enrols participants at 7 paediatric EDs in 5 European countries (Germany, Greece, Spain,

79 Switzerland and the United Kingdom). Enrolment started in July 2021 (trial start date: 1st July 2021) and is

80 expected to be complete in early 2024 (planned end date – last patient last visit: 31st March 2024).

81 **Trial population**

82 Inclusion criteria for the trial consist of two sets, with the first describing respiratory tract infections in

83 paediatric patients and the second describing the situation of potential management uncertainty in which

84 test results may immediately affect management decisions. Few exclusion criteria were introduced to

85 increase generalisability of the trial results. The full eligibility criteria are listed in table 1.

Inclusion criteria (all must be fulfilled)
1. ARI presentation Children of any age presenting to the ED with an acute illness (present for 14 days or less) with temperature ≥38.0°C measured at presentation or parental report of fever within the previous 72 hours AND at least two of the below: • Cough • Abnormal sounds on chest auscultation (crackles, reduced breath sounds, bronchial breathing, wheezing) • Clinical signs of dyspnea (chest indrawing, nasal flaring, grunting) • Signs of respiratory dysfunction: tachypnoea for age (as per hospital standard) or decreased oxygen saturation (<92% in room air) • Signs of reduced general state: poor feeding, vomiting or lethargy/drowsiness
2. Management uncertainty At time of screening • Patient has undergone first assessment by managing clinical team (doctor or nurse, incl. triage) • Hospitalisation is not yet determined, i.e., neither by clinical presentation definitely requiring hospitalisation (e.g., per local guideline) nor by fixed decision of managing clinical team; admission to a short-stay unit or surveillance unit is not considered a hospitalisation for this trial • Antibiotic treatment or hospitalisation is being considered by the managing team • The rapid syndromic diagnostic test result can be awaited up to 4 hours before the decision to discharge the patient or to initiate antibiotic treatment is made
Exclusion criteria (none may be fulfilled)
1. Development of acute respiratory infection more than 48 hours after hospital admission (hospital acquired);

2. Patients with a severe underlying medical condition dictating management decisions including hospitalisation and/or antibiotic treatment (e.g., cystic fibrosis, immunosuppression);
3. Hospitalisation for at least 24 hours within the last 14 days (healthcare-associated);
4. Confirmed pregnancy or breastfeeding;
5. Any clinically significant abnormality identified at the time of screening that in the judgment of the Investigator would preclude safe completion of the study or constrain endpoints assessment such as major systemic diseases or patients with short life expectancy;
6. Inability to obtain informed consent;
7. Alternative noninfectious diagnosis that explains clinical symptoms.

86   Table 1: Eligibility criteria

87   **Screening, recruitment and consent**

88   During working hours of study staff, patients in the emergency department or short-stay unit are screened

89   for eligibility by study staff. In most instances, screening takes place as soon as possible after initial triage

90   but screening at any later stage within the emergency department was possible. Informed consent is

91   sought from all patients meeting the eligibility criteria at the time of screening. The health status of

92   patients might rapidly deteriorate between screening and randomisation. Therefore, all eligibility criteria

93   are be re-evaluated and confirmed by trained and delegated trial staff prior to the decision to randomise

94   the patient.

95   Screening failures are defined as patients who were found eligible per screening but have either not given

96   informed consent, or have deteriorated between screening and randomisation, and therefore no longer

97   fulfil eligibility criteria. Screening failures are recorded anonymously on a screening log detailing the reason

98   for screening failure and are not randomised. No diagnostic procedures are performed for the purpose of

99   checking eligibility criteria specifically, i.e., any procedures indicated for the standard of care patient

100 management will be performed but none will be added to check eligibility criteria.

101 **Randomisation and blinding**

102 Participants are randomised with equal probability into two allocation groups: (a) the control group,

103 receiving the current standard of care at the respective trial site, which may include rapid diagnostic testing

104 for specific pathogens or syndromic testing with results reported after a longer time than four hours, or (b)

105 the intervention group, receiving the standard of care plus immediately a nasopharyngeal swab tested with

106 the BIOFIRE® Respiratory Panel 2.1*plus* (RP2.1*plus*). The intervention is a multiplexed nucleic acid test for

107 the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic

108 acids in nasopharyngeal swabs obtained from patients suspected of respiratory tract infections. The assay is

109 licensed in CE marked and FDA cleared, for the use intended in this trial. The pathogens included in the

110 assay are adenovirus, coronaviruses (229 E, HKU1, NL63, OC43, SARS-CoV-2), human metapneumovirus,

111 human rhinovirus/enterovirus, influenza A, including subtypes H1, H1-2009, and H3, influenza B, middle

112 east respiratory syndrome coronavirus (MERS-CoV), parainfluenza virus (1, 2, 3, 4), respiratory syncytial

113 virus, *Bordetella parapertussis*, *Bordetella pertussis*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*.

114 After all eligibility criteria have been verified and informed consent has been obtained, randomisation is

115 performed using the built-in randomisation module of the electronic Case Report Form system (Research

116 Online). Allocation is concealed until the moment of randomisation. To this end, block randomisation is

117 used with variable blocks of size 2, 4 and 6. Randomisation is stratified by centre. In the intervention group,

118 a URT swab is obtained by trained trial or clinical staff and submitted to the panel assay test with as little

119 delay as possible. After the decision to randomise the subject is made, subjects will not be excluded from

120 the trial. Due to the nature of the intervention, blinding is not possible. If the allocated intervention is not

121 applied for any reason, this will be recorded and follow-up for the participant will be completed.

122 **Outcome measures and assessments**

123 The co-primary study endpoints are:

124 1. Days alive out of hospital within 14 days after study enrolment

125 2. Days on Therapy (DOT) with antibiotics within 14 days after study enrolment

126 14 days were selected over 30 days as time window for the primary endpoints because a potential superior

127 effect would be expected to be more immediate, and a shorter window resulted in a small gain in power.

128 Furthermore, delayed effects will still be captured in the secondary endpoints.

129 The secondary endpoints are listed in table 2.

Non-inferiority safety endpoint:

- For initially hospitalised patients: i) any readmission, ii) ICU admission => 24 hours after

  hospitalisation, or iii) death, within 30 days after study enrolment

- For initially non-admitted patients: any admission or death within 30 days after study enrolment. Direct costs and indirect costs within 30 days after enrolment, specifically cost of healthcare within 30 days after enrolment, including hospital and ICU days, utilisation of non-hospital services and cost of anti-infective and concomitant medication, and cost of workdays lost within 30 days, including days for childcare

Change in quality of life as determined by EQ-5D-5L (or suitable alternative for age), days away from usual childcare routine or school and healthcare utilisation on day 1, 14, and 30 after enrolment.

Proportion of participants with an identified respiratory pathogen in both study groups on randomisation day samples.

