

A prospective, multi-center, randomized trial of fecal microbiota transplantation (FMT) delivered by capsule vs colonoscopy in the management of recurrent *Clostridium difficile* infection (CDI)

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## List of Abbreviations and Acronyms

|        |  |
|--------|--|
| BA     | bile acid  |
| BSC    | biological safety cabinet  |
| C&S    | culture and sensitivity  |
| CDI    | <i>Clostridium difficile</i> infection                                       |
| CEGIIR | Center of Excellence for Gastrointestinal Inflammation and Immunity Research |
| CRP    | C reactive protein   |
| ER     | Emergency Room   |
| FMT    | fecal microbiota transplantation   |
| HAV    | hepatitis A virus  |
| HBV    | hepatitis B virus  |
| HBT    | human biotherapy   |
| HCV    | hepatitis C virus  |
| Hgb    | hemoglobin   |
| HIV    | human immunodeficiency virus   |
| HTLV   | human T lymphotropic virus   |
| IBD    | inflammatory bowel disease   |
| IBS    | irritable bowel syndrome   |
| LFT    | liver function test  |
| O&P    | ovum and parasite  |
| PLT    | platelet   |
| RCDI   | recurrent <i>Clostridium difficile</i> infection                             |
| UAH    | University of Alberta Hospital   |
| WBC    | white blood cell   |

## Study Synopsis

|  |  |
|--|--|
| Title  | A prospective, multi-center, non inferiority, randomized trial of fecal microbiota transplantation (FMT) delivered by capsule vs colonoscopy in the management of recurrent Clostridium difficile infection (CDI)  |
| Investigational Product and Indication for Use | FMT for recurrent CDI  |
| Study Purpose                                  | To compare efficacy and safety of FMT delivered by capsule vs colonoscopy in the treatment of recurrent CDI  |
| Study Design                                   | Prospective, multi-center, randomized study with 1:1 (capsule: colonoscopy) ratio  |
| Study Population                               | Patients with at least 3 episodes of CDI within 9 months   |
| Sample Size                                    | 116  |
| Dosing   | FMT by capsule: 40 capsules<br>FMT by colonoscopy: 360 cc of filtered fecal slurry   |
| Masking  | None   |
| Study Outcomes                                 | <p><b>Primary outcomes</b></p> <ul style="list-style-type: none"> <li>a) Proportion of patients without recurrent CDI in each arm at week 12.</li> </ul> <p><b>Secondary outcomes</b></p> <ul style="list-style-type: none"> <li>a) All serious adverse events up to and including week 12. A serious adverse event is any event which results in any of the following: <ul style="list-style-type: none"> <li>i) Death</li> <li>ii) Colonic perforation</li> <li>iii) Proven infection related to FMT</li> </ul> </li> <li>b) Minor adverse events up to and including week 12, including: <ul style="list-style-type: none"> <li>i) Nausea</li> <li>ii) Vomiting</li> <li>iii) Abdominal pain</li> <li>iv) Fevers</li> <li>v) Technical difficulty retaining FMT slurry or swallowing capsules</li> </ul> </li> <li>c) Mortality rate directly attributable to CDI at week 12 in each arm.</li> <li>d) Patient satisfaction and preference with mode of delivery (capsule vs colonoscopy)</li> <li>e) Changes in quality of life pre and post FMT.</li> </ul> <p><b>Exploratory outcomes</b></p> <ul style="list-style-type: none"> <li>a) Changes in intestinal microbiome pre and post FMT in serial stool samples up to 48</li> </ul> |

|                    |   |
|--------------------|---|
|                    | <p>weeks.</p> <ul style="list-style-type: none"> <li>b) Changes in urine metabolomics pre and post FMT in serial urine samples.</li> <li>c) Medical costs for RCDI treatment, including drugs, emergency room visits, physician visits, and hospitalization.</li> <li>d) Costs associated with FMT delivered by capsule and colonoscopy.</li> <li>e) IBD flare post FMT</li> <li>f) Changes in bile acid composition pre and post FMT in serial stool samples.</li> </ul>   |
| Inclusion Criteria | <ol style="list-style-type: none"> <li>1. Age <math>\geq 18</math> and <math>\leq 90</math> years at the time of Screening.</li> <li>2. Diagnosis of at least 3 episodes of recurrent CDI, with each episode defined as presence of diarrhea (<math>\geq 3</math> unformed stools/24 hours) associate with positive stool <i>Clostridium difficile</i> toxin, occurring within 3 months of each other.</li> <li>3. CDI infection under symptomatic control with <math>\leq 3</math> loose/unformed BM's per 24 h period for at least 2 consecutive days before procedure.</li> <li>4. Those with ability to provide informed consent.</li> <li>5. Females of childbearing potential who are sexually active with a nonsterilized male partner must use at least one method of effective contraception and must agree to continue using such precautions for the duration of the trial.</li> <li>6. Nonsterilized males who are sexually active with a female partner of childbearing potential must use at least one method of effective contraception and must not donate or bank sperm for fertilization purpose during the trial.</li> </ol> |
| Exclusion Criteria | <ol style="list-style-type: none"> <li>1. Those with complicated CDI, defined as WBC <math>&gt;35</math> or <math>&lt;0.5 \times 10^9/L</math>, significant abdominal pain and distension with evidence of toxic megacolon or pseudomembranous colitis, hypotension defined as systolic blood pressure <math>&lt; 90</math> mmHg unresponsive to fluid resuscitation, end organ failure, or requiring intensive care unit admission.</li> <li>2. Those with chronic diarrheal illness, such</li> </ol>  |

|                |  |
|----------------|--|
|                | <p>as irritable bowel syndrome or inflammatory bowel disease unless they are in remission for at least 3 months prior to enrollment.</p> <ol style="list-style-type: none"> <li>3. Those taking or planning to take an investigational drug within 3 months of enrollment.</li> <li>4. Those taking chemotherapy or radiation treatment.</li> <li>5. Those with oropharyngeal or significant esophageal dysphagia</li> <li>6. Those with ileus or small bowel obstruction.</li> <li>7. Those with colostomy or ileostomy.</li> <li>8. Those with subtotal colectomy.</li> <li>9. Those who are pregnant or plan to be pregnant within 3 months of enrollment.</li> <li>10. Those who are breastfeeding or plan to breast feed during the trial.</li> <li>11. Those with an active infection requiring antibiotic therapy.</li> <li>12. Those who cannot discontinue <i>Saccharomyces boulardii</i> or another probiotic.</li> <li>13. Those with life expectancy &lt; 3 months.</li> </ol> |
| Study Duration | 48 weeks   |
| Study Sponsor  | <p>Dina Kao<br/>University of Alberta<br/>Edmonton, Alberta</p>  |

