Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

CMI
CLINICAL
MICROBIOLOGY
AND INFECTION

ESCMID

Review

How to: Establish and run a stool bank[☆]

E.M. Terveer [1, *, 12], Y.H. van Beurden [2, 3], A. Goorhuis [4], J.F.M.L. Seegers, M.P. Bauer [5],
E. van Nood [6], M.G.W. Dijkgraaf [7], C.J.J. Mulder [3], C.M.J.E. Vandenbroucke-Grauls [2],
H.W. Verspaget [8], J.J. Keller [9, 10, **, 11], E.J. Kuijper [1, 11, 12]

[1]) *Dept. of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands*
[2]) *Dept. of Medical Microbiology & Infection Control, VU University Medical Center, Amsterdam, The Netherlands*
[3]) *Dept. of Gastroenterology, VU University Medical Center, Amsterdam, The Netherlands*
[4]) *Dept. of Internal Medicine, Academic Medical Center, Amsterdam, The Netherlands*
[5]) *Dept. of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands*
[6]) *Dept. of Internal Medicine, Havenziekenhuis, Rotterdam, The Netherlands*
[7]) *Clinical Research Unit, Academic Medical Center, Amsterdam, The Netherlands*
[8]) *Dept. of Biobanking and Gastroenterology, Leiden University Medical Center, Leiden, The Netherlands*
[9]) *Dept. of Gastroenterology, MC Haaglanden, The Hague, The Netherlands*
[10]) *Dept. of Gastroenterology, Leiden University Medical Center, Leiden, The Netherlands*

ARTICLE INFO

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. **E.M. Terveer, Clin Microbiol Infect 2017;23:924**

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 1
Overview of currently existing donor faeces banks

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

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.
[a] Until 1 April 2017.
[b] Commercial, social enterprise.

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 fulfil 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 the 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 sense 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/m^2 is also an exclusion criterion, as

Table 2
Donor screening by laboratory screening of faeces and serum

Laboratory screening serum	Laboratory screening faeces
• Hepatitis A (IgM + IgG) • Hepatitis B (HBsAg + anti-Hbcore) • Hepatitis C (anti-HCV) • Hepatitis E (IgM + IgG) • HIV (anti-HIV, type 1 and 2) • Lues; *Treponema pallidum* (Ig) • Cytomegalovirus (IgM + IgG) • Epstein Barr Virus (IgM + IgG) • *Strongyloïdes* (IgG1/IgG4)[a]	• *Clostridium difficile* (PCR) • *Helicobacter pylori* (antigen test) • Bacterial gastroenteritis: (PCR, followed by culture) *Salmonella* spp. *Campylobacter* spp., *Campylobacter jejuni*, *C. coli*, *Shigella* spp., *Yersinia enterocolitica* and *Y. pseudotuberculosis*, *Aeromonas* spp., *Plesiomonas shigelloides*, and Shiga Toxin-producing *E. coli* • Antibiotic-resistant bacteria (culture); ESBL and/or carbapenemase-producing bacteria, vancomycin-resistant enterococci, and methicillin-resistant *Staphylococcus aureus* • Viral pathogens (PCR): Norovirus serotype I+II, Astrovirus, Sapovirus, Rotavirus, Adenovirus 40/41, Adenovirus non–40/41, Enterovirus, Parechovirus, Hepatitis E • Parasites (PCR): *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium parvum* and *C. hominis*, *Microsporidium* spp, *Strongyloïdes*[a] • Microscopy for ova, cysts, and larvae [69]: e.g. *Blastocystis hominis*

Questionnaire: 1 day before donation of faeces
Stool frequency/pattern, general health, use of antibiotics, travel history, sexual behaviour

When donors pass the questionnaire, faeces is first screened for the presence of *Dientamoeba fragilis* and *Blastocystis hominis.* When negative, other pathogens are investigated, after which screening of serum is performed.
[a] If travel history to Middle and South America, Africa, or Asia.

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-transmittable 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 <60 years, substantial comorbidity, various medications, autism [22,64,65], autoimmune disorders [66], neurological disease [67,68].

