CONSENSUS DOCUMENT ONT-AEMPS ABOUT DONATION, COLLECTION, PROCESSING AND TRANSPLANTATION OF FECAL MICROBIOTA FOR THE TREATMENT OF CLOSTRIDIOIDES DIFFICILE INFECTION

Adopted by the Transplant Commission of the Interterritorial Council of the National Health Care System.

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This document has been prepared by the Agencia Española del Medicamento y Productos Sanitarios (AEMPS) and the Organización Nacional de Trasplantes (ONT) to facilitate the incorporation of fecal microbiota transplantation into clinical practice in Spain, taking into account the development of this therapy and the entry into force of *Regulation (EU) 2024/1938 of the European Parliament and of the Council of 13 June 2024 on standards of quality and safety for substances of human origin intended for administration to humans and repealing Directives 2002/98/EC and 2004/23/EC* . The document is intended to serve as a reference for healthcare professionals involved in this activity, establishments responsible for the preparation of fecal microbiota for clinical use, and competent authorities in the field of substances of human origin. It will be subject to updates in accordance with the scientific, technical, and regulatory advances that occur after its publication. This document was adopted by the Transplant Commission of the Interterritorial Council of the National Health System at its regular meeting held on May 21st, 2025.

LIST OF ABBREVIATIONS

ACG: American College of Gastroenterology

EDQM: European Directorate for the Quality of Medicines & HealthCare

ESCMID: European Society of Clinical Microbiology and Infectious Disease

SE-F: Establishment authorized for the activities established in current legislation. Applied to feces.

LDL: Low-density lipoprotein

FM: Fecal Microbiota

R-CDI: Recurrent Clostridioides difficile infection

SoHO: Substances of Human Origin

FMT: Fecal Microbiota Transplant

1. INTRODUCTION

Fecal microbiota transplantation (FMT) involves applying fecal matter from a healthy donor to a recipient, with the goal of restoring or modifying their fecal microbiota (FM).[1] FMT is performed by administering the processed feces product from one or more donors into the recipient's gastrointestinal tract (via colonoscopy, enemas, jejunal tube, or capsules).

Recolonization of the gastrointestinal tract with transferred bacteria has demonstrated efficacy in the treatment of recurrent *Clostridioides infection* (mainly toxigenic *Clostridioides difficile*) of the gastrointestinal tract resistant to antibiotic treatment, improving the symptoms of the associated disease and preventing new episodes, by modifying the human intestinal microbiota. There is evidence of its efficacy based on observational studies, randomized experimental studies and meta-analyses.

This document focuses on the process from obtaining FM to its clinical use specifically for the treatment of **recurrent or refractory *Clostridioides difficile (C. difficile)* infection (R-CDI) in adults**. The document does not address its use in other patient populations, diseases, or disorders.

2. REGULATORY FRAMEWORK

2.1. Regulation on Substances of Human Origin and FMT

Since the entry into force of *Regulation (EU) 2024/1938 of the European Parliament and of the Council of 13 June 2024 on quality and safety standards for substances of human origin intended for administration to humans and repealing Directives 2002/98/EC and 2004/23/EC,* [2] hereinafter *SoHO Regulation* , which will be mandatory to implement in 2027, FM has the regulatory consideration of Substance of Human Origin (SoHO). For this reason, it is necessary to apply to the activities related to it, fundamental aspects related to the protection of donor and recipient (including the requirement of a specific informed consent for both) contemplated in the *SoHO Regulation*, as well as to comply with its guidelines in aspects related to registration, authorization and inspection of the centers that participate in these activities, coding, traceability, biovigilance and authorization of FM preparations different from the one addressed in this document (modifications in its preparation or in its clinical indication).

Until the date of implementation of the *SoHO Regulation* and the entry into force of the national norms that complement the aforementioned Regulation, it is necessary to articulate the guarantees of this SoHO through the existing regulations in Spain for this purpose, that is, *Royal Decree-Law 9/2014 of July 4, which establishes the quality and safety standards for the donation, obtaining, evaluation, processing, preservation, storage and distribution of human cells and tissues and approves the coordination and operating standards for their use in humans.* [3]

Unlike the Directives it repeals, the *SoHO Regulation* does not contain technical guidelines, but instead draws on certain international guidelines, in particular the *Guideline on the Quality and Safety of Tissues and Cells for Clinical Use* of the European Directorate for the Quality of Medicines and Healthcare (EDQM) and the guidelines of the European Center for Disease Control (ECDC). However, the SoHO Regulation allows Member States to adopt other guidelines, provided that these are considered equivalent to the aforementioned international guidelines. The 5th Edition of the *EDQM Guideline* addresses aspects related to FMT; in addition to the technical requirements for ensuring the quality and safety of donated stool, it also includes data on and uses of FMT based on the available scientific evidence. Both the *SoHO*

Regulation and the *EDQM Guideline* establish the voluntary and unpaid nature of SoHO donation, and consequently of stool donation, as a fundamental ethical principle.

2.2. Competent Authorities and Supervisory Activities

Verifying compliance with the *SoHO Regulation* through SoHO monitoring activities is of fundamental importance to ensure that the objectives set out in the Regulation are effectively achieved throughout the European Union, while respecting the internal healthcare organization requirements set out in the national legislation of each Member State.

These oversight activities aim to verify the correct application of the *SoHO Regulation*, i.e., that SoHO entities comply with the provisions of the *SoHO Regulation* and that SoHO preparations meet the requirements for authorization by the SoHO Competent Authorities (SCA).

In carrying out oversight activities, national SCA, such as the Organización Nacional de Trasplantes (ONT) and the designated regional SCA, must act independently and impartially. It is important that their oversight functions be separate and independent from the activities of SoHO and any SoHO entities or external influences, that they be adequately equipped and staffed with human resources, that they offer guarantees of professionalism and transparency, and that they always prioritize transparency over confidentiality in their actions to mitigate breaches that affect the protection of donors or recipients. However, professional and legal rights must be protected by ensuring the confidentiality of information provided during inspections and other oversight activities.

When a serious risk to human health is detected that leads SCA to take enforcement action, they should prioritize transparency over confidentiality. Circumstances such as the detection of an entity offering services to the public without the required registration and without complying with standards for the protection of SoHO recipients, such as infectious disease testing, should be considered a serious risk to human health, and such information should be made publicly available.

Finally, SCA should maintain channels of communication and cooperation with the competent authorities of other regulatory frameworks that may be involved in innovative SoHO-based product developments.

2.3. Fecal Microbiota intended for the manufacture of products regulated by other European Union legislation

The *SoHO Regulation* defines a **SoHO** as "*any substance collected from the human body, whether or not it contains cells and whether those cells are living or not, including SoHO preparations resulting from the processing of such substance"*.

In turn, the **SoHO preparation** is defined as "*a type of SoHO that has been subjected to processing and, where appropriate, to one or more other activities related to SoHO that have a direct impact on its quality, safety and efficacy, has a specific clinical indication and is intended for application in humans in a SoHO recipient or is intended for distribution"* .

It is important to note that the *SoHO Regulation* defines **processing** as *"any operation involved in the handling of SoHO, including, but not limited to, washing, shaping, separation, decontamination, sterilization, preservation and packaging, except for the preparatory handling of SoHO for immediate*

human application during a surgical intervention, without the SoHO being removed from the surgical field before they are applied."

