Online Supplement 1

Study Protocol and Study Procedures

Study Protocol

1. Introduction
   1.2 The principal research questions to be addressed
   1.3 Evidence from the literature
      1.3.1 Preliminary data
   1.4 Rational for conducting this trial
   1.5 Potential risks to the safety of subjects involved in the trial

2. The Proposed Trial
   2.1 The proposed design of the study
   2.2 The planned trial interventions
   2.3 The proposed methods for protection against sources of bias and subject allocation to trial groups
   2.4 The planned inclusion/exclusion criteria for donors and patients
      2.4.1 Patient inclusion criteria
      2.4.2 Patient exclusion criteria
      2.4.3 Donor inclusion
      2.4.4 Donor exclusion
   2.5 The proposed duration of treatment period
   2.6 The proposed frequency and duration of follow-up
   2.7 The proposed primary and secondary outcome measures
   2.8 Measurement of outcome at follow-up
   2.9 Proposed sample size and the justification for the assumptions underlying the power calculations
   2.10 The planned recruitment rate, period and strategy
   2.11 Centres for the study
   2.12 The proposed type of statistical and metagenomic analyses
   2.13 The proposed frequency of analyses
   2.14 Planned subgroup analyses
   2.15 Pilot study using this design
   2.16 Trial steering and the data safety and monitoring committee

3. Trial Organization
3.1 Investigators
3.2 Data Management and Analysis Centre
3.3 Trial Training

References
1. Introduction

*Clostridium difficile* (*C. difficile*) infection (CDI) is a growing concern due to treatment failures with the standard therapy and a number of deaths directly related to this infection [1]. It is also problematic as it can lead to chronic diarrhea and has been the source of outbreaks in many hospitals [2]. One of the major risk factors for developing this infection is taking an oral or intravenous antibiotic. The healthy bacteria which reside within the colon are the major defense against the growth of *C. difficile* within the large intestine. Antibiotics kill these bacteria and allow *C. difficile* bacteria to multiply, produce the toxins and cause disease. The only available treatment in Canada for this infection is administering metronidazole or oral vancomycin. The efficacy of these antibiotics is limited as it consists of treating an infection which also suppresses the growth of anaerobic bacteria such as *Bacteriodes fragilis* group which protect against *C. difficile*. This may in part explain the reason for recurrences following a course of treatment with these antibiotics.

Patients frequently experience recurrent CDI following the completion of treatment using metronidazole or vancomycin. Recurrent CDI is a major clinical problem and public health crisis, however, there has not been any randomized controlled clinical trials assessing the efficacy of any treatment modalities for recurrent disease. To date, there are only case cohort and case series evaluating efficacy of different antibiotic regimens for the treatment of recurrent CDI [3]. An alternative to antibiotic therapy for CDI, especially for recurrent diseases is to infuse millions of healthy gut bacteria directly into the colon of infected patients to combat *C. difficile* by a procedure known as fecal microbiota transplant (FMT), also termed, fecal transplantation. FMT is a process in which a healthy donor’s stool is diluted with water and the liquid portion of the specimen is rectally administered to an affected patient. FMT serves to reconstitute the altered colonic flora, in contradistinction to antibiotics, which further disrupt the microbiome. There has been a growing acceptance of this innovative therapy by both patients and healthcare providers, [4]. The literature reveals a cumulative clinical success rate of 89% (98/110 patients) that utilize FMT in laboratory confirmed recurrent CDI cases [5-7, 8-21]. A recently published study supports the use of frozen FMT for the management of recurrent CDI [22]. The feasibility of using frozen FMT offers a number of advantages such as immediate availability, minimize donor screening and the ability to provide consistent treatment across different centres. The purpose of this investigation is to formally conduct a randomized controlled trial of comparing fresh vs. frozen-and-thawed human biotherapy for recurrent *Clostridium difficile* infection.

