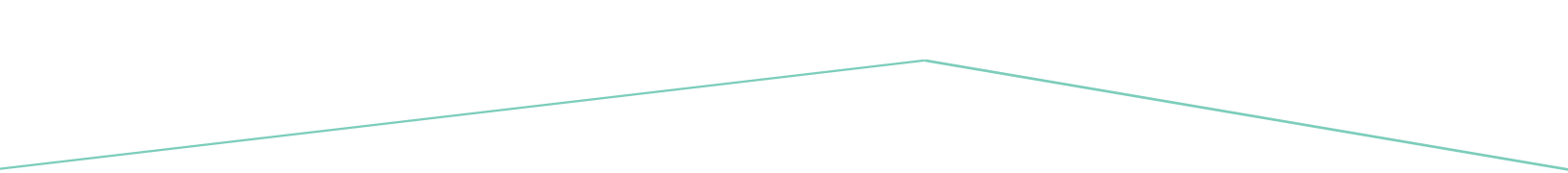




From Stool Donation to Fecal Microbiota Preparation

The Logistics of Fecal Microbiota Preparation Manufacturing
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Abstract

Fecal microbiota transplantation (FMT) preparations comprise minimally processed stool that retain a broad fraction of the stool donor’s complete microbiome. In clinical trials and real-world settings, FMT has been shown to treat approximately 80% of antibiotic-resistant *C. difficile* infections and is the standard of treatment for recurrent *C. difficile*. This paper, using OpenBiome as an example, reviews important considerations for manufacturing FMT preparations including safety and operational measures.

Note: Considerations for donor screening are covered separately in a white paper titled [“Clinical Considerations for Donor Selection”](#)

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Overview of Manufacturing

Although clinical trials and data from real world settings demonstrate that Fecal Microbiota Transplantation (FMT) resolves 80-90% of antibiotic-resistant *C. difficile* infections, there is no standard formulation for FMT Preparations.

FMT material has been delivered in the form of fresh, frozen, lyophilized, and cryopreserved stool.¹ The active ingredient in FMT preparations has not yet been identified and each of these forms of preparation creates different selection pressure on the donor's microbiome, resulting in different consortiums of bacteria. Additionally, the amount of donor bacteria that a patient receives depends not only on the form of the preparation but also on the delivery modality which include upper delivery (i.e., nasoenteric/gastric tube or EGD), lower delivery (i.e., colonoscopy, sigmoidoscopy, or enema), and orally vial capsules.

The manufacturing processes described here have been used by OpenBiome to manufacture over 60,000 FMT preparations that were delivered to approximately 1,300 health-care facilities in the United States. In 2016, an analysis of follow-up data from 482 healthcare facilities found that, across all formulations and delivery modalities, OpenBiome material has an 84% clinical cure rate.² When possible, we've taken care to emphasize aspects of our manufacturing processes that are broadly applicable to the field of stool banking.

Product

OpenBiome manufactures two liquid FMT preparations:

- FMP (Fecal Microbiota Preparation) 250: A 250 milliliter formulation for lower delivery via colonoscopy, sigmoidoscopy, or enema.
- FMP30: A 30 milliliter formulation designed for upper delivery via nasoenteric/gastric tube or EGD.

Process

For both preparations, the manufacturing process is divided into three sections (Figure 1):

- **Stool Examination:** Donor stool is obtained in a controlled setting. Donors and their stool are checked for potential health risks to patients before the manufacturing process begins. (For more on donor selection and donor screening, please see the white paper titled "[Clinical Considerations for Donor Selection](#)"). In OpenBiome's experience, about one-third of stool donations are rejected for being underweight (not having enough material to process into FMT preparations) or having potentially pathological morphology.

- **Processing:** Saline and glycerol buffer is added to the stool and the resulting mixture is minimally processed to remove fiber and nonsoluble material while leaving the microbiome intact.
- **Storage:** Newly manufactured FMT preparations are aliquoted into uniquely identified bottles and stored long-term in a temperature-controlled, -80-degree Celsius freezer.