Furthermore, the *SoHO Regulation* defines SoHO activities as those that have an impact on the quality, safety and effectiveness of SoHO:

- ✓ SoHO Donor Registration
- ✓ SoHO donor history review and medical examination
- ✓ Testing of SoHO donors or of persons from whom SoHO are collected for autologous or within-relationship use;
- ✓ Collection
- ✓ Processing
- ✓ Quality control
- ✓ Storage
- ✓ Release
- ✓ Distribution
- ✓ Import
- ✓ Export
- ✓ Human application
- ✓ Clinical-outcome registration

Based on the consideration of FM as SoHO, the aforementioned activities must follow the measures provided for in the *SoHO Regulation*, which establishes high levels of quality and safety, as well as the need to demonstrate effectiveness, for all SoHO intended for application in humans.

Although everything included in this document in relation to FM and its preparations for clinical application in humans is considered SoHO, in the future there may be situations in which the development of new preparations from FM may lead to doubts about its consideration as a medicine, being necessary a thorough prior evaluation of each particular case by the Innovation Evaluation Committee of the ONT (https://www.ont.es/wp-content/uploads/2024/04/FUNCIONES-COMITE-EVALUACION-DE-INNOVACION-CON-CyTH-WEB.pdf*)* and the Innovation Office of the Spanish Agency for Medicines and Health Products (AEMPS) (https://www.aemps.gob.es/medicameONTs-de-uso-humano/oficina-de-apoyo-a-la-innovacion-y-conocimieONT-sobre-m edicameONTs/). **Any questions regarding the regulatory classification of a product developed from FM must be directed to these bodies, who will coordinate the evaluation of the product and its regulatory status (innovacion.ont@sanidad.gob.es).**

Should FM be used in the future to manufacture products regulated by other EU legislation and intended for human use, the provisions of the *SoHO Regulation* must also be taken into account with regard to certain activities, in particular:

- ✓ If FM is obtained for the purpose of manufacturing the aforementioned products, the provisions of the *SoHO Regulation* regarding donor registration, donor history review and medical examination, SoHO donor testing, collection and release will always apply. The *SoHO Regulation* will also apply to the storage, distribution, import, and export of FM if these are carried out before distribution to a manufacturer regulated by other EU legislation.
- ✓ Where FM is used to manufacture products regulated by other EU legislation but the resulting product is intended exclusively for therapeutic use in the person from whom the FM was

obtained, the *SoHO Regulation* will apply to activities relating to testing and procurement of the person from whom the FM is obtained for autologous use.

2.4. FMT for the treatment of *Clostridioides difficile* infection and potential future indications

Although FMT is well-established for the treatment of *Clostridioides difficile* infection (CDI), there are other potential future indications, some of which are under investigation. Regarding established uses, the *EDQM Guidelines* will be followed, and the recommendations of scientific societies will be taken into account (*The European Faecal Microbiota Transplantation Network of Academics*, https://eurfmt.com/).

There are issues regarding the risks inherent in multi-donor preparations or pooling of FM. The *EDQM Guide* does not recommend pooling of tissues and cells in general, although it recognizes that it may be necessary when it is the only method to ensure their availability or clinical effectiveness, providing specific FM profiles. In fact, pooling is a common practice in the field of donated breast milk and platelet transfusion. In these cases, an appropriate risk assessment should be performed and the guidelines in the *EDQM Guide* should be followed to ensure traceability, regardless of the number of donations used in the final FM preparation.

3. USE OF FMT IN THE TREATMENT OF CLOSTRIDIOIDES DIFFICILE INFECTION

C. difficile was initially named *Bacillis difficile* due to the difficulty of isolating it in culture media. It was later renamed *Clostridioides difficile,* and in 2016, due to its phylogenetic distance from the genus *Clostridium*, it was reclassified as *Clostridioides difficile*. Some variants of *C. difficile* produce toxins A and B that are pathogenic in humans.

C. difficile is able to form spores that are resistant to a wide range of disinfectants and persist in the environment for long periods of time; as a result, it is a highly transmissible microorganism associated with healthcare-associated infections. It typically manifests as mild diarrhea or severe pseudomembranous colitis, which can be fatal.[4]

An episode of CDI is defined by the presence of compatible clinical findings (including diarrhea, ileus, or megacolon) and microbiologic evidence of free *C. difficile toxins* detected by enzyme immunoassay without reasonable evidence of other causes of diarrhea, **OR** a clinical picture compatible with CDI and a positive nucleic acid amplification test, preferably with a low cycle threshold (Ct) value, or a positive toxigenic *C. difficile* culture, **OR** pseudomembranous colitis diagnosed endoscopically, after colectomy, or at autopsy, in combination with a positive test for toxigenic *C. difficile*. [5]

The high sensitivity of molecular tests for the detection of *C. difficile toxins* has, however, come with a risk of overtreatment of merely colonized patients.[6] Furthermore, a significant proportion of patients adequately treated for CDI present with persistently positive tests for toxigenic *C. difficile* weeks later. Therefore, an adequate clinical assessment of patients and a reasonable exclusion of symptoms that are not due to other causes are essential.

Mortality directly attributable to CDI is estimated to be between 4% and 7% and increases progressively with age.[7] Although most patients respond well to treatment, some develop a **severe, complicated or fulminant clinical picture**, defined by the presence of at least one of the following signs attributed to CDI:

hypotension, septic shock, elevated serum lactate, ileus, toxic megacolon, intestinal perforation or any fulminant evolution.[5]

On the other hand, around 20% of those affected experience disease **recurrences**, and some of them experience multiple episodes that are difficult to control.[8] The same criteria for diagnosing recurrence are used to diagnose recurrence as for the first episode.[5] Recurrence of CDI is associated with high hospital readmission rates, increased costs, and higher mortality.[9] Most recurrences occur within 8 weeks of diagnosis, and this period is therefore usually used to standardize the definition of recurrence. For the diagnosis, not only microbiological confirmation of toxigenic *C. difficile* in stool is essential, but also the reappearance of symptoms after previous resolution with treatment.

Finally, there is a situation called **refractory CDI**, defined as CDI that does not respond to antibiotic therapy, i.e. does not show a response after 3-5 days of antibiotic treatment.[5] This situation probably encompasses different conditions such as persistence of diarrhea due to dysbiosis, presence of other undiagnosed causes of diarrhea, very early recurrences or simple variability in the response to antibiotic treatment of CDI.

With these considerations, it is worth reviewing the data and uses of the FMT in the different situations previously described.[5, 8, 10]

3.1. Recurrent CDI

Recurrent CDI is the clinical situation for which the greatest degree of evidence of the therapeutic efficacy of FMT is available. However, some authors have criticized the available studies, suggesting that they have significant limitations that prevent the efficacy and safety of the procedure from being established.[11] The most recent versions of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) clinical guidelines for the treatment of CDI [5]place fidaxomicin as the treatment of choice in first episodes of CDI and first recurrences (second episodes) when available and recommend the use of FMT (or bezlotoxumab together with conventional antibiotic treatment) for the treatment of second and subsequent recurrences.[5] The American College of Gastroenterology (ACG) clinical guidelines also recommend the use of FMT for second and subsequent recurrences,[8] provided that the patient has been treated with conventional antibiotic therapy in previous episodes.[12]

The use of FMT in this setting requires a multidisciplinary approach to adequately assess the benefit/risk balance for each patient. Furthermore, patient preferences and the healthcare setting (including the availability of FMT preparations) must be taken into account.