Proportion of participants on non-first-line anti-infective regimens (as defined by local guidelines)

Time to de-escalation and time to stop of anti-infective therapy

Proportion of hospitalised participants with detection of cephalosporin-, carbapenem- or quinolone-resistant Enterobacteriaceae on any standard of care samples >7 days after randomisation

Hours in individual or cohort isolation in hospitalised participants

130   Table 2: Secondary endpoints

131   Primary endpoints were adapted after a decision to terminate the recruitment of adult patients on a

132   partner protocol on 3rd May 2022. The adult partner trial was terminated mainly because of slow

133   recruitment and because of management workflows for patients having changed during the Covid-19

134   pandemic in ways that additionally impeded patient inclusions. Prior to this adaptation, the non-inferiority

135   safety endpoint was considered a third co-primary endpoint. Because mortality in the study population in

136   high-resource settings is extremely low, and secondary admission rates among children initially managed in

137   the community as well as re-admission and secondary ICU admission rates among primarily admitted

138   children are likely to be in the range of below 5%, this endpoint was judged to unlikely be relevant or

139   appropriate for the paediatric population. Additionally, secondary admissions will still provide safety

140   information on the first co-primary endpoint.

141   Participants are followed up until 30 days after randomisation. Standard of care clinical and microbiological

142   data are collected. The participant data set summarises the illness episode and outcome, microbiological

143   testing, antimicrobial use, use of healthcare facilities including hospitalisations and return to normal

144 activity, childcare arrangements and quality of life. Data is entered into case report forms in a GCP-

145 compliant database held at the Julius Center, UMC Utrecht. Follow-up information including data for health

146 economic analysis is collected on day 14 (visit window: day 12 – 16) and on day 30 (visit window: day 28 –

147 32) after randomisation. Parents or participants themselves (where age-appropriate) are contacted by

148 study staff for the follow-up visits, usually via telephone but in case of hospitalisation or hospital

149 attendance during the visit window face-to-face visits are acceptable. Quality of life is measured by EQ-5D,

150 using age-appropriate versions including proxy versions that are emailed to families. In case of failure to

151 successfully contact families at the end of trial participation, the participant's general practitioner is

152 contacted to complete information on the primary endpoints.

153 **Sample size and power**

154 A reduction of one day in antibiotic treatment or increase of one day in days alive out of hospital were

155 chosen for a clinically relevant reduction in antibiotic prescribing and a reduction in hospital costs,

156 respectively. In children, the co-primary superiority endpoints are likely to be dominated by the DOT with

157 antibiotics, as ambulatory exposure to antibiotics is likely to be common in the absence of hospital

158 admission, whereas many admitted children would be expected to be treated with antibiotics as well.

159 The sample size estimation was performed for this endpoint. From a recent publication on variations in

160 antibiotic prescribing in febrile children presenting to European EDs, the standard deviation for days on

161 antibiotic treatment was estimated as 3.7 days. Based on this, recruitment of 170 children per arm (total of

162 340 children) will be sufficient to detect a difference of one day in this endpoint (power 80%, alpha 0.05).

163 To account for uncertainty about the variability in both co-primary endpoints in the paediatric study

164 population, we adopt a highly conservative approach aiming to recruit 252 evaluable children per arm

165 (total of 504 children), resulting in adequate power to detect a difference in one day in both endpoints

166 (table 3), with the calculations performed for the "antibiotic prescribing" endpoint

SD	Delta	Alpha	Beta	Correction	Sample size per arm
2.5	1	0.025	0.2	1	99
3.0	1	0.025	0.2	1	142
3.5	1	0.025	0.2	1	193

3.7	1	0.025	0.2	1	215
4.0	1	0.025	0.2	1	252
4.5	1	0.025	0.2	1	318
5.0	1	0.025	0.2	1	393

167 Table 3: Sample sizes for Days on antibiotic treatment (paediatric) using different assumptions

168 **Analysis plan**

169 The analysis will be performed by the trial statistician using the R language and environment for statistical

170 computing (version 3.6 or higher). Reporting will follow the CONSORT guidelines.

171 Both co-primary endpoints will be tested separately, and superiority is confirmed if either one or both are

172 superior in terms of the primary analysis.

173 To investigate differences between the two arms for each endpoint separately, a two-sample t-test of the

174 log transformed mean time (in hours) on antibiotic treatment or alive out of hospital comparing those on

175 the standard of care arm (control) and the intervention arm will be performed, assuming a pooled variance

176 estimate.

177 An adjusted linear mixed effects model will be fitted with log transformed days on antibiotic treatment or

178 days alive out of hospital as dependent variable, and an indicator variable for the randomised arm, age

179 groups (<5y, 5-17y) and comorbidities (stratified according to modified Charlson comorbidity index: 0, 1,

180 +1) as independent variables. Further independent variables will be considered in post hoc analyses. The

181 model will include a random intercept for each country (and potentially, emergency department in country

182 if cluster sizes allow), accounting for clustering on these variables. Zero-inflated or similar models will be

183 considered if data are heavily skewed.

184 We anticipate days alive out of hospital data to be heavily right skewed in the full analysis set, and

185 therefore suitable transformations or modelling approaches will be considered as appropriate.

186 Subgroup analyses of the primary endpoints will include

187   • by age groups (<5, >5)

188   • by admission at baseline (yes/no)

189   • by receipt of antibiotics at baseline (yes/no)

- for those on antibiotic therapy at baseline, we will dichotomise days on treatment into two groups (0="1-5 days", 1=">5 days"), and fit a (mixed effects) logistic model with this grouping as dependent variable, adjusting as above.

- by country

- by emergency department (if the number of patients allows).

A detailed analysis plan for all secondary objectives will be finalised before the trial's database closure and will be under version control at the Paediatric Research Centre, University of Basel Children's Hospital.

Sub-study and biobanking

The sub-study will have its own analysis plan which will be finalised before the respective database is locked.

The aim of the microbiology study, located at the University of Antwerp, is to use suitable methods, including metagenomic sequencing, to characterise changes in microbiological colonisation and antimicrobial resistance patterns dependent on treatment with antibiotics. In a subset of study sites and participants (up to 150 participants), additional oropharyngeal samples are obtained from participants. One sample is obtained on the day of randomisation and one sample on day 30 (visit window: day 28 – 32) after randomisation. Specific procedures for collection and processing are provided to sites. After receiving specific instructions, the day 30 swab can be obtained at home and sent to the local study site via mail.

Inclusion in the microbiology study will require separate informed consent.

Biological samples obtained for the study (including leftovers from the specimens obtained for the intervention and for the microbiology study) are be stored at all sites and shipped to the University of Antwerp for inclusion in a biobank, subject to the condition that separate informed consent for biobanking is given.

Participation in the main study does not depend on consent for the microbiology study or for biobanking.

Monitoring

Representatives of the trial management team and a designated study monitor conducted a remote site initiation visit at each study site to verify qualifications of the local investigators and inform the local teams

216 of responsibilities and the procedures for ensuring adequate and correct documentation and use of the

217 electronic data capture system as well as providing training on implementing all trial activities.

218 Sites are requested to enter data in the eCRF within 5 working days following each subject's visit. The

219 monitor ensures that data is entered in a timely manner. When queries regarding the data entered in the

220 eCRF are raised, the site is expected to resolve them within 10 working days.

