SPONSOR: JENNIFER GRANT, MD

NORTHSHORE UNIVERSITY HEALTHSYSTEM

847-570-1502 (DESK)
847-657-5764 (FAX)

Fecal Microbiota Transplantation versus Standard Medical Therapy for Initial Treatment of Recurrent Clostridium difficile Infection

Product:	Fecal Microbiota Transplantation (FMT) Treatment Protocol
Proposed Indication:	Recurrent *Clostridium difficile* Infection
Date:	7/11/2016 (Revision 4)

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1. PRODUCT NAME AND APPLICATION NUMBER

Fecal Microbiota Transplantation (FMT) for Relapsing *Clostridium difficile* Infection
Application number: NA

*Note: This application was modeled after IND 15310 from Dr. Colleen Kelly with her permission. There are no major deviations from her protocol.

*Since July 2013, the FDA approved our Investigational New Drug Application. All research procedures will be completed under IND 15668.

2. CHEMICAL NAME AND STRUCTURE

Not applicable.
Biologically active human fecal material

3. PROPOSED INDICATION(S)

Recurrent *Clostridium difficile* infection

4. DOSAGE FORM, ROUTE OF ADMINISTRATION AND DOSING REGIMEN

Dosage form:	Solution of filtered human donor stool (approximately 50 grams) homogenized in sterile normal saline (500 mL).
Route:	Infused into the colon as a retention enema
Dosing:	200-300 mL x 1 dose.

5. BACKGROUND

<u>*Clostridium difficile*</u>

Clostridium difficile infection (CDI) has increased in incidence and severity over the last decade and is associated with poor outcomes including increased morbidity, mortality, and healthcare costs (1-8). Relapse occurs in 15-35% of patients after the first episode of CDI and 45-65% of patients who have one relapse will experience a subsequent relapse (9, 10). Dysbiosis - decreased diversity of the fecal microbiome – is thought to contribute to the high rate of relapse (11). FMT quickly and successfully restores normal intestinal microorganisms of the diseased patient via infusion of a liquid stool preparation from a healthy donor. FMT resulted in disease resolution in ~90% of cases reported in a systematic review and meta-analyses without any significant adverse events noted (12, 13). A cost-effectiveness analysis examining treatments for recurrent CDI demonstrated that administration of FMT via colonoscopy was the most cost effective approach in managing the first recurrence of CDI (14). Colonoscopy was selected as the delivery method of choice due to the highest reported success rate with a single donor infusion. If less expensive FMT methods using standardized frozen fecal preparations delivered via enema or duodenal infusion could be employed with cure rates of 85%, such approaches

could be more cost effective than FMT colonoscopy (14). Successful treatment of recurrent CDI (~90%) using a standardized frozen preparation obtained from a "universal" donor has been described (15, 16). The use of frozen stool preparations obtained from a "universal" donor reduced costs associated with donor screening, allowed for the timely treatment of patients without delays due to identifying and screening a donor and improved the overall aesthetic experience for patients. We aim to compare the effectiveness of FMT using a frozen fecal preparation delivered via enema to standard antibiotic therapy on clinical outcomes in patients with their initial CDI recurrence.

Fecal Microbiota Transplantation (FMT)

Most commonly, CDI is associated with use of antimicrobial agents that are thought to alter the normal bacterial flora of the gastrointestinal tract to permit colonization and subsequent proliferation and toxin elaboration by *C. difficile*[11]. Though the precise mechanisms by which this occurs are still incompletely understood, depletion of physiologic microflora, in particular members of the *Bacteroidetes* and *Firmicutes* phyla are thought to play an important role[13-15]. The specific antimicrobials used to treat CDI also may predispose patients to further relapses through the maintenance of perturbed intestinal flora, and may contribute to the emerging problems of drug resistance. Indeed, both metronidazole and vancomycin have broad-spectrum activity against Gram-negative and Gram-positive organisms respectively.

Fecal microbiota transplantation (FMT) (also known as fecal bacteriotherapy, fecal flora reconstitution or stool transplant) involves administration of fecal material from a healthy individual (donor) into a sick patient (with relapsing CDI) to restore the phylogenetic diversity of normal stool and along with this diversity, the "colonization resistance" that enables normal intestinal flora resist overgrowth of *C. difficile*[16]. Numerous case reports and retrospective case series have demonstrated benefit of FMT in patients with severe or recurrent CDI with cure rates as high as 100% and a mean cure rate of 89% for the approximately 300 cases reported in the world literature[13-31]. Methods used to administer FMT have included fecal suspensions given via nasogastric and nasoduodenal tubes, by upper tract endoscopy (EGD), as retention enemas, or through the colonoscope at the time of colonoscopy[13-30]. Colonoscopic administration has the advantage of direct infusion of the transplant throughout the colon, while also allowing for direct examination of the colonic mucosa, which is often necessary in patients with recurrent CDI to exclude any coexistent colonic pathology, such as IBD.

FMT appears to be safe, with no adverse effects or complications directly attributed to the procedure yet reported in the medical literature. Certainly, the possibility of bias exists in the current literature, as unsuccessful treatment attempts or adverse events related to FMT might be underreported. A well-designed clinical trial would help to determine whether or not FMT is effective in the treatment of recurrent *C. difficile* colitis and would establish safety data. I am presently conducting an NIH-approved open-label randomized controlled trial of donor stool and recipient stool in the treatment of recurrent CDI. Because transmission of infectious agents is a major potential concern, as part of our routine clinical use of FMT, we screen all stool donors for common pathogens (bacteria and parasites), similar to existing FDA guidelines for donors of human cells, tissues, and cellular

and Tissue-Based Products (HCT/Ps). This treatment protocol uses the same process of donor eligibility determination and screening as the study IND for our clinical trial.

Proposed Use and Rationale

Despite overwhelmingly positive anecdotal experience from the growing number of physicians who have performed this procedure and reported success in case-studies and small series, and a larger number of physicians who have resorted to FMT in cases of resistant, recurrent, relapsing CDI, FMT is not yet routinely performed; its acceptance is growing, however. It has now become a therapeutic alternative for resistant cases of CDI and is recommended as such by the CDI Guidelines Committee of the American College of Gastroenterology. As you know, patients suffering from relapsing *C. difficile* are often desperate for a cure and seek guidance from online communities[33-35]. Many are willing to travel great distances to undergo FMT. Some have even resorted to performing home-enemas of donor stool when they are unable to find a physician who is willing or able to perform FMT. Offering this treatment within an FDA approved IND protocol would assure that FMT is performed as safely as possible and allows of necessary monitoring for safety issues.

6. CHEMISTRY, MANUFACTURING AND CONTROLS INFORMATION

Stool is a heterogeneous substance, composed primarily of bacteria and water, but also containing viral and fungal organisms, metabolic products of these organisms, undigested foods, bile, bilirubin, cholesterol, inorganic salts, dead cells, and mucus from the lining of the intestinal wall. The exact composition of human stool varies from person to person and from day to day. The process of donor eligibility determination and screening, stool processing and infusion defines the FMT "product" which is analogous to transplant in that it restores phylogenetic diversity to the recipient's (previously deficient) intestinal microbiota[36]. Carefully devised donor screening and testing guidelines and stool processing protocol (as detailed in this protocol) ensures that FMT is performed safely and effectively.

7. CLINICAL DATA SUMMARY

7.1 Proposed Indication

Recurrent *Clostridium difficile* infection

7.2 Background of the Disease

Clostridium difficile is a common nosocomial pathogen. By far the most common cause of infectious diarrhea in hospitalized patients in North America, there has been an alarming rise in incidence of *C. difficile* infection (CDI) since 2000. This rise has been accompanied by increasing rates of severe disease resulting in colectomy and/or death. Equally as concerning has been the increased incidence of community-associated CDI which has been reported in populations previously believed to be low risk. A common management problem in CDI is relapse, which occurs in up to 25% of patients after treatment of the initial infection. Patients who experience one recurrence have up to a 45%

risk of further relapse and those with two or more episodes face up to a 65% risk. While the first relapse is generally treated with a second course of metronidazole or vancomycin, current guidelines recommend a tapering course of oral vancomycin, which is typically given over 4-8 weeks at a cost of $3500, after a second recurrence. Unfortunately, treatment options are limited for those patients who develop further recurrences. Patients treated with Fidaxomicin currently are faced with an even more expensive cost of therapy. A number of patients become "vancomycin dependent," developing CDI relapse whenever this antibiotic is stopped. Antimicrobial agents are thought to alter normal bacterial flora of the gastrointestinal tract so as to permit colonization and/or proliferation and toxin elaboration by *C. difficile*. The specific antimicrobials used to treat CDI (metronidazole, vancomycin, etc.) also may predispose patients to further relapses through the maintenance of perturbed intestinal flora and may contribute to the emerging problems of drug resistance.

