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ORIGINAL ARTICLE

Establishing a donor stool bank for faecal microbiota transplantation: methods and feasibility

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Abstract

Faecal microbiota transplantation (FMT) is a promising treatment, but donor selection and implementation in clinical practice are difficult. Here, we describe the establishment of a donor stool bank based on the Tissue Act. Stool donors were recruited among blood donors and asked to donate five times in a month. A screening questionnaire, a medical interview and testing of blood and stool were conducted before and after donations. Donations were made at home and transported to the lab, where 50 g of stool was suspended and filtered in saline and 20-mL glycerol (final concentration of 10%) to a volume of 170 mL. The processed stool was assigned a batch number, frozen within 2 h after defecation and stored at − 80 °C for up to 1 year. All steps were documented and cross-checked before donor stool were released for clinical use. Thirteen donors were eligible at the first interview and started donations. Two donors were excluded due to a positive *Helicobacter pylori* test, two withdrew consent and one was lost to follow-up. One donor took a single dose of NSAIDs 2 days prior to a donation, which was discarded. There were no other excluding findings at the second interview or testing. Eight of the 13 donors were approved as stool donors. All donated five times with each donation yielding 1–6 portions. Eighty-four portions were released for clinical use. Recruiting stool donors among blood donors is safe and effective. The Tissue Act yields an appropriate regulative framework for FMT.

Keywords Faecal microbiota transplantation · FMT · Donor stool bank · Donor selection · The tissue act · Blood donors

Introduction

Faecal microbiota transplantation (FMT) is a treatment method, where donor stool is introduced in the gut of a patient with the purpose of restoring a disturbed gut microbiota to improve health [1]. FMT has been proven to be an effective treatment for recurrent *Clostridioides difficile* infection (CDI, former *Clostridium difficile* [2]) [3–5], but it might also be effective for other diseases and currently, there is a large research activity on the subject [1].

However, FMT is difficult to implement in a clinical setting. Especially, there are difficulties in selecting the right stool donors in an effective and safe manner.

Selecting stool donors should seek to eliminate the risk of transferring diseases—both infectious and non-infectious. Thus, a thorough screening and testing program of potential donors is needed [1, 6].

Historically, individual donors related to the patient have most often been used [5], but some patients are not able to point out eligible relatives and some relatives may have information about their health, they do not wish to share. This raises ethical issues in this method of donor recruitment but can be avoided by using universal donors, i.e. unrelated anonymous volunteers. Using universal donors can furthermore increase the cost-effectiveness by reducing the need for testing because each donor can donate to several patients.

Keeping donor stool frozen allows easy and quick access to FMT when needed [6, 7]. Frozen donor stool has been shown to be as effective as fresh donor stool for FMT [8–10], thus making banking of donor stool very attractive.

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Several non-profit stool banks have emerged during the last years, including stool banks in the US, Netherlands, UK, France, Australia, Spain, Austria, Hong Kong, Germany, Denmark and more are coming [11, 12].

Donor stool used for FMT consists of several components, including microorganisms, microbial metabolites, food remnants, cell debris and metabolic waste. The mechanism(s) of action of FMT are unknown, though there are several theories. Other than introducing a diverse donor microbiota, also microbial metabolites such as secondary bile acids, short-chain fatty acids and bacteriocins may be crucial for treating recurrent CDI. The mechanism(s) of action of FMT might be different when treating other diseases, such as inflammatory bowel disease [13]. Nevertheless, there is no definition of the active substance(s) in donor stool and there is an inherent variation between stools. These features make it difficult to characterise donor stool and FMT within present legislation. Some argue that it should be considered as a drug or as a tissue, while others suggest that FMT should be regulated as a category for itself, like blood donation [14, 15].

In Europe, there have been no general announcements on the regulation of FMT and for now, this issue remains in the hands of the national health authorities [11, 14, 16].

Establishment of a donor stool bank should focus on traceability and safety of the donor stool. It should not only include strict screening and testing for infectious and non-infectious diseases, but also include a system for handling and reporting adverse events and potential long-term effects.

In Denmark, there is currently no legislation on FMT, but the health authorities urge clinicians to follow the principles of The Danish Tissue Act [17]. The Danish Tissue Act derives from the European Union Tissue and Cells Directives and is also used for regulating stem cells, sperm cells, bone and other human cells and tissues. Here, we describe a setup with strict procedures based on the principles from this Act.