221 The monitor visits a site at least once during the course of the study, when at least 3 subjects are

222 randomised and completed data collection in the eCRF up to at least Day 30. Depending on the subject

223 enrollment rate and any site-specific issues, the total number of on-site monitoring visits may be increased.

224 The visits include Source Data Verification (SDV) for selected variables: 100% SDV is performed on all

225 Informed Consent Form (ICF) versions and consent process in the source; a total of 10% of subjects (always

226 including the first 3 randomised subjects, thereafter randomly selected) have SDV performed on the

227 primary and secondary endpoint CRFs. 100% serious adverse events (S)AEs, serious adverse device effects

228 (S)ADEs and device deficiencies (DD) that are reported in accordance with the study protocol, including

229 potential unreported events for these subjects reviewed.

230 In accordance with ICH GCP guidelines,[12] audits may be performed by the ethics committees and

231 competent authorities during the course of the study.

232

ETHICS AND DISSEMINATION

Ethical and regulatory compliance

235 Prior to study conduct, the protocol, proposed patient information, consent form and other study-specific

236 documents were approved by all local ethics committees, with the first approval received in Switzerland in

237 June 2021 (Ethikkommission Nordwest- und Zentralschweiz (2021-00713)). The current protocol version is

238 4.0, approved between October 2022 and March 2023 for the respective trial sites. Changes compared to

239 the first version are mainly concerned with the primary endpoint as explained above and do not include

240 changes in the trial conduct. The trial is sponsored by the Penta Foundation, Corso Stati Uniti, 4, 35127

241 Padova, Italy. The industry partner bioMérieux supplied equipment, consumables and logistical support for

242 the trial.

243 Before commencement of the trial, a risk classification following the ISO 201916 standard and ICH-GCP E6

244 guidelines was carried out. The risk classification of the ADEQUATE Study is defined as negligible, because

245 participation in the intervention group has no significant additional risks compared to the standard of care.

246 This study is registered on https://clinicaltrials.gov (NCT04781530) since 1st March 2021

247 The study is carried out according to the protocol and with principles enunciated in the current version of

248 the Declaration of Helsinki,[13] the guidelines of Good Clinical Practice (GCP) issued by ICH,[12] in case of

249 medical device: the European Directive on medical devices 93/42/EEC and the ISO Norm 14155,[14].

250

Patient and Public Involvement

252 This protocol was written without patient involvement. Patients or guardians were not invited to comment

253 on the study design or to contribute to the writing or editing of this document for readability or accuracy.

254

Dissemination of results

256 The data from all centres will be analysed together and published as soon as possible in peer-reviewed

257 journals, as well as being presented at national or international conferences.

258   The results of this trial will be submitted for Open Access publication in high impact peer-reviewed journals

259   likely to be read by health professionals in the management of ARI in children in Europe. The work will be

260   presented at key medical conferences. To maximise the impact of the trial across Europe, its findings will be

261   disseminated more widely through abstracts for oral and poster presentations submitted to some of the

262   main relevant national and international conferences.

263   Findings will further be distributed through activities of the VALUE-Dx consortium's workpackage 6,

264   including press releases, the consortium website and educational activities and materials. The social media

265   presence of the organisations involved will also be used to highlight news about the trial.

266   Datasets generated from the trial will be made accessible in line with regulatory requirements on request

267   to the trial consortium through the corresponding author.

268   **TRIAL STATUS AND DISCUSSION**

269   Currently, 421 children have been enrolled in the trial. Follow-up has been completed for 388 and 22 have

270   missed the 14d and 28d follow-up visit, but data on primary endpoints may still be completed following GP

271   enquiry. Recruitment accrual is at 80% of target.

272   Following strictly pragmatic trial design decisions, the trial will have limited ability to elucidate the potential

273   mechanism that enables the test to be effective, or prevents it from being effective. For example, the

274   protocol does not provide guidance on interpretation of test results. Clinicians' perceptions about the

275   positive and negative predictive values of the test results for any specific aetiology are therefore not

276   controlled in our trial. In clinical practice, these may change with longer-term trends of changing incidences

277   of pathogens and the trial results may potentially be less applicable under these circumstances. On balance,

278   we believe that this is outweighed by the gain in external validity that a pragmatic trial offers, namely that

279   we expect the trial results to be broadly generalisable because we aimed to reduce introduction of

280   selection bias.

281   The trial assesses the effectiveness of the diagnostic test in a specific setting, namely used close to the

282   point of care in the emergency department. Patients in the trial's control group may have received the

283   same or similar tests as long as results were only received after more than four hours. The effectiveness of

284   the test may therefore be lower compared to a scenario in which the test was only compared to patients

285   with no respiratory panel assay data available.

286   A limitation of the rapid syndromic test used is that it is does not cover *S. pneumoniae* or other bacteria

287   considered typical causes of acute lower respiratory tract infection. The trial does not offer any insight into

288   whether such an assay might be effective in the same setting.

289   A 2014 Cochrane Review found a trend towards reduced antibiotic use with use of rapid syndromic tests in

290   paediatric EDs.[15] Since then, two single-centre randomised controlled trials (RCT), one from Finland and

291   one from the US, found no effect of a similar test as used in our trial on antibiotic prescribing in EDs.[16, 17]

292   Both trials employed a similar strategy of approaching children at an early point in time and before clinical

293   assessment. Our trial differs in that children were not eligible if decisions on their hospitalisation had

294   already been made, including through a fixed treatment guideline or standard operating procedure.

295   Additionally, children were excluded when it was deemed obvious from the start by clinicians that neither

296   antibiotics nor hospitalisation were considered. Also, both trials did not investigate duration of antibiotics,

297   thereby potentially missing an effect on antibiotic use if results from the test would make clinicians more

298   likely to stop antibiotics early. Finally, both trials were designed to show a difference in antibiotic

299   prescribing but did not complement this with decisions to hospitalise patients. Thus, our trial adds to the

300   previous literature

301   -   by employing the same protocol across a range of different settings,

302   -   by studying the intervention in a population in which clinicians express an initial degree of

303   uncertainty about management,

304   -   by treating hospitalisation and its duration as equally important effects of a rapid syndromic test as

305   treatment with antibiotics,

306   -   and by capturing delayed effects of the test on both

307   The trial's primary endpoint was adapted after the start of the trial. Although this is often considered

308   acceptable, it is still a decision that needs careful deliberation and explanation. The ADEQUATE trial was

309   initially designed as two partner trials in EDs, one in the adult and one in the paediatric population. The

310   primary outcomes were planned to be analysed together, thus a safety non-inferiority endpoint with high

311   relevance mainly for the adult population was introduced into the primary endpoints. Because of the low

312   risk of meeting this endpoint, demonstrating non-inferiority was dominating the sample size estimation for

313   the paediatric trial. Following the obligation to restrict the number of individuals in clinical trials to the

314   number necessary to generate robust findings, we decided to move the non-inferiority endpoint to the

315   secondary endpoints as soon as the adult trial was terminated due to changes in routine care making the

316   trial unfeasible.

