Serial fecal microbiota transplant plus fidaxomicin in the treatment of severe or fulminant *Clostridium difficile* infection (CDI)

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Study product: Human biotherapy
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List of Abbreviations and Acronyms

BA	bile acid
BID	twice a day
BSC	biological safety cabinet
C&S	culture and sensitivity
CDI	*Clostridium difficile* infection
CRP	C reactive protein
FMT	fecal microbiota transplantation
HAV	hepatitis A virus
HBV	hepatitis B virus
HCV	hepatitis C virus
Hgb	hemoglobin
HIV	human immunodeficiency virus
HTLV	human T lymphotrophic virus
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
LFT	liver function test
O&P	ovum and parasite
PLT	platelet
RIW	Resource Intensity Weights
SCDI	Severe *Clostridium difficile* infection
SOP	Standard Operating Procedure
UAH	University of Alberta Hospital
WBC	white blood cell

Study Synopsis

Title	Serial fecal microbiota transplant (FMT) plus fidaxomicin in the treatment of severe or fulminant *Clostridium difficile* infection (CDI)
Investigational Product and Indication for Use	FMT and fidaxomicin for severe or fulminant CDI
Study Purpose	To determine the safety and efficacy of serial FMT and fidaxomicin in the treatment of severe or fulminant CDI
Study Design	Prospective, open label, multi-center study
Study Population	Patients with severe or fulminant CDI
Sample Size	30
Dosing	Each cycle consists of: 1. Serial FMT: 720 cc (1st treatment), 360 cc (2nd Rx), and 180 cc (3rd Rx) of fecal slurry 2. Fidaxomicin: 200 mg oral BID x7-10 days
Masking	None
Study Outcomes	Primary outcomes CDI resolution, defined as < 3 unformed bowel movements/ 24 hrs or a return to baseline bowel habit, 2 weeks after the last FMT treatment Secondary outcomes 1. Sustained CDI resolution, defined as a lack of CDI recurrence 8 weeks after the last FMT treatment 2. All serious adverse events up to and including week 8. A serious adverse event is any event which results in any of the following after the final FMT: a. Death b. Colonic perforation c. Proven infection related to FMT d. Subsequent hospitalization due to CDI within study period 3. Need for colectomy

	Exploratory outcomes 1. All direct medical costs for CDI treatment 2. Changes in microbial composition, metabolomics and host immune response after treatment
Inclusion Criteria	1. Age > 18 years with severe[1] or fulminant[2] CDI, without an adequate response to metronidazole IV 500 mg q8H and vancomycin 500 mg PO q6h for at least 2 days or after a single Fecal Microbiota Transplant (FMT). An adequate response is defined as a decrease in stool frequency or inflammatory markers (WBC or C reactive protein) by at least 10% over 48 hours 2. Those with ability to provide informed consent or an alternative decision maker providing consent [1]- Severe CDI defined as WBC > 15,000 cells/mm3 or serum creatinine level >1.5mg/dL or 1.5x premorbid level [2]- Fulminant CDI defined as defined as having any of the following attributable to CDI: Hypotension or shock, ileus, megacolon. An abdominal CT scan should be strongly considered to rule out perforation
Exclusion Criteria	1. Those with bowel perforation 2. Those taking chemotherapy or radiation treatment with absolute neutrophil count of < 1000 cells/mm^3 3. Those with colostomy or ileostomy, or colonic strictures 4. Those with subtotal colectomy or planning to have a colectomy 5. Those with significant ileus
Study Duration	Approximately 20 weeks
Study Sponsor	University of Alberta Edmonton, Alberta

1.0 Introduction

Clostridium difficile infection (CDI) is the leading cause of hospital-acquired infectious diarrhea, representing a significant healthcare burden worldwide. Severe CDI is associated with a high morbidity and mortality rate despite medical and/or surgical intervention(1). Around 8% develop fulminant CDI resulting in toxic megacolon, multi-organ failure and death (2). In a meta-analysis of primary studies published in 2002 and 2007, the annual cost of CDI in the United States adjusted to 2012 dollars was estimated at $1.5 billion (3). Recent surveillance data from Alberta Health Services (AHS) found that Edmonton has the highest rate of healthcare associated CDI and the highest CDI mortality in the province of Alberta (Figure 1).

Mild and moderate cases of CDI respond very well to vancomycin, which has recently become the first line therapy(4). Unfortunately, vancomycin is not a narrow spectrum antibiotic, and could also promote the development of vancomycin resistant Enterococcus (VRE) (5). Another antibiotic fidaxomicin, a narrow spectrum antibiotic, is non inferior to vancomycin in clinical efficacy in the treatment of mild to moderate infection and is shown to significantly reduce the rate of CDI recurrence (6); however, the high cost prohibits its use as a first line therapy. Furthermore, it has not been used in treating patients with severe or fulminant CDI. Other therapies including monoclonal antibodies against C Diff Toxin B, bezlotoxumab, have been shown to reduce recurrence in CDI but the mechanism of action and long term effects are still not understood (7). Tigecycline is another broad spectrum antibiotic of the glycylcycline class that has been shown to significantly reduce the toxin production in hypervirulent strains of *C difficile*, however it can lead to further disruption to the gut microbiome (7). Overall these newer therapies are expensive with little knowledge of the mechanism of action in the treatment of CDI (7).

