Review

How to: Establish and run a stool bank*


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Abstract

Background: Since 2013, several stool banks have been developed following publications reporting on clinical success of ‘faecal microbiota transplantation’ (FMT) for recurrent Clostridium difficile infections (CDI). However, protocols for donor screening, faecal suspension preparation, and transfer of the faecal suspension differ between countries and institutions. Moreover, no European consensus exists regarding the legislative aspects of the faecal suspension product. Internationally standardized recommendations about the above mentioned aspects have not yet been established.

Objective: In 2015, the Netherlands Donor Feces Bank (NDFB) was founded with the primary aim of providing a standardized product for the treatment of patients with recurrent CDI in the Netherlands. Standard operation procedures for donor recruitment, donor selection, donor screening, and production, storage, and distribution of frozen faecal suspensions for FMT were formulated.

Results and discussion: Our experience summarized in this review addresses current donor recruitment and screening, preparation of the faecal suspension, transfer of the faecal microbiota suspension, and the experiences and follow-up of the patients treated with donor faeces from the NDFB.

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Background

Clostridium difficile, recently reclassified as Clostridioides difficile [1] is capable of inducing diarrhoeal disease (C. difficile infection, CDI) through production of secreted toxins [2]. After CDI treatment, the risk of a recurrence within 8 weeks is 15–25%, which rises to 40–65% in patients with multiple recurrences [2,3]. Recurrences are associated with clinically severe diarrhoea and persistent disturbance of the colonic microbiota [4]. Faecal microbiota transplantation (FMT) is therefore a very effective treatment for...
recurrent CDI, with cure rates close to around 85% [5,6]. Large-scale implementation of FMT in daily clinical practice is hampered by lack of easily available donor faeces and safety concerns. A centralized stool bank can overcome these hurdles.

**Aim and structure of a non-profit stool bank**

The overall and primary aim of a stool bank is to provide on a (inter)national or regional level, ready to use, high-quality donor faeces solutions to treat patients with recurrent or refractory CDI. Second, a central stool bank should enable careful monitoring of treatment outcome, side effects, and long-term effects of FMT. Therefore, the stool bank should preferably be facilitated by a well-equipped biobank to store an aliquot of the donor faeces, and samples of all delivered faecal suspensions, to guarantee traceability in case of adverse events. A stool bank is ideally entwined with a clinical microbiological department as the expertise and equipment to perform both various screening tests, and to process faecal suspensions is already present. As FMT is not yet an approved, treatment modality by the European Medicines Agency (EMA) or US Food and Drug Administration (FDA), commercial stool banks are not the preferred suppliers. A stool bank working group should consist of experts in the fields of microbiology, infectious diseases, gastroenterology, biobanking, and methodology, and if donor faeces is considered to be a drug, pharmacology. An overview of the currently existing donor faeces banks is depicted in Table 1. Similar to the NDFB, most of the donor banks are non-profit and primarily use FMT for treatment of patients with recurrent CDI.

**Legislation of a donor faeces bank**

There is still considerable confusion about the regulatory aspects of FMT [7–10]. The FDA dictates that adequate informed consent must be obtained before use of FMT products [11]. In the