317   Paediatric ARI is a common condition with diverse aetiology. A diagnostic intervention reducing length of

318   hospital stay and antibiotics has a high potential to (a) reduce strain on healthcare resources, (b) reduce

319   evolution of antimicrobial resistance and (c) improve children's and parents' well-being. The ADEQUATE

320   trial will provide conclusive evidence on the effectiveness of a rapid syndromic test for this purpose.

321

STATEMENTS

A. contributorship statement

Members of the ADEQUATE Paediatric Trial Group contributed in the following roles:

Conceptualisation: Cristina Prat Aymerich, Malte Kohns Vasconcelos, Andrew Atkinson, Henri van Werkhoven, Surbhi Malhotra-Kumar, Herman Goossens, Marc Bonten, Julia A. Bielicki

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358   Writing – review & editing: all authors

359

B. competing interests

361   Benjamin Hommel, Marie Tessonneau, Sophie Vandepitte, Jean-Louis Tissier, Florence Allantaz and Philippe

362   Cleuziat are employees of bioMérieux, the manufacturer of the diagnostic tool under study in this trial.

363   They were involved in the administration of the trial, provided resources and monitored the trial progress.

364   They were not involved in the design or analysis of the trial. The other authors have no potential conflict of

365   interest to disclose.

366

C. funding

368   This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant

369   agreement No 820755. This Joint Undertaking receives support from the European Union's Horizon 2020

370   research and innovation programme and EFPIA and bioMérieux SA, Janssen Pharmaceutica NV, Accelerate

371   Diagnostics S.L., Abbott, Bio-Rad Laboratories, BD Switzerland Sàrl, and The Wellcome Trust Limited.

372

373 **D. data sharing statement**

374 The article does not contain a report on analysed data.

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SPIRIT ✓

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title <mark>✓</mark>	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration <mark>✓</mark>	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version <mark>l225</mark>	3	Date and version identifier
Funding<mark>✓</mark>	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors <mark>contributor statement and acknowledgements</mark>
	5b	Name and contact information for the trial sponsor named <mark>II225-6</mark>
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities <mark>contributor statement and acknowledgements</mark>
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) <mark>contributor statement and acknowledgements</mark>
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention <mark>II36-61, 65-7, 255-7</mark>
	6b	Explanation for choice of comparators <mark>II56-61</mark>
Objectives	7	Specific objectives or hypotheses <mark>II69-72</mark>

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| Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) ll69,96,145 |

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained ll73-6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) table 1, ll84-8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered ll100-12
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) n/a
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) n/a
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial ll96-9
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended respective section (l116ff)
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) ll130-41, we decided against a diagram because the structure of FU is very simple in this trial
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations respective section (l142ff)
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size n/a

Methods: Assignment of interventions (for controlled trials)

2

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions <mark>ll108-11</mark>
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned <mark>ll108-11</mark>
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions <mark>ll108-11</mark>
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how <mark>ll113-4</mark>
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial <mark>n/a</mark>

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol <mark>ll130-41</mark>
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols <mark>ll139-41</mark>
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol <mark>ll133-4, 202ff</mark>
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol <mark>respective section (l158ff)</mark>
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) <mark>referred to SAP</mark>

| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) ==referred to SAP== |

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed ==acknowledgements==
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial ==n/a==
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct ==table 2 and ll216-7==
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor ==ll218-9==

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval ==respective section (l222ff)==
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) ==respective section (l222ff)==
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) ==respective section (l83ff)==
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable ==ll197-210==
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial ==ll133-4, 202ff==
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site ==funding and competing interests statements==

Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators <mark>data sharing statement</mark>
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation <mark>n/a</mark>
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions <mark>respective section (l239ff)</mark>
	31b	Authorship eligibility guidelines and any intended use of professional writers <mark>n/a</mark>
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code <mark>none available yet</mark>

Appendices

| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates <mark>supplement</mark> |
| Biological specimens | 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable <mark>ll186ff</mark> |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

**Randomised multi-centre effectiveness trial of rapid
syndromic testing by panel assay in children presenting to
European emergency departments with acute respiratory
infections – trial protocol for the ADEQUATE Paediatric trial**

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Randomised multi-centre effectiveness trial of rapid syndromic testing by panel assay in children presenting to European emergency departments with acute respiratory infections – trial protocol for the ADEQUATE Paediatric trial

ADEQUATE Paediatric Trial Group

Keywords:

pragmatic trial, acute respiratory infection, syndromic testing, multiplex PCR, point-of-care testing

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1 **ABSTRACT**

2 **Introduction:** Syndromic panel assays, i.e. using one test to simultaneously target multiple pathogens with

3 overlapping signs and symptoms, have been integrated into routine paediatric care over the past decade,

4 mainly for more severely ill and hospitalised patients. Their wider availability and short turnaround times

5 open the possibility to apply them to non-hospitalised patients as well. In this context, it is important to

6 trial how clinicians make use of pathogen detection data and if their early availability influences

7 management decisions, particularly antibiotic use and hospitalisation.

8 **Methods and analysis:** ADEQUATE is an individually-randomised, controlled, open-label effectiveness trial

9 comparing the impact of a respiratory pathogen panel assay (BIOFIRE® Respiratory Panel 2.1*plus*) used as a

10 rapid syndromic test on nasopharyngeal swabs in addition to the standard of care versus standard of care

11 alone. The trial will 1:1 randomise 520 participants under the age of 18 at 9 paediatric emergency

12 departments in 6 European countries. Inclusion criteria for the trial consist of two sets, with the first

13 describing respiratory tract infections in paediatric patients and the second describing the situation of

14 potential management uncertainty in which test results may immediately affect management decisions.

15 Enrolment started in July 2021 and is expected to be complete in early 2024. We will perform a two-sample

16 t-test assuming a pooled variance estimate to compare the log transformed mean time on antibiotic

17 treatment (in hours) and number of days alive out of the hospital within 14 days after study enrolment

18 between the control and intervention arms.

19 **Ethics and dissemination:** The trial protocol and materials were approved by research ethics committees in

20 all participating countries. The respiratory pathogen panel assay is CE marked and FDA cleared for

21 diagnostic use. Participants and caregivers provide informed consent prior to study procedures

22 commencing. The trial results will be published in peer-reviewed journals and at national and international

23 conferences. Key messages will also be disseminated via press and social media where appropriate.

24 **Trial registration number:** NCT04781530

25

26

27 **Strengths and limitations of this study (max 5 bullet points):**

28 - The eligibility criteria in this trial are tailored to include a patient population where decisions are

29 pending and test results may impact initial management decisions.

30 - The trial's setting spans European countries with some difference in available resources and the

31 results will therefore likely be generalisable to other high-income country settings.

32 - The panel assay used in the trial is assessed as a test close to the point of care in the emergency

33 department and use of the test in other scenarios may result in different estimates for

34 effectiveness.

35 - Due to the pragmatic design with minimised interference with routine procedures and clinician

36 judgement, results may lose applicability with major changes in the respective health system.

