Supplementary Digital Content 1

To paper: Řehořová et. al: Multi-donor Faecal Microbial Transplantation for Critically Ill Patients: Rationale and Standard Operating Procedure. Biology 2021; XX(X):XXX-XXX

Faecal microbial transplantation for critically ill patients

**STANDARD OPERATING PROCEDURE**

**Disclaimer**

This Standard Operating Procedures (SOPs) are intended to unify and standardise the process of multi-donor faecal microbial transplantation (FMT) for critically ill patients with diarrhoea. This document was created to maximise the safety and efficacy whilst reflecting specific logistical challenges in critically ill patients.

The SOPs reflect state-of-the-art at the time of its publication and regular updates are expected as new data emerge. FMT for critically ill patients remains an experimental procedure and **should not be used outside the context of clinical trials** adherent to principles of Good Clinical Practice.

**Donor selection and investigation**

Donor candidates shall be selected from healthy volunteers who fulfil following criteria:

Inclusion criteria:

* Age 18-60 years
* Altruistic motivation to donate
* Informed consent

Exclusion criteria:

* Works in healthcare or shares household with a person who works in healthcare
* Antibiotic exposure during last 6 months
* History of gastrointestinal disease including but not limited to inflammatory bowel disease, malignancies, polypes)
* Risk of having a transmittable disease including but not limited to: symptoms and signs of an acute infectious disease or vaccination during last two months, more than 1 sexual partner during last 12 months, history of drug abuse, travel outside Europe or North America during last 6 months.
* BMI >30 or <20kg/m2
* The use during last two months of drugs that may influence microbiota such as proton pump inhibitors, steroids, cytostatic etc.
* Allergies including food allergies
* History of autoimmune, metabolic, neurodegenerative or psychiatric diseases.

These requirements are compliant with Czech FMT guidelines (version May 2018, issued by the Czech Society for Infectious Diseases, <https://www.infekce.cz/DPFMT18.htm>).

The candidate will be informed in detail by the examiner on the FMT method, stool donation, and project outline. After obtaining written Informed consent from the candidates, they file a pre-specified questionnaire (See below) to check for exclusion criteria. Subsequently, the donor undergoes a physical examination and a detailed history.

Afterwards, the standardized donor workup will include:

* Blood: full blood count incl. WBC differential, Sedimentation Rate, CRP, liver function tests (ALT, AST, ALP, GMT, bilirubin), creatinine, albumin and level of glucose, HIV (p24 and anti-HIV-1 and 2), syphilis serology (RRR or TPPA), and hepatitis panel (HBsAg, anti-HBc IgM, and IgG, HBeAg, anti-HBc total, anti-HCV, anti-HAV, anti-HEV)
* Stool examination: *C.dif*. (GDH and toxin A and B), cultivation to detect *Shigella, Campylobacter, Listeria, Pseudomonas aeruginosa, Enteropathogenic E.coli (EPEC, EAEC, ETEC, EIEC), Yersinia and Salmonella sp*., Vibrio cholerae, MDRO (VRE, ESBL, CRE), standard microscopy parasitological exam (*Giardia lamblia, Cryptosporidium parvum, Isospora, Microsporidia, Entamoeba histolytica*) and from virological exam will be performed PCR *CMV, Rotavirus, Norovirus*. Faecal occult blood test.
* Nasopharyngeal swab – PCR to rule out COVID-19 infection and culture-based assay MRSA

The basic blood test aims to confirm the patient's good clinical condition. Other tests aim to prevent the transmission of latent infection from the donor to the recipient.

This complete examination of the donor is to be repeated after 2 months throughout all donation periods. Donors are encouraged to report any change of their clinical condition.

If the following examination is also found to be physiological (ie without signs of the donor's infectious disease), the stool donated by the donor during these 2 quarantine months (frozen in the stool bank) will be considered non-infectious and only then can be used for FMT.

***NOTE:*** *It is the ultimate responsibility of the physician to accept or reject donor. Indeed, clinical judgement is required and some laboratory abnormalities (e.g. ALP below normal range) may not represent contraindication, whilst others always do (e.g. HIV positivity). The test described above are the mandatory minimum, that can be extended as per doctor’s judgement. Yet, there will always be a small but not negligible risk of transmission of infections for which the donor is not investigated (e.g. HPV, HSV-2, chlamydia, gonorrhoea, or other disease including those that are yet unknown to medical science). These should be mentioned in the informed consent.*

**Stool sample collection**

For better control of the timing of defecation, the donor may use a glycerine suppository. The stool must be solid, blood-free, and urine-free.



**Figure S1: Example of stool collection container (Disposable Commode Specimen Collection Systems, BMP Medical, Boston, MA, USA)**

Upon collection, date and time are noted. In a plastic transportation container, the sample is delivered to the place of processing as soon as possible, but no later than four hours from the defecation. Upon collection, its date and time are noted. During sample hand oper, the donors are again asked on symptoms of infectious disease, diarrhoea, travel abroad, use of medication and risky sexual behaviour.

**Preparation of graft**

The stool is processed no later than 6 hours from defecation in a three-step process – homogenization, filtration, and freezing.