## 1.0 Introduction

*Clostridium difficile* infection (CDI) is one of the most common hospital acquired infections in the developed countries, with increasing incidence, severity and mortality over the last decade linked to the emergence of NAP1/BI/027 strain. CDI outbreaks have been reported not only in hospitals but also in long term care facilities. Hospital acquired CDI increases healthcare cost by 4-fold compared to matched hospitalization, which translates into additional cost of \$1 billion dollars annually in the United States, and \$100 million dollars annually in Canada.<sup>1, 2</sup>

Following a course of standard antibiotic therapy for CDI, approximately 20-30% of patients will experience a recurrence within 8 weeks of symptom resolution. The risk of recurrence continues to rise with each subsequent episode, approaching at least 60% after the 3<sup>rd</sup> episode. Managing these patients with RCDI is a major clinical challenge, as there is currently no approved therapy which is highly effective, resulting in increased health care burden and patient suffering, and potentially contributing to outbreaks in hospitals and nursing homes.

Fecal microbiota transplantation (FMT), which aims to restore intestinal microbiota and colonization resistance, has emerged as the most effective therapy for recurrent CDI, although the long term safety is still not known.<sup>3-5</sup> Four systematic reviews of case series published to date showed FMT to be highly effective, with an overall cure rate of at least 80%, depending on the route of delivery and volume of stool infused. The efficacy was higher (over 90%) when FMT was infused by the lower route and when the volume of infusate was higher when compared to the upper route (80%) and lower volume of stool slurry.<sup>6-9</sup> Furthermore, no significant adverse events attributed to FMT have been reported in the literature, with over 1000 cases to date. The first randomized controlled trial in 2013 compared FMT by duodenal infusion to oral vancomycin and found FMT to be superior to vancomycin in eradicating recurrent CDI (81% vs 31%,  $p < 0.001$ ) after one infusion, and the success rate was further increased to 93.8% after the second infusion.<sup>10</sup> Transient abdominal pain, bloating, belching, diarrhea and constipation are potential minor side effects. A recent multi-center case series of 61 adult and 5 pediatric patients even found FMT to be safe in immunocompromised individuals.<sup>11</sup>

There is also evidence suggesting that both fresh and frozen-and-thawed FMT offer similar efficacy in RCDI cure rate.<sup>12</sup> In addition, there is data supporting the use of volunteer or universal donors since patient directed stool donors do not confer any advantage in treatment efficacy.<sup>7, 13</sup> Therefore, standardized stool processing of universal donors in the laboratory makes it possible to bank frozen processed fecal slurry which is ready to use when required, which significantly simplifies the practical aspects of FMT without loss of efficacy in clearing recurrent CDI.<sup>13</sup> However, there is no consensus on how to select and screen a stool donor, although most experts agree on a minimal set of exclusion criteria based on clinical history, gastrointestinal comorbidities, systematic medical conditions, medication use, and laboratory parameters.<sup>13, 14</sup>

A recent study examined the cost effectiveness of competing strategies for RCDI using 4 treatment strategies for first-line treatment of RCDI which included metronidazole, vancomycin, fidaxomicin and FMT, and modeled up to 2 additional recurrences following the initial episode, with willingness-to-pay threshold set at \$50,000 per quality-adjusted life-year. It found that FMT delivered via colonoscopy was

the most cost-effective strategy, with an incremental cost-effectiveness ratio of \$17,016 relative to oral vancomycin.<sup>15</sup>

Although highly effective, the precise mechanisms by which FMT cures recurrent CDI remain largely unknown. Emerging data showed that the presence of Bacteroidetes is one of the indicators of re-establishment of anaerobic microbial flora and prevents further RCDI.<sup>16</sup> Animal and human studies also suggest that the obligate anaerobic microbes in the *Firmacutes* and *Bacteroidetes* phyla are involved in colonization resistance, thereby restoring defense against *C. difficile*.<sup>17</sup> Another line of evidence suggests normalization of bile acid metabolism through transplanted fecal bacterial community may also be a potential key mechanism.<sup>18</sup> It is known that gut microbiota is able to alter bile acid composition by deconjugation and dehydroxylation. Weingarden et al was able to demonstrate that while pre-FMT fecal samples in patients with RCDI contained high concentrations of primary bile acids (BA), which have pro-germinant activity for *Clostridium difficile*, post-FMT fecal samples from these patients contained mostly secondary BA, which have an inhibitory effect on *Clostridium difficile*.<sup>18</sup> Furthermore, the changes in the bile acid composition can in turn affect intestinal microbial community, since some intestinal bacteria (eg. *Bacteroides wadsworthia*, *E coli* and *Listeria*) are BA tolerant and their growth may even be favored in the presence of BAs, which conversely can suppress other symbiotic commensals. However, it is not clear which bacteria are critical in the pathways of bile acid metabolism.

### 1.1 Rationale for study

Delivery of FMT by upper route, including gastroscopy, nasogastric/ nasojejunal tube, and lower route, including retention enema, sigmoidoscopy, or colonoscopy have all been utilized successfully.<sup>5, 19-21</sup> A small pilot study randomized 20 patients with RCDI to frozen-and-thawed FMT by either nasogastric tube (NGT) or colonoscopy, and achieved cure in 6/10 in NGT group and 8/10 in colonoscopy group (P= 0.628).<sup>22</sup> However, there is no consensus on the ideal mode for FMT delivery. All these means of FMT administration have potential risks of patient discomfort, perforation or aspiration, either directly or indirectly related to procedures. Furthermore, endoscopic delivery requires significant health care utilization and associated cost. Therefore, it is extremely desirable if FMT can be infused by a non invasive modality, which would significantly reduce patient discomfort, procedure related risks and health care costs, while offering similar efficacy to colonoscopic delivery in the range of 90%.

### 1.2 Preliminary data

Dr. Louie at the University of Calgary has developed an innovative way of delivering FMT by oral capsules, and has treated 40 patients over a 3 year period with a success rate of 96%. Using either a healthy related donor or unrelated healthy volunteer donors, fresh stools obtained within 48 h of processing have been used as a starting material. After sedimentation by high speed centrifugation, fecal microbes are resuspended to allow pipetting into #1 gelatin capsules, over encapsulated twice with #0 and #00 capsules, followed by immediate ingestion. Several patients have received FMT via flash frozen capsules stored at -70°C for up to 2 months, and have also been cured of recurrent CDI. Serial fecal analyses of these patients had shown near instantaneous recovery of the main components of the microbiome 48 and 72 hours post FMT, and the microbial compositions remained stable 1 week, 1 month, 3 months and 6 months post FMT.



