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How do I Establish a Stool Bank for Fecal Microbiota Transplantation within the Blood- and Tissue Transplant Service?

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to TRANSFUSION.

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ABSTRACT

Worldwide, there is a rising demand for thoroughly screened, high quality fecal microbiota transplantation (FMT) products that can be obtained at a reasonable cost. In the light of this evolving therapeutic area of the intestinal microbiota, both private and public stool banks have emerged. However, some of the larger difficulties when establishing stool banks are caused by the absence of or international disagreement on regulation and legislative formalities. In this context, the establishment of a stool bank within a non-profit blood- and tissue transplant service has several advantages. Especially, this setting can ensure that every step of the donation process, laboratory handling, and donor-traceability is in agreement with the current expert guidelines and meets the requirements of the European Union’s regulative directives on human cells and tissues. Although safety and documentation are the top priority of the stool bank setup presented here, cost-effectiveness of the production is possible due to a high donor screening success rate and the knowhow, infrastructure, facilities, personnel, and laboratory- and quality management systems that were already in place. Overall, our experience is that a centralized, non-profit, blood- and tissue transplant service is an ideal and safe facility to run a stool bank of high quality FMT products that are based on stool donations from volunteer, unpaid, healthy, blood donors.

KEY WORDS

Faecal microbiota transplantation
Stool donor
Clostridioides difficile / Clostridium difficile
Chronic inflammatory diseases
Blood- and tissue transplant service
ABBREVIATIONS

FMT = Fecal microbiota transplantation
CDI = *Clostridioides difficile* infections
LIS = Laboratory information system
FDA = Food and Drug Administration
EU = European Union
SOPs = Standard operating procedures
AEs = Adverse events
DIN = Donation identification number
INTRODUCTION

Fecal microbiota transplantation (FMT) encompasses the transfer of gut microbiota obtained from donor stool into a recipient’s intestine with the principal aim of restoring a disturbed microbial community (dysbiosis).¹ In recent years, FMT has experienced a renaissance within the health community due to its tremendous efficacy in the treatment of recurrent Clostridioides difficile infection (CDI).² With a concomitant rising attention towards the composition and function of the intestinal microbiota and its proposed role in the pathophysiology of various cardiometabolic- and chronic inflammatory diseases, trials exploring new indications and applications of FMT are currently being undertaken.³,⁴ This booming of FMT worldwide has created an expanding demand for easy and rapid access to high quality and safe FMT while still maintaining reasonable costs.⁵ To overcome the logistic barriers of such a large-scale donor-dependent operation, the idea of banking feces within public blood banks has recently been put forward.⁶

Here we describe how a stool bank of frozen feces successfully was established within a non-profit blood- and tissue transplant service by making use of the existing infrastructure, the certified laboratory facilities, and the trained personal who are experienced in biosafety procedures of handling other “substances of human origin”. Furthermore, the in-place quality management systems and laboratory information systems (LIS) provided a crucial basis for complying with the standards of quality, safety, and transplant traceability which are laid down in the regulations of human cells and tissues products. Our experience is that this setup has several advantages including quick and easy implementation, access to donor recruitment within a well-known population of healthy, trust-worthy individuals and a resulting high donor screening success rate, complete documentation and traceability during the production and distribution, timely and securely delivery to FMT treatment centers, high cost effectiveness, and available supportive research logistics and resources.
LEGISLATION AND REGULATION

A major challenge to safeguard patients and donors when trying to establish FMT into clinical practice is the complex nature of feces. Moreover, FMT is a non-standardized and still-evolving treatment and as such, the regulation governing the use of FMT is in nascent stages. Hence, no international regulatory harmonization or common legal framework concerning the donation of human feces exist. Indeed, the spectrum of regulatory statuses ranges from strictly regulated to non-existing. For example, the United States Food and Drug Administration (FDA) has declared that FMT is a biological drug product which must be administered under the regulation of an investigational new drug (FDA enforcement policy [FDA-2013-D-0811]). In contrast, according to the legal opinion of the European Union (EU) feces is not a medical drug or a medical device (EU Commission statement [CASoHO E01718 1641864]). Nor does it fall within the scope of the current EU Tissues and Cells Directive (2004/23/EC) as the human tissues and cells contained therein are not the active component of the substance. Consequently, individual European member states are free to regulate FMT on a national level (European Directorate for the Quality of Medicines & HealthCare guidelines [Guide to the quality and safety of tissues and cells for human application; 4th Edition 2019]).