The purpose of this article is thus to describe and address the feasibility of the establishment of a frozen donor stool bank in eastern Denmark, i.e. Centre for Faecal Microbiota Transplantation Zealand (CFMT Zealand). The donor stool bank has been developed by experts in the fields of clinical microbiology, clinical immunology, infectious diseases and gastroenterology.

Methods

Donor selection and testing

Potential stool donors were recruited among healthy active Danish blood donors aged 18–65 years. Thus, stool donors were recruited among an altruistic background population with no significant chronic diseases or infectious risks. Only donors with normal weight (BMI 18.5–25.0 kg/m^2), normal bowel habits (1–2 daily of normal consistency) and with no use of antimicrobial therapy in the past 6 months [18, 19] were eligible for stool donation. There was no economic compensation involved.

Potential stool donors were contacted at a visit in the blood bank and given oral and written information. To ensure eligibility, they filled in a screening questionnaire, including questions on medical history, medication use and risk of infectious disease as known from screening before blood donation. These questions were supplemented by questions about bowel habits, BMI, use of antimicrobial therapy and MRSA screening questions (Table 1). A medical doctor conducted an interview based on the answers. If potential donors had one or more positive answers to these questions, details on the circumstances were obtained. Potential implications of the circumstance were judged using the criteria for blood donation complemented by literature on different factors' impact on the gut microbiome. The donor was immediately excluded if the situation could inflict any safety risk or risk of harmful impact on the gut microbiome. If a blood donor was eligible at the medical interview, donation could begin.

Donors were requested to donate five times in a month. Blood and stool tests were performed when donations started and repeated at the last donation (Table 2).

If there were any abnormal findings in the tests, the donor was excluded and the received donations discarded. Before consenting, the donors were informed about the risk of having an abnormal finding. In case of findings needing further investigation or treatment, the donor was referred to relevant specialists by the responsible medical doctors.

Donors were instructed to contact the doctor in case of any changes in health, but all donors were also re-interviewed by phone after the last donation to uncover any health changes during the donation period.

All donated material was kept in quarantine until the final approval of the donor and until the material could be released for clinical use (Fig. 1).

Donation

At recruitment, donors received equipment and instructions for donating stool at home. At-home-donation was chosen for convenience for the donors. Donors were offered a mild laxative if needed (10-mg oral bisacodyl in the evening before donation or 5-mL rectal sodium citrate+sodium lauryl sulfoacetate just before donation).

Donations were made directly into a plastic container (RPC Superfos SuperLift®, product no: 8032), which fitted in the toilet bowl. The container was labelled with a unique donor-id and their social security number. Furthermore, donors provided the label with date and exact time of defaecation and signed the donation to confirm their identity. The donation container was packed in a plastic bag and placed into a box fitted for the

Table 1 Screening questions for potential stool donors before and after donations

Questions derived from screening before blood donations
Since the last blood donation…
- Have you been sick?
- Have you been examined or treated by a doctor?
- Have you travelled outside of Denmark, Scandinavia and Germany?
- Have you received a blood transfusion?
- Have you been vaccinated?
- Have you had icterus, infectious hepatitis, malaria or syphilis?
- Have you taken any medication in the past 2 months? (Which medication)
- Have you taken any pain killer medication in the past week? (Which medication)
- Have you been pregnant? (Date of birth/abortion)
- Have you had your ears or any other body part pierced?
- Have you received acupuncture, been tattooed or had scarification done?
- Have you been exposed to HIV infection (as described in the information material for blood donors from the Danish Health Authorities)?
- Does anyone in your household have infectious hepatitis?
- Have you been to the dentist in the last 2 weeks?
- Have you ever used intravenous drugs or shared a syringe or needle with others?
- Have you ever had sexual contact with another man? (Question only for men)
- Do you feel completely healthy?
Supplementing questions specific for stool donors
- Do you have 1–2 daily bowel movements?
- Is the consistency of your stool normal (smooth and shaped like a sausage)?
- Have you taken any antibiotics in the past 6 months?
- Height and weight (for calculating BMI)
Screening questions for MRSA:
- Have you ever been tested positive for MRSA?
- Have you had any contact with a person with MRSA in the last 6 months?
- Have you been admitted and/or treated at a hospital or clinic abroad (outside Scandinavia)?
- Have you or a household member had weekly or more frequent contact with live pigs in the last 6 months?