1.2 The principal research questions to be addressed.

The primary goal of this proposal is to study the outcome of patients with recurrent CDI treated with fresh FMT vs. frozen FMT in a randomized controlled trial. The specific objectives are to evaluate the safety of both types of FMT and to compare the clinical response, treatment failure and relapse rate in patients treated with fresh FMT compared to those treated with frozen-and-thawed FMT; to assess the functional health and well-being of patients in each arm using the validated tool, and to determine the feasibility of providing standardized FMT in multiple centres across Canada, including community
hospitals. The metagenomics will also be conducted from the stool samples collected from select patients from each arm: pre and post treatment and the matching donors. The metagenomics data will be used to determine the bacteria which may have contributed to the cure of CDI.

1.3 Evidence from the literature

CDI is the most frequent cause of healthcare-associated infectious diarrhea in industrialized countries and affects over 300,000 patients each year in the United States [24,25]. The incidence of CDI has nearly tripled between 1996 and 2005 (from 31 to 84 per 100,000 patient-days) in the United States, [1]. The rise in incidence has been accompanied by an increase in disease severity, with mortality in up to 6.9% of cases, [2]. According to the Canadian Nosocomial Infection Surveillance Program study conducted from November 1, 2004 through April 30, 2005, the incidence rate of healthcare-associated CDI for adult patients admitted to Canadian hospitals is 65 per 100,000 patient-days. The same study identified that the overall and attributable mortality of patients with CDI is 16.3% and 5.7%, respectively in Canada, which is similar to the US data [1, 25]. The associated economic burden has also been significant. Nosocomial CDI increases the cost of otherwise matched hospitalizations by four-fold, translating to greater than $1 billion/year (United States), [26, 27]. Since the implementation of mandatory reporting of CDI cases in September 2008 in Ontario, more than 13 healthcare facilities declared CDI outbreak in Ontario [28]. There were a number of deaths directly due to CDI in these outbreaks. The management of each outbreak is very costly. The direct attributable costs associated with the outbreak management alone per episode per institution exceeded $1 million (direct communication with a hospital chief financial officer).

There is a growing concern regarding failure of standard antimicrobial therapy. The treatment failure rates for metronidazole, which is the first line therapy for uncomplicated CDI, have risen from 2.5% to greater than 18% since 2000, [1, 29, 30]. Recurrence rates are higher among the elderly, and exceed 50% for those over the age 65 [31]. Recurrence rates exceed 60% for patients who have failed 3 or more episodes of standard antimicrobial therapies, [25]. The vanB gene, which is responsible for conferring vancomycin resistance in Enterococcus has been isolated in clostridia, potentially threatening the future use of vancomycin in CDI, [32].

Given the high failure and recurrence rates using the standard therapy, the principal investigator (PI) of this research proposal has been offering FMT for patients who experienced CDI for longer than 6 months despite multiple courses of metronidazole and oral vancomycin therapy. She began treating patients with recurrent CDI with FMT for the following reasons. First, the patients were not responding to the antibiotic treatment. Second, patients may experience intolerance to metronidazole due to metallic taste, significant nausea and loss of appetite, which can lead to further weight loss as patients with CDI experience considerable weight loss [5, 6]. Also, some patients develop irreversible peripheral neuropathy (nerve damage) with long term use of metronidazole [7]. Third, some of the patients with refractory CDI could not afford to continue with oral vancomycin. The cost of oral vancomycin was prohibitive and they were not routinely reimbursed by the public health plan. A 14-day course of oral vancomycin costs $600 and a number of the patients were on this antibiotic for 6 – 18 months at a cost
of $7,200 to 21,600 (personal communication with St. Joseph’s Healthcare Outpatient pharmacist). The cost of one FMT is approximately $100, which includes the laboratory screening test and the nurse’s administration time.