7.3 Rational for use of an Unapproved Product

Clostridium difficile is an increasingly common infection. In addition to occurring more frequently, there is an epidemic of serious cases which are more refractory to therapy and which have high rates of colectomy and death. Most patients with *C. difficile* respond to a course of oral metronidazole or vancomycin, however, up to 25% of patients relapse after initial treatment. A number of patients do not respond to these antibiotics or develop further recurrences when they are stopped. Unfortunately, treatment options are limited for those patients who develop recurrences. Use of antimicrobials to treat *C. difficile* colitis may predispose these patients to further relapses through the maintenance of disturbed intestinal flora and may contribute to the emerging problem of drug resistance. FMT has been shown to restore the phylogenetic richness and diversity to the intestinal microbiome thereby restoring colonization resistance.

The potential risks associated with this treatment appear to be minimal. Although there is a very low risk for complications related to endoscopic administration and theoretical risks related to FMT including transmission of infection or risk factors for other diseases, the potential benefits of treatment in this group of patients are great, notably resolution of *C. difficile* infection. Thus, the potential benefits of FMT outweigh the potential risks of the treatment in these patients who have not responded to standard antimicrobial therapy.

7.4 Summary of Previous Human Experience

7.4.1 Previously Published Case Reports and Case Series

Table 1. Experience with Fecal Microbiota Transplantation 1958-2010

Author	Indication	No. Patients	Method of FMT	Outcome
Eiseman et al[24]	Pseudomembranous colitis	4	Fecal enema	Symptoms resolve in all within 48 hours
Collins[39]	Pseudomembranous colitis	12	Fecal enema	"Favorable reaction in 10/12"
Fenton et al[40]	Pseudomembranous colitis	1	Enema	Patient Cured
Bowden et al[25]	Pseudomembranous colitis	16	Fecal enema (15); enteric tube (1)	Symptoms resolved within 1-12 days in 13/16 patients

Schwan et al[17]	Relapsing *C. difficile*	1	Fecal enema	Prompt & complete normalization of bowel function
Tvede and Rask Madsen[13]	Relapsing *C. difficile*	2	Rectal infusion	Symptomatic cure in patient 1, patient 2 improved after infusion of bacterial mixture
Flotterod and Hopen[27]	Refractory *C. difficile*	1	Duodenal tube	Diarrhea resolved after infusion
Paterson et al[41]	Chronic *C. difficile*	7	Rectal tube	Rapid symptom relief and no relapse
Lund-Tonnesen[42]	C. difficile diarrhea	18	Colonoscopy (17), gastrostomy (1)	15/18 cured with no relapses
Persky and Brandt[18]	C. difficile diarrhea	1	Colonoscopy	Symptomatic relief within 6 hours
Faust et al[43]	Recurrent pseudomembranous colitis	6	Method unstated	All patients responded and 4/6 became C. difficile toxin negative
Aas et al[19]	Recurrent C. difficile	18	Nasogastric tube	15/18 had no recurrence
Jorup-Ronstrom et al[44]	C. difficile diarrhea	5	Fecal lavage (3), enema (1), not stated (1)	4/5 cured
Wettstein et al[45]	C. difficile diarrhea	16	Colonoscopy, enema	15/16 cured
Borody et al[46]	C. difficile in refractory IBD	6	Enema	Negative culture and toxin assay at 8 weeks in all
Louie et al[47]	C. difficile diarrhea	45	Rectal catheter	43/45 cured
Nieuwdorp et al[48]	C. difficile diarrhea	7	Colonoscopy	5/7 cured
You et al[20]	Fulminant C. difficile diarrhea	1	Enema	Within 36 hours bowel function returned and abdominal distension decreased
Hellemans et al[49]	C. difficile diarrhea	1	Colonoscopy	Patient cured
MacConnachie et al[50]	Recurrent C. difficile	15	Nasogastric tube	12/15 became symptom free
Khoruts et al[15]	C. difficile diarrhea	1	Colonoscopy	Patient cured
Yoon and Brandt[22]	Recurrent/refractory C. difficile	12	Colonoscopy	Symptomatic cure in all 12
Rohlke et al[23]	Recurrent C. difficile	19	Colonoscopy	18/19 became asymptomatic immediately; 1 patient relapsed; 3 had recurrence during follow-up period after antibiotics
Silverman et al[21]	Chronic C. difficile	7	At-home fecal enema	All responded
Garborg et al[51]	Recurrent C. difficile	40	EGD (38), colonoscopy (2)	83% success rate
Kelly and deLeon[28]	Recurrent C. difficile	12	Colonoscopy	All patients responded
Russell et al[52]	Refractory C. difficile	1	Nasogastric tube	Symptom resolution within 36 hours in 2 year old girl

Total 275 Response 246/275=89%
EGD, esophagogastroduodenoscopy; IBD, Inflammatory Bowel Disease

7.4.2 Investigator's Published and Continuing Experience

Dr. Smith is board-certified in Infectious Diseases and has a special interest in Clostridium difficile infection (CDI). She has previously published on the epidemiology of community-acquired and healthcare-associated CDI. She has experience in treating patients with recurrent CDI using fecal microbiota transplantation (FMT). Dr. Smith, Dr. Eugene Yen, and several members of the Division of Infectious Diseases at NorthShore University HealthSystem have performed FMT for 18 patients with recurrent CDI. Table 2 summarizes our clinical experience to date. Our primary clinical outcome, prevention of CDI relapse and/or significant diarrhea requiring vancomycin within 8 weeks after FMT was achieved in 17/18 patients (94.5%) after one infusion of donor stool. Three patients

relapsed (2/3 following receipt of antibiotics). One of those 3 patients responded to a course of oral vancomycin and did not relapse. The other 2 patients have had multiple relapses and are currently awaiting repeat FMT following IND approval. We have successfully and without adverse events treated patients with immunodeficiency including CLL, solid organ malignancy, and hypogammaglobulinemia.

TABLE 2: Patients (18) Treated by Drs. Yen (GI), Smith (ID), Kaplan(ID), Schrantz (ID), Semel (ID), Nega (ID). GI=Gastroenterology, ID=Infectious Diseases. ABX = Antibiotics.

Number	Patient (Sex, Age)	Donor Relation-ship	Inciting Event	CDI Duration (Months)	Other Treatments Prior to FMT	Route of Instillation	Colonoscopy Findings	Primary Clinical Outcome	FMT Date	CDI Recurrence	Date	Adverse Events
1	F, 81	Husband	ABX	11	Metronidazole x 1 Vancomycin taper x 2 Rifaximin 'chaser' x 1 Fidaxomicin x 1 Vancomycin	EGD	N/A	Cure	10/10/11	No	N/A	None *Died due to Metastatic pancreatic ca 1/30/12 (diagnosed during eval for recurrent CDI)
2	M, 84	Wife	ABX	9	Metronidazole x 2 → rash Vancomycin taper x 2	Colonoscopy	Sessile polyp Diverticuli Internal Hemorrhoids	Cure	11/14/12	No	N/A	None *Died on Hospice for underlying severe CHF >1 month after procedure. Last office visit notes diarrhea resolved and no GI symptoms
3	M, 83	Daughter	ABX/GI surgery	6	Vancomycin x 1 Metronidazole x 4 Cholestyramine Fidaxomicin	Colonoscopy	Sigmoid circumferential mass (had known colon CA) and diverticuli	Cure	2/20/13	No	N/A	None
4	F, 78	Unrelated	No Antibiotics	11	Metronidazole x 1 Vancomycin x 2 Vancomycin taper Rifaximin taper Fidaxomicin IVIG	EGD	N/A	Cure	5/15/12	No	N/A	None
5	M, 75	Daughter	No Antibiotics Caregiver for wife who was frequently hospitalized and in Rehab	4	Vancomycin x 1 Vancomycin taper x 2	EGD	N/A	Cure	1/27/12	No	N/A	None