Severe CDI is defined as white blood cell count > 15,000 cells/mm^3 and fulminant CDI is defined as having any of the following attributable to CDI: hypotension or shock, ileus, and toxic megacolon (4). Severe and fulminant CDI require combined metronidazole with vancomycin; surgical intervention is indicated when medical therapy fails (4).

Fecal microbiota transplantation (FMT), which restores intestinal dysbiosis, is highly efficacious in treating mild to moderate recurrent CDI. There is also emerging evidence that FMT may be effective in severe or fulminant CDI, a situation where the only alternative is colectomy (8). FMT can be administered by difference routes, including enema, colonoscopy and oral capsules (9). However, Health Canada does not allow oral capsule to be administered outside the context of a clinical trial. Although few studies have described the efficacy of FMT in severe and fulminant CDI, it appeared efficacious and safe. For example, our group has successfully treated 6 patients with fulminant CDI with serial FMT plus vancomycin over the past year (Figure 2.0). These patients had avoided colectomy as a result. In addition, Fischer and colleagues demonstrated 17/19 (91%) clinical cure rate in these critically ill patients using combined serial FMT and vancomycin (10). In this protocol, Fischer described using colonoscopy-delivered FMT to treat fulminant CDI after failing at least 5 days of combined metronidazole and vancomycin. At the time of colonoscopy to deliver FMT, if pseudomembranes were noted, vancomycin was restarted for at least 5 days before the second colonoscopic FMT was administered. If pseudomembranes persisted, vancomycin was again given for 5 more days

before the third FMT was administered, and this cycle of FMT and vancomycin continued until pseudomembranes resolved. However, there are questions and uncertainties with this approach. First, vancomycin is not a narrow spectrum antibiotic, and it seems counterintuitive to give FMT and vancomycin at the same time. Second, the presence of pseudomembranes cannot be used for daily clinical monitoring, as it requires an invasive test. Furthermore, it remains unknown how many FMT each patient requires, what the most convenient or safest way to administer the FMT is, what the best strategies for monitoring is, or what the optimal frequency of FMT is. The success of Fischer's protocol thus also highlights the gaps in knowledge in mechanisms of action for FMT in severe or fulminant CDI.

In this prospective, open-label, multi-center study, we aim to determine the efficacy and safety of using a combined serial FMT by enema plus fidaxomicin to treat patients who have severe or fulminant CDI not responding to maximal medical therapy. Our hypothesis is the combination of FMT plus fidaxomicin can reduce the number of FMT required and hospital length of stay compared to FMT plus vancomycin from a historical cohort.

2.0 Objectives

Primary Objectives: To determine the clinical efficacy of combined serial FMT and Fidaxomicin in severe or fulminant CDI 2 weeks after the last FMT

Secondary Objectives:

a) To determine sustained CDI resolution, defined as a lack of CDI recurrence 8 weeks after the last FMT treatment
b) To describe serious adverse events up to and including week 8 after the final FMT. A serious adverse event is any event which results in any of the following:
    i) Death
    ii) Colonic perforation
    iii) Proven infection related to FMT
    iv) Subsequent hospitalization due to CDI within study period
c) To determine colectomy rate in this patient population

Exploratory Objectives:

a) All direct Medical costs for CDI treatment
b) Changes in microbial composition, metabolomics and host immune response after combined serial FMT plus fidaxomicin

3.0 Methods

3.1 Study Design

Prospective, open-label, multi-center study. Sites include:

- University of Alberta Hospital, Edmonton AB

- Foothills Medical Centre, Calgary AB
- Royal Jubilee Hospital, Victoria BC

3.2 Study Setting, Patient Recruitment and Sample Size

In-patients with severe or fulminant CDI who fulfill study criteria will be screened for potential enrollment into this study. We aim to recruit 30 patients over 2 years. As this is a pilot study with a unique patient population, the sample size is based on estimate of patient volume seen previously at all participating sites combined.

3.3 Study Population

Patients admitted to hospital who fulfill the inclusion/exclusion criteria will be considered.

3.31 Inclusion Criteria

- Age > 18 years with severe[1] or fulminant[2] CDI, without an adequate response to metronidazole IV 500 mg q8H and vancomycin 500 mg PO q6h for at least 2 days or after Fecal Microbiota Transplant (FMT). An adequate response is defined as a decrease in stool frequency or inflammatory markers (WBC or C reactive protein) by 10% over 48 hours
- Those with ability to provide informed consent or an alternative decision maker providing consent

[1]- Severe CDI defined as WBC > 15,000 cells/mm3 or serum creatinine level >1.5mg/dL or 1.5x premorbid level

[2]- Fulminant CDI defined as defined as having any of the following attributable to CDI: Hypotension or shock, ileus, megacolon (4). An abdominal CT scan should be strongly considered to rule out perforation

3.32 Exclusion Criteria

- Those with bowel perforation
- Those taking chemotherapy or radiation treatment with absolute neutrophil count of < 1000 cells/mm3
- Those with colostomy or ileostomy or colonic strictures
- Those with subtotal colectomy or planning to have a colectomy
- Those with significant ileus

3.4 Stool donor selection and testing

Five universal stool donors registered with the Edmonton FMT program will provide the starting material, which is raw stool for patients recruited in Edmonton. These donors do not work in healthcare fields, and are known personally to the investigators. They are instructed not to provide a stool collection if they have any symptoms suggestive of an infection as per donor collection SOP and checklist in Appendix B.

They are rescreened every 2 months. The other sites included in the study have established stool donors in their facility and will provide their own FMT using identical protocol for donor screening and FMT manufacturing.