More importantly, no serious adverse events have been observed. No instances of vomiting have been recorded, although less than 10% of patients experienced transient nausea. Loose bowel motions have been noted during the first 24 hours after FMT by less than 10% of patients.

## **2.0 Objectives**

- a) Primary objective: to compare the efficacy of FMT delivered by capsule vs colonoscopy at week 12
- b) Secondary objectives:
  - i) To determine mortality rate directly attributable to recurrent CDI in each arm
  - ii) To determine serious and minor adverse events in each arm
  - iii) To determine patient satisfaction and preference in FMT delivery (capsule vs colonoscopy).
  - iv) To determine changes in quality of life following FMT as measured by RAND 36 questionnaire.
- c) Exploratory objectives:
  - i) To determine the changes in fecal microbial composition associated with cure by stool metagenomics.
  - ii) To determine the rate of IBD flares
  - iii) To compare the fecal microbial composition post FMT between capsule and colonoscopic delivery by metagenomics up to 48 weeks.
  - iv) To evaluate medical cost differentials between those who receive timely FMT (ie after 3 episodes) vs delayed FMT (> 4 episodes)
  - v) To compare the cost between FMT delivery by capsule vs colonoscopy
  - vi) To determine the durability of transplanted intestinal microbiome in the recipients over time.
  - vii) To identify microbial or metabolic profile predictive of treatment success and failure.
  - viii) To determine long term safety of FMT up to 48 weeks.
  - ix) To examine changes in serum and stool bile acid composition before and after FMT.

## **3.0 Methods**

### **3.1 Study design**

This prospective, multi center, non inferiority, randomized trial will enroll 116 patients with recurrent CDI to receive FMT by oral capsule or colonoscopy (1:1).

### **3.2 Study setting and patient recruitment**

Patients who are referred to the Divisions of Infectious Diseases and Gastroenterology at the University of Calgary and University of Alberta for treatment of recurrent CDI will be approached for potential recruitment. These patients will be screened and assessed at the at the Zeidler Ledcor Center, University of Alberta Hospital (UAH) or Royal Alexandra Hospital in Edmonton, or Foothills Hospital, Peter Lougheed Center, Rockyview General Hospital, and the Microbial Health Clinic ID/GI clinic at the Foothills Hospital in Calgary.

### 3.3 Study population

Participants with the following inclusion criteria and none of the exclusion criteria will be enrolled in the study.

#### 3.3.1 Inclusion criteria

- a) Age  $\geq 18$  and  $\leq 90$  years at the time of screening.
- b) Diagnosis of at least 3 episodes of recurrent CDI, with each episode defined by
  - i) presence of diarrhea ( $\geq 3$  unformed stools/24 hours)
  - ii) positive stool *Clostridium difficile* toxin
  - iii) each episode occurring within 3 months of one another
  - iv) recurrence of diarrhea after a period of symptom resolution following at least a 10 d course of metronidazole, vancomycin or fidaxomicin
- c) CDI infection under symptomatic control with  $\leq 3$  loose/unformed BMs per 24h period for at least 2 consecutive days before the procedure
- d) Those with ability to provide informed consent.
- e) Females of childbearing potential who are sexually active with a nonsterilized male partner must use at least one method of effective contraception and must agree to continue using such precautions for the duration of the trial.
- f) Nonsterilized males who are sexually active with a female partner of childbearing potential must use at least one method of effective contraception and must not donate or bank sperm for fertilization purpose during the trial.

#### 3.3.2 Exclusion criteria

- a) Those with complicated CDI, defined as WBC  $>35$  or  $<0.5 \times 10^9/L$ , significant abdominal pain and distension with evidence of toxic megacolon or pseudomembranous colitis, hypotension defined as systolic blood pressure  $< 90$  mmHg unresponsive to fluid resuscitation, end organ failure, or requiring intensive care unit admission
- b) Those with chronic diarrheal illness, such as irritable bowel syndrome or inflammatory bowel disease, unless the condition is under control or in remission 3 months prior to enrollment.
- c) Those taking or planning to take an investigational drug within 3 months of enrollment.
- d) Those taking chemotherapy or radiation treatment.
- e) Those with oropharyngeal or significant esophageal dysphagia so that they are unable to swallow capsules.
- f) Those with ileus or small bowel obstruction.
- g) Those with colostomy or ileostomy.
- h) Those with subtotal colectomy.
- i) Those who are pregnant or plan to be pregnant within 3 months of the study.
- j) Those who are breastfeeding or plan to breast feed during the trial.
- k) Those with an active infection requiring antibiotic therapy during study period.
- l) Those who cannot discontinue *Saccharomyces boulardii* or another probiotic
- m) Those with life expectancy  $< 3$  months.

### **3.4 Stool donor selection and testing**

Four universal stool donors registered with the Edmonton FMT program and three universal stool donors registered with the Calgary FMT program will provide the starting material, which is raw stool. These donors work in the healthcare related fields, and do not receive financial compensation for stool donation. Donor inclusion, exclusion criteria, screening and testing are outlined in detail in appendix A. They are rescreened every 4 months. 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.

#### **3.4.1 stool collection and processing**

Each donor will provide a fresh stool specimen, weighing approximately 100g, as per donor stool collection SOP. The stool specimen is stored at 4-8°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 Appendix C1 for colonoscopic delivery and C2 for capsule delivery.

### **3.5 Trial intervention and follow up**

This study will randomize 116 patients with mild to moderate recurrent CDI in a 1:1 ratio and stratified by age ( $\geq 65$  vs  $< 65$  years), presence or absence of immunosuppression as well as city (ie Calgary vs Edmonton) to either FMT delivered by capsules or colonoscopy.

Screening visit: baseline characteristics including age, gender, duration of RCDI, previous CDI therapy, medical history and medication use will be collected. Blood work including CBC, electrolytes, creatinine, AST, ALT, ALP, albumin, C-reactive protein, INR, HIV, and viral hepatitis serology will be drawn at screening. Urine, stool and serum samples will also be collected.

