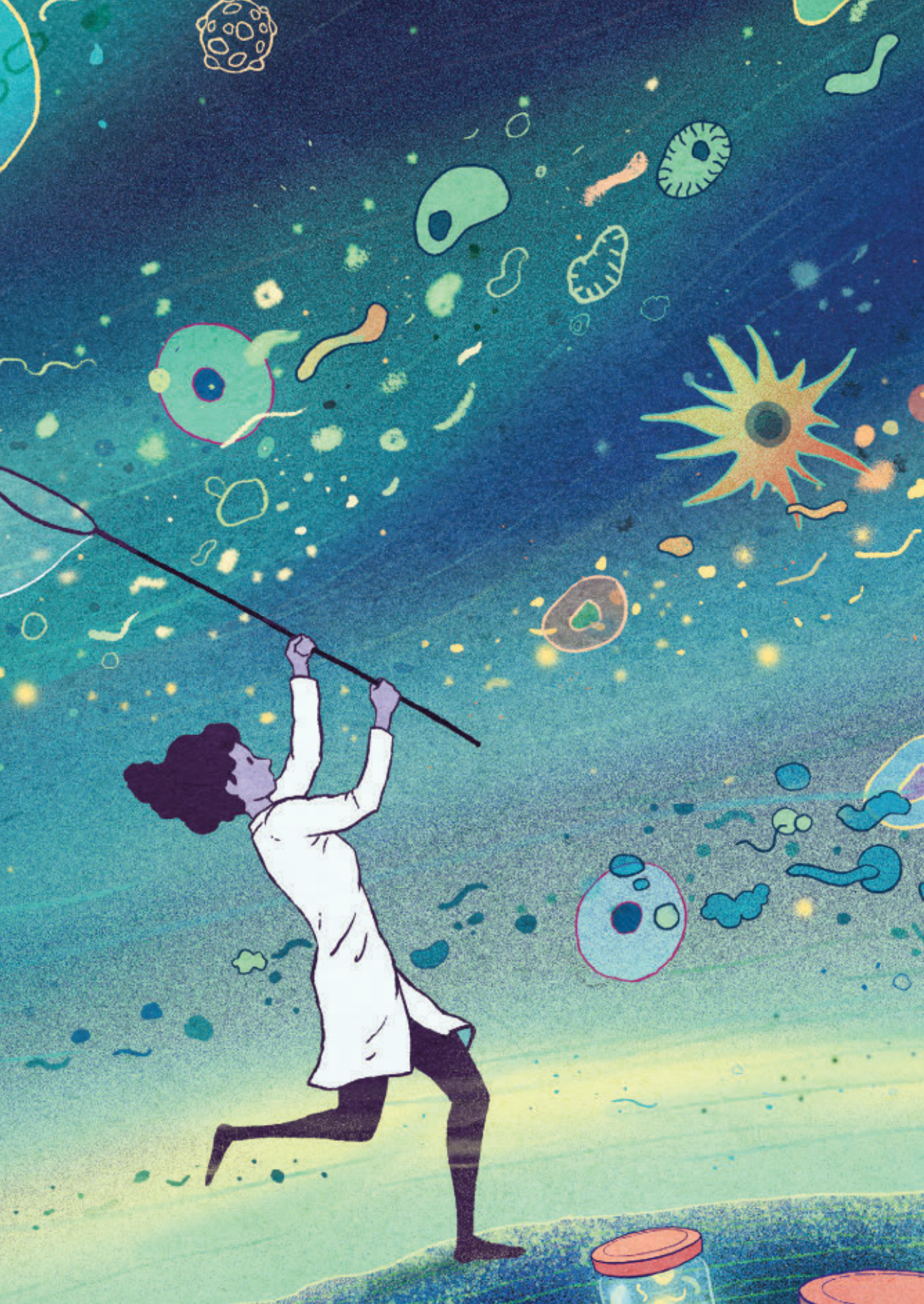
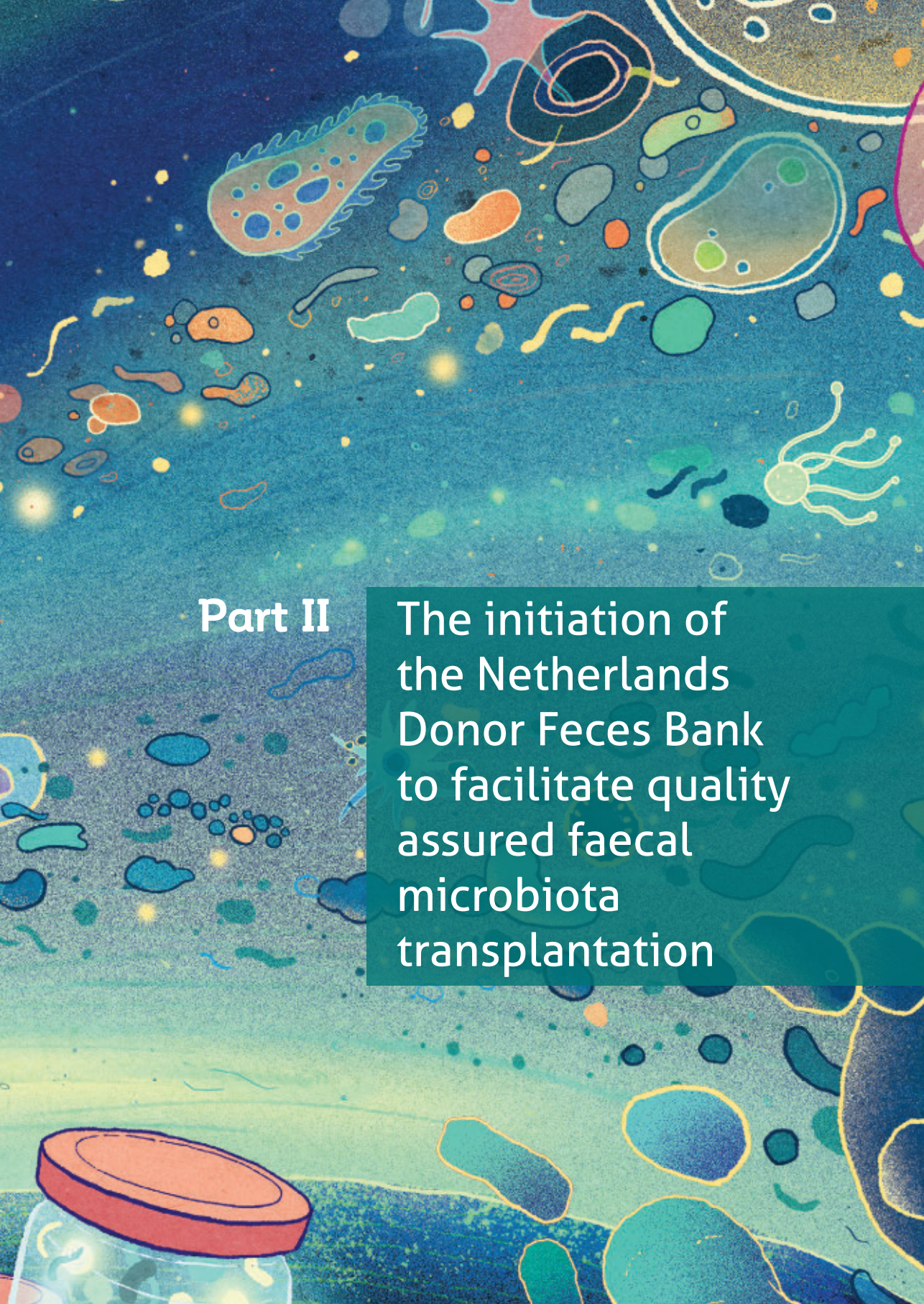
Exploring the role of the microbiota: in defence against Clostridioides difficile and multidrug resistant Gram-negatives