3.41 Donor stool collection and processing

Each donor will provide a fresh stool specimen, weighing approximately 100g, as per donor stool collection SOP (Appendix B). The stool specimen is stored at 4-8^{0}C after collection and brought into the lab within 12 hours of collection. Donor stools should have the appearance of type 2-5 on the Bristol Stool Scale, and be free of blood, mucus or urine contamination. No pooling of stools will occur. Once received by the lab, it will be processed as per protocol for enema. Each donation of 100 g of stool will produce approximately 360 cc of fecal slurry, which will be stored frozen at -80^{0}C as per our manufacturing protocol.

See Appendix A for donor screening SOP, Appendix C for donor questionnaire and Appendix D for donor consent.

3.5 Intervention and follow up

Patients who fulfill inclusion and exclusion criteria will be screened. Once consent has been obtained, metronidazole and vancomycin will be discontinued and 2L of Golytely will be administered the night prior to FMT. Each participant will undergo a large volume enema of 720cc delivered FMT on day 1, consisting of 200g of donor stool followed by two more days of small volume FMT enema on day 2 (360 cc) and day 3 (180 cc). This constitutes as cycle 1. In the event that a colonoscopy is indicated to rule out other pathology, then the scheduled enema can be delivered at the time of colonoscopy. On day 1, the patient will also start a 7-10 day course of fidaxomicin, as long as no clinical deterioration or complication such as bowel perforation has occurred. Following the first cycle of FMT treatment and fidaxomicin, careful clinical monitoring including vitals, requirement for vasopressors (if applicable), abdominal pain and distension, stool frequency and inflammatory markers including white blood cell (WBC) and C reactive protein (CRP) will be performed daily (Figure 3.0). If the patient has improvement in clinical parameters and inflammatory markers, and has reached a plateau without further changes over 24-48 hours after the first cycle, a second cycle of enema delivery of FMT will be administered. The FMT and fidaxomicin combined cycles will continue until clinical resolution of diarrhea and/or return of inflammatory markers to baseline prior to CDI. At that point, a final FMT 180cc enema will be administered. The maximal number of cycles is 4. At any time when a patient's conditions worsens, the study team will refer patient for surgery.

Each patient will receive FMT from a single donor with multiple collections obtained over time.

Baseline characteristics including age, sex, duration and response to CDI therapy, medical history (including inflammatory bowel disease, irritable bowel syndrome, baseline bowel habit, bowel resection, chemotherapy or radiation, number of CDI in the previous 12 months) and medication use will be collected. Blood work including CBC, electrolytes, creatinine, ALT, ALP, albumin, CRP, INR, HIV, and viral hepatitis serology will be drawn at screening visit (Figure 4.0). In addition, baseline collection of blood, and stool samples will be collected and

biobanked. Repeat stool and blood samples will be collected after each cycle of treatment, and again at week 1, 2, 4 and 8 after the final FMT.

Clinical status as well as monitoring for adverse events will be assessed daily up to 2 weeks after the final FMT while a patient is hospitalized, then weekly to week 8 after the final FMT. Patients are instructed to call as soon as there is recurrence of persistent diarrhea, and an unscheduled visit will take place if needed. Given COVID-19 pandemic, study follow-up may be conducted by phone if participants are unable to or do not want to attend study visits or if the visit is deemed non-essential by the principal investigator (PI). Due to this some of these sample collections may not be possible.

3.6 Subject Withdraw or Termination

Subjects will be discontinued from the trial in the following situations:

- Withdrawal of consent. The subject is free to withdraw from the trial without prejudice to further medical care
- Need for urgent colectomy

3.7 Outcome Measures

3.71 Primary Outcome

CDI resolution, defined as < 3 unformed bowel movements/ 24 hrs or a return to baseline bowel habit, 2 weeks after the last FMT treatment

3.72 Secondary Outcomes

a) Sustained CDI resolution, defined as a lack of CDI recurrence 8 weeks after the last FMT treatment

Clinical status and biological samples will be taken to ensure that CDI has not recurred at 8 weeks

b) Adverse events

All serious adverse events will be recorded up to and including week 8 after the final FMT. A serious adverse event is any event which results in any of the following:

- Death
- Colonic perforation
- Proven infections related to FMT
- Subsequent hospitalization due to CDI within study period

c) Proportion of patients requiring colectomy

3.73 Exploratory Outcomes

a) Costs for CDI Treatment

Direct Medical costs for CDI treatment will be obtained from administrative database to include length of hospital stay, procedures and tests performed, ICU stay and medication use.

b) Stool microbial composition analysis by metagenomics

Stool samples will be subjected to physical disruption using a bead-beating kit and microbial DNA extracted using the Qiagen QIAamp DNA stool kit and stored at -20^{o}C. DNA will be used as input for the Illumina Nextera® XT DNA Sample Preparation Kit to construct indexed paired-end DNA libraries. The pooled and indexed library set will be denatured and sequenced on an Illumina MiSeq.

