Supplementary appendix:

Standardization of stool banking for faecal microbiota transplantation: a consensus report of the multidisciplinary UEG working group

1. **Supplementary file 1:** Working plan project group; predefined questions per subgroup, based on the process of stool banking and clinical application of faecal microbiota transplantation (FMT)

2. **Supplementary file 2.** Statements and recommendations

3. **Supplementary file 3.** Initial donor screening questionnaire for faecal microbiota transplantation (FMT)

4. **Supplementary file 4:** Short questionnaire for donors prior to faeces donation and at repeat (interval) screening

5. **Supplementary file 5:** Additional donor screening for immunocompromised patients

6. **Supplementary file 6:** Example of informed consent form for healthy donors

7. **Supplementary file 7**: Structured questionnaire to collect information about adverse events in patients treated with FMT

8. **Supplementary file 8**: Templates of Standard operational procedures
    1. *SOP INITIAL DONOR SCREENING*
    2. *SOP ADDITIONAL SCREENING OF DONORS AND FMT PRODUCTS FOR IMMUNOCOMPROMISED PATIENTS*
    3. *SOP PROCESSING AND STORAGE OF DONOR MATERIAL*
    4. *SOP APPLICATION AND APPROVAL OF DISTRIBUTION*
    5. *SOP FAECAL MICROBIOTA TRANSPLANTATION*
    6. *SOP handling and reporting of adverse events*

Supplementary file 1. Predefined questions per subgroup, based on the process of stool banking and clinical application of faecal microbiota transplantation (FMT)

Subgroup 1: Donor selection and screening (including statement about reimbursement, retesting /window phase).

To address:

- Redefining minimum criteria for stool screening
- Development of a standardized donor questionnaire
- Requirements for data management concerning donor screening
- Which tests should be repeated to exclude presence of infectious disorders in the window phase?
- When to reimburse donors?
- Should we exclude donors with an (familial) increased risk for the development of microbiota associated disorders?
- Use of medication by donors

Subgroup 2: Processing and storage of faeces preparations

To address:

- Minimum amount of donor faeces? Final volume of suspension?
- Saline? Other dilutant?
- Anaerobic preparation required?
- Is there a need for additional quantative description of suspension?
- Is there a need for a cryoprotectant (glycerol)? Concentration?
- Protocols for processing + tips
- Storage: -80?
- Biobank required?
- Database management of preparations?
- Need for GMP/GLP facilities?
- How should donor faeces preparations be shipped?
- How to store donor faeces preparations after delivery?
- How should donor faeces preparations be handled before use (thawing?)

Subgroup 3: Protocols for FMT

To address:

- How to organise consultation by an expert center/stool bank be
- *C. difficile* treatment:
    - Pretreatment? Antibiotics? Bowel lavage?
    - Upper versus lower GI?
    - Infusion time?
- General statement about protocols for other indications possible?

Subgroup 4: Contraindications and special circumstances: severely ill or immunocompromised patients, food allergy, children, dysmotility

To address:

- Contraindications?
- Severely ill patients?
- Immunocompromised patients: additional testing of donor?

- Food Allergy
- Dysmotility?
- FMT in children

Subgroup 5: Follow-up, regulation and quality assurance

To address:

- What is the minimum required length of FU?
- Standardized FU questionnaire
- Minimum durations of storage of donor data?
- Do we need to store a donor faeces sample? How long?
- How to report outcome and safety data? To whom?
- How to manage SAE?
- How to organize quality auditing?
- How should the production of FMT preparations be regulated?
- How could stool banks be organized? Non-profit vs profit

Supplementary file 2. Statements and recommendations.
The GRADE system (Grades of Recommendation Assessment, Development and Evaluation) was used to grade the strength of evidence (high/ moderate/ low/ very low) and strength of recommendation (strong/ weak). Statements addressing organisational aspects of stool banking were based on expert opinion or law governed.

Classification and organization

- FMT is a medical treatment that should be carried out by registered specialists.
  Quality of evidence: moderate
  Grade of recommendation: strong
- The procurement and processing of donated faeces is best covered within the EU Tissue and Cells Directive (2004/23/EC) with national oversight by national, competent authorities.
  Quality of evidence: n/a
  Grade of recommendation: strong
- Any modifications to the donated faeces other than those necessary for the conservation of the microbial community renders the product made of the donated faeces comparable to a drug that from the applied modification step and forward is best covered within the European directive for medicinal products intended for human use (2001/83/EC). Such modifications include but are not restricted to:
  - Isolation or enrichment of specific components
  - Standardization of the faecal preparation by multi-donor compilation
  - Development into an industrially manufactured product
  Quality of evidence: n/a
  Grade of recommendation: strong
- The responsible person for an FMT center should be a physician with specialist registration.
  Quality of evidence: low
  Grade of recommendation: strong
- A traceable, quality system that relies on the principles of good clinical practice (ICH) should be instated.
  Quality of evidence: law governed
  Grade of recommendation: strong
- All documentation needed to ensure traceability should be kept for a minimum 30 years. Samples from the applied fecal suspensions should be kept for 10 years.
  Quality of evidence: law governed
  Grade of recommendation: strong
- An organizational plan should detail all the intended activities and involved personnel to provide FMT as a treatment.
  Quality of evidence: low
  Grade of recommendation: strong
- The faecal donor should be anonymous to the recipient
  Quality of evidence: low
  Grade of recommendation: strong
- An FMT center should maintain a register to document patient flow, performance, clinical outcome, and safety measures.