*** For bacterial viability reasons, stool samples must be processed within 24 hours of donation.**

An internal study by OpenBiome demonstrated that, when stool samples are stored at 2-8 degrees Celsius with an oxygen scavenger for 48 hours, there is no significant change in total number of cells, percent viable cells, or bacterial community composition. To be conservative and provide additional safety margin, OpenBiome decided on a 24-hour processing time limit.

Personnel

The manufacturing process requires coordination between two teams:

- **The Donor Operations Teams** made up of clinical staff and donation coordinators who monitor stool donor health and manage the process of receiving stool donations.
- **The Biomanufacturing Team** made up of laboratory technicians who process stool donations into FMT preparations.

During the manufacturing process, chain of custody should be maintained from the moment the stool donation is provided. Chain of custody begins with the Donor Operations Team and ends with the Biomanufacturing Team.

Manufacturing within the Larger Context of Stool Banking

The manufacturing process is an integral part of a stool bank's Quality and Safety program alongside Donor Assessment, Quality Assurance, and Pharmacovigilance (Figure 2).

Liquid FMT Preparation Manufacturing Protocol

Listed below are step-by-step directions describing the process used by OpenBiome to manufacture FMP250s and FMP30s. These directions are also summarized in Figure 1. This protocol is meant to illustrate basic manufacturing principles that should be adapted based on a stool bank's own FMT formulations, safety guidelines, and facilities.

Stool Examination

Overall Goals and Responsibilities

- The overall goal of stool examination is to verify the quality of the stool before it goes through the FMT manufacturing process.
- During the stool examination phase, the **Donor Operations Team** receives stool provided by the donor and reviews donor health for potential safety concerns. This donor health review at the time of each donation is part of a program of donor health surveillance that is described in more detail in “The Logistics of Donor Screening”. The stool sample is then transported to the **Biomanufacturing Team**, which visually inspects the stool for any abnormalities.
- **Additional Reading:** For more information on how stool banks evaluate and monitor patient health, read our papers titled “[Clinical Considerations for Donor Screening](#)” and “[When is an FMT Treatment Ready for Use? Health Monitoring and Material Release](#).”

Protocol

- A qualified stool donor provides a stool sample at a controlled stool collection site.
- The Donor Operations Team provides donors access to a bathroom that is cleaned between each use and a collection container for their stool.
- The sample is placed in a tamper-evident enclosure and marked with donor identifiers and time of passage.
- The donor completes a short health questionnaire (Appendix 1) to note any changes to their health that may potentially affect the quality or safety of their stool. The questionnaire is reviewed by the donor operations team.
- Any abnormal observations are escalated to the clinical staff, which will determine if the sample will be destroyed and if follow-up with the donor is necessary.
- If there is an abnormal result, the Donor Operations Team will mark the donation with a “Destroy” or “Hold” label to instruct the Biomanufacturing Team on how to handle the sample.
- “Destroy” donations will be destroyed as biohazardous waste according to institutional guidelines. “Hold” donations will not be manufactured and also be destroyed unless clinical staff communicate their approval.
- Two biomanufacturing technicians verify that the tamper evident seal is maintained and that all associated documentation is present.
- The stool sample (within its collection container) is transferred into a UV-sterilized biosafety cabinet that has been cleaned with a sporicidal agent, dedicated for sample processing, and isolated from any other processes or materials within the stool bank’s facility.

- Stool donations are weighed and then inspected by biomanufacturing technicians in a biosafety cabinet for weight, pathology, and Bristol score. Signs of pathology include melena, hematochezia, and mucus. Any sample with concerns for pathology is discarded, triggering a comprehensive clinician-led assessment to re-evaluate donor eligibility.
- Stool samples are weighed by placing the collection container on a scale and then subtracting the weight of an empty collection container from the total weight.
- If a donation does not meet the weight acceptance criteria, the donation is visually inspected for signs of abnormality, pathology, and Bristol score and then immediately discarded. The minimum weight of stool donations should be set to ensure there is enough material to make an FMT preparation and associated retain aliquots required for retrospective testing if needed (See Step 6 in “Processing” below for more information on retain aliquots.)
- If a donation meets the weight criteria, the donation is inspected for signs of abnormality, pathology, and Bristol score. A Bristol score of 3 or 4 is required to continue processing. If the visual inspection does not meet acceptable criteria, the donation is rejected and discarded following hazardous waste procedures.
- The results of the inspection described in step 6 are recorded in a donation tracking form.