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 gastrointestinal diarrhoea and are therefore also screened [29]. Enterovirus and Parechovirus are usually asymptomatic but can cause skin disease (and foot-and-mouth disease), pleurodynia, 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 donorship 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 guarantee 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 requestor 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 intravenously administered humanized monoclonal antibodies against *C. difficile* toxin B (bezlotoxumab) protect against (multiple) recurrent 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 re-frozen, 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], colonoscope [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

Table 3
Experiences of the NDFB with donor screening

Potential donors	Action	Exclusion reasons[a]	Excluded (n)	Suitable donors[b] (n)
165 ⇩	Request of information by email	62% age >50 years, 26% unable to deliver faeces <2 hour after defecation, 6% BMI >25, 6% other	94 (57%)	71 (43%)
71 ⇩	Extended questionnaire	17.2% age >50 years, 27.1% BMI >25, 14.3% (history of) depression, 8.5% comorbidity/medicine use, 7.1% profession of healthcare worker[c], 7.1% inability to deliver faeces <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	50 (70.4%)	21 (12.7%)
21 ⇩	First faeces screening	42.9% *D. fragilis*, 4.8% *D. fragilis* and *B. hominis*, 4.8% *D. fragilis* and *C. jejuni*, 4.8% *E. histolytica*[f]	11 (52.3%)	10 (6.1%)
10 ⇩	Serum screening	None	0 (0%)	10 (6.1%)
10	Repeated faeces screening	20% *B. hominis*, 10% ESBL *E. coli*, 30% donor withdrawal (after 0, 2, and 6 months) Temporarily excluded: acute diarrhoea (for 3 months), rotavirus carriership (for 2 weeks)	6 (60%)	4 (2.4%)

[a] Some volunteers have multiple exclusion criteria.
[b] 1 minus cumulative proportion of excluded donors.
[c] Higher risk of temporary carriership of pathogens.
[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 contra-indicated (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 diarrhoea. 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 fidax-omicin 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) dona-tion 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, advi-sory), 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, drop-ping to €785 in case of 400 patients yearly to account for econo-mies of scale.

Transparency declaration

Acknowledgements

References

[1] Lawson PA, Citron DM, Tyrrell KL, Finegold SM. Reclassification of *Clostridium difficile* as Clostridioides difficile (Hall and O'Toole 1935) Prévot 1938. Anaerobe 2016;40:95–9.

[2] Smits WK, Lyras D, Lacy DB, Wilcox MH, Kuijper EJ. *Clostridium difficile* infection. Nat Rev Dis Primers 2016;2:16020.

[3] Keller JJ, Kuijper EJ. Treatment of recurrent and severe *Clostridium difficile* infection. Annu Rev Med 2015;66:373–86.

[4] Seekatz AM, Young VB. *Clostridium difficile* and the microbiota. J Clin Investig 2014;124(10):4182–9.

[5] van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. New Engl J Med 2013;368(5):407–15.

[6] Cammarota G, Masucci L, Ianiro G, Bibbo S, Dinoi G, Costamagna G, et al. Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent *Clostridium difficile* infection. Aliment Pharmacol Ther 2015;41(9):835–43.

[7] Ratner M. Fecal transplantation poses dilemma for FDA. Nat Biotechnol 2014;32(5):401–2.

[8] Moore T, Rodriguez A, Bakken JS. Fecal microbiota transplantation: a practical update for the infectious disease specialist. Clin Infect Dis 2014;58(4):541–5.

[9] Hecht GA, Blaser MJ, Gordon J, Kaplan LM, Knight R, Laine L, et al. What is the value of a food and drug administration investigational new drug application for fecal microbiota transplantation to treat *Clostridium difficile* Infection? Clin Gastroenterol Hepatol 2014;12(2):289–91.

[10] Vyas D, Aekka A, Vyas A. Fecal transplant policy and legislation. World J Gastroenterol 2015;21(1):6–11.

[11] U.S. Food and Drug Administration. Guidance for industry: enforcement policy regarding investigational new drug requirements for use of fecal microbiota for transplantation to treat *clostridium difficile* infection not responsive to standard therapies. 2013. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm361379.htm.

[12] Smith MB, Kelly C, Alm EJ. Policy: how to regulate faecal transplants. Nature 2014;506(7488):290–1.

[13] Kassam Z, Lee CH, Yuan Y, Hunt RH. Fecal microbiota transplantation for *Clostridium difficile* infection: systematic review and meta-analysis. Am J Gastroenterol 2013;108(4):500–8.

