Title	**Prospective, open-label trial to evaluate efficacy of fecal microbiota transplantation for treatment of chronic gastrointestinal dysbiosis or clearance of antimicrobial resistant organism.**
Protocol	GID.FMT.1
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ABBREVIATIONS

CD	Crohn's Disease
CDAI	Crohn's Disease Activity Index
CDI	*Clostridioides difficile* infection
DSMC	Data Safety Monitoring Committee
FMT	Fecal Microbiota Transplantation
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
IBS-SSS	Irritable Bowel Syndrome Severity Scoring System
LYO-FMT	Lyophilized Fecal Microbiota Transplant
MC	Microscopic Colitis
PROM	Patient Reported Outcome Measure
UC	Ulcerative Colitis

Study Rationale and Background

Fecal Microbiota Transplantation (FMT), which had been predominantly utilized by the veterinarians until late 1990's has generated a significant interest for its potential use in various gastrointestinal, psychiatric, neurologic and metabolic disorders within the past few years. Since 2010, there has been an explosion of research, publications and media coverage related to the high efficacy range, 80 – 90% for treatment of recurrent *Clostridioides (Clostridium) difficile* infection (rCDI). The exact mechanisms of its success in curing CDI are yet to be discovered. However, growing evidence suggests that it likely is a synergistic interaction of metabolites, bacteriophages, glycoproteins and bacterial, fungal components of microbiome, which mediate the effects of FMT. Metagenomic studies have shown that patients with rCDI lack protective and diverse colonic microbiome and remain in a state of chronic dysbiosis. Following a successful FMT, the microbiome of a patient with rCDI resembles that of the donor's and remains as such overtime. There is no precise and agreed definition of dysbiosis. For the purpose of this study, dysbiosis is defined as perturbation of host-microbial interactions which results in compositional changes in the fecal microbiota as determined by clinical criteria of constellation of symptoms, including change in the bowel function (diarrhea, constipation or bloating) in which an alteration of the microbiota is either known based on molecular or culture-based profiling or suspected according to the history, which includes but is not limited to repeated or prolonged use of antibiotics or gastrointestinal infection.

The cause of inflammatory bowel disease (IBD) is unknown but studies have shown that IBD is a chronic inflammatory disease with altered and decreased microbiota diversity of the gastrointestinal tract when compared to the healthy individuals. Canada has the highest incidence of IBD in the world. The annual total (direct and indirect) health costs is estimated to $2.8 billion or $11,900 per person per year.[17] IBD includes Crohn's Disease (CD) and Ulcerative Colitis (UC). While these diseases are collectively referred as IBD, there are distinct differences - most notably the area of the intestinal tract affected and the extent of the inflammation. UC typically affects the colon; the disease usually starts at the anus and may progress upward, and may even involve the entire colon. While in CD, the inflammation tends to occur in patches and may involve any area throughout the entire intestinal tract; however, it most often affects the terminal ileum of the small intestine. Inflammation due to UC involves only the inner intestinal mucosa, while the inflammation in CD disease can extend through the entire thickness of the bowel wall.

The management of CD is challenging due to extra-intestinal manifestations and overlapping symptomology with other inflammatory disorders. Treatment typically targets symptom relief, but and patients' ability to tolerate therapy also plays a key role. UC is characterized by lifelong relapsing and remitting colorectal inflammation. The cause of UC is unknown, but is thought to result from an aberrant immune response to environmental factors in genetically predisposed individuals. Metagenomic studies have shown that both patients with UC and recurrent *Clostridiodes difficile* infection (rCDI) lack diversity and richness of their colonic microbiota and remain in a state of chronic dysbiosis. While current drug treatments and surgery to remove the colon and rectum can reduce symptoms, they are costly, associated with adverse effects, and do not promote the restoration of healthy gut bacteria. Recent studies have shown that fecal microbiota transplant (FMT) is effective in treating IBD. Recent case series in CD and clinical trials in UC patients have shown FMT to be a potential therapy to induce and maintain clinical remission in subset of IBD patients.