Whole-genome shotgun sequencing will be performed and taxonomic classification of reads from each library will be conducted with Kraken. The database used consisted of all bacteria, archaea, viruses, fungi, and protozoa full-length genomes from NCBI RefSeq, the human genome assembly GRCh38, and reference bacterial assemblies from the Human Microbiome Project. Read assignments were filtered with Kraken-filter using a threshold of 10%.

c) Stool Metabolomics analysis

Stool samples will be lyophilized for 24 hours using a VirTis Benchtop BTP 8ZL freeze dryer (BPS, UK). Dried samples will be weighed, and bile acids extracted using a 2:1:1 (vol) mixture of water, acetonitrile and 2-propanol in a Biospec bead beater with 1.0 mm Zirconia beads. Following centrifugation (16,000 x g, 20 minutes) the supernatant will be filtered using 0.45 μm microcentrifuge filters (Costar, Corning). Quality control (QC) samples will be prepared using equal parts of the fecal filtrates. QC samples will be used as an assay performance monitor, and as a proxy to remove features with high variation. QC samples will also be spiked with mixtures of bile acid standards (55 bile acid standards including 36 non-conjugated, 12 conjugated with taurine, seven conjugated with glycine (Steraloids, Newport, RI, USA)) and will be analyzed along with the stool samples to determine the chromatographic retention times of bile acids and to aid in metabolite identification.

Bile acid analysis of fecal extracts will be performed using ACQUITY UPLC (Waters Ltd, Elstree, UK) coupled to a Xevo G2 Q-ToF mass spectrometer equipped with an electrospray ionization source operating in negative ion mode (ESI-), using the method described by Sarafian and colleagues. Waters raw data files will be converted to NetCDF format and data will be extracted using XCMS (v1.50) package with R (v3.1.1) software. Probabilistic quotient normalisation6 will be used to correct for dilution effects and chromatographic features with coefficient of variation higher than 30% in the QC samples will be excluded from further analysis. The relative intensities of the features will be corrected to the dry weight of the fecal samples.

d) Blood Immunological profiling

Immunophenotypic analysis of peripheral immune cell populations through peripheral blood mononuclear cells (PBMCS) that will be isolated from buffy coats and stained with optimized combinations of fluorochrome-conjugated mAbs. Frequencies of T- and B-regulatory cells, as well as Th1, Th2 and Th17 populations will be ascertained using established flow cytometric antibody panels and isotype controls. Relevant signature cytokines and transcription factors will

also be included in an already established 9-colour FACS panel. We will also examine effector and memory B and T cell populations, in addition to plasma and dendritic cell populations plus their relevant signature cytokines and transcription factors (all pending sufficient availability of cells).

4.0 Proposed Analysis

The primary outcome, defined as CDI resolution at week 2, will be analyzed using the per protocol approach for all patients. Adverse events will be tabulated in each predetermined outcome.
To measure the direct medical costs, the utilization of different health care services, including pharmaceuticals, physician services, Emergency Department visits, inpatient hospitalizations, and Intensive Care Unit time will be summed for each individual patient. In order to attempt to attach a dollar value to the resource utilization, CIHI's Resource Intensity Weights (RIW) will be used. The RIW methodology derives estimated resource utilization compared to an 'average typical hospital stay', the cost of which has been calculated for the Edmonton Zone in each fiscal year from 2010/11 through 2014/15. RIW values are calculated based on the patient's Comprehensive Ambulatory Classification System (CACS) group, age and based on the patient's Case Mix Group (CMG) code, age, and comorbidity level.

5.0 Data Safety and Monitoring

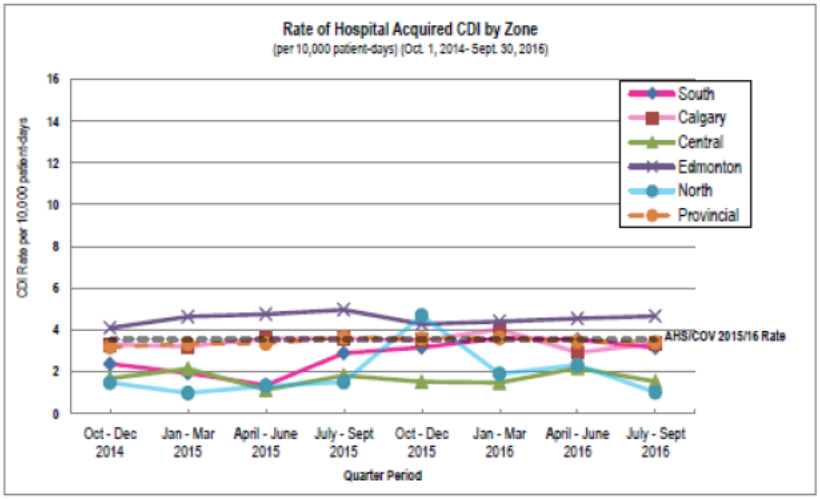
An independent data safety monitoring board (DSMB) will be appointed and following the project till completion. The members of the DSMB are not involved in this trial, and will not have potential patients who might be enrolled in this study.

6.0 Significance

This will be the first study examining the clinical efficacy of combined FMT and fidaxomicin in the treatment of severe or fulminant CDI refractory to medical therapy. Longitudinal clinical samples collected during this study will undergo detailed analysis to gain better understanding of the complex interaction between microbiome and human host in this context. Results from this study have the potential to stimulate further studies aimed at elucidating mechanisms of action of FMT in order to develop rational microbiome based therapy. The knowledge gained during this trial will help us optimize treatment algorithm for patients suffering from treatment refractory CDI, and ultimately improve patient experiences and outcomes while simultaneously decrease health care costs.

Figure 1. Provincial rates of health care associated CDI and attributable mortality to CDI by zone between 2014 and 2016. Alberta Health Services Quality Performance Report 2015-2016. Hospital-acquired Clostridium difficile infections. http://www.albertahealthservices.ca/assets/about/publications/ahs-pub-pr-2015-16-q2-detail-hospital-acquired-infections.pdf

The provincial rate of HA-CDI is 3.4 cases per 10,000 patient-days.