FMT serves to reconstitute the altered colonic flora, in contradistinction to antibiotics, which further disrupt the microbiome. There has been a growing acceptance of this innovative therapy by both patients and healthcare providers, [4]. A review of the literature reveals a cumulative clinical success rate of 89% (98/110 patients) in 14 small, uncontrolled studies that utilize FMT in laboratory confirmed CDI [4-21]. These reports are limited by heterogeneity in delivery modalities, publication bias and small sample sizes, [12, 13]. There have been three methods of providing FMT: nasogastric tube, colonoscopy or by retention enema, [11]. Currently, there is no consensus on the ideal delivery modality for FMT. Most published reports have evaluated FMT via nasogastric tube or colonoscopy [14, 18], which are both cumbersome and costly. Retention enema is economical, minimally invasive and can be utilized in both the hospital and outpatient care settings. Additionally, this modality averts the risk of serious complications such as gastrointestinal perforation, which has been reported with nasogastric intubation and colonoscopy, [11, 33]. Despite its advantages there is a paucity of data on FMT via enema. Given the low cost, no reported adverse outcomes and the potential for high cure rates, FMT using retention enema needs to be further explored and scientifically substantiated with a randomized clinical trial. Also, a study using Bacteroides species as a marker of the status of the normal microbiota showed that oral vancomycin may continue to suppress the growth of B. fragilis group even at 4 weeks of completion of the antibiotic [34]. Our preliminary stool metagenomics of patients cured of CDI also showed that the presence of Bacteroidetes was one of the indicators of re-establishment of anaerobic microbial flora and prevented further recurrence of CDI [35].

1.3.1 Preliminary data

To date, approximately 90 FMT’s using retention enema have been performed at St. Joseph’s Healthcare Hamilton (SJHH) which is affiliated with McMaster University. Both fresh and frozen and thawed FMT have been used in patients with apparent similar efficacy, however, a formal evaluation of efficacy between the two groups has not been performed. The following summarizes a formal evaluation of the first twenty-seven patients who underwent FMT via retention enema at SJHH for recurrent CDI [37]. All medical records were independently reviewed by the members of the current team. The inclusion criteria for this report consisted of the following: laboratory-confirmed presence of C. difficile toxin using enzyme immunoassay and no other etiology for diarrhea; refractory CDI defined as ongoing diarrhea despite antimicrobial treatment or recurrent CDI defined as complete resolution of diarrhea for at least 3 days followed by new onset of diarrhea; and complete clinical and laboratory documentation by chart or telephone review.

Three healthy healthcare workers and family members of the patients volunteered as donors. They underwent complete medical history and were screened for transmissible pathogens: human immunodeficiency virus (HIV) 1/2, viral hepatitis B, C, syphilis, human T-lymphotrophic virus (HTLV) 1/II and Helicobacter pylori. Their stool sample was also screened for ova and protozoan, enteric bacterial pathogens and C. difficile
toxin. FMT donors did not take antibiotics for four months prior to the donation of stools and they were not on any medications. The patients discontinued CDI therapy at least twenty-four hours prior to FMT. Approximately 50 g of fresh stool (collected within 3 hours prior to the procedure) was emulsified in 300 mL of commercially-bottled water. The sample was then left to settle for 5–10 minutes and the supernatant component of the sample was aspirated into a 60 ccc syringe. The specimen was refrigerated at 2-5 degrees Celsius until the time of FMT. Patients were instructed to hold the infusate for at least thirty minutes and to remain supine to minimize the urge to defecate. If diarrhea, consistent with CDI recurred, the procedure was repeated until the patient was cured. Instructions were also given to patients regarding decontamination of bathroom facilities and use of antibiotics in order to prevent re-infection. The demographic characteristics and the disease burden of the 27-patient cohort are summarized in Table 2 [37]. The mean patient age was 69.4 years (range 26–87) with 14 (51.9%) male subjects and 22 (81.5%) in-patients. Patients had received antibiotics for a mean length of 10.8 days prior to CDI for a number of primary infections, most commonly cellulitis (n = 5) and urinary tract infections (n = 5). Patients had a mean duration of diarrhea of 152.6 days (range 22–672), with a maximum of 11.2 stools per day (range 6–25). Fever and abdominal pain were documented in 29.6% and 74.1% of patients, respectively.