3.2. Severe and treatment-refractory CDI

Although the evidence currently available in this clinical situation is less than that available in recurrent CDI, the latest clinical guidelines published by the ESCMID[5]and the ACG[8]recommend considering FMT in patients with severe (or fulminant) CDI who do not respond to antibiotic therapy, especially when patients are not considered suitable for surgery.

In these patients, a benefit/risk balance assessment is recommended on a case-by-case basis, taking into account the feasibility of the surgical approach, the availability of FMT, and the medical team's experience with this type of transplant.

3.3. Available data on FMT safety

Regarding the safety of FMT, several clinical studies[13,14]as well as a meta-analysis of such studies published in 2016[15], show that the most frequently reported adverse reactions are abdominal pain/bloating, diarrhea, flatulence, and fatigue. In one case series, the most frequently reported adverse reaction was worsening of arthritis, a fact that the authors linked to a higher prevalence of joint disease in the older patients in the study.[16]

The potential long-term adverse reactions associated with the administration of fecal matter are unknown, both due to the potential transmission of unknown pathogens and the functional or metabolic changes that the interaction with the new microbiota may induce in the recipient[10]or the impact on the transmission of procarcinogenic bacteria. Therefore, long-term results would be necessary to determine the safety profile of FMT in these patients.[17]

It is worth noting that two recent reviews of FMT adverse reactions showed that the majority are related to the administration procedure.[18,19] In this regard, although more data are needed, particularly from randomized clinical trials, it is possible to speculate that FMT performed by oral administration of capsules may have fewer side effects than FMT by other traditional administration routes, since they involve more invasive procedures and therefore carry a higher risk, inherent to the administration procedure itself. In published studies of FMT by capsules, the adverse reactions identified were mostly mild, the most frequent being nausea, abdominal discomfort, bloating and irregular bowel movements.[20,21,22] However, in 2019, two cases of transmission of extended-spectrum beta-lactamase-producing *E. coli* were reported to immunosuppressed patients who had received FMT, one of whom died.[23] Subsequently, in 2020, the presence of enteropathogenic *E. coli* and Shiga toxin-producing[24]*E. coli* in the product used for FMT in 6 patients was reported, 4 of whom required hospitalization.[25] In addition, the deaths of two patients who had received FMT from the same donor were reported, although it could not be confirmed that this infection contributed to their death. The identification of these cases related to the transmission of pathogenic microorganisms has led to the modification of donor screening protocols.

In a recent review,[26] which included a meta-analysis of 20 randomized clinical trials and 109 non-randomized clinical trials, it was observed that 19% of patients had experienced some adverse reaction, the most frequent being diarrhea (10%), abdominal discomfort (7%), nausea, vomiting or flatulence (3.3%).

Regarding serious adverse reactions, bacteremia and death were observed in 0.09% of patients.

4. EVALUATION AND SELECTION OF STOOL DONORS FOR FMT

4.1 General aspects

According to current legislation, the donation of any SoHO must be a **voluntary and unpaid act**, which contributes to the safety and protection of human health and respects human dignity. Notwithstanding the foregoing, the procedure related to fecal donation will in no case be burdensome for the living donor.

On the other hand, if an institution intends to carry out any promotional or advertising activity in support of FM donation, this will always be done in a general manner, without seeking a benefit for specific individuals, and will emphasize its voluntary and altruistic nature, in addition to requesting prior authorization from the SCA. The existence and/or persistence of false, misleading, or biased advertising

and promotion will be incompatible with the authorization of activities related to obtaining, preserving, processing, distributing, or applying FM in Spain by the center, institution, unit, or SoHO establishment for feces (SE-F) that issued such advertising or has contractual relations with the institution that issued it.

Informed consent must be obtained prior to FM donation. To this end, potential stool donors for FMT must receive written information in understandable language about the donation process, the final destination of the donated stool, the consequences and risks of donation, the nature of the studies involved in their evaluation as a donor, and the possibility of revoking this consent, as well as its limitations. The informed consent document must include, at a minimum, information about the selection process, the results of tests performed that may be relevant to the donor's health, the possibility of multiple donations, the inclusion of data in the corresponding registry, and the possibility of unscheduled contact with the donor should any adverse event or reaction occur.

The donor **recruitment process** influences donor selection and safety. Selection rates vary between 3% and 30% depending on whether campaigns target the general population or specific donor groups. There are different types of donors depending on their relationship to the recipient: blood relatives, individuals with close contact with the patient (partners and other cohabitants), or healthy volunteers unrelated to the recipient. The evidence available at the time of writing this document does not support the superiority of related donors compared to unrelated donors when FMT is used for the treatment of CDI.[¡Error! Marcador no definido.] In fact, anonymous donors are preferred to maintain separation between donor and recipient and ensure the altruistic nature of the donation.

Donor selection aims to protect the recipient, avoiding adverse reactions associated with the transfer of pathogenic microorganisms from fecal matter and ensuring the effectiveness and safety of FMT.

Since advanced age is associated with changes in FM, it is preferable to select potential **donors aged 18–50 years, or up to 60 years in the case of a negative colorectal cancer screening test**. Although there may be more suitable donors than others whose FM is associated with better outcomes for different indications, currently there are no criteria for defining the optimal donor. Regarding the treatment of CDI, a recent study of 6,053 patients treated with FMT from 249 donors has shown that the "donor effect" in this indication is not clinically relevant.[27]

The evaluation and selection of potential donors must be carried out by a **multidisciplinary team** that includes specialists in different areas (gastroenterologists, internal medicine physicians, or physicians specializing in the diagnosis and treatment of infectious diseases), from a centralized consultation room for the FMT. Once the exclusion criteria have been ruled out and the donor's informed consent form has been signed, a general history, clinical examination, and a specifically designed questionnaire will be conducted. If no contraindications are identified after the initial clinical evaluation, blood and stool samples will be obtained for laboratory analysis.

4.2. Donor Selection Criteria

The Catalan Society of Digestive Disease, the Catalan Society of Infectious Diseases and Clinical Microbiology and the GEMBIOTA group of the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) has established recommendations regarding FM donor selection criteria and tests to be performed (**Annex 1**).[28] This document is considered aligned with the provisions of Chapters VI and VII of the *SoHO Regulation* due to its equivalence with the *EDQM Guide* and is therefore **assumed as the reference standard in Spain**. According to the aforementioned document, the pre-donation study or first

screening must be carried out at most 2 weeks before donation and, to guarantee the safety of the recipients, a second screening must be carried out 8 weeks later. During this period of time, donations will remain in quarantine, frozen between -60ºC and -80ºC to be subsequently released for use. This procedure confirms that the donor has not experienced any significant changes in their health during the donation period.

There are small differences in the selection processes included in the various study protocols published in the literature,[10] but all of them emphasize the importance of the interview with the donor to carry out a detailed anamnesis and the use of questionnaires to evaluate the donor's health status and lifestyle habits, which help to identify risk factors.