Processing

Overall Goals and Responsibilities

- The overall goal of processing is to collect samples of raw stool as well as safety aliquots of the FMT preparation for retrospective testing in the event of an adverse event, and to process stool donations into FMT preparations.
- During processing, the **Biomanufacturing Team**, homogenizes stool donations in buffer and filters out insoluble material.

Protocol

(The stool sample is located in the collection container within the biosafety cabinet from step 6 of **Stool Examination**)

- A pre-determined number, usually three, samples of raw stool for retains is taken by one Biomanufacturing technician and verified by a second.
- The raw stool retains is a pea-sized portion of the donated stool sample weighing approximately three grams.
- A sterile-filtered diluent consisting of 12.5% glycerol in normal saline buffer (0.90% w/v sodium chloride in water) is added to the filter bag containing a 30-micron filter. The volume of buffer added depends on the weight of the donation and type of FMT preparation:
- For **FMP250s** (a 250-milliliter liquid formulation designed for lower delivery): 10 grams of buffer is added to the filter bag per gram of stool.

- For **FMP30s** (a 30-milliliter liquid formulation designed for upper delivery): 2.5 grams of buffer is added to the filter bag per gram of stool.
The amount of buffer is calculated by one Biomanufacturing technician and verified by a second.
- Within the biosafety cabinet, the stool sample (meeting the specifications for starting material as described in steps 1-6 of Stool Examination) is transferred to the sterile filter bag containing buffer.
- The transfer of stool is facilitated by a sterile, disposable spatula
- All stool material is added to the same side of the membrane in the filter bag.
- The filter bag is sealed, removed from the biosafety cabinet, and introduced to secondary containment within a homogenizer blender for approximately 180 seconds (or longer, if needed) to homogenize the stool and buffer.
- The filter bag is returned to the biosafety cabinet and unsealed. The sample filtrate is pushed through the bag filter; one side of the filter contains fiber and remaining solids and the other contains filtered sample; this is the FMT-preparation substance.
- The sample is aliquoted into sterile bottles using sterile, disposable serological pipettes.
- For **FMP250s** (a 250-milliliter liquid formulation designed for lower delivery): FMT preparation is aliquoted into 250 milliliter bottles.
- For **FMP30s** (a 30-milliliter liquid formulation designed for upper delivery): FMT preparation is aliquoted into 30 milliliter bottles.
At this point, safety aliquots of FMT preparation substance are also collected. For both FMP250s and FMP30s, 18 milliliters of the final FMT preparation are saved as follows:
- Four 1.5 milliliter aliquots (for a total of 6 milliliters). These aliquots are set aside for pharmacovigilance purposes and enable retrospective testing in the event that FMT material is suspected to be involved in an adverse response.
- Two 1.0 milliliter aliquots (for a total of 2 milliliters). These aliquots are used by Quality Control to test for potency in the form of viable cells per milliliter of FMT preparation.
- Five 2.0 milliliter aliquots (for a total of 10 milliliters). These aliquots are set aside for pharmacovigilance purposes and enable retrospective testing in the event that FMT material is suspected to be involved in an adverse response.
- Bottles are labeled with a unique identifier and barcode for tracking purposes.
- Unique identifiers should contain three pieces of information:
 - The identity of the stool donor
 - The lot number identifying the particular bowel movement providing the stool sample (A stool donor may provide multiple stool samples)
 - The subunit of the sample (A single stool sample may provide enough material for multiple FMT preparations).