The CDI treatment of each patient and the clinical outcome following FMT are as follows. The mean cumulative treatment using metronidazole or vancomycin monotherapy each was 24.9 days and 54.6 days, respectively; 13.6 days of vancomycin taper protocol and 9.9 days of metronidazole and vancomycin combination therapy prior to FMT. One patient was treated with intravenous immunoglobulin and three were treated with probiotics. After FMT, 25 of 27 (92.6%) patients experienced clinical resolution of diarrhea. Of these, 22 (88%) resolved within 24 hours. Five patients underwent a second FMT within 24 hours due to ongoing diarrhea, and three of these patients resolved. Two patients (patient 15 and 27) were deemed treatment failures as they continued to have ongoing diarrhea despite two FMT. There were no relapses or adverse events in the cohorts that successfully underwent FMT with mean follow-up at 427.3 days post-transplant.

### 1.4 Rational for conducting this trial

Determining the efficacy and safety of FMT for CDI through a controlled, comparative clinical trial has important implications for policy making as the results of this study will be instrumental in demonstrating to the scientific and healthcare communities the role of FMT as a treatment modality for recurrent CDI. Curing CDI will restore the health and quality of life not just at the individual patient level but to the healthcare communities as well. Patients with refractory CDI require prolonged hospital admission, which increases the organism burden within the healthcare facilities. This in turn leads to the spread of the infection to other vulnerable patients. If FMT proves to be safe and effective in curing patients with CDI in a well-designed clinical trial, it will reduce the risk of severe complications in each patient and reduce transmission of CDI to other susceptible patients. All these will also reduce healthcare costs. In addition, if the efficacy and safety of frozen and thawed FMT is equivalent to fresh FMT then this treatment will be readily available and consistent across multiple centres in Canada.
1.5 Potential risks to the safety of subjects involved in the trial.

There may be a very rare risk of transmitting potential pathogens through FMT. To minimize the risk, each donor is rigorously screened with questions for any risk behaviours, physically examined for any markers of underlying disease, and tested for blood and stool pathogens. To date, there have not been any reports of significant discomfort or adverse outcomes directly associated with FMT administered by enema.

2. The Proposed Trial

2.1 The proposed design of the study

The design of this research is a prospective, randomized multi-site trial using fresh vs. frozen-and-thawed human biotherapy conducted at St Joseph’s Healthcare Hamilton (SJHH) and Kingston General Hospital. Stratification will be performed according to the following: age (≥65 versus <65); primary onset (community- versus hospital-associated CDI); and the number of recurrent CDI episodes (≥ 2 versus < 2). Further details are provided in section 2.3.

2.2 The planned trial interventions

The study subjects will consist of patients over the age of 18 years who are able to consent and comply with treatment. Subjects will be required to have laboratory or pathology-confirmed diagnosis of recurrent CDI based on the Society for Healthcare Epidemiology of America (SHEA) definition, [26], where recurrence is defined as return of diarrhea and positive stool test after a period of symptom resolution within 8 weeks of the first episode and has received at least a 10-day course of oral vancomycin.

The subjects will be randomly assigned to receive either fresh FMT or frozen and thawed FMT using a specimen prepared from healthy, screened donor(s). Stratified randomization will be performed using a computer-generated list and the allocation will be concealed by using password protected file. For the frozen-and-thawed FMT, the specimens will be kept frozen at -20 °C for up to 30 days with daily temperature monitoring. Thawing of FMT will be done at 5 °C overnight prior to the procedure. Eligible patients who are receiving antibiotic for CDI at the time of screening will discontinue the antibiotic 24 - 48 hours prior to the scheduled FMT. Subjects will undergo retention enema(s) (supernatant of specimen prepared from screened donor) on day 1 and repeat on day 5 through day 8 following randomization if there is recurrence or no improvement of diarrhea by day 4. An unblinded, independent laboratory technologist will prepare the retention enema according to the treatment arm to which the patient is assigned. In situations where the patient requires a second FMT, the technologist will provide the same type of FMT the patient is originally allocated to. The patient and the investigators will be blinded as to the treatment allocation.