Quality of evidence: low
Grade of recommendation: strong

- Clinical trials should be conducted according to the principles for good clinical practice, including the documentation of adverse events.
  Quality of evidence: low
  Grade of recommendation: strong

Recruitment, selection and screening of donors

- Stool donation is a voluntary act. All donors should be informed about the associated risks and benefits and provide written informed consent that covers the provision of personal information, the screening processes, the provision of multiple donations, the storage of donor data in a donor registry and future unscheduled contacts by the stool bank in case of adverse events or for research purposes.
  Quality of evidence: low
  Grade of recommendation: strong
- Unpaid donations should be preferred, as they reduce the risk applicants providing false information during the screening process. Reimbursement for travel to the donation center can be offered.
  Quality of evidence: low
  Grade of recommendation: weak
- Donor screening should be conducted in accordance with a locally approved set of standard operating procedures (SOP) and performed by staff and during timeslots specifically assigned to this task. Meetings between the donor screening staff and the rest of the stool bank team need to be scheduled on a regular basis, in order to identify problems with respect to availability of donations well in advance.
  Quality of evidence: low
  Grade of recommendation: strong
- Data collected during the donor selection process should be documented in a pre-specified set of forms that facilitates the structured display of results obtained during the screening process (oral and analyses) and includes documentation of donor clearance. Paper-based documentation systems are acceptable, but secure electronic systems should be preferred that are auditable, traceable and retrievable.
  Quality of evidence: low
  Grade of recommendation: strong
- All information collected during donor screening, including identity and results, should be stored for at least 30 years. Storage must be in accordance with local data protection regulations.
  Quality of evidence: low
  Grade of recommendation: strong
- Preferably, the entire process of donor screening should be conducted by the stool bank team. Outsourcing to a commercial vendor with a financial interest is possible, as long as the stool bank maintains oversight of all processes.
  Quality of evidence: low
  Grade of recommendation: strong
- Universal donors are be preferred to patient-selected donors. In individual cases, a patient-selected donor may be accepted, if he or she fulfills all criteria determined in the screening process for universal donors and if donor and patient understand the

associated risks, benefits and alternatives.
Quality of evidence: low
Grade of recommendation: strong

- During the first step of the screening process, the medical history and risk behavior of potential donors should be assessed by use of a dedicated questionnaire. The results should be evaluated by a physician. To objectify evaluation, there should be a document clearly identifying the consequence of a specific response for the screening process. The questionnaire should cover the items listed in table 1
  Quality of evidence: low
  Grade of recommendation: strong
- Once a potential donor has been found suitable for further evaluation based on the donor questionnaire and the physical examination, he or she should undergo blood and faeces screening for transmissible pathogens. Screening should cover the tests listed in table 2.
  Quality of evidence: low
  Grade of recommendation: strong
- Once a donor has been approved for donation, he or she should complete a second questionnaire assessing the occurrence of any event that may have occurred between donor approval and the donation. The questionnaire should cover all aspects covered in the initial questionnaire.
  Quality of evidence: low
  Grade of recommendation: strong
- Complete donor screening based on blood and faecal analyses should be repeated at minimum every 3 months
  Quality of evidence: low
  Grade of recommendation: weak
- FMT products should be placed under quarantine until the donor has been found acceptable in a repeat screen.
  Quality of evidence: low
  Grade of recommendation: strong
- If donor screening is performed as recommended in this document, direct testing of the FMT preparations is not mandatory. It may, however, be necessary according to local regulation.
  Quality of evidence: low
  Grade of recommendation: weak

<u>Processing of preparations:</u>

- Donor stool collection and preparation for FMT should follow a standard protocol.
  Quality of evidence: low
  Grade of recommendation: strong
- Stool should be collected in a clean container.
  Quality of evidence: low
  Grade of recommendation: strong
- Stool should be processed to a faecal suspension within 6 hours.
  Quality of evidence: low
  Grade of recommendation: weak

- Pooling of donor faeces during processing is not recommended.
  Quality of evidence: low
  Grade of recommendation: weak
- Aerobically and anaerobically prepared faecal suspensions are considered suitable when preparing FMT.
  Quality of evidence: moderate
  Grade of recommendation: strong
- It is preferred to use ≥ 50 g of stool to prepare a faecal suspension for rCDI treatment. However, concerning cost-effectivity use of 25 gram of faeces could be considered.
  Quality of evidence: moderate
  Grade of recommendation: weak
- Sterile 0.9% saline should be used as diluent to prepare the faecal suspension.
  Quality of evidence: moderate
  Grade of recommendation: weak
- Faeces should be diluted, homogenized and filtered using sterile or clean material (autoclaved or disposable) when applicable.
  Quality of evidence: low
  Grade of recommendation: strong
- Glycerol in an end concentration of 10% should be added to a faecal suspension prior freezing.
  Quality of evidence: moderate
  Grade of recommendation: strong
- Thawing of FMT suspensions at ambient temperature or overnight in the refrigerator is the preferred option. Alternatively, warm water bath with fresh (at least food grade quality) water can be used. Quality of evidence: low
  Grade of recommendation: weak
- Thawed FMT suspensions should be infused the same day, and should NOT be refrozen.
  Quality of evidence: moderate
  Grade of recommendation: strong

<u>Storage</u>

- A completely traceable system should be in place to accurately trace the faecal preparation and current inventory.
  Quality of evidence: law governed,
  Grade of recommendation: strong
- All newly processed faecal preparations should be stored in distinct quarantine sections until all donor screening tests are present.
  Quality of evidence: law governed
  Grade of recommendation: strong
- A registry linking the faecal preparations to the recipients must be in place.
  Quality of evidence: law governed
  Grade of recommendation: strong
- The storage at -80°C or lower is preferred, although short term storage at -20°C is acceptable.
  Quality of evidence: low
  Grade of recommendation: moderate

- Faecal preparations stored at -80°C should be regarded as having a patent shelf life of 24 months (in the future possibly longer).
  Quality of evidence: low
  Grade of recommendation:moderate
- The date of expiration should be registered on the product.
  Quality of evidence: law governed
  Grade of recommendation: strong
- Samples from the donated fecal material or the applied faecal preparation should be stored for 10 years following the application to enable testing in case of AE of SAEs.
  Quality of evidence: low
  Grade of recommendation: strong