[14] Bakken JS, Borody T, Brandt LJ, Brill JV, Demarco DC, Franzos MA, et al. Treating *Clostridium difficile* infection with fecal microbiota transplantation. Clin Gastroenterol Hepatol 2011;9(12):1044–9.

[15] Youngster I, Sauk J, Pindar C, Wilson RG, Kaplan JL, Smith MB, et al. Fecal microbiota transplant for relapsing *Clostridium difficile* infection using a frozen inoculum from unrelated donors: a randomized, open-label, controlled pilot study. Clin Infect Dis 2014;58(11):1515–22.

[16] World Health Organization. Blood transfusion safety. http://www.who.int/bloodsafety/voluntary_donation/en/.

[17] Anand R, Song Y, Garg S, Girotra M, Sinha A, Sivaraman A, et al. Effect of aging on the composition of fecal microbiota in donors for FMT and its impact on clinical outcomes. Dig Dis Sci 2017;62(4):1002–8.

[18] Reijnders D, Goossens GH, Hermes GD, Neis EP, van der Beek CM, Most J, et al. Effects of gut microbiota manipulation by antibiotics on host metabolism in obese humans: a randomized double-blind placebo-controlled trial. Cell Metab 2016;24(1):63–74.

[19] Alang N, Kelly CR. Weight gain after fecal microbiota transplantation. Open Forum Infect Dis 2015;2(1). ofv004.

[20] Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy har-vest. Nature 2006;444(7122):1027–31.

[21] Michail S, Durbin M, Turner D, Griffiths AM, Mack DR, Hyams J, et al. Alter-ations in the gut microbiome of children with severe ulcerative colitis. Inflamm Bowel Dis 2012;18(10):1799–808.

[22] Rosenfeld CS. Microbiome disturbances and autism spectrum disorders. Drug Metab Dispos 2015;43(10):1557–71.

[23] Brandt LJ, Aroniadis OC. An overview of fecal microbiota transplantation: techniques, indications, and outcomes. Gastrointest Endosc 2013;78(2): 240–9.

[24] Mattila E, Uusitalo-Seppala R, Wuorela M, Lehtola L, Nurmi H, Ristikankare M, et al. Fecal transplantation, through colonoscopy, is effective therapy for recurrent Clostridium difficile infection. Gastroenterology 2012;142(3):490–6.

[25] Hamilton MJ, Weingarden AR, Sadowsky MJ, Khoruts A. Standardized frozen preparation for transplantation of fecal microbiota for recurrent Clostridium difficile infection. Am J Gastroenterol 2012;107(5):761–7.

[26] Woodworth MH, Carpentieri C, Sitchenko KL, Kraft CS. Challenges in fecal donor selection and screening for fecal microbiota transplantation: a review. Gut Microbe 2017;8(3):225–37.

[27] Cammarota G, Ianiro G, Tilg H, Rajilic-Stojanovic M, Kump P, Satokari R, et al. European consensus conference on faecal microbiota transplantation in clinical practice. Gut 2017;66(4):569–80.

[28] Kazerouni A, Burgess J, Burns LJ, Wein LM. Optimal screening and donor management in a public stool bank. Microbiome 2015;3:75.

[29] Lion T. Adenovirus infections in immunocompetent and immunocompromised patients. Clin Microbiol Rev 2014;27(3):441–62.

[30] Pons-Salort M, Parker EP, Grassly NC. The epidemiology of non-polio enteroviruses: recent advances and outstanding questions. Curr Opin Infect Dis 2015;28(5):479–87.

[31] Harvala H, Simmonds P. Human parechoviruses: biology, epidemiology and clinical significance. J Clin Virol 2009;45(1):1–9.

[32] Bowles NE, Ni J, Kearney DL, Pauschinger M, Schultheiss HP, McCarthy R, et al. Detection of viruses in myocardial tissues by polymerase chain reaction. evidence of adenovirus as a common cause of myocarditis in children and adults. J Am Coll Cardiol 2003;42(3):466–72.

[33] Hogema BM, Molier M, Sjerps M, de Waal M, van Swieten P, van de Laar T, et al. Incidence and duration of hepatitis E virus infection in Dutch blood donors. Transfusion 2016;56(3):722–8.

[34] Garcia LS. Dientamoeba fragilis, one of the Neglected intestinal Protozoa. J Clin Microbiol 2016;54(9):2243–50.

