Phase 1/2 Placebo Controlled, Partially-Blinded Clinical Trial to Assess the Safety and Efficacy of Microbial Restoration by Enema with Banked and Thawed Processed Stool in Individuals with One or More Recurrences of *Clostridium difficile* Associated Disease (CDAD)

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Statement of Compliance

The study will be carried out in accordance with Good Clinical Practices (GCP) as required by the following:

- United States Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 54, 21 CFR Part 56, and 21 CFR Part 312);
- International Conference on Harmonization (ICH) E6; 62 Federal Register 25691 (1997);
- National Institutes of Health (NIH) Clinical Terms of Award, as applicable.

Compliance with these standards provides public assurance that the rights, safety and well-being of study subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki.

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

Signature Page

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Site Investigator Signature:

Signed: _____ Date: _____

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List of Abbreviations

AE	Adverse Event/Adverse Experience
AESI	Adverse Event of Special Interest
ALT	Alanine Aminotransferase
BM	Bowel movement
CDAD	Clostridium *difficile* Associated Disease
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CPT	Cell Preparation Tube
CRE	Carbapenem-Resistant Enterobacteriaceae
CRF	Case Report Form
CRO	Contract Research Organization
CSR	Clinical Study Report
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
EDTA	Ethylenediaminetetraacetic acid
ESBL	Extended Spectrum Beta-Lactamase
FDA	Food and Drug Administration
FWA	Federalwide Assurance
FMPE	Fecal Microbiota Preparation Enema
FMPP	Fecal Microbiota Preparation Placebo
FMT	Fecal Microbiota Transplant
GCP	Good Clinical Practice
HDL	High-Density Lipoproteins
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
IBS	Irritable Bowel Syndrome
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board

ISM	Independent Safety Monitor
JAMA	Journal of the American Medical Association
LAR	Legally Authorized Representative
LDL	Low-Density Lipoproteins
MedDRA®	Medical Dictionary for Regulatory Activities
MDRO	Multi-Drug Resistant Organisms
MOP	Manual of Procedures
MRSA	Methicillin-Resistant Staphylococcus Aureus
N	Number (typically refers to subjects)
NCI	National Cancer Institute, NIH, DHHS
NDA	New Drug Application
NEJM	New England Journal of Medicine
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
NOCMC	New-Onset Chronic Medical Condition
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PCR	Polymerase Chain Reaction
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
SST	Serum Separator Tube
TG	Triglycerides
TNF	Tumor Necrosis Factor
US	United States
VRE	Vancomycin-Resistant Enterococcus
WHO	World Health Organization

Protocol Summary

Title:	Phase 1/2 Placebo Controlled, Partially-Blinded Clinical Trial to Assess the Safety and Efficacy of Microbial Restoration by Enema with Banked and Thawed Processed Stool in Individuals with One or More Recurrences of *Clostridium difficile* Associated Disease (CDAD).
Phase:	1/2
Population:	162 (108 in the fecal microbiota transplant (FMT) group, 54 in the placebo group) male or female subjects, 18 years or older, previously diagnosed with recurrent *Clostridium difficile*-Associated Disease (CDAD).
Number of Sites:	Multi-center study, up to 3 VTEU sites
Study Duration:	3 years
Subject Participation Duration:	Approximately 1 year.
Description of Agent or Intervention:	Fecal Microbiota Preparation-cryopreserved filtered human feces.
Objectives:	**Study Objectives** To investigate the safety and efficacy of fecal microbiota transplantation (FMT) delivered via enema after thawing of frozen, banked fecal microbiota to patients with recurrent *Clostridium difficile*-Associated Disease (CDAD). **Primary Objectives:** • To evaluate the safety of FMT(s) delivered by enema vs. placebo delivered by enema. • To determine efficacy of FMT delivered by enema vs. placebo delivered by enema.

Secondary Objectives:

- To evaluate the sustained clinical response rate of FMTs delivered by enema vs. placebo delivered by enema.
- To evaluate the rate of recurrent CDAD.
- To evaluate the time to recurrent CDAD.

Exploratory Objectives:

- To evaluate changes in the fecal microbiome as a function of clinical response and CDAD recurrence.
- To determine the rate of development of metabolic syndrome.

Primary Safety Outcome Measures:

- Number of subjects with an AE through 30 days after completing treatment for recurrent CDAD.
- Number of subjects with a SAE through 365 days after completing treatment for recurrent CDAD.
- Number of subjects with a new onset of related chronic medical condition through 365 days after completing treatment for recurrent CDAD.
- Number of subjects with newly acquired transmissible infectious diseases which are considered adverse events of special interest (AESI) through 365 days after completing treatment for recurrent CDAD.

Primary Efficacy Outcome Measures:

- Proportion of subjects with clinical response through Day 30 (±3) after randomization.

  Clinical response is defined as those subjects who have no recurrence of CDAD through Day 30 (±3) after completing treatment for recurrent CDAD..

  CDAD is defined as bowel movements as determined by ≥3 unformed stools (soft or watery; e.g., take the shape of

the container in which collected) within 24 consecutive hours with a positive PCR test for *Clostridium difficile*.

Secondary Efficacy Outcome Measures:

- Proportion of subjects with sustained clinical response through Day 60 (±5) after randomization.

- Sustained clinical response is defined as those subjects who responded by Day 30 with no recurrence of CDAD through Day 60 after randomization

- Number of recurrences of CDAD through Days 30 and 60 after completing treatment for recurrent CDAD.

  The date of "completing treatment for recurrent CDAD" is the date of the effective enema OR if there is no effective enema then the date of last ineffective enema. An effective enema is defined as the enema followed by no diarrhea by Day 8 OR with diarrhea at Days 5-8 but without a positive PCR test for *C. difficile*. An ineffective enema is defined as the enema followed by diarrhea at Days 5-8 but with a positive PCR test for *C. difficile*. The number of enemas allowed differs between the placebo and the FMT group as shown in the schema shown in Figure 1.

- Time (in days) from randomization until the study day when first CDAD reoccurred (through 60 days).

Exploratory Outcome Measures:

- Changes in gut microbial diversity through 365 days after completing treatment for recurrent CDAD.

- Number of subjects with abnormal clinical laboratory tests (hematology, biochemistry) through 30 days after completing treatment for recurrent CDAD.

- Number of subjects with new onset metabolic syndrome through 365 days after completing treatment for recurrent CDAD.

  Clinical manifestations of Metabolic Syndrome are: Hypertension, Hyperglycemia, Hypertriglyceridemia, Reduced HDL-C, abdominal obesity.

- Agreement of PCR test results and toxin assay results in subjects with a clinical suspicion of CDAD

Description of Study Design:

This multi-center, randomized, partially blinded trial will compare the safety and efficacy of FMT given by enema in subjects 18 years of age or older with recurrent CDAD (i.e. ≥2 episodes of CDAD within the previous 12 months, including the last episode). Subjects must meet inclusion criteria, no exclusion criteria, and diarrheal symptoms must be controlled (<3 unformed stools per a 24 consecutive hour period) off treatment prior to randomization. For the most recent episode subjects must have completed, prior to enrollment, a course of at least 10 days of oral vancomycin, oral/IV metronidazole or oral fidaxomicin.

Subjects will then be randomized in a 2:1 ratio at each site to 1 of 2 treatment groups:

- FMT by enema: 100 grams of thawed processed stool diluted into 250 ml of saline and delivered by retention enema (target dwell time of 1-3 hours) given 1-3 hours after loperamide 4 mg po x 1.

- Placebo by enema: 250 ml of saline and delivered by retention enema (target dwell time of 1-3 hours) given 1-3 hours after loperamide 4 mg po x 1.

Subjects will be evaluated through 60 days after randomization for clinical response (see definition under endpoints/outcome measures above).

Subjects with a clinical response by Day 8 and 30 after randomization CDAD will be evaluated for sustained clinical response through 60 days after randomization.

Subjects without a clinical response between Day 5-8 after randomization will have

- FMT group: 10-14 days of vancomycin followed by a washout period of at least 2 but no more than 4 days followed by FMT+ enema. If there is no clinical response at days 5-8 after a second FMT, the subject will be referred for treatment from locally available options but will be followed long-term (up to 1 year) only for safety.

- Placebo group: 10-14 days of vancomycin followed by a washout period of at least 2 but no more than 4 days followed by FMT+ enema. If no clinical response at days 5-8 after FMT+ enema, subject will receive one additional FMT by enema after 10-14 days of vancomycin with a washout period of at least 2 but no more than 4 days. If there is no clinical response at days 5-8 after a second FMT, the subject will be referred for treatment from locally available options but will be followed long-term (up to 1 year) only for safety.

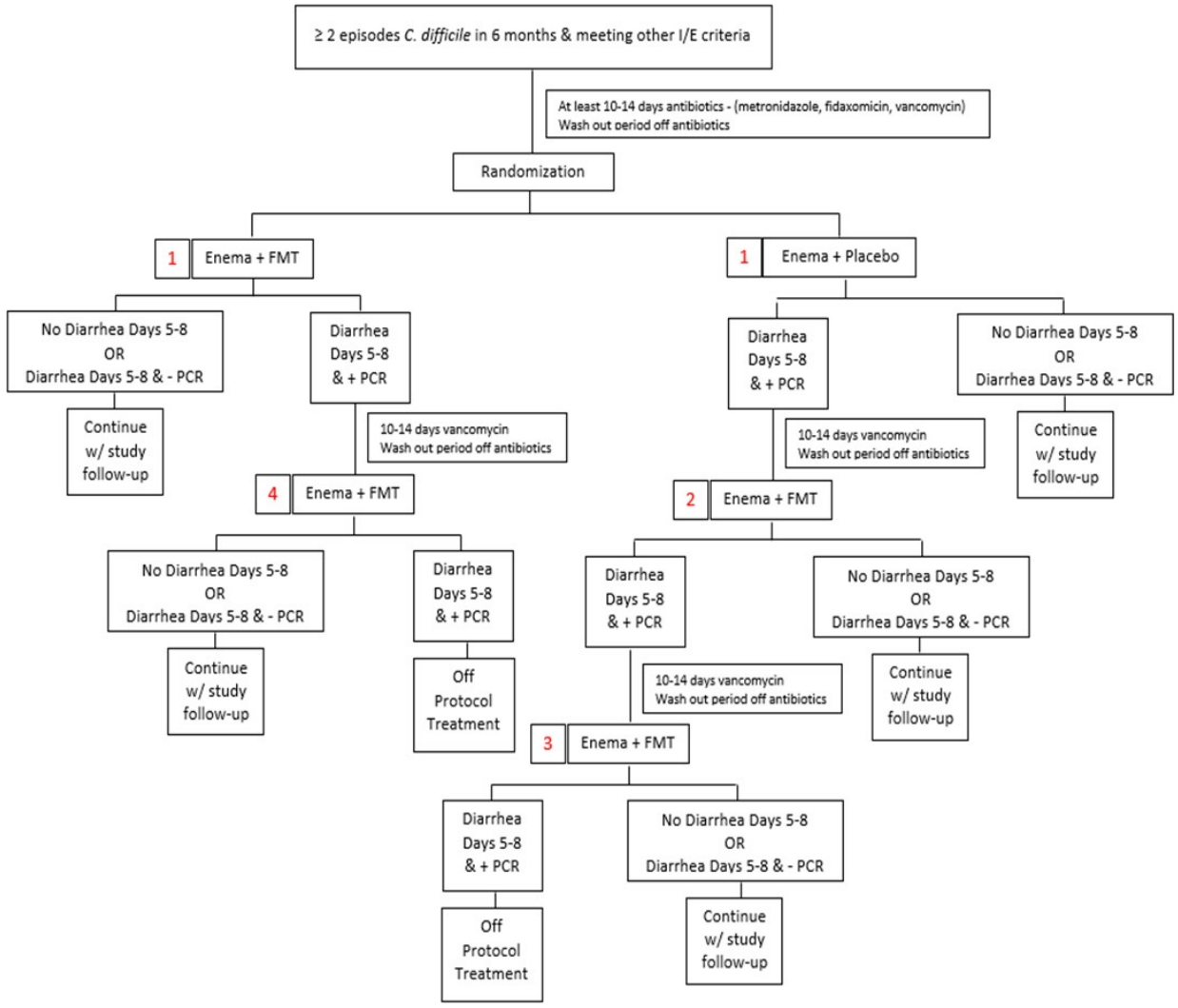
Subjects will be followed for safety for 1 year after completing treatment for recurrent CDAD (FMT and Placebo).

Stool samples will be collected at screening, enrollment and at Days 9, 30, 60 and 365 after completing treatment for recurrent CDAD and at the time of recurrence (for subjects who experience a recurrence). Stool samples will be collected if the subject experiences recurrence of CDAD at any time through 60 days after completing treatment for recurrent CDAD. Samples will be used for *C. difficile* PCR and toxin testing (when symptoms of CDAD are present), microbiome determination and other exploratory microbiological endpoints. An aliquot of stool will also be stored for determination of adverse events of special interest.

Information regarding the co-administration of any oral or parenteral antibacterials or probiotics, any acid blockers (H2 blockers and PPIs), NSAID, antineoplastic, lipid lowering agents, antihistamines or any antiperistaltics will be recorded through 365 days after completing treatment for recurrent CDAD.