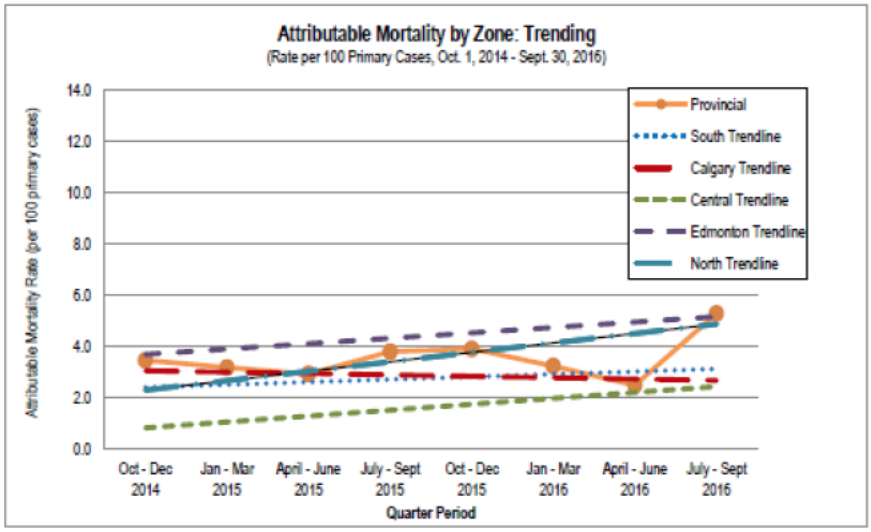


Figure 2.0- Pilot data:
Over the past year, we have successfully treated 6 patients with fulminant CDI with serial FMT plus vancomycin at the University of Alberta. These patients had avoided colectomy as a result. Below are endoscopic images of before (image on the left, showing severe pseudomembranous colitis) and after serial FMT treatment (image on the right, showing normal colon).

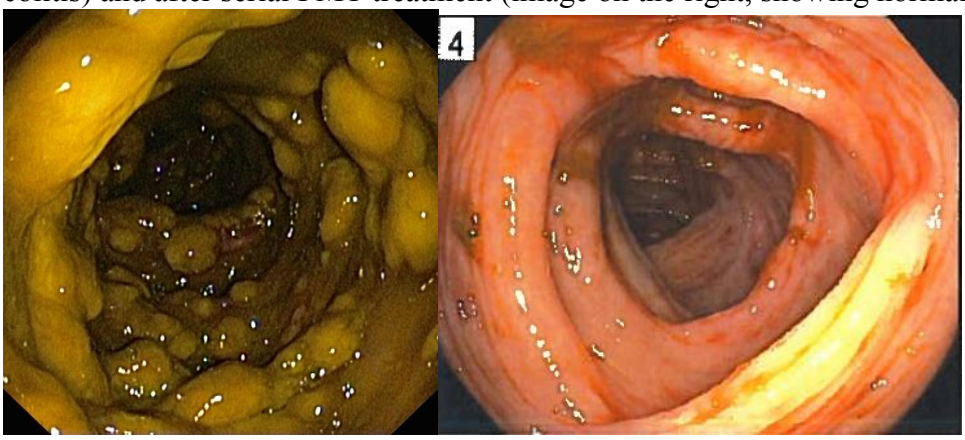


Figure 3.0
Sequential fecal microbiota transplant protocol proposed.