Storage

Overall Goals and Responsibilities

- The overall goal of storage is to store FMT preparation for long-term at -80 degrees Celsius until the preparations pass through quarantine and are delivered for clinical use.
- During storage, the Biomanufacturing Team transfers bottles containing newly manufactured FMT preparation material from the biosafety cabinet to a temperature-monitored storage freezer. The locations of each FMT preparation are traced in an inventory management software system.
- **Additional Reading:** For more information on how units are released from quarantine, read our paper titled "[When is an FMT Treatment Ready for Use? Health Monitoring and Material Release.](#)"

Protocol

(The newly manufactured FMT preparation is located in a sterile, uniquely labelled bottle within the biosafety cabinet from step 7 of **Processing**)

- Bottles are moved to and initially stored at -20 degrees Celsius in a temperature-monitored freezer. At the end of the manufacturing shift, and no later than 24 hours from the stool sample time of passage, FMT units are transferred to a temperature-monitored, -80-degree Celsius freezer.
- FMT units in freezers are segregated onto trays on the freezer shelves. Transfer of material occurs in a controlled manner and product movement is tracked in a database to maintain an electronic record of its location.
- Post manufacturing, Biomanufacturing technicians clean the biosafety cabinet using ethanol, sporicidal agent, and UV light.

Materials List

Manufacturing FMT preparations from donated stool requires the following materials and facilities:

Stool Examination

- Controlled collection site with a bathroom that is cleaned between each use
- Stool sample collection container that fits over the toilet
- Donor health questionnaire
- Tamper-evident enclosure
- Biosafety cabinet dedicated to processing stool
- Laboratory personal protective equipment

Processing

- Sterile-filtered diluent consisting of 12.5% glycerol in normal saline buffer (0.90% w/v sodium chloride in water)
- 300-micron filter bags
- Sterile, disposable spatulas
- Homogenizer
- Sterile serological pipettes
- FMT preparation bottles

Storage

- Labels and bar codes
- Temperature controlled, -80 degrees Celsius freezer
- Electronic database and record of FMT preparations' location