BMI, body mass index; *MRSA*, methicillin-resistant *Staphylococcus aureus*

purpose. Thus, the stool did not need to be transferred and the multilayer packaging prevented contamination.

Immediately after defaecation, donors notified the lab and a taxi transported the donation. The maximum allowed time from defaecation to freezing of processed stool was 2 h. Recruited donors lived in proximity to the lab (< 30-min drive) to minimize transportation time.

Processing of donor stool

Dedicated and chlorine-disinfected lab facilities were used.

At arrival, the donation was inspected, confirming correct and intact packaging, clear identification and signature of the donor. If any irregularities were noticed, it was documented, and if there was any risk that the quality and safety might have been compromised, the donation was discarded. The time for defaecation was checked and the procedure performed to allow freezing within the 2-h window.

The procedure of processing donor stool was adapted from Satokari et al. [8]. Instead of the reported 30 g of donor stool per portion, we used 50 g per portion. Processing of the donor stool is shown in Fig. 2. The procedure was performed in a LAF bench. To avoid cross-contamination, only single-use equipment was used, except for the scale for measuring stool, disinfection with chlorine of this and the lab facilities was repeated after processing and only stool from a single donor was handled at a time.

The processed donor stool was labelled with an assigned batch number and an expiry date. The batch number included the unique donor-id and date of processing to secure traceability.

Sampling

Samples for testing were retrieved before processing of the donor stool from the first and the last donation. Furthermore, safety samples of donor stool and from plasma were frozen. These are kept in case a recipient is diagnosed with a pathogen and the donor stool is suspected to be the source. Safety samples are kept for 30 years according to the Tissue Act.

Storage of processed donor stool

Processed donor stool was stored at − 80 °C for up to 1 year.

Donor stool in quarantine was kept separate from donor stool released for use. Each portion was clearly labelled with a yellow quarantine-label, while portions released for clinical use were marked with a green released label.

Access for the − 80 °C freezer was limited by lock and logged. The freezer temperature was monitored and secured by an electronic alarm system.

An inventory list was kept, including the status for each portion.

Releasing donor stool for clinical use in FMT

Before donor stool was released for clinical use, a two-step process was conducted by two appointed medical doctors cross-checking everything.

The first step was final approval of the donor based on interviews and testing. Both screening and testing

Table 2 Tests for potential stool donors before and after donations

Blood tests	
- Leukocytes, differential count, platelets, haemoglobin	
- Immunoglobulin A (IgA)	
- HbA1c	
- Hepatitis A (HAV-IgM), Hepatitis B (HBsAg and Anti-Hbc) and Hepatitis C (anti-HCV)	
- HIV-1 and HIV-2 (anti-HIV-1,2)	
- NAT-screening for HIV, HBV and HCV	
- Syphilis (screening: WR and RPR)	
- Cytomegalovirus, CMV (CMV-IgM and CMV-IgG)	
- Epstein-Barr virus, EBV (VCA IgM, VCA-IgG and EBNA IgG)	
Stool tests	
Enteropathogenic bacteria	*Clostridioides difficile*, *Salmonella*, *Shigella*, *Campylobacter coli/jejuni*, *Yersinia enterocolitica*, *Aeromonas*, diarrheagenic *Escherichia coli* (*E. coli*): Shiga toxin-producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC) and Attaching and Effacing *E. coli* (A/EEC)
Enteropathogenic viruses	Adenovirus, Rotavirus, Norovirus, Astrovirus[a] and Sapovirus[a]
Enteropathogenic parasites	*Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum*[a], *Cryptosporidium hominis*[a] and worms
Multiresistant bacteria	*Extended-spectrum beta-lactamase* (ESBL)-producing *E. coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*, Vancomycin-resistant enterococcus (VRE) and Carbapenemase-producing organisms (CPO)[b]
Other	*Helicobacter pylori* antigen