<u>Clinical application of FMT:</u>

- Consultation may be offered by stool banks, making use of an expert panel
  Quality of evidence: moderate
  Grade of recommendation: weak
- Before FMT, patients with rCDI are generally pre-treated 4-10 days vancomycin (or fidaxomicin). Antibiotics should be stopped on the day before FMT (> 24 hours before infusion.
  Quality of evidence: moderate
  Grade of recommendation: strong
- Bowel lavage is in general also prescribed before upper GI delivery.
  Quality of evidence: low
  Grade of recommendation: weak
- The choice for a route of delivery of FMT preparations should be based on local preferences and patient characteristics if applicable. Administration via a duodenal tube (upper GI delivery) or colonoscopy (lower GI delivery) appears equally effective. Administration by capsules, nasogastric tube or enemas is also possible.
  Quality of evidence: moderate
  Grade of recommendation: weak
- The infusion of the donor faeces suspension is straight forward and can be performed by by an appropriately qualified individual.
  Quality of evidence: low
  Grade of recommendation: strong
- FMT is indicated in patients with recurrence of CDI following appropriate antibiotic treatment.
  Quality of evidence: strong
  Grade of recommendation: strong
- FMT can be a life saving rescue treatment in patients with severe CDI. Stool banks should have a protocol to facilitate emergency FMT.
  Quality of evidence: moderate
  Grade of recommendation: strong
- FMT for other indications should preferably be limited to the research setting or, in the absence of alternative therapeutic options, compassionate use.
  Quality of evidence: low
  Grade of recommendation: weak

Follow-up after FMT

- Patients should be followed for at least 8 weeks following the FMT.
  Quality of evidence: high
  Grade of recommendation: strong
- Short term (within 24 hours) and long term AE and SAE should be registered and reported to local, national authorities according to established agreements.
  Quality of evidence: law governed
  Grade of recommendation: strong
- An FMT center should be fully equipped to handle all AE and SAE related to FMT.
  Quality of evidence: law governed
  Grade of recommendation: strong
- A (national) registry to collect outcome data and adverse events, and to conduct long term follow-up after FMT seems required to assess the long term safety of FMT for various conditions
  Quality of evidence: low
  Grade of recommendation: strong

Supplementary file 3. Initial donor screening questionnaire for faecal microbiota transplantation (FMT).
This questionnaire is meant to be an example and should be adapted according to local guidelines and risk assessment

Question	Consequence
Risk of infectious diseases	
• Is your current job associated with the risk of contact with human blood or other excretions ? • Is your current job associated with frequent animal contacts (e.g. veterinarian, animal attendant, gamekeeper)? • Are you/have you ever been infected with any of the following infectious diseases? • HIV • Brucellosis • Hepatitis A, B, C, D or E • Tuberculosis • Sexually transmitted diseases (e.g. syphilis, gonorrhea) • Helicobacter pylori • Strongyloidiasis • Malaria • Babesiosis • Leishmaniasis • Leprosy • Trypanosomiasis • Chagas disease • Did you suffer from any infectious disease within the last six months? If yes, please specify. • Were you tattooed or pierced within the past 6 months? If yes, in which country? • Did you receive acupuncture treatment within the past 6 months? If yes, in which country? • Did you experience a needle stick injury within the past 6 months? If yes, what were the consequences with respect to potential infection? • Do you suffer from anal lesions? • Did you receive any blood products (e.g. packed red cells, plasma, platelets, immunoglobulins) within the past 6 months? • Have you ever undergone a tissue/organ transplantation? • Did you undergo any surgery, a biopsy or any other invasive procedure within the past 6 months or is it scheduled? • Have you ever been imprisoned? • Have you ever injected drugs? • Have you ever suffered from alcoholism? • Did you ever receive injections that have not been prescribed by a physician (e.g. muscle building supplements)? • Did you have unprotected sexual contact (vaginal, oral, anal without condom) with a new partner within the past 6 months? • Did you ever have sexual contacts with men having sex with men? • Did you ever have sexual contacts with a person coming from a foreign country? If yes, from which country? • Did you ever have sexual contacts with a person suffering from HIV, HTLV, syphilis or hepatitis?	• Persons reporting previous infection with HIV, TBC, trypanosomiasis or Chagas disease should always be excluded. • For all other responses, exclusion is at the physician's discretion. In their judgement, physicians should consider that diseases that have been adequately treated and cured (except those mentioned above) should not necessarily lead to donor exclusion.

• Did you ever have sexual contacts with a person abusing drugs intravenously? • Did you ever have sexual contacts with a person treated for hemophilia? • Have you ever received payment or other considerations in turn for sexual contacts? • Where were you born?	
Intestinal health	
• Have you ever undergone surgery on your gastrointestinal tract? If yes, which type of surgery?	
• Do/did you suffer from any of the following symptoms within the past 3 months? o vomiting o diarrhea o fever or rash o constipation o bloody stools o mucous stools o abdominal pain	Exclusion is at the physician's discretion.
• Have you ever lived abroad for more than 6 months? If yes, where and when? • Did you travel to a tropical country within the last 6 months? If yes, where?	Exclusion is at the physician's discretion
Comorbidities	
• Have you ever suffered from any of the following diseases? o Gastrointestinal disease o Hepatic disease o Endocrinological/metabolic disease o Cardiovascular disease o Rheumatological/immunological disease o Hematological/oncological disease o Neurological or psychiatric disease o Allergies or atopy o Creutzfeldt Jakob disease (including family history) o Any other chronic disease not listed above? If yes, please specify	Exclusion is at the physician's discretion.
• Were you hospitalized within the last 4 months? If yes, why?	Exclusion is at the physician's discretion.
Possible residues of drugs/medication	
• Are you taking any regular medication or nutritional supplements? If yes, please list.	Contraceptives are accepted as regular medications. For all other medications, exclusion is at the physician's discretion. In this case, care should be taken to exclude drugs and nutritional supplements that

	are likely to be excreted in the faeces.
• Were you treated with antibiotics within the past 3 months? • Did you receive chemotherapy within the last 3 months or is it scheduled?	A positive response to at least one of these questions should lead to exclusion.
• Have you been vaccinated within the past 8 weeks? If yes, which vaccination?	Exclusion is at the physician's discretion.
Age and weight	
• How old are you? • How tall are you? • What is your weight?	Exclusion if: age <18 and >60 years BMI < 20 and > 25 kg/m^2 *
Other	
• For women: Are you pregnant? • If you are found as an eligible donor, is it possible to deliver your faeces within 2 hours after defecation at the stool bank?	Pregnancy should lead to exclusion.