The entire grafty preparation process is performed in the laminar flow box or biohazard box, dedicated exclusively for the donor stool processing. All the equipment used in the process is either sterilized before every use or disposable. The surroundings are sanitized according to the local recommendations. The surfaces are disinfected by washing, wiping, or immersing in disinfectants. Every workplace must have its protocol to control the sanitation, which compliant with the internal guidelines of the healthcare institution and local legislation. The control process must include recording sanitation events and schedule. The complete sanitation of the workplace and the equipment resterilization is performed always after a single donor sample processing.

STEP 1 Homogenization is performed after the visual control and weighting of the stool, which must be solid, blood-free, and urine-free, and of a minimum weight of 100 g. Smaller samples are discarded. First, the stool is diluted in sterile normal saline (0.9% NaCl) solution in a weight/weight ratio of approximately 1:3. The resulting mixture is homogenized with a dedicated immersion lab blender at 18 000 rev/min for 2 min. The viscosity is important for the resulting product, with a mixing time of approximately 2 minutes. The material for administered via enema must have a density that corresponds to a thinner slurry.

STEP 2 Filtration. The homogenized material is filtered through 2 layers of sterile gauze to remove all solid parts. Two gauze layers are recommended. Using one layer only results in insufficient filtration, whilst more than two layers led to low yields of filtrate due to retention of large specimen fraction on the gauze. The resulting filtrate must have a viscosity that corresponds to a homogenous cream soup, free of any solid particles.

STEP 3 Freezing. Finally, the filtrate is mixed with glycerol in a 9:1 volume/volume ratio. Glycerol prevents the formation of large ice crystals, and hence protects the bacteria. Then, the sample is divided into 50mL equivalents and deep-frozen at -80 C in labelled sterile containers. Each transplant aliquot is labelled with the following: unique numbering for this stool sample, volume - batch number, composition, time and date of processing, address of the processing facility, donor ID, storage conditions, due date, and warning: ‘Use immediately after thawing!’. The labelled samples are stored at -80 °C for 3 to 12 months.

**Graft quarantine and safety precautions**

A frozen stool sample cannot be used until after the donor has had second negative blood tests and passed examination. The period between tests is two months and it follows that each sample is quarantined for 2-4 months before use. Nonetheless, sample should be used as soon as cleared safe for use and storage time should not exceed 12 months, due to the gradual decrease of the living content and concomitant reduction of the efficiency of the FMT.

Reference sample from each frozen batch is clearly labelled and stored separately for at least 6 months after the last use of that batch.

**Final preparation of multi-donor transplant**

To maximize the diversity of the transplant, the final specimen for the FMT is defined as a mixture of seven processed stool samples from seven donors. Two hours before the FMT, the samples are taken from the freezer and are thawed at 37 °C water bath for 2 hours. The water bath is disinfected before use, according to a disinfection protocol, established in each healthcare institution. Then it is filled with sterile water and heated to 37 °C. The frozen samples are inserted into the bath in such a manner so that the water level only reaches below the screw cap of the vessel. After 1 hour, the samples are wiped with the disinfection wipes and the content is examined visually, whether completely thawed, without opening the vessel. If the sample still contains a frozen fraction, another 30 minutes interval in the bath is added. After the ample completely thaws, the vessel is opened, and its temperature is checked (using a dedicated contactless thermometer) to be between 36.0 and 37.3 °C. The content is transferred into a 1000 mL sterile bottle, where all 7 transplants are mixed. The resulting mixture is sucked into four 100ml syringes and used immediately but never later than two hours from preparation. Re-freezing of the thawed or partially thawed extract is not permitted

**Recipient preparation and transplantation delivery**

Enteral nutrition will be stopped 12h before the procedure. If considered safe, the patient may receive loperamide 2 mg p.o. or into nasogastric tube 2 hours before FMT.

Carefully check the identity and informed consent form for each patient. Provide second check of exclusion criteria – such as bowel perforation, recent colonic surgery or neutropaenia.

Before processing, check and document:

* Physical examination incl. vital function and temperature (it is assumed that in most ICU patients these will be monitored continuously)
* SOFA score (a set of laboratory results and examinations estimating organ function).
* Abdominal X-ray free of pneumoperitoneum or megacolon.
* Baseline samplings (such as recipients blood and stool) done as per study protocol

Then 350mL of thawed donor stool (stool suspension) will be delivered by retention enema into the patient’s colon by a semirigid single-use rectal catheter of an appropriate size (such as Peristeen Anal Irrigation, Coloplast Ltd., or similar). The patient will be positioned in the left semilateral Trendelenburg's position for 15 minutes and then in the right semilateral Trendelenburg’s position for another 15 minutes. Fecal management system (e.g. FlexiSeal©) will be inserted and clamped for 2hours after FMT. Body temperature, heart rate, oxygen saturation, and blood pressure will be continuously monitored for at least 6 hours after the procedure and thereafter as per the physician’s discretion.

Check for and document carefully any adverse events.