HbA1c haemoglobin A1c, *HIV* humane immune deficiency virus; *NAT* nucleic acid amplification technique; *HBV* hepatitis B virus; *HCV* hepatitis C virus; *WR* Wassermann's reaction; *RPR* rapid plasma reagin test; *IgM* immunoglobulin M; *IgG* immunoglobulin G

[a] Added in January 2019 due to expansion of the analytical repertoire in the clinical microbiology lab

[b] Added in January 2019 due to increased prevalence in Denmark

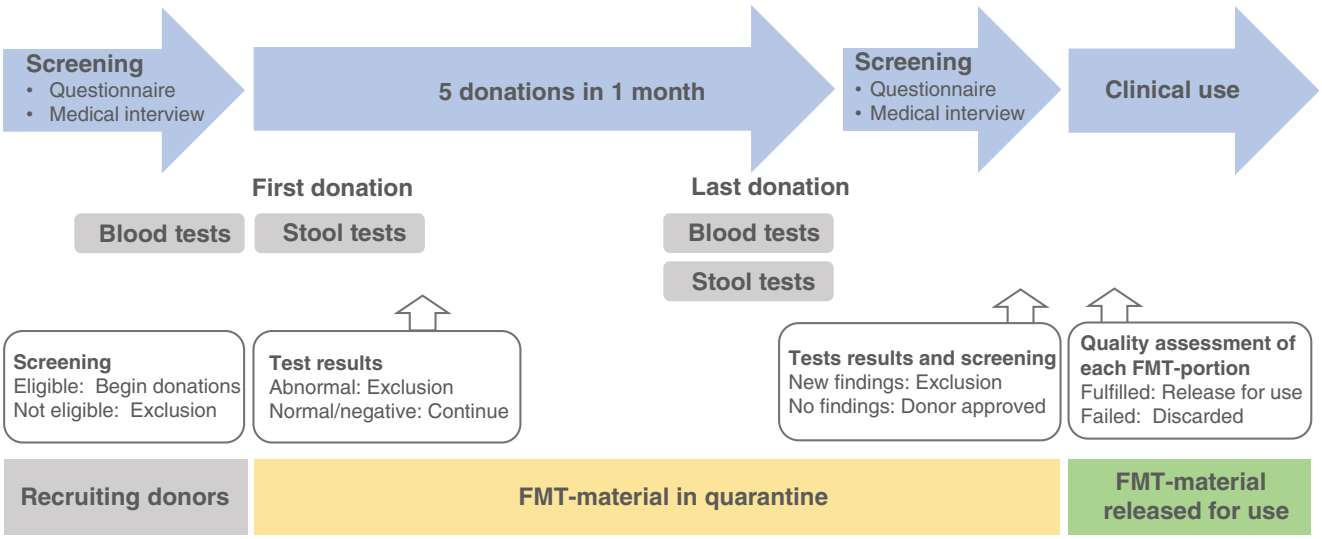

Fig. 1 Algorithm for donor selection and testing. All processed materials are kept in quarantine until the donor can be approved after repeat screening and testing and until the FMT material can be released for clinical use

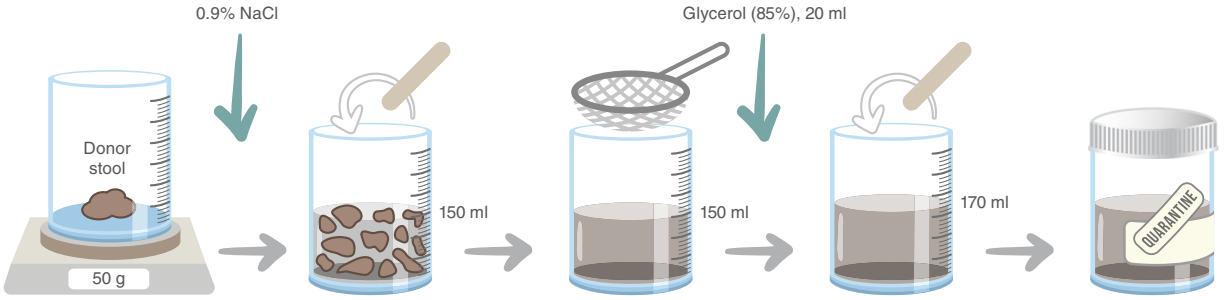

Fig. 2 Processing of donor stool. Protocol adapted from Satokari et al. [8]

results had to be normal. Furthermore, donors could not be approved if they reported any health changes or any events that might compromise the quality and safety of the donor stool. If a donor was not approved, all the donated quarantined stool was immediately discarded.