<table>
<thead>
<tr>
<th>Location, founded</th>
<th>Legislation</th>
<th>Donors</th>
<th>Products</th>
<th>Indications</th>
<th>No. of issued products</th>
<th>Contact address and website</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leiden University Medical Centre, The Netherlands 2015</td>
<td>Allowed for CDI, no legal guideline</td>
<td>Healthy unrelated donors, unpaid</td>
<td>Fresh frozen stool samples</td>
<td>Recurrent/ refractory CDI Pilot study for IBS Clinical trial for MDR bacteria CDI not responding to standard therapies Clinical trials for all other indications</td>
<td>31</td>
<td><a href="mailto:info@NDFB.nl">info@NDFB.nl</a> <a href="http://www.ndfb.nl/">http://www.ndfb.nl/</a></td>
</tr>
<tr>
<td>OpenBiome, Somerville, MA, USA 2012</td>
<td>Regulated as an investigational biologic, ‘enforcement discretion’ permits use of FMT for rCDI without IND</td>
<td>Rigorously screened universal donors; compensated $40 per donation</td>
<td>Fresh frozen stool samples in three delivery formats: upper delivery, lower delivery, and oral delivery (capsules)</td>
<td>Recurrent/ refractory CDI</td>
<td>23 000</td>
<td><a href="mailto:Info@openbiome.org">Info@openbiome.org</a> <a href="http://www.openbiome.org/">http://www.openbiome.org/</a></td>
</tr>
<tr>
<td>Birmingham, UK 2015</td>
<td>MHRA manufacturers’ licence needed for clinical trial use Special licence for CDI Officially under MHRA as a medicinal product</td>
<td>Healthy unrelated donors, unpaid</td>
<td>Fresh frozen stool samples</td>
<td>Recurrent/ refractory CDI</td>
<td>&gt;200</td>
<td>PHE Public Health Laboratory Birmingham <a href="mailto:bhs-tr.HP@bhs.net">bhs-tr.HP@bhs.net</a></td>
</tr>
<tr>
<td>Portsmouth, UK 2013</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>70</td>
<td><a href="mailto:fmt@porthosp.nhs.uk">fmt@porthosp.nhs.uk</a></td>
</tr>
<tr>
<td>Saint-Antoine Hospital, AP-HP, Paris, France 2014</td>
<td>Allowed for CDI (considered as a drug) Clinical trial for other indications</td>
<td>Healthy related or unrelated donors, unpaid (paid for clinical trial)</td>
<td>Fresh frozen stool samples (frozen since July 2015)</td>
<td>Recurrent CDI Clinical trial for Crohn’s disease</td>
<td>55</td>
<td>Prof. Dr. Harry Sokol Gastroenterology Department, Saint-Antoine hospital <a href="mailto:harry.sokol@aphp.fr">harry.sokol@aphp.fr</a> Clinical Microbiome Research Group, Dr. Maria J.G.T. Vehreschild Department of International Medicine, University Hospital, Cologne Dr. López-Sanromán, Gastroenterology, Hospital Ramon Y Cajal, 28034 Madrid</td>
</tr>
<tr>
<td>University Hospital Cologne, Germany 2014</td>
<td>No legal guideline</td>
<td>Healthy, unrelated donors, unpaid</td>
<td>Frozen preparations for endoscopic application, enema or in capsules</td>
<td>Recurrent CDI</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>Hospital Ramón y Cajal, Madrid, Spain 2016</td>
<td>No legal guideline</td>
<td>Healthy related or unrelated donors, unpaid</td>
<td>Fresh frozen stool samples</td>
<td>Recurrent CDI, in principle local patients only</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Medical University Graz, Austria 2012</td>
<td>Allowed for CDI based on national guideline Other indications need ethics committee board approval</td>
<td>Healthy related and unrelated volunteers. Clinical trials compensated with €50.-/donation</td>
<td>Fresh and frozen faecal samples ready to use for lower GI endoscopy</td>
<td>Recurrent CDI Severe CDI Idiopathic colitis Colitis in critical ill patients Clinical trials for UC, IBS, GVHD</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>Asia Microbiota Bank, Hong Kong 2016</td>
<td>No legal guideline</td>
<td>Healthy unrelated donors, paid</td>
<td>Frozen processed microbiota samples (no fresh or whole stool samples available clinically)</td>
<td>Recurrent CDI Primary CDI Clinical trial for IBS, IBD and MDR bacteria</td>
<td>In process, to be determined</td>
<td><a href="mailto:health@asiabiobank.com">health@asiabiobank.com</a> <a href="http://www.asiabiobank.com">www.asiabiobank.com</a></td>
</tr>
</tbody>
</table>

CDI, *Clostridium difficile* infection; IBS, irritable bowel syndrome; MDR, multi-drug-resistant; IND, investigational new drug; MHRA, Medicines & Healthcare products Regulatory Agency; UC, ulcerative colitis; GvHD, graft versus host disease.

* Commercial, social enterprise.

* Until 1 April 2017.
European Union (EU), a standardized policy is lacking and each member state is allowed to have its own policy. In The Netherlands, FMT is currently regarded as an unclassified treatment approach, which is allowed (if applied safely), for patients with recurrent CDI, or in the context of an approved investigational study protocol.