* some stool banks accept donor with BMI > 18 and < 30 kg/ m2

Supplementary file 4: Short questionnaire for donors prior to faeces donation and at repeat (interval) screening

Question	**Answer**
General Questions	
• Have you suffered from diarrhoea since the previous screening? If yes: - How often a day? - For how many days? - Did you have any other complaints? - (E.g. fever, abdominal pain, nausea, vomiting) - If yes, please specify: - Is there any plausible explanation for the symptoms? - If yes, please specify:	Yes/No …. Times …. Days Yes/No
• Have you been ill since previous screening? If yes: - Did you have fever? - Did you have jaundice? - Did you have swollen glands? - Did you have a sore throat? cough? / rhinitis - Other complaints	Yes/No Yes/No Yes/No Yes/No Yes/No Yes/No
• Have you used antibiotics since previous screening?	Yes/No
• Have you been abroad since previous screening? If yes, where?	Yes/No
• Did you have sexual intercourse with men having sex with men since the previous screening?	Yes/No
Donor Information	
Donor code: Time and date of defecation: Time and date of faeces delivered:	
General Information	
Hereby I declare to have filled all questions truthfully: Name: Date of birth: Signature: Date:	Yes/No

Supplementary file 5: Additional donor screening for immunocompromised patients:

Screening from Blood

Viruses	**Parasites**
Cytomegalovirus (CMV) Epstein-Barr Virus (EBV)	Toxoplasma gondii

Serology: EBV IgM/IgG*, CMV IgM/IgG*, Toxoplasma gondii IgM

* EBV- and CMV-tests aim to assess sero-concordance with recipient, but donors seropositive for CMV will be excluded

Screening from Faeces:

Bacteria	**Viruses**	**Parasites**
Plesiomonas shigelloides	Adenovirus Parechovirus Astrovirus Enterovirus (excl. Rhinovirus) Sapovirus	Microsporidia

Supplementary file 6: example of *Informed Consent Healthy Donors*, provided by the Cologne Stool Bank.

Dear Sir/Madam,

You are interested in becoming a feces donor for medical purposes. In this document, we will provide you with information that will help you to make an informed decision on your willingness to undergo screening with the aim of eventually donating your feces. If you feel that this document does not provide all the information you need to provide informed consent, please feel free to request further information from the stool bank personnel.

Why is there a Donor Feces Bank?

Our intestine harbors millions of bacteria, viruses and other microorganisms. These organisms, also referred to as microbiota, are highly important in maintaining different bodily functions in balance. This includes the body's ability to resist invasion of bacteria that can make us sick. In patients who have received antibiotics, this balance can be disturbed, allowing for overgrowth of a bacterium called *Clostridium difficile* in the gut of these patients. These bacteria are able to produce toxins which can cause diarrhea and an inflammation of the gut wall, a so-called colitis. Most of these patients can be treated without further complications, but for some, a "fecal microbiota transplantation (FMT)" remains the only available treatment option. The aim of a Donor Feces Bank is to make safe and efficient FMT products available to these patients.

Another important role of a Donor Feces Bank is to provide FMT products for clinical research. The aim of this research is to further improve the efficacy and safety of FMT products and to identify other situations in which FMT products could help to treat patients. This research may be carried out in collaboration with other institutions or companies.

How does the screening process work?

To become a feces donor, you will need to pass several levels of screening:

1. You will be extensively screened for risk factors of potentially transmissible diseases by use of a questionnaire. It is important for the safety of the patients treated with FMT products derived from your donations, that you complete these questionnaires carefully and truthfully. Otherwise, you may be putting their health at risk. Please be not disappointed, if you are excluded on the basis of this questionnaire. Since FMT is a rather new procedure, we are very careful in selecting our donors, and most of the people who fill in the questionnaire are found unsuitable for further screening.

2. If the questionnaire shows that your lifestyle and health record are suitable for a feces donor, you will be seen by a physician and be subjected to stool and blood analyses, in order to ensure that you do not carry any organisms that could cause diseases in the recipients of FMT products. In detail, the following sampling schedule will be applied for screening:

Insert local standard

3. If the stool and blood tests do not identify any reasons for excluding you as a feces donor, you may start donating feces. Each time you make a feces donation, you will be given another questionnaire assessing any irregularities in your health since the last donation. Just as for the initial questionnaire, it is important that you complete these questionnaires carefully and truthfully.

4. Every *Insert local standard*, the stool and blood tests will be repeated, in order to ensure that your donations are still safe for our patients.

What are the potential risks of donating feces?

During the screening process, you will undergo repetitive blood collections. There is a certain risk associated with every blood sample collection, i.e. pain, secondary bleeding, venous thrombosis or infection at the puncture site. The probability, however, that a blood collection will cause a serious complication, is extremely low.

Screening for bacteria and viruses implies that an infection or a carrier status that was previously unknown to you will be discovered. In this case, you will be informed personally about the results by one of the physicians involved. This physician will, if necessary and desired, also guide you in finding appropriate treatment options. This information will only be disclosed to you and if you wish to your treating physicians. However, in some cases, if we identify a pathogen that requires a report to the the Municipal Health Services, we will be obliged to file such a report.

Follow-up contacts

We might contact you during your time as a feces donor or even after you are no longer donating. The reason for such a contact could be side effects that occurred in a patient who has been treated with FMT preparations based on your donor feces.

Recompensation

Insert local standard

How will my personal data be protected?

As a donor, your name will be kept confidential. Only the staff of the Donor Feces Bank and inspectors of government agencies have access to the medical files regarding donation data (questionnaire and screening results). Donating feces as a volunteer means that you give permission to this access. At the same time, we guarantee that your personal data will not be disclosed to any other persons.

Insert local standard concerning data protection laws

Who can I contact if I have any questions?

Insert local contact

Herewith,I confirm that I have read and understood this document and am aware of the screening procedures and about the implications of becoming a feces donor for medical purposes. I agree to these terms and conditions.