37 -

38   **INTRODUCTION**

39   Community-acquired acute respiratory infections (ARI) are the most frequent reason for unscheduled

40   healthcare visits and at the same time, the most frequent cause of inappropriate antibiotic use.[1, 2] While

41   most ARI cause mild symptoms and are self-limiting, lower respiratory tract infections, including

42   pneumonia, globally cause more than half a million deaths in <5 year old children per year.[3] Especially

43   since the wide roll-out of conjugate vaccines, most of these infections in children do not require treatment

44   with antibiotics. Antibiotic consumption is a driver of development of antimicrobial resistance (AMR) and

45   where use of antibiotics in the individual is not warranted, the ecological and economic cost of

46   antimicrobial resistance per antibiotic consumed is considerable.[4-6]

47   Determining which pathogen is the likely cause of an infectious episode is one common approach for

48   clinicians to decide on the probability of antibiotic treatment being beneficial in a patient. In paediatric

49   routine care, pathogen testing is usually limited to upper respiratory tract (URT) samples.[7] A wide range

50   of common respiratory pathogens that may cause more severe disease are frequently present in the URT of

51   asymptomatic children as well, thereby making it more difficult to determine the causative pathogen of an

52   episode.[8] While for some viral pathogens, especially RSV, influenza virus, parainfluenza virus and human

53   metapneumovirus, there is a high probability that their detection explains the cause of an episode of

54   severe ARI, for others, including *Streptococcus pneumoniae* and human rhinovirus, the association is much

55   weaker.[9] Detection of a viral pathogen does not exclude a bacterial aetiology of an illness episode and

56   uncertainty of aetiology may increase the probability of antibiotic prescriptions.[10]

57   Children hospitalised for ARI stay in hospital for a median of 2 to 3 days and resolution of symptoms takes

58   much longer.[3, 11] Interventions reducing hospital stays have a high potential to reduce psycho-social

59   costs for families and economic costs for the health system.

60   Syndromic panel assays, i.e. using one test to simultaneously target multiple pathogens with overlapping

61   signs and symptoms, have been integrated into routine paediatric care including in emergency

62   departments over the past decade, mainly for more severely ill and hospitalised patients. Their wider

63   availability and short turnaround times open the possibility to apply them to non-hospitalised patients as

64 well. In this context, it is important to trial how clinicians make use of pathogen detection data and if their

65 early availability influences management decisions.

66 VALUE-Dx is the first Innovative Medicines Initiative project initiated by six in vitro diagnostic companies

67 who joined forces with 20 non-industry partners to combat AMR and improve patient outcomes. The

68 multidisciplinary consortium involves clinicians, microbiologists, health economists, social scientists, and

69 industry. The trial described here is a part of this VALUE-Dx project. It aims to determine if the integration

70 of a rapid syndromic test at an early point in time in the management workflow in paediatric emergency

71 departments can influence the decisions to treat a patient with antibiotics or to hospitalise them.

METHODS AND ANALYSIS

ADEQUATE is an individually randomised, controlled, open-label superiority effectiveness trial comparing

the impact of a respiratory pathogen panel assay used as a rapid syndromic test in addition to the standard

of care versus standard of care alone on antibiotic use and hospitalisations in paediatric patients with ARI

presenting to EDs. The trial is part of workpackage 4 of the VALUE-Dx consortium.

Trial setting

The trial enrols participants at 7 paediatric EDs in 5 European countries (Germany, Greece, Spain,

Switzerland and the United Kingdom). Enrolment started in July 2021 (trial start date: 1st July 2021) and is

expected to be complete in early 2024 (planned end date – last patient last visit: 31st March 2024).

Trial population

Inclusion criteria for the trial consist of two sets, with the first describing respiratory tract infections in

paediatric patients and the second describing the situation of potential management uncertainty in which

test results may immediately affect management decisions. Few exclusion criteria were introduced to

increase generalisability of the trial results. The full eligibility criteria are listed in table 1.

Inclusion criteria (all must be fulfilled)
1. ARI presentation Children of any age (under the age of 18) presenting to the ED with an acute illness (present for 14 days or less) with temperature ≥38.0°C measured at presentation or parental report of fever within the previous 72 hours AND at least two of the below: • Cough • Abnormal sounds on chest auscultation (crackles, reduced breath sounds, bronchial breathing, wheezing) • Clinical signs of dyspnea (chest indrawing, nasal flaring, grunting) • Signs of respiratory dysfunction: tachypnoea for age (as per hospital standard) or decreased oxygen saturation (<92% in room air) • Signs of reduced general state: poor feeding, vomiting or lethargy/drowsiness
2. Management uncertainty At time of screening • Patient has undergone first assessment by managing clinical team (doctor or nurse, incl. triage) • Hospitalisation is not yet determined, i.e., neither by clinical presentation definitely requiring hospitalisation (e.g., per local guideline) nor by fixed decision of managing clinical team; admission to a short-stay unit or surveillance unit is not considered a hospitalisation for this trial • Antibiotic treatment or hospitalisation is being considered by the managing team • The rapid syndromic diagnostic test result can be awaited up to 4 hours before the decision to discharge the patient or to initiate antibiotic treatment is made
Exclusion criteria (none may be fulfilled)

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> 1. Development of acute respiratory infection more than 48 hours after hospital admission (hospital acquired);
> 2. Patients with a severe underlying medical condition dictating management decisions including hospitalisation and/or antibiotic treatment (e.g., cystic fibrosis, immunosuppression);
> 3. Hospitalisation for at least 24 hours within the last 14 days (healthcare-associated);
> 4. Confirmed pregnancy or breastfeeding;
> 5. Any clinically significant abnormality identified at the time of screening that in the judgment of the Investigator would preclude safe completion of the study or constrain endpoints assessment such as major systemic diseases or patients with short life expectancy;
> 6. Inability to obtain informed consent;
> 7. Alternative noninfectious diagnosis that explains clinical symptoms.

14 86 Table 1: Eligibility criteria

15
16
17 87 **Screening, recruitment and consent**
18
19
20 88 During working hours of study staff, patients in the emergency department or short-stay unit are screened
21
22 89 for eligibility by study staff. In most instances, screening takes place as soon as possible after initial triage
23
24 90 but screening at any later stage within the emergency department was possible. Informed consent is
25
26 91 sought from all patients meeting the eligibility criteria at the time of screening. The health status of
27
28 92 patients might rapidly deteriorate between screening and randomisation. Therefore, all eligibility criteria
29
30 93 are be re-evaluated and confirmed by trained and delegated trial staff prior to the decision to randomise
31
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33 94 the patient.
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35
36 95 Screening failures are defined as patients who were found eligible per screening but have either not given
37
38 96 informed consent, or have deteriorated between screening and randomisation, and therefore no longer
39
40 97 fulfil eligibility criteria. Screening failures are recorded anonymously on a screening log detailing the reason
41
42 98 for screening failure and are not randomised. No diagnostic procedures are performed for the purpose of
43
44 99 checking eligibility criteria specifically, i.e., any procedures indicated for the standard of care patient
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47 100 management will be performed but none will be added to check eligibility criteria.
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50 101 **Randomisation and blinding**
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53 102 Participants are randomised with equal probability into two allocation groups: (a) the control group,
54
55 103 receiving the current standard of care at the respective trial site, which may include rapid diagnostic testing
56
57 104 for specific pathogens or syndromic testing with results reported after a longer time than four hours, or (b)
58
59 105 the intervention group, receiving the standard of care plus immediately a nasopharyngeal swab tested with
60