Although FMT appears to be a typical transplantation product to most experts in the field [12], it does not fulfill the criteria for guidance by the EU tissue and cell transplantation act, because the cellular component of FMT appears not to be the active substance. Furthermore, human excretions are excluded by the US act for tissue and cell transplantations. As a consequence, several European countries considering donor faeces to be a drug (Table 1), which has major regulatory implications negatively influencing future availability and pricing of donor stool solutions for FMT. Application as a drug has the consequence that the proposed drug would have to be identical in active ingredient, dosage form, route of administration, quality, and performance characteristics. However, the complexity of the microbial community in stool and the variability across stool samples makes it impossible to guarantee contents from batch to batch. Furthermore, it would have the consequence of putting faecal material for use in FMT under the jurisdiction of hospital pharmacies, requiring storage of the faecal product in the pharmacy itself. In this regard, common and consultation of the experts in the field may hopefully result in adjustment of the EU law in concordance with the rapid scientific developments, enabling future status of donor faeces as a transplantation product.

**How to recruit donors?**

Historically, FMT donors were conveniently selected among close relatives and friends of patients with the underlying idea that they would have at least a partially shared microbiome, increasing the chances of success [13], and limiting the risk of pathogen transmission [13,14]. However, later evidence showed that FMT with donor faeces from unrelated donors was as effective [5,15]. This finding provided an opportunity for a better standardized, safer, faster, and cheaper method of donor selection, screening, and faecal suspension preparation.

The NDFB acquired many potentially interested donors after announcing the opening of the first Dutch stool bank via local and national media (e.g. paper, national news). One of several options for recruitment of faeces donors is from established blood donors, as this has the advantage of previously screened, healthy, and motivated volunteers. An important difference in donor recruitment in The Netherlands and most other European countries (except Germany) compared with the USA is that it is prohibited to offer a paid reimbursement for blood (or stool) donations. This prohibition is in line with the blood donating advice of the World Health Organization, which states that the safest blood donors are voluntary, non-remunerated donors [16]. As it is important to limit the time between defecation and delivery of the faeces, to preserve as many anaerobes as possible, donors should be recruited in the near proximity of stool banks, such as non-healthcare workers of the hospital and personnel of companies in the neighbourhood.

**Donor screening by questionnaire and interview**

All potential donors are extensively screened by a questionnaire and a personal interview concerning risk factors for transmissible diseases and factors influencing the intestinal microbiota (Table 2). The NDFB has applied an arbitrary age limit of 18 to 50 years, assuming that above the age of 50 years a significant increase of comorbidities with a less stable microbiota can be present [17]. A body mass index (BMI) > 25 kg/m² is also an exclusion criterion, as obesity may also be associated with a specific microbiota composition [18]. Moreover, one case-report, and an experimental animal study suggesting new-onset obesity after infusion of donor faeces of an overweight donor have been reported [19,20]. Any other gastrointestinal disorder (e.g. irritable bowel syndrome (IBS), Crohn’s disease, or ulcerative colitis) also qualifies as an exclusion criterion of donation [21]. Other exclusion criteria that have been shown to be related to aberrant microbiota composition are depicted in Box 1 [22]. The list of exclusion criteria is likely to expand in the future when other conditions are found to be associated with an altered microbiota composition.

**Box 1**

**Aim and exclusion criteria of the donor screening by questionnaire**

**Aim:** Risk assessment of faecal- and/or blood-transmitted diseases and illnesses associated with a disturbed microbiota

**Exclusion criteria:** Age < 18 or ≥ 50, BMI < 18.5 or > 25 [19,20], high risk faecal- and/or blood-transmissible diseases, recent antibiotic use (<6 months) [57,58], gastrointestinal complaints (e.g. diarrhoea, obstipation, or irritable bowel-like symptoms) [59–61], recent travel to endemic areas of gastrointestinal pathogens, (first-degree relative with) inflammatory bowel disease [62], GI malignancy [63], first-degree relative with a GI malignancy < 50 years, substantial comorbidity, various medications, autism [22,64,65], autoimmune disorders [66], neurological disease [67,68].