Terveer, E.M.





Part II

The initiation of the Netherlands Donor Feces Bank to facilitate quality assured faecal microbiota transplantation

Chapter

5

How to: Establish and run a stool bank

Development of the Netherlands Donor Feces Bank

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Elisabeth M. Terveer[1], Yvette H. van Beurden[2,3], Abraham Goorhuis[4], Jos F.M.L. Seegers[5], Martijn P. Bauer[6], E. van Nood[7], Marcel G.W. Dijkgraaf[8], Chris J.J. Mulder[3], Christina M.J.E. Vandenbroucke-Grauls[2], Hein. W. Verspaget[9], Josbert J. Keller[*10,11], Ed J. Kuijper[*1]

[*] both authors contributed equally to this manuscript

1 Dept. of Medical Microbiology, Leiden University Medical Center, Leiden, the Netherlands and also representative of the European Study Group of C. difficile (ESGCD)
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 Unaffilitated
6 Dept. of Internal Medicine, Leiden University Medical Center, Leiden, the Netherlands
7 Dept. of Internal Medicine, Havenziekenhuis, Rotterdam, the Netherlands
8 Clinical Research Unit, Academic Medical Center, Amsterdam, the Netherlands
9 Dept. of Biobanking and Gastroenterology, Leiden University Medical Center, Leiden, the Netherlands
10 Dept. of Gastroenterology, MC Haaglanden, The Hague, the Netherlands
11 Dept. of Gastroenterology, Leiden University Medical Center, Leiden, The Netherlands

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Chapter 5. How to: Establish and run a stool bank

Abstract

Since 2013, several stool banks have been developed following publications reporting on clinical success of "Fecal Microbiota Transplantation" (FMT) for recurrent *Clostridium difficile* infections (CDI). However, protocols for donor screening, fecal suspension preparation and transfer of the fecal suspension differ between various countries and institutions. Moreover, no European consensus exists regarding the legislative aspects of the fecal suspension product. Internationally standardized recommendations about the above mentioned aspects have not yet been established. In 2015, the Netherlands Donor Feces Bank (NDFB) was founded with the primary aim to provide 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 fecal suspensions for FMT were formulated. Our experience summarized in this review addresses current donor recruitment and screening, preparation of the fecal suspension, transfer of the fecal microbiota suspension and the experiences and follow-up of the patients treated with donor feces of the NDFB.

Background

Clostridium difficile, recently reclassified as *Clostridioides difficile* [1] is capable of inducing diarrheal disease (*C. difficile* infection, CDI) due to the production of secreted toxins [2]. After CDI treatment, the risk of a recurrence within eight 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]. Fecal 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 feces 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 feces solutions to treat patients with recurrent or refractory CDI. Secondly, 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 feces, and samples of all delivered fecal 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 fecal suspensions is already present. Since 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, Methodology, and if donor feces is considered as a drug; Pharmacology. An overview of the currently existing donor feces banks is depicted in Table 1. Similar as to the NDFB, most of the donor banks are non-profit and primarily use FMT for treatment of patients with recurrent CDI.

Table 1. Overview of currently existing donor feces 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 Massachusetts, 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 3 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 Dept. Saint-Antoine hospital harry.sokol@aphp

Abbreviations; 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

Table 1. continued

Location, founded	Legislation	Donors	Products	Indications	No.of issued products [a]	Contact address and website
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 Dept. 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 Microbiotab 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

Abbreviations; 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

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Legislation of a donor feces 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 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 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 are considering donor feces as 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 fecal material for use in FMT under the jurisdiction of hospital pharmacies, requiring storage of the fecal 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 a future status of donor feces as 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

feces 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 fecal 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 feces donors are amongst established blood donors, as this has the advantage of previously screened, healthy and motivated volunteers. An important difference in the donor recruitment in the Netherlands and most other European countries (except Germany) compared to 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 feces, to preserve as much anaerobes, donors should be recruited in the near proximity of stool banks, such as non-health care 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, since 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 feces of an overweight donor has been reported [19, 20]. Any other gastrointestinal disorder (e.g. irritable bowel syndrome (IBS), Crohn's disease and 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 will probably 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 fecal- 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 fecal- and or blood transmittable diseases, recent antibiotic use (<6 months) [23, 24], gastrointestinal complaints (for example diarrhoea, obstipation or irritable bowel like symptoms) [25-27], recent travel to endemic areas of gastrointestinal pathogens, (first degree relative with) inflammatory bowel disease [28], GI malignancy [29], first degree relative with a GI malignancy < 60 years, substantial comorbidity, various medication, autism [22, 30, 31], auto-immune disorders [32], neurological disease [33, 34]