Estimated Time to Complete Enrollment: 2 years

Figure 1: Schematic of Study Design



Scenarios:

1= Receipt of placebo or FMT with no diarrhea or diarrhea with negative *C. difficile* testing by Day 8 after randomization.

2= Receipt of placebo with diarrhea and positive *C. difficile* testing Days 5-8 after randomization followed by no diarrhea or diarrhea with negative *C. difficile* testing by Day 8 after FMT.

3= Receipt of placebo with diarrhea and positive *C. difficile* testing Days 5-8 after randomization followed by diarrhea and positive *C. difficile* testing 5-8 days after first FMT.

4= Receipt of FMT with diarrhea and positive *C. difficile* testing Days 5-8 after randomization

Note: If diarrhea develops after Day 8 and is *C. difficile* positive, a referral to standard medical care will be initiated and patient will follow up as needed

Washout period is defined as at least 2 days without CDAD treatment

1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Clostridium difficile is an anaerobic, gram-positive, spore-forming, toxin-producing bacillus acquired through ingestion of spores after disruption of normal gut flora typically from exposure to antimicrobials.

Clostridium difficile associated disease (CDAD) can be mild, but can progress to a severe disease with pseudomembranous colitis and toxic megacolon requiring colectomy (Dallal et al., 2002) and even resulting in death.

In recent years, there has been a change in the epidemiology of CDAD. Currently, CDAD is the leading nosocomial infection in the United States (Miller et al., 2011). The Centers for Disease Control and Prevention estimate that there are 453,000 cases annually in the US resulting in 29,000 deaths (Lessa et al., 2015). Based on Ohio estimates where CDAD reporting is mandatory, there are 333,000 initial and 145,000 recurrent healthcare facility-onset CDAD cases occurring annually in the US (Campbell et al., 2009). Also, CDAD is increasingly reported in populations thought previously to be at low risk for infection such as young, healthy persons living in the community and peripartum women (Dial et al., 2005, Chitnis et al., 2013, Rouphael et al., 2008). The attributable mortality increased from 5.7 to 23.7 deaths per 1 million persons from 1999 to 2004 (Redelings et al., 2007). CDAD results in $4.8 billion in excess costs in US acute care facilities annually (Dubberke et al., 2012); each episode costing $6,774-$10,212 for CDAD requiring admission, $2,992-$29,000 for hospital-acquired CDAD (Gabriel et al., 2014). The increase in severity and incidence of CDAD is due mostly to the emergence of an epidemic strain designated as North American PFGE pulse-field type 1 [NAP1]/restriction endonuclease analysis type BI/PCR ribotype 027 or NAP1/BI/027 (McDonald et al., 2005). The epidemic strain is characterized by fluoroquinolone resistance and higher levels of toxin production (Warny et al., 2005) than conventional strains, causing a 3-fold higher mortality rate than matched controls infected with less virulent strains (Pepin et al., 2005).

Permanent cure of CDAD requires some combination of immunity and restoration of normal colonic microflora, needed to eliminate or suppress CDAD. Antibiotic treatment of *C. difficile* helps to ameliorate symptoms while allowing gradual restoration of the normal gut flora, but may in itself further alter the colonic microbiota. Most initial episodes respond to either fidaxomicin (Louie et al., 2011), vancomycin (Zar et al., 2007), or metronidazole (Wenisch et al., 1996, Teasley et al., 1983) courses. However, CDAD recurrence is common (Johnson, 2009) with 15%–30% of patients experiencing a relapse in symptoms after effective initial therapy, usually in the first few weeks after treatment is discontinued.

Treatment of recurrent CDAD is challenging. The first recurrence can be treated with the same antibiotic used for the initial episode. Unfortunately, a significant rate of further episodes follows a first recurrence (35-45% (Kelly et al. 2008) and up to 65%, in one study (McFarland et al., 2002)). Management strategies for recurrent *C. difficile* infection include tapered and pulsed dosing of vancomycin (Tedesco et al., 1985), occasionally followed by rifaximin chase (Johnson et al., 2007), or use of probiotics (Surawicz et al., 2000), as well as IVIG (Abougergi et al., 2011) as well as human monoclonal antibodies (Wilcox et al., 2017; Lowy et al., 2010) , all with variable success rates. While fidaxomicin was shown to reduce initial relapse rate of CDAD, it failed to alter the recurrence rate of CDAD caused by the hypervirulent strain (Louie et al., 2011). Since the pathogenesis of CDAD is related to alteration of intestinal microbiota commonly caused by the use of broad spectrum antibiotics, restoration of the intestinal flora should offer a durable cure in recurrent CDAD (Lawley et al., 2012). Marked reduction in the bacterial species diversity is seen in patients with CDAD, particularly those with recurrent infections. The reduction is seen at the phylum level with Bacteriodetes, normally one of the two dominant phyla in the colon, replaced by minor constituents of the colon microbiota such as members of the Proteobacteria and Verrucomicrobia phyla (Chang et al., 2008). Using deep sequencing of the 16S rRNA gene, clinical cure of CDAD was associated with an increase in diversity and richness after fecal microbiota transplantation (FMT) in which a healthy ecosystem is transplanted directly into the colon (Shahinas et al., 2012, Hamilton et al., 2013), providing evidence on why FMT would be the ideal strategy for the treatment of recurrent CDAD.

2.2 Rationale

Fecal microbiota transplantation (FMT), the reconstitution of normal flora by a "stool transplant" from a healthy individual to a *C. difficile* -infected recipient, was first described in 1958 (Eiseman et al., 1958). The procedure is quite effective and can be done through different routes: enema (the most common technique used until 1989) nasogastric or nasojejunal tube, upper tract endoscopy since 1991 and colonoscopy since 1998 (Moore et al., 2014). In the first randomized clinical trial, there was resolution of recurrent CDAD in 81% of patients after one infusion with donor feces versus 31% of patients treated with vancomycin alone and 23% of patients receiving vancomycin and bowel lavage (Van Nood et al., 2013). Four out of five other randomized clinical trials showed 80-93% efficacy for FMT against recurrent CDAD (Cammarota et al., 2014, Youngster et al., 2014, Lee et al., 2016, Kelly et al., 2016). Only one clinical trial (Hota et al., 2017) was terminated at interim analysis for lack of efficacy; however, the study offered a single FMT administration with longer timeframe for FMT preparation, randomized patients with active CDAD, did not use a bowel preparation, and 37.5% of patients were not able to retain at least 80% of the enema therefore receiving less than 40 grams of transplanted stools. Another larger trial using the enema route for FMT administration enrolled 232 patients who received 1 to 5 FMT either from frozen or fresh stools with an efficacy of more than 83% per protocol for

both arms. However the study had a short follow up period (Lee et al., 2016). A recent systemic review of 536 patients treated with FMT showed 87% resolution of diarrhea. Diarrhea resolution rates varied according to the site of infusion: 81% in the stomach; 86% in the duodenum/jejunum; 93% in the cecum/ascending colon; and 84% in the distal colon (Cammarota et al., 2014) and amount of fecal material transplanted.

Despite its efficacy, FMT has been initially slow to be widely adopted in the treatment of recurrent CDAD due to safety and acceptability concerns but also logistical challenges and lack of standardization (Bakken et al., 2011).

FMT is generally well tolerated and safe with transient abdominal discomfort and bloating after the procedure. However little data on long-term safety exist; the majority of patients receiving FMT were typically being followed for more than 2 months but less than a 1 year.

Although reluctance initially present, the aesthetically unpleasing aspect of the procedure did not alter the patients' interest or willingness to try FMT (Zipursky et al, 2012) and currently physicians are also more willing to refer patients with recurrent CDAD for FMT evaluation (Jiang et al., 2013).

Centralization of the screening and processing steps would make the treatment cheaper, safer, less variable and more convenient with a readily available pool of donors (Bakken et al., 2011). Though no head-to-head trial has compared different routes of FMT administration, transplant by lower route seems to be superior in efficacy. Colonoscopy, with its ability to deliver enough volume of FMT throughout the entire colon is appealing; however, the procedure is time consuming, resource intensive and may be unsafe in active CDAD by increasing the risk of perforation or with the risk of sedation associated with the procedure. Rectal enema on the other hand is a simple and cheap procedure not requiring an endoscopist, or anesthetist. However, since enemas only reach the splenic flexure, there may be a need for repeated instillation.

Further research is needed to explore the optimal preparation, route, timing, and number of administrations as well as the long term safety of FMT.

The current study will use standardized frozen fecal samples transplanted by enema route after bowel preparation, allowing multiple FMT administrations and looking both at the efficacy and long term safety of FMT in patients with recurrent CDAD.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

In the first randomized controlled trial on FMT (van Nood et al, 2013), mild diarrhea (94%), abdominal cramping (31%) and belching (19%) were observed on the day of colonoscopic infusion. These symptoms resolved within 3 hours. During follow-up, three patients who were treated with donor feces (19%) had constipation. No other adverse events related to the study treatment were reported. The other randomized clinical trials yielded similar findings. The long-term follow up related to FMT is not well known. Only one retrospective multi-center study on 77 patients looked at the long term safety profile of FMT performed by colonoscopy, with a follow up varying between 3 to 68 months (Brandt et al., 2012). Seven deaths were noted, none related to FMT (Brandt et al., 2012). Autoimmune diseases (Vrieze et al., 2013) including peripheral neuropathy, Sjogren's syndrome, idiopathic thrombocytopenic purpura and rheumatoid arthritis have been reported in four patients seen in long-term follow-up (Brandt et al., 2012). Interestingly, two patients had an improvement in their pre-existing allergic sinusitis and arthritis associated with their FMT. Another concern is the possibility of developing metabolic syndrome (Vrieze et al., 2012). Transmission of potential infectious agents to the recipient is a concern, and though not definitively reported in the past (Schwartz et al., 2013) the FDA is now aware of bacterial infections caused by multi-drug resistant organisms (MDROs) that occurred due to transmission of a MDRO from use of investigational FMT. Two immunocompromised adults who received investigational FMT developed invasive infections caused by extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli (E.coli)*. One of the individuals died. (Center for Biologics Evaluation and Research 6/13/2019). The FDA provided additional guidelines for donor screening that specifically address risk factors for colonization with MDROs, and individuals at higher risk for colonization with MDRO's must be excluded from donation.The FDA also requires FMT donor stool testing include MDRO testing to exclude use of stool that tests positive for MDRO. The MDRO tests should at minimum include extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, vancomycin-resistant enterococci (VRE), carbapenem-resistant Enterobacteriaceae (CRE), and methicillin-resistant *Staphylococcus aureus* (MRSA).

In addition to the above testing routine screening of donors for the following agents should decrease any potential future risk: HIV type 1 and 2, Hepatitis A, -B, -C, Treponema pallidum, HTLV-1, -2, *C. difficile*, Cyclospora, Salmonella, Shigella, Campylobacter, E. coli 0157:H7, Shiga-toxin producing E. coli, Ova and enteric parasites including Isospora, Helicobacter pylori, Rotavirus, Adenovirus, Norovirus, Vibrio, Giardia lamblia, Cryptosporidium, and Microsporidia. In addition, culture based stool testing is performed on the following organisms: vancomycin-resistant enterococcus (VRE), extended spectrum beta-lactamase (ESBL), and carbapenemase producing gram-negative rods. A nasal swab culture is also preformed to rule out methicillin-resistant *Staphylococcus aureus* (MRSA).

Enema is usually well tolerated. Enema can cause nausea, vomiting, abdominal pain and diarrhea. Rarely enema can cause severe complications such intestinal bleeding, perforation or infection. For barium enema as an example, the perforation rate is less than colonoscopy (0.02 to 0.23%) but still carries a high mortality rate of 35-50% (deFeiter et al, 2006). However, a recent case series of 27 patients using FMT delivered by enema reported no complications (Kassam et al, 2012). A follow up case series (n=94) from the same group showed that 10% of the patients experienced transient constipation and excess flatulence post-FMT(s) (Lee et al., 2014). The largest FMT trial using the enema route showed that within 24 hours of the procedure 70% of subjects experienced diarrhea, 10% abdominal cramps and 5% nausea with up to 20% experiencing constipation at follow up (Lee et al, 2016).

Oral vancomycin (Vancocin® package insert, 2011) will be used after randomization and prior to repeating the FMT. Nephrotoxicity (e.g., reports of renal failure, renal impairment, blood creatinine increased) has occurred following oral Vancocin® therapy in randomized controlled clinical studies, and can occur either during or after completion of therapy. The risk of nephrotoxicity is increased in patients >65 years of age. Ototoxicity has occurred in patients receiving vancomycin. It may be transient or permanent. It has been reported mostly in patients who have been given excessive intravenous doses, who have an underlying hearing loss, or who are receiving concomitant therapy with another ototoxic agent, such as an aminoglycosides. However, subjects with concomitant antibiotic use are excluded from this study. The most common (>10%) adverse reactions associated with the use of Vancocin® in clinical trials included: nausea (17%), abdominal pain (15%) and hypokalemia (13%). Other adverse events (>5%) included peripheral edema, fatigue, fever, diarrhea, vomiting, flatulence, urinary tract infection, back pain and headache. Important/life-threatening side effects are rare (<1%) and include vasculitis, thrombocytopenia, nephrotoxicity, neurotoxicity and ototoxicity.