**Table 2**

<table>
<thead>
<tr>
<th>Laboratory screening serum</th>
<th>Laboratory screening faeces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis A (IgM + IgC)</td>
<td>Clostridium difficile (PCR)</td>
</tr>
<tr>
<td>Hepatitis B (HBsAg + anti-Hbc)</td>
<td>Helicobacter pylori (antigen test)</td>
</tr>
<tr>
<td>Hepatitis C (anti-HCV)</td>
<td>Bacterial gastroenteritis: (PCR, followed by culture) Salmonella spp., Campylobacter jejuni, C. coli, Shigella spp., Versinia enterococctica and Y. pseudotuberculosis, Aeromonas spp., Plesiomonas shigelloides, and Shiga Toxin-producing E. coli</td>
</tr>
<tr>
<td>HIV (anti-HIV, type 1 and 2)</td>
<td>Antibiotic-resistant bacteria (culture); ESBL and/or carbapenemase-producing bacteria, vancomycin-resistant enterococci, and mexitillic-resistant Staphylococcus aureus</td>
</tr>
<tr>
<td>Lues; Treponema pallidum (Ig)</td>
<td>Viral pathogens (PCR): Norovirus serotype I-III, Astrovirus, Rotavirus, Adenovirus 40/41, Adenovirus non-40/41, Enterovirus, Parechovirus, Hepatitis E</td>
</tr>
<tr>
<td>Cytomegalovirus (IgM + IgG)</td>
<td>Parasites (PCR): Giardia lamblia, Entamoeba histolytica, Cryptosporidium parvum and C. hominis, Microsporidium spp., Strongyloides⁴</td>
</tr>
<tr>
<td>Epstein Barr Virus (IgM + IgG)</td>
<td>Microscopy for ova, cysts, and larvae [69]: e.g. Blastocystis hominis</td>
</tr>
<tr>
<td>Strongyloides (IgG1/IgG4)⁴</td>
<td>⁴ If travel history to Middle and South America, Africa, or Asia.</td>
</tr>
</tbody>
</table>
Donor screening by laboratory tests

Extensive laboratory analysis should be performed to identify potential pathogens transmissible by faecal transfusion. An overview of all tests performed by the NDFB is shown in Table 2. The pathogens included in the blood-screening programme correspond with the screening protocols for blood donors and are generally agreed among the different stool banks [14,15,23–27]. However, screening protocols for detection of specific microorganisms in the intestinal tract differ between stool banks, and evolve with time and new insights, as there is no consensus guideline. This applies, for example, to screening for the presence of multi-drug-resistant (MDR) organisms, including ESBL- and carbapenemase-producing bacteria, vancomycin-resistant enterococci, and methicillin-resistant *Staphylococcus aureus*. Screening for the (asymptomatic) presence of rotavirus is not routinely performed by stool banks, but as rotavirus is often found in asymptomatic donors, especially in winter, we included this in our protocol [26,28]. Adenovirus type 40/41, Sapovirus and Astrovirus are associated with mild gastro-intestinal diarrhea and are therefore also screened [29]. Enterovirus and Parechovirus are usually asymptomatic but can cause skin disease (and foot-and-mouth disease), pneumoconiosis, myocarditis, and meningitis [30,31]. Adenovirus non-40/41 can cause myocarditis [32]. In addition, faeces is screened for hepatitis E, which is often found in asymptomatic (blood) donors [33]. To prevent transmission and development of systemic infections, potential donors are screened with PCR for all the above-mentioned viruses (see also Table 2 for the total list of pathogens).

The significance of *Dientamoeba fragilis* and *Blastocystis hominis* as enteropathogens is less clear [34–37]. *D. fragilis* and *B. hominis* are commonly found in faecal samples of both symptomatic and asymptomatic individuals [37,38]. Prevalence varies considerably depending on geographic location, the group studied, and diagnostic methods used [34]. The cell wall of *B. hominis* is fragile and disrupts easily; storage of microscopically positive stool samples in 10% glycerol at −80°C results in complete lysis and negative microscopy after the samples are thawed and reinvestigated (unpublished observation). Despite the uncertainty of *B. hominis* and *D. fragilis* pathogenicity, colonization may be considered an indicator of a suboptimal microbiota composition [39]. Therefore, positive individuals are excluded from donation for NDFB.

The serostatus of the donor is determined for Epstein–Barr virus (EBV) and cytomegalovirus (CMV). Immunocompromised patients will be matched accordingly for safety reasons. However, the risk of transmission is not established and we await the results of ongoing study regarding the risk of CMV transmission resulting from FMT (TRANSDECMV Clin Trial Gov: NCT02694484).