Potential feces donor:

_____ _____

Place, Date First and family name

Supplementary file 7: Structured questionnaire to collect information about adverse events in patients treated with FMT

Question	Answer
General Questions	
• What was your defecation pattern in the period preceding your CDI complaints?	
• What was your defecation pattern before faeces transplantation?	
• Do you rate your current defecation pattern has improved/is similar/or is worsened in compared to the period before the first CDI episode:	Yes/No
• Have you been hospitalized in the period following FMT? If yes, why?	Yes/No
• Did you develop an infection after FMT? If yes: - What kind of infection? - Did you receive antibiotics?	Yes/No
Adverse Events on the Day of FMT	
• Did you vomit or experience nausea? If yes: - Did it stop you from eating? - How often did you vomit? - Did you choke?	Yes/No Yes/No Yes/No Yes/No
• Did you experience bloating? If yes: Mild/Moderate/Severe	Yes/No
• Did you experience belching? If yes: - Did it stop you from eating?	Yes/No Yes/No
• Did you experience abdominal cramps? If yes: - On a scale 1-10, how severe? (1=very mild 10=unbearable)	Yes/No
• Did you have diarrhea? If yes: - How often? - How was the consistency (watery, mushy) - Was blood present?	Yes/No ….. Yes/No
• Did you have any other complaints? If yes, general description of complaints:	Yes/No
Adverse Events on Day 2-6 Days Post-FMT	
• Did you vomit or experience nausea? If yes: - Did it stop you from eating?	Yes/No Yes/No

- How often did you vomit? - Did you choke?	Yes/No Yes/No
• Did you experience bloating? If yes: Mild/Moderate/Severe	Yes/No
• Did you experience belching? If yes: - Did it stop you from eating?	Yes/No Yes/No
• Did you experience abdominal cramps? If yes: - On a scale 1-10, how severe? (1=very mild 10=unbearable)	Yes/No
• Did you have diarrhea? If yes: - How often? - How was the consistency (watery, mushy) - Was blood present?	Yes/No ….. Yes/No
• Did you have any other complaints? If yes, general description of complaints: • If you experienced abdominal complaints after FMT, were they already present before the first symptoms of CDI? • If Yes, did they worsen / improve?	Yes/No Yes/No
Adverse events weeks/months after FMT (week 1 – follow up patient)	
• Did you vomit or experience nausea? If yes: - Did it stop you from eating? - How often did you vomit? - Did you choke?	Yes/No Yes/No Yes/No Yes/No
• Did you experience bloating? If yes: Mild/Moderate/Severe	Yes/No
• Did you experience belching? If yes: - Did it stop you from eating?	Yes/No Yes/No
• Did you experience abdominal cramps? If yes: - On a scale 1-10, how severe? (1=very mild 10=unbearable)	Yes/No
• Did you have diarrhea? If yes: - How often? - How was the consistency (watery, mushy) - Was blood present?	Yes/No ….. ….. Yes/No
• Did you have any other complaints? If yes, general description of complaints:	Yes/No

• Did you suffer from constipation? If yes: - Was it different from prior to your faeces transplantation? - Did you use laxatives? • If you experienced abdominal complaints after FMT, were they already present before the first symptoms of CDI? • If Yes, did they worsen / improve?	Yes/No Yes/No Yes/No Yes/No

Supplementary file 8: Templates for standard operational procedures (SOPs) used for stool banking and faecal microbiota transplantation (FMT). Based on the SOPs provided by the Netherlands Donor Feces Bank (NDFB) and the Stool bank of Aarhus University Hospital, Aarhus N, Denmark

1. *SOP INITIAL DONOR SCREENING3*

2. *SOP ADDITIONAL SCREENING OF DONORS AND FMT PRODUCTS FOR IMMUNOCOMPROMISED PATIENTS*

3. *SOP PROCESSING AND STORAGE OF DONOR MATERIAL*

4. *SOP APPLICATION AND APPROVAL OF DISTRIBUTION*

5. *SOP FAECAL MICROBIOTA TRANSPLANTATION*

6. *SOP HANDLING AND REPORTING OF ADVERSE EVENTS*

1. SOP Donor screening

Rigorous donor screening is mandatory to uphold the highest safety standards, and assure high quality donor faecal preparations. Here we provide the minimal requirements for donor screening

Initial screening should be a 3-step model:
1. medical history and risk assessment
2. faecal sample analysis
3. blood sample analysis

Subsequent screening is performed
1. by interview before each donation (short questionnaire)
2. at three months intervals (or earlier)
    a. using the short questionnaire similar to the questionnaire used before each donation
    b. repeating all stool and blood tests
3. on indication based on complains or travel history of the donor

Intitial donor screening:

Anamnesis / Questionnaire

The following topics should be at least addressed during the initial screening. A template questionnaire is included as separate appendix.

Infectious diseases:
o History or exposure to infectious diseases with chronic activity: particularly human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus (HCV), non-successfully eradicated *Helicobacter pylori*, syphilis, malaria, trypanosomiasis, tuberculosis, Chagas disease, strongyloidiasis. o Any currently active infection or those of relevance within the last 6 months o Life attenuated vaccine within the last 8 weeks o Country of birth
At-risk behavior:
o Current or previous intravenous drug use o Ongoing high-risk sexual behavior o Travel to high-risk foreign countries within the last 6 months o Current occupation in a setting facilitating acquisition of potential pathogens (e.g. veterinarian, animal attendant, gamekeeper, prison worker) o Tattoo, piercing or acupuncture within the last 6 months

o Major surgery within the past 6 months o Contact with human blood (e.g. accident, needle stick injury) within the last 6 months o Previous prison term o Previous tissue/organ transplantation o Transfusion of blood products (e.g. packed red cells, plasma, platelets, immunoglobulins) within the last 6 months
Medical history
o Chronic diseases o (Risk of) Creutzfeldt Jacob disease o Allergies or atopy (e.g. food or drug allergies, asthma) o Hospitalization within the last 4 months o Ongoing pregnancy o Antibiotic treatments, scheduled or received within the last 3 months o Regular medication or nutritional supplements o BMI (accepted if ≥20 and ≤25 kg/m2)[a] o Age (accepted if ≥18 and ≤ 60 years)
Intestinal health
o Previous or scheduled gastrointestinal surgery, except for appendectomy o Gastrointestinal symptoms within the last 3 months (e.g. diarrhoea, constipation, hematochezia, vomiting, abdominal pain), or (removed) adenomatous polyps or sessile serrated lesions o Any other relevant clinical sign or symptom within the last 3 months (e.g. fever or rash)

[a] Some stool banks accept donors with a BMI > 18 and < 30 kg/m2

Minimally recommended faecal sample analysis

The below are recommended; however, testing should be tailored to local pathogen epidemiology