106 the BIOFIRE® Respiratory Panel 2.1*plus* (RP2.1*plus*). The intervention is a multiplexed nucleic acid test for

107 the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic

108 acids in nasopharyngeal swabs obtained from patients suspected of respiratory tract infections. The assay is

109 licensed in CE marked and FDA cleared, for the use intended in this trial. The pathogens included in the

110 assay are adenovirus, coronaviruses (229 E, HKU1, NL63, OC43, SARS-CoV-2), human metapneumovirus,

111 human rhinovirus/enterovirus, influenza A, including subtypes H1, H1-2009, and H3, influenza B, middle

112 east respiratory syndrome coronavirus (MERS-CoV), parainfluenza virus (1, 2, 3, 4), respiratory syncytial

113 virus, *Bordetella parapertussis*, *Bordetella pertussis*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*.

114 After all eligibility criteria have been verified and informed consent has been obtained, randomisation is

115 performed using the built-in randomisation module of the electronic Case Report Form system (Research

116 Online). Allocation is concealed until the moment of randomisation. To this end, block randomisation is

117 used with variable blocks of size 2, 4 and 6. Randomisation is stratified by centre. In the intervention group,

118 a URT swab is obtained by trained trial or clinical staff and submitted to the panel assay test with as little

119 delay as possible. After the decision to randomise the subject is made, subjects will not be excluded from

120 the trial. Due to the nature of the intervention, blinding is not possible. If the allocated intervention is not

121 applied for any reason, this will be recorded and follow-up for the participant will be completed.

Outcome measures and assessments

123 The co-primary study endpoints are:

124 1. Days alive out of hospital within 14 days after study enrolment

125 2. Days on Therapy (DOT) with antibiotics within 14 days after study enrolment

126 14 days were selected over 30 days as time window for the primary endpoints because a potential superior

127 effect would be expected to be more immediate, and a shorter window resulted in a small gain in power.

128 Furthermore, delayed effects will still be captured in the secondary endpoints.

129 The secondary endpoints are listed in table 2.

Non-inferiority safety endpoint:

- For initially hospitalised patients: i) any readmission, ii) ICU admission => 24 hours after hospitalisation, or iii) death, within 30 days after study enrolment

- For initially non-admitted patients: any admission or death within 30 days after study enrolment.

Direct costs and indirect costs within 30 days after enrolment, specifically cost of healthcare within 30 days after enrolment, including hospital and ICU days, utilisation of non-hospital services and cost of anti-infective and concomitant medication, and cost of workdays lost within 30 days, including days for childcare

Change in quality of life as determined by EQ-5D-5L (or suitable alternative for age), days away from usual childcare routine or school and healthcare utilisation on day 1, 14, and 30 after enrolment.

Proportion of participants with an identified respiratory pathogen in both study groups on randomisation day samples.

Proportion of participants on non-first-line anti-infective regimens (as defined by local guidelines)

Time to de-escalation and time to stop of anti-infective therapy

Proportion of hospitalised participants with detection of cephalosporin-, carbapenem- or quinolone-resistant Enterobacteriaceae on any standard of care samples >7 days after randomisation

Hours in individual or cohort isolation in hospitalised participants

130 Table 2: Secondary endpoints

131 Primary endpoints were adapted after a decision to terminate the recruitment of adult patients on a

132 partner protocol on 3rd May 2022. The adult partner trial was terminated mainly because of slow

133 recruitment and because of management workflows for patients having changed during the Covid-19

134 pandemic in ways that additionally impeded patient inclusions. Prior to this adaptation, the non-inferiority

135 safety endpoint was considered a third co-primary endpoint. Because mortality in the study population in

136 high-resource settings is extremely low, and secondary admission rates among children initially managed in

137 the community as well as re-admission and secondary ICU admission rates among primarily admitted

138 children are likely to be in the range of below 5%, this endpoint was judged to unlikely be relevant or

139 appropriate for the paediatric population. Additionally, secondary admissions will still provide safety

140 information on the first co-primary endpoint.

141 Participants are followed up until 30 days after randomisation. Standard of care clinical and microbiological

142 data are collected. The participant data set summarises the illness episode and outcome, microbiological

143 testing, antimicrobial use, use of healthcare facilities including hospitalisations and return to normal

144 activity, childcare arrangements and quality of life. Data is entered into case report forms in a GCP-

145 compliant database held at the Julius Center, UMC Utrecht. Follow-up information including data for health

146 economic analysis is collected on day 14 (visit window: day 12 – 16) and on day 30 (visit window: day 28 –

147 32) after randomisation. Parents or participants themselves (where age-appropriate) are contacted by

148 study staff for the follow-up visits, usually via telephone but in case of hospitalisation or hospital

149 attendance during the visit window face-to-face visits are acceptable. Quality of life is measured by EQ-5D,

150 using age-appropriate versions including proxy versions that are emailed to families. For children under the

151 age of three years, no validated version of the EQ-5D exists. Therefore, the the global rating scale on the

152 existing EQ-5D proxy version validated for children from three years of age onwards is used here.In case of

153 failure to successfully contact families at the end of trial participation, the participant's general practitioner

154 is contacted to complete information on the primary endpoints.

Sample size and power

156 A reduction of one day in antibiotic treatment or increase of one day in days alive out of hospital were

157 chosen for a clinically relevant reduction in antibiotic prescribing and a reduction in hospital costs,

158 respectively. In children, the co-primary superiority endpoints are likely to be dominated by the DOT with

159 antibiotics, as ambulatory exposure to antibiotics is likely to be common in the absence of hospital

160 admission, whereas many admitted children would be expected to be treated with antibiotics as well.

161 The sample size estimation was performed for this endpoint. From a recent publication on variations in

162 antibiotic prescribing in febrile children presenting to European EDs, the standard deviation for days on

163 antibiotic treatment was estimated as 3.7 days.[12] Based on this, recruitment of 170 children per arm

164 (total of 340 children) will be sufficient to detect a difference of one day in this endpoint (power 80%, alpha

165 0.05).

166 To account for uncertainty about the variability in both co-primary endpoints in the paediatric study

167 population, we adopt a highly conservative approach aiming to recruit 252 evaluable children per arm

168 (total of 504 children), resulting in adequate power to detect a difference in one day in both endpoints

169 (table 3), with the calculations performed for the "antibiotic prescribing" endpoint. Accounting for potential

170 loss to follow-up, we set a total recruitment target of 520 children.