Approximately 2 months after the initial screening, a new donor sample of faeces and blood are screened again, using similar tests as applied at entry of the programme (see Table 2), except for CMV and EBV which are repeated once a year (in case of a negative serostatus). After a successful second screening, the donor faecal suspensions collected until 2 weeks prior to the second screening are released for patient treatment. This quarantine period minimizes the risk for transmissible diseases.

Collection, preparation, and storage of donor faeces suspensions

It is generally believed that a high viability of bacteria in stools increases the chance of successful FMT. As the majority of faecal bacteria are anaerobic, faeces must be processed within 6 hours of defecation [5,6]. To prevent environmental contamination, faeces is collected by the donor in a faecal container (e.g. Fecotainer). For suspension, approximately 60 g of donor faeces is used based on the data of a systematic review suggesting a decreased cure rate with <50 g [40]. The faeces is homogenized with saline using a mortar and pestle, whereas some laboratories use a commercial blender [15,25]. Disadvantages of blenders are difficulties with appropriate sterilization and aerosolization of the faeces. A metal sieve (mesh 300 μm) is used to remove undigested food fragments. The faecal suspension is then concentrated by centrifugation (15 min, 6000 g) [25] and glycerol is added as cryoprotectant to a final concentration of 10% in a total end volume of 200 mL. A recent study showed that frozen faecal suspension is equally effective as a fresh faecal suspension for the treatment of CDI [41]. This allows stool samples to be stored at −80°C for a longer period of time until the donor has been retested prior to actual use of the donor faeces. Clinical success of frozen suspensions is reported until 5 to 6 months of storage at −80°C, but could be much longer, in theory. Like OpenBiome, the NDFB uses a storing period of 2 years.

How to apply safety measures and include quality controls

At the Leiden University Medical Center (LUMC), storage of the FMT suspensions is accommodated by the certified centralized biobanking facility in a specific −80°C freezer with connected alarm notification to guaranteed continuous registration of the storage. In addition, the biobanking facility uses a dedicated biobanking information and management system (BIMS SampleNavigator for coding, registration, tracking, and tracing of the biosamples. FMT suspensions, in combination with a small portion of the original faeces and a 2 cc portion of the FMT suspension, are stored under a unique donor code with a successive suffix number for donation time and date for retrospective quality assessment. Information on the FMT suspension labels includes donor code, suspension number, production and expiration date, volume, and storage temperature instruction. Distribution of the FMT upon granted request by the NDFB is provided by dry-ice shipment through a certified Biologistic Courier service. Registration in a BIMS-related database for the shipped FMT suspensions, including recipient institution and requesstor information, is provided so that biovigilance tracing can be performed in cases of adverse events.

An important aim of the NDFB is to recognize complications of FMT. Therefore, systematic follow-up of both patients and donors is performed with signed informed consents. The NDFB collects recipients’ faeces and clinical data on the day of FMT and approximately 3 weeks after the procedure. Furthermore, clinical information including abdominal complaints, development of diarrhoea, and adverse events (e.g. nausea, bloating, abdominal pain, belching, vomiting) is collected. No systematic long-term follow-up has been scheduled yet to register development of autoimmune diseases, malignancies, and other potentially microbiome-associated syndromes both in donors and patients. However, all faeces and serum samples have been stored in the biobank and remain available for analysis.

How to determine eligibility of patients with recurrent *Clostridium difficile* infection for FMT

As the effectiveness of FMT has only been recognized by the authorities for recurrent CDI, it is extremely important to diagnose recurrent CDI both with the presence of clinical symptoms and positive microbiological tests. Therefore, written requests for FMT treatment with a standardized form are evaluated by at least two clinical members of the NDFB board to determine eligibility of the patient. It is required that patients have a laboratory-documented episode of recurrent CDI following at least one course of adequate CDI antibiotic therapy (>10 days 125 mg vancomycin QID; > 10 days metronidazole 500 mg TID; 10 days 200 mg fidaxomicin BID).
Recurrent CDI is defined as the re-appearance of diarrhoea (at least three unformed stools per 24 hours for 2 consecutive days; or at least eight unformed stools per 48 hours) within 8 weeks of cessation of antibiotic therapy in combination with a positive diagnostic test for C. difficile. We strongly recommend a two-stage testing algorithm, as recently advised by the C. difficile ESCMID diagnostic guideline [42]. In particular, a positive test for the presence of free toxins in faeces samples (e.g. by EIA) is a prerequisite, especially for patients with comorbidity of the intestinal tract, such as inflammatory bowel disease (IBD). If laboratories only use PCR to detect toxin genes of C. difficile, we advise to sending a fresh faeces sample to a reference laboratory for toxin detection, as C. difficile (spores) can persist after successful treatment and may reflect colonization.