Microscopic colitis (MC) is a chronic inflammatory disease of the colon as manifested by chronic, watery, non-bloody diarrhea. MC usually occurs in middle-aged individuals with a female preponderance. Currently, there are limited treatment options for MC; budesonide may be effective for short-term treatment of MC and can improve quality of life. However, up to 80% will experience symptomatic relapse following cessation of budesonide. Routine maintenance treatment with budesonide is controversial as long-term treatment may increase the risk of steroid-related side effects.

IBS is characterized by chronic, relapsing abdominal discomfort and altered bowel movements – constipation, diarrhea or mixed (diarrhea and constipation). IBS affects approximately 15-20% of Canadians and its economic and social burden is estimated to be over $6.5 billion per year in healthcare costs, work productivity losses, and reduced quality of life (QoL). The etiology and pathophysiology of IBS are not yet established, but appear to be a complex interplay between the host and environment factors. Currently, there are no evidence-based therapies available to cure IBS. Studies have shown that fecal microbiota transplantation (FMT) may be an effective IBS treatment. Given the lack of safe and effective treatment for IBD and IBS which are thought to be due to gastronintestinal dysbiosis, this study was conducted.

There is also some evidence in the literature showing efficacy of FMT in clearance of antimicrobial resistant organism (ARO) such as extended-spectrum of beta-lactamases (ESBLs), carbapenem resistant or vancomycin resistant enterococci from the gastrointestinal tract of colonized patients.[11-13] There have been requests from clinicians to utlize FMT for eradication of ARO in rectally colonized patients admitted to an acute care facility in order to facilitate their discharge to a longterm care facility as they were otherwise eligible to be transferred.

Study Objectives and Outcome Measures

The objective of this study is to assess the efficacy of FMTs via enema for 1) symptom improvement in individuals with a formal diagnosis of dysbiosis due to active inflammatory bowel disease (CD, UC), microscopic colitis or irritable bowel syndrome; 2) clearance of antimicrobial resistant organism from the gastrointestinal tract.

Primary Outcome Measurements:
For the participants with chronic GI dysbiosis, the efficacy of FMT treatment will be based on improvement in participant's symptom score from baseline (pre-transplant) to intervention (post-transplant) 4 weeks, 12 weeks and 1 year following FMT. For participants with ARO colonization, determination of clearance of ARO based on stool culture for ARO at baseline, day 7, day 30 and 3 months following last FMT.

Secondary Outcome Measurements for participants with IBD, MC or IBS:

1. Evaluate efficacy and safety of FMT in patients with chronic gastrointestinal dysbiosis and antimicrobial resistant organism in the gut

2. Evaluate the Ulcerative Colitis Disease Activity Index from baseline and following each FMT using partial-MAYO score.
3. Evaluate Crohn's Diseases Activity Index (CDAI) change from baseline 4 weeks, 12 weeks and 1 year post-FMT.
4. Evaluate Microscopic colitis response to FMT using physician's global assessment and number of unformed bowel movements per 24 hours
5. Evaluate Irritable Bowel Syndrome Severity Scoring System (IBS-SSS) grade assessment change from baseline, 4 weeks, 12 weeks and 1 year post-FMT.

Ulcerative Colitis

The efficacy of FMT for UC will be determined based on changes to partial-MAYO score from baseline and at weeks 4, 12 and year 1, following the treatment. Partial-MAYO is a validated scoring system to determine the activity of UC. It uses the three non-invasive components of the full Mayo Score (stool frequency, rectal bleeding and Physician's global assessment) as perceived by the patient, to score ulcerative colitis patient's disease severity score. The index considers these three clinical parameters, each of which is assigned a score from 0 to 3 according to the clinical evaluation, with a total possible score of 9. Higher the score, more severe the disease; score of 0 to 1 is considered in remission; 2 – 4 mild; 5 – 7; moderate; > 7 severe colitis.

Crohn's Disease

CDAI is a validated instrument used in adults with active Crohn's disease. The index consists of eight factors, 2 of which are subjective: stool habits; pain; general well being; features of extra intestinal disease; use of opiates for diarrhea; abdominal mass; hematocrit (hct); and percentage of body weight below standard. Scores range from 0 to ~ 600: > 450 is severe disease; 220 – 450 moderately active disease; 150 – 219 mildly active disease. Clinical remission is defined as a CDAI score <150, clinical response is either a CDAI score <150 or a CDAI reduction of ≥100 from baseline.