SD	Delta	Alpha	Beta	Correction	Sample size per arm
2.5	1	0.025	0.2	1	99
3.0	1	0.025	0.2	1	142
3.5	1	0.025	0.2	1	193
3.7	1	0.025	0.2	1	215
4.0	1	0.025	0.2	1	252
4.5	1	0.025	0.2	1	318
5.0	1	0.025	0.2	1	393

171 Table 3: Sample sizes for Days on antibiotic treatment (paediatric) using different assumptions

172 **Analysis plan**

173 The analysis will be performed by the trial statistician using the R language and environment for statistical

174 computing (version 3.6 or higher). Reporting will follow the CONSORT guidelines.

175 Both co-primary endpoints will be tested separately, and superiority is confirmed if either one or both are

176 superior in terms of the primary analysis.

177 To investigate differences between the two arms for each endpoint separately, a two-sample t-test of the

178 log transformed mean time (in hours) on antibiotic treatment or alive out of hospital comparing those on

179 the standard of care arm (control) and the intervention arm will be performed, assuming a pooled variance

180 estimate.

181 An adjusted linear mixed effects model will be fitted with log transformed days on antibiotic treatment or

182 days alive out of hospital as dependent variable, and an indicator variable for the randomised arm, age

183 groups (<5y, 5 to <18y) and comorbidities (stratified according to modified Charlson comorbidity index: 0,

184 1, +1) as independent variables. Further independent variables will be considered in post hoc analyses. The

185 model will include a random intercept for each country (and potentially, emergency department in country

186 if cluster sizes allow), accounting for clustering on these variables. Zero-inflated or similar models will be

187 considered if data are heavily skewed.

188 We anticipate days alive out of hospital data to be heavily right skewed in the full analysis set, and

189 therefore suitable transformations or modelling approaches will be considered as appropriate.

190 Subgroup analyses of the primary endpoints will include

191 • by age groups (<5, >5)

192 • by admission at baseline (yes/no)

193 • by receipt of antibiotics at baseline (yes/no)

194 • for those on antibiotic therapy at baseline, we will dichotomise days on treatment into two groups

195 (0="1-5 days", 1=">5 days"), and fit a (mixed effects) logistic model with this grouping as

196 dependent variable, adjusting as above.

197 • by country

198 • by emergency department (if the number of patients allows).

199 A detailed analysis plan for all secondary objectives will be finalised before the trial's database closure and

200 will be under version control at the Paediatric Research Centre, University of Basel Children's Hospital.

201 **Sub-study and biobanking**

202 The sub-study will have its own analysis plan which will be finalised before the respective database is

203 locked.

204 The aim of the microbiology study, located at the University of Antwerp, is to use suitable methods,

205 including metagenomic sequencing, to characterise changes in microbiological colonisation and

206 antimicrobial resistance patterns dependent on treatment with antibiotics. In a subset of study sites and

207 participants (up to 150 participants), additional oropharyngeal samples are obtained from participants. One

208 sample is obtained on the day of randomisation and one sample on day 30 (visit window: day 28 – 32) after

209 randomisation. Specific procedures for collection and processing are provided to sites. After receiving

210 specific instructions, the day 30 swab can be obtained at home and sent to the local study site via mail.

211 Inclusion in the microbiology study will require separate informed consent.

212 Biological samples obtained for the study (including leftovers from the specimens obtained for the

213 intervention and for the microbiology study) are be stored at all sites and shipped to the University of

214 Antwerp for inclusion in a biobank, subject to the condition that separate informed consent for biobanking

215 is given.

216 Participation in the main study does not depend on consent for the microbiology study or for biobanking.

217 **Monitoring**

218 Representatives of the trial management team and a designated study monitor conducted a remote site

219 initiation visit at each study site to verify qualifications of the local investigators and inform the local teams

220 of responsibilities and the procedures for ensuring adequate and correct documentation and use of the

221 electronic data capture system as well as providing training on implementing all trial activities.

222 Sites are requested to enter data in the eCRF within 5 working days following each subject's visit. The

223 monitor ensures that data is entered in a timely manner. When queries regarding the data entered in the

224 eCRF are raised, the site is expected to resolve them within 10 working days.

225 The monitor visits a site at least once during the course of the study, when at least 3 subjects are

226 randomised and completed data collection in the eCRF up to at least Day 30. Depending on the subject

227 enrollment rate and any site-specific issues, the total number of on-site monitoring visits may be increased.

228 The visits include Source Data Verification (SDV) for selected variables: 100% SDV is performed on all

229 Informed Consent Form (ICF) versions and consent process in the source; a total of 10% of subjects (always

230 including the first 3 randomised subjects, thereafter randomly selected) have SDV performed on the

231 primary and secondary endpoint CRFs. 100% serious adverse events (S)AEs, serious adverse device effects

232 (S)ADEs and device deficiencies (DD) that are reported in accordance with the study protocol, including

233 potential unreported events for these subjects reviewed.

234 In accordance with ICH GCP guidelines,[13] audits may be performed by the ethics committees and

235 competent authorities during the course of the study.

236

237 **ETHICS AND DISSEMINATION**

238 **Ethical and regulatory compliance**

239 Prior to study conduct, the protocol, proposed patient information, consent form and other study-specific

240 documents were approved by all local ethics committees, with the first approval received in Switzerland in

241 June 2021 (Ethikkommission Nordwest- und Zentralschweiz (2021-00713)). The current protocol version is

242 4.0, approved between October 2022 and March 2023 for the respective trial sites. Changes compared to

243 the first version are mainly concerned with the primary endpoint as explained above and do not include

244 changes in the trial conduct. The trial is sponsored by the Penta Foundation, Corso Stati Uniti, 4, 35127

245 Padova, Italy. The industry partner bioMérieux supplied equipment, consumables and logistical support for

246 the trial.

247 Before commencement of the trial, a risk classification following the ISO 201916 standard and ICH-GCP E6

248 guidelines was carried out. The risk classification of the ADEQUATE Study is defined as negligible, because

249 participation in the intervention group has no significant additional risks compared to the standard of care.

250 This study is registered on https://clinicaltrials.gov (NCT04781530) since 1st March 2021

251 The study is carried out according to the protocol and with principles enunciated in the current version of

252 the Declaration of Helsinki,[14] the guidelines of Good Clinical Practice (GCP) issued by ICH,[13] in case of

253 medical device: the European Directive on medical devices 93/42/EEC and the ISO Norm 14155,[15].

254

255 **Patient and Public Involvement**

256 This protocol was written without patient involvement. Patients or guardians were not invited to comment

257 on the study design or to contribute to the writing or editing of this document for readability or accuracy.

258

259 **Dissemination of results**

260 The data from all centres will be analysed together and published as soon as possible in peer-reviewed

261 journals, as well as being presented at national or international conferences.

262   The results of this trial will be submitted for Open Access publication in high impact peer-reviewed journals

263   likely to be read by health professionals in the management of ARI in children in Europe. The work will be

264   presented at key medical conferences. To maximise the impact of the trial across Europe, its findings will be

265   disseminated more widely through abstracts for oral and poster presentations submitted to some of the

266   main relevant national and international conferences.

267   Findings will further be distributed through activities of the VALUE-Dx consortium's workpackage 6,

268   including press releases, the consortium website and educational activities and materials. The social media

269   presence of the organisations involved will also be used to highlight news about the trial.