The processing and the storage of the fresh and frozen products for the FMT will be standardized using the standard operating procedure. All sites will establish designated
donors based on the donor inclusion/exclusion criteria prior to commencement of the trial at each respective site to ensure efficient process. For subjects requesting to use the specimen from the donor of his/her choice for the FMT then the potential donor(s) will undergo physical examination, complete the donor health questionnaire, consent and undergo screening blood work and their stool samples will be tested for the pathogens described on the consent form. The eligible “relative” donor will collect the stool as per SOP, and submit the freshly collected stool sample at least 48 hours prior to FMT to allow freezing and also in the morning of the scheduled FMT to the laboratory to ensure that the participant receives the allocated FMT and the designated donor’s stool sample. This will also allow maintenance of blinding of the participant and the investigator.

All subjects will be assessed at day 1 and day 10 (+/-4) with medical history (including concurrent medications and recent antibiotic use), physical examination, blood work and stool collection. They will be assessed and a repeat FMT will be performed on day 5 (+3) if there is no improvement of diarrhea by day 4 or recurrence of diarrhea during the follow-up period. The subjects will be requested to record the number and type of bowel movements per 24-hour period using Bristol Stool Chart, presence of abdominal discomfort, intolerance to treatment between day 1 and 12 using the diary provided. In addition, the subjects will be assessed for clinical response, treatment failure and adverse reactions by history, physical examination, complete blood count and chemistry panel will be performed and a stool sample collected and kept frozen at -20 °C at day 10 (+/-4); weeks 5 and 13 for metagenomics and future typing of the strain. Following the second clinic visit, there will be weekly communication for 12 weeks subsequent to the completion of treatment for any evidence of relapse, adverse event and overall health. When there is ongoing or recurrence of diarrhea, the participant(s) will return for medical history, physical examination, complete blood count, chemistry panel and stool for C. difficile toxin by EIA or PCR for tcdB gene. Study procedures are outlined in Appendix 1. For treatment failures or relapse within the study period in either regimen, subjects will be offered repeat FMT(s) or standard antibiotic therapy. Subjects will be requested to complete RAND Health Survey and collect stool at day 1 (baseline), day 10, weeks 5, 13 following the last FMT.

The research coordinator and research assistant(s) will be responsible for coordinating the recruitment, screening of potential subjects using the inclusion/exclusion criteria and reviewing with the PI. The research assistant will randomize the patient using a computer generated code. Blood and stool collection will be done at outpatient laboratories or in clinical research offices for outpatients; for inpatients they will be collected and processed as per inpatient policy. The physical examination will be performed by the PI or a fully trained licensed healthcare provider. The trained research staff will administer the enema, arrange for follow-ups and perform daily and weekly telephone contacts as outlined. All blood work and stool tests results will be reviewed by the investigator to ensure the eligibility of the subject and to monitor the patients’ health. Any abnormal results will be assessed by the investigator to determine if the subject should remain in the study. This will be done electronically in order to ensure that none of the required tests are missed. If the required tests are not done locally, a paper copy of the results will be reviewed by the investigator and filed in the patient’s binder.
The data will be collected by the research staff. The data analyses will be done by the statistician and the statistical research team.

2.3 The proposed methods for protection against sources of bias and allocation of subjects to trial groups

In order to mitigate bias, both the patient and study staff will be blinded to the allocation of the treatment. An un-blinded, independent laboratory technologist will prepare the retention enema according to the treatment arm to which the patient is assigned. Patients will be stratified based on age ≥ 65 versus < 65, number of recurrences as these are risk factors for recurrent CDI. Stratifying the subjects according to age will also provide better proportional sampling. Stratification based on number of recurrences, e.g., 1st recurrence versus ≥ 2 recurrences following treatment for CDI will also reduce potential source of bias since multiple recurrences may indicate infection due to more virulent C. difficile or underlying host factors predisposing to recurrent infections or non-response to treatment. In addition, age > 65 and ≥ 2 recurrences are known risk factors for recurrent CDI. Finally, subjects will be stratified with respect to whether the development of the primary episode of CDI was in the community versus in a hospital.