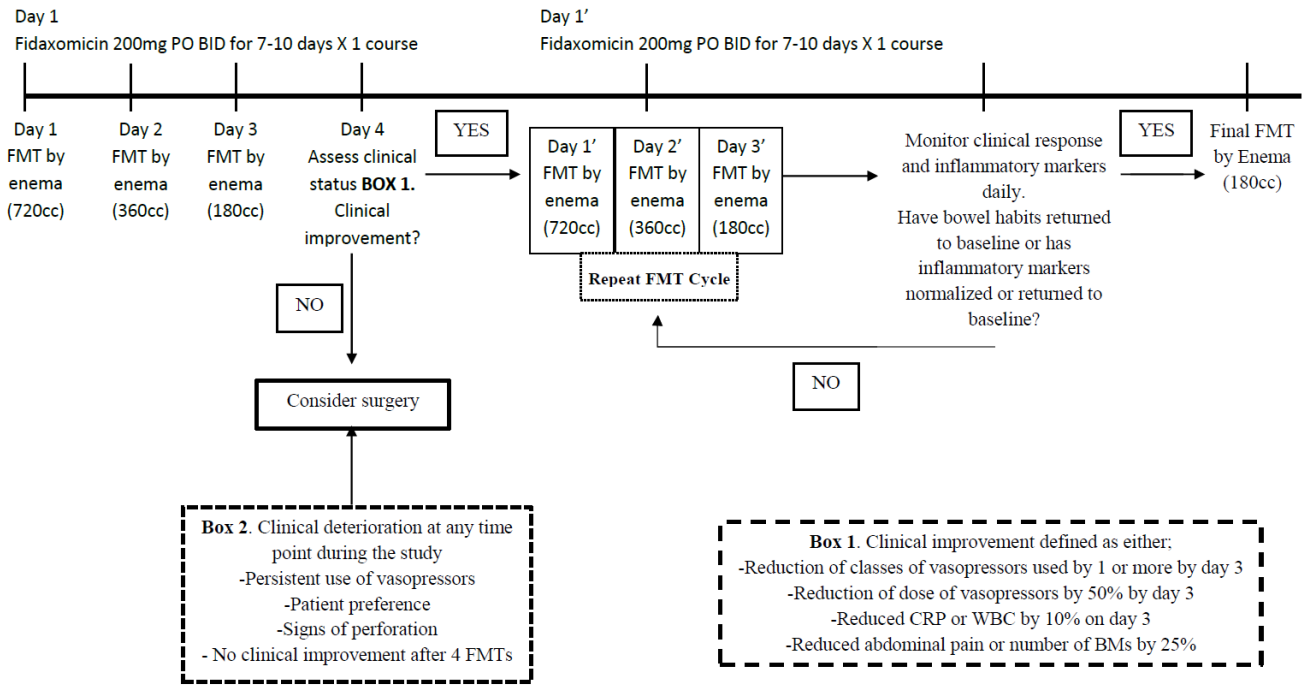
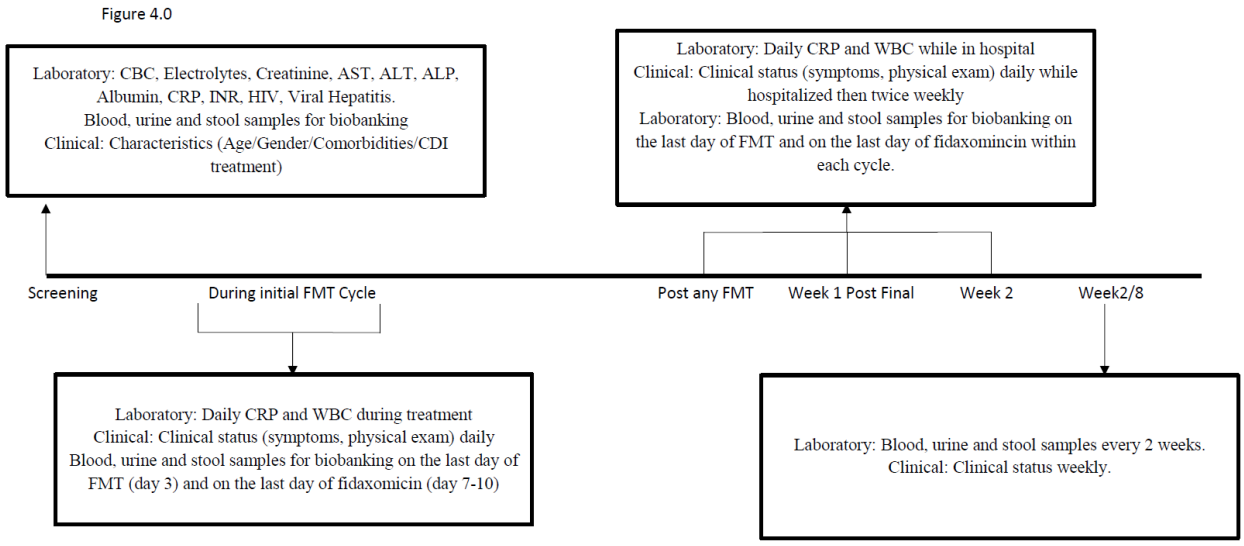


Figure 4.0

Proposed sampling for each patient given the protocol seen in Figure 3.0



Appendix A: Universal stool donor testing/screening SOP

Universal stool donors will provide the raw material for FMT. They are screened at baseline and then every 2 months at a minimum for the duration of the trial. They underwent initial detailed history and physical exam, and had been screened with a donor questionnaire, which did not identify any high risk behaviors. They tested negative for all the following potential infections as listed below. They have provided stools for over 300 patients since 2012, and none of the recipients have developed any known infectious complications. All donors are personally known to the investigators. It is simply not practical or cost effective to keep testing them each and every time when there is a scheduled FMT. Since there is no consensus on the mandatory required tests for stool donor, we have chosen the recommendations published by Khoruts et al in the American Journal of Gastroenterology in 2012 as well as the FMT Safety Notice from Health Canada dated July 16,2019 pertains to MDRO testing, while Mar 25, 2020 pertains to COVID-19

These universal donors have been in the same positions for years are unlikely to move away or stop donating. We have not encountered a situation when a universal donor could not donate on a day when HBT was scheduled to treat patients with recurrent Clostridium difficile infections since Oct 2012. In the unlikely event that he or she can no longer donate, a second donor will be assigned to a particular patient.

We will hold donor FMT materials in quarantine until a donor has passed 2 consecutive screening tests.

On initial history, the donors must fulfill the following inclusion and exclusion criteria:

Donor inclusion criteria:

1. Able to provide and sign informed consent (Appendix D).
2. Able to complete and sign the stool donor questionnaire (Appendix C).
3. Able to adhere to fecal transplantation stool collection standard operating procedure (appendix B).

Donor exclusion criteria:

1. History of any type of active cancer or autoimmune disease (eg multiple sclerosis, connective tissue disease), metabolic syndrome, chronic pain syndrome, and atopic diseases.
2. History of risk factors for acquisition of HIV, syphilis, Hepatitis B, Hepatitis C, prion or any neurological disease as determined by the donor questionnaire (Appendix 2).
3. Gastrointestinal comorbidities, e.g., inflammatory bowel disease, irritable bowel syndrome, chronic constipation or diarrhea, gastrointestinal malignancy or known polyposis.
4. Tattoo or body piercing within 6 months of stool donation.
5. Incarceration or history of incarceration.
6. Receipt of blood transfusion from a country other than Canada in preceding 6 months.
7. Antibiotic use, systemic immunosuppressive or biological agents, systemic antineoplastic agents and exogenous glucocorticoids in the preceding 3 months prior to stool donation.
8. Receipt of any type of live vaccine within 3 months prior to stool donation.