Loperamide is a synthetic antidiarrheal for oral use. The adverse events with an incidence of 1.0% or greater are constipation, nausea, and abdominal cramping.

GoLYTELY is an osmotic laxative, and nausea, abdominal fullness and bloating are the most common adverse reactions (occurred in up to 50% of patients) to administration of GoLYTELY. Abdominal cramps, vomiting and anal irritation occur less frequently. These adverse reactions are transient and usually subside rapidly Additionally, isolated cases of urticaria, rhinorrhea, dermatitis and (rarely) anaphylactic reaction have been reported which may represent allergic reactions.

Phlebotomy is usually well tolerated. Some subjects may get lightheaded or faint during or just after having blood drawn. Having blood drawn can be painful and can cause bruising. Bruising can be prevented or reduced by putting pressure on the site for a few minutes after the blood is drawn. Further, there is a very small risk of getting an infection at the site where the blood is

drawn. To reduce the risk of infection, sterile equipment will be used and the area where the blood will be drawn should be cleaned with an antiseptic (e.g. alcohol).

2.3.2 Known Potential Benefits

FMT efficacy has been shown in one randomized clinical trial long with many other case series and case reports to be effective in treating recurrent CDAD with an efficacy rate of 80-93% (Cammarota et al., 2014, Youngster et al., 2014, Lee et al., 2016, Kelly et al., 2016)). The use of frozen samples has also been shown to be 83-90% effective (Hamilton et al., 2012) in a small case series (n=21 patients with recurrent CDAD) and in other recent publications (n=20) (Youngster et al., 2014), (n=232) (Lee et al., 2016). A recent clinical trial showed similar efficacy for frozen versus fresh FMT in refractory or recurrent CDAD (Lee et al., 2016) using the enema route. For FMT by the enema route, 86% had no further CDAD at 6 months follow up in the largest case series to date of 94 recurrent/refractory CDAD patients (Lee et al, 2014).

3 OBJECTIVES

3.1 Study Objectives

To investigate the safety and efficacy of fecal microbiota transplantation (FMT) delivered via enema after thawing of frozen, banked fecal microbiota to patients with recurrent *Clostridium difficile*-Associated Disease (CDAD).

Primary Objectives:

- To evaluate the safety of FMT(s) delivered by enema vs. placebo delivered by enema.
- To determine efficacy of FMT delivered by enema vs. placebo delivered by enema.

Secondary Objectives:

- To evaluate the sustained clinical response rate of FMTs delivered by enema vs. placebo delivered by enema.
- To evaluate the rate of recurrent CDAD.
- To evaluate the time to recurrent CDAD.

Exploratory Objectives:

- To evaluate changes in the fecal microbiome as a function of clinical response and CDAD recurrence.
- To determine the rate of development of metabolic syndrome.

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

Primary Safety Outcome Measures:

- Number of subjects with an AE through 30 days after completing treatment for recurrent CDAD.
- Number of subjects with a SAE through 365 days after completing treatment for recurrent CDAD.
- Number of subjects with a new onset of related chronic medical condition through 365 days after completing treatment for recurrent CDAD.

- Number of subjects with newly acquired transmissible infectious diseases which are considered AESI through 365 days after completing treatment for recurrent CDAD.

Primary Efficacy Outcome Measure:

- Proportion of subjects with clinical response through Day 30 (±3) after randomization.

  Clinical response is defined as those subjects who have no recurrence of CDAD through Day 30 (±3) after completing treatment for recurrent CDAD.

  CDAD is defined as bowel movements as determined by ≥3 unformed stools (soft or watery; e.g., take the shape of the container in which collected) within 24 consecutive hours and a positive PCR test for *Clostridium difficile*.

3.2.2 Secondary Outcome Measures

Secondary Efficacy Outcome Measures:

- Proportion of subjects with sustained clinical response through Day 60 (±5) after randomization.

  Sustained clinical response is defined as those subjects who responded by Day 30 with no recurrence of CDAD through 60 days after randomization.

- Number of recurrences of CDAD through Days 30 and 60 after completing treatment for recurrent CDAD.

The date of "completing treatment for recurrent CDAD" is the date of the first effective enema OR if there is no effective enema then the date of last ineffective enema. An effective enema is defined as the enema that was followed by no diarrhea by Day 8 OR with diarrhea at Days 5-8 but without a positive PCR test for *Clostridium difficile*. An ineffective enema is defined as the enema followed by diarrhea at Days 5-8 but with a positive PCR test for *Clostridium difficile*. The number of enemas allowed differs between the placebo and the FMT group as shown in the schema shown in Figure 1.

- Time (in days) from randomization until the study day when first CDAD reoccurred (through 60 days).

3.2.3 Exploratory Outcome Measures:

- Changes in gut microbial diversity through 365 days after completing treatment for recurrent CDAD.

- Number of subjects with abnormal clinical laboratory tests (hematology, biochemistry) through 30 days after completing treatment for recurrent CDAD.
- Number of subjects with new onset metabolic syndrome through 365 days after completing treatment for recurrent CDAD.

  Clinical manifestations of Metabolic Syndrome are: Hypertension, Hyperglycemia, Hypertriglyceridemia, Reduced HDL-C, abdominal obesity.
- Agreement of PCR test results and toxin assay results in subjects with a clinical suspicion of CDAD

4 STUDY DESIGN

This is a multi-center, randomized, placebo controlled, partially blinded trial comparing the safety and efficacy of fecal microbiota transplantation versus placebo both delivered by rectal enema in subjects 18 years of age or older with recurrent CDAD (i.e., ≥ 2 episodes of CDAD within the previous 12 months, including the last episode). 162 (108 in the FMT group, 54 in the placebo group) male or female subjects will be enrolled in the study. CDAD is defined as bowel movements as determined by ≥3 unformed stools (soft or watery) within 24 consecutive hours with a positive PCR test for *Clostridium difficile*. Subjects must have had treatment for most recent CDAD with at least 10 days of either metronidazole po/IV (500 mg tid), oral vancomycin (at least 125 mg qid), or oral fidaxomicin (200 mg bid) and have no diarrheal symptoms (<3 unformed stools per 24 hour period) off antibiotics during the washout period.

Once enrolled, subjects will be randomized at each site to receive either FMT by enema or placebo by enema in a 2:1 ratio. Subjects in the FMT arm will receive FMT by enema, but if CDAD develops between Days 5-8 after the enema then the subject will receive 10-14 days of oral vancomycin . After a washout period of at least 2 but no more than 4 days without diarrheal symptoms (<3 unformed stools per 24 consecutive hour period) another FMT by enema will be administered. If subject fails the second FMT by enema, then he/she will be referred for treatment from locally available options but will be followed long-term (up to 1 year) only for safety.

The subjects in the placebo arm will crossover and receive FMT by enema if CDAD is present between Days 5-8 after receiving placebo. The subject might then receive one more FMT by enema depending on clinical response as detailed above. If the subject fails the second FMT by enema, then he/she will be referred for treatment from locally available options but will be followed long-term (up to 1 year) only for safety.

Also, if a subject in the placebo group or in the FMT arm fails after Day 8, he/she will be referred for treatment from locally available options and will be followed long-term (up to 1 year) only for safety.

The study is described as partially blinded because by design subjects are to be blinded only to a certain point such that after the second ineffective enema the subject will become aware as to which group they were originally assigned. Moreover, all subjects following scenarios 2, 3, or 4 will receive a second enema containing active FMT (FMPE) and thus will not be blinded to the nature of the second enema.

Subjects will be followed for clinical response (efficacy) and safety. The clinical response is defined as those subjects who have no recurrence of CDAD by Day 30 after randomization.

Sustained clinical response is defined as those subjects who have responded by Day 30 with no recurrence of CDAD through 60 days after randomization.

AEs will be collected from enrollment through 30 days after completing treatment for recurrent CDAD. New-onset Adverse Events of Special Interest (AESI) will be collected up to 365 days after completing treatment for CDAD (Appendix E) . SAEs will be collected through the whole study period. Investigators will look for signs and symptoms of CDAD from enrollment until 60 days after treatment of recurrent CDAD. If there is no recurrence of CDAD 60 days after treatment, subjects will be contacted every 2 months by telephone calls until 365 days after treatment of recurrent CDAD.

Blood samples will be obtained for assessment of safety and for exploratory endpoints (metabolic syndrome markers & infectious agents). The blood samples will be obtained at screening, enrollment and Days 9, 30, 60, and 365, after completing treatment for recurrent CDAD. Leftover blood specimens from the assays will be stored and may be used for future research.

Stool samples will be obtained at screening, enrollment and Days 9, 30, 60 and 365 after completing treatment for recurrent CDAD and at the time of recurrence (for subjects who experience a recurrence). Samples will be used for *C. difficile* PCR and toxin testing (when symptoms of CDAD are present), assessment of safety (infectious agents) as well as microbiome determination and other exploratory endpoints. Leftover stool specimens will be stored and may be used for future research.

Information regarding the co-administration of any oral or parenteral antibacterials or probiotics, any acid blockers (H2 blockers and PPIs), NSAID, antineoplastic, lipid lowering agents, antihistamines or any antiperistaltics will be recorded through 365 days after completing treatment for recurrent CDAD.

Note: The date of "completing treatment for recurrent CDAD" is the date of the first effective enema OR if there is no effective enema then the date of last ineffective enema. An effective enema is defined as the enema that was followed by no diarrhea by Day 8 OR with diarrhea at Days 5-8 but without a positive PCR test for *Clostridium difficile*. An ineffective enema is defined as the enema followed by diarrhea at Days 5-8 and with a positive PCR test for *Clostridium difficile*. The number of enemas allowed differs between the placebo and the FMT group as shown in the schema shown in Figure 1.

5 STUDY ENROLLMENT AND WITHDRAWAL

This study will be conducted in male and female subjects 18 years of age or older who are diagnosed with recurrent CDAD. This study is a multi-site study enrolling 162 (108 in the FMT group, 54 in the placebo group) subjects. Subjects will be recruited from within the larger health care system surrounding the clinical sites (inpatient and outpatient).

Only subjects who meet all inclusion criteria and no exclusion criteria will be eligible for enrollment into this study.

5.1 Subject Inclusion Criteria

Subjects eligible to participate in this study must meet the following inclusion criteria:

1. Providing permission to access the medical record.

2. Male or non-pregnant female 18 years or older at the time of enrollment.

3. Able to provide signed and dated informed consent.

4. ≥2 episodes of CDAD in the past 12 months, including the last episode if present at screening[1].

[1]Defined by ≥1 confirmed positive CDAD by diagnostic methods and another occurrence substantiated by medical history.

5. Completed treatment course of at least 10 days of oral vancomycin, oral/IV metronidazole, or oral fidaxomicin for the most recent episode prior to enrollment.

6. Controlled diarrheal symptoms (<3 unformed stools per 24 consecutive hour period).

7. Deemed likely to survive for 1 year after enrollment.

8. Women of childbearing potential[2] in sexual relationships with men must use an acceptable method of contraception[3] from 30 days prior to enrollment until 4 weeks after completing study treatment.

[2]Not sterilized via tubal ligation, bilateral oophorectomy, salpingectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with documented radiological confirmation test at

least 90 days after the procedure, and still menstruating or <1 year of the last menses if menopausal. Also includes females who are postmenopausal <1 year.

[3] Includes, but is not limited to, barrier with additional spermicidal foam or jelly, intrauterine device, hormonal contraception (started at least 30 days prior to study enrollment), intercourse with men who underwent vasectomy.

9. Males must agree to avoid impregnation of women between Day 1 and 28 days following each administration of the study product.

10. Negative urine or serum pregnancy test within 24 hours of enrollment and randomization.

11. Is able to provide blood and fecal specimens.

12. Is able to complete a test of comprehension.

5.2 Subject Exclusion Criteria

1. Previous FMT within the previous 12 months prior to study enrollment .

2. Any heart, lung, pancreas, or intestinal transplant recipient or any HIV positive transplant recipient[1].

[1] not excluded from the trial are subjects who are kidney, liver, or liver/kidney transplant recipients AND are more than 6 months from transplantation AND have not had a rejection episode in the past 6 months AND have been stable on immunosuppressive regimen for the past 6 months (any prescription change that is due to change of health care provider, insurance company, etc., or that is done for financial reasons, will not be considered a deviation of this criterion).

3. Requiring antibiotics in the past 2 weeks prior to receiving the enema for a condition other than CDAD or scheduled to be used in the upcoming 2 weeks.

4. Unable to tolerate enema for any reason.

5. Any GI cancer in the past 6 months or any actively treated malignancy[2,3].

[2]Not excluded from the trial are subjects with actively treated basal and squamous cell cancers without any systemic treatment.

[3]Subjects with recently treated malignancy (past 2 months) should have an absolute neutrophil count ≥ 1000 /µL since treatment. Subjects with leukemia can not be enrolled in the study.

6. Patients with a history of severe anaphylactic food allergy.

7. Patients with decompensated cirrhosis[4].

[4] Decompensated cirrhosis is defined as cirrhosis with any history of the following: variceal hemorrhage, ascites, spontaneous bacterial peritonitis, hepatocellular carcinoma, hepatorenal syndrome, or hepatopulmonary syndrome.