4.3. Donor Evaluation

Donor selection involves two phases: a first phase in which a thorough medical history is taken to assess potential risks, and a second phase in which specific blood and stool tests are performed.

The **medical history and risk assessment** are based on an interview and a questionnaire, which must then be evaluated by a qualified healthcare professional. The questionnaire includes questions designed to rule out risk factors for infectious diseases, identify individuals with acute or chronic illnesses, and detect substance use that may affect FM.

The **tests to be performed on donor blood and stool** are listed in **Annex 1.** These tests should be updated regularly based on knowledge of risk factors for disease transmission and always in accordance with ECDC recommendations (https://www.ecdc.europa.eu/en).

In the case of FM, the list of microorganisms that, if identified in cells or tissues, require their exclusion **does not apply** (except in specific cases of hematopoietic stem cell transplantation after a careful risk-benefit assessment or if they can be eliminated through a validated sterilization process). In particular, the presence of *Staphylococcus aureus*, *Staphylococcus lugdunensis*, *Streptococcus* spp., *Enterococcus* spp., *Clostridium* spp., Enterobacteriaceae (e.g. *Escherichia coli*, *Enterobacter* spp.), yeasts and filamentous fungi (molds) does not entail the exclusion of feces.

The *EDQM Guide* establishes that, in the case of recipients with severe immunosuppression, additional safety measures are necessary, including the determination of Cytomegalovirus (CMV) and Epstein-Barr Virus (EBV) in both the donor and the recipient. However, the document of the Catalan Society of Digestive Medicin*e*, assumed as the reference standard in Spain, establishes the indication that all donors should undergo such additional measures regardless of the recipient's status.

Additionally, a second specific questionnaire must be used before each donation to assess any condition that has arisen or become evident and is known between donor selection and the time of donation (**Annex 1**).

Complete donor screening (general blood and stool tests) should be performed every two months. Thus, the donation period should have a defined maximum duration, not exceeding two months, and should begin and end with a complete donor screening. Performing a new complete screening after the donation period before any use of the preparations eliminates the need for blood screening for each donation.

5. OBTAINING FM

It is essential to obtain **specific informed consent** prior to beginning the donation process.

Defecation may take place at the donor's home, but it is preferable to do so at an authorized SE-F. The sample must be collected in a specially prepared, single-use, tightly sealed container. For sample acceptance, a visual inspection must be performed, and, following the Bristol scale,[29] only Bristol 3-4 samples should be accepted, not from donors with diarrhea or constipation.

In order to maintain the viability of the anaerobic microbial population, although not strictly necessary, it is advisable to use a container that preserves anaerobiosis or to incorporate an anaerobic system into the container to maintain these conditions until processing.

The sample must be transported to the SE-F where processing will be carried out in a thermal bag to keep it refrigerated and within the time established by the *EDQM Guidelines* for processing (less than 6 hours). In cases where processing is not carried out within this time period, the sample must be kept refrigerated at 4 °C in special containers under anaerobic conditions (Gut Alive^{R}) until delivery within a maximum period of 24 hours. Prior to processing or freezing the sample, control aliquots must be collected from each sample to be frozen and to assess the need to analyze the bacterial load of that specific sample at any time by culture.

The amount of feces required to perform a FMT is 50 g. If the collected sample is larger, it can be separated into 50-g doses and processed in parallel.

6. FM PROCESSING

As a general rule, processing will follow the technical specifications outlined in the *EDQM Guide.*[¡Error! Marcador no definido.] The processing methods employed must guarantee the quality and safety of the FM and its clinical effectiveness. To ensure compliance with these objectives, processing methods must be validated before implementation and periodically according to a risk assessment that will determine their frequency.

In turn, SE-F facilities must comply with the requirements defined in the *EDQM Guide* to minimize the risk of contamination during processing. In particular, through the use of the EDQM *Contamination Risk Assessment Tool* (MiRCA) (https://www.edqm.eu/en/-/mirca-an-edqm-tool-to-enhance-safe-use-of-substances-of-human-origin), capable of identifying contamination risks associated with the methods of obtaining or processing SoHO, alerting the level of severity and advising on the measures to be adopted to minimize these risks.

FMT was traditionally performed with fresh feces and minimal processing. However, numerous studies have shown that freezing does not affect the quality of the final preparation, and this is currently the method recommended by the *EDQM Guide.*[¡Error! Marcador no definido.]

Preparation **with frozen feces** can be done in two ways:

- ✓ **Immediate processing and subsequent freezing:** The sample is diluted in 250 ml of sterile saline solution (0.9%) or, failing that, sterile water, until a homogeneous mixture is obtained. During sample processing, protective gloves and a face mask should be worn. The preparation can be performed under aerobic conditions; greater efficacy in the treatment of refractory CDI has not been demonstrated with the anaerobic preparation. After homogenization, the volume must be

filtered (or centrifuged) until a liquid sample is obtained that does not contain traces that could clog the tube through which the administration will be performed. After filtration, 10% sterile 99% glycerol is added. The product is then collected in a sterile container and frozen at -80°C, properly labeled until use and in a manner that allows correct traceability. Once the sample is required for administration, the volume must be thawed for 14–18 hours at 4°C and then for 1 hour at room temperature. Once thawed, it can be used to perform FMT via either of the two administration routes described below. A stool sample must have a single freeze/thaw cycle.

- ✓ **Direct freezing:** This involves freezing the sample directly between -60°C and -80°C without any manipulation. Once the sample is needed, it is thawed progressively, going from -80°C to -20°C in 24 hours and then to 4°C in another 24 hours. Finally, the process described above begins at room temperature and the FMT is performed immediately.

The stability of frozen FM at -80°C is unknown, so the maximum storage time for samples is not well established. Most authors agree on a maximum recommended storage period of 24 months at this temperature. After this time, samples must be disposed of via standard means. Studies analyzing sample stability at different time intervals are needed to determine the maximum storage time.

6.1. Volume according to the route of administration of the FM preparation

The final volume of the sample depends on the route of administration:[30]

- ✓ **Upper gastrointestinal tract:** The final volume to be administered with 50 g of feces should not exceed 200 ml, with a very slow infusion rate (10–25 ml/minute) to avoid the risk of nausea, regurgitation and/or aspiration.
- ✓ **Lower gastrointestinal tract:** The final volume to be administered with 50 g of feces can be between 250 and 500 ml, to be deposited in the cecum or in the most proximal section reached with the colonoscope (if the cecum cannot be reached).

6.2 Protocol for processing stool samples to obtain lyophilized FM capsules

As a general rule, the environmental conditions for processing must meet the requirements defined in the *EDQM Guide* **¡Error! Marcador no definido.**.

The freeze-drying and encapsulation process consists of four parts:

1. The weighed sample is processed in a Class II laminar flow cabinet using sterile equipment and diluted with saline solution to a volume of 1:10. It is recommended to allow the sample to hydrate for at least 20 minutes to facilitate the subsequent mixing step. For this purpose, an automatic homogenizer such as the Stomacher 400 Circulator (Seward Ltd., Sussex, UK) is preferably used for 1 minute at 230 rpm.
2. After homogenization, the tubes are centrifuged at 200 g for 10 minutes at 4 °C, and the supernatant obtained is subjected to a second centrifugation at 600 g for 15 minutes at 4 °C. It is recommended that the supernatant be filtered prior to the second centrifugation to remove traces of organic matter. After the second centrifugation, the supernatant is decanted and discarded, retaining the pellet that will contain the FM. Before starting the freezing process, lyo/cryoprotective agents such as mannitol or trehalose must be incorporated in proportions of 5 to 10%. This pellet will be frozen at -80 °C for at least 1 hour in containers suitable for the lyophilization process.