[35] Holtman GA, Kranenberg JJ, Blanker MH, Ott A, Lisman-van Leeuwen Y, Berger MY. Dientamoeba fragilis colonization is not associated with gastrointestinal symptoms in children at primary care level. Fam Pract 2017;34(1):25–9.

[36] Turkeltaub JA, McCarty 3rd TR, Hotez PJ. The intestinal protozoa: emerging impact on global health and development. Curr Opin Gastroenterol 2015;31(1):38–44.

[37] Bruijnesteijn van Coppenraet LE, Dullaert-de Boer M, Ruijs GJ, van der Reijden WA, van der Zanden AG, Weel JF, et al. Case-control comparison of bacterial and protozoan microorganisms associated with gastroenteritis: application of molecular detection. Clin Microbiol Infect 2015;21(6). 592.e9–19.

[38] Windsor JJ, Macfarlane L, Hughes-Thapa G, Jones SK, Whiteside TM. Incidence of Blastocystis hominis in faecal samples submitted for routine microbiological analysis. Br J Biomed Sci 2002;59(3):154–7.

[39] Lepczynska M, Bialkowska J, Dzika E, Piskorz-Ogorek K, Korycinska J. Blastocystis: how do specific diets and human gut microbiota affect its development and pathogenicity? Eur J Clin Microbiol Infect Dis 2017 Mar 22. http://dx.doi.org/10.1007/s10096-017-2965-0 [Epub ahead of print].

[40] Gough E, Shaikh H, Manges AR. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent Clostridium difficile infection. Clin Infect Dis 2011;53(10):994–1002.

[41] Lee CH, Steiner T, Petrof EO, Smieja M, Roscoe D, Nematallah A, et al. Frozen vs fresh fecal microbiota transplantation and clinical resolution of diarrhea in patients with recurrent Clostridium difficile infection: a randomized clinical trial. JAMA 2016;315(2):142–9.

[42] Crobach MJ, Planche T, Eckert C, Barbut F, Terveer EM, Dekkers OM, et al. European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for Clostridium difficile infection. Clin Microbiol Infect 2016;22(Suppl. 4):S63–81.

[43] Bagdasarian N, Rao K, Malani PN. Diagnosis and treatment of Clostridium difficile in adults: a systematic review. JAMA 2015;313(4):398–408.

[44] Beurden van YH, Nieuwdorp M, Berg van de PJEJ, Mulder CJJ, Goorhuis A. Current challenges in the treatment of severe Clostridium difficile infection: early treatment potential of fecal microbiota transplantation. Ther Adv Gastroenterol 2017;10(4):373–81.

[45] Wilcox MH, Gerding DN, Poxton IR, Kelly C, Nathan R, Birch T, et al. Bezlotoxumab for prevention of recurrent Clostridium difficile infection. New Engl J Med 2017;376(4):305–17.

[46] Saeedi BJ, Morison DG, Kraft CS, Dhere T. Fecal microbiota transplant for Clostridium difficile infection in a pregnant patient. Obstet Gynecol 2017;129(3):507–9.

[47] Satokari R, Mattila E, Kainulainen V, Arkkila PE. Simple faecal preparation and efficacy of frozen inoculum in faecal microbiota transplantation for recurrent Clostridium difficile infection–an observational cohort study. Aliment Pharmacol Ther 2015;41(1):46–53.

[48] Gweon TG, Kim J, Lim CH, Park JM, Lee DG, Lee IS, et al. Fecal microbiota transplantation using upper gastrointestinal tract for the treatment of refractory or severe complicated Clostridium difficile infection in elderly patients in poor medical condition: the first study in an Asian Country. Gastroenterol Res Pract 2016;2016:2687605.

[49] Aas J, Gessert CE, Bakken JS. Recurrent Clostridium difficile colitis: case series involving 18 patients treated with donor stool administered via a nasogastric tube. Clin Infect Dis 2003;36(5):580–5.

[50] Postigo R, Kim JH. Colonoscopic versus nasogastric fecal transplantation for the treatment of Clostridium difficile infection: a review and pooled analysis. Infection 2012;40(6):643–8.

[51] Terveer EM, van Beurden YH, van Dorp S, Keller JJ, Kuijper EJ. Is the lower gastrointestinal route really preferred over the upper gastrointestinal route for fecal microbiota transfer? J Clin Gastroenterol 2016;50(10):895.