6	M, 76	Daughter	ABX	21	Vancomycin x 1 Vancomycin taper x 4 Probiotics Rifaximin	EGD	N/A	Cure	9/11/12	No	N/A	None
7	F, 62	Husband	ABX	12	Metronidazole Vancomycin x multiple courses Vancomycin taper x multiple courses	Retention enema	N/A	Cure	8/16/12	Yes	1/6/13	None *Note prior Hx lymphocytic colitis and hypogammaglobulinemia on IVIG
8	F, 77	Husband	ABX	5	Vancomycin x 1 Vancomycin taper x 2 Fidaxomicin Metronidazole intolerance	EGD	N/A	Cure	11/14/12	No	N/A	None
9	F, 61	Daughter	ABX	3	Metronidazole x 2 Vancomycin Taper x 1	Retention enema	N/A	Cure	2/15/13	Yes	4/18/13	None * Note patient with ALS who would develop resp failure requiring ventilator with each episode
10	F, 34	Husband	ABX	10	Metronidazole x 1 Vancomycin x 1 Vancomycin Taper x 2	EGD	N/A	Cure	3/22/13	No	N/A	None
11	M, 76	Son	ABX	10	Metronidazole Vancomycin x 1 Vancomycin Taper x 2 Fidaxomicin	Colonoscopy	Diverticuli Polyps Hemorrhoids	Cure	8/22/12	No	N/A	None *Note patient had advanced dementia and died 11/28/12 (not due to CDI)
12	F, 89	Unrelated	ABX	10	Metronidazole x 2 Vancomycin x 1 Vancomycin Taper x 2	Colonoscopy	Diverticuli Hemorrhoids	Cure	11/7/12	No	N/A	None

13	M, 86	Daughter	ABX	19	Metronidazole x 3 Vancomycin taper x 3 Kefir Probiotics	Colonoscopy	Polyps Diverticuli	Cure	10/11/12	No	N/A	None
14	F, 28	Husband	ABX	36	Multiple vancomycin tapers (16 episodes over 3 years)	EGD	Chronic Inflammation no IBD	Cure	5/19/10	No	N/A	None
15	F, 82	Husband	ABX	14	Vancomycin x multiple Vancomycin Taper Maintenance dose vanco	EGD	N/A	Cure	12/14/11	No	N/A	None
16	F, 29	Mother	ABX	2	Vancomycin x 1 Fidaxomicin x 2	EGD	N/A	Cure	8/16/12	No	N/A	None *Note history of Ulcerative colitis
17	F, 86	Cousin	ABX/GI Surgery	10	Vancomycin x 1 Vancomycin taper x 3	Colonoscopy	Diverticuli Hemorrhoids	Cure	5/10/12	No	N/A	None
18	F, 84	Son	ABX	4	Metronidazole x 1 Vancomycin x 1 Vancomycin taper x 1 Fidaxomicin Tigecycline	Colonoscopy	Colitis (bx negative for other etiology)	Cure	3/14/13	Yes	3/25/13	None * Patient had UTI and was treated with Cefpodoxime by PCP then relapsed within days

Safety

All patients tolerated the procedure well without any procedure (colonoscopy, EGD, or retention enema) – related complications. Three of the patients died, as noted, within 1 year following the procedure, none of which were directly related to the procedure or *C. difficile* infection (CDI) but rather related to underlying severe comorbid conditions. Documentation in the medical record conforms resolution of diarrhea and no infectious symptoms to suggest active CDI at the time of death. There were no other significant complications or adverse outcomes.

8. TREATMENT PROTOCOL

Study Design

A one-year, open-label randomized controlled trial from 1/1/15-12/31/15 at all NorthShore hospitals.

Patient Selection

All hospitalized patients in the NorthShore system >18 years of age who are diagnosed with active CDI, defined as >3 diarrheal stools per day and a positive *C. difficile* PCR assay, will be evaluated for inclusion in the study. Hospitalized patients presenting with a relapse of CDI occurring at least 15 days after an index episode of CDI will be eligible for enrollment. Exclusion criteria will include pregnancy, neutropenia (absolute neutrophil count <1000/μl), contraindication for retention enema, toxic megacolon or health-threatening food allergy not controlled for in the donor diet. Eligible patients will undergo written informed consent followed by randomization into intervention and control groups.

Outcomes

The primary outcome will be clinical resolution of diarrhea without recurrence of CDI within 90 days. Secondary outcome measurements will include the time to clinical resolution of symptoms (number of daily bowel movements, daily white blood cell count, daily creatinine, and temperature), hospital length of stay (LOS), ICU admission and LOS, total charges, 90-day mortality, 90-day readmission, colectomy, antibiotic use, co-colonization of the gastrointestinal (GI) tract with other multidrug-resistant organisms (MDROs), and the presence of adverse events.

Study Procedures

Patients will be eligible for enrollment within 48 hours of the positive *C. difficile* PCR or EIA test. Subjects will be randomized with a 1:1 ratio to either the FMT or the standard antibiotic treatment arms by computer-generated randomization numbers. There will be a separate randomization for the subjects on their first relapse vs. their second or greater relapse since these two patient populations may respond differently to FMT and/or antibiotics.

Patients who are randomized to the intervention group will have antimicrobials targeting *C. difficile* discontinued at least 6 hours prior to undergoing an FMT via retention enema. A second FMT via retention enema will be administered at 24-48 hours if diarrhea and clinical symptoms persist. Patients randomized to the control group will be treated with

antimicrobials targeting *C. difficile* according to the Society for Healthcare Epidemiology of America Clinical Practice Guidelines for CDI (18). FMT will be offered to the control group after 90 days if they experience relapsing CDI. If a patient in the control arm progresses to refractory CDI, FMT will be offered (which will be accounted for with the ITT statistical analysis). Donor and recipient stool will be collected before and after the FMT procedure to be cultured later for future research studies. Collected stool will stored in the Department of Pathology and will be under the supervision of the Infectious Disease physicians overseeing the research study. All samples will be coded with subject-ID numbers that are created by the research staff. In addition, the date/time of collection will be included on the stool sample label. Lastly, the severity of each patients CDI episode will be recorded based on Society for Healthcare Epidemiology of America Clinical Practice Guidelines for CDI (18). Please note that severity of C. difficile will not determine randomization status.

8.1. Indications

This is a protocol for use of fecal microbiota transplantation (FMT) in adult (≥18 year old) hospitalized patients suffering from recurrent *C. difficile* infection (CDI). FMT will be used to treat CDI in the following circumstances:

1. Recurrent or relapsing CDI (first relapse)
    a. At least 3 diarrheal stools per day and a positive C. difficile PCR assay
    b. Hospitalized patients presenting with their first relapse of CDI occurring at least 15 days after an index episode of CDI

2. Recurrent or relapsing CDI (second or greater relapse)
    a. At least 3 diarrheal stools per day and a positive C. difficile PCR assay
    b. Hospitalized patients presenting with their second or greater relapse of CDI occurring at least 15 days after an index episode of CDI

Definitions

- *Clostridium difficile* infection: as per SHEA-IDSA guidelines, at least three unformed stools over 24 hours for two consecutive days and either positive stool testing (ELISA or PCR) for *C. difficile* toxins or pseudomembranes on colonoscopy.

8.2 Donor Eligibility Determination

I. Donor selection and screening: Two healthy "universal" donors who have previously donated fecal material for FMT have expressed willingness to participate in the study. Donors will complete the American Association of Blood Banks donor questionnaire for exposure to infectious agents as well as undergo serologic and stool testing for communicable diseases or pathogenic bacteria/viruses as previously described (17).

A. Donor Interview: Potential donors will have a brief interview to determine the presence of systemic medical conditions, which will preclude donation.

1. Known communicable disease
2. Systemic autoimmunity or atopic diseases
3. Chronic pain syndromes (for example: fibromyalgia, chronic fatigue)

4. Neurologic, neurodevelopmental or neurodegenerative disorders
5. Malignancy
6. Diarrheal disorder (IBS, IBD, celiac disease)
7. Use of antibiotics for any indication within the past 3 months

B. Donor Screening Questionnaire. Potential donors will undergo informed consent for screening tests and if they wish to participate will be interviewed using a questionnaire based on the Donor History Questionnaire (DHQ) materials prepared by the AABB Donor History Task Force for use in screening blood donors. The DHQ is especially important to identify risks for diseases and conditions for which there are no laboratory tests, for which tests are not sensitive enough to detect infectious disease agents, and for which tests are unable to identify early stage or window period infections. Additional questions which are felt relevant to FMT, including recent use of antimicrobials, and history of relevant medical conditions have been added to this questionnaire (appendix 1).