3. The pellet is then freeze-dried, which can be carried out in various commercial freeze-dryers that must reach a freeze-drying temperature of at least -50°C. The freeze-drying process is carried out under vacuum for approximately 18 hours (O/N), or until the product is completely freeze-dried.
4. The freeze-dryer is slowly decompressed to prevent powder dispersion. The product can then be encapsulated directly in gastro-resistant capsules. It is recommended to carry out the encapsulation process in a hood, but taking care to avoid air flow. The capsules should be kept at room temperature or refrigerated at 4°C, but always in the absence of moisture. It is recommended to add drying agents to the storage container, considering the expiration date .

Capsules should be carefully inspected before administration and should have an intact surface and should be discarded if cracked.

7. LABELING AND PACKAGING

Labelling and packaging conditions must comply with the specifications in force for human tissues and cells.[2, 3] Primary and secondary containers must be correctly identified:[¡Error! Marcador no definido.]

- ✓ Primary container:
  - Donation code/donation identification
  - Type of preparation
  - Expiration date
- ✓ Secondary container:
  - Type of preparation
  - Unique identification code or number
  - Batch number if applicable
  - SE-F Identification
  - Expiration date
  - Final destination of the preparation, particularly if it is for a specific recipient
  - Unique EU Code (SEC) in case of distribution for human administration in a SoHO entity other than the SE-F

  If there is not enough space on the container label, the information can be included in documents accompanying the sample, ensuring at all times that they are not separated.

The label or accompanying documentation must include the following information:

- ✓ Description and final volume of the FM preparation
- ✓ Date of distribution of the FM preparation
- ✓ Tests performed on the donor and their results, if relevant
- ✓ Storage and conservation recommendations
- ✓ Instructions for opening containers and handling the FM preparation
- ✓ Expiration date once the container is opened

8. STORAGE

Frozen FM preparations must be stored in sealed and properly labeled containers to ensure traceability. Freezers must maintain temperatures between -60°C and -80°C, have continuous monitoring, an alarm

system, and specific compartments for quarantined FM preparations, released FM preparations, and aliquots of FM preparations.

Fresh preparations must be quarantined in specific freezer compartments and moved to the released preparation compartments once they have passed the quality controls that allow their release. Quality criteria will be defined in the operating procedures of the SE-F quality management system. Samples of all FM preparations must be retained in case additional testing of a FM preparation is required. Only released preparations may be used for FMT. Storing frozen preparations between -60°C and -80°C ensures their clinical usefulness for up to 24 months after freezing.

9. QUALITY AND RELEASE CONTROLS

Quality control aims to verify that materials and processes, as well as the final preparation, meet predetermined specifications before release. Procedures must define the various specifications that, for FMT, must include everything from donation to the end of processing, including all documentation that must accompany each step (donor evaluation, donation, processing, and release), verification of the intact appearance of the FM preparation, packaging, weight of the donation and the final FM preparation, as well as storage conditions.

The specifications attributable to the final FM preparation will be subject to the provisions of *Royal Decree-Law 9/2014* [3]until the date of implementation of the *SoHO Regulation,*[2] from which point onwards they will be required to comply with its content. In particular, those referring to exclusions related to the presence of pathogens, diversity of the final preparation, and quantity and viability of the bacteria present in the final FM preparation. To ensure a high level of quality and safety of the final FM preparation, these specifications must be met as part of the quality control of the FM product for release by the person responsible for the SE-F.

10. DISTRIBUTION-ASSIGNMENT

The distribution of a FM preparation to a FMT center from an SE-F will be carried out based on an agreement between both entities, which will include the responsibilities of each entity, as established in current regulations.[3,2] The FM preparation will be requested from the SE-F by a physician or healthcare personnel authorized by them. Only FM preparations authorized by a physician in accordance with the consolidated uses listed in this document and to entities authorized to carry out the FMT by the competent authority of the corresponding Autonomous Region will be distributed.

The distribution of the FM preparation must be carried out using a validated transport system, in containers containing dry ice, unless the preparations are to be used immediately, in which case they can be transported at a temperature between 4-8ºC.

For the distribution of FM preparations, the SE-F must have operating procedures that include at least[¡Error! Marcador no definido.]

- ✓ Transport conditions (e.g. temperature, time limit) to ensure that the properties of the FM preparation are maintained
- ✓ Transport container specifications. All containers must be validated.
- ✓ Agreement with the transportation company/service describing its responsibilities

- ✓ Procedure and measures to be taken by qualified SE-F personnel in case of withdrawal
- ✓ Procedure for handling returned preparations and criteria for inclusion in the inveONTry

11. HUMAN APPLICATION

The FMT may only be carried out in entities specifically authorized by the competent authority of the corresponding Autonomous Region.

The clinical administration of the FM preparation and patient follow-up is the responsibility of the physician performing the FMT.

FMT can be performed through the lower gastrointestinal tract, by retention enema or colonoscopy, or through the upper gastrointestinal tract, by gastroscope or tube (nasogastric, nasoduodenal or nasojejunal), or by oral capsules, which may contain frozen or lyophilized material.

FMT is an effective treatment for recurrent *C. difficile infection* (R-CDI), regardless of the route of administration[¡Error! Marcador no definido.]. The preferred method of administration may vary depending on the patient's clinical situation. In the context of R-CDI treatment, the most common practice is to administer *C. difficile*- specific antibiotics a few days before administering the FM preparation.[10,13,31]

Regardless of the route of administration, it is recommended that any antibiotic treatment be discontinued or terminated (at least 24-48 hours beforehand) and, whenever possible, that any medication that may interfere with the integrity and composition of the FM be discontinued.[10] [10]A new medical evaluation along with laboratory testing is recommended before performing the FMT[10,32.]

FMT procedures requiring enteral tube placement or endoscopy must be performed after fasting for 6-8 hours. Administration of new antibiotics after the procedure should be avoided as much as possible. Consultation with physicians specializing in the diagnosis and treatment of infectious diseases is recommended whenever FMT recipients have a long-term antibiotic indication or an antibiotic indication within 8 weeks of FMT.

Before administering a frozen solution, it must be thawed. A stool sample must undergo a single freeze/thaw cycle.

The specifics of the procedure for the different routes of administration are detailed below.

11.1. Upper gastrointestinal tract

11.1.1. FMT via gastroscope or nasogastric, nasojejunal, or gastrostomy tube

FMT can be performed through the working channel of a **gastroscope or through a nasogastric, nasojejunal, or gastrostomy tube**. It is recommended that patients remain in a 45° upright position for 4 hours after the infusion to prevent aspiration of the instilled material.

This route should be used with caution in patients at risk for regurgitation and/or those with swallowing disorders. This route is not recommended for ileus.

Prokinetics and proton pump inhibitors have been frequently used pre-intervention in the literature.[32,33,34] This procedure should be used with caution due to its potential effect on the modification of the intestinal microbiota.[10] However, the studies demonstrating this effect of proton pump inhibitors were

conducted in subjects with continuous use of the same, not on a one-off basis.[35,36] It does seem reasonable to restrict their use after FMT, whenever possible, to preserve the composition of the infused FM.