First 30 subjects will be allocated to fresh or frozen arm using computer generated simple randomization. The subjects will be stratified by age (≥ 65 versus < 65), number of recurrence of CDI (≥ 2 versus < 2), and hospital-acquired vs. community-associated CDI. Subjects will be allocated to a specific treatment arm based on these strata using computer generated block randomization following the analyses of the first 30 subjects and their strata distribution. Participating sites will provide the information needed for stratification to the central site (St. Joseph’s Healthcare) for block randomization and the treatment allocation will be forwarded to the un-blinded research staff.

2.4 The planned inclusion/exclusion criteria for donors and patients

2.4.1 Patient inclusion criteria

1. Age 18 years or older.
2. Able to provide informed consent
3. Laboratory or pathology: confirmed diagnosis of recurrent CDI with symptoms (defined below) within the previous 180 days.
4. ≥ 2 episodes of CDI within 6 months.

Symptoms of CDI include: diarrhea defined as: 3 or more unformed bowel movements in 24 hours for a minimum of 2 days with no other causes for diarrhea

2.4.2 Patient exclusion criteria

1. Planned or actively taking an investigational product for another study.
2. Patients with neutropenia with absolute neutrophil count <0.5 x 10^9/L
3. Evidence of toxic megacolon or gastrointestinal perforation on abdominal x-ray
4. Peripheral white blood cell count > 30.0 x 10^9/L AND temperature > 38.0 °C
5. Active gastroenteritis due to *Salmonella, Shigella, E. coli* 0157H7, *Yersinia* or *Campylobacter*.
6. Presence of colostomy.
7. Unable to tolerate FMT or enema for any reason.
8. Anticipated requirement for systemic antibiotic therapy for more than 7 days.
9. Actively taking *Saccharomyces boulardii*.
10. Severe underlying disease such that the patient is not expected to survive for at least 30 days.
11. Any condition that, in the opinion of the investigator, that the treatment may pose a health risk to the patient.

### 2.4.3 Donor inclusion
1. Able to provide and sign informed consent.
2. Able to complete and sign the donor questionnaire
3. Able to adhere to fecal transplantation stool collection standard operating procedure.

### 2.4.4 Donor exclusion
1. 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 vancomycin resistant Enterococcus (VRE), methicillin resistant *S. aureus* (MRSA), *Salmonella, Shigella, E.coli* O157 H7, *Yersinia* and *Campylobacter*
2. Detection of ova, parasites, *C. difficile* toxin, norovirus, adenovirus, rotavirus on stool examination
3. History of any type of active cancer or autoimmune disease
4. History of risk factors for acquisition of HIV, syphilis, Hepatitis B, Hepatitis C, prion or any neurological disease as determined by the donor questionnaire.
5. History of gastrointestinal comorbidites, e.g., inflammatory bowel disease, irritable bowel syndrome, chronic constipation or diarrhea
6. Receipt of blood transfusion from a country other than Canada in preceding 6 months
7. Antibiotic use or any systemic immunosuppressive agents in the 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 behaviours which in the opinion of the investigator may pose risk to the recipients or the donor

### 2.5 The proposed duration of treatment period
Patients will receive FMT enema on day 1 and repeat on day 5 -8 following randomization if there is no improvement of diarrhea by day 4. To date, none of the
patients who underwent FMT reported discomfort or any other adverse effects associated with enema.