Microscopic Colitis

Physician's global assessment and number of unformed bowel movements per 24 hours were employed to assess response to FMT as these parameters used to determine MC treatment in clinical trials and care.

Irritable Bowel Syndrome

The Irritable Bowel Syndrome Symptom Severity Scale (IBS-SSS) is a validated instrument with a scoring system which produces a meaningful value that is both reproducible and sensitive to change. The instrument contains five questions across the following domains: pain; distension; bowel score and quality of life. Each question can generate a maximum score of 100 using prompted visual analogue scales; the maximum achievable score is 500. IBS-SSS is mild for scores 75 – 175; moderate 176 – 300 and severe if > 300.

The Proposed Trial

The proposed design of the study

The design of this research is a prospective open-label trial using lyophilized or frozen FMT to evaluate efficacy of fecal microbiota transplantation for treatment of chronic gastrointestinal (GI) dysbiosis and clearance of antimicrobial resistant organism (ARO) from the GI tract of the ARO colonized individuals.

The planned trial interventions

Recruitment

The research coordinator will be responsible for coordinating the recruitment, screening of potential participants using the inclusion/exclusion criteria and reviewing with the PI. The PI will make the final determination of a potential participants' eligibility. Fifty participants with chronic GI dysbiosis will be recruited. Twenty patients with confirmed ARO will be recruited to take part in this study; the ARO study results will be used to determine the feasibility of further study.

Study Population

The participants will consist of patients over the age of 18 years who or their legally authorized representative consent and comply with study procedures. Participants will be required to have a formal diagnosis of chronic gastrointestinal dysbiosis by a referring gastroenterologist or known to be colonized with ARO based on positive rectal cultures for same organism on 2 separate occasions (each culture 1 month apart) and must have a positive culture within 14 days of enrollment.

ARO Participants

Following informed consent and prior to receiving FMT, enrolled ARO participants will have their blood collected for Hepatitis BsAg, Hepatitis C Ab, HIV, Syphilis serologies. Blood is collected for these pathogens to determine their serostatus at the time of study enrollment. Participants are advised through the study consent that if their blood tests positive for any of these diseases, the researchers are required to notify the public health department and that the public health department may contact them.

At baseline, ARO participants will have medical history, physical exam done by the physician-researcher, and information on concomitant medications will be collected. The participants will receive a total of two lyophilized FMT (LYO-FMT) or frozen FMT enemas using stool samples collected from healthy, screened donor(s); the participant will receive the first FMT treatment week 1 and a second FMT will be administered within three weeks of first treatment. Participant's stool will be retested one week, one month and 3 months following the second FMT to determine whether the ARO has been eradicated.

GI Dysbiosis Participants

Following informed consent and pre-transplant, enrolled participants will have their blood collected for Hepatitis BsAg, Hepatitis C Ab, HIV, Syphilis serologies unless these serologies were performed within 3 months of enrollment. Blood is collected for these pathogens to determine their serostatus at the time of study enrollment and prior to receiving FMT.

All participants will be assessed at day 1 by the physician-investigator. A medical history and physical examination will be performed. In addition, optional blood and stool samples may be collected from IBD/IBS participants who consented for these samples prior to the FMT and at weeks 4, 12 and 6 months from FMT.

The participants will receive lyophilized FMT (LYO-FMT) or frozen FMT enemas using stool collected from healthy, screened donor(s). Participants with will receive FMT treatment via retention enema(s) twice weekly for 4 weeks. If there is symptom improvement after 4 weeks of treatment, then the participants will receive FMT treatment once weekly for 4 additional weeks. If the participant does not experience symptom improvement after the initial 4 weeks of treatment, the physician-investigator will discuss options with the participant and a mutual decision will be reached to pursue an additional 4 weeks of FMT treatment or withdraw from the study and pursue usual care. When continued improvement is seen then the participants will receive FMT maintenance; the frequency of FMT treatment is participant dependent and is at the discretion of the physician-investigator and is based upon clinical exam and symptomatic improvement. If no improvement is seen after the 8th FMT, then the participant will be withdrawn from the study and s/he reverts to usual care for his/her condition. Participants can opt to receive maintenance FMT in the clinic or be trained by study staff for home administration.