9. Ingestion of nut or shell fish 3 days preceding donation if the recipient has known allergies to these food.
10. Any current or previous medical or psychosocial condition or behaviors which in the opinion of the investigator may pose risk to the recipients or the donor.
11. Travel to areas of the world where diarrheal illnesses or BSE/TSE are endemic (within 6 months of stool donation).
12. High risk of multi-drug resistant organisms, including healthcare workers, recent hospitalization and medical tourism.
13. Travel history outside of Canada within 14 days of screening
14. Exposure to individuals diagnosed with or suspected to have COVID-19 within 14 days of screening
15. History of a fever, cough, sore throat or any other signs of respiratory or other infectious illness in the past 14 days
16. Diagnosis of COVID-19 within the past 3 months

Initial blood work and stool testing, which will be repeated at a minimum of every 2 months.. However, to minimize COVID-19 transmission risk, the quarantine period is at least 5 weeks as per Health Canada directions.

Blood:

- CBC, electrolytes, creatinine, AST, ALT and ALP
- Human Immunodeficiency virus (HIV) 1/2
- hepatitis A IgM Ab
- hepatitis B: HBVsAg, HBVsAb, HBVc Ab (IgM and IgG)
- hepatitis C antibody
- RPR (syphilis)
- human T- lymphotrophic virus (HTLV) I/II

Nasal Swab:

- MRSA

Stool:

- enteric pathogens: *Salmonella*, *Shigella*, *E.coli* O157 H7, *Yersinia*, *Campylobacter (see Appendices 3-8 for lab SOP)*
- *C. difficile* toxin by EIA
- ova and parasites
- VRE
- CRE
- ESBL

Appendix B: Stool collection instruction for donors (to be filled out with each stool collection)

Stool collection for human biotherapy/ fecal microbiota transplantation

1. Do not take any medications (laxatives, anti-diarrheal drugs, mineral oil, bismuth, magnesium, or kaolin) for at least 3 days prior to and during the specimen collection. If you have been taking antibiotics in the last 90 days, you should not be collecting stool.

2. Make sure you have not had fever, vomiting, diarrhea or other symptoms of infection within the last 30 days. **Inform the investigator if you have any of these symptoms, as you should not donate stool. Please hand in this form with your stool collection with each donation.**

Day of FMT Procedure/donor Checklist
Donor Name:
Date:

	Yes	No	
Do you feel well and healthy today?			
Have you had any fever, vomiting, diarrhea, cough, sore throat or other symptoms of infection within the last 30 days?			
If yes, specify:			
Antibiotics within past 90 days?			
Any medications (laxatives, anti-diarrheal drugs, mineral oil, bismuth, or magnesium, kaolin) in the past 3 days			
Since your last donation have you started working as a healthcare worker or worked in a long term care facility?			
Since your last donation have you been hospitalized or been treated in a long term care facility?			
Since your last donation, have you had medical treatment requiring frequent, regular attendance at outpatient medical or surgical clinics? If yes, please list dates and reason:			

Since your last donation, have you engaged in medical tourism i.e. travelled outside Canada for medical treatment?			
Since your last donation, have you had contact with anyone who has been diagnosed or suspected to have COVID-19?			
Since your last donation, have you travelled outside of Canada?			
Since your last donation, have you been diagnosed with COVID-19?			

3. Collect the specimen into the commode specimen collection system provided (Fisher 07544208) at home. This system fits under the toilette seat in center of rear of bowl. Do not mix with urine, paper or water. Once stool has been collected, snap on the lid provided to seal the container. Make sure you collect at least 100 g of stool, which is about the size of 4 Timbits donuts.

4. Place the container in the plastic bag supplied by the designate. Seal the bag tightly.

5. Mark date and time of collection and your initials on the lid.

6. Wash hands with soap and water when finished.

7. Maintain stool sample at 2^0C to 8^oC until specimen can be delivered to University of Alberta Hospital. Keep an ice pack in the bag with stool sample to keep it cold during delivery.

8. Please return specimen within 12 hours of collection and give to the hospital staff designate.

Appendix C: Donor Questionnaire for human biotherapy/fecal microbiota transplantation

	Yes	No
Are You		
1. Feeling healthy and well today?	☐	☐
2. Have you experienced a fever, cough, sore throat or any other sign of respiratory or other infectious illness in past 14 day?	☐	☐
3. Currently taking an antibiotic?	☐	☐
4. Currently taking any other medication for an infection?	☐	☐
5. Currently taking any immunosuppressant medication by mouth or injection?	☐	☐
6. Have you taken any antibiotics in the past 6 months?	☐	☐
7. Have you had any fevers, vomiting, diarrhea or other symptoms of infection within the past 4 weeks?	☐	☐
Do you have		
8. History of chronic diarrhea persisting > 10 days?	☐	☐
9. History of blood in stool not related to hemorrhoid?	☐	☐
10. History of change in bowel habit, alternating from constipation to diarrhea?	☐	☐
11. Any type of active cancer that is not cured?	☐	☐
12. Any active autoimmune diseases?	☐	☐
In the past **12 weeks** have you		
13. Have you been in contact with anyone who has been diagnosed or suspected to have COVID-19?	☐	☐
14. Have you travelled outside Canada?	☐	☐
15. Had any vaccinations? If yes, please indicate which one(s)	☐	☐
16. Had contact with someone who had a smallpox vaccination?	☐	☐
17. taken antibiotics, systemic immunosuppressive or biological agents, systemic antineoplastic agents and exogenous glucocorticoids? If you have, you should not be a stool donor.	☐	☐
In the past **16 weeks** have you		
18. Lived with a person who has hepatitis A?	☐	☐
19. If yes, have you received vaccine against hepatitis A?	☐	☐
In the past **12 months** have you		
20. Had a blood transfusion?	☐	☐