It is advisable to check with an X-ray that the tube is correctly inserted, and after the procedure, it is recommended to remove it 30 minutes after the end of the infusion.

In general, total volumes of bacterial suspension administered by this route are lower compared with administration into the lower gastrointestinal tract. Depending on the infusion site, different amounts have been used; for the stomach, a median of 25 g (range, 5–30 g) of feces in a suspension with a median volume of 68 ml (range, 10–150 ml); for the duodenum/jejunum, a median of 63 g (range, 10–150 g) of feces in a suspension with a median volume of 252 ml (range, 60–500 ml).[13]

11.1.2 Administration by capsules of frozen or lyophilized contents

Recently, administration of FM via **capsules, both frozen and lyophilized**, has been incorporated into this route. Studies published to date indicate that FMT via capsules has a similar efficacy (87%-100%),[19,20, 32, 37, 38,39] better tolerance and is associated with fewer adverse reactions than through other routes.[19,20, 37, 39]

In the medical literature we can find at least 8 studies, two of them randomized clinical trials, that document FMT through capsules, either in frozen form [20, 40, 41.42] or in lyophilized form.[19,37,38,42]

Capsules should be carefully inspected before administration and should have an intact surface and discarded if cracked.

11.1.3 FMT by capsules vs. FMT by traditional methods

Two randomized trials with capsules have shown no significant differences between this and other traditional forms of administration, such as enema or colonoscopy. FMT using frozen capsules was compared with FMT administered by colonoscopy and no significant differences were found between the groups with respect to efficacy, with a 96.2% resolution rate of C. *difficile infection* in both the capsule group (51/53) and the colonoscopy group (50/52).[20] The capsule FMT administration group performed better in terms of tolerance of the FMT process, compared with the colonoscopy group.[20] On the other hand, another trial compared FMT using lyophilized capsules and FMT via enema, in which no significant differences were found in terms of efficacy rate, since they had a resolution in 26 of 31 (84%) subjects who received lyophilized capsules and in 30 of 34 (88%) who received FMT via enema (p = 0.76).[19]

Therefore, current data show that administration via either frozen or lyophilized capsules presents no differences in efficacy compared to traditional FMT procedures. Future studies evaluating the efficacy of capsules in routine clinical practice, as well as specific administration protocols, are needed.

11.1.4 Capsules with lyophilized contents vs. frozen contents

There is still uncertainty about the number of capsules administered for FMT (ranging from 2 to 40), as it varies greatly. In the available studies, the number of patients who received a low number of capsules is very limited, but this does not appear to have an impact on the efficacy rate.[19,29, 40-44 43] The limited evidence available shows that bacterial viability data for lyophilized forms appear to be comparable to those for frozen forms.[19]

In the case of FMT using frozen capsules, the most commonly used doses have been 20 to 40 capsules. However, the most recent studies with capsules with lyophilized content have managed to reduce doses

to 2-4 capsules for single administration.[40,41] Precisely one of the advantages of lyophilized encapsulated forms compared to frozen encapsulated forms is the volume of product that can be packaged in lyophilized forms, which would lead, in theory, to a reduction in the number of capsules required to ingest a given dose and therefore, to improved tolerance and compliance with the process.[19,40,41] On the other hand, freeze-dried powder has a less noticeable odor or flavor than the frozen product.[19,40] Finally, it should be noted that storage of freeze-dried capsules is simpler than that of frozen capsules since they do not require an ultra-freezer for preservation.[40,41]

11.2. Lower Gastrointestinal Tract

11.2.1. FMT by colonoscopy

FMT by colonoscopy is one of the methods with the best resolution rates, generally above 90%, with a range between 71% and 100%.[18,44]

For this route of administration, it is recommended to first perform a laxative bowel wash similar to that conventionally used for any colonoscopy. Preparation with 4 liters of polyethylene glycol has frequently been used, although low-volume preparations are now available that significantly improve patient tolerance.[33,35]

Sometimes patients with CDI who are candidates for FMT are elderly and/or frail, and colonoscopy poses an additional risk to consider. The contraindications for this procedure are the same as for a conventional colonoscopy.

It is recommended to perform it under the usual sedation regimen, following the hospital's clinical practice. The sample was traditionally instilled using prefilled syringes through the colonoscope channel or, currently, with the use of a water pump, which makes the procedure faster and simpler. The sample should preferably be deposited in the right colon, reaching the terminal ileum or cecum whenever possible, as this appears to result in a higher efficacy rate. In cases of severe colitis, the fecal suspension may be released into the left colon at the physician's discretion for safety reasons.[10,14,33, 45, 46, 47]

It is recommended that, after the procedure, the patient remains in the right lateral decubitus position for 45-60 minutes and follow the usual post-colonoscopy instructions.[36] Administration of loperamide or another antiperistaltic drug may be helpful to prevent excretion for at least 4 hours.

11.2.2. FMT by enema

FMT via enema has also been shown to be effective.[46 48, 49] However, the efficacy rate appears to be lower than that of other routes (63.3%–86.2%), and for optimal disease resolution, it must be performed repeatedly.[10,46,54,50] Its main advantages are its simplicity of administration and low cost. The amount of fecal suspension used per enema generally ranges from 150 to 500 ml.

As with colonoscopy, it may be helpful to administer a single dose of loperamide or another antiperistaltic drug after the procedure. It is recommended to remain supine for at least 30 minutes.

12. TRACEABILITY

The FMT traceability requirements will be subject to the SoHO legislation in force in Spain.[2,3]

SE-F must have a traceability system that uniquely associates each FM donor with the processed FM and with all documents and samples, FM preparations and SoHO entities associated with the FMT at each step.

All samples will be identified by their collection date and with a specific alphanumeric code, which must be linked to the donor code for each donation and processing, and whose information is only available to the responsible personnel. Once the FMT is performed, the sample code must be linked to a specific code for the recipient. This way, the donor's anonymity is maintained without losing sample traceability over time, allowing the donor and recipient to be identified for each donation and processing.

Samples from all donations should be collected for analysis of FM by sequencing and culture methods for future research (e.g., adverse reactions in the recipient). It would also be advisable to conduct implantation studies, i.e., to identify the microorganisms colonizing the recipient's intestinal tract. For this purpose, serial samples should be taken from the recipient (at 1 month and 3 months) to ensure microbiological control of the process.

The code of the administered preparation with the batch number must be recorded in the recipient's medical history.

The traceability system must be able to:

- ✓ Identify the FM donor and the SE-F that releases the FM preparation;
- ✓ Identify the FM recipient at the center performing the FMT;
- ✓ Locate and identify all relevant data on the quality and safety of the FM and all materials or equipment used that may affect its quality or safety.

According to current Spanish SoHO legislation,[2,3] the data required for full traceability must be retained for a minimum of 30 years after clinical use. Data may be archived electronically, and if the SE-F ceases to operate, these data must be transferred to another SE-F, with the prior knowledge of the corresponding SoHO Competent Authority, until the deadline expires.

In the event of non-use of the released product sent for use by a specific recipient, the reason and final destination of that product (return, if this option is contemplated, or destruction) must be stated.