2.6 The proposed frequency and duration of follow-up
All subjects will be assessed at day 1, and day 10 (+/-4) with medical history, physical examination, blood work and stool collection. They will be assessed and a repeat FMT will be performed on day 5 (+3) if there is no improvement of diarrhea by day 4. Patients will also be provided with a diary to record any discomforts and bowel movements and stool type using Bristol Stool Chart. They will be informed to contact the research team whenever they experience > 3 loose and watery bowel movements within 24 hours. A C. difficile infection fact sheet will be provided to the patients with the pertinent information and instruction in case of relapse. When there is ongoing or recurrence of diarrhea, the participant(s) will return for clinical and laboratory evaluation, including C. difficile toxin by enzyme immunoassay (EIA) or by polymerase chain reaction (PCR) targeting tcdB on the stool sample. A one year follow-up phone call will be conducted in order to assess clinical resolution. Study procedures are outlined in Appendix 1.

2.7 The proposed primary and secondary outcome measures
The primary outcomes are:
1. The evaluation of the safety of FMT.
2. Assessment for adverse reactions in each study group by history, physical examination, complete blood count and chemistry panel at baseline, day 12, week 5 and at completion (week 13) of the study period.
3. To determine the cure rate in each group defined by complete clinical cure without CDI recurrence at 13 weeks from the last FMT.

The secondary outcomes are:
1. To determine the relapse rate of clinical and laboratory evidence of CDI within the 13-week study period in subjects treated with fresh FMT in comparison to frozen thawed FMT. The relapse is defined as recurrence of diarrhea and positive C. difficile toxin by EIA or tcdB gene by PCR within the study period following the initial cure.
2. Assessment of the functional health and well-being of patients in each arm using the self-administered RAND questionnaire at day 10, week 5 and week 13

Additional objectives of the study:
1. Collection and analysis of stool metagenomics of subjects and donors at baseline and day 10 and at week 13 for select subjects from each group.
2. Measurement of C. difficile toxin A- antibody (IgG) level of subjects at baseline, day 10 and at week 13.
3. To determine the feasibility of providing standardized FMT in multiple centres across Canada, including community hospitals

2.8 Measurement of outcome at follow-up
To determine clinical response, treatment failure and adverse reaction, the subjects will be requested to record the number of bowel movements per 24-hour period, abdominal discomfort, intolerance to treatment between day 1 and 12. In addition, they will be assessed with medical history, physical examination, complete blood count, chemistry
panel and EIA or PCR on stool for *C. difficile* toxin/gene at day 10; weeks 5 and 13. There will be weekly communication for following the last FMT for any evidence of relapse or adverse events. Whenever there is evidence of recurrent diarrhea during the study period, the participant will return for full history, physical examination, complete blood count, chemistry panel and *C. difficile* toxin by EIA or PCR for *tcdB* gene on the stool.

2.9 Proposed sample size and the justification for the assumptions underlying the power calculations

A non-inferiority trial of fresh (control) versus frozen-and-thawed (treatment) FMT will be conducted in which the success rate will be assumed to be 85% in both arms with a prescribed maximum difference of 15%. Pursuing this at the usual 5% significance level, 80% power and allowing for a 10% attrition rate, the sample size will be 156 patients in total, 78 patients per each arm.

2.10 The planned recruitment rate, period and strategy

Approximately 6 – 8 subjects per week will be recruited and it will take 18 to 24 months to recruit 136 subjects. The PI receives 3 – 4 patient referrals each week with refractory/recurrent CDI, which will equate to 200 patients from her practice alone over a 24-month period. A notice of this trial will be circulated to the physicians in Hamilton, Kingston and to the Southwestern Ontario region which will increase the recruitment rate.

2.11 Centres for the study

Three centres will be involved in this trial: St Joseph’s Health Care Hamilton, Hamilton Health Sciences and Kingston General Hospital.

2.12 The proposed type of statistical and metagenomics analyses

The primary statistical analysis will be the standard binomial (proportion) test for comparing two populations. Other more advanced techniques such as logistic regression, generalized linear models and generalized estimating equations will be used to simultaneously test for treatment differences and covariate parity in the two populations. In addition, with the recurrence data we will also undergo longitudinal survival data analysis using parametric and non-parametric methods. Furthermore, attention to the likely attrition will be addressed using techniques such as the bootstrap, EM-algorithm, and Lasso type estimators to verify robustness.