Following randomization: All patients will have been treated with a course of vancomycin at least for 10 days at the dosage of 125 mg PO four times a day until resolution of diarrhea, and will need to be maintained at vancomycin at 125 mg PO twice a day. Vancomycin will be discontinued 24 hours prior to FMT.

Patients who are randomized to colonoscopy-delivered FMT will receive 360 ml of frozen-and-thawed fecal slurry, infused entirely in the cecum. During the colonoscopy insertion phase, 2 colonic biopsies will be taken from cecum, transverse colon, descending colon, sigmoid colon and rectum.

Patients who are randomized to FMT by capsule will receive 40 frozen FMT-containing capsules in the lab. Written instructions will be provided. No colonic biopsies will be taken.

Follow-up: All patients will be seen in the clinic 1 week (+/- 2 days) after FMT. A telephone follow up will be conducted by the study nurse 2 weeks (+/- 2 days) after FMT. Clinic follow-up will then occur at weeks 4 (+/- 2 days) and 12, 24 and 48 weeks (+/- 7 days) post FMT. Urine and stool samples will be collected at baseline, and at each follow up visit. At each follow-up, the stool frequency diary will be reviewed.

The RAND 36 questionnaire will be administered at screening visit and again at week 4. The patient satisfaction and preference questionnaire will be administered at screening and at week 1.

### **3.6 CDI recurrence after FMT**

If there is recurrence of diarrhea, patients are instructed to call the study nurse and submit a stool sample for *C. difficile* toxin testing. They will be evaluated in the clinic for potential CDI recurrence. Should *C. difficile* toxin becomes positive, they will be treated again with vancomycin at 125 mg PO four times a day for at least 10 days, till resolution of diarrhea, followed by 125 mg PO bid until they can be offered a second FMT.

Following resolution of diarrhea, the subject will be given a second FMT by colonoscopy from the same stool donor if they are in the colonoscopy group. Vancomycin will be discontinued 24 hours prior to FMT. For oral capsule recipients, the patient will be similarly evaluated and treated, with vancomycin and be given FMT by capsule from the same donor.

### **3.7 Subject withdraw or termination**

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

- a) Withdrawal of consent or lost to follow-up. The subject is free to withdraw from the trial without prejudice to further medical care
- b) Pregnancy
- c) Need for urgent colectomy

### **3.8 Outcome measures**

#### **3.8.1 Primary outcomes**

Proportion of patients without recurrent CDI 12 weeks post FMT in each group.

#### **3.8.2 Secondary outcomes**

- a) Mortality rate directly attributable to CDI at week 12 in each arm.
- b) Patient satisfaction and preference with mode of delivery (capsule vs colonoscopy)
- c) Changes in quality of life pre and 4 weeks post FMT assessed by SF-36.
- d) All serious adverse events up to and including week 12. A serious adverse event is any event which results in any of the following:
  - i. Death
  - ii. Colonic perforation
  - iii. Proven infections related to FMT
- e) Minor adverse events up to and including week 12, including:
  - i) Nausea
  - ii) Vomiting
  - iii) Abdominal pain
  - iv) Fevers
  - v) Technical difficulty retaining FMT or swallowing capsules

#### **3.8.3 Exploratory outcomes**

- a) Changes in intestinal microbiome as analyzed by metagenomics pre and post FMT in serial stool samples up to week 48.
- b) IBD flare
- c) Changes in urine metabolomics pre and post FMT in serial urine samples up to week 48.
- d) Costs associated with FMT delivered by capsule and colonoscopy.
- e) Medical costs for RCDI treatment, including antibiotics, emergency room visits, and hospitalization
- g) Changes in bile acid composition pre and post FMT in serial serum and stool samples.
- h) Determine the presence of mucosal-associated microbes in intestinal biopsies.
- i) Changes in short chain fatty acid composition pre and post FMT in serial serum and stool samples.

### **3.9 Statistical consideration and analysis**

#### **3.9.1 Sample size calculation**

A non-inferiority trial of capsule versus colonoscopy delivered FMT will be conducted where the difference of success rate between the two modalities is prescribed at 15%, with the success rate of colonoscopy estimated at 91.2% based on the systematic review by Kassam et al.<sup>7</sup> Pursing this at the usual 5% significance level, 80% power and allowing for a 15% attrition rate will require 116 patients in total, with 58 in each arm.

#### **3.9.2 Stool microbial analysis**

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°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. Sequencing parameters consist of paired-end 250bp dual index sequencing chemistry using a MiSeq Reagent Kit-v2 (500 cycle) and the FASTQ Only workflow. Produced FASTQ files are then subjected to bioinformatics analysis. The raw FASTQ files are first filtered for adapter sequences and end trimmed of bases with quality less than 15. Reads are then aligned against a set of ribosomal and mitochondrial databases with SOAPalign, with unaligned reads passed to each successive alignment. Unaligned reads are re-aligned to human, bacterial, and viral nucleotide databases, also with SOAPalign. Microbial genes are classified using the web-based MG-RAST (MetaGenomics Rapid Annotation using Subsystem Technology) to provide the metabolic potential of the gut microbiota. Spec1 (<http://vm-lux.embl.de/~mende/spec1/>) will be used to determine species composition in the samples, and HUMAnN (<http://huttenhower.sph.harvard.edu/humann>) for the characterization of microbial pathways in the communities. Community diversity across samples will be assessed through distance metrics such as Bray-Curtis and Morisita-Horn, while alpha-diversity characterization will be conducted with metrics such as the Shannon-Wiener diversity index. Relative abundance data will be transformed [ $\log(X+1)$ ] prior to generation of the resemblance matrix. Hierarchical clustering analysis using Spearman rank correlation with complete linkage and groups identified based on correlations >0.5 will be used.

### **3.9.3 Urine metabolomics analysis**

Urine samples will be obtained in a standard urine collection jar containing sodium azide to prevent bacterial growth, and frozen after collection. Urine samples will be taken in the morning in patients who are fasting in order to avoid the potential effects that time of day and diet may have on the metabolic profile. Urine samples will be prepared for NMR analysis and run on a 4-channel Varian INOVA 600 MHz NMR spectrometer. <sup>1</sup>H-NMR analysis using Chenomx NMR Suite software will allow simultaneous identification of up to 300 small molecules. For those potential metabolites that are not in the Chenomx library, spectral binning on subtracted NMR spectra will be performed to determine whether any other metabolites exist that have not been identified. In cases where an unknown metabolite is found, a combination of <sup>1</sup>H-NMR and Mass Spectrometry can be used to aid in identification. Parametric and/or non-parametric testing will be done using STATA<sup>®</sup> and Matlab<sup>®</sup> software packages. Methods specifically designed for repeated measure longitudinal studies (RM-ANOVA, Linear/Nonlinear multi-level Mixed Models) will be used, with due consideration for correction for multiple comparison using False Discovery Rate calculations. For purposes of uncovering more general cross-sectional multivariate metabolite profiles will be examined using a selection of multivariate regression analyses, including logistic regression, linear discriminate analysis, canonical variate analysis, and partial least squares discriminant analysis. Confidence Intervals and Receiver Operating Characteristic curves will be derived for all predictive statistical models.