ONT is, in accordance with current legislation, responsible for reporting the existence of serious adverse events that could affect other Member States through the notification system established by the European Commission. It will also notify the regional transplant coordination units where affected SE-F are located, or where a serious adverse event that occurred in another country could be affected, of all information related to said event. Likewise, the ONT maintains a system that supports the coding system, accessible through https://portal.ont.es/dana-na/auth/url_default/welcome.cgi all authorized SE-F, as well as for the transplant coordination units of the Autonomous Regions.

13. BIOVIGILANCE OBLIGATIONS

The notification of adverse events (AE) and adverse reactions (AR) and their management will be carried out based on what is specified in the SoHO legislation in force in Spain[2,3]and in the *National Biovigilance System* (https://www.ont.es/wpcontent/uploads/2023/08/Sistema_de_Biovigilancia.tejidos.pdf).

Recipients should be adequately monitored for the development of FMT-related complications.

In addition, a treatment and clinical outcome monitoring plan must be in place at centers authorized for FMT for patients with R-CDI, adjusted for severity and comorbidities. This plan will include indications for hospitalization in cases with underlying pathologies and based on clinical condition and diagnosis. There is no consensus on the observation period for patients after FMT, which depends on the route of administration, comorbidities, and the patient's clinical status.

Patient monitoring will be conducted to identify possible AR. The AR described in the literature are mostly mild and self-limited, of gastrointestinal nature: abdominal pain, dyspepsia, diarrhea, fever, or constipation. Some are longer-lasting, such as weight gain or intestinal bacterial overgrowth.

Occasional serious ARs (SAR) have been reported, including viral or bacterial infections, transient recurrence of inflammatory bowel disease, and even death. In line with current Spanish SoHO legislation, [2, 3] it is essential to report any suspected AE or AR as specified in the *National Biovigilance System*, in order to initiate an investigation and case management for the adoption of preventive, therapeutic, and corrective measures deemed appropriate.

All SE-F will also designate a biovigilance officer and develop operating procedures for the management of AE and AR.

Any AE occurring from donation to the release and distribution of FM preparations must be appropriately notified to the competent authority immediately. Likewise, the SAR must be notified immediately to the competent authority. The SE-F is responsible for ensuring that the vigilance case is notified to the competent authority and that the necessary corrective measures are implemented. Operating procedures must include the withdrawal and disposal of FM preparations, if necessary. ONT is responsible for reporting the existence of serious AE that could affect other Member States through the notification system established by the European Commission. Likewise, ONT will notify the regional transplant coordination units where the affected SE-F are located or that could be affected by a serious AE occurring in another country, of all information related to said event.

14. REGISTRATION, AUTHORIZATION AND INSPECTION OF CENTERS

Aspects relating to the registration of SE-F and FM procurement centers or units, and FMT centers, as well as their authorization and inspection, shall be governed by the provisions of Royal Decree-Law 9/2014, of July 4. The transplant coordination units of the Autonomous Regions shall be responsible for providing information to the ONT on the SE-F and FM procurement centers or units and FMT centers authorized for such activities within their scope of competence.

14.1 Requirements for authorization of sample preparation for FMT in SE-F

SE-F wishing to obtain-process-release and distribute samples for FMT must be expressly authorized by the competent authority of their corresponding Autonomous Region to carry out said activities, in addition to complying with the requirements specified in *Royal Decree-Law 9/2014* [3] and in the *SoHO Regulation* [4] when applicable, and its implementing regulations, both Spanish and those from European institutions.

SE-F must have at least:

- ✓ Adequate facilities for the selection and screening of stool donors, confidential information for donors, and for the processing and storage of FM preparations, minimizing the risk of

pathogen contamination during processing or cross-contamination. Generally speaking, a dedicated laboratory is required for FM processing.

- ✓ Qualified personnel and designation of a responsible person, a release officer and a doctor whose skills comply with the provisions of the SoHO Regulation.
- ✓ Quality management system with operating procedures to ensure compliance with the good practice guidelines for tissue establishments defined in the *EDQM Guide.*[1]
- ✓ Computerized document management system that ensures record keeping throughout the entire process and compliance with EU and national data protection legislation[51]

14.2. Requirements for the authorization of centers for FMT

It is essential to have centers capable of offering this treatment safely and effectively to selected patients. However, since the number of patients eligible for FMT is not very high, it is currently not necessary to have this resource in most healthcare centers. Although the necessary technology is not particularly complex, aspects related to safety and proper indication for its administration make it advisable to centralize it in referral centers with proven experience that guarantee the adequate quality of this service.

The FMT must be carried out in health centers or services that are duly authorized in accordance with current regulations by the corresponding competent authority of the respective Autonomous Regions.

15. ANNEX 1 EXCLUSION CRITERIA AND ANALYTICAL TESTS TO BE PERFORMED

Table 1. Exclusion criteria in donor selection.

Exclusion criteria		
Demographics	**Age** : < 18 years or > 50 years	
	BMI : < 18.5 kg/m^2 or > 30 kg/m^2	
Medical history	**Relevant** : neoplasia, communicable diseases	
	History of : autoimmune diseases, atopy, asthma, diabetes *mellitus* , treatment with immunomodulatory agents, chronic pain syndromes, neurological or neurodevelopmental disorders, psychiatric disorder, or metabolic syndrome	
	Family history of : colorectal cancer, polyposis syndrome, inflammatory bowel disease, celiac disease, autoimmune diseases	
	Major gastrointestinal or non-gastrointestinal surgery (last 4 months: major fracture, joint replacement, etc.)	
Gastrointestinal history	**Gastrointestinal diseases** : inflammatory bowel disease, irritable bowel syndrome, chronic constipation, chronic diarrhea, previous history of *C. difficile infection* and/or gastrointestinal bleeding	
	Fever or digestive symptoms : diarrhea, nausea, vomiting, constipation, abdominal pain, etc.	
	Taking **medications** that, when excreted in feces, pose a risk to the recipient or cause changes in the intestinal microbiota or dysbiosis (PPI, etc.)	
Infectious history	Positive result for any pathogen determined in the microbiological study in **Table 3** , **Table 4** and **Table 5**	
	Taking **antimicrobials** (antibiotics, antivirals, and antifungals) or **probiotics** in the last 6 months	
	SARS-CoV-2 or contact with a positive case in the last 4 weeks	
Risk factors	**Communicable diseases**	**sexual** behavior in the last 6 months (multiple partners, HIV positive, intravenous drug use, etc.)
		Tattoo , ***piercing*** and/or **acupuncture** in the last 6 months
		Imprisonment
		Travel in the last 6 months to tropical countries, countries with endemic diarrheal diseases, or high risk of traveler's diarrhea
		Needlestick accident
		Work with animals (**zoonosis**)
		Creutzfeldt-Jakob disease
	Colonization by multi-resistant microorganisms	**Healthcare** workers
		People in contact with the **health system** (hospitalization, etc.)
Others	**Smoking** habit (> 10 cigarettes/day)	
	Drug use	
	Having received **blood products** in the last 6 months	
	Having received **attenuated and/or live vaccines** in the last 6 months	

PPI : proton pump inhibitor; **BMI** : body mass index; **SARS-CoV-2** : severe acute respiratory syndrome coronavirus 2; **HIV** : human immunodeficiency virus

Up to 60, if you have a negative colorectal cancer screening test

Table 2. Donor selection questionnaire.