Although the regulative requirements differ depending on the location of the FMT center, expert guidelines addressing the screening process, the donor recruitment procedure, the laboratory preparation, the clinical application, and the requirements of safety and documentation are available. Indeed, both European and international expert recommendations of stool banking and FMT methodology have been published. In addition, a complete clinical application framework for FMT that complies with the European Tissue Act has been suggested. Such expert guidelines and clinical frameworks are currently the best starting point to decide on the practical setup when establishing a stool bank. Especially in countries lacking active regulative oversight of FMT, the blood- and tissue transplant service facilities can ensure that precautionary measures...
and rigorous safety standards of every step in the handling of donor feces are being addressed and properly managed and documented in accordance with the regulative directives of human cells and tissues (e.g. the European directive 2004/23/EC). Other stool bank setups and the implementation of an FMT service in a blood centre has previously been described. Here we provide a further detailed description of the practical implementation of a centralized stool bank within an existing non-profit blood- and tissue transplant service.

HOW TO GET STARTED
The storage of frozen, allogenic feces donated from anonymous, healthy individuals is the fundament of our stool bank. This setup enables a thorough screening a head of time as well as renders flexibility and stability of the deliveries for both planned and semi-acute FMT. Furthermore, the use of anonymous donors secures that the confidentiality and impartiality of the donor and the recipient can be maintained. Last but not least, donor anonymity removes the risk of coercion that patient-selected donors may experience when asked about helping a friend or relative. A logistical challenge that should be addressed early on when planning to establish a stool bank within a blood- and tissue transplant service is that the handling of unsterile fecal donations must be performed in designated rooms to separate these from the remaining production. Most of the other practicalities and equipment that needs to be in place when starting up a stool bank is already close at hand within the existing facility or can easily be purchased at low costs.

Our methods for material processing and safety precautions adhere to the basic principles for documentation and safe preparation of human material, including detailed protocols in securing the materials, maintenance of standard operating procedures (SOPs) for the laboratory preparation, use of certified laboratory testing, definition of quality control tests, and standards for the release of the final product. We found that such requirements can easily be met in the current organization of blood and tissue transplant services where LIS for the traceability of the products
from the donor to the recipient is mandatory, and where SOPs, registration of equipment, and documentation of competences for the personnel involved in the stool bank can be handled in quality management systems that are already in place.

SCREENING CRITERIA

The donor screening program forms the cornerstone of minimizing the risk of transferring harmful substances during FMT. However, the definition of a ‘healthy’ stool donor and what comprises a healthy gut microbiota from an inventory standpoint remain unclear. Therefore, setting up a centralized stool bank in an already existing non-profit blood- and tissue transplant service should be a collaborative effort involving a multi-disciplinary expert panel consisting of specialists from various medical fields including transfusion and/or tissue transplantation medicine, medical gastroenterology, clinical microbiology and/or infection medicine. The screening criteria should adhere to the national regulatory guidelines, if such exist, or follow the most updated international expert consensus guiding principles addressing both known infectious diseases as well as potential microbiota-associated diseases. In addition, other relevant screening markers should be considered according to the local infectious pressure including the prevalence of antibiotic-resistance bacteria strains.14

Furthermore, to enhance the chances of selecting a donor with a healthy, stable, and diverse fecal microbiota, we suggest that age and weight criteria (body mass index) as well as factors indicating a healthy lifestyle such as no stress, no extreme diet or dietary supplements, no smoking, no alcohol intake, no medication or non-prescription drugs, and no antibiotic use within a certain period prior to donation should be considered as relevant screening criteria in accordance with the best available evidence. Such reflections could especially prove vital if the donation is intended for patients with non-CDI disease where the microbiota might be less dysbiotic and more resistant towards the introduction of a new microbial community.
RECRUITMENT STRATEGY

Regardless of the exact context of the screening program, our experience is that making only blood- and plasma donors eligible for screening has several benefits. Firstly, regular blood donors are motivated to donate voluntary and without receiving any compensation. Secondly, blood donors usually live healthy lives and will often be able to meet the lifestyle requirements stated in the screening program thereby providing a sufficient recruitment base. Thirdly, recruitment of stool donors among healthy individuals who are being screened frequently increases the success rate of passing the laboratory screening tests. Finally, the trust in unpaid, volunteer donors is high which is of great importance since a substantial part of the screening process is based on health- and lifestyle-related questionnaires.