Our modified DHQ will be used to exclude donors with these and other risk factors:

1. High risk sexual behaviors (examples: sexual contact with anyone with HIV/AIDS or hepatitis, men who have sex with men, sex for drugs or money)
2. Known exposure to HIV or viral hepatitis within the previous 12 months.
3. Being held in a correctional facility for more than 72 hours in the last 12 months
4. Use of intravenous drugs or intranasal cocaine
5. Recent tattoo or body piercing
6. Recent transfusion, transplant or skin graft
7. Risk factors for variant Creutzfeldt-Jakob disease

DHQ will be completed within 30 days of FMT. Use of antibiotics will preclude donation for a period of 3 months.

C. Donor Laboratory Testing. HIV 1 & 2 testing will be performed within 2 weeks of donation for FMT. The other serologic and stool screening will be performed within one month of donation for FMT. (appendix 2).

1. Stool:
    - *Clostridium difficile* toxin by PCR
    - Routine bacterial culture for enteric pathogens (E coli, Salmonella, Shigella, Yersinia, Campylobacter)
    - Culture for *Listeria monocytogenes* and *Vibrio* (*parahaemolyticus and cholerae*)
    - Fecal *Giardia* antigen
    - Fecal *Cryptosporidium* antigen
    - Acid-fast stain for *Cyclospora* and *Isospora*
    - Ova and parasites
    - Stool for Rotavirus via EIA
    - Helicobacter pylori stool antigen

- Norovirus by PCR

2. Blood:
   - HIV, type 1 and 2
   - HAV IgM
   - HBsAg, anti-HBc (both IgG and IgM), and anti-HBs.
   - HCV Ab
   - RPR

D. Day of donation: On the day of the stool donation, donor will be questioned regarding the following:

1. Fever, vomiting, diarrhea or other symptoms of infection within the last 30 days.

8.3 Patient eligibility determination

A. Potential FMT patients will undergo a medical interview to determine eligibility for the treatment and a physical exam will be performed.

B. Only patients ≥18 years old will be treated under this FMT protocol.

C. Serologic Testing will be done on all patients to document baseline status prior to FMT including: HIV 1 & 2, Hepatitis A total, Hepatitis B surface Ag, surface Ab and core Ab, Hepatitis C Ab, and RPR.

D. Pre-treatment Medications

1. Relapsing CDI patients will have completed at least a 10 day course of vancomycin (or other antibiotics with activity against CDI including metronidazole, fidaxomicin and rifaximin) for their initial episode of CDI (the one prior to hospital stay) prior to undergoing FMT.

2. To control disease while awaiting FMT, vancomycin will be continued by relapsing subjects until at least 6 hours prior to scheduled procedure and by refractory subjects until the evening prior to the procedure. Other antibiotics with activity against CDI are also permissible while awaiting FMT including metronidazole, fidaxomicin and rifaximin.

E. Female patients

1. Females of childbearing potential will have a urine pregnancy test on the day of the FMT procedure (to ensure eligibility). Patients who are pregnant will not be permitted to receive FMT.

2. Female subjects should not become pregnant nor breast-feed an infant within 4 weeks of treatment by FMT. In order to reduce the risk of pregnancy, patient or her partner should use an acceptable method of birth

control listed below, regularly and consistently. Acceptable methods of birth control (continuing pre-FMT and for one month after treatment) include:

- An approved oral contraceptive (birth control pill)
- Intra-uterine device (IUD)
- Hormone implants
- Contraceptive injection (Depo-Provera)
- Barrier methods (diaphragm with spermicidal gel or condoms)
- Transdermal contraceptives (birth control patch)
- Vaginal contraception ring (birth control ring)
- Sterilization (tubal ligation, hysterectomy or vasectomy)
- Abstinence

3. If patients become pregnant or suspect that they are pregnant, or if they make someone pregnant, within 4 weeks of treatment with FMT, they will be instructed to immediately inform the treating physician. If patients suspect that they are pregnant within 4 weeks of FMT, a pregnancy test will be done. If conception occurs within the 30-day post-FMT period, the study physician will assist the patient in getting obstetrical care and will follow the progress of the pregnancy. The study physician will request access to patients and/or infants medical records for up to at least eight weeks after delivery.

8.4. Preparation of Stool for FMT Infusion

A. Collection and handling

1. Donors will be supplied a toilet hat and clean, sealable plastic containers for collection and transport of stool specimens. Containers will be labeled with the name, date of birth and date/time of stool collection.

2. Collected stool will be immediately processed and stored at -80C for future use.

B. Location & preparation of processing

- Stool will be processed in a designated area at each site. This is typically within the endoscopy unit or area where the FMT will be performed.
- Universal precautions will be used during processing (gown, gloves, eye protection).
- Clean counter surface will be covered with a Chux® pad.
- After the FMT, all surfaces will be wiped with hospital-approved disinfectant solution.

C. Preparation materials

- 1 liter bottle of sterile, nonbacteriostatic normal saline
- 60 cc disposable slip (catheter)-tip syringes
- Clean gauze
- Clean plastic spoon

D. Preparation method

Fecal microbiota preparations will be handled as described by Hamilton et al (15).

- 500 cc of saline will be poured out of the 1-liter bottle and discarded.
- RA will suspend at least 50 grams (or available quantity) of stool in 250 mL of sterile, non-bacteriostatic normal saline within 2 hours of collection.
- The slurry will be filtered, centrifuged, and re-suspended in sterile, non-bacteriostatic normal saline and sterile pharmaceutical grade glycerol to a final concentration of 10%
- The final product will be stored at -80ºC for up to 8 weeks
- When needed for use, the material will be thawed in an ice bath over 2-4 hours and then diluted to 250 mL sterile, non-bacteriostatic normal saline before infusion into the recipient.
- The fecal suspension will be drawn into five 60 cc slip (catheter) tip syringes for infusion.

8.5. FMT Procedure.

A. Patient preparation

1. Patients will have completed at least a 10-day course of vancomycin (or other anti-CDI therapy such as fidaxomicin or metronidazole) for the most recently diagnosed acute CDI prior to undergoing FMT (e.g. their first episode)

2. To prevent disease relapse while awaiting FMT, anti-CDI therapy will be continued by patients until at least 6 hours prior to scheduled procedure.

3. Subject will be ordered to take no food or liquids (NPO) at least 4 hours prior to the procedure.

4. 1-2 hours before retention enema, the patient may take 2 loperamide tablets to aid in retention of administered donor stool.

B. Methods of FMT infusion.

1. Procedure will be performed in the patient's hospital room (NorthShore University HealthSystem).

2. The method used to deliver the FMT will be a retention enema:

- A rectal tube will be placed by the staff nurse.
- The donor specimen will be infused into the rectum via the rectal tube in place. Once the sample has been instilled, the tube will be clamped for 1 hour.
- Vitals signs will be collected every 15 minutes by clinical staff (nursing staff) and recorded in patient's EMR.

- After one hour the rectal tube will be unclamped and removed. The rectal tube will be kept in place only if medically necessary.

- If patient has not improved based on clinical parameters, a 2nd FMT will be completed within 24- 48 hours.

3. The physician will administer ~250-300 mL of the fecal suspension in aliquots of 60 mL via retention enema.

4. The patient is encouraged to retain stool for as long as possible (optimally 1 hour).

8.6. Follow up

A. Plan for clinical follow up:

- Patients will be encouraged to contact the research team immediately if they experience recurrence of diarrhea so that stool can be tested for *C. difficile* toxins A & B and treatment with anti-CDI therapy can be initiated if necessary.
- Patients will be contacted daily by telephone or in-person while hospitalized after the FMT procedure. In addition, research staff will complete chart review to collect vital signs, lab results, and medication information. If patient is unavailable or unable to respond to questions, research staff will speak with nurse assigned to patient.
- Patients will be asked to submit a stool sample 7 days post-FMT for *C. difficile* toxin testing as well as co-colonization of the GI tract with multi-drug resistant organisms (MDROs).
- In addition, patients will be contacted via telephone by a member of the research staff 7 days post FMT (+ 3 days).
- Regardless of symptoms, all subjects will be scheduled in the office for follow up visit at 30 days post-FMT (+30 days). During this time, patients will submit stool specimens for *C. difficile* toxin testing as well as co-colonization of the GI tract with multi-drug resistant organisms (MDROs)
- Patients will also provide a third stool sample at their 4 week follow up visit.
- Patients will be contacted via telephone by a member of the research staff 3 months, 6 months, and annually post-FMT (all contacts with a window of ±14 days). The annual follow-up calls will occur indefinitely.
- Patients will be instructed to inform the treating physician of any infectious symptoms or new medical conditions which develop after FMT. Adverse events will be elicited at follow up telephone contacts and follow up visits and all patients will be contacted via telephone by research staff member 3 months, 6 months, and annuallyafter the last treatment to record any SAEs, new medical conditions/diagnoses or changes in medical conditions/medications since last clinical contact.