8.Untreated HIV disease[5].

[5]If no HIV screening results are available in the medical record from within the last six months, a HIV screening test will be performed during screening.

9. Other severe immunosuppression or immunodeficiency conditions[6].

[6]not excluded from the trial are, subjects who take daily dose of systemic corticosteroid equivalent to <20mg prednisone for any duration, or ≥20 mg prednisone for <14 days, or alternate-day corticosteroid therapy at any dose, OR methotrexate ≤0.4 mg/kg/week, OR azathioprine ≤3 mg/kg/day, OR 6-mercaptopurine ≤1.5 mg/kg/day.

10. Severe OR acute disease at the time of enrollment[7]

[7]Temperature >100.4˚F (38.0˚C) or heart rate less than 45 bpm or greater than 130 bpm, or systolic blood pressure less than 80 mm Hg or greater than 155 mm Hg, or diastolic blood pressure greater than 100 mm Hg, or at the discretion of the investigator

11. Major surgery of the GI tract in the past 2 months.

12.Having a non tolerance[8] to or any component of vancomycin, loperamide or GoLYTELY.

[8]tolerance is defined as the absence of immunoglobulin E-mediated allergy (e.g., urticaria, angioedema, bronchospasm, or anaphylaxis) and the absence of severe allergy (e.g., Stevens-Johnson syndrome/toxic epidermal necrolysis)

13. Active[9] inflammatory bowel disease (IBD) including ulcerative colitis, Crohn's disease, indeterminate colitis or celiac disease.

[9]Active IBD is defined as any IBD requiring any steroid use in the past 6 months OR any increase in dose or frequency of medications in the past 6 months (any prescription change that is due to change of health care provider, insurance company, etc., or that is done for financial reasons, will not be considered a deviation of this criterion).

14. Uncontrolled irritable bowel syndrome (IBS)[10] or any active uncontrolled gastrointestinal disorders or diseases[11]

[10] Uncontrolled IBS refers to any IBS with diarrhea on average more than once a week for the past 3 months prior to last CDAD episode.

[11]GI obstruction, ileus, gastric retention, bowel perforation, toxic colitis or toxic megacolon, persistent infectious gastroenteritis, persistent or chronic diarrhea of unknown etiology, or refractory/severe Clostridium difficile infection (severe CDAD identified as leukocytosis with a white blood cell count greater than 15,000 cells/mL or an increase in the serum creatinine level to 1.5 times the premorbid level), chronic diarrhea of unknown cause for 6 weeks or more.

15. Unable to comply with protocol requirements.

16. Participation in any other clinical drug research trial within 30 days prior to enrollment or for 1 year after enrollment that might interfere with the safety and efficacy assessment.

17. A condition that would jeopardize the safety or rights of the subject, would make it unlikely for the subject to complete the study, or would confound the results of the study.

5.3 Treatment Assignment Procedures

5.3.1 Randomization Procedures

Randomized treatment assignments will be generated by a statistician at The Emmes Corporation, the statistical and data coordinating center (SDCC) for this study, in a 2:1 ratio for the group receiving FMT and the group receiving placebo, respectively. A stratified, permuted block randomization will be used. Stratification will be by enrolling site.

Subjects will be randomized using The Emmes Corporation's Internet Data Entry System (IDES).Upon entry of demographic data and confirmation of eligibility for the trial, IDES will assign each subject a treatment code from the list. The unblinded pharmacist at each site will be provided with a treatment key, which links the treatment code to the actual treatment assignment, which will be kept in a secure place.

Instructions for use of the enrollment module are included in the IDES User's Guide. Manual back-up randomization procedures are provided in the MOP for use in the event that the site temporarily loses access to the Internet or the online enrollment system is unavailable.

5.3.2 Masking Procedures

This is a partially blinded study where the subject and the investigator will be blinded to the treatment assignment. The enemas will be packaged to appear identical and the placebo enema fluid itself will be colored to match the active study product. For the first enema visit, an unblinded RN under the supervision of an unblinded investigator will perform the enema. The unblinded staff and investigator will not be involved in any subsequent study assessment.

The randomization schedule will be maintained by the Emmes Corporation. Study subjects or (legally authorized representative (LAR) will remain blinded and not be provided any information until all subjects have completed the trial and the database has been locked. However, the study is designed to be blinded only to a certain point because after the second ineffective enema the subject will become aware as to which group they were originally assigned (FMPE vs. FMPP).). Moreover, all subjects assigned to scenarios 2, 3, or 4 will receive a second enema containing active FMT (FMPE) and thus will no longer be blinded. The analysis for FMPE efficacy will include clinical response at Day 30 (primary) and Day 60 (secondary) after randomization. The cross-over study design impacts blinding and the primary efficacy analysis will only include results after a single enema.

5.3.3 Reasons for Withdrawal

A subject may withdraw from the study at any time for any reason, without any consequence. Subjects may also withdraw voluntarily from receiving the study product for any reason. A subject may withdraw or be withdrawn from the study by the Investigator for the following reasons:

- Adverse event that may make it no longer in the best interest of the subject to continue participation in the study
- Subject choice (withdrawal of consent)
- Protocol violation/non-compliance
- Pregnancy
- Lost to follow up
- Other reasons as determined by the investigator

5.3.4 Handling of Withdrawals

The primary reason for withdrawal from the study will be recorded on the Study Status CRF page.Subjects will be encouraged to complete the Early Termination Visit. The Early Termination Visit procedures are listed in Section 7.5. Although subjects are free to withdraw at any time, subjects will be encouraged to remain in the study for follow-up safety evaluation. Every attempt should be made to follow all AEs and SAEs ongoing at the time of early withdrawal to resolution or until stabilized.

Subjects who discontinue the study early will not be replaced.

5.3.5 Termination of Study

Although the study Sponsor has every intention of completing the study, it reserves the right to terminate the study at any time for clinical or administrative reasons.

In addition, this study may be halted early based on interim safety and efficacy analyses as per the DSMB charter or FDA recommendations.

5.3.6 Sharing treatment assignments

Study treatment assignments will be shared with participants to aide with future medical care and treatment of CDAD. Treatment assignments will be shared after the database is locked but prior to CSR finalization. Study team will contact participants via phone using an IRB-approved phone script and will be given the option to choose whether or not to receive study treatment assignment (i.e., placebo or FMT). For participants who choose to receive study treatment assignment, an IRB-

approved addendum to the ICF will be presented orally over the telephone to verbally consent the participant prior to unblinding. The ICF addendum will outline the rationale for unblinding as stated above. After reviewing the addendum and receiving verbal consent from the participant, the study team will proceed with the unblinding process to reveal the study treatment assignment.

Treatment assignment will be provided by mail or encrypted e-mail depending on participant's preference. The participant will be provided with site PI contact information in case there are further questions.

Family of deceased participants will not be contacted.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

Fecal Microbiota Preparation Enema (FMPE)

Fecal microbial transplant (FMT) is the process by which the reconstitution of normal flora is delivered by a "stool transplant" from a healthy individual to an individual who has experienced ≥2 episodes of CDAD. The FMPE will be used for the FMT process and is described as human feces filtered to 330 microns suspended in sterile Sodium Chloride (0.9% USP), Glycerol (12.5%, USP) and deionized water.

All donors are screened for Multidrug-resistance Organism (MDRO) including the extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, vancomycin-resistant enterococci (VRE), and carbapenem-resistant *Enterobacteriaceae* (CRE), and Methicillin-resistant *Staphylococcus aureus* (MRSA).

Fecal Microbiota Preparation Placebo (FMPP)

The FMPP by enema will be Sodium Chloride (0.9%, USP), Glycerol (12.5%, USP), 8-12 drops brown food coloring (AmeriColor 204) (<1%) and deionized water.

Vancomycin

Vancomycin is a tricyclic glycopeptide antibiotic and is a product of the organism *Amycolatopsis orientalis*. The bactericidal action of vancomycin against the vegetative cells of *C. difficile* results primarily from inhibition of cell-wall biosynthesis. In addition, vancomycin alters bacterial cell membrane permeability and RNA synthesis.

Loperamide

Loperamide hydrochloride, 4-(p-chlorophenyl)-4-hydroxy-N,N-dimethyl-a,a-diphenyl-1-piperidinebutyramide monohydrochloride, is a synthetic antidiarrheal for oral use. Loperamide prolongs the transit time of the intestinal contents. It reduces daily fecal volume, increases the viscosity and bulk density, and diminishes the loss of fluid and electrolytes.

GoLYTELY

GoLYTELY oral solution is a combination of polyethylene glycol 3350, osmotic laxative, and electrolytes. It will be used for the bowel preparation and is indicated for bowel cleansing prior to colonoscopy and barium enema X-ray examination.

6.1.1 Acquisition

FMPE and FMPP will be provided by OpenBiome and upon request by DMID, will be transferred to the following address:

DMID-Clinical Materials Services (CMS)
Fisher BioServices

Vancomyin, loperamide, GoLYTELY will be supplied by Fisher BioServices. Study product(s) will be shipped to the investigational site upon request and approval by DMID.

6.1.2 Formulation, Packaging, and Labeling

Fecal Microbiota Preparation

- **Enema (FMPE)**

  The FMT study product, FMPE, will be formulated as 100 grams of human feces homogenized in 250 mL of sterile Sodium Chloride (0.9%, USP), Glycerol (12.5%, USP) and deionized water. The active product will be packaged in a PET Nalgene Bottle with HDPE screw-cap.

- **Placebo (FMPP)**

  The placebo, FMPP, will be formulated at 250 mL sterile Sodium Chloride (0.9%, USP), Glycerol (12.5%, USP), brown food coloring (<1%) (AmeriColor 204 or similar), and

deionized water. The placebo product will be packaged in an identical container as the active product.

Vancomycin

Vancomycin will be supplied as 125-mg capsules for oral administration.

Loperamide

Loperamide will be supplied as 2-mg capsules for oral administration.

GoLYTELY

GoLYTELY solution contains: 236 grams of polyethylene glycol (PEG) 3350, 22.74 grams sodium sulfate (anhydrous), 6.74 grams of sodium bicarbonate, 5.86 grams of sodium chloride, and 2.97 grams of potassium chloride. It will be supplied as a 4-L jug containing white powder for reconstitution with water.

All study products will be labeled according to manufacturer specifications and include the statement "Caution: New Drug – Limited by Federal (or United States) Law to Investigational Use."

6.1.3 Product Storage and Stability

Fecal Microbiota Preparation (FMPE)

Store FMPE immediately at -80°C +/- 10°C. Prior to shipment to the clinical research site, Fecal Microbiota Preparation for FMT will be stored at -80°C +/- 10°C. Upon receipt locally at each participating VTEU, product will be stored in a -80°C +/- 10°C freezer. Each unit bottle is labeled with an expiration date if stored at -80°C +/- 10°C. Once thawed, material may NOT be refrozen and may remain for up to 4 additional hours at room temperature or 8 hours refrigerated. Material may be stored for 24 months at -80°C +/- 10°C. This -80°C +/- 10°C storage expiration date is found on the unit ID label affixed to each bottle. Use FMPE prior to the expiration date.

Fecal Microbiota Preparation Placebo (FMPP)

Store FMPP immediately at -80°C +/- 10°C . Prior to shipment to the clinical research site, Placebo (FMPP) for FMT will be stored at -80°C +/- 10°C . Upon receipt locally at each participating VTEU, product will be stored in a -80°C +/- 10°C freezer. Each unit bottle is labeled with an expiration date if stored at -80°C +/- 10°C . Once thawed, material may NOT be

refrozen and may remain for up to 4 additional hours at room temperature or 8 hours refrigerated.

FMPP Expiration Dates: Store placebo treatment units at 80°C +/- 10°C for up to 24 months from the date of production. This -80°C +/- 10°C storage expiration date is found on the unit ID label affixed to each bottle. Use FMPP prior to the expiration date.

Loperamide

Loperamide capsules must be stored at 20° to 25°C (68° to 77°F).

Vancomycin

Vancomycin capsules must be stored at 20° to 25°C (68° to 77°F).

GoLYTELY

GoLYTELY must be stored in sealed container at 15° to 30°C (59° to 86°F). Upon reconstitution, keep solution refrigerated. Use within 48 hours of reconstitution. Discard unused portion.

6.2 Dosage, Preparation and Administration of Study Intervention/Investigational Product

FMPE and Placebo (FMPP) must be prepared on the same day as administration. To avoid contamination and risk, standard protocols for handling biohazardous material will be employed. Additionally, during material transfer peri-procedure, sterile microbiological technique will be employed.

6.2.1 FMPE by Enema

FMPE by enema: Bowel preparation with GoLYTELY is performed one day prior to administration of enema.On the day of FMT, loperamide 4mg administered orally as a single dose will be given 1-3 hours prior to administration of the enema.

The FMPE is thawed over the course of 1 hour in a warm water bath (approximately 30°C) or at room temperature for 4.5 hours. After thawing, swirl moderately for 10 seconds to resuspend particulates and transfer to an enema container.

The FMPE will be administered rectally by retention enema (target dwell time of 1-3 hours).

The procedure is performed by nursing staff under investigator's supervision.