Bacteria	**Test suggestion**	**Exclusion** [d]
Clostridioides difficile	PCR	Yes
Salmonella spp.	PCR	Yes
Campylobacter jejuni and coli	PCR	Yes
Shiga-like toxin-producing e. coli (STEC) stx1/stx2 [a]	PCR	Yes
Shigella spp.	PCR	Yes
Yersinia enterocolitica	PCR	Yes
Helicobacter pylori	Antigen test	Yes

Vibrio species [b]	Culture	Yes
Antibiotic resistant bacteria		
Multidrug resistant Gram-negative bacteria (MRGN), including Extended spectrum beta-lactamase producing bacteria (ESBLs) and Carbapenemase-producing organisms (CPO)	Culture	Yes
Vancomycin resistant *Enterococci* (VRE)	Culture	Yes
Methicillin resistant *Staphylococcus aureus* (MRSA)	Culture	Yes
Viruses		
Norovirus	PCR	Yes
Rotavirus	PCR	Yes
SARS-CoV-2 [c]		Yes
Parasites		
Blastocystis hominis [e]	Microscopy (not PCR)	Yes
Dientamoeba fragilis	Microscopy/PCR	yes
Giardia lamblia	PCR	Yes
Entamoeba histolytica	PCR	Yes
Cryptosporidium parvum	PCR	Yes
Cryptosporidium hominis	PCR	Yes
Strongyloïdes stercoralis [f]	PCR	Yes
Helminths	Microscopy	Yes

[a] Enteropathogenic Escherichia coli (EPEC) testing may be considered in some countries
[b] if visited or residing in tropical country within the last 6 months
[c] COVID (SARS-CoV-2) testing of asymptomatic donors combining serology and stool testing requires further validation
[d] temporary exclusion. For *Entamoeba histolytica* and *Strongyloides stercoralis* additional treatment is needed. For the other indications a rescreening can be performed after 1-6 months.
[e] colonization with *Blastocystis hominis* is not considered an exclusion criterium. However, the working group advises to monitor for the effects of transmission
[f] If residing in or foreign travel to tropical country within the last 6 months

Minimally recommended blood sample analysis

Pathogen	**Test**
Hepatitis A	IgM
Hepatitis B	HBsAg
Hepatitis C	Anti-HCV
Hepatitis E	IgG / IgM
HIV 1/2	Combined HIV antigen/Antibody test
SARS-CoV-2*	
Treponema pallidum	TPHA
General laboratory	CRP, creatinine, ALT, bilirubin, blood cell count

* COVID-19 (SARS-CoV-2) testing of asymptomatic donors combining serology and stool testing requires further validation
several stool banks also include HTLV 1 and 2 testing

Approval

If a donor is approved after interview and extensive analysis of stool and blood, stool donation may start.

Subsequent testing, quarantine period

Upon each donation a short questionnaire should be filled out by the donor to assess the risk of possible pathogens (see separate file in Supplementary Appendix).

A short questionnaire in combination with rescreening with (all) feces and blood tests should be performed every 3 months.

If the donor passes the rescreening all the donated material can be released from quarantine and be used for patient care, taking a window phase of 4 weeks into account. New donations after a repeat screening round are quarantined again until this process has been repeated.

Based on new information provided by the short questionnaire a rescreening could be performed sooner if deemed required by the donor screening staff.

2 SOP: Additional screening of donors and FMT products for immunocompromised patients

Categories:

A (severely immunocompromised):
-Current or foreseeable neutropenia within the next 14d, defined as <500 Neutrophils/µl -Scheduled for allogeneic SCT or having received allogeneic SCT within 100d -Active Graft versus Host Disease requiring immunosuppressive treatment
B (moderately immunocompromised):
-Patients with <200 CD4 T-cells/µl -Prolonged use of corticosteroids at a mean dose of ≥ 0.3mg/kg/d of prednisone equivalent for > 3 weeks -Treatment with other recognized T-cell immunosuppressants, such as cyclosporine, TNF-alpha blockers, specific monoclonal antibodies (e.g. alemtuzumab), MTX or nucleoside analogues during the last 90 days -Inherited severe immunodeficiency (e.g.chronic granuloumatous disease or severe combined immunodeficiency)

Recipients in Group A carry the highest risk of developing complications after transfer of potential pathogens. This risk is considered less pronounced for Group B. However, there is only limited data available on the risks of FMT in patients at different levels of immunosuppression, we recommend that this extensive screening approach should be applied to donors scheduled to provide FMT products to recipients in group A, and should be considered for Group B. At the same time, we highly encourage adverse event documentation in immunocompromised patients. Based on future evidence, categories and associated screening requirements may be re-evaluated.

Additional Screening panel:

Screening from blood

Viruses	**Parasites**
Cytomegalovirus (CMV) Eppstein-Barr Virus (EBV)	*Toxoplasma gondii*

Serology: EBV IgM/IgG*, CMV IgM/IgG*, Toxoplasma gondii IgM

* EBV- and CMV-tests aim to assess seroconcordance with recipient, but donors seropositive for CMV will be excluded

Screening from faeces:

Bacteria	**Viruses**	**Parasites**
Plesiomonas shigelloides	Adenovirus Parechovirus Astrovirus Enterovirus (excl. Rhinovirus) Sapovirus	Microsporidia

3 SOP Processing and storage of donor material

Suggested outline processing of faecal suspensions

Amount of faeces	50 gram, alternatively: 25-30 gram
Processing	aerobic or anaerobic
Diluent	NaCl 0.9%
Cryoprotectant	Glycerol 10%
End volume	50-60 gram: 200 cc 30 gram: 100 cc
Storage	- 80°C, maxium 2 years Temporarily <1 month at -20°C
Timeframe between collection and storage	< 6 hours (rapid processing preferred)

Methods

Reagents

- Sterile saline (NaCl 0.9%)
- Glycerol 50% (as 100% glycerol is too viscous to handle)
- Disinfectants (for example Virkon 1%)
- Aquadest

Material

- Class II Laminar Flow Cabinet
- Homegenizer stomacher: Bagmixer ® (or blender, or mortar and pestle or spatula)
- Bagmixer ® filter bags (mesh between 200-300 µm)
- If additional filtration is needed (in case of other homogenization than bagmixer) than seeve: for example stainless steel sieve (mesh size ±200-300 µm)
- 500ml sterile measuring cup for collection of the sieved faecal suspension
- Vortex
- Scale
- 500ml/100ml sterile measuring cup
- Sterile wooden spatulas
- Sterile 100 ml measuring cup
- Pipetboy
- 1000 µl pipet
- Sterile screw cap tube/scintiliation vial 2-4ml for storage of QC
- 250ml plastic container (for example Nalgene container) suitable for -80°C (select a container which can withstand high pressure, to prevent leakage; for example 12 PSI (pounds per square inch))
- Safety bag
- Labels, suitable for -80°C storage

Donor material

Fresh donor faeces, received within 2 hours after defecation.