The second step included a separate release of each single donor stool portion from approved donors. The processing, labelling and storage of each portion of donor stool were cross-checked for deviations or adverse events. Only if all these stages were passed, the portion of donor stool was released for clinical use.

Distribution of donor stool and thawing before FMT

Before use, the donor stool was distributed in batches to predefined treatment sites at clinical wards that perform the FMT procedure. If the donor stool was distributed to another hospital, it was transported temperature-logged on dry ice by personnel from CFMT Zealand. The maximum allowed temperature variation during transport was − 80 to −60 °C. Donor stool was kept at the treatment sites under the same conditions as in the lab.

Before clinical use in FMT, the donor stool was thawed in a warm water bath at 37 °C. The warm water bath was emptied, dried and disinfected after each use to avoid contamination. After thawing, donor stool was transferred to two 100-mL syringes, ready for use.

Documentation and quality control

CFMT Zealand is located in a department of clinical microbiology, which secures that appropriate lab facilities and experience in handling biological material is in place. CFMT has its own dedicated lab in the department and only a few specially trained personnel are involved in the process to secure the needed expertise.

The donor stool bank is based on the principles of the Tissue Act [17]. The setup includes a stringent quality assessment system with detailed standard operating procedures for all steps in the process. All steps are clearly documented in an electronic clinical trial registration and data capture system with automatic backup and audit trail.

The setup focuses on clear donor-recipient traceability, but also traceability of critical equipment (defined as all equipment with direct contact to the donor stool) and traceability of involved personnel and processing. All documentation and safety samples will be kept for 30 years to secure prolonged traceability.

The setup further involves procedures for the treatment sites, including procedures on storage, thawing and administrating the FMT. Only a few dedicated treatment sites offer the treatment in close collaboration to CFMT. There are procedures on handling adverse events (i.e. events occurring in the recipient of the donor stool, events that might affect the quality and safety of the donor stool or events that put the centre's personnel at risk). If necessary, also procedures for withdraw of any donor stool suspected to be involved in risk of disease are in place.

Results

At the establishment of the donor stool bank, 13 donors were eligible after the first medical interview and started donations immediately. The exclusion process is shown in Fig. 3.

The blood bank staff performed short information and prescreening. We do not have any numbers for how many blood donors were invited or evaluated by them. The staff reported that some donors declined due to aesthetic reasons and others due to lack of time. Many blood donors had a BMI

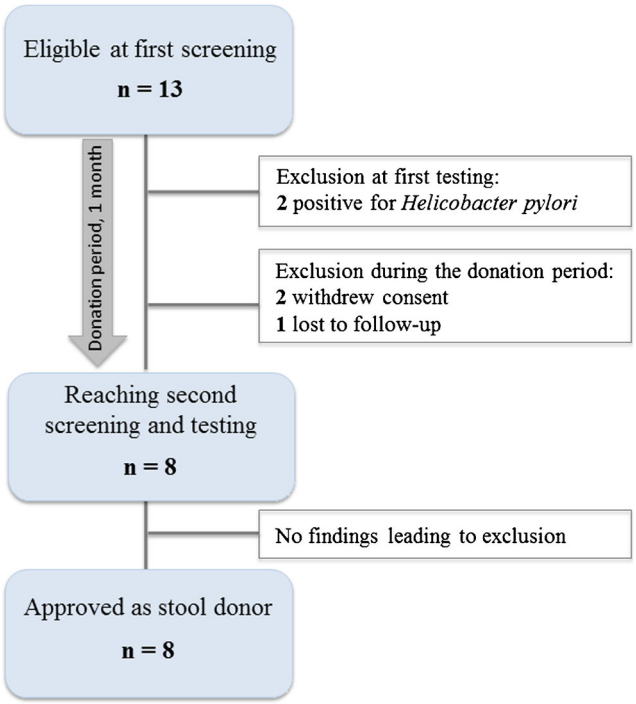

Fig. 3 Flowchart of donor selection and testing

larger than 25 kg/m^2, some did not report normal bowel habits and others had recently received antibiotics or antiemetics.