6.2.2 Placebo (FMPP) by Enema

Placebo (FMPP) by enema: Bowel preparation with GoLYTELY is performed one day prior to administration of enema. On the day of FMT, loperamide 4mg administered orally as a single dose will be given 1-3 hours prior to administration of the enema.The placebo (FMPP) is thawed over the course of 1 hour in a warm water bath (approximately 30 C) or at room temperature for 4.5 hours. After thawing, swirl moderately for 10 seconds to resuspend particulates and transfer to an enema container.

The placebo will be administered rectally by retention enema (target dwell time of 1-3 hours).

The procedure is performed by nursing staff under investigator's supervision.

6.2.3 Loperamide

Loperamide 4 mg will be administered orally as two-2mg capsules given as a single dose at least 1-3 hours prior to the administration of the enema.

6.2.4 Vancomycin

Vancomycin 125 mg will be administered orally as one 125mg capsule four times daily for 10-14 days, if CDAD is present at 5-8 days after fecal/placebo enema.

6.2.5 GoLYTELY

Fill the supplied container with lukewarm water (to facilitate dilution) to the 4 liter fill line. The solution is clear and colorless when reconstituted to a final volume of 4 liters. After capping the container, shake vigorously several times to ensure the ingredients are dissolved. Instruct subject to drink a total of up to 4 liters at a rate of 240 mL (8 oz.) every 10 minutes, until 4 liters are consumed or the rectal effluent is clear. Rapid drinking of each portion is preferred to drinking small amounts continuously.

6.3 Modification of Study Intervention/Investigational Product for a Participant

If the subject is unable to retain more than approximately 50% of the enema within 1-3 hours, then the procedure is repeated the same day, after additional antiperistaltic agent is given. No further enema will be given if the second enema is incomplete or ineffective. Subjects who are unable to retain the first or second enema will not be replaced and will continue as study subjects

but future specimens won't be collected from these subjects but they will be followed long-term (up to 1 year) only for safety.

6.4 Accountability Procedures for the Study Intervention/Investigational Product(s)

The principal investigator (PI) (or designee) will maintain an accurate record of the receipt of the test materials as shipped by the Sponsor (or designee), including the date received. One copy of this receipt will be returned to the Sponsor when the contents of the test material shipment have been verified. In addition, the unblinded pharmacist (or designee) will maintain a log of all clinical trial materials (FMPE/placebo(FMPP)/vancomycin/loperamide/GoLYTELY) dispensed. Clinical trial materials for each subject will be inventoried and accounted for throughout the trial. Upon receipt of the study product, the PI is responsible for distribution of the study products, and has ultimate responsibility for drug accountability. Logs of study product receipt, temperature and storage conditions, maintenance, documentation of study drug dispensation, return and disposal must be maintained in the study file. This clinical trial material accountability record will be available for inspection at any time. At the completion of the study, the original drug accountability record will be available for review by the Sponsor upon request.

At the completion of the study, all unused drug supplies will be returned to the Sponsor (or designee) or disposed of by study site, after study monitoring occurs and per the Sponsor's (or designee's) written instructions.

6.5 Assessment of Subject Compliance with Study Intervention/Investigational Product

- Assessment by study staff of the retention enema is performed according to the MOP. Briefly, nursing staff will administer the study treatment by retention enema. If the subject is unable to retain more than 50% of the enema with 1-3 hours, then the procedure is repeated the same day, after additional Loperamide is given. No further enema will be given if the second enema is incomplete.
- Assessment by study staff of the completion of the vancomycin course will be done by pill count per MOP. Briefly, fifty-six (56) 125mg capsules will be required for each subject prior to the enema procedure. The capsules will be stored at the sponsor's repository and a bulk supply will be provided to the site pharmacy to dispense as needed.

6.6 Concomitant Medications/Treatments

The following concomitant medications (by prescription or over the counter) will be recorded, ranging from 90 days prior to screening and for 365 days after completing treatment for recurrent CDAD:

- Anti-infectives
- Any probiotics
- Any medications used in the treatment of CDAD
- Any acid blockers (H2 blockers and PPIs)
- Any antiperistaltics
- Any NSAID
- Any Antihistamines
- Lipid Lowering Agents
- Antineoplastic agents
- Glucocorticoids

There are no prohibited treatments/medications except for FMT outside the current study and antibiotics other than to treat CDAD in 30 days prior to receiving enema.

7 STUDY SCHEDULE

The Schedule of Events is included as Appendix A.

7.1 Screening: Day (-21 to -1)

The following assessments and procedures will be performed and noted in each subject's chart or record during Screening:

- Obtain an informed consent: following a full explanation of the study protocol, each subject must give written consent. The consent form must be signed prior to performance of any study-related activity.

- Obtain a medical history: Significant medical histories including past three years self-reported weight history, if this is not available in the medical record, will be collected from each subject before study enrollment.

- Assess inclusion/exclusion criteria: each subject will be assessed for inclusion and exclusion* criteria before study enrollment.

  *If no HIV screening results are available in the medical record from a recent time point, within the last six months, a HIV screening test will be performed during screening. If the HIV test is positive this will be reported to the Public Health Department as required by state law. The protocol investigators will privately discuss the results with the subject and notify the subject's primary physician upon request.

- Administer test of comprehension.

- Collect prior and concomitant medications.

- Evaluate for signs and symptoms of CDAD by a clinician.

- Perform a detailed physical exam: each subject will receive a physical exam including vital signs (blood pressure, pulse, weight, height and body temperature).

- Perform serum pregnancy test on females of childbearing potential.

- Obtain blood samples for laboratory tests including hemoglobin, WBC, neutrophil count, platelets, (2 mL in EDTA), ALT, creatinine, potassium (5 mL in SST) for clinical labs.

- Obtain a stool sample for *C. difficile* PCR and toxin assay, for microbiome determination, for baseline safety assessment of AESIs related to newly acquired transmissible infectious agents and for future use.

- Instruct subject on bowel preparation (GoLYTELY) on the day before enrollment and use of Loperamide the day of enrollment. The dietary guidelines will be provided for procedures at home one day before, day of and following each enema.

7.2 Enrollment Baseline Day (1)

The following assessments and procedures will be performed and noted in each subject's chart or record during enrollment:

Pre enema procedures

- Confirm inclusion/exclusion criteria: each subject will be re-assessed for inclusion and exclusion criteria before randomization.

- Obtain an interim medical history.

- Review prior and concomitant medications.

- Evaluate for absence of signs and symptoms of CDAD by a clinician.

- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight, waist circumference, and body temperature).

- Obtain blood samples for laboratory tests including metabolic syndrome markers (TG, HDL, LDL, HLD/LDL ratio, fasting glucose) (5 mL in SST), hemoglobin, WBC, neutrophil count, platelets. (2 mL in EDTA), ALT, creatinine, potassium (5 mL in SST) for clinical labs. Additional blood samples will be stored to check for infectious agents (10 mL in SST and 20 mL in EDTA) and for future use (48 mL in CPTs).

- Perform a serum or urine pregnancy test on females of childbearing potential.

- If possible, obtain stool sample for microbiome determination, for safety assessment following AESI related to newly acquired transmissible infectious agents and for future use.
- Randomize to treatment arm.
- Enema procedure per MOP.

Post enema procedures

- Collect and record adverse events during and 30 minutes following rectal enema by interviewing subject and direct observation.

- Provide a memory aid. In addition, subjects will be provided with instructions regarding description of stool form and frequency along with other symptoms.
- Inform subject to contact the site for any severe symptoms prior to the Day 4 (-1) assessment by phone. The subject will be seen in the clinic for an assessment based on the clinical judgment of the investigator.

7.3 Follow-up

7.3.1 Study Day 4 (-1) (telephone contact):

Collect and record adverse events.

- Evaluate for symptoms of CDAD (≥3 unformed stools (soft or watery) within 24 consecutive hours) by study staff and determination of clinical response by investigator.

- Review memory aid.

- Review concomitant medications.

- Instruct subject to call clinic if gastrointestinal symptoms (including diarrhea) occur between days 5-8.

7.3.2 Interim visit (Study Days 5 through 8)

Subjects who have called the clinic complaining about diarrhea in any of study Day 5, 6, 7, or 8 will be instructed to return to the clinic and:

- Confirm inclusion/exclusion criteria: each subject will be re-assessed for inclusion and exclusion criteria.

- Obtain an interim medical history.
- Collect concomitant medications.
- Evaluate for signs and symptoms of CDAD by a clinician and determination of clinical response by investigator.
- Perform a physical exam: each subject will receive a physical exam depending on interim history including vital signs (blood pressure, pulse, weight, and body temperature).
- Collect and record adverse events, including adverse events of special interest (Appendix E).
- Review memory aid.
- Perform a urine or serum pregnancy test on females of childbearing potential.
- Obtain stool samples for *C. difficile* PCR and toxin assay, for microbiome determination, for safety assessment following AESIs related to newly acquired transmissible infectious agents (as applicable per Appendix E) and for future use.
- Provide 10-14 days of oral vancomycin. If the clinical suspicion is high, vancomycin should be dispensed and started immediately and if the PCR test is positive then vancomycin should be continued.But, if the clinical suspicion is low, vancomycin will be dispensed but won't be started until the PCR results are back and subject is notified. If the subject received placebo and the PCR assay is positive, continue on to scenario 2 (see 7.3.4). If the subject received FMT and the PCR assay is positive, continue on to scenario 4 (see 7.3.5). If the PCR test is negative, continue on to scenario 1 (see 7.3.3).
- For the GoLYTELY® and loperamide - the subject will come and pick them up from the clinic at least one day prior to the enema procedure.
- If no diarrhea after completion of vancomycin, instruct subject to be off vancomycin for a washout period of at least 2 but no more than 4 days prior to next enema + FMT visit. Instruct subject on bowel preparation and use of antiperistaltic agent the day of fecal enema.
- If diarrhea persists after completion of vancomycin, instruct participant to call clinic.

7.3.3 Scenario 1: Receipt of placebo or FMT with no diarrhea or diarrhea with negative *C. difficile* testing at study days 5 through 8 after randomization (Appendix A-1):

7.3.3.1 Study Day 9 (+3) clinic visit

- Collect and record adverse events, including AESI (Appendix E) and SAEs.
- Evaluate for signs and symptoms of CDAD by a clinician and if present obtain a stool sample for PCR to determine clinical response by the investigator.
- Review memory aid.
- Obtain an interim medical history.
- Review concomitant medications.
- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight and body temperature).
- Obtain blood samples for laboratory tests: hemoglobin, WBC, neutrophil count, platelets. (2 mL in EDTA), ALT, creatinine, potassium (5 mL in SST), and additional blood samples will be stored for future use (48 mL in CPTs).
- Obtain stool samples for microbiome determination, for safety assessment following AESI related to newly acquired transmissible infectious agents (as applicable per Appendix E) and for future use.
- Instruct subject to call clinic if gastrointestinal symptoms (including diarrhea) occur.

7.3.3.2 Weekly (D15±2 and D22±2) telephone contact

- Collect and record adverse events, including AESIs (Appendix E).
- Evaluate for signs and symptoms of CDAD by a clinician and if present obtain a stool sample for PCR to determine clinical response by the investigator.
- Review memory aid.

- Review concomitant medications.
- Instruct subject to call clinic if gastrointestinal symptoms (including iarrhea) occur.

7.3.3.3 Study Day 30 (±3) clinic visit

- Collect and record adverse events, including AESIs (Appendix E).
- Evaluate for signs and symptoms of CDAD by a clinician and if present obtain a stool sample for PCR to determine clinical response by the investigator.
- Review memory aid.
- Obtain an interim medical history.
- Review concomitant medications.
- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight and body temperature).
- Obtain blood samples for laboratory tests: hemoglobin, WBC, neutrophil count, platelets. (2 mL in EDTA), ALT, creatinine, potassium (5 mL in SST). Additional blood samples will be stored for future use (48 mL in CPTs).
- Obtain stool samples for microbiome determination, for safety assessment following AESI related to newly acquired transmissible infectious agents (if applicable per Appendix E) and for future use.
- Instruct subject to call the clinic if diarrhea occurs.

7.3.3.4 Study Day 60 (±5) clinic visit:

- Collect and record serious adverse events, AESIs (Appendix E) and new onset of related chronic medical conditions.
- Evaluate for signs and symptoms of CDAD by a clinician and if present obtain a stool sample for PCR to determine clinical response by the investigator.
- Obtain an interim medical history.

- Review concomitant medications.

- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight, waist circumference and body temperature).

- Obtain blood samples for laboratory tests: hemoglobin, WBC, neutrophil count, platelets. (2 mL in EDTA), ALT, creatinine, potassium (5 mL in SST), metabolic syndrome markers (5 mL in SST). Additional blood samples will be stored for future use (48 mL in CPTs).

- Obtain stool samples for microbiome determination and for future use.

- Instruct subject to call clinic if an interim diagnosis of CDAD is made or an occurrence of SAE or if there is an occurrence of new onset of related chronic medical condition.

7.3.3.5 Every 2 months (D120±7, D180±7, D240±7, D300±7) telephone contact:

- Collect and record serious adverse events, occurrence of new onset of related chronic medical condition and AESIs (Appendix E).

- Review concomitant medications.

- Inquire if interim diagnosis of CDAD has been made.