By making use of the in-place infrastructure, we have succeeded in recruiting donors through advertisement posted in the donor waiting area of the blood bank. Here, the staff provides information about being a stool donor. If the donor consents to take part in the screening process, a blood bank physician performs the initial risk assessment and medical interview. Our experience is that nearly one third of the donors who responds to the stool donor advertisement at our blood bank facility ends up fulfilling all stool donor requirements. Similar eligibility rates ranging from 20-30% have been reported in other selected subpopulations. These rates are substantially higher than the screening results of the general public that have been reported to be as low as 3-10%. However, due to a substantial variability in donor recruitment and selection protocols, no firm conclusion on the best recruitment strategy can be drawn based on these studies.

SCREENING INTERVALS

In contrast to blood-, plasma- and tissue donations, each stool donation is not linked to a complete screening performed on the donation date. Instead, the current expert guidelines recognize that an
initial screening followed by a rescreening at the end of the donation period combined with a health statement signed by the donor in connection with each donation is sufficient to document a safe interval without changes in the screening criteria. Currently, there exists no international consensus on the optimal duration of one donation cycle. Indeed, some stool bank facilities operate with a certain number of donations before performing the rescreening procedure while other sites operate with a donation cycle of a predefined duration.\textsuperscript{10,11} However, as all donations in a cycle must be discarded in case of any positive tests following the last donation or in case the donor reports any changes in health status during the donation period, we recommend a relative short period between the first and final screening. This is in line with data from an American public stool bank (OpenBiome) revealing that a decrease in the screening interval from 60 to 36 days reduced the costs by 10%.\textsuperscript{19} At our stool bank facility, a donation cycle of 30 days with an estimated donation rate of eight to ten donations per cycle has proven cost-effective.

DONATION AND LABORATORY PREPARATION

Each donation is assigned a unique donation identification number (DIN) using ISBT 128, the international standard for the terminology, identification, coding and labelling of substances of human origin. Using the LIS of the facility (ProSang, CSAM, Stockholm), the DIN and product codes are connected to the complete record of the donor. In connection with each donation, our laboratory personnel verify and document the donor identity and that the packaging, transportation and labelling requirements have been met. In addition, the donor signs a short statement confirming no changes in health status or other screening criteria and verifies date and time of defecation. To ensure traceability, all critical equipment is registered within the LIS in connection with the collection and preparation of each donation. Most of the equipment is single-use, and the reusable equipment is cleaned and autoclaved prior to the stool preparation to minimize the risk of cross contamination.
Our current laboratory practice for processing donor stool and preparing the material for CDI FMT is described in a detailed manufacturing protocol that follows the most updated consensus expert guidelines which has been described elsewhere. In short, feces is suspended in sterile saline (0.9%) and then blended and filtrated before a cryoprotectant (e.g., glycerol) is added up to a final concentration of 10%. However, the detailed practical guidance on how to process donated stool will not be described in detail here as these recommendations are regularly updated and may vary depending on the disease indication and the choice of the end product type (capsules or liquid solutions) and FMT method. Instead, we will stress several critical parameters of the preparation that could prove vital for the success rate of FMT. Such elements include the total time from defecation to the stool bank freezer, the ambient temperature, and exposure to oxygen during transport and laboratory handling.

Over the years, a six-hour FMT protocol describing the timing of the manual stool handling from defecation to freezing has been widely applied because of the resulting transplant’s high curing rate in the treatment of CDI. However, due to new automatic purification systems such as the GenFMTer, the preparation time can be shortened to one hour. Still, the clinical impact of this shorter production time has yet to be enlightened. Equally, the most optimal room temperature during preparation remains to be established. Next, homogenization of stool in ambient air has been shown to significantly reduce bacteria viability and the ability of microbiota to produce short-chain fatty acids such as butyrate. This finding might especially be important when targeting inflammatory diseases. Likewise, some bacteria may die or be destroyed during a prolonged blending process while other bacteria will benefit from this approach due to less need for filtration and the resulting maintenance of a larger amount of fibrous material within the transplant solution. Finally, although banking of processed frozen donations that requires only minimal preparation before transplantation simplifies the practical handling and logistics, freezing could potentially harm the effects of FMT for non-CDI diseases.
Overall, there is a great demand for more research into the effect mechanism(s) of FMT, strategies to facilitate long-lasting engraftment (if desirable), and the development of quick-tests that can characterize the donor microbiota in terms of viability, diversity and functionality which may further assist the matching of donor and recipient. Filling this knowledge gap will expectedly pave the way for individualized laboratory handling based on the characteristics of the donor, the patient, and the disease target with the potential to improve the clinical outcome of FMT. Consequently, central stool banks should have the resources to be at the forefront of the FMT practice and be able to meet new product demands.