Donor Questionnaire		
Demographics	Sex	
	Weight	
	Height	
	Race	
	Country of birth	
Toxic habits	Do you drink alcohol regularly? How much?	
	Do you smoke? How much?	
	Do you use or have you used any drugs by injection, inhalation, snorting, or any other route in the last 12 months?	
Personal medical history	Do you suffer or have you suffered from any major illness?	
	Are you being monitored by a specialist for any health problems?	
	Do you suffer from or have you been diagnosed with any of the following diseases: diabetes *mellitus* , cancer, obesity, high blood pressure, autoimmune disease, atopic disease, chronic pain syndrome, neurological/neurodegenerative or psychiatric disease?	
	Are you allergic to any food or medication?	
	Have you ever had any surgery ?	
	Do you regularly take any medication? If so, please specify.	
Infectious history	Have you ever suffered from malaria, Chagas disease, leishmaniasis, tuberculosis, or babesiosis (parasites that affect the blood)?	
	Have you ever received a blood transfusion? When?	
	Have you ever received a hair, tissue, organ, skin, bone, dura mater, or bone marrow graft?	
Infectious history	Have you received the vaccines listed in the vaccination schedule? Have you received any attenuated and/or live vaccines in the last 6 months?	
	Have you had any sexually transmitted infections?	
	Have you been diagnosed with HIV, HCV or HBV infection?	
Gastrointestinal history	Do you have any digestive conditions? (IBD, liver cirrhosis, irritable bowel syndrome, chronic diarrhea, chronic constipation, blood in stool, celiac disease, hemorrhoids, frequent flatulence, cramps, or recurring abdominal pain)	
	Have you taken any of the following medications in the last 6 months: antimicrobial, PPI, immunosuppressant, gastric acid suppressant, laxatives, or antidiarrheals?	
Family history	Digestive system diseases: polyps, intestinal/colon cancer, IBD, Crohn's disease, ulcerative colitis, irritable bowel syndrome	
	Do any of your relatives have Creutzfeldt-Jakob disease?	
	Are there any hereditary diseases?	
Risk factors	**Colonization by multi-resistant microorganisms**	Have you been admitted to a hospital in the last 6 months?
	Contraction of communicable diseases	Have you engaged in risky sexual behaviors in the past 12 months? Multiple partners, HIV, HBV, HCV, or any sexually transmitted infection, hemophilia, frequent partner changes, or a past injecting drug user or sex worker.
		Does your regular partner have any infectious disease?

		Have you been in contact with another person's blood or pricked yourself with any material that could be contaminated with another person's blood or fluids?
		Between 1980 and 1996, did you reside in the UK?
Others		

Table 3. Blood laboratory tests for donor selection.

Blood tests	
General analytics	Gomerular filtration
	Liver profile
	C-reactive protein
	TSH
	T4
	Antitransglutaminase antibodies
	Lipid profile
Bacteria	Serology against *Treponema pallidum*
Virus	Serology against CMV (IgG), EBV (IgG) and HSV-1 and HSV-2 (IgG [)]
	Serology against HAV (IgM), HBV (HBsAg, IgM and anti-HBc IgG), HCV (HCV Ac) and HEV (IgM and IgG)
	Serology against HIV-1, HIV-2 and HTLV-1 and HTLV-2 (Ac)
	Serology against SARS-Cov-2 (IgM and IgG)
Parasites	Serology against *Strongyloides stercolaris* (IgG) and *Toxoplasma gondii* (IgG)

HCV Ac : hepatitis C virus antibody; **HBSAg** : hepatitis B virus surface antigen; **anti-HBc** : hepatitis B virus core antibody; **CMV** : cytomegalovirus; **HTLV-1** : human T-lymphotropic virus type I; **HTLV-2** : human T-lymphotropic virus type II; EBV : Epstein-Barr virus ; **HAV : hepatitis** A virus; HBV: hepatitis B virus; **HCV** : hepatitis C virus; **HEV** : hepatitis E virus; **HSV -1** : herpes simplex virus type 1; **HSV -2** : herpes simplex virus type 2; **HIV-1 :** human immunodeficiency virus type 1; HIV **-2** : human immunodeficiency virus type 2; **IgG** : immunoglobulin G; **IgM** : immunoglobulin M; **SARS-CoV-2** : severe acute respiratory syndrome coronavirus 2; **T4** : thyroxine; **TSH** : thyroid-stimulating hormone.

[a] *It is recommended to rule out donors with positive serology for CMV, EBV, HSV-1, HSV-2 and Toxoplasma gondii for seronegative immunocompromised recipients*

Table 4. Laboratory tests on stool for donor selection.

Stool analysis	
General analytics	Detection of occult blood in [feces]
	Calprotectin
Bacteria [b]	Study for toxigenic *Clostridioides difficile*
	Detection of gastrointestinal pathogens: *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Vibrio cholerae , detection of Escherichia coli* pathotypes (enterotoxigenic, enteroaggregative, enterohemorrhagic, enteropathogenic, enteroinvasive) and *Helicobacter pylori* [c]
	Plesiomonas and *Aeromonas*
	Detection of multi-resistant bacteria: ESBL-producing Enterobacteriaceae, vancomycin-resistant Enterococcus, carbapenem-resistant Enterobacteriaceae, and [MRSA]
Virus [b]	*Norovirus, Rotavirus, Adenovirus, Enterovirus* and SARS-CoV-2
Parasites [b]	*Giardia lamblia, Cryptosporidium* spp., *Entamoeba histolytica, Blastocystis hominis* and *Dientamoeba fragilis*
Mushrooms [b]	Microsporidia [and]

ESBL : extended spectrum beta-lactamases; **MRSA** : Methicillin-resistant *Staphylococcus aureus ;* **SARS-CoV-2** : *severe acute respiratory syndrome coronavirus 2* .

a If the donor is 40 years of age or older.

b The detection technique is at the discretion of each microbiology laboratory.

c Only in those cases in which the FMT is to be performed by upper gastrointestinal endoscopy.

d In case the donor reports contact with MRSA carriers.

e Practice in case the FMT recipient is an immunosuppressed patient.

Table 5. Nasopharyngeal swab laboratory tests for donor selection.

Nasopharyngeal swab	
Bacteria [a]	SARM [b]
Virus [a]	SARS-Cov-2

MRSA : Methicillin-resistant Staphylococcus aureus; **SARS-CoV-2** : severe acute respiratory syndrome coronavirus 2.

The detection technique is at the discretion of each microbiology laboratory.

b If the donor reports contact with MRSA carriers.

Table 6. Aspects to re-evaluate the donor on the day of donation.

Questionnaire to re-evaluate the donor on the day of donation	Yeah	No
New intestinal symptoms: diarrhea, nausea, vomiting, abdominal pain, jaundice		
Diarrhea (more than 3 loose or liquid stools) in cohabitants, including children, in the previous month		
Signs or symptoms of recent illness (e.g. fever, odynophagia, lymphadenopathy)		
Use of antibiotics or other drugs with the potential to alter the microbiota		
New sexual partners		
Travel abroad		
Travel to tropical areas		
Contact with blood or high-risk sexual practices		

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