21. Had a transplant such as organ, tissue or bone marrow?	☐	☐	
22. Had a graft such as bone or skin?	☐	☐	
23. Come into contact with someone else's blood?	☐	☐	
24. Had an accidental needle-stick?	☐	☐	
25. Had sexual contact with anyone who has HIV/AIDS or has had a positive test for the HIV/AIDS virus?	☐	☐	
26. Had sexual contact with a prostitute or anyone else who takes money or drugs or other payment for sex?	☐	☐	
27. Had sexual contact with anyone who has ever used needles to take drugs or steroids, or anything <u>not</u> prescribed by their doctor?	☐	☐	
28. Had sexual contact with anyone who has hemophilia or has used clotting factor concentrates?	☐	☐	
29. Female donors: Had sexual contact with a male who has ever had sexual contact with another male? (Males: check "I am male")	☐	☐	☐ I am male
30. Had sexual contact with a person who has hepatitis?	☐	☐	
31. Had a tattoo?	☐	☐	
32. Had ear or body piercing?	☐	☐	
33. Had or been treated for syphilis, gonorrhea or Chlamydia?	☐	☐	
34. Been in juvenile detention, lockup, jail, or prison for more than 72 hours?	☐	☐	
In the past **three years** have you			
35. Been outside the United States or Canada? If yes, list the places(s)	☐	☐	
From **1980 to the present,** have you			
36. Receive a blood transfusion in the United Kingdom or France? (Review list of countries in UK.)	☐	☐	
From **1977 to the present,** have you			
37. Received money, drugs, or other payment for sex?	☐	☐	
38. Male donors: had sexual contact with another male, even once? (Females: check "I am female")	☐	☐	☐ I am female
Have you **EVER**			
39. Had a positive test for the HIV/AIDS virus?	☐	☐	
40. Used needles to take drugs, steroids, or anything <u>not</u> prescribed by your doctor?	☐	☐	
41. Used clotting factor concentrates?	☐	☐	
42. Had hepatitis?	☐	☐	
43. Had malaria?	☐	☐	
44. Had Chagas' disease?	☐	☐	

45. Had babesiosis?	☐	☐
46. Received a dura mater (or brain covering) graft?	☐	☐
47. Had sexual contact with anyone who was born in or lived in Africa?	☐	☐
48. Have any of your relatives had Creutzfeldt-Jakob disease?	☐	☐
49. Have you ever had any of the following gastrointestinal diseases or problems: Irritable bowel syndrome Crohn's disease Ulcerative colitis Gastrointestinal cancers Celiac disease	☐	☐
50. Do you have any autoimmune diseases, for example rheumatoid arthritis, multiple sclerosis or lupus? If yes, please list:	☐	☐
51. Do you have any neurological diseases, for example Parkinsons, autism, ALS? If yes, please list:	☐	☐
52. Are you a healthcare worker or do you work in long term care facilities?	☐	☐
53. Have you ever engaged in medical tourism? That is, travelled outside of Canada for medical treatment?	☐	☐
54. Have you ever had medical treatment requiring frequent, regular attendance at outpatient medical or surgical clinics? If yes, please describe reason and timing:	☐	☐
55. Have you ever been hospitalized or been treated in a long term care facility? If yes, please list reason and date:	☐	☐

This information will remain strictly confidential
To the best of my knowledge, the above information is accurate and true.

Donor's Signature: **Date:**

Appendix D: CONSENT TO DONATE STOOL for FMT (fecal microbiota transplantation)

I, _____consent to undergoing collection of blood and stool for potential donation of my stool for FMT now and as requested. I agree to undergoing blood and stool tests, including Complete Blood Count, creatinine, sodium, potassium, chloride, alanine transaminase, alkaline phosphatase, Hepatitis A, B and C, HIV, HTLV I/II, RPR (Syphillis), MRSA and stool culture and susceptibility for enteric ova and parasites, VRE, CRE, ESBL and Clostridium difficile toxin. I have completed the FMT donor questionnaire truthfully and to the best of my knowledge.

I understand that I will have to bring in fresh stool sample within 5 hours of collection to UAH laboratory for processing when requested. I will notify Dr. Dina Kao whenever there is a change to my health status. I will refrain from the donation when I experience fever, sore throat and/or diarrhea and any changes to my social status which may pose any potential risk to the recipient.

I also agree for my stool samples to undergo culture and molecular testing to determine the type of bacteria and products they contain. This information along with any related research can be submitted for presentation and publication. I understand that no information which discloses my identity will be released or published.

I have had the opportunity to ask questions about the process and have had my questions answered to my satisfaction. I declare that I have read this form and understand it.

Signature of Donor Print Name of Donor Date (DD/MMM/YYYY)

Signature of Health Practitioner Print Name Date (DD/MMM/YYYY)

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