### **3.9.4 Stool bile acid composition analysis**

Bile acid composition will be measured in stool samples via high pressure liquid chromatography (HPLC). Once bile acids have been extracted from stool samples, they are diluted at 1:40 ratio with HPLC-grade methanol. Each sample is then placed in a microfuge tube and spun in a microfuge at ~14,000 rpm for 10 minutes to remove particulates. The supernatant is then transferred to Agilent-compatible autosampler vials with spring inserts, capped and stored at -20°C prior to analysis. When ready for analysis, 5-10 µL from each sample are injected into the HPLC machine.

## **4.0 Significance**

Results gained from this trial will allow us to standardize FMT methodology and delivery in order to successfully implement this treatment protocol throughout hospitals across Alberta. The study would also allow us to determine the safest and cost effective way of delivering FMT. Furthermore, it will also allow us to understand the durability of transplanted microbiome and gain insight into the mechanisms of action of FMT.

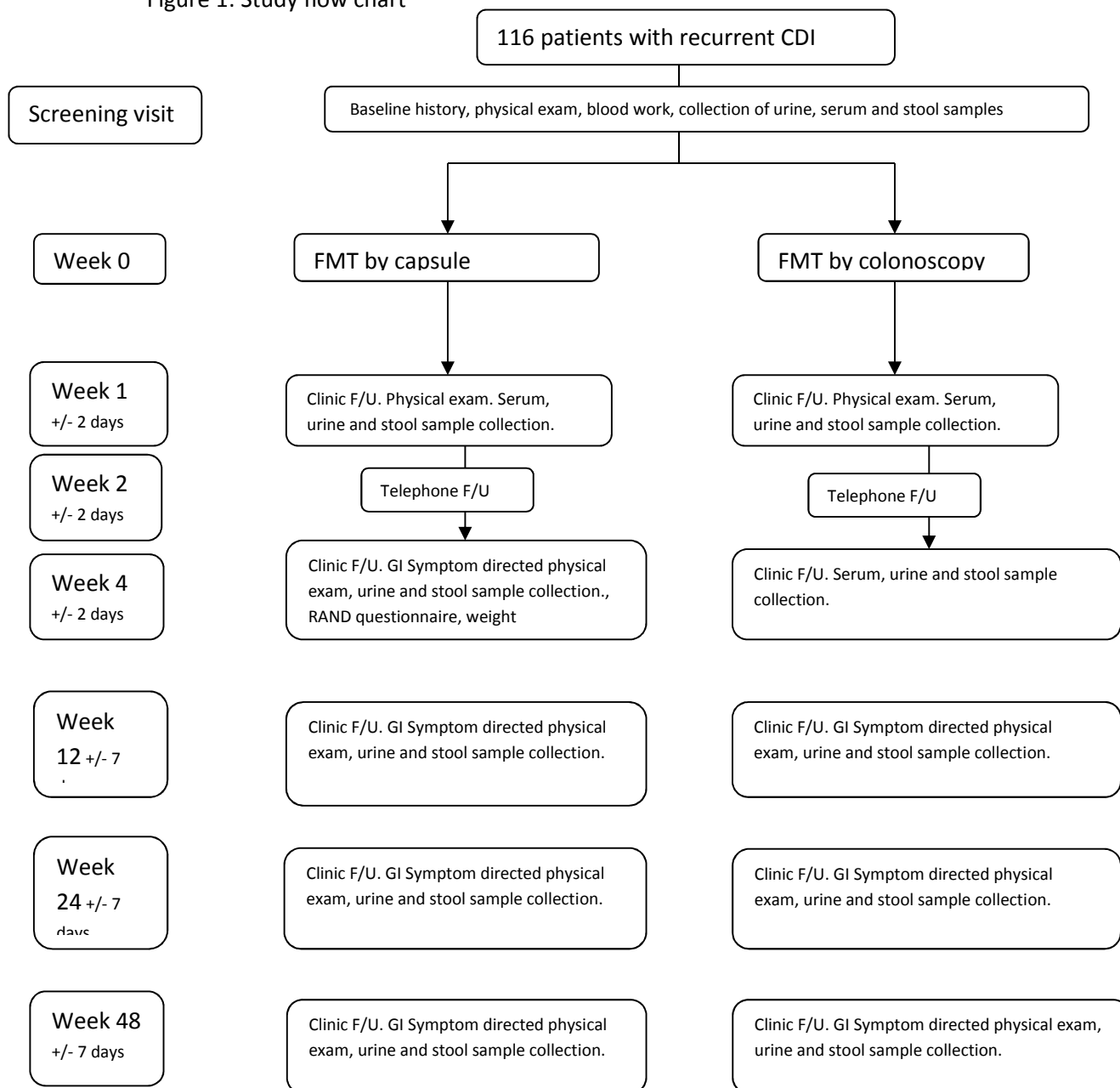
## **5.0 Data safety monitoring**

An independent data safety monitoring board (DSMB), consisting of Dr. Geoff Taylor and Dr. Christina Surawicz has been appointed, and will continue to oversee the trial 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.

The DSMB will review data every 4 months, and then again at the conclusion of enrollment. They will also receive each report of a serious event (SAE), with an investigator assessment of causality/attribution possibly related to the study. They will review and evaluate the results and report to the investigators.

If the DSMB decides that there is a pattern consistent with an important increase in frequency of the occurrence of one or more types of SAEs within one or two of the treatment arms, not reasonably explained by events clearly unrelated to study participation, they will provide a recommendation to the investigators 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 any of the investigators. Recommend that enrollment in the study be halted if: 1) as many as three subjects that received treatment with FMT are reported to experience the same or substantially similar types of SAE and he concluded that such SAEs could be reasonably related, or 2) if any one subject who receives FMT is reported to experience an SAE that had fatal or life-threatening outcome and the DSMB concluded that such SAE could be reasonably related to FMT.