Donor questionnaire

CONFIDENTIAL

Name: DOB: Unique donor identification number:

|  |  |
| --- | --- |
| Current weigh …kg, height…. cm, |  |
| Do you or people you share a common household work in healthcare? | Text  Description automatically generated with medium confidence |
| Have you been using antibiotics or gut disinfectants during last three months?  If yes, please give details: | Text  Description automatically generated with medium confidence |
| Have you been using drugs affecting the immune system (such as steroids, cyclosporin, immune suppressants, biological therapy)? If yes or unsure, please discuss with the doctor. | Text  Description automatically generated with medium confidence |
| Do you have any allergies? | Text  Description automatically generated with medium confidence |
| Are you regularly using any medicines?  If yes, please indicate the name and dose. | Text  Description automatically generated with medium confidence |
| Have you ever had chemotherapy or radiotherapy? | Text  Description automatically generated with medium confidence |
| Have you ever received blood transfusion or been treated with a blood product such as fresh frozen plasma?  If yes, please indicate date and circumstances. | Text  Description automatically generated with medium confidence |
| Do you have diabetes mellitus, heart disease, or autoimmune disease?  If yes, please indicate | Text  Description automatically generated with medium confidence |
| Have you been in the period of last three months vaccinated?  If yes, please indicate the vaccine and exact date of application | Text  Description automatically generated with medium confidence |
| Do you smoke?  If yes, please indicate how much and for how long | Text  Description automatically generated with medium confidence |
| How many units of alcohol do you drink on an average day?  1 unit = 0.5 L beer = 0.2 L of wine = 0.04 L of spirit | unit/week |
| How many hours do you normally sleep? | Text  Description automatically generated with medium confidence |
| Have you been diagnosed with any of the below listed diseases?  Alzheimer disease and other dementia, Parkinson disease, Prion diseases Motoneuron disease, Huntington disease, Spinocerebral ataxia, Spinal muscle atrophy | Text  Description automatically generated with medium confidence |
| Have you been diagnosed depression and given antidepressant drugs?  If yes, please provide details. | Text  Description automatically generated with medium confidence |
| Have you been treated for inflammatory bowel disease such as ulcerous colitis, Crohn disease or irritant bowel? | Text  Description automatically generated with medium confidence |
| Did you undergo surgery of the gastrointestinal tract – bowel, liver, pancreas, gall bladder etc.?  If yes, please give time and details. | Text  Description automatically generated with medium confidence |
| Have you been treated for another gastrointestinal tract disease?  If yes please indicate. | Text  Description automatically generated with medium confidence |
| Have you been treated for gastrointestinal tract tumour? | Text  Description automatically generated with medium confidence |
| Have you been treated for parasitic infection, Giardia lambia or other microorganisms affecting the gastrointestinal tract?  If yes, please indicate the exact name of the infection agents, date of symptoms emergence, date of symptoms disappearance, exact name of the medicines used, date of treatment termination | Text  Description automatically generated with medium confidence |
| Were you diagnosed with polyps or diverticula of the gut wall? | Text  Description automatically generated with medium confidence |
| Have you been examined for gastrointestinal tract symptoms such as constipation, diarrhea, bloody stool etc.?  If yes, please indicate when and what. | Text  Description automatically generated with medium confidence |
| Have you had fever, diarhoea or flu-like symptoms in the last four week?  If yes, please give more details | Text  Description automatically generated with medium confidence |
| Has any person close to you had fever, diarhoea or flu-like symptoms in the last four week?  If yes, please give more details |  |
| Have you been tested positive for Covid-19 in last three months? | Text  Description automatically generated with medium confidence |
| Has any person close to you tested positive for Covid-19 in last two weeks? | Text  Description automatically generated with medium confidence |
| Have you been hospitalized in last three months? | Text  Description automatically generated with medium confidence |
| Have you had piercing, beauty surgery or any other skin penetration procedure during last three months?  If yes, please indicate what, where and when (exact treatment date(s)) | Text  Description automatically generated with medium confidence |
| Have you ever been identified carrier of or had infection caused by multi-resistant bacteria (such as MRSA, VRE, ESBL E. Coli etc)? | Text  Description automatically generated with medium confidence |
| Are you treated or observed for hepatitis type A,B,C or HIV/AIDS infection? | Text  Description automatically generated with medium confidence |
| Did you come into contact with a patient with confirmed infectious hepatitis or HIV/AIDS? | Text  Description automatically generated with medium confidence |
| Were you in a close contact with a person with a high risk of sexually transmitted diseases (prostitute, drug addict)? | Text  Description automatically generated with medium confidence |
| Have you had parallel relations with more than 1 sexual partners over last twelve months? | Text  Description automatically generated with medium confidence |
| Have you ever used any illicit drugs?  If yeas, please provide details | Text  Description automatically generated with medium confidence |
| Did you travel outside Europe/North America over last 6 months  If yes, give details: | Text  Description automatically generated with medium confidence |
| Are you aware of any other condition, which – in your opinion – may change your ability to safely donate stool?  If yes, give details: | Text  Description automatically generated with medium confidence |

References: See Řehořová et. al: Multi-donor Faecal Microbial Transplantation for Critically Ill Patients: Rationale and Standard Operating Procedure. Biomolecules 2021; XX(X):XXX-XXX