- Instruct subject to call clinic if interim diagnosis of CDAD made or occurrence of SAE or if there is an occurrence of new onset of related chronic medical condition.

7.3.3.6 Study Day 365 (±14) clinic visit

- Collect and record serious adverse events and occurrence of new onset of related chronic medical condition and AESIs (Appendix E).

- Review concomitant medications.

- Obtain an interim medical history.

- Inquire if interim diagnosis of CDAD made.

- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight, waist circumference, and body temperature).

- Obtain blood samples for laboratory tests: hemoglobin, WBC, neutrophil count, Platelets. (2 mL in EDTA), ALT, creatinine, potassium (5 mL in SST), metabolic syndrome markers (TG, HDL, LDL, HDL/LDL ratio, fasting glucose) (5 mL in SST). Additional blood samples will be stored for future use (48 mL in CPTs).

- Obtain stool samples for microbiome determination and for future use.

7.3.4 Scenario 2: Receipt of placebo with diarrhea and positive *C. difficile* testing Days 5 through 8 after randomization followed by no diarrhea or diarrhea with negative *C. difficile* testing at study days 5 through 8 after FMT (Appendix A-2)

7.3.4.1 Enema + FMT visit (12-18 days post last enema treatment)

Pre enema procedures

- Confirm inclusion/exclusion criteria: each subject will be re-assessed for inclusion and exclusion criteria before enema +FMT.

- Obtain an interim medical history.

- Collect concomitant medications.

- Evaluate for absence of signs and symptoms of CDAD by a clinician.

- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight, waist circumference and body temperature).

- Obtain blood samples for laboratory tests including metabolic syndrome markers (TG, HDL, LDL, HDL/LDL ratio, fasting glucose) (5 mL in SST), Hemoglobin, WBC, Neutrophil Count, Platelets. (2 mL in EDTA), ALT, Creatinine, potassium (5 mL in SST). Additional blood samples will be stored to check for infectious agents (10 mL in SST and 20 mL in EDTA) and future use (48 mL in CPTs).

- Perform a urine or serum pregnancy test on females of childbearing potential.

- If possible, obtain stool samples for microbiome determination for safety assessment following AESI related to newly acquired transmissible infectious agents and for future use.

Enema procedure per MOP: enema + FMT

Post enema procedures

- Collect and record adverse events during and 30 min following rectal enema by interviewing subject and direct observation.

- Provide a memory aid. In addition, subjects will be provided with instructions regarding description of stool form, frequency and other symptoms.

- Inform subject to contact the site for any severe symptoms prior to the Day 4 (-1) assessment by phone. The subject will be seen in the clinic for an assessment based on the clinical judgment of the investigator.

7.3.4.2 Study Day 4 (-1) (post last enema treatment telephone contact):

- Collect and record adverse events

- Evaluate for symptoms of CDAD (≥3 unformed stools within 24 consecutive hours).

- Review memory aid.

- Review concomitant medications.

- Instruct subject to call clinic if gastrointestinal symptoms (including diarrhea) occur between days 5-8 after treatment of recurrent CDAD.

7.3.4.3 Interim visit (5 through 8 days post last enema treatment)

Subjects who have called the clinic complaining about diarrhea in any of study Day 5, 6, 7, or 8 will be instructed to return to the clinic and:

- Confirm inclusion/exclusion criteria: each subject will be re-assessed for inclusion and exclusion criteria.
- Obtain an interim medical history.
- Collect concomitant medications.
- Evaluate for signs and symptoms of CDAD by a clinician.
- Perform a physical exam: each subject will receive a physical exam depending on interim history including vital signs (blood pressure, pulse, weight, and body temperature).
- Collect and record adverse events, including AESIs (Appendix E).
- Review memory aid.
- Perform a urine or serum pregnancy test on females of childbearing potential.
- Obtain stool samples for *C. difficile* PCR and toxin assay, for microbiome determination, for safety assessment following AESI related to newly acquired transmissible infectious agents (as applicable per Appendix E) and for future use.
- Provide 10-14 days of oral vancomycin. If the clinical suspicion is high, vancomycin should be dispensed and started immediately and if the PCR test is positive then vancomycin should be continued and the subject moves to scenario 3 (see 7.3.6). But if the clinical suspicion is low, vancomycin will be dispensed but won't be started until the PCR results are back and subject is notified. If the PCR is negative, the subject continues in scenario 2.
- If no diarrhea after completion of vancomycin, instruct subject to be off vancomycin for a washout period of at least 2 but no more than 4 days prior to next enema + FMT visit. Instruct subject on bowel preparation and use of antiperistaltic agentthe day of fecal enema.
- If diarrhea persists after completion of vancomycin, instruct participant to call clinic.

7.3.4.4 Study Day 9 (+3) post last enema treatment clinic visit

- Collect and record adverse events, including newly acquired transmissible infectious diseases (Appendix E).

- Evaluate for signs and symptoms of CDAD by a clinician and if present obtain a stool sample for PCR to determine clinical response by the investigator.

- Review memory aid.

- Obtain an interim medical history.

- Review concomitant medications.

- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight and body temperature).

- Obtain blood samples for laboratory tests: Hemoglobin, WBC, Neutrophil Count, Platelets. (2 mL in EDTA), ALT, Creatinine, potassium (5 mL in SST), and additional blood samples will be stored for future use (48 mL in CPTs).

- Obtain stool samples for microbiome determination, for safety assessment following AESI (as applicable per Appendix E) related to newly acquired transmissible infectious agents and for future use.

- Instruct subject to call clinic if gastrointestinal symptoms (including diarrhea) occur.

7.3.4.5 Weekly (D15±2 and D22±2) telephone contact

- Collect and record adverse events, including AESIs (Appendix E).

- Evaluate for symptoms of CDAD by study staff.

- Review memory aid.

- Review concomitant medications.

- Instruct subject to call clinic if gastrointestinal symptoms (including diarrhea) occur.

7.3.4.6 Study Day 30 (±3) post last enema treatment clinic visit

- Collect and record adverse events, including AESIs(Appendix E).
- Evaluate for signs and symptoms of CDAD by a clinician.
- Review memory aid.
- Obtain an interim medical history.
- Review concomitant medications.
- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight and body temperature).
- Obtain blood samples for laboratory tests: Hemoglobin, WBC, Neutrophil Count, Platelets. (2 mL in EDTA), ALT, Creatinine, potassium (5 mL in SST). Additional blood samples will be stored for future use (48 mL in CPTs).
- Obtain stool samples for microbiome determination, for safety assessment following AESI related to newly acquired transmissible infectious agents (as applicable per Appendix E) and for future use.
- Instruct subject to call the clinic if diarrhea occurs.

7.3.4.7 Study Day 60 (±5) post last enema treatment clinic visit:

- Collect and record serious adverse events, including AESIs (Appendix E) and new onset of related chronic medical conditions.
- Evaluate for signs and symptoms of CDAD by a clinician
- Obtain an interim medical history.
- Review concomitant medications.
- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight, waist circumference and body temperature).

- Obtain blood samples for laboratory tests: Hemoglobin, WBC, Neutrophil Count, Platelets. (2 mL in EDTA), ALT, Creatinine, potassium (5 mL in SST), metabolic syndrome markers (5 mL in SST). Additional blood samples will be stored for future use (48 mL in CPTs).

- Obtain stool samples for microbiome determinationand for future use.

- Instruct subject to call clinic if an interim diagnosis of CDAD is made or an occurrence of SAE or if there is an occurrence of new onset of related chronic medical condition.

7.3.4.8 Every 2 months (D120±7, D180±7, D240±7, D300±7) telephone contact:

- Collect and record serious adverse events, occurrence of new onset of related chronic medical condition and AESIs(Appendix E).

- Review concomitant medications.

- Inquire if interim diagnosis of CDAD has been made.

- Instruct subject to call clinic if interim diagnosis of CDAD made or occurrence of SAE or if there is an occurrence of new onset of related chronic medical condition.

7.3.4.9 Study Day 365 (±14) post last enema treatment clinic visit

- Collect and record serious adverse events and occurrence of new onset of related chronic medical condition and newly acquired transmissible infectious diseases (Appendix E).

- Review concomitant medications.

- Obtain an interim medical history.

- Inquire if interim diagnosis of CDAD made.

- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight, waist circumference, and body temperature).

- Obtain blood samples for laboratory tests: Hemoglobin, WBC, Neutrophil Count, Platelets. (2 mL in EDTA), ALT, Creatinine, potassium (5 mL in SST), metabolic

syndrome markers (TG, HDL, LDL, HDL/LDL ratio, fasting glucose) (5 mL in SST). Additional blood samples will be stored for future use (48 mL in CPTs).

- Obtain stool samples for microbiome determinationand for future use.

7.3.5 Scenario 4: Receipt of FMT with diarrhea and positive *C. difficile* testing Days 5 through 8 after randomization (Appendix A-2)

7.3.5.1 Enema + FMT visit (12-18 days post last enema treatment)

Pre enema procedures

- Confirm inclusion/exclusion criteria: each subject will be re-assessed for inclusion and exclusion criteria before enema +FMT.

- Obtain an interim medical history.

- Collect concomitant medications.

- Evaluate for absence of signs and symptoms of CDAD by a clinician.

- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight, waist circumference and body temperature).

- Obtain blood samples for laboratory tests including metabolic syndrome markers (TG, HDL, LDL, HDL/LDL ratio, fasting glucose) (5 mL in SST), Hemoglobin, WBC, Neutrophil Count, Platelets. (2 mL in EDTA), ALT, Creatinine, potassium (5 mL in SST). Additional blood samples will be stored to check for infectious agents (10 mL in SST and 20 mL in EDTA) and future use (48 mL in CPTs).

- Perform a urine or serum pregnancy test on females of childbearing potential.

- If possible, obtain stool samples for microbiome determination, for safety assessment following AESI related to newly acquired transmissible infectious agents and for future use.

Enema procedure per MOP: enema + FMT

Post enema procedures

- Collect and record adverse events during and 30 min following rectal enema by interviewing subject and direct observation.

- Provide a memory aid. In addition, subjects will be provided with instructions regarding description of stool form, frequency and other symptoms.

- Inform subject to contact the site for any severe symptoms prior to the Day 4 (-1) assessment by phone. The subject will be seen in the clinic for an assessment based on the clinical judgment of the investigator.

7.3.5.2 Study Day 4 (-1) (post last enema treatment telephone contact):

- Collect and record adverse events .

- Evaluate for symptoms of CDAD (≥3 unformed stools within 24 consecutive hours).

- Review memory aid.

- Review concomitant medications.

- Instruct subject to call clinic if gastrointestinal symptoms (including diarrhea) occur between days 5-8 after treatment of recurrent CDAD.

7.3.5.3 Interim visit (5 through 8 days post last enema treatment)

- Confirm inclusion/exclusion criteria: each subject will be re-assessed for inclusion and exclusion criteriaObtain an interim medical history.

- Obtain an interim medical history

- Collect concomitant medications.

- Evaluate for signs and symptoms of CDAD by a clinician.

- Collect and record adverse events, including AESIs (Appendix E).

- Review memory aid.

- Perform a physical exam: each subject will receive a physical exam depending on interim history including vital signs (blood pressure, pulse, weight, and body temperature).

- Perform a urine or serum pregnancy test on females of childbearing potential.

- Obtain stool samples for *C. difficile* PCR and toxin assay, for microbiome determination, for safety assessment following AESI related to newly acquired transmissible infectious agents (if applicable per Appendix E) and for future use.

- Provide 10-14 days of oral vancomycin. If the clinical suspicion is high, vancomycin should be dispensed and started immediately and if the PCR is positive then vancomycin should be continued. But if the clinical suspicion is low, vancomycin will be dispensed but won't be started until the PCRresults are back and subject is notified.

- Instruct subject on bowel preparation and use of antiperistaltic agentthe day of fecal enema.

- If diarrhea persists after completion of vancomycin, instruct participant to call clinic.

7.3.5.4 Study Day 9 (+3) post last enema treatment clinic visit

- Collect and record adverse events, including AESIs (Appendix E).

- Evaluate for signs and symptoms of CDAD by a clinician and if present obtain a stool sample for PCR to determine clinical response by the investigator.

- Review memory aid.

- Obtain an interim medical history.

- Review concomitant medications.

- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight and body temperature).

- Obtain blood samples for laboratory tests: Hemoglobin, WBC, Neutrophil Count, Platelets. (2 mL in EDTA), ALT, Creatinine, potassium (5 mL in SST). Additional), and additional blood samples will be stored for future use (48 mL in CPTs).

- Obtain stool samples for microbiome determination, for safety assessment following AESI (as applicable per Appendix E) related to newly acquired transmissible infectious agents and for future use.

- Instruct subject to call clinic if gastrointestinal symptoms occur.

7.3.5.5 Weekly (D15±2 and D22±2) telephone contact

- Collect and record adverse events, including AESIs (Appendix E).

- Evaluate for symptoms of CDAD by study staff.

- Review memory aid.

- Review concomitant medications.

- Instruct subject to call clinic if gastrointestinal symptoms (including diarrhea) occur.

7.3.5.6 Study Day 30 (±3) post last enema treatment clinic visit

- Collect and record adverse events, including AESIs (Appendix E).