At the time of each study visit and follow up, participants will be assessed for clinical response, including bowel movements and adverse reactions. Any participant who reports worsening symptoms or AE/SAE will return to the clinic for assessment by the physician investigator. At that time, the participant will undergo a physical exam. Additional tests may be ordered (i.e blood work) at the discretion of the treating physician investigator.

From the previous UC FMT trial, it was discovered that the participants who responded to the initial series of FMT, required ongoing maintenance FMT to remain UC symptom-free. Following the completion of 6-month FMTs, participants will have the option of continuing with the maintenance FMT which can range from one FMT every 2 weeks to every 6 months depends on the individual. In the event that it is not possible for the participant to return to the clinic or requires significant time/travel commitments to receive the FMTs, either the participant or a household member will be trained to store and to administer the lyophilized FMT at home, if the participant is willing to do so.

The home maintenance FMT will be offered only when all the following conditions are met:

1. There is clinical evidence of improvement following the initial FMT series.
2. Participant agrees to continue with maintenance FMT
3. Participant or a household can safely administer FMT at home as assessed by the research staff.
4. There is availability of lyophilized FMT for distribution

5. The participant or delegate is willing to notify the research staff of the progress and request for lyophilized FMT to be available either by picking them up or be shipped 3 weeks prior to needing additional lyophilized FMT.
6. Participant agrees to pay for the shipping supplies and the shipment of FMTS

Inclusion/exclusion criteria for patients and donors

Participant inclusion criteria

- Age 18 years or older.
- Able to provide informed consent.
- Willing and able to comply with all the required study procedures.
- Must have at least one of the following conditions
    - Underlying inflammatory bowel disease (ulcerative colitis, Crohn's disease), or microscopic colitis diagnosed by a gastroenterologist
    - Irritable bowel syndrome as defined by Rome IV diagnostic criteria*:
        - Recurrent abdominal pain or discomfort** at least 3 days/month in last 3 months associated with two or more of the following:
            - Improvement with defecation
            - Onset associated with a change in frequency of stool
            - Onset associated with a change in form (appearance) of stool

          * Criterion fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis

          ** "Discomfort" means an uncomfortable sensation not described as pain.
    - Chronic severe constipations/dysbiosis as diagnosed by a gastroenterologist
- Colonized with ARO based on positive rectal cultures for same organism on 2 separate occasions (each culture 1 month apart) and must have a positive culture within 14 days of enrollment.

Particpant exclusion criteria

- Planned or actively taking another investigational product
- Patients with neutropenia with absolute neutrophil count $<0.5 \times 10^9$/L
- Evidence of toxic megacolon or gastrointestinal perforation on abdominal x-ray
- Peripheral white blood cell count $> 30.0 \times 10^9$/L AND temperature > 38.0 ºC
- Active gastroenteritis due to *Salmonella, Shigella,* shiga toxin-producing *E. coli*, *Yersinia* or *Campylobacter*.
- Unable to tolerate FMT or enema for any reason.
- Requiring systemic antibiotic therapy at the time of FMT.
- Actively taking *Saccharomyces boulardii* or other probiotic; yogurt is allowed
- Severe underlying disease such that the patient is not expected to survive for at least 30 days.
- History of severe allergy to any food

Donor inclusion

- Able to provide and sign informed consent.
- Able to complete and sign the donor questionnaire
- Able to adhere to fecal transplantation stool collection standard operating procedure.