B. Patients who relapse

1. In the event of treatment failure (defined as CDI recurrence requiring additional anti-infective therapy), the treating physician will have the following options.
    - Patient may undergo a second FMT using the same or an alternate donor. This second FMT will be completed within 24-48 hours of the first FMT if diarrhea persists.
    - Patient may choose not to receive further FMT, but may continue to be treated in accordance with the current standard of care. The patient will continue to be followed for closely for AEs with telephone calls and clinic visits as per this protocol.
    - Alternatively, patients who chose not to receive further FMT may decide to be treated at another facility of their choosing. They will still be followed as per protocol through the 30 day post FMT period and asked to receive a 3 month and 6 month follow-up telephone call to assess for late SAEs or changes in medical conditions/diagnoses or medications.

Patients who chose to undergo a second FMT will have the identical procedure, including donor eligibility determination, follow up, and safety monitoring.

C. Withdrawal of patients

Patients who withdrawal from treatment protocol will be characterized as follows:

1. Patients who receive FMT and are then lost to follow up: Every reasonable effort will be made to contact these patients to ensure they are receiving appropriate follow up care with documentation of any SAEs/AEs or changes in medical conditions/diagnoses or medications.
2. Patients withdrawn because of SAE/AE or at the discretion of the physician will continue to receive treatment (outside this protocol) in accordance with current standard of care. Patients having AEs will be monitored with relevant clinical assessments and laboratory tests, as determined by the treating physician.

D. Adverse Events

We will specifically document and report to FDA:

- Complications related to the colonoscopy or sigmoidoscopy (sedation related, perforation, bleeding)
- Complications related to FMT (infection, inflammatory or allergic reaction)
- Solicited and unsolicited AEs, including fever, will be assessed at follow up telephone contacts and clinic visits.
- Development of new symptoms/diagnoses (irritable bowel syndrome, inflammatory bowel disease, autoimmune disorder, neurologic disorder) which may be related or unrelated to FMT will be elicited at the 6 month follow up telephone call, documented and reported to the FDA.
- Subjects who have an SAE that is possibly, probably or definitely related to FMT will not receive a second FMT, if, after review of the SAE, it is determined that they are at risk for another SAE if a second FMT is performed. For example, if the subject develops an allergic reaction post FMT and it is determined that the donor

inadvertently ingested the known allergen prior to donation, the subject may qualify for a second FMT after subsequent donor screening minimizes exposure to the allergen.

- The sponsor will notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information.
- We will notify the FDA within 7 days if an infectious disease is possibly, probably or definitely transmitted to a patient via FMT.
- All SAEs will be reported within 15 days to the FDA
- All other AEs will be reported in an annual report to the FDA.

1. Patient reporting of AEs:

Patient will be instructed to contact the treating physician/research staff at any time point post-FMT to report symptoms experienced. The following AEs will be solicited at telephone contacts (daily case report forms, 1-week follow up, 3-month follow up, and 6-month follow). and at 4-week clinic visit along with the intensity of each.

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
ESTIMATING SEVERITY GRADE				
	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death
SYMPTOM SPECIFIC SEVERITY GRADE				
Fever (oral)	(99.9-100.5°F)	(100.6-102.5°F)	(102.6-104°F)	> 104°F
Diarrhea	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 – 6 stools over baseline per 24-hour period	Bloody diarrhea OR Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g., hypotensive shock)
Nausea	Transient (< 24 hours) or intermittent nausea with no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 – 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Fatigue Malaise	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Incapacitating fatigue/ malaise symptoms causing inability to perform basic self-care functions

Vomiting	Transient or intermittent vomiting with no or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Headache	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Headache with significant impairment of alertness or other neurologic function
Distension/bloating, abdominal discomfort	Asymptomatic	Symptomatic, but not interfering with GI function	Symptomatic, interfering with GI function	---
Abdominal Pain	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Disabling pain causing inability to perform basic self-care functions OR Life threatening consequences (i.e. acute peritonitis)
Dehydration	Increased oral fluids indicated; dry mucous membranes; diminished skin turgor	IV fluids indicated <24 hours	IV fluids indicated >24 hours	Life-threatening consequences (e.g. hemodynamic collapse)
Dizziness	With head movements or nystagmus only; not interfering with function	Interfering with function, but not interfering with ADL	Interfering with ADL	Disabling
Colitis Symptoms	Asymptomatic, pathologic or radiographic findings only	Abdominal pain; mucus or blood in stool	Abdominal pain, fever, change in bowel habits with ileus; peritoneal	Life-threatening consequences (e.g. perforation, bleeding, ischemia, necrosis, toxic megacolon)
Weight Loss	5 to <10% from baseline; intervention not indicated	10-<20% from baseline; nutritional support indicated	≥20% of baseline	---
Rash	Macular or popular eruption or erythema without associated symptoms	Macular or popular eruption or erythema with pruritus or other associated symptoms; localized desquamation or other lesions covering <50% of body surface area (BSA)	Severe, generalized erythroderma or macular, popular, or vesicular eruption; desquamation covering ≥50% BSA	Generalized exfoliative, ulcerative, or bullous dermatitis
Dysphagia	Symptomatic; able to eat regular diet	Symptomatic and altered eating/swallowing	Symptomatic and severely altered eating/swallowing	Life-threatening consequences (e.g. obstruction, perforation)

		(e.g., altered dietary habits, oral supplements); IV fluids indicated <24hrs	(e.g. inadequate oral caloric or fluid intake); IV fluids, tube feedings, or TPN indicated ≥24hrs	

9. Adverse Event Reporting

A. Definitions

1. Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or clinical trial subject administered FMT that does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a FMT, whether or not related to the FMT.

• AEs may be new events or may be pre-existing conditions that have become aggravated or have worsened in severity or frequency.

• AEs may be clinically significant changes from baseline in physical examination, laboratory tests, or other diagnostic investigation (e.g., laboratory results, x-ray findings).

Pregnancy is not an AE; however, if a female subject becomes pregnant during 4-week period post-FMT, the treating physician will notify the FDA.

2. Serious Adverse Event (SAE)

A serious adverse event is any adverse experience occurring during or after FMT that results in any of the following outcomes:

• Death;

• Life-threatening experience;

Note: An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

• Requires inpatient hospitalization or prolongation of existing hospitalization.

Note: Adverse events requiring hospital admissions that are less than 24 hours in duration do not meet this criterion. A scheduled hospitalization for a pre-existing condition that has not worsened during participation in the study does not meet this criterion. Pre-planned hospitalizations for an elective medical/surgical procedure or routine check-ups do not meet this criterion;

• Results in persistent or significant disability or incapacity;

• Is a congenital anomaly or birth defect;

• Is considered to be an important medical event.

Note: Important medical events are those that may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above.

3. Planned Hospitalization

A hospitalization planned prior to FMT is to be considered a therapeutic intervention and not the result of a new SAE. If the planned hospitalization or procedure is executed as planned, it will be recorded in the subject's medical history or procedures. However, if the event/condition worsens during the trial, it must be reported as an AE**.**

4. *Adverse reaction*
An adverse reaction means any adverse event caused by FMT. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that FMT caused the event.

5. *Suspected Adverse Reaction*
Suspected adverse reaction means any adverse event for which there is a reasonable possibility that FMT caused the adverse event. For the purposes of IND safety reporting, reasonable possibility" means there is evidence to suggest a causal relationship between FMT and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

6. *Unexpected*
An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

B. Monitoring
1. Monitoring of Adverse Events
Each patient will be monitored for the occurrence of AEs, including SAEs, beginning immediately after FMT. Patients will be questioned at each follow up time-point regarding stool form/frequency, presence of abdominal pain, fevers and subjective well-being and/or examined (at 4-week follow up visits) by the treating physician for evidence of AEs. The questioning of subjects with regard to the possible occurrence of adverse events will be generalized such as, "How have you been feeling since your last visit?"

- Patients having AEs will be monitored with relevant clinical assessments and laboratory tests, as determined by the treating physician.
- AEs, actions taken as a result of AEs, and follow-up results must be recorded in the patient's medical record.
- Patients will receive a follow up phone call 6 months post-transplant to record any SAEs, new medical conditions/diagnoses or changes in conditions or diagnoses since last study contact.