Principal component analysis will be used for metagenomic analysis.

2.13 The proposed frequency of analyses

The analyses will be conducted following the data collection stage at the end of year 2. An interim analysis will be performed after 30 patients have been recruited to assess safety and ongoing trial feasibility. The study will continue beyond this point as recommended by the independent data safety monitoring committee.
2.14 Planned subgroup analyses
We will perform subgroup analyses of the effect of the intervention in high-risk subjects who are over 65 years of age. In particular, the analyses proposed in 2.12, will be carried out on this sub-population and the 65 years of age and under group in order to obtain clinical information in terms of recurrence and clinical response by age category. Further subgroup analyses will be performed according to number of recurrences, inpatient versus outpatient and the strain type, NAP1/BI/027 versus non-NAP 1.

2.15 Pilot study using this design
We have conducted a retrospective study for FMT in CDI from which this trial was designed[36]. In addition, the results of the metagenomics study has been reported in [35] showing a redistribution of Bacteroidetes and Firmicutes phyla following FMT. This is explained in greater detail in [36].

2.16 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 DMC members are as follows:

- Fiona Smaill MD (Chair of DMC)- Medical Microbiologist and Infectious Diseases Physician Chair, Department of Pathology and Molecular Medicine, McMaster University
- Shariq Haider, MD- Infectious Diseases Physician Department of Medicine, McMaster University
- Keith Tsoi, MD – Gastroenterology, Department of Medicine, McMaster University
- Gerald Evans, MD - Chair, Division of Infectious Diseases & Associate Professor, Department of Medicine, Queens University

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/attribution 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.

3 Trial Organization

3.1 Investigators
Christine Lee, Principal Investigator
Elaine Petrof
Mark Ropeleski
Marek Smieja
Theodore Steiner
3.2 Data Management, Treatment Allocation and Analysis Centre
Research Office
50 Charlton Ave, L424-2
Hamilton, Ontario
L8N4A6
905.522.1155 x 34982

3.3 Trial Training
Prior to participant recruitment sites are trained and provided with manufacturing and handling SOPs, by teleconference. Sites are then trained in FMT preparation by real time video conference. Copies of delegation logs documenting site staff responsibilities and training dates will be retained at St. Joseph’s Healthcare Hamilton.

3.4 Preparation Locations for FMT
FMT will be prepared at each centre’s designated microbiology laboratory with daily temperature monitoring of the laboratory, refrigerator and freezer, in which the FMT will be prepared and stored.
References


[23] RAND Health SF-36 Quality of Life Survey.


[26] Cohen SH, Gerding DN, Johnson S, Kelly CP, MD, Loo VG, McDonald LC, Pepin J, Wilcox MH. *Clinical Practice Guidelines for Clostridium difficile Infection in Adults: 2010 Update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA).* Infection Control and Hospital Epidemiology 2010;31(5):431-55.


[28] [http://patientsafetyontario.net/Reporting/En/ViewReports.aspx?rpt=1](http://patientsafetyontario.net/Reporting/En/ViewReports.aspx?rpt=1)


Patients consented and randomized
Baseline blood and stool test

**Fresh HBT Arm**
- **Day 1**
  - Physical examination
  - Blood work and stool
  - Completion of Health Survey
  - HBT Enema

**Frozen-and-Thawed HBT Arm**
- **Day 5 - 8**
  - If recurrent/ongoing diarrhea repeat
  - HBT Enema

**Day 10 (+/-4)**
- Physical examination
- Blood work and stool
- Completion of Health Survey

**Week 5**
- Blood work and stool
- Completion of Health Survey

**Week 13**
- Blood work and stool
- Completion of Health Survey

**One Year**
- Stool Collection
- Completion of Health Survey