For a first recurrence of CDI, it is advised to first treat the patient with another course of antibiotics. Fidaxomicin could be considered because of its potentially relapse-reducing effect resulting from its narrow antibiotic spectrum [43]. In general, FMT is advised in patients with multiple recurrences. However, in some cases of severe, therapy refractory CDI, FMT could be considered for a first recurrence [27,44]. A recently completed study suggests that intravenous bezlotoxumab (a monoclonal antibody against toxin B) protect against (multiple) first recurrence of CDI. However, it is yet unclear which patients really benefit from this very expensive treatment strategy [45].

Pregnancy, severe food allergy, and antibiotic usage other than for C. difficile on the day of expected infusion are exclusion criteria for FMT treatment. Although, recently, the first case report of successful and safe FMT in a pregnant patient was published [46]. All potential risks, benefits, logistics, and procedural details are discussed with the patient by the treating physician.

### What is the procedure of FMT?

If the patient is eligible for treatment with FMT, donor faeces suspension is transported to the referring hospital on dry ice. Prior to transplantation, the faeces suspension is thawed (overnight in a 4°C refrigerator or during 5 hours at room temperature), based on literature and our expert opinion [15,41,47]. The donor faeces suspension may be kept at room temperature for up to 3 hours or refrigerated at 4°C for up to 6 hours. Samples should never be refrozen, because freeze-thaw cycles may compromise stability and efficacy of the sample, possibly because of loss of viability. To eradicate vegetative cells of C. difficile, prior to FMT (until 1 day before the procedure), patients receive vancomycin (125–250 mg QID) for a minimum of 4 days, followed by 2 L of bowel lavage 1 day prior to FMT [5]. Whether bowel lavage can be excluded from the protocol is currently a matter of discussion, as recent reports have shown similar efficacy for FMT without bowel lavage [48–50]. The treating physician is advised to avoid antibiotics in FMT patients during the first month after FMT unless strictly necessary, and preferably keep doses as small as possible. FMT is generally performed by infusion of a donor faeces solution through a gastric or duodenal tube [5], colonoscopy [6], or enema [40]. All infusion routes have advantages and disadvantages, and the ideal method should be evaluated for each individual. The FMT procedure can be performed by the treating physician and does not justify standard referral to a specialized centre. Physicians are instructed how to perform FMT, and if necessary, FMT training sessions are offered. In The Netherlands, FMT via duodenal tube is preferred because it is generally well tolerated by patients, and is less invasive compared with colonoscopy, especially in an inflamed bowel as with severe CDI [5,51,52]. On the day of FMT treatment, a duodenal tube is