Figure 1: Study flow chart





#### Appendix A: Universal stool donor testing/screening SOP

Since only 5 universal stool donors (3 in Edmonton and 2 in Calgary) will provide the raw material for FMT, we propose that we rescreen them at the start of this trial and then every 4 months 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 80 patients since 2012, and none of the recipients have developed any known infectious complications. All donors work in the healthcare related fields and 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. We propose rescreening these donors every 4 months. We believe this is an adequate rescreening interval, adapted by other centers with a universal donor program, since each donor goes through the donor collection SOP with each stool donation. A rescreening /retesting frequency of less than 4 months is inconvenient, costly (\$1000 per donor screening and testing) and likely of no added benefit. The average incubation period is 28 days for HAV, 120 days for HBV, 45 days for HCV, and up to 3-6 months for HIV (although the antibody may turn positive as soon as 2-8 weeks from the time of exposure). Therefore, we believe that retesting universal donors every 4 months is a reasonable and practical interval.

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.

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 1).
2. Able to complete and sign the stool donor questionnaire (Appendix 2).
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).

Initial blood work and stool testing, which will be repeated every 4 months, as per FDA guidelines

<http://www.fda.gov/BiologicsBloodVaccines/guidanceComplianceRegulatoryInformation/Guidances/Transfusion/ucm073964.htm>, are as follows:

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- lymphotropic virus (HTLV) I/II

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
- MRSA and VRE
- ESBL will not be tested, since these organisms are likely of no clinical relevance. *Vibrio* and *polio* will not be tested in asymptomatic individuals unless there is a known outbreak in the donor community, a highly unlikely possibility, since all donors were born in Canada and reside in Calgary or Edmonton.

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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.

|  |     |    |  |
|--|-----|----|--|
| <b>Day of FMT Procedure/donor Checklist</b>  |     |    |  |
| <b>Donor Name:</b>   |     |    |  |
| <b>Date:</b>   |     |    |  |
|  | Yes | No |  |
| Do you feel well and healthy today?  |     |    |  |
| Have you had any fever, vomiting, diarrhea 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 |     |    |  |

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°C to 8°C 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.

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#### Appendix C1: Stool processing for colonoscopy delivery.

Once received by the processing laboratory, stool will be held in a cold room at 2°C-8°C, for no longer than 30 min, until visual inspection and HBT processing occur.

Each batch of human biotherapy (HBT) is obtained from a single donor, and processed within 8 hours of collection. No pooling of stools will occur. Only one HBT from a single individual will be processed at a time. Each processed batch will contain approximately 100 g of stool mixed with 200 cc of 0.9% normal saline (NS) as a diluent. This will provide approximately 200 cc of filtrate, which will have 20 cc of 100% glycerol added and be frozen at -70°C as a single dose. The thawed dose will be diluted with approximately 160cc of 0.9% NS and a total volume of 360 cc will be administered by colonoscopy as a single dose.

Disinfect the all inner surfaces of the class 2 biological safety cabinet (BSC) following Sterilization SOP (Appendix D) before and after each processing. The total stool inspection and processing time is less than 30 minutes.

The inspection and entire processing takes place within a BSC:

1. Each stool collection is weighed, and only 100 g of stool is retained. The specimen is visually inspected to ensure it contains no urine, mucus or blood. Discard if there is contamination with blood, mucus or urine. Ensure the stools have consistency between Bristol Stool Scale type 2-5 (the most common stool consistency of healthy, asymptomatic individuals, and unlikely to represent an infectious process), otherwise discard. Collect a quality control sample by taking approximately a 1 mL aliquot and storing it in a 1.5 mL eppendorf tube at -70°C.
2. One hundred grams of stool is then placed into a stomacher bag (7"x12", VWR CA89085-572) on one side of the filter mesh, to which 200 cc of 0.9% NS (for irrigation) is added.
3. Gently remove most of the air in the stomacher bag by draping the top of the bag over the heat sealer (Fisher Scientific, 14816237). Alternatively, use a reusable bag clamp (Fisher Scientific, 0100262) instead of heat-sealing to seal the bag for homogenization and filtration.
4. Close the heat sealer (set to 8 heat setting) across the open end of bag and wait for 1 second then release. The bag should be well sealed with no leaks and few air bubbles.
5. Mash and squish the bag with hands until liquid is homogeneous (3 to 5 minutes).
6. Find the side of the bag that has the filtered liquid.
7. Place filtered side up and pinch the outside plastic (using plastic clamping forceps) to create an air pocket in which to slice a hole with a disposable sterile blade. The hole should be 2 cm in diameter.
8. Allow the liquid stool slurry to drain into clean cups (16oz Eco-cup; Real Canadian Wholesale club, 18770800027).
9. Visually inspect the filtrate to ensure it maintains the usual brown color spectrum of stool filtrate.
10. Collect a quality control sample by taking approximately a 1 mL aliquot and storing it in a 1.5 mL eppendorf tube at -70°C.
11. To the slurry (should be approximately 200cc), mix in 20ml of sterile 100% glycerol and transfer the solution to 250ml storage bottles (Fisher Scientific, 02-896-1D) or non-filtered stomacher

- bag (VWR, 11216-900) for freezing at  $-70^{\circ}\text{C}$  for up to 2 months. Leave air space for expansion in either freezing vessel. Label the storage bottle or stomacher bag with the Lot Number created according to the date of specimen (dd/mm/yy) preparation and the 2 initials of donor (first and last names), followed by the time of processing (hour:minute). For example, if processing occurs on May 1, 2014 at 9:15 am and the donor's initials are JB, the Lot Number will be 050114JB915.
12. When required, thaw the bottles or sealed bags at  $2-8^{\circ}\text{C}$  overnight. Check the production date to ensure that the specimen has not expired, ie greater than 2 months of storage.
  13. Once thawed, add approximately 160ml of 0.9% NS to bring the total volume to 360 cc.
  14. The HBT is then aspirated into sterile 60 cc slip-tip syringes (VWR CAB309653; components: polypropylene, polyethylene, synthetic isopropylene), which are then sealed with caps (VWR CAB305819). Each batch should contain approximately 360 mL, or 6 syringes.
  15. Label syringes with the Lot Number listed in step 11, followed by the date and time of frozen-and-thawed processing (dd/mm/yy/hour/minute). For example, if the initial manufacturing occurs on May 1, 2014 at 9:15 am, the donor's initials are JB, and the sample has been frozen till June 2 and thawed out on June 3 when the final processing occurs at 10:30, the Lot Number will be 050114JB915:0602141030.
  16. Discard each batch if not used within 2 months of processing.
  17. None of the supplies other than the heat sealer and weigh scale are reused. Discard all supplies in a biohazard waste bin and sterilize the heat sealer and scale with 10% Bleach for 10 min. Heat sealer and weigh scale are exclusive use for HBT processing.