270   Datasets generated from the trial will be made accessible in line with regulatory requirements on request

271   to the trial consortium through the corresponding author.

TRIAL STATUS AND DISCUSSION

Currently, 421 children have been enrolled in the trial. Follow-up has been completed for 388 and 22 have missed the 14d and 28d follow-up visit, but data on primary endpoints may still be completed following GP enquiry. Recruitment accrual is at 80% of target.

Following strictly pragmatic trial design decisions, the trial will have limited ability to elucidate the potential mechanism that enables the test to be effective, or prevents it from being effective. For example, the protocol does not provide guidance on interpretation of test results. Clinicians' perceptions about the positive and negative predictive values of the test results for any specific aetiology are therefore not controlled in our trial. In clinical practice, these may change with longer-term trends of changing incidences of pathogens and the trial results may potentially be less applicable under these circumstances. On balance, we believe that this is outweighed by the gain in external validity that a pragmatic trial offers, namely that we expect the trial results to be broadly generalisable because we aimed to reduce introduction of selection bias.

The trial assesses the effectiveness of the diagnostic test in a specific setting, namely used close to the point of care in the emergency department. Patients in the trial's control group may have received the same or similar tests as long as results were only received after more than four hours. The effectiveness of the test may therefore be lower compared to a scenario in which the test was only compared to patients with no respiratory panel assay data available.

A limitation of the rapid syndromic test used is that it is does not cover *S. pneumoniae* or other bacteria considered typical causes of acute lower respiratory tract infection. The trial does not offer any insight into whether such an assay might be effective in the same setting.

A 2014 Cochrane Review found a trend towards reduced antibiotic use with use of rapid syndromic tests in paediatric EDs.[16] Since then, two single-centre randomised controlled trials (RCT), one from Finland and one from the US, found no effect of a similar test as used in our trial on antibiotic prescribing in EDs.[17, 18] Both trials employed a similar strategy of approaching children at an early point in time and before clinical assessment. Our trial differs in that children were not eligible if decisions on their hospitalisation had already been made, including through a fixed treatment guideline or standard operating procedure. Additionally, children were excluded when it was deemed obvious from the start by clinicians that neither

300 antibiotics nor hospitalisation were considered. Also, both trials did not investigate duration of antibiotics,

301 thereby potentially missing an effect on antibiotic use if results from the test would make clinicians more

302 likely to stop antibiotics early. Finally, both trials were designed to show a difference in antibiotic

303 prescribing but did not complement this with decisions to hospitalise patients. Thus, our trial adds to the

304 previous literature

305   -   by employing the same protocol across a range of different settings,

306   -   by studying the intervention in a population in which clinicians express an initial degree of

307   uncertainty about management,

308   -   by treating hospitalisation and its duration as equally important effects of a rapid syndromic test as

309   treatment with antibiotics,

310   -   and by capturing delayed effects of the test on both

311 The trial's primary endpoint was adapted after the start of the trial. Although this is often considered

312 acceptable, it is still a decision that needs careful deliberation and explanation. The ADEQUATE trial was

313 initially designed as two partner trials in EDs, one in the adult and one in the paediatric population. The

314 primary outcomes were planned to be analysed together, thus a safety non-inferiority endpoint with high

315 relevance mainly for the adult population was introduced into the primary endpoints. Because of the low

316 risk of meeting this endpoint, demonstrating non-inferiority was dominating the sample size estimation for

317 the paediatric trial. Following the obligation to restrict the number of individuals in clinical trials to the

318 number necessary to generate robust findings, we decided to move the non-inferiority endpoint to the

319 secondary endpoints as soon as the adult trial was terminated due to changes in routine care making the

320 trial unfeasible.

321 Paediatric ARI is a common condition with diverse aetiology. A diagnostic intervention reducing length of

322 hospital stay and antibiotics has a high potential to (a) reduce strain on healthcare resources, (b) reduce

323 evolution of antimicrobial resistance and (c) improve children's and parents' well-being. The ADEQUATE

324 trial will provide conclusive evidence on the effectiveness of a rapid syndromic test for this purpose.

325

STATEMENTS

A. contributorship statement

Members of the ADEQUATE Paediatric Trial Group contributed to the conceptualisation, data curation,

funding acquisition, investigation, methodology, project administration, formal analysis, software,

resources, supervision and writing of the original draft. All group members contributed to review and

editing of the manuscript.

B. competing interests

Benjamin Hommel, Marie Tessonneau, Sophie Vandepitte, Jean-Louis Tissier, Florence Allantaz and Philippe

Cleuziat are employees of bioMérieux, the manufacturer of the diagnostic tool under study in this trial.

They were involved in the administration of the trial, provided resources and monitored the trial progress.

They were not involved in the design or analysis of the trial. The other authors have no potential conflict of

interest to disclose.

C. funding

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D. data sharing statement

The article does not contain a report on analysed data.

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STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title ✓	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration ✓	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version l225	3	Date and version identifier
Funding✓	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors contributor statement and acknowledgements
	5b	Name and contact information for the trial sponsor named ll225-6
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities contributor statement and acknowledgements
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) contributor statement and acknowledgements
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention ll36-61, 65-7, 255-7
	6b	Explanation for choice of comparators ll56-61
Objectives	7	Specific objectives or hypotheses ll69-72

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| Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) ll69,96,145 |

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained ll73-6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) table 1, ll84-8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered ll100-12
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) n/a
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) n/a
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial ll96-9
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended respective section (l116ff)
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) ll130-41, we decided against a diagram because the structure of FU is very simple in this trial
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations respective section (l142ff)
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size n/a

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions ll108-11
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned ll108-11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions ll108-11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how ll113-4
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial n/a

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol ll130-41
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols ll139-41
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol ll133-4, 202ff
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol respective section (l158ff)
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) referred to SAP

20c   Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) ==referred to SAP==

Methods: Monitoring

Data monitoring   21a   Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed ==acknowledgements==

21b   Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial ==n/a==

Harms   22   Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct ==table 2 and ll216-7==

Auditing   23   Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor ==ll218-9==

Ethics and dissemination

Research ethics approval   24   Plans for seeking research ethics committee/institutional review board (REC/IRB) approval ==respective section (l222ff)==

Protocol amendments   25   Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) ==respective section (l222ff)==

Consent or assent   26a   Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) ==respective section (l83ff)==

26b   Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable ==ll197-210==

Confidentiality   27   How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial ==ll133-4, 202ff==

Declaration of interests   28   Financial and other competing interests for principal investigators for the overall trial and each study site ==funding and competing interests statements==

Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators <mark>data sharing statement</mark>
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation <mark>n/a</mark>
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions <mark>respective section (l239ff)</mark>
	31b	Authorship eligibility guidelines and any intended use of professional writers <mark>n/a</mark>
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code <mark>none available yet</mark>

Appendices

| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates <mark>supplement</mark> |
| Biological specimens | 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable <mark>ll186ff</mark> |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.