For all SAEs and AEs, relevant clinical assessments and laboratory tests will be repeated as clinically appropriate, until final resolution or stabilization of the event(s).

C. Assessment of Adverse Events
1. Assessment of Severity

The severity of AEs will be assessed according to the following definitions:
•**Mild**: the AE is noticeable to the patient and/or the Investigator, but does not interfere with routine activity.
•**Moderate**: the AE interferes with routine activity, but responds to symptomatic therapy or rest.
•**Severe**: the AE significantly limits the patient's ability to perform routine activities despite symptomatic therapy.

2. Assessment of Causality
The physician must assess the relationship of any AE (including SAEs) to FMT, as related or not related, based on clinical judgment and using all available information, and may include consideration of the following factors:
• Possible alternative causes of the AE, including the disease under treatment, pre-existing conditions, concomitant use of other drugs, and presence of environmental or genetic factors.
• The temporal association between FMT exposure and onset of the AE.
• Whether the manifestations of the AE are consistent with known actions or theoretical toxicity of FMT.

The causal relationship between FMT and the AE will be assessed using one of the following categories:
Not Related: An AE is not associated with FMT if:
• Temporal relationship is lacking (e.g., the event did not occur within a reasonable time frame following administration of FMT); or
• Other causative factors more likely explain the event (e.g. pre-existing condition, other concomitant treatments);
Related: An AE is attributed to FMT if:
• There is a positive temporal relationship (e.g., the event occurred within a reasonable time frame following FMT); and
• The AE is more likely explained by FMT than by another cause

D. Reporting Safety Observations by the Investigator to the Sponsor

1. Reporting of Nonserious AEs
All AEs, regardless of seriousness, severity, or causal relationship to FMT, will be recorded in the patient's medical record.

2. Reporting of FMT Exposure during Pregnancy
If a female patient or the female partner of a male patient becomes pregnant during 4-week period post-FMT, the physician is required to follow up on the pregnancy until it has completed. The outcome of the pregnancy and the status of the newborn (if applicable) will be reported within 24 hours of becoming aware. If the female partner of a male subject becomes pregnant, the physician must obtain consent to collect pregnancy information (including the status of the newborn, if applicable).

3. Reporting of Safety Observations by the Physician

Any occurrence of the following events or outcomes a patient treated under this IND must be reported expeditiously by the treating physician to the FDA.

1. SAE
2. Death of a subject

The investigator is to report any safety observations from the list above to the FDA within 7 days of becoming aware of the event. Any observation that is also an AE will be recorded in the medical record along with any actions taken. If not all information is available at the time of initial report, follow up SAE reports will be completed and submitted.
The investigator is required to follow SAEs until resolution. Resolution is defined as:

- Resolved with or without residual effects
- Return to baseline for a pre-existing condition
- Fatal outcome; if autopsy is performed, the autopsy report must be provided to the sponsor.

4. Protocol-Specific Exceptions to (Serious) Adverse Event Reporting
A suspected clinical endpoint event, regardless of when the event occurs, is not to be reported as an AE or SAE or reported in an expedited manner as an SAE.
The suspected clinical endpoint event includes:
Recurrent CDAD: The patient meets all of the following:
• Initial response to therapy with cure of CDI at end of treatment (vancomycin) pre-FMT; and
• A minimum of three unformed bowel movements over a 24-hour period
and
• A positive result for *C. difficile* toxin by EIA or PCR.

E. Monitoring and submitting safety reports.
The sponsor will notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information. The sponsor will notify the FDA in an IND safety report of potentially serious risks as soon as possible, but no later than 15 calendar days after the sponsor receives the safety information and determines that the information qualifies for reporting. *Participating providers* include all persons treating patients under the sponsor's IND. In addition, the sponsor will identify in each IND safety report, all IND safety reports previously submitted to FDA concerning a similar suspected adverse reaction and will analyze the significance of the suspected adverse reaction in light of previous, similar reports or any other relevant. The sponsor will evaluate a suspected adverse reaction in the context of other related reports or adverse events. Sponsor will maintain records (binder: database) documenting AEs. Physician will periodically review and analyze the entire safety database for IND safety reporting purposes. An IND safety report will be submitted when any of the following criteria are met:

A. Serious and unexpected suspected adverse reaction
B. Findings from other sources

The sponsor will also report expeditiously any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings that suggest a significant risk in humans exposed to FMT.

C. Increased occurrence of serious suspected adverse reactions

The sponsor will report any clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure

F. Annual Safety Reports.

Although this is an expanded access IND, the sponsor will provide the following for the annual report under 21 CFR 312.33(b);

- percentage of patients with at least one AE (within pre-specified time periods)
- percentage of patients with at least one SAE (within pre-specified time periods)
- percentage of patients who did/did not experience relapse in the 8 weeks post first FMT
- percentage of patients who received a second FMT within 8 weeks
- other pertinent statistics which would help to evaluate the safety and effectiveness of FMT

10. Risks Associated with FMT

Based on the existing literature (>800 case reports to date over a 60 year period) and the sponsors previous experience, the risks from FMT are felt to be low. This protocol details and minimizes risks to patients through the following methods.

1. Expanded access IND from FDA obtained for using FMT in patients with relapsing or refractory CDI.
2. Patients will be given informed consent using the standard consent process for retention enema. Additional risks related to FMT, such as transmission of infectious agents, allergens, and other diseases and conditions will also be discussed. Patients will sign a separate informed consent form (appendix 6.) for the FMT procedure.
3. Donor will be tested for most common infectious agents. Individuals with communicable disorders or a history of high-risk behaviors will not be permitted to donate. Patient privacy rules will be applied to donor screening and testing.
4. Protections against risk are provided in detail below.

10.1. Potential Risks to Patients

There are 3 areas of risk to patients associated with treatment. These include:

1) Physical risks related to the retention enema.
2) Theoretical risks (infectious and otherwise) related to FMT and
3) Psychological or other risks related to confidentiality and loss of privacy.

Standard retention enema risks include possible damage to the colonic membrane that lines the rectum and overload. Overload occurs when too much fluid is absorbed into the bloodstream resulting in cardiopulmonary complications. Many adverse effects of retention enema resolve shortly after the procedure has been completed, but in some cases abdominal discomfort and gaseous pain side can persist for several hours. There have been no infectious complications directly attributable to FMT reported in the literature to date. However, since the process

involves infusion of one person's "body fluids" into another person, transmission of an infectious agent or other disease or condition remains a theoretical possibility. From infusion of donor microflora, they could potentially acquire antibiotic resistance or risk factors for chronic diseases such as diabetes, inflammatory bowel disease, or colon cancer. Risks will be minimized by a rigorous donor-selection process and evaluative studies on stool and blood of the donor to exclude transmission of infectious agents prior to FMT. Donors will not have history of systemic autoimmunity, chronic pain syndromes, neurologic or neurodegenerative disorders, malignancy, diarrheal disorder or use of antibiotics within 3 months. Patients will be informed that the treatment or procedure may involve these theoretical risks and additional risks that are currently unforeseeable. Infusion of fecal material containing a potential agent to which the subject is allergic is also a concern and so patients with a history of severe (anaphylactic) food allergy will be excluded from this study and donors will be queried regarding ingestion of potential allergens during the 5-day period preceding donation.

Physical Risks to Patient:
Very Likely
• Mild to moderate abdominal pain or gaseous discomfort during/after retention enema
• Fatigue the day of the retention enema from sedatives
• Blood drawing: pain, bruising, feeling faint, slight risk of infection
Less Likely
• Nausea with possible vomiting from ingestion of colon prep solution
Less Likely But Serious
• Contracting infection from donor specimen
• Allergic reaction to unknown antigen present in donor stool
• Risks and side effects related to the retention enema including overload and adverse cardiopulmonary events related to sedation
• Acquisition of antibiotic resistance or risk factors for chronic diseases such as diabetes, inflammatory bowel disease, obesity, or colon cancer

10.2. Potential Risks to Donor
This protocol also involves individuals (donors) recruited to donate stool for FMT. There are 3 areas of risk to the potential stool donor. These include:

1) Physical risks related to laboratory testing and the stool collection protocol and
2) Psychological risks related to revealing sensitive information during donor screening process
3) Risks related to confidentiality and loss of privacy.