Once processed, each batch will be kept with an ice pack in the sealed plastic tool box during transport to the endoscopy unit, to maintain temperature of  $2-8^{\circ}\text{C}$ . The transport process from the lab to the University of Alberta Hospital endoscopy unit takes no more than 15 minutes. The transport process from the lab to the Royal Alexandria Hospital takes no more than 30 minutes.

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## Appendix C2: Stool processing for capsule

Once received by the processing laboratory, stool will be held in a cold room at 2°C-8°C, for no longer than 30 min, until visual inspection and human biotherapy (HBT) processing occur.

Each batch of HBT is obtained from a single donor, and processed within 8 hours of collection. No pooling of stools will occur. Only one HBT from a single individual will be processed at a time. Each processed batch will contain approximately 20 capsules.

Disinfect the all inner surfaces of the class 2 biological safety cabinet (BSC) following Sterilization SOP (Appendix D) before and after each processing. The total stool inspection and processing time is 2 hours.

The inspection and entire processing takes place within a BSC:

1. Each stool collection is weighed, and only 100 g of stool is retained. The specimen is visually inspected to ensure it contains no urine, mucus or blood. Discard if there is contamination with blood, mucus or urine. Ensure the stools have consistency between Bristol Stool Scale type 2-5 (the most common stool consistency of healthy, asymptomatic individuals, and unlikely to represent an infectious process), otherwise discard. Collect a quality control sample by taking approximately a 1 mL aliquot and storing it in a 1.5 mL eppendorf tube at -70°C.
2. One hundred grams of stool is then placed into a stomacher bag (7"x12", VWR CA89085-572) on one side of the filter mesh, to which 200 cc of 0.9% NS (for irritation) is added.
3. Gently remove most of the air in the stomacher bag by draping the top of the bag over the heat sealer (Fisher Scientific, 14816237). Alternatively, use a reusable bag clamp (Fisher Scientific, 0100262) instead of heat-sealing to seal the bag for homogenization and filtration.
4. Close the heat sealer (set to 8 heat setting) across the open end of bag and wait for 1 second then release. The bag should be well sealed with no leaks and few air bubbles.
5. Mash and squish the bag with hands until liquid is homogeneous (3 to 5 minutes).
6. Find the side of the bag that has the filtered liquid.
7. Place filtered side up and pinch the outside plastic (using plastic clamping forceps) to create an air pocket in which to slice a hole with a disposable sterile blade. The hole should be 2 cm in diameter.
8. Allow the liquid stool slurry to drain into clean cups (16oz Eco-cup; Real Canadian Wholesale club, 18770800027).
9. Visually inspect the filtrate to ensure it maintains the usual brown color spectrum of stool filtrate.
10. Collect a quality control sample by taking approximately a 1 mL aliquot and storing it in a 1.5 mL eppendorf tube at -70°C.
11. To the slurry (should be approximately 200cc), mix in 40ml of sterile glycerol and transfer the solution to 50 ml conical screw top tubes and spun in a centrifuge at room temperature at 400xg for 20 minutes. The supernatant is decanted into high speed centrifuge vessels and the filtrate containing suspended organisms is centrifuged at 1000xg for 30 minutes at 4-8°C.
12. The supernatant is discarded and the final sediment is mixed to incorporate residual liquid to allow pipetting of the aggregated microbial sludge. The final volume is approximately 10 mL.
13. Using either a microtiter template or by individual hand held half capsules, #1 gelatin capsules

are filled to brim at 0.47 ml, closed, then over encapsulated twice with #0 and #00 capsules and then flash frozen at -55°C on dry ice and stored in a clear plastic bag at -70°C. Approximately 40 capsules are manufactured from one processing originating from a single donor.

14. Label the plastic bag with the Lot Number created according to the date of specimen (dd/mm/yy) preparation and the 2 initials of donor (first and last names), followed by the time of processing (hour:minute). For example, if processing occurs on May 1, 2014 at 9:15 am and the donor's initials are JB, the Lot Number will be 050114JB915.
15. Discard each batch if not used within 2 months of manufacturing.
16. None of the supplies other than the heat sealer and weigh scale are reused. Discard all supplies in a biohazard waste bin and sterilize the heat sealer and scale with 10% bleach for 10 min. Heat sealer and weigh scale are exclusive use for HBT processing.

Frozen capsules are taken out of -70°C freezer and thawed for 5-7 minutes at room temperature prior to ingestion. Check the production date to ensure the capsules have not expired (ie after 2 months of production). Patients are instructed to come to the lab and ingest these capsules in a single sitting over 20-30 minutes. They are also instructed not to eat or drink for 2 hours afterwards to allow capsules sufficient time to enter the small bowel.



## Appendix D: BSC Sterilize

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Purpose: This procedure should be used to clean and sterilize any biological safety cabinet (BSC) before and after use.

Materials required:

- Household vinegar or 2% acetic acid.
- 70% denatured alcohol.
- Paper towels

Procedure:

### Before BSC use

1. Wipe all internal surfaces (including glass) with vinegar until dry.
  - a. Lift work surface and wipe underneath with vinegar as well.
2. Spray all internal surfaces (including glass and underneath work surface) with 70% denatured alcohol.
3. Load any consumables and apparatus that can stand UV light into BSC.
4. Close safety glass on BSC.
5. Turn on UV light for 15 min.
6. After 15 min, turn off UV, turn on blower and lift safety glass.
7. After 15 min begin to work in BSC.

### After BSC use

1. Remove all equipment (after you clean them) and consumables from BSC.
2. Wipe all internal surfaces (including glass) with vinegar until dry.
  - a. Lift work surface and wipe underneath with vinegar as well.
3. Spray all internal surfaces (including glass and underneath work surface) with 70% denatured alcohol.
4. Close safety glass on BSC.
5. Turn on UV light for 15 min.
6. Turn off UV light.