Figure 1: Manufacturing Overview

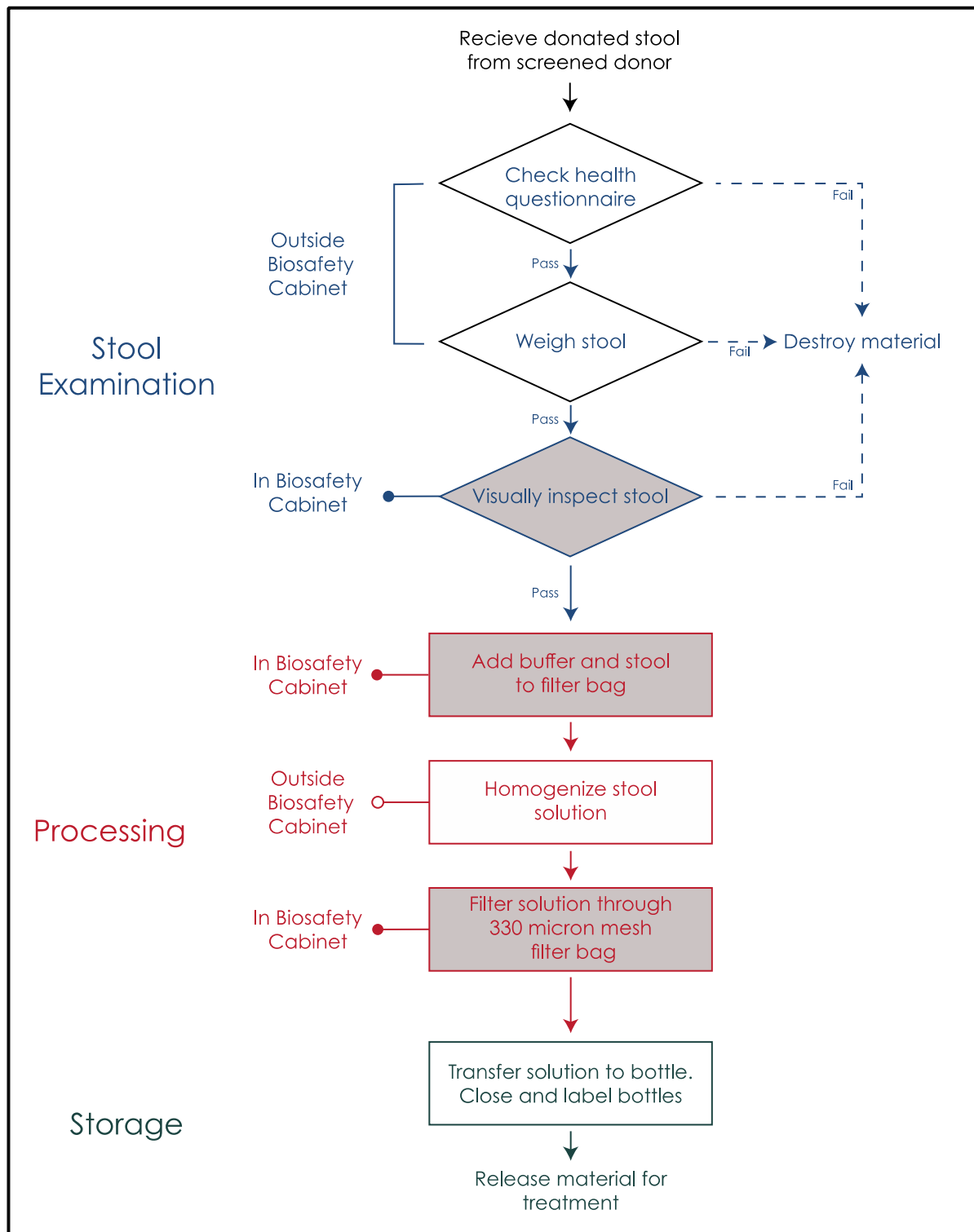


Figure 2: Stool Banking Operations

Overview of OpenBiome's Quality and Safety Program including its manufacturing process.

Donor Assessment	Manufacturing	Quality Assurance	Pharmacovigilance
Clinical Assessment Prospective candidates undergo clinical evaluation that includes medical histories, behavioral risks, and current health status Laboratory Screening Prospective candidates are screened for over 30 stool and serological tests. Less than 3% qualify to become donors.	Standardized Stool Examinations Lab technicians evaluate every stool sample based on Bristol type and stool pathology Processing Controls All stool processing occurs under a Class II biosafety cabinet that is UV-sterilized and cleaned with a sporicidal agent. All equipment is sterilized and/or disposable. Storage and Shipping Controls All samples are stored in a glycerol buffer at -80°C, sealed with tamper-evident bands, and transported on dry ice with temperature verification.	Continuous Donor Requalification Donors are under medical monitoring throughout the entire donation window and fully rescreened every 60 days. Quarantine Procedure Prior to release, donated material is quarantined for 60 days in between two full panel screens at a CLA-verified laboratory Safety Aliquots Multiple samples of all material are preserved for a minimum of 24 months, enabling retesting as needed.	Material Tracking Clinical partners complete Material Tracking Logs to evaluate unit-specific inventory regularly, enabling response coordination, and proactive system-wide recalls if necessary. Efficacy Monitoring Partners complete FMT Follow-Up Forms for each patient treated with OpenBiome material, reporting de-identified patient outcome data. Adverse Event Reporting All adverse events are reported to OpenBiome and evaluated using a standardized consensus-based decision-making algorithm.

Appendix 1

Description of OpenBiome Short Health Questionnaire (SHQ) that is completed by donors before each stool donation.

The SHQ aims to capture any changes in donor health or exposures that could impact the potential for new infectious or non-infectious disease risk factors. The SHQ covers;

- Recent sickness
- Changes in diet
- Changes in bowel consistency or frequency
- Travel
- Medical visits
- Antibiotic, antifungal or antiviral exposure
- Other new medications or over the counter supplements
- Other risk factors for multi-drug (e.g. hospital admission, medical tourism)
- Contacts or symptoms of SARS-CoV-2 (e.g. fever, cough, shortness of breath)