At the first testing of blood and stool, two donors had an abnormal finding and were excluded—both due to a positive *Helicobacter pylori* antigen test. The two donors were informed of the finding, but since they were both asymptomatic, no further actions were taken.

Two donors withdrew consent because of logistic difficulties, while one donor was lost to follow-up.

At the second medical interview, one donor reported intake of a single dose of NSAIDs 2 days prior to a donation. This was not a reason for excluding the donor, but the donation received a few days after intake was discarded because NSAIDs might affect the gut microbiome [20]. No other excluding findings were made at the second medical interview of the donors and no donors had any abnormal blood or stool test results after the five donations.

Thus, eight donors were finally approved as stool donors. Of these, most were women (75%, $n = 6$), the age ranged between 21 and 52 years (median = 38.5 years), and their BMI ranged between 19.9 and 25.0 kg/m^2 (median = 23.5 kg/m^2).

All eight finally approved donors donated five times each.

Each donation yielded 1–6 portions of processed donor stool (median = 2). Thus, each donor contributed with 7–16 portions (median = 10.5).

Time from defaecation to freezing ranged between 45 and 118 min (median 69 min, IQR = 61–90 min). Thus, the 2-h window was met for all donations.

In total, 98 FMT portions were processed and frozen. Twelve of these were from the two excluded donors and thus destroyed immediately at exclusion. The remaining 86 FMT portions were kept in quarantine until potential release. After discarding the two portions donated just after NSAIDS intake, 84 portions of donor stool were released for clinical use.

There were no deviations in the procedures on processing, labelling and storage of the donor stool and no donors experienced any significant health changes in the donation period.

Donor stool from CFMT has been distributed for use in FMT at two treatment sites with no deviations in the procedures of distributing or transporting the donor stool to the treatment sites, nor deviations in storage or thawing before FMT.

The donor stool has primarily been used for treating recurrent CDI in clinical practice and in a randomized clinical trial. This study compares FMT to rectal bacteriotherapy (i.e. rectal application of a fixed mix of 12 gut bacteria) and to the standard treatment with vancomycin. Results from this trial will be published separately. (ClinicalTrials.gov: NCT02774382).

Discussion

Here, we describe the establishment of a donor stool bank with pretested frozen donor stool for FMT. We recruited stool donors among established blood donors with a setup that included dual medical interviews and testing of blood and stool before and after a donation period of 1 month. Donor stool was kept in quarantine until it could be released for clinical use. The setup includes thorough procedures for safety, traceability, quality assessment and documentation, based on the principles of the Tissue Act, which regulates stem cells, sperm cells and other human cells and tissues.

A common feature in the experience of donor stool banks is a very low rate of finally approved stool donors, ranging from 2.4 to 31% [7, 11, 12, 21–23]. Our experience is not directly comparable with these numbers, because we do not know how many blood donors were invited and prescreened by the blood bank staff. We could finally approve 62% of the donors that were eligible after the first medical interview, where other stool banks report a frequency of 19–63% (from reported absolute numbers) [7, 11, 12, 21, 23].

The difference in the frequency of approved donors can be due to some variations not only in exclusion criteria and testing panel, but also in the background population and, for some banks, a large number of lost-to-follow-up. Some criteria differing between donor stool banks include limits for age and BMI. Also, the testing spectrums for infectious agents vary, e.g. for intestinal parasites [7, 11, 12, 21–23].

The upper age limit differs between donor stool banks with arbitrary cut-offs from 50 to 65 years [7, 11, 22–24]. Studies have shown that the composition and diversity of the gut

microbiota change with age. A study showed that this change was associated with "frailty" rather than with chronological age [25, 26]. When comparing the efficacy of FMT for recurrent CDI using donors < 60 years to donors ≥ 60 years, a study found no difference in the clinical effect and no difference in diversity, even though there were some compositional changes in the elderly donor group [27]. Thus, in a healthy donor population, it seems reasonable to include donors up to the age of 65 years. Donors older than 65 years will probably have a higher risk of known/unknown morbidity.