- Evaluate for signs and symptoms of CDAD by a clinician.

- Review memory aid.

- Obtain an interim medical history.

- Review concomitant medications.

- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight and body temperature).

- Obtain blood samples for laboratory tests: Hemoglobin, WBC, Neutrophil Count, Platelets. (2 mL in EDTA), ALT, Creatinine, potassium (5 mL in SST). Additional blood samples will be stored for future use (48 mL in CPTs).

- Obtain stool samples for microbiome determination, for safety assessment following AESI related to newly acquired transmissible infectious agents (as applicable per Appendix E) and for future use.

- Instruct subject to call the clinic if diarrhea occurs.

7.3.5.7 Study Day 60 (±5) post last enema treatment clinic visit:

- Collect and record serious adverse events, including AESIs (Appendix E) and new onset of related chronic medical conditions.

- Evaluate for signs and symptoms of CDAD by a clinician.

- Obtain an interim medical history.

- Review concomitant medications.

- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight, waist circumference and body temperature).

- Obtain blood samples for laboratory tests: Hemoglobin, WBC, Neutrophil Count, Platelets. (2 mL in EDTA), ALT, Creatinine, potassium (5 mL in SST), metabolic syndrome markers (5 mL in SST). Additional blood samples will be storedfor future use (48 mL in CPTs).

- Obtain stool samples for microbiome determinationand for future use.

- Instruct subject to call clinic if an interim diagnosis of CDAD is made or an occurrence of SAE or if there is an occurrence of new onset of related chronic medical condition.

7.3.5.8 Every 2 months (D120±7, D180±7, D240±7, D300±7) telephone contact:

- Collect and record serious adverse events, occurrence of new onset of related chronic medical condition and AESIs (Appendix E).

- Review concomitant medications.
- Inquire if interim diagnosis of CDAD has been made.
- Instruct subject to call clinic if interim diagnosis of CDAD made or occurrence of SAE or if there is an occurrence of new onset of related chronic medical condition.

7.3.5.9 Study Day 365 (±14) post last enema treatment clinic visit

- Collect and record serious adverse events and occurrence of new onset of related chronic medical condition and AESIs (Appendix E).
- Review concomitant medications.
- Obtain an interim medical history.
- Inquire if interim diagnosis of CDAD made.
- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight, waist circumference, and body temperature).
- Obtain blood samples for laboratory tests: Hemoglobin, WBC, Neutrophil Count, Platelets. (2 mL in EDTA), ALT, Creatinine, potassium (5 mL in SST), metabolic syndrome markers (TG, HDL, LDL, HDL/LDL ratio, fasting glucose) (5 mL in SST). Additional blood samples will be stored for future use (48 mL in CPTs).
- Obtain stool samples for microbiome determination and for future use.

7.3.6 Scenario 3: Receipt of placebo with diarrhea and positive *C. difficile* testing Days 5-8 after randomization followed by diarrhea and positive *C. difficile* testing 5-8 days after first FMT (Appendix A-3):

7.3.6.1 Enema + Second FMT visit (12-18 days post last enema visit)

Pre enema procedures

- Confirm inclusion/exclusion criteria: each subject will be re-assessed for inclusion and exclusion criteria before enema + FMT.

- Obtain an interim medical history.
- Collect concomitant medications.
- Evaluate for absence of signs and symptoms of CDAD by a clinician.
- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight, waist circumference and body temperature).
- Obtain blood samples for laboratory tests including metabolic syndrome markers (TG, HDL, LDL, HDL/LDL ratio, fasting glucose) (5 mL in SST), Hemoglobin, WBC, Neutrophil Count, Platelets. (2 mL in EDTA), ALT, Creatinine, potassium (5 mL in SST). Additional blood samples will be stored to check for infectious agents (10 mL in SST and 20 mL in EDTA) and for future use (48 mL in CPTs).
- Perform a urine or serum pregnancy test on females of childbearing potential.
- If possible, obtain stool samples for for microbiome determination, for safety assessment following AESI related to newly acquired transmissible infectious agents and for future use

Enema procedure per MOP: enema + FMT

Post enema procedures

- Collect and record adverse events during and 30 min following rectal enema by interviewing subject and direct observation.
- Provide a memory aid. In addition, subjects will be provided with instructions regarding description of stool form, frequency and other symptoms.
- Inform subject to contact the site for any severe symptoms prior to the Day 4 (-1) assessment by phone. The subject will be seen in the clinic for an assessment based on the clinical judgment of the investigator.

7.3.6.2 Study Day 4 (-1) (post last enema treatment telephone contact):

- Collect and record adverse events.
- Evaluate for symptoms of CDAD by study staff.
- Review memory aid.
- Review concomitant medications.

7.3.6.3 Interim visit (Study Days 5 through 8)

- Obtain an interim medical history.
- Collect concomitant medications.
- Evaluate for signs and symptoms of CDAD by a clinician.
- Collect and record adverse events, including newly acquired transmissible infectious diseases
- Review memory aid.
- Perform a physical exam: each subject will receive a physical exam depending on interim history including vital signs (blood pressure, pulse, weight, and body temperature). Perform a urine or serum pregnancy test on females of childbearing potential.
- Obtain stool samples for *C. difficile* PCR and toxin, for microbiome determination, for safety assessment following AESI related to newly acquired transmissible infectious agents (if applicable per Appendix E) and for future use.

7.3.6.4 Study Day 9 (+3) post last enema treatment clinic visit

- Collect and record adverse events, including AESIs (Appendix E).
- Evaluate for signs and symptoms of CDAD by a clinician and if present obtain a stool sample for PCR to determine clinical response by the investigator.
- Review memory aid.

- Obtain an interim medical history.
- Review concomitant medications.
- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight and body temperature).
- Obtain blood samples for laboratory tests: Hemoglobin, WBC, Neutrophil Count, Platelets. (2 mL in EDTA), ALT, Creatinine, potassium (5 mL in SST), and additional blood samples will be stored for future use (48 mL in CPTs).

  Obtain stool samples for microbiome determination, for safety assessment following AESI related to newly acquired transmissible infectious agents and (if applicable per Appendix E) for future use.
- Instruct subject to call clinic if gastrointestinal symptoms including diarrhea occur.

7.3.6.5 Weekly (D15±2 and D22±2) telephone contact

- Collect and record adverse events, including AESIs (Appendix E).
- Evaluate for symptoms of CDAD by study staff.
- Review memory aid.
- Review concomitant medications.
- Instruct subject to call clinic if gastrointestinal symptoms (including diarrhea) occur.

7.3.6.6 Study Day 30 (±3) post last enema treatment clinic visit

- Collect and record adverse events, including AESIs (Appendix E).
- Evaluate for signs and symptoms of CDAD by a clinician.
- Review memory aid.
- Obtain an interim medical history.

- Review concomitant medications.

- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight and body temperature).

- Obtain blood samples for laboratory tests: Hemoglobin, WBC, Neutrophil Count, Platelets. (2 mL in EDTA), ALT, Creatinine, potassium (5 mL in SST), metabolic syndrome markers (5 mL in SST). Additional blood samples will be stored for future use (48 mL in CPTs).

- Obtain stool samples for microbiome determination, for safety assessment following AESI related to newly acquired transmissible infectious agents (if applicable per Appendix E) and for future use.

- Instruct subject to call the clinic if diarrhea occurs.

7.3.6.7 Study Day 60 (±5) post last enema treatment clinic visit:

- Collect and record serious adverse events, including AESIs (Appendix E) and new onset of related chronic medical conditions.

- Evaluate for signs and symptoms of CDAD by a clinician.

- Obtain an interim medical history.

- Review concomitant medications.

- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight, waist circumference and body temperature).

- Obtain blood samples for laboratory tests: Hemoglobin, WBC, Neutrophil Count, Platelets. (2 mL in EDTA), ALT, Creatinine, potassium (5 mL in SST), metabolic syndrome markers (5 mL in SST). Additional blood samples will be storedfor future use (48 mL in CPTs).

- Obtain stool samples for microbiome determinationand for future use.

- Instruct subject to call clinic if an interim diagnosis of CDAD is made or an occurrence of SAE or if there is an occurrence of new onset of related chronic medical condition.

7.3.6.8 Every 2 months (D120±7, D180±7, D240±7, D300±7) telephone contact:

- Collect and record serious adverse events, occurrence of new onset of related chronic medical condition and newly acquired transmissible infectious diseases (Appendix E).
- Review concomitant medications.
- Inquire if interim diagnosis of CDAD has been made.
- Instruct subject to call clinic if interim diagnosis of CDAD made or occurrence of SAE or if there is an occurrence of new onset of related chronic medical condition.

7.3.6.9 Study Day 365 (±14) post last enema treatment clinic visit

- Collect and record serious adverse events and occurrence of new onset of related chronic medical condition and AESIs (Appendix E).
- Review concomitant medications.
- Obtain an interim medical history.
- Inquire if interim diagnosis of CDAD made.
- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. However all subjects will have their vital signs checked (blood pressure, pulse, weight, waist circumference, and body temperature).
- Obtain blood samples for laboratory tests: Hemoglobin, WBC, Neutrophil Count, Platelets. (2 mL in EDTA), ALT, Creatinine, potassium (5 mL in SST), metabolic syndrome markers (TG, HDL, LDL, HDL/LDL ratio, fasting glucose) (5 mL in SST). Additional blood samples will be stored to check for future use (48 mL in CPTs).
- Obtain stool samples for microbiome determinationfor future use.

7.4 Final Study Visit

As stated above.

7.5 Early Termination Visit

- The following assessments will be performed and noted in each subject's chart or record during the Early Termination Visit:
- Collect and record adverse events, including AESIs (Appendix E).
- Review concomitant medications.
- Evaluate for signs and symptoms of CDAD by a clinician and determination of clinical response by investigator.
- Review memory aid if prior to Day 30 after last CDAD treatment.
- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. All subjects will have their vital signs checked (blood pressure, pulse, weight, waist circumference and body temperature).
- Draw blood samples for laboratory tests including metabolic syndrome markers (TG, HDL, LDL, HDL/LDL ratio, fasting glucose) (5 mL in SST), Hemoglobin, WBC, Neutrophil Count, Platelets. (2 mL in EDTA), ALT, Creatinine, potassium (5 mL in SST). Additional blood samples will be stored for future use (48 mL in CPTs).
- Obtain stool samples for *C. difficile* PCR and toxin assay; for microbiome determination, for safety assessment following AESI related to newly acquired transmissible infectious agents (if applicable per Appendix E) and for future use.

7.6 Unscheduled Visit for Recurrence

Any subject who has a suspected recurrence of CDAD prior to Day 60 post last CDAD treatment shall return to have all unscheduled visit assessments performed.

This visit is scheduled to be conducted as an 'in person' office visit; however SAE, concomitant medication and CDAD status assessments may be performed by telephone if an office visit cannot be performed.

At any unscheduled visit for recurrence, the Investigator will conduct the following evaluations:

- Collect and record adverse events (if prior to Day 30 after completing treatment for recurrent CDAD) or serious adverse events, AESI (Appendix E) and new onset of related chronic medical conditions (if after Day 30 from completing treatment for recurrent CDAD).

- Review concomitant medications.

- Evaluate for signs and symptoms of CDAD by a clinician and determination of clinical response by investigator.

- Review memory aid if prior to Day 30 after last CDAD treatment.

- Perform a physical exam: each subject may receive a physical exam depending on interim medical history. All subjects will have their vital signs checked (blood pressure, pulse, weight, body temperature and waist circumference).

- Obtain stool samples for *C. difficile* PCR and toxin assay; for safety assessment following AESI related to newly acquire transmissible infectious agents (if applicable per Appendix E) and for future use. Despite no additional visit is planned after completing vancomycin/after washout period, if the clinical picture is unclear prior to the next enema and at the discretion of the PI, participant might be asked to return to clinic for another unscheduled visit.

- Obtain blood samples for laboratory tests including metabolic syndrome markers (TG, HDL, LDL, HDL/LDL ratio, fasting glucose) (5 mL in SST), Hemoglobin, WBC, Neutrophil Count, Platelets. (2 mL in EDTA), ALT, Creatinine, potassium (5 mL in SST) for clinical labs.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

Detailed medical history will be obtained by direct interview. This includes the past three years self-reported weight history, if this is not available in the medical record, will be collected from each subject before study enrollment.

A detailed physical examination will be conducted at the screening visit and history driven physical exams as needed at enrollment and follow up visits depending on interim medical history. A history driven physical examination is distinguished from a complete physical exam as all assessments are not required (e.g., pelvic, rectal, etc.). This exam will include assessment of each of the following body systems: abdomen, cardiovascular/heart, extremities, general appearance, genitourinary, HEENT, lymph nodes, musculoskeletal, neck, neurological, pulmonary/chest, and skin. Thirty (30) minutes post enema administration the systemic reaction will be assessed.

Vital sign assessments including systolic and diastolic blood pressure [sitting or lying], heart rate (pulse), oral temperature and weight will be obtained at each visit. In addition, waist circumference will be obtained prior to enrollment and at subsequent enemas, Day 60, and Day 365 after last CDAD treatment.

Subjects will be queried for adverse events through 30 days after last CDAD treatment. New-onset Adverse Events of Special Interest (AESI) will be collected up to 365 days (Appendix E) after randomization.