Healthy related and volunteer donors will be recruited at each site. In order to exclude donors at high risk of passing on infection, a donor-screening questionnaire (appendix 1) will be administered. This questionnaire does contain sensitive and potentially embarrassing questions about incarceration, drug use, and high-risk sexual behaviors. Additionally, they will be asked questions about their baseline health status and co-morbidities. Laboratory tests drawn as part of the screening process will include testing for HIV, viral hepatitis and syphilis. These serologic results, if found to be abnormal, could

cause psychological distress to the donor. However, the benefit of being made aware of a previously undiagnosed infectious condition outweighs this risk. Potential donors may experience psychological distress if they are excluded from donating stool based on these screenings. To facilitate proper stool consistency, donors will take a single dose of an osmotic laxative on the night prior to donation.

Physical Risks to Donor:

Very Likely

- Blood drawing: pain, bruising, feeling faint, slight risk of infection

Less Likely

- Mild nausea or abdominal discomfort from ingestion of an osmotic laxative (if needed)

Nonphysical Risks to Donor:

Less Likely

- Embarrassment or psychological distress from answering questions regarding drug use and sexual habits asked during the donor screening process.
- Psychological distress (guilt and embarrassment) if screening questionnaire or laboratory test results exclude the person from donation.

Unlikely

- There is also risk of compromising donor privacy through breach of data confidentiality of sensitive protected health information such as results of HIV or viral hepatitis testing.

11. Adequacy of Protection against Risks

A. Recruitment of Donors

Two healthy "universal" donors who have previously donated fecal material for FMT have expressed willingness to participate in the study. Recruits will be informed of the commitment including a detailed description of the donor screening process and a stool donation protocol. The research staff will explain that a thorough medical history interview, focused physical exam and screening questionnaire will be completed. They will be informed that the questionnaire asks potentially sensitive questions regarding incarceration, drug use, and sexual habits. They will be informed of the requirements to have laboratory blood work (including HIV testing) and stool studies and to submit stool specimen on the day of the fecal transplant procedure.

B. Protection against risk

Additionally, the risks described above will be minimized by the following procedures:

1. We will minimize potential risks due to loss of confidentiality by having all information collected and handled by research staff trained to deal appropriately with sensitive clinical issues. Potential donors will also be informed about the risk of being ineligible to donate stool due to positive results on screening questionnaires or laboratory testing. Results of donor medical interview, screening and laboratory testing will be kept separate from patient data and will not be available to the patient at any time. All information will be treated as confidential material and will be available only to clinical staff. Computer data

files will be available only to authorized personnel and no names or obvious identifying information will be stored in data files. No participant will be identified in any report to the FDA. Further, when contacting participants for follow-up, no identifying information other than the first name of the caller will be used when leaving messages or speaking to anyone other than the patient him/herself. Written consent will be obtained to contact other persons for the purpose of locating the participant for follow-up and participants can refuse or revoke such consents. No information about participants will be released without their permission or where required by law.

2. We will minimize the theoretical risks of infectious disease or other condition possibly transmitted through FMT by using donor-screening protocols modeled after blood banks and organ transplant programs. Potential volunteer donors will undergo thorough screening to determine eligibility for donation. The primary purpose of the donor examination and interview is to ensure that the donor is in good health, and to identify risk factors for diseases transmissible by stool. The donor interview will be used to identify risks for diseases and conditions for which there are no laboratory tests, for which tests are not sensitive enough to detect infectious disease agents, and for which tests are unable to identify early stage or window period infections. Potential donors will be interviewed using a donor screening questionnaire (appendix item 1) based on the Donor History Questionnaire materials prepared by the AABB Donor History Task Force for use in screening blood donors. Additional questions which are felt relevant to fecal microbiota transplantation including recent use of antimicrobials, and history of malignancy, gastrointestinal, neurologic or autoimmune disorders have been added to this questionnaire. Donor screening and serologic testing for relevant communicable diseases will be performed based on relevant portions of FDA guidelines for donors of human cells, tissues, and cellular and tissue-based products (HCT/Ps). This includes testing donors for HIV types 1 and 2, Hepatitis A, Hepatitis B, Hepatitis C, and *Treponema pallidum.* Donor stool will be tested for bacterial culture, Culture for *Listeria monocytogenes* and *Vibrio* (*parahaemolyticus and cholerae*), Rotavirus via EIA, *Clostridium difficile* toxins A&B, Fecal *Giardia* & *Cryptosporidium* antigens, *Cyclospora, Isospora* and Ova and Parasites. Subjects will be tested for HIV 1 & 2, Hepatitis A, B and C and Syphilis to confirm baseline status and prevent future questions about disease transmission. Potential donors will undergo a thorough medical history and examination to exclude any diseases which could (theoretically) be transmitted through the microflora including gastrointestinal diseases (IBD, IBS, chronic diarrhea or constipation), autoimmune or atopic conditions, malignancy or neurologic/neurodegenerative disease. Donors will be questioned on the day of donation for FMT and those who report fever, vomiting, diarrhea or other symptoms of infection within the last 30 days or ingestion of potential allergen where the recipient has a known allergy to the agent will not be permitted to donate.

3. We will minimize the risk of severe *C. difficile* relapse by maintaining close clinical contact with all patients. Patients will be encouraged to contact the research staff if they experience recurrence of diarrhea, fever or abdominal pain so that stool can be tested for *C. difficile* toxins A & B and antibiotics can be resumed if necessary. Patients will be contacted via telephone by a research staff member daily while hospitalized 7 days, 3 months, and 6 months after the treatment. They will have follow-up visits in the clinic 4 weeks post-FMT.

12. Safety monitoring protocol

Safety monitoring plan

Solicited and unsolicited AEs will be elicited and reviewed daily while patients are hospitalized, at 1-week phone calls, 30-day clinic visits, and 3 month and 6 month telephone calls. Patients will be contacted via telephone by a member of the research staff approximately 6 months after the FMT to record any SAEs, new medical conditions/diagnoses or changes in medical conditions/medications since last study contact.

The safety of patients will be monitored during each contact with patients. Both anticipated and unanticipated adverse events and problems will be formally monitored and recorded. Unanticipated serious adverse events or problems will be reported to the hospital and university IRBs (as per local reporting requirements), the FDA (within 15 days; or 7 days for unexpected fatal or life threatening events or transmission of infectious agent). Anticipated and less serious adverse events will be submitted annually in reports to the FDA. The Sponsor will be responsible for monitoring the safety and efficacy of this treatment and complying with the reporting requirements.

The sponsor will convene a safety monitoring board independent of this protocol (composed of ID and/or GI specialists) who will review the outcomes of patients treated under this protocol every 6 months. This board will have the power to halt the treatment of patients under this protocol if it is determined that safety concerns exist. The FDA will be notified within 48 hours if the expanded access IND is halted for review.

Appendix 1.

FMT Donor History Questionnaire

Are you:

1. Feeling healthy and well today? Yes No
2. Currently taking any medication for infection? Yes No

Have you:

3.Taken any antibiotics within the past 6 months? Yes No
4. Had any fevers, vomiting, diarrhea or other symptoms of infection within the past 4 weeks? Yes No

In the past 8 weeks have you:

5. Had any vaccinations or other shots? Yes No
6.Had contact with someone who has had the Smallpox vaccine? Yes No

In the past 12 months have you:

7. Had a blood transfusion? Yes No
8. Had a transplant (organ, tissue, bone marrow, dura mater- brain covering)? Yes No
9. Had a skin or bone graft? Yes No
10. Come into contact with someone else's blood? Yes No
11. Had an accidental needle stick? Yes No
12. Had sexual contact with anyone who has HIV/AIDS? Yes No
13. Had sexual contact with a prostitute or anyone else who takes money or drugs as payment for sex? Yes No
14. Had sexual contact with anyone who has ever used needles to take drugs or steroids, or anything NOT prescribed by their doctor? Yes No
15. Had sexual contact with anyone who has hemophilia or has used clotting factor concentrates? Yes No
16. *Female donors*: Had sexual contact with a male who has ever had sexual contact with another male (male donors circle "I am male)? Yes No I am male
17. Had sexual contact with a person who has hepatitis? Yes No
18. Lived with a person who has hepatitis? Yes No
19. Had a tattoo? Yes No
20. Had an ear or body piercing? Yes No
21. Been treated for syphilis or gonorrhea? Yes No
22. Been in lockup, jail or prison for >72 hours? Yes No

In the past three years have you:

23. Been outside the United States or Canada? Yes No
    List location/time spent:_____

From 1980 through 1996:

24. Did you spend time that adds up to three (3) months or more in the United Kingdom? Yes No

25. Were you a member of the U.S. military, a civilian military employee or a dependent member of the U.S. military? Yes No