Donor screening by laboratory tests

An extensive laboratory analysis should be performed to identify potential pathogens transmissible by fecal transfusion. An overview of all tests performed by the NDFB is shown in Table 2. The pathogens included in the blood-screening program correspond with the screening protocols for blood donors and are generally agreed upon between the different stool banks [14, 15, 35-39]. However, screening protocols for detection of specific microorganisms in the intestinal tract differ between stool banks, and evolve with time and new insights, since there is no consensus guideline. This applies for example to the screening for the presence of multidrug 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 since rotavirus is frequently found in asymptomatic donors, especially in winter, we included this in our protocol [38, 40]. Adenovirus type 40/41, Sapovirus and Astrovirus are associated with mild gastro-intestinal diarrhoea and are therefore also screened [41]. Enterovirus and Parechovirus are usually asymptomatic but can cause skin disease (and-foot-and-mouth disease), pleurodynia, myocarditis and meningitis [42, 43]. Adenovirus non-40/41 can cause myocarditis [44]. In addition, feces is screened for Hepatitis E, which is frequently found in asymptomatic (blood)

donors [45]. To prevent transmission and development of systemic infections, potential donors are screened with PCRs for all the above mentioned viruses (see also Table 2 for the total list of pathogens).

Table 2. Donor screening by laboratory screening of feces and serum.

When donors pass the questionnaire, feces 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.

• **Laboratory screening serum**	**Laboratory screening feces**
• 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 gastro-enteritis: (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 [46]: for example: *Blastocystis hominis*
Questionnaire: One day before donation of feces Stool frequency/pattern, general health, use of antibiotics, travel history, sexual behaviour	

a If travel history to Middle and South America, Africa or Asia

The significance of *Dientamoeba fragilis and Blastocystis hominis* as enteropathogens is less clear [47-50]. *D. fragilis* and *B. hominis* are commonly found in fecal samples of both symptomatic and asymptomatic individuals [50, 51]. Prevalence varies considerably depending on geographic location, the group studied, and diagnostic methods used [47]. 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, colonisation may be considered an indicator of a suboptimal microbiota composition [52]. Therefore, positive individuals are excluded from donorship for NDFB.

The serostatus of the donor is determined for Epstein-Barr virus (EBV) and Cytomegalo virus (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 due to FMT (TRANSDECMV Clin Trial Gov: NCT02694484).

Approximately two months after the initial screening, a new donor sample of feces and blood are screened again, using similar tests as applied at entry of the program (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 fecal suspensions collected until two 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 feces suspensions

It is generally believed that a high viability of bacteria in stools increases the chance of successful FMT. Since the majority of fecal bacteria are anaerobic, feces needs to be processed within six hours after defecation **[5, 6]**. To prevent environmental contamination, feces is collected by the donor in a fecal container (for instance Fecotainer™). For suspension, approximately 60 gram of donor feces is used based on the data of a systematic review suggesting a decreased cure rate with < 50 gram **[53]**. The feces is homogenized with saline using a mortar and pestle, whereas some laboratories use a commercial blender **[15, 37]**. Disadvantages of blenders are difficulties with appropriate sterilisation and aerosolization of the feces. A metal sieve (mesh 300µm) is used to remove undigested food fragments. The fecal suspension is then concentrated by centrifugation (15 minutes, 6000g) **[37]** 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 fecal suspension is equally effective as a fresh fecal suspension for the treatment of CDI **[54]**. 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 feces. Clinical success of frozen suspensions is reported until five to six months of storage at -80°C, but could be much longer, in theory. Like OpenBiome, the NDFB uses a storing period of two 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 a 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 bio samples. FMT suspensions, in combination with a small portion of the original feces 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 in order to be able to perform biovigilance tracing 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' feces and clinical data on the day of FMT and approximately three weeks after the procedure. Furthermore, clinical information including abdominal complaints, development of diarrhea 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 auto-immune diseases, malignancies and other potentially microbiome-associated syndromes both in donors and patients. However, all feces 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

Since 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 (≥3 unformed stools per 24 hours for two consecutive days; or ≥8 unformed stools per 48 hours) within eight weeks after 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 **[55]**. In particular, a positive test for the presence free toxins in feces 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 a PCR to detect toxin genes of *C. difficile*, we advise to send a fresh feces sample to a reference laboratory for toxin detection, since *C. difficile* (spores) can persist after successful treatment and may reflect colonisation.