Quality control

An aliquot (1-2 cc) of the faecal suspension and the original donor stool are separately stored for quality assurance (in case of suspected transmission of an infectious agents via the faecal suspension).

Delivery of donor stool

On weekdays from.. (time)… the donor delivers his/her faeces to the appropriate desk of the stool bank. Alternatively an employee of the stool bank can collect the faeces from the donor at home. The faeces is collected in a clean "Fecotainer" (http://www.fecotainer.eu/en/) and additionally packaged in a plastic (odor-free) bag. The faeces must arrive at the laboratory within 2 hours after defecation. If it takes more than 30 minutes to hand in the collected fae-ces, storage in a cooler bad or refrigerator is preferred.

The employee of the administration desk:

- Accepts the delivered faeces.
- Supplies the donor with new materials (fecotainer, plastic bag, short questionnaire).
- Informs the technician within 10 minutes that donor faeces is arrived for processing.
- Checks if additional feces screening are requested and arranges this if necessary.

Preparation of faeces suspensions

- Check if donor code corresponds with name.
- After delivery, the donor faeces is immediately processed in a class II Flow Cabinet in semi-sterile conditions.
- Before and after working in the flow cabinet, the cabinet must be cleaned with disinfectants (for example a 1% Virkon solution) and rinsed with aquadest.
- Weigh the faeces and calculate how many faecal suspensions can be prepared. Define in this SOP the amount of faeces needed for each sample. For example:

Total amount of feces	Faecal suspension*	Faeces	Diluent	QC feces	QC fecal suspension	Research sample**
31-61 gram	1	30 gram	80 ml	1 of 1 gram	1 of 1 cc	0-4 of 1 gram
61-91 gram	2	60 gram	160 ml	1 of 1 gram	2 of 1 cc	0-4 of 1 gram
92-121 gram	3	90 gram	240 ml	Consider 2 of 1 gram	3 of 1 cc	0-4 of 1 gram
122-154 gram, and so on…..	4	120 gram	320 ml	Consider 2 of 1 gram	4 of 1 cc	0-4 of 1 gram

*1 faecal suspension, consists of 80ml faecal suspension and 20ml glycerol (50%) (end volume 10%)

**Consider adding glycerol to (part of) research sample for culture

- Weigh …. gram of faeces in a sterile measuring cup.
- Transfer the faeces into a Bagmixer® filter bag, and add a total of …. ml physiological saline (NaCl 0.9%) to the filterbag.
- Seal the bag with a clip, transfer the bag to the Bagmixer®.
- Describe the speed and time settings of Bagmixer® in this SOP. Set the mixing time of the Bagmixer®.
- Transfer the homogenized and sieved faecal suspension with a pipet to the storage container. Describe an accepted volume (…cc) range of the faecal suspension for instance (79-101ml). If this volume is not reached, add the required amount of volume in saline to the faecal residual product and repeat mixing and transfer to the container.
- Add 20 ml glycerol (50%) to the faecal suspension (end concentration 10%)

- Vortex the suspension for 30 seconds.
- For quality control: transfer 1 ml of the faecal suspension with a 1000 µl pipet into screw cap tube. Fill 1 sterile screw cap tubes with 1-2 grams of faeces.
- Prepare … identical labels with: the donor code, lotnumber / identifying code, date of defecation/expiration date, material type (faecal suspension, QC faecal suspension, QC faeces, research sample etc.).
- Put the container with the faecal suspension in a safety bag and seal. Apply also a label on the safety bag.
- Store each sample type in a dedicated (compartment) of a -80 °C freezer (describe for each sample type the dedicated freezer location).
- Record in a donor database the following: data of short questionnaire and manufacturing details (time and date of defecation and storage, Bristol stool scale, amount of faeces, volume faecal suspension, quality controls collected, deviations from SOP etc.)

Transfer from quarantine to biobank:

- Describe the procedure of relocation after the faecal suspensions has been cleared after a repeat screening, and found suitable for patient use.
- Person X gives the sign which faecal suspensions (by identifying code) are cleared
- Relocate cleared faecal suspension to freezer X

4 SOP Application and approval of distribution

A stool bank should have a working group of experts available for consultation and approval of distribution of donor faecal preparations for FMT. Preferably this group consists of at least one gastroenterologist, medical microbiologist, and infectious disease specialist. This group can approve the distribution of donor faecal preparations by the stool bank, and can provide consultation on FMT treatment in patient care. Below a proposed workflow is described for such a competent body.

Indication, mission for issuance and approval of issuance

1. The stool bank will be approached by a practitioner by phone or email regarding the need for an FMT.
2. The requesting physician will be requested to fill in an application form. An individual FMT number will be provided to the practitioner, to handle the patient data anonymously. After a *patient code* is provided, the FMT request can be sent by secured e-mail to the stool bank coordinator
3. If recurrent CDI is the indication (i) and (ii) there are no relative contra indications, the coordinator asks the stool bank affiliated physicians for approval of the issuance. Fast handling of applications is necessary. Experts could discuss via e-mail and should provide a definitive advice within 48 hours.

   Clearly state in this SOP who is responsible (persons / title) for approving the request, and what criteria should be fulfilled for approval.
4. For indications other than recurrent *C. difficile,* or if there are relative contra-indications, an external expert could be approached before the donor faeces may be issued.

Instruction practitioner and collecting patient records

1. The coordinator sends the practitioner the “SOP FMT” by secured email, and aurally describes: responsibility, pre-treatment and preferred route of administration.
2. The practitioner will be advised to do a check up on the patients (at the outpatient clinic) 8-12 weeks after the FMT, and preferably also 6 months after FMT.