[52] Beurden van YH, Groot de PF, Nood van E, Nieuwdorp M, Keller JJ, Goorhuis A. Complications, effectiveness, and long term follow-up of fecal microbiota transfer by nasoduodenal tube for treatment of recurrent Clostridium difficile infection. United Eur Gastroenterol J 2016:1–12. http://dx.doi.org/10.1177/2050640616678099 (in press).

[53] Lee CH, Belanger JE, Kassam Z, Smieja M, Higgins D, Broukhanski G, et al. The outcome and long-term follow-up of 94 patients with recurrent and refractory Clostridium difficile infection using single to multiple fecal microbiota transplantation via retention enema. Eur J Clin Microbiol Infect Dis 2014;33(8):1425–8.

[54] Paramsothy S, Borody TJ, Lin E, Finlayson S, Walsh AJ, Samuel D, et al. Donor recruitment for fecal microbiota transplantation. Inflamm Bowel Dis 2015;21(7):1600–6.

[55] Tariq R, Weatherly R, Kammer P, Pardi DS, Khanna S. Donor screening experience for fecal microbiota transplantation in patients with recurrent C. difficile infection. J Clin Gastroenterol 2016;0(0) [Epub ahead of print].

[56] Burns LJ, Dubois N, Smith MB, Mendolia GM, Burgess J, Edelstein C, et al. Donor recruitment and eligibility for fecal microbiota transplantation: results from an international public stool bank. Gastroenterology 2015;148(4): S96–7.

[57] Willing BP, Russell SL, Finlay BB. Shifting the balance: antibiotic effects on host-microbiota mutualism. Nat Rev Microbiol 2011;9(4):233–43.

[58] Robinson CJ, Young VB. Antibiotic administration alters the community structure of the gastrointestinal micobiota. Gut Microbe 2010;1(4):279–84.

[59] Codling C, O'Mahony L, Shanahan F, Quigley EM, Marchesi JR. A molecular analysis of fecal and mucosal bacterial communities in irritable bowel syndrome. Dig Dis Sci 2010;55(2):392–7.

[60] Hold GL, Smith M, Grange C, Watt ER, El-Omar EM, Mukhopadhya I. Role of the gut microbiota in inflammatory bowel disease pathogenesis: what have we learnt in the past 10 years? World J Gastroenterol 2014;20(5): 1192–210.

[61] Tedjo DI, Smolinska A, Savelkoul PH, Masclee AA, van Schooten FJ, Pierik MJ, et al. The fecal microbiota as a biomarker for disease activity in Crohn's disease. Sci Rep 2016;6:35216.

[62] Fischer M, Bittar M, Papa E, Kassam Z, Smith M. Can you cause inflammatory bowel disease with fecal transplantation? A 31-patient case-series of fecal transplantation using stool from a donor who later developed Crohn's disease. Gut Microbe 2017;8(3):205–7.

[63] Borges-Canha M, Portela-Cidade JP, Dinis-Ribeiro M, Leite-Moreira AF, Pimentel-Nunes P. Role of colonic microbiota in colorectal carcinogenesis: a systematic review. Rev Esp Enferm Dig 2015;107(11):659–71.

[64] de Theije CG, Wopereis H, Ramadan M, van Eijndthoven T, Lambert J, Knol J, et al. Altered gut microbiota and activity in a murine model of autism spectrum disorders. Brain Behav Immun 2014;37:197–206.

[65] Kraneveld AD, Szklany K, de Theije CG, Garssen J. Gut-to-Brain Axis in autism spectrum disorders: central role for the microbiome. Int Rev Neurobiol 2016;131:263–87.

[66] Purchiaroni F, Tortora A, Gabrielli M, Bertucci F, Gigante G, Ianiro G, et al. The role of intestinal microbiota and the immune system. Eur Rev Med Pharmacol Sci 2013;17(3):323–33.

[67] Parashar A, Udayabanu M. Gut microbiota: implications in Parkinson's disease. Parkinsonism Relat Disord 2017;38:1–7.

[68] Tremlett H, Bauer KC, Appel-Cresswell S, Finlay BB, Waubant E. The gut microbiome in human neurological disease: a review. Ann Neurol 2017;81(3): 369–82.

[69] Allen AV, Ridley DS. Further observations on the formol-ether concentration technique for faecal parasites. J Clin Pathol 1970;23(6):545–6.