LABELING, STORAGE AND QUARANTINE HANDLING

Following the laboratory preparation, we apportion the FMT product obtained from one donation (batch) into tubes (splits) containing approximately 10 g of feces each. Each tube is clearly labeled with the donation number of the specific batch (DIN) and the product code including an additional split number. For this purpose, the ISBT 128 coding system has proven very suitable. Indeed, we use the internationally defined product code for fecal microbiota, namely W0002 (Figure 1). By linking this product code to the rest of the donation number, the end user of the FMT get all relevant information about the exact donation site and date, overall donation data, and product specification. The generation of a corresponding bar code allow us to scan the complete donation code into our electronic LIS.

The EU regulations of Blood and of Tissues and Cells define strict traceability demands that need to be met: patient, donor, and the product must be recorded in a traceable manner for at least 30 years after the treatment has been performed. In order to fulfill these requirements, the ISBT128 code handled in an electronical LIS is particularly appropriate. Another advantage of the donation management in the LIS is that with strict rules and algorithms for the release of products, this system clearly distinguishes between products in quarantine and released
products. Furthermore, the storage system itself (i.e. the freezers) also secures a clear distinction between quarantined donations awaiting approval of the complete rescreening process and released donations that are delivered within a verified safe donation cycle. Finally, our storage facilities are strictly controlled, and the temperature of the freezers is monitored and secured by a centralized electronic alarm system with the possibility to track all temperature data linked to each specific donation.

STORAGE LIFE
To date, no studies have specifically examined the timely microbial durability of frozen feces dispersed in saline and 10% glycerol at −80 °C. Nor has it been verified how donation viability tests as well as other proposed donation quality tests relate to the clinical efficacy of FMT in various diseases. Consequently, the optimal storage life for frozen donations has yet to be clarified. The current practice for defining an expiration date of frozen feces is based on clinical experience of efficacy and safety of FMT performed in patients with CDI. Unfortunately, although the effect of FMT may be affected by the storage time of the transplant, this aspect is seldomly reported in randomized controlled trials of FMT. Nevertheless, it is widely accepted that the transplant material can be stored at −80 °C for one year, and most likely much longer, without any notable reduction in its curative abilities of CDI. Interestingly, as part of a clinical quality assurance study to document the effect of the fecal transplant produced at our stool bank facility, we observed clinical and microbiological resolution of CDI following FMT applied by colonoscopy in patients with recurrent disease treated with material that have been stored for more than two years at −80 °C (data not published). Still, more research is needed to investigate a possible overall reduction in efficacy rates over time.

RELEASE AND DISTRIBUTION
The release of the final product adheres to the standards of tissue and blood donation. Only products that have been documented to fulfill the release criteria within the LIS can generate the required transplant record. The transplant record contains the unique donation number (DIN) and product code that links to information of the specific donation, the laboratory preparation, the FMT recipient, and the time of the procedure. Based on this record data, each FMT is registered in the LIS. At our FMT center, five tubes (splits) containing a total of 50 g of feces are being transferred to the recipient during one FMT. When preparing the final FMT solution, we seek to pool splits from the same batch. However, if there are less than five splits remaining from the same donation date, we allow that splits from the same donor collected on different dates in the same donation cycle can be used. Like other donation procedures, we generate a transplant record for each split that must be checked against the label on the split tube. Moreover, the person who perform the transplantation must verify that the identification of the FMT recipient matches the transplant records.

RISK MANAGEMENT AND EFFECT MONITORING

One of the main concerns about the use of FMT is that multiple recipients could be adversely affected by a currently undetectable infection or transmissible process. Hence, principles for control and surveillance of blood- and tissue products should be followed, such as the EU Guideline to Good Manufacturing Practice, Annex 20 on Quality Risk Management. This includes systems capable of addressing risk identification and performing risk analysis, risk evaluation as well as risk control. An approach to risk management could be the mapping of all processes on a fishbone diagram (https://www.skymark.com/resources/tools/cause.asp).