From 1980 to the present:
26. Did you spend time that adds up to five (5) or more years in Europe? Yes No
27. Receive a blood transfusion in the United Kingdom or France? Yes No

From 1977 to the present, have you:
28. Received money, drugs, or other payment for sex? Yes No
29. *Male donors*: had sexual contact with another male, even once (female donors circle "I am female")? Yes No I am female

Have you EVER:
30. tested positive for HIV/AIDS virus? Yes No
31. used needles to take drugs or steroids or anything NOT prescribed by your doctor? Yes No
32. used clotting factor concentrates? Yes No
33. had viral hepatitis? Yes No
34. had any type of cancer (including leukemia)? Yes No
35. had sexual contact with anyone who was born or lived in Africa? Yes No
36. been in Africa? Yes No
37. had sex for drugs or money? Yes No
38. had any of the following gastrointestinal diseases or problems?
    - Irritable bowel syndrome? Yes No
    - Crohn's disease? Yes No
    - Ulcerative Colitis? Yes No
    - Chronic diarrhea? Yes No
    - Gastrointestinal cancers? Yes No
    - Celiac disease? Yes No
39. received growth hormone made from human pituitary glands? Yes No
40. Have any of your relatives had Creutzfeldt-Jakob disease? Yes No

General Medical History
41. Do you have any autoimmune diseases (for example: Rheumatoid arthritis, Multiple Sclerosis, Lupus) Yes No
    If yes, please list:_____
42. Do you have any neurologic diseases (example: Parkinson's, Autism, ALS)? Yes No
    If yes, please list: _____

Appendix 2.

Donor Testing

Serologic:

1. HIV, type 1 (FDA-licensed screening test either for anti-HIV-1 or combination test for anti-HIV-1 and anti-HIV-2)

2. HIV, type 2 (FDA-licensed screening test either for anti-HIV-2 or combination test for anti-HIV-1 and anti-HIV-2)
3. HAV IgM (FDA-licensed screening test for anti-HAV IgM)
4. HBV (FDA-licensed screening test for Hepatitis B surface antigen (HBsAg) and for total antibody to Hepatitis B core antigen (anti-HBc) (IgG and IgM)
5. HCV (FDA-licensed screening test for anti-HCV;and FDA-licensed screening NAT test for HCV, or combination NAT)
6. *Treponema pallidum* (FDA-cleared screening test for syphilis or FDA-cleared diagnostic serologic test for syphilis). As an exception for syphilis test results under § 1271.80(d)(1), one may determine to be eligible a donor whose specimen tests positive or reactive on a non-treponemal (VDRL, RPR) screening test for syphilis and negative or nonreactive on a specific treponemal confirmatory test (e.g., fluorescent treponemal antibody with absorption test (FTA-ABS), so long as all other required testing and screening are negative or nonreactive. A donor whose specimen tests positive or reactive on either a specific treponemal confirmatory test for syphilis or on a treponemal screening test is not eligible.

Confirmatory tests: Confirmatory tests will be performed when a positive or reactive screening test result is received for such purposes as donor counseling or investigating discordant test results. However, negative or nonreactive results on a confirmatory test would not override a positive or reactive screening test (except for syphilis tests as previously described in this section).

Hepatitis B surface antibody (anti-HBs) test: If you obtain a positive or reactive anti-HBs test and other markers for Hepatitis B infection are negative or non-reactive, the donor may be eligible. The presence of anti-HBs alone would not disqualify the donor, because it usually is an indication of vaccination against Hepatitis B. However, in this situation, if the anti-HBc were also positive or reactive, the donor is ineligible. Data suggests that such results can be associated with infectivity.

Stool:

1. Stool culture for enteric pathogens (routine pathogens tested include: E coli 0157:H7, Salmonella, Shigella, Yersinia, Campylobacter)
2. Culture for *Listeria monocytogenes*
3. Culture for *Vibrio* (*parahaemolyticus and cholerae*)
4. *Clostridium difficile* toxins A&B (PCR)
5. Fecal Giardia and Cryptosporidium antigens
6. Microscopic examination for Ova and Parasites including *Cyclospora* and *Isospora*.
7. Testing for Rotavirus via EIA
8. Helicobacter pylori stool antigen

Appendix 3.
Collection and Preparation of Stool for FMT Infusion

1. Donors will be supplied a toilet hat and clean, sealable plastic containers for collection and transport of stool specimens. Containers will be labeled with the name, date of birth and date/time of stool collection.
2. Collected stool will be delivered to the outpatient lab where it will be processed within 2 hours of delivery.

B. Location & preparation of processing

- Stool will be processed in the Evanston Microbiology lab.
- Universal precautions will be used during processing (gown, gloves, eye protection).
- Clean counter surface will be covered with a Chux® pad.
- After the FMT, all surfaces will be wiped with hospital-approved disinfectant solution.

C. Preparation materials

- 1 liter bottle of sterile, nonbacteriostatic normal saline
- blender
- Stainless steel filters
- Glycerol
- Storage tubes

D. Preparation method

Fecal microbiota preparations will be handled as described by Hamilton et al (15).

- 50g of fecal material homogenized with 250 mL of sterile, non-bacteriostatic normal saline within 2 hours of collection.
- The slurry will be filtered, centrifuged, and re-suspended in sterile, non-bacteriostatic normal saline and sterile pharmaceutical grade glycerol to a final concentration of 10%
- The final product will be stored at -80ºC for up to 8 weeks in the Pathology Department at NorthShore University HealthSystem with subject ID number
- When needed for use, the material will be thawed in an ice bath over 2-4 hours and then diluted to 250 mL sterile, non-bacteriostatic normal saline before infusion into the recipient.
- The fecal suspension will be drawn into five 60 cc slip (catheter) tip syringes for infusion.

Appendix 4.
Table of Visits and Interventions (Donor)

	Initial Visit
informed consent	x
medical interview	x
clinic visit	x
HIV 1 & 2 testing	x
serologic testing[2]	x
stool testing[3]	x
DHQ administered	x
Day of donation checklist[4]	At time of donation

[2]HAV IgM, HBsAg, anti-HBs, HCV Ab, RPR

[3] C. diff toxin by PCR, routine bacterial culture for enteric pathogens, culture for listeria monocytogenes and vibrio (parahaemolyticus and cholerae), fecal giardia antigen, fecal cryptosporidium antigen, acid-fast stain for cyclospora and isospora, ova and parasites, rotavirus EIA, *Helicobacter pylori* antigen (stool)

[4]Symptoms of infection, ingestion of allergens, recent use of antibiotics, verification of DHQ and screening

Appendix 5.
Table of Visits and Interventions (Patient)

	Initial Visit	≥ 6 hours prior to FMT	Day of FMT	Daily while hospitalized post FMT	7 days post FMT	30 days post FMT	3 months post FMT	6 months post FMT
Medical Interview	x		x					x
Physical exam	x		x					
Symptoms elicited[1]	x		x	x	x	x	x	x
Retention Enema			x					
Telephone Contact				x	x		x	x
Clinic visit						x		
Serologic testing[2]	x							
Stool testing[4]					x	x		
Urine pregnancy test (females)	x		x					
Stop antibiotic medication		x	x					
Adverse events				x	x	x	x	x
Changes in medical conditions, diagnoses or medications elicited				x	x	x	x	x

[1]Stool form/frequency, presence of abdominal pain, fevers, subjective well being

[2]HIV 1 & 2, Hepatitis A total, Hepatitis B surface Ag, Hepatitis B surface Ab, Hepatitis core Ab, Hepatitis C Ab, RPR

[3]Stool may also be collected for C. difficile PCR at any time if dictated by patient symptoms

Appendix 6: Criteria to define severity of CDI episode and antimicrobials targeting *C. difficile* according to the Society for Healthcare Epidemiology of America Clinical Practice Guidelines for CDI

Clinical Definition	Supportive Clinical Data	Recommended Treatment
Mild or Moderate	WBC ≤ 15,000 **AND** Creatinine ≤ 1.5x pre-morbid level	Vancomycin 125 mg po q 6 hours x 14 days
Severe	WBC > 15,000 **OR** Creatinine > 1.5x pre-morbid level	Vancomycin 125 mg po q 6 hours x 14 days
Severe, Complicated*	WBC > 15,000 **OR** Creatinine > 1.5x pre-morbid level **OR** Hypotension /Shock *patients are excluded from the study if they have toxic megacolon	• Vancomycin 125 mg po q 6 hours x 14 days • Metronidazole 500 mg IV q8 hours • If ileus, may add rectal vancomycin if okay with GI/surgical service

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