

UMN Quality Metrics

MTP-101LR is intended to be used for treatment of recurrent *C. difficile* infections. The vast majority of clinical experience with this product uses colonoscopy as a route for delivery, although it has also been used anecdotally via upper endoscopy and G-tube.

- The dose ($\geq 5 \times 10^{11}$ bacteria) is measured in the number of bacteria rather than grams of stool. The clinical efficacy, which is defined as no spontaneous relapse of *C. difficile* infection within 2 months of follow-up, is $\sim 90\%$ in patients without underlying inflammatory bowel disease (IBD). Relapse of *C. difficile* infection is defined as increased diarrhea with confirmed *C. difficile* positive by PCR. Early experience in patients with underlying IBD suggested lower efficacy ($\sim 75\%$) and greater incidence of late (> 2 months) relapses. The severity of inflammation is likely an important variable.
- Common post-FMT symptoms include excessive intestinal gas, bloating, and irregular bowel movements in the initial weeks after administration.
- Dose titration experience showed that clinical potency is decreased when the dose is lowered to 1×10^{11} bacteria.
- The dose is formulated as a suspension of bacteria in 35 mL of saline with 10% glycerol, contained in cryobags. Once thawed, the cryobag is accessed with a plastic 22g cannula (provided) that is mounted on a slip tip syringe. The contents are withdrawn and diluted with the desired amount of normal saline (no preservatives). The typical final volume is ~ 60 -90 mL, which is administered via the biopsy channel of the colonoscope into the ileum or cecum. The channel is then flushed with an extra dose of saline into the colon.
- The product should be stored long-term in a -80°C freezer. Stability of the product has been validated with viability assays to 7 years.
- Thawing is done in an ice bath and product stability has been validated to 24 hours (on ice). The product should be kept on ice until the start of the colonoscopy. Syringes with the material are laid out at room temperature at the start of the procedure. The product should be used following thawing on the same day (we recommend within 4 hours). Refreezing is assumed to result in loss of potency.

MTP-101LF is intended to be used for treatment of fulminant *C. difficile* infection via colonoscopy. It is identical in appearance, storage requirements, and handling to MTP-101R. The only difference is the dose, which is $\geq 2.5 \times 10^{11}$ bacteria. Treatment of fulminant *C. difficile* infection requires repeated administrations, although in most cases MTP-101LF can be used as an initial dose only. We can provide the specific recommended protocol developed at the University of Minnesota.



MTP-101LR and MTP-101LF. The label will be coordinated with OpeBiome.

MTP-101C is a freeze-dried, double-encapsulated preparation of microbiota. The dose is identical to MTP- 101LR ($\geq 5 \times 10^{11}$), which is contained in 3-5 capsules, 00 size. The preparation is easy to handle and can be administered in clinic or dispensed to home, depending on clinic protocols.

- The preparation is stable at a range of temperatures (- 80oC to room temperature).
- Stability testing was done up to 2 years so far at -80oC, 1 year at -20oC, 6 months at 4oC (laboratory refrigerator), 1 month home refrigerator (~ 4oC with fluctuations), and 4 days at room temperature.
- The capsules are bottled in sealed containers with an included desiccant capsule.

*All microbiota products using cGMP protocols described in our IND 15071 in the Molecular and Cellular Therapeutics facility at the University of Minnesota. The facility itself is registered with the FDA and operates under its own Type V Drug Master File 12975.



MTP-101C is normally bottled in a sealed opaque container. The label will be coordinated with OpenBiome.

There are three elements in manufacturing these products:

- (1) the donor program
- (2) the actual manufacturing
- (3) quality assurance and material release.

Below is a brief summary of these steps:

1. Donor program

Donor screening and testing is a continuous process. Physical exams and blood tests are conducted in the research clinic at 717 Delaware St. S.E (DCRU), Minneapolis, MN 55455. The donor evaluation includes **screening** and **testing elements**.

A. Donor Screening

- Initial screening is done with extensive questionnaires. Briefly, stool donors must qualify as blood donors, but also be in excellent health and:
 - have no history of antibiotic exposure for at least 6 months;
 - take no prescription medications;
 - have no gastrointestinal disorders, e.g., irritable bowel syndrome, inflammatory bowel disease, food intolerances, etc.;
 - have no allergies or atopic conditions;
 - have no metabolic disorders or any individual diagnostic criteria for metabolic syndrome (e.g., increased blood pressure);
 - have no neurologic or psychiatric disorders;
 - have no high-risk travel history;
 - do not have regular contact with patients a hospital, ambulatory clinic, nursing home, hospice, or a similar healthcare facility as part of their work or a living situation.
- Medical history and physical examination are conducted by a physician initially and every 3 months for all active donors.
- Brief Health Questionnaires accompany every stool donation.
- Individuals taking care of sick people at home or having contact with sick patients in the hospital or care facilities are excluded.

B. Donor testing

- Serologic and NAAT testing for HIV, HAV, HBV, HCV, CMV, EBV are done during initial donor qualification, every 3 months, and at least 15 days after the last stool donation used for material production. MHA-TP test for syphilis is done following the same schedule.
- Metabolic testing is done at inclusion and every 6 months. This includes a lipid panel, fasting glucose, high sensitivity CRP level, and liver function tests. In addition, FANA is done as part of the evaluation for autoimmunity risk.
- Enteric pathogen testing is done at the initial evaluation AND every lot of fecal microbiota.

- NAAT-based testing for enteric pathogens includes: *C. difficile*, *Campylobacter*, *Plesiomonas shigelloides*, *Vibrio*, *Salmonella*, *Shigella*, Shiga toxins (STEC), enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroaggregative *E. coli*, Enteroinvasive *E. coli*, *Yersinia enterocolitica*, *Cyclospora*, *Isospora*, *Giardia duodenalis*, *Cryptosporidium*, *Entamoeba histolytica*, Adenovirus, Rotavirus, Norovirus I and II; Sapovirus (I, II, IV, V), Astrovirus, ova and parasites microscopic evaluation.
- Culture-based testing for enteric pathogens includes: *Listeria*, *Aeromonas*, *Campylobacter*, *E. Coli* 0157, *Plesiomonas*, *Salmonella*, *Shigella*, *Vibrio*.
- In addition to enteric pathogens, the stool going into microbiota production is tested by culture for presence of MRSA, ESBL, CRE, and VRE.
- COVID-19 screening/SARS-CoV-2 testing
 - Daily diary of symptoms, temperature, and potential COVID-19 contacts starting at least 28 days prior to donation and continuing at least through 14 days following donation.
 - Testing for SARS-CoV-2 that is done via nasopharyngeal or nasal swabs every 14 days starting 14 days before donation and continuing through at least 14 days following donation. We also anticipate to be able to test the stool directly for SARS-CoV-2 once the FDA authorizes such a test.

2. Manufacturing

We follow standardized chain of custody and cGMP protocols in our manufacturing, the essential elements of which include:

- Clean, inspected production facility with environmental controls.
- Consistent manufacturing protocols.
- Standardized dosing in terms of number of bacteria and viability, as measured by membrane dye permeability assays.
- Unit tracking and retained samples from each production lot.

3. Material release

The material release involves independent review of all donor testing results and production batch records to ensure compliance with the IND protocols.

Aliquots of each donation are saved for potential future testing.