Issuing faeces for FMT

The stool bank will plan a transplantation date with the practitioner. The following schedule could be applied:
day 1: register via biobank at the shipping company
day 2: faeces preparation will be retrieved
day 3: faces preparations will be delivered on dry ice to the applicant.
day 4: faeces transplantation

Stool banks need to describe a procedure for emergency release.

Storage after transportation

Once preparations are transferred to a ward or an endoscopy unit for administration, temporary storage at – 20°C is acceptable.

5 SOP Faecal Microbiota Transplantation

Below is described how to perform a FMT in patients suffering from recurrent CDI. For possible future indications deviations from this protocol could be made. In individual cases the stool bank expert team can also advise to deviate from this protocol for patients suffering from rCDI. For severe CDI, the route of administration is based on the clinical judgement of the treating physician.

FMT is the transfer of faecal microbiota from a healthy donor into the gastrointestinal tract of a recipient. The following mild adverse events are not uncommon after FMT but are transient in nature:

- Loose stools immediately after FMT (~ 95%)
- Abdominal cramps (~ 30%) and belching (~ 20%)
- Constipation (~ 20%)

In case of suspicion of transfer of infectious agent via FMT, the stool bank should be immediately notified.

Patient preparation

- Treat the patient with oral vancomycin 125-250mg qid (or fidaxomidin 200mg bid) for 4-10 days prior to FMT. Antibiotics should be stopped the day before FMT (≥ 24 hours prior to FMT).
- A) Upper GI-administration:
    - Treat the patient with 2 liters macrogol (e.g. Klean-prep) the day before the FMT. After the 2 litres macrogol the patient may eat a light meal.
    - The patient should be sober at the day of FMT to place the duodenal probe. This probe can be positioned with the help of an electromagnetic sensory system (CortrakTM) or duodenoscopy. A 50 cc luer-lock syringe should fit on the probe.
    - If the duodenal probe is placed by a duodenoscopy, the position of the duodenal probe could be confirmed with an X-ray.
- B) Colonoscopic administration: In case of colonoscopic delivery the local protocol for bowel lavage can be used.
- C) Administration of capsules: If capsules are used, a bowel lavage is not necessarily required.

Faeces for FMT preparation

- The donor faeces suspension (… cc) for FMT is supplied in plastic containers on dry ice. *Capsules are usually supplied in tubes, usually 30 capsules (based on local protocols) per treatment.*
- Instructions for thawing should take the volume of the faecal preparation into account.
  Thawing of a 198 cc suspension: overnight at 4°C (refrigerator) or for 5 hours at room temperature.
  Capsules do not require more than 15 min. of thawing time.
- If the donor faeces suspension is thawed in the refrigerator or in cold water, the suspension should adjust to room temperature before infusion. Thawing of capsules in water baths is not feasible.
- After thawing the faeces suspensions can be kept up to 3 hours at room temperature and up to 6 hours in the refrigerator. Capsules should be administered as soon as possible after removal from the freezer.
- The donor faeces preparation can NOT be refrozen after thawing.

FMT application

Administration of FMT suspensions

- Draw up the faecal suspension into 50cc syringes which can be connected to the feeding tube e.g. luer-lock, or fits to the working channel of the colonoscope.
- Release all air from the syringes, place a luer-lock cap on the syringe and, if desired, wrap aluminium foil around the syringe so the patient will not see the faeces suspension.
    - A) Upper GI-administration: Consider placing the patient's bed in slight anti-Trendelenburg position. Inject the donor faeces suspension through the duodenal probe slowly (about 2 minutes per 50cc syringe). Inject the faeces suspension slowly in 20 minutes, taking short breaks after each injection. Patients may drink tap water during the procedure. Flush the tube with tap water after FMT and leave the tube in situ for at least 30 minutes after FMT.
      …….. or alternatively, infuse the donor feces suspension through a gastroscope
    - B) Colonoscopic administration: In case of colonoscopic delivery the syringes can be rapidly infused into the work channel of the colonoscope when the most distal point of the endoscopy is reached. Preferably the faecal suspension is delivered in the terminal ileum. Monitor the patient for at least 2 hours after FMT (check p/RR/T for 30 minutes)
- O Monitor the patient for at least 2 hours after FMT (check p/RR/T for 30 minutes)
- The patient should be advised to use the bathroom before leaving the hospital; after FMT loose stools may occur.

Administrion by Capsules

- The patient should be offered sufficient time (usually up to 48h) for ingestion of 30 capsules. As a first step, a small number of capsules should be offered to the patient to familiarize the patient with the preparations. Yogurt or water may be taken together with the capsules to facilitate ingestion. Depending on the ease of capsule intake, a higher number of capsules at a time may be delivered to the patient until all 30 capsules have been ingested.

Follow up:

FMT services should inform the stool bank about treatment outcome and adverse reactions. A structured questionnaire as provided as separate file in the supplementary appendix can be used.

6 SOP Handling and reporting of (Serious) Adverse Reactions

All SAEs and AEs should be reported to the stool bank who provided the faecal suspension for FMT. The treating physician is responsible for reporting all AEs. In general, SAEs are reported within 48 hours after occurring, even if deemed unrelatable to FMT. The stool bank should provide consultation for further handling of the SAE.
SAEs should be discussed within the stool bank and reported to appointed competent body. If an SAE is possibly related to the FMT, all fecal suspension from the same donor will be quarantined until the SAE has been discussed by the stool bank.

The below form can be used to document and report SAEs. Adverse events can be reported using the follow-up form provided in supplementary file 7

Serious Adverse Event Form	**Answer**
Reporting (Treating Physician)	
• Hospital and department: • Name of treating physician: • Report ID: • Report date: • Serious adverse event date: • FMT date: • Type of serious adverse event: • Details of serious adverse event:	
Conclusions (Stool Bank or Steering Committee)	
• Stool bank: • Report ID: • Date Report received: • Date Report discussed: • Donor ID used in FMT preceding serious adverse event: • Suspension ID used in FMT preceding serious adverse event:	

• Serious adverse event confirmed? If no, why? If yes: - Clinical outcome? - Related to FMT? - Test results? - Conclusions? • Recommendations to for future prophylaxis?	

References:

1. Directive 2004/23/ec of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells: European Union, 2004.
2. Guide to the quality and safety of tissues and cells for human application, 4th Edition. Strasbourg: European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM), 2019.