Donor exclusion

- Tested positive for any of the following: Human Immunodeficiency virus (HIV) 1/2, hepatitis IgM, hepatitis B (HBsAg), hepatitis C antibody, syphilis, human T-lymphotrophic virus (HTLV) 1/II and COVID-19 Detection of vancomycin resistant *Enterococcus* (VRE), methicillin resistant *S. aureus* (MRSA), Extended-spectrum beta-lactamase (ESBL) or carbapenem-resistant enterobacteriaceae (CRE); *Salmonella*, *Shigella*, shiga toxin-producing *E.coli*, *Yersinia* and *Campylobacter*, ova, parasites, *C. difficile* toxin, norovirus, adenovirus, rotavirus on stool examination
- Physical contact with COVID-19 positive individual within 14 days of stool donation
- History of any type of active cancer or autoimmune disease
- History of risk factors for acquisition of antibiotic resistant organisms (MRSA, VRE, ESBL, CRE) HIV, syphilis, Hepatitis B, Hepatitis C, prion or any neurological disease as determined by the donor questionnaire, appendix A.
- History of gastrointestinal comorbidites, e.g., inflammatory bowel disease, irritable bowel syndrome, chronic constipation or diarrhea
- Receipt of blood transfusion from a country other than Canada in preceding 6 months
- Antibiotic use or any systemic immunosuppressive agents in the 3 months prior to stool donation
- Receipt of any type of live vaccine within 3 months prior to stool donation
- *(participant with history of severe allergy to any food is excluded)History of depression, anxiety or panic disorder
- History of any type of active cancer or autoimmune disease
- Body mass index > 29
- Failed any one of the donor screening questionnaire at the time of donor screening
- Any current or previous medical or psychosocial condition or behaviours which in the opinion of the investigator may pose risk to the recipients or the donor

Adverse Event Reporting

All adverse drug event (ADE) and serious adverse events (SAE) will be tracked in the participant's study file and REDCap and reported to Island Health's Clinical Research Ethics Board (CREB). As per Health Canada, a SAE is defined as "an adverse drug reaction that requires inpatient hospitalization or prolongation of existing hospitalization, that causes congenital malformation, that results in persistent or significant disability or incapacity, that is life threatening or that results in death". Each SAE that is attributed to FMT is subject to expedited reporting to Health Canada and should be reported individually in accordance with the data element(s) specified in the Health Canada/ICH Guidance Document E2A: "Clinical Safety Data Management: Definitions and Standards for Expedited Reporting". Expedited reports are required for events that meet all of these three criteria: serious, unexpected and a suspected causal relationship; this is defined as any untoward medical occurrence that at any dose results in

any one of the following: results in death; is life-threatening; requires inpatient hospitalization or prolongation of existing hospitalisation; results in persistent or significant disability/incapacity or is a congenital anomaly/birth defect. An event reaction causality scoring system will be used to determine the probability of the relationship of the event to study intervention. Source documents will be used to capture AEs and SAEs. All AEs will be reported to the CREB within 24 hours of the PI being aware of the event.

Known side effects of FMT are described in table 6.0; these potential effects are listed in the informed consent and participants are advised to report these and any other effects that they feel is relevant or causing concern.

FMT Side Effects

Possible FMT Side Effects	Frequency that the side effect has been reported
Hard stool	10%
Increase in flatulence or passing gas	10%
Stomach discomfort	8%
Increase appetite	3%
Nausea	2%
Minor temporary pressure or discomfort in the rectum	1%

There has been a report of transmission of multi-drug resistant organism (MDRO) to patients who received FMT from a donor, whose stool was not screened for the MDRO.

There may be a small risk of transmission and infection due to MDRO. However, the donor's stool samples used at Vancouver Island Health Authority routinely undergo screening for MDRO. The donor's blood and stool are regularly tested for transmissible infectious organisms that may cause disease. If a donor and/or their stool sample shows abnormal result(s), such stool samples will not be considered for FMT processing.

Proposed sample size and the justification for the assumptions

Fifty participants will be recruited for the chronic GI dysbiosis component of the study based on the 5% response in placebo group and 24% in FMT arm with significance level 5% and power of 80% in the FMT trial of ulcerative colitis. For the ARO participants, the sample size will be 20 as this will be a pilot project to determine the feasibility of further study.

The proposed type of statistical analyses

The clinical response rate will be determined using standard binary outcome protocols. Also, logistic regression and survival analysis methods will be performed. The primary endpoints of clinical response, and treatment failure will be analyzed by logistical regression.

The proposed frequency of analyses
The analyses will be conducted following the data collection stage at the end study. An interim analysis will be performed after 15 patients have been recruited to assess safety and ongoing trial feasibility for the dysbiosis group.

Trial steering and the data safety and monitoring committee

An independent data monitoring committee (DMC) will be constituted prior to the conduct of the trial and this committee will continue to serve to oversee until the completion of the trial.