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**Table 3**

<table>
<thead>
<tr>
<th>Potential donors</th>
<th>Action</th>
<th>Exclusion reasons[^a]</th>
<th>Excluded (n)</th>
<th>Suitable donors[^b] (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>165</td>
<td>Request of information by email</td>
<td>62% age &gt;50 years, 26% unable to deliver faeces &lt;2 hour after defecation, 6% BMI &gt;25, 6% other</td>
<td>94 (57%)</td>
<td>71 (43%)</td>
</tr>
<tr>
<td>71</td>
<td>Extended questionnaire</td>
<td>17.2% age &gt;50 years, 27.1% BMI &gt;25, 14.3% (history of) depression, 8.5% comorbidity/medicine use, 7.1% profession of healthcare worker[^c], 7.1% inability to deliver faeces &lt;2 hour, 7.1% (close relative with) IBD, 4.3% anorexia, 2.9% recent use of antibiotics[^d], 2.9% autism, 2.9% (risk factors for) colon carcinoma[^e], 2.9% profession with frequent travelling, 2.9% abundant flatulence</td>
<td>50 (70.4%)</td>
<td>21 (12.7%)</td>
</tr>
<tr>
<td>21</td>
<td>First faeces screening</td>
<td>42.9% D. fragilis, 4.8% D. fragilis and B. hominis, 4.8% D. fragilis and C. jejuni, 4.8% E. histolytica[^f]</td>
<td>11 (52.3%)</td>
<td>10 (6.1%)</td>
</tr>
<tr>
<td>10</td>
<td>Serum screening</td>
<td>None</td>
<td>0 (0%)</td>
<td>10 (6.1%)</td>
</tr>
<tr>
<td>10</td>
<td>Repeated faeces screening</td>
<td>20% B. hominis, 10% ESBL E. coli, 30% donor withdrawal (after 0, 2, and 6 months[^g]), Temporarily excluded: acute diarrhoea (for 3 months), rotavirus carrier ship (for 2 weeks)</td>
<td>6 (60%)</td>
<td>4 (2.4%)</td>
</tr>
</tbody>
</table>

[^a] Some volunteers have multiple exclusion criteria.
[^b] 1 minus cumulative proportion of excluded donors.
[^d] Antibiotic use in the previous 6 months.
[^e] Close relative with colon carcinoma, onset below age of 60.
[^f] Treated, included as donor 6 months later.
placed through duodenoscopy, is radiologically guided, or placed by use of an electromagnetic imaging system (e.g. Cortrak TM). The thawed faeces solution of approximately 200 mL is slowly infused through the duodenal tube with a 50 cc syringe, at a rate of 10 cc/min, after which the tube is flushed with 50 mL tap water. Thirty minutes after FMT, the duodenal tube is removed and patients are monitored for 2 hours. If FMT through a duodenal tube is contraindicated (i.e. because of hampered bowel passage or higher risk of aspiration), FMT is performed via colonoscopy. We generally do not recommend enemas, because of the need for repeated FMTs to achieve a high cure rate with enemas [53].

**NDFB experience during May-January 2017**

In March 2016, the opening of NDFB was reported in various local and national newspapers and broadcast on radio and television programmes, accompanied by an invitation for volunteers to register as donors. Subsequently, 165 volunteers registered and were informed by email about the procedure and asked to complete an online questionnaire. After this evaluation only 21 potential donors (12.7%) were screened for the presence of transmissible diseases (Table 3). Nine (5.5% of initial responders) volunteers passed the screening and were invited to donate. This percentage is low, although in line with earlier reports on donor screening [29,54–56]. The faecal suspensions were quarantined for 2 months, after which the donors were re-screened. Two volunteers had to temporarily stop donating for 3 months because of an episode of acute diarrhea. Four donors did not pass a re-screening: two carried *B. hominis*, one an ESBL-positive *E. coli* (exclusion for at least 6 months), and one donor a rotavirus (indication for re-screening of the previous donated samples and exclusion for 2 weeks); this underlines the importance of a quarantine period. As a substantial portion of donors only donate temporarily, donor recruitment is a continuous process.

In May 2016, the first FMT with a donor faeces suspension of the NDFB was performed. In the first 9 months after its opening, 31 faeces suspensions to 18 different hospitals throughout The Netherlands have been distributed for treatment with FMT. We noted a cure rate of 84%, which is in line with the earlier reported randomized controlled trials [5,6].

**Business plan**

In The Netherlands, disease entities are reimbursed regardless of the given treatment (e.g. for recurrent CDI; vancomycin or fidaxomicin or FMT) when the patient is treated in daycare. A business case to calculate the break-even point of producing safe faeces samples for FMT was determined for the NDFB. We considered (i) recruitment, screening, and selecting of suitable donors, (ii) donation of faeces by donors and periodic rescreening, (iii) assessment of eligibility of patients’ demand for FMT, (iv) supply of a safe faecal suspension, and (v) post-treatment monitoring. Costs covering hospital staff involved (medical, technical, administrative, advisory), laboratory tests, storage, and biobanking amount to a unit cost per patient to be treated (including 10% re-treatment in case of initial non-response) of €899 in case of 100 patients yearly, dropping to €785 in case of 400 patients yearly to account for economies of scale.

**Transparency declaration**

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