Some donor stool banks allow BMI to be up to 28–30 kg/m^2 [22–24]. Research suggests that the gut microbiome differs between normal weight and overweight individuals [28]. In mice studies, FMT with stool from obese donors lead to an increase in body weight in recipient mice compared with mice receiving FMT from a lean donor [29]. A single case report described a large weight gain in a mother after FMT from her obese daughter, whereas a study on 173 patients receiving FMT from normal weight, overweight or obese donors reported no difference in the post-FMT weight between the three groups [30, 31]. Thus, the evidence to suggest that an overweight phenotype can be transferred through the gut microbiota is very limited, but it still seems sensible to retain the upper limit of 25 kg/m^2, despite the challenge of recruiting normal weight donors in a healthy western blood donor population.

Some donor stool banks test for the parasites *Blastocystis hominis* and *Dientamoeba fragilis* and exclude the donor if these are found. This counts for a substantial part of the excluded donors in these banks [11, 22, 23]. Several newer studies in Danish and European populations have shown a high prevalence of *Blastocystis* in the general background population, a higher prevalence of *Blastocystis* and *D. fragilis* among healthy controls compared with patients with either irritable bowel syndrome or inflammatory bowel disease and an association between these parasites and high richness and diversity in the gut microbiome [32–36]. Furthermore, eradicating children with *D. fragilis* with metronidazole did not improve their gastrointestinal symptoms compared with placebo in a double-blinded RCT, supporting that this parasite is unlikely to be pathogenic [37]. The practice of excluding potential donors carrying these parasites, thus, might lead to exclusion of healthy good donors.

At CFMT Zealand, we recruit stool donors among blood donors instead of advertising in the general population. Furthermore, donors receive no economic compensation for either blood or stool donations, which, according to the World Health Organization, yields the safest (blood) donors [38]. As reported, only a few donors deemed eligible at the first medical interview had test results or health changes in the donation period that precluded them from participation. Thereby, we could approve a very high rate of these donors. This is probably because active blood donors represent an altruistic and healthy subpopulation compared with donors recruited from the general population through advertisement.

Furthermore, we used a direct approach with contact during visits in the blood bank, which decreased the lost to follow-up rate often seen when using advertisement. Donors eligible at screening begin donation immediately and before testing, but safety is ensured by quarantining all received material. This approach bares an inherent risk of having to discard some processed material, but this has only been necessary for a few portions. Thus, recruitment of stool donors among blood donors appears to be an advantage with more effective access to suitable donors.

A potential limitation to this description is the limited number of donors. Furthermore, we have not registered how many blood donors were invited or prescreened by blood bank staff before the medical interview. Thus, we cannot directly compare the setup to other published stool banks. Even though donors are recruited among blood donors, only a small proportion fulfil the criteria for stool donation with BMI > 25 kg/m^2 as the most frequent exclusion criteria. The process could probably be more effective if potential stool donors are prescreened by the blood bank personnel as an integrated part of the daily reception of blood donors before passing a potential stool donor to a medical doctor. Furthermore, it is possible to invite approved donors for additional donation periods to increase donations per donor.

The applied method of screening and testing potential stool donors was based on recommendations and experiences from other protocols available at the time of establishment [3, 39, 40]. There are currently no international standards on the selection or testing of stool donors. After the establishment of the current donor stool bank, some consensus reports and guidelines on FMT and donor selection procedures have been published [1, 6, 41, 42]. The setup of our stool bank is in line with these recommendations with some minor deviations, including small differences in testing. Our setup was modified in accordance with expert knowledge on local prevalence rates of infectious agents, the background population of blood donors and to comply with criteria from the Tissue Act.

It is important that donor stool banks continuously evaluate the quality and safety of their material and develop their protocols with current knowledge. For example, our centre has recently extended the stool tests to include carbapenemase-producing organisms, because of increasing prevalence in Denmark. Currently, a working group associated with the United European Gastroenterology is in the process of developing a European standard for donor stool banks, which will provide needed guidance [43].

Donors were offered a single dose of an oral stimulant laxative or a rectal osmotic laxative before donation if needed.

We can not exclude that this can affect the microbiota. Bowel lavage with high-dose oral osmotic laxative before colonoscopy can affect the gut microbiome, but to our knowledge, the impact of stimulant laxatives alone or rectal osmotic laxatives have not been studied. [44–46] We do not know to what extent the offered laxative was used, but believe it to be very limited.