For example, to enable the tracking down of a potential transmitted pathogen, we register all critical equipment in the LIS and store archive samples of donor feces collected before and after the laboratory processing. This procedure is crucial in the case of suspected infectious
adverse events (AEs), where we must be able to conduct a ‘look-back’ exercise to identify if the donor or the laboratory processing was the source of the infection. If severe AEs is believed to be attributable to FMT, the national health authorities (or other applicable regulatory agencies) must be informed. At present, there exists no international control and surveillance system of AEs in relation to FMT.

In addition to these requirements, we store additional fecal samples for quality and quantitative characterization, which can be coupled to a data management setup allowing a structured follow-up of patients that have received FMT. This quality assurance setup will help us generate more knowledge about potential long-term side effects as well as assist the identification of effect mechanisms and prognostic markers. Finally, the implementation of LIS registrations throughout the entire process enables the production of annual activity reports for active, regulatory governing and accrediting inspections by health authorities.

COSTS
A recent study calculating the total costs related to FMT performed at a publicly funded hospital revealed that the high effectiveness and reduction in hospital costs the year following FMT - when performed in patients with recurrent CDI - more than make up for the extra costs of a single FMT compared to antibiotic treatment. Still, keeping the costs of FMT low is important to make FMT an attractive and accessible alternative to standard medical treatment. The costs of the complete screening per donation cycle (first and final screening) at our stool bank facility are approximately USD 6000. Due to the high screening success rate that we have obtained within the population of blood- and plasma donors, less time is used for screening and less costs are related to the hematologic, serologic and microbiologic analyses performed on candidates that do not pass the screening. Furthermore, we can perform most of the non-fecal analyses at our own laboratory at relative low costs due to high sample quantities collected from both blood-, plasma- and stool
donors. Moreover, when using frozen feces, one donor can serve for multiple FMT donations. Finally, we do not compensate the donors which further reduces the costs.

Naturally, the overall costs to produce FMT material will vary between donors and between different donation cycles of the same donor due to variation in donation frequency, and size and content of the donation. In this respect, the selection and retention of donors with stable deliveries are crucial. Nevertheless, if 1000-1500 g of stool is collected during a cycle of 30 days, the costs for producing one transplant product containing 50 g of feces will be approximately USD 590 (Table 1). This is substantially below the costs reported from other nonprofit stool banks such as the American OpenBiome facility (USD 1595 per dose),25 and the Netherlands Donor Feces Bank (USD 1155 per dose (EUR 1050)).11,26

CONCLUSION
The demand for FMT is rising worldwide. New treatment indications and novel treatment delivery regimens comprising frequent administration as well as individualized, disease-directed FMTs might become the future. This rapid evolvement of the field of FMT requires that legislation and clinical consensus guidelines are regularly updated followed by fast clinical implementation. To enable an upscaling of the production process and improve the safety, quality and innovation of next-generation (customized) FMT products while keeping costs low, a flexible setup, great logistic resources including access to LIS and quality management systems, accumulation of expertise, a considerable product flow, systematic data collection, and high-level research facilities are required. Our experience is that a centralized, non-profit, blood- and tissue transplant service is an ideal facility to accomplish this task. Finally, transparency and access to efficacy and safety data for regulators and providers are essential. Hence, a common international regulatory framework which can ensure an active regulatory oversight should be pursued. Likewise, an international control and surveillance system of adverse effects of FMT is highly needed.
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laboratory preparations of the donations.
REFERENCES

FIGURE LEGENDS

Figure 1. Transplantation record and product label.

Table 1. Average costs of one FMT product (50 g feces). All amounts are in USD.

<table>
<thead>
<tr>
<th>RESOURCES</th>
<th>COSTS PER FMT PRODUCT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruitment, donor information and medical interview (worktime)</td>
<td>8</td>
</tr>
<tr>
<td>Hematologic, serologic and microbiologic screening‡</td>
<td>246</td>
</tr>
<tr>
<td>Utensils and reagents</td>
<td>89</td>
</tr>
<tr>
<td>Registration, laboratory processing and issuing (worktime)</td>
<td>81</td>
</tr>
<tr>
<td>Quality assurance (worktime)</td>
<td>5</td>
</tr>
<tr>
<td>Overhead costs including facilities, storage and other indirect costs∫</td>
<td>161</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>590</strong></td>
</tr>
</tbody>
</table>

* All amounts have been converted from DKK to USD using an exchange rate of 655 DKK per USD 100.
‡ The microbiologic screening analyses underlying the cost calculation have previously been reported.27
∫ Overhead also include costs related to failed screening, product waste and information to rejected donors.