Appendix 1: Donor Questionnaire for human biotherapy/fecal microbiota transplantation

|  | Yes                      | No                       |
|--|--------------------------|--------------------------|
| <b>Are You</b>   |                          |                          |
| 1. Feeling healthy and well today?   | <input type="checkbox"/> | <input type="checkbox"/> |
| 2. Currently taking an antibiotic?   | <input type="checkbox"/> | <input type="checkbox"/> |
| 3. Currently taking any other medication for an infection?   | <input type="checkbox"/> | <input type="checkbox"/> |
| 4. Currently taking any immunosuppressant medication by mouth or injection?  | <input type="checkbox"/> | <input type="checkbox"/> |
| <b>Do you have</b>   |                          |                          |
| 5. History of chronic diarrhea persisting > 10 days?   | <input type="checkbox"/> | <input type="checkbox"/> |
| 6. History of blood in stool not related to hemorrhoid?  | <input type="checkbox"/> | <input type="checkbox"/> |
| 7. History of change in bowel habit, alternating from constipation to diarrhea?  | <input type="checkbox"/> | <input type="checkbox"/> |
| 8. Any type of active cancer that is not cured?  | <input type="checkbox"/> | <input type="checkbox"/> |
| 9. Any active autoimmune diseases?   | <input type="checkbox"/> | <input type="checkbox"/> |
| <b>In the past 12 weeks have you</b>   |                          |                          |
| 10. Had any vaccinations? If yes, please indicate which one(s)   | <input type="checkbox"/> | <input type="checkbox"/> |
| 11. Had contact with someone who had a smallpox vaccination?   | <input type="checkbox"/> | <input type="checkbox"/> |
| 12. taken antibiotics, systemic immunosuppressive or biological agents, systemic antineoplastic agents and exogenous glucocorticoids?<br>If you have, you should not be a stool donor. | <input type="checkbox"/> | <input type="checkbox"/> |
| <b>In the past 16 weeks have you</b>   |                          |                          |
| 13. Lived with a person who has hepatitis A?   | <input type="checkbox"/> | <input type="checkbox"/> |
| 14. If yes, have you received vaccine against hepatitis A?   | <input type="checkbox"/> | <input type="checkbox"/> |
| <b>In the past 12 months have you</b>  |                          |                          |
| 15. Had a blood transfusion?   | <input type="checkbox"/> | <input type="checkbox"/> |
| 16. Had a transplant such as organ, tissue or bone marrow?   | <input type="checkbox"/> | <input type="checkbox"/> |
| 17. Had a graft such as bone or skin?  | <input type="checkbox"/> | <input type="checkbox"/> |
| 18. Come into contact with someone else's blood?   | <input type="checkbox"/> | <input type="checkbox"/> |
| 19. Had an accidental needle-stick?  | <input type="checkbox"/> | <input type="checkbox"/> |
| 20. Had sexual contact with anyone who has HIV/AIDS or has had a positive test for the HIV/AIDS virus?   | <input type="checkbox"/> | <input type="checkbox"/> |
| 21. Had sexual contact with a prostitute or anyone else who takes  | <input type="checkbox"/> | <input type="checkbox"/> |

|  |                          |                          |   |
|--|--------------------------|--------------------------|---|
| money or drugs or other payment for sex?   |                          |                          |   |
| 22. Had sexual contact with anyone who has ever used needles to take drugs or steroids, or anything <u>not</u> prescribed by their doctor? | <input type="checkbox"/> | <input type="checkbox"/> |   |
| 23. Had sexual contact with anyone who has hemophilia or has used clotting factor concentrates?  | <input type="checkbox"/> | <input type="checkbox"/> |   |
| 24. Female donors: Had sexual contact with a male who has ever had sexual contact with another male? (Males: check "I am male")            | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/><br>I am male   |
| 25. Had sexual contact with a person who has hepatitis?  | <input type="checkbox"/> | <input type="checkbox"/> |   |
| 26. Had a tattoo?  | <input type="checkbox"/> | <input type="checkbox"/> |   |
| 27. Had ear or body piercing?  | <input type="checkbox"/> | <input type="checkbox"/> |   |
| 28. Had or been treated for syphilis, gonorrhea or Chlamydia?  | <input type="checkbox"/> | <input type="checkbox"/> |   |
| 29. Been in juvenile detention, lockup, jail, or prison for more than 72 hours?  | <input type="checkbox"/> | <input type="checkbox"/> |   |
| In the past <b>three years</b> have you  |                          |                          |   |
| 30. Been outside the United States or Canada? If yes, list the places(s)   | <input type="checkbox"/> | <input type="checkbox"/> |   |
| From <b>1980 to the present</b> , have you   |                          |                          |   |
| 31. Receive a blood transfusion in the United Kingdom or France? (Review list of countries in UK.)   | <input type="checkbox"/> | <input type="checkbox"/> |   |
| From <b>1977 to the present</b> , have you   |                          |                          |   |
| 32. Receive money, drugs, or other payment for sex?  | <input type="checkbox"/> | <input type="checkbox"/> |   |
| 33. Male donors: had sexual contact with another male, even once? (Females: check "I am female")   | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/><br>I am female |
| Have you <b>EVER</b>   |                          |                          |   |
| 34. Had a positive test for the HIV/AIDS virus?  | <input type="checkbox"/> | <input type="checkbox"/> |   |
| 35. Used needles to take drugs, steroids, or anything <u>not</u> prescribed by your doctor?  | <input type="checkbox"/> | <input type="checkbox"/> |   |
| 36. Used clotting factor concentrates?   | <input type="checkbox"/> | <input type="checkbox"/> |   |
| 37. Had hepatitis?   | <input type="checkbox"/> | <input type="checkbox"/> |   |
| 38. Had malaria?   | <input type="checkbox"/> | <input type="checkbox"/> |   |
| 39. Had Chagas' disease?   | <input type="checkbox"/> | <input type="checkbox"/> |   |
| 40. Had babesiosis?  | <input type="checkbox"/> | <input type="checkbox"/> |   |
| 41. Received a dura mater (or brain covering) graft?   | <input type="checkbox"/> | <input type="checkbox"/> |   |
| 42. Had sexual contact with anyone who was born in or lived in Africa?   | <input type="checkbox"/> | <input type="checkbox"/> |   |
| 43. Have any of your relatives had Creutzfeldt-Jakob disease?  | <input type="checkbox"/> | <input type="checkbox"/> |   |

This information will remain strictly confidential

To the best of my knowledge, the above information is accurate and true.

---

**Donor's Signature:**

**Date:**

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Appendix 2: 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, bilirubin, Hepatitis A, B and C, HIV, HTLV I/II, VDRL/RPR (Syphilis) and stool culture and susceptibility for enteric pathogens, ova and parasites, and Clostridium difficile toxin. I have completed the FMT donor questionnaire as 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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