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 potentially relapse reducing effect due to its narrow antibiotic spectrum **[56]**. 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 **[39, 57]**. A recently completed study suggests that intravenously administered humanised monoclonal antibodies against *C. difficile* toxin B (bezlotoxumab) protects against (multiple) recurrent CDI. However, it is yet unclear which patients really benefit from this very expensive treatment strategy **[58]**.

Pregnancy, severe food allergy, and antibiotic usage other than for *C. difficile* at 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 has been published [59]. 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 feces suspension is transported to the referring hospital on dry ice. Prior to transplantation, the feces suspension is thawed (overnight in a 4°C refrigerator or during five hours at room temperature), based on literature and our expert opinion [15, 54, 60]. The donor feces suspension may be kept at room temperature for up to three hours or refrigerated at 4°C for up to six hours. Samples should never to be re-frozen, because freeze-thaw cycles may compromise stability and efficacy of the sample, possibly due to loss of viability. To eradicate vegetative cells of *C. difficile*, prior to FMT (until one day before the procedure), patients receive vancomycin (125-250 mg QID) for a minimum of four days, followed by two litres of bowel lavage one day prior to FMT [5]. Whether bowel lavage can be excluded from the protocol is currently a matter of discussion, since recent reports have shown similar efficacy for FMT without bowel lavage [61-63]. The treating physician is advised to avoid antibiotics in FMT patients during the first month after FMT unless strictly necessary, and preferably as small as possible. FMT is generally performed by infusion of a donor feces solution through a gastric or duodenal tube [5], colonoscope [6], or enema [54]. All infusion routes have advantages and disadvantages, and in every patient the ideal method should be evaluated. The FMT procedure can be performed by the treating physician and does not justify standard referral to a specialised 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 to colonoscopy, especially in an inflamed bowel as with severe CDI [5, 64, 65]. On the day of FMT treatment, a duodenal tube is placed through duodenoscopy, radiological guided, or with use of an electromagnetic imaging system (e.g. Cortrak TM). The thawed feces solution of approximately 200 ml is slowly infused through the duodenal tube with a 50 cc syringe, at a rate of 10 cc/minute, 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 two hours. If FMT through a duodenal tube is

contra-indicated (i.e. due to a hampered bowel passage or higher risk of aspiration), FMT is performed via colonoscopy. We generally do not advice enemas, because of the need of repeated FMT's to achieve a high cure rate with enemas **[66]**.

NDFB experience during May-January 2017

In March 2016, the opening of NDFB was reported in various local and national newspapers and broadcasted in radio and television programs, accompanied by an invitation for volunteers to register as donor. Subsequently, 165 volunteers registered and informed by email about the procedure and were requested 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, though in line with earlier reports on donor screening **[40, 67-69]**. The fecal suspensions were quarantined for two months after which the donors were re-screened. Two volunteers had to temporarily stop donating for three 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 donates temporarily, donor recruitment is a continuous process.

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

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 feces < 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 health care worker [c], 7.1% inability to deliver feces < 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 feces 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 feces 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 six months
e Close relative with colon carcinoma, onset below age of 60
f Treated, included as donor six months later

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 feces samples for FMT was determined for the NDFB. We differentiated between (i) recruitment, screening and selecting of suitable donors (ii) donation of feces by donors and periodic rescreening, (iii) assessment of eligibility of patients' demand for FMT (iv) supply of a safe fecal suspension, and (v) post-treatment monitoring. The costs covering involved hospital staff (medical, technical, administrative, advisory), laboratory

tests, storage and bio-banking amounts 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.

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