Each donation received yielded up to six portions and on average each donor thus contributed with 10.5 portions within a month. Costello et al. reported a similar experience with a single donation resulting in 0–8 FMT portions [23]. To our knowledge, no other donor stool banks have reported the number of portions yielded per donation, per donor or per testing.

The present protocol on processing stool donations was based on the published protocol by Satokari et al. [8]. This group developed and validated a simple preparation procedure with minimal manipulation with manual mixing and filtering through a sieve. We used an amount of 50-g donor stool per portion, instead of 30 g used by Satokari et al., as a review indicated that the success rate of FMT might be smaller if using less than 50 g per treatment [47].

We chose a 2-h time limit between defaecation and freezing of processed stool. Some other published protocols use a limit of 6 h [11, 23, 24]. Studies investigating the viability of gut microbiota have shown diverging results from none to some degradation of diversity within 8 h when stored at room temperature. These studies have not investigated the functions of the microbiota [48, 49] and there are no studies on the impact of time before freezing on the clinical effect of frozen donor stool. Thus, we have chosen the lowest logistic possible time limit between defaecation and freezing.

The processing of donor stool is not performed under anaerobic conditions. Some argue that using anaerobe conditions might be needed to retain the strict anaerobe bacteria [6, 23, 50], while Satokari et al. and other protocols find it unnecessary for treating recurrent CDI. These protocols with preparation in ambient air are clinically validated for treatment of recurrent CDI and have demonstrated transfer of a donor-like microbiome, including anaerobes [3, 8, 10, 51]. However, it is possible that anaerobic processing and preservation of strict anaerobes in donor stool can be pivotal for treating inflammatory bowel disease (IBD), where *Faecalibacterium prausnitzii*—an obligate anaerobe with anti-inflammatory properties—is considered crucial [50, 52, 53]. Only one in three of the RCTs that demonstrated that FMT is more effective than placebo for achieving remission in ulcerative colitis prepared the stool under anaerobic conditions [53–55]. This study achieved a similar effect as the others, but with a less intensive dose regimen, which could be due to increased preservation of anaerobes [53]. Nevertheless, more studies are needed to determine the role of strict anaerobes and anaerobic preparation of donor stool for FMT for different clinical indications.

In CFMT Zealand, processed and released donor stool are stored for up to 1 year. Even though storage for up to 2 years does not affect the clinical effect according to the experience of some large donor stool banks [11], validation studies on duration of storage are lacking. To our knowledge, the use of frozen donor stool for FMT has only been clinically validated for storage up to 10 months and the culturable viability of the donor stool has been validated for up to 6-month storage [9]. Thus, we chose a compromise with storage duration of 1 year.

Guidelines for standardisation on donor stool processing and storing are lacking. There is very little evidence in the field and limited validation of the methods both in vitro and in vivo. In addition, current knowledge is based on FMT for recurrent CDI. There might be other considerations if used for other indications. More studies are needed to address and compare different protocols on donor stool processing and storing to yield the optimal methods to obtain the best clinical effect of FMT.

Even though donor stool banks are now established around the world with strict criteria and other safety measures for donor selection and stool processing, it is not possible to entirely eliminate the risk of transferring disease, especially non-infectious diseases associated to gut microbiota changes. These diseases can be asymptomatic at the time of donation or microbiota changes can exist many years before debut. This is important to keep in mind when choosing an indication of FMT and judging risks versus benefits for patients. Further work is necessary to refine FMT and identify active substances to allow more specific treatments such as bacterial cultures or treatment with relevant microbiotic metabolites.

Conclusions

Recruitment of stool donors among blood donors is feasible and safe. Establishment of a donor stool bank with pretested frozen material allows effective and quick access to FMT, which is preferable in daily clinical practice. A setup based on the principles of the Danish Tissue Act and the European Union Tissue and Cells Directives yields an appropriate framework for quality assessment, safety and traceability of donor stool.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The project was approved by The Regional Ethics Committee for Region Zealand, Denmark (SJ-478). The project was conducted in accordance with the Helsinki Declaration (1964) and its later amendments.

Informed consent Informed consent was obtained from all stool donors at recruitment.

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