The DSMC will convene on a periodic basis; normally, after data on the first 20% of participants have been enrolled into a study, and at the conclusion of enrollment. The committee will be advised on a regular basis of participant enrollment; receive (verbatim) each report of a serious adverse event (SAE), with an investigator assessment of causality/attributability of at least possibly related to study that is provided from the investigator and follow-up SAE reports will be promptly forwarded to the Chair.

Enrollment stopping rules

If the DMC decided that there were a pattern consistent with an important increase in frequency of the occurrence of one or more types of SAEs then a review by the DMC Chair will be requested.

The DMC, upon review of the participant-specific reported SAEs, will: Convene a meeting of the full DMC for the purpose of providing a recommendation to the investigator regarding cessation or continuation of enrollment in the study. Such recommendation will be formally provided within 24 hours of such meeting and will not be influenced by actions or opinion of the any of the investigators; Recommend that enrollment in the study be halted if as many as three (3) participants that received treatment with FMT were reported to experience the same or substantially similar types of SAE and the DMC concluded that such SAEs could be reasonably related to treatment with FMT; Recommend that enrollment in the study be halted if any one participant who received treatment with FMT were reported to experience an SAE that had fatal or life-threatening outcome and the DMC concluded that such SAE could be reasonably related to FMT.

Trial Organization

Data Management, Treatment Allocation and Analysis Centre

Vancouver Island Health Authority (VIHA)

Royal Jubilee Hospital

Trial Training
Prior to patient recruitment, all research laboratory staff will be trained and provided with manufacturing of FMT suspension and the lyophilization and reconstitution for delivery of FMT by enema to patients. Copies of delegation logs documenting site staff responsibilities and training dates will be retained at VIHA.

References

1. Van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. N Engl J Med 2013;368:407–15.
2. Lee Ch, Belanger JE, Kassam Z, et al. The outcome and long-term follow-up of 94 patients with recurrent and refractory Clostridium difficile infection using single to multiple fecal microbiota transplantation via retention enema. Eur J Clin Microbiol Infect Dis 2014;33;1425-1428.
3. Kassam Z, Lee CH, Yuan Y. et al. Fecal microbiota transplantation for Clostridium difficile infection: systematic review and meta-analysis. Am J Gastroenterol 2013;108:500-508.
4. Song Y, Garg S, Girotra M, et al. Microbiota dynamics in patients treated with fecal microbiota transplantation for recurrent *Clostridium difficile* infection. Plos ONE 2013;8:e81330.
5. Hamilton M, Weingarden A, Unno T, Khoruts A, Sadowsky M. High-throughput DNA sequence analysis reveals stable engraftment of gut microbiota following transplantation of previously frozen fecal bacteria. Gut Microbes 2013;4:125–133.
6. Weingarden A, Chen C, Bobr A, et al. Microbiota trans- plantation restores normal fecal bile acid composition in recurrent *Clostridium difficile* infection. Gastronint Liver Physiol 2014;306:310–9.
7. Borody TJ, Warren EF, Leis S. et al. Treatment of ulcerative colitis using fecal bacteriotherapy. J Clin Gastroenterol 2003;27:42-47.
8. Moayyedi P, Surette MG, Kim PT, et al. Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized, controlled trial. Gastroenterology 2015; 149:102-109.
9. Rossen NG, Fuentes S, van der Spek MJ, et al. Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis. Gastroenterology 2015;149:110-118.
10. http://thepowerofpoop.com/epatients/fecal-transplant-instructions/
11. Singh R, Nieuwdorp IJ, Berge JM, Bemelman FJ, Geerlings SE. The potential beneficial role of faecal microbiota transplantation in diseases other than *Clostridium difficile* infection. Clin Microbio Inf 2014;20:1119-1125.
12. Lagier JC, Million M, Fournier PE, et. al. Faecal microbiota transplantation for stool decolonization of OXA-48 carbapenemase-producing Kelbsiella pneumonia. J Hosp Infect 2015;90:171-174.
13. https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/important-safety-alert-regarding-use-fecal-microbiota-transplantation-and-risk-serious-adverse.