Serious adverse events will be collected through the duration of the study. New onset related chronic medical conditions will be collected from the time the first enema is administered through the duration of the study.

CDAD recurrence will be evaluated any time prior to Day 60 after last CDAD treatment in clinic and by phone through the duration of the study.

Clinical Response Evaluation:

The clinical evaluation will include a quantification of the symptoms of CDAD to assess clinical response by Day 8 and through Day 30 after completing CDAD treatment and an evaluation of sustained clinical response through 60 days after completing CDAD treatment. . Clinical response will be determined by the Investigator based on the memory aid and PCR test.

Clinical response is defined as those subjects who have no recurrence of CDAD. Subjects will be provided with a memory aid (through 30 days after completing CDAD treatment) to record information on the number and consistency of the stools passed and any other symptoms that they may be experiencing.

Subjects not meeting the definition of clinical response at Days 5-8 after randomization are considered for additional enemas. Any subject with recurrence of CDAD from the time of randomization to the specified time frame will be considered a failure. Treatment failures will require no further clinical efficacy evaluation beyond the point of failure, although safety reporting shall continue for subjects receiving FMT(s) or placebo by enema through the duration of the study. The number of recurrences after treatment failure shall also continue up to Day 60 after the last enema.

For subjects who are categorized as treatment failures in either arm, the PIs will inform their primary care physicians (PCPs), share all pertinent information related to the study and facilitate referral to locally available treatment options but will be followed long-term (up to 1 year) only for safety. Study information will be provided in the form of a letter to the study subject which includes the PI and study staff contact information.

Recurrence is defined as the re-establishment of diarrhea (frequency of passed unformed stools $\geq$3 unformed stools within 24 consecutive hours) with positive PCR test for *C. difficile*.

Sustained clinical response is defined as those subjects who have initial response by Day 8 and through Day 30 after randomization with no recurrence of CDAD through 60 days after randomization.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

Blood and urine samples for clinical laboratory tests will be obtained at different visits as per the schedule of events (Appendix A1, A2, and A3). The clinical lab tests will include biochemistry, hematology, serum or urine pregnancy test (if applicable) as well as HDL, LDL, HDL/LDL ratio, TG, fasting glucose.Additional blood samples will be obtained for future research and also stored to check for infectious agents.

For additional details refer to the MOP.

Solicited laboratory values will be entered in the Clinical Labs eCRF.

The clinical laboratory tests will be performed by the clinical laboratory at each individual site and by the central laboratory (depending on the type of test).

8.2.2 Special Assays or Procedures

Stool and blood samples will be collected at different visits as per the schedule of events. If a stool sample cannot be obtained during the visit, then the subject will be allowed to bring in a stool sample at the study visit (+1 day). See MOP for details.

Stool samples may be split into five aliquots: (1) for *C. difficile* PCR, (1)toxin assay (at screening and for clinical suspicion of CDAD) , (1) for microbiome determination, (1) for safety assessment following AESI related to newly acquired transmissible infectious agents (Appendix E) and (1) for future use.

If HIV screening results are not available in the medical record within the last six months, a HIV screening test will be performed during screening.

PCR and toxin testing will only be done if the signs and symptoms of CDAD are present.

If the fecal sample collected for PCR and toxin testing is not available, an additional fecal sample should be collected and aliquoted prior to enrollment.

If the fecal sample collected at screening is unformed or liquid it will be analyzed for presence of *C. difficile* using PCR and toxin testing at the site's local laboratory within 48 hours of sample collection.

The fecal and blood (serum and plasma) aliquots collected for safety assessment will be stored and only analyzed for select infectious agents following a report of an AESI related to newly acquired transmissible infectious agents (Appendix E). The assays for individual infectious agents will be performed at a local lab.

In addition, after enrollment has been completed stool samples will be analyzed from a subset of qualifying subjects, who provided a complete set of samples, to identify and measure the major constituents of the intestinal microbiota (i.e. microbiome determination) organisms.

Blood collected in CPT tubes will be used to isolate peripheral blood mononuclear cells (PBMCs) for future use.

8.2.3 Specimen Preparation, Handling, and Shipping

8.2.3.1 Instructions for Specimen Preparation, Handling, and Storage

Blood will be collected at the clinic and sent to the laboratory for processing prior to storage. After collection, blood specimen tubes are required to sit at room temperature for 30-120 minutes, and for up to 8 hours, if necessary (e.g., off-site collection), until the blood has clotted before centrifugation. Note: Blood tubes may be refrigerated overnight before aliquoting. Every effort should be made to freeze the sera within 24 consecutive hours after collection.

Temperatures required for storage following processing:

- All serum specimens should be stood upright in a freezer set at -20°C or colder until frozen.
- All plasma specimens should remain at -80°C or colder after processing.
- All PBMC specimens should be placed in room temperature isopropanol-filled cell freezer ("Mr. Frosty") or equivalent. The apparatus should then be placed into a freezer that is -80°C or colder.
- All stool specimens should be stored at -80ºC or colder.

Stool samples will be collected using specially designed stool collection kits. Subjects will be provided with stool collection kits and the instructions for collection and storage before delivering it to the clinical site.

Subjects are required to submit stool samples within 8 hours of the clinic visit. Upon receiving the stool sample, Clinical Center staff will immediately aliquot, weigh, and label the sample for storage and/or transport. All samples from each cohort will be batched shipped in dry ice to Fisher Repository and then distributed to the laboratory for testing.

Note that stools that are to be tested for the presence of CDAD must be processed within 48 hours of collection or storage at 2-8°C using Biosafety Level Two practices.

Further details regarding the specimen preparation, handling, and storage are described in the MOP.

8.2.3.2 Specimen Shipment

Do not include any subject identifiers on specimen tubes – each tube should only be labeled with the barcode at the time of shipment.

Serum, plasma, and stool specimens must be sent frozen and on dry ice.

Each subject's specimens for each visit must be sent in two separate shipments – one half of the specimens in each – to avoid loss of all samples if a shipping error occurs. Priority Overnight shipment with a "no later than 10:30 am" delivery time is required for all specimens.

Further details regarding specimen shipment are described in the MOP.

9 ASSESSMENT OF SAFETY

Regulatory requirements including the FDA regulations, ICH Guidelines for GCP, and European Union (EU) Clinical Trials Directive set forth safety monitoring and reporting responsibilities of sponsors and investigators to ensure the safety and protection of human subjects participating in clinical trials.

Responsibilities

Investigators participating in this clinical trial are responsible for and will:

- Instruct subjects in the reporting of AEs;
- Evaluate subject safety including assessment of AEs and AESI's for seriousness, severity, and causality;
- Notify the sponsor (DMID) of SAEs immediately;
- Provide detailed written reports, including necessary documentation requested by the sponsor or Institutional Review Board (IRB)/Independent Ethics Committee (IEC), promptly following immediate initial reports; and
- Inform the IRB/IEC of AEs as required by applicable regulatory requirements.

9.1 Specification of Safety Parameters

Safety will be assessed by the frequency and severity of:

Adverse events

Solicited adverse events occurring from the time of each study enema through 8 days after the last study enema:

- Fever
- Chills
- Nausea
- Diarrhea
- Vomiting
- Constipation
- Abdominal Cramps
- Abdominal bloating

- Flatulence
- Malaise, fatigue
- Loss of appetite

Bowel movements will be recorded through 30 days after the last study enema

Clinical Safety Laboratory Adverse Events

Clinical laboratory teststo be evaluated include: Potassium, Creatinine, Liver Function Tests (ALT), Hemoglobin, WBC, Neutrophil Count, Platelets. Only Grade 3 abnormal clinical laboratory tests through 30 days after completing treatment for recurrent CDAD will be reported as AEs.

Unsolicited Adverse Events

Unsolicited are non-serious adverse events that will be reported through 30 days after completing treatment for recurrent CDAD.

New-Onset Related Chronic Medical Condition (NOCMC)
New onset of related chronic medical conditions through 365 days after completing treatment for recurrent CDAD will be reported. NOCMCs are defined as any new ICD-10 diagnosis that is applied to the subject during the duration of the study, after receipt of the study agent, that is expected to continue for at least 3 months and requires continued health care intervention.

Serious Adverse Events

Serious adverse events through 365 days after completing treatment for recurrent CDAD will be reported.

Adverse Events of Special Interest

New-onset Adverse Events of Special Interest (AESI) will be collected through 365 days after completing treatment for recurrent CDAD (Appendix E). AESI are defined as newly acquired transmissible infectious agents or infectious diseases related to study product and the following agents: HIV type 1 and 2, Hepatitis A, B, C, *Treponema pallidum*, HTLV-1, -2, *Cyclospora*, *Salmonella*, *Shigella*, *Campylobacter*, *E. coli* 0157:H7, Shiga-toxin producing *E. coli*, Ova and enteric parasites including *Isospora*, Vancomycin-resistant *Enterococcus* (VRE), extended spectrum beta-lactamase (ESBL), carbapenemase producing gram-negative rods, methicillin-resistant *Staphylococcus aureus* (MRSA), *Helicobacter pylori*, Rotavirus, Adenovirus, Norovirus, *Vibrio*, *Giardia lamblia*, *Cryptosporidium*, and *Microsporidia*.

New onset metabolic syndrome

New onset metabolic syndrome will be reported through 356 days after completing treatment for recurrent CDAD (Refer to MOP).

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

An AE is defined as any noxious, pathologic, or unintended change in anatomic, physiologic, or metabolic functions, as indicated by physical signs, symptoms, and/or laboratory changes occurring in any phase of the clinical trial, regardless of their relationship to investigational product.

AEs not meeting the criteria for "serious adverse events" should be captured on the appropriate CRF. Information to be collected includes event description, date of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and date of resolution/stabilization of the event. AEs occurring while on study must be documented appropriately regardless of relationship. AEs will be followed to adequate resolution or until the event or abnormality stabilizes to the investigator's and/or Sponsor's satisfaction.

Any medical condition that is present at the time that the patient is screened should be considered as baseline and not reported as an AE. However, if it worsens at any time during the study, it should be recorded as an AE.

Adverse events (AE) will be collected through 30 days after completing treatment for recurrent CDAD.

Adverse Events of Special Interest are those events thought to be potentially related with the investigational compound under study. An adverse event of special interest (serious or non-serious) is one of scientific and medical concern specific to the product, for which ongoing monitoring and rapid communication by the investigator to the DMID can be appropriate. Such an event might warrant further investigation in order for it to be characterized and understood. Reporting on Adverse Events of Special Interest is an emerging and ever more critical aspect related to characterizing the safety profile of the compound.

Treatment failure and recurrence will not be recorded on the AE CRFs, as these efficacy endpoints will be recorded elsewhere on the CRF.

Surgical procedures are not AEs; they are therapeutic measures for conditions that require surgery. However, the condition for which the surgery is required is an AE, if it occurs or is detected during the study. Planned surgical measures and the condition(s) leading to these measures are not AEs if the condition(s) was (were) known before the start of study treatment.In the latter case, the condition should be reported as medical history.

AEs must be graded for severity and relationship to study product.

Severity of the Event:

AEs will be assessed by the clinician using a protocol defined grading system (see Appendix C).For events not included in the protocol defined grading system, the following guidelines will be used to quantify intensity.

- Mild:events require minimal or no treatment and do not interfere with the patient's daily activities.
- Moderate:events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe:events interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

Relationship to Study Products:

The clinician's assessment of an AE's relationship to test article is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study product assessed using the terms: related or not related. In a clinical trial, the study product must always be suspect.To help assess, the following guidelines are used.

- **Related** – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- **Not Related –** There is not a reasonable possibility that the administration of the study product caused the event

9.2.2 Solicited Adverse Events

The stool log, distributed as part of the Memory Aid will be used to record the number and consistency of bowel movements (BM), 30 days after enema, as well as solicited (only through Day 8) and unsolicited adverse events. Subjects will be instructed to call the clinic anytime if they have three or more loose BMs within 24 consecutive hours between Day 30 and Day 60.

9.2.3 Serious Adverse Events

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death;
- Life-threatening adverse event*;
- Inpatient hospitalization or prolongation of existing hospitalization;
- A congenital anomaly/birth defect.
- Persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life function, or;

*Life-threatening adverse event. An adverse event 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 which, had it occurred in a more severe form, might have caused death.

Any adverse event or suspected adverse reaction that meets the criteria for a serious adverse event will be:

- recorded on the appropriate SAE CRF
- followed through resolution by a study clinician
- reviewed and evaluated by a study clinician

9.2.4 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

Any clinical lab value Grade 3 only (unless present at baseline see Protocol Section 9.2.1) will be reported as an AE. Grade 1 or 2 clinical lab values will not be reported as AEs.

Any abnormal test result that is determined to be an error does not require reporting as an AE.

Any abnormal test finding that meets the criteria for an SAE (described above) should be reported as such.

9.3 Reporting Procedures

9.3.1 Serious Adverse Events

The following procedures will apply to all serious adverse events:

- Recorded on the appropriate serious adverse event report form and sent to DMID Pharmacovigilance Group
- Reported by the investigator to the site's Independent Safety Monitor
- Reviewed and followed to resolution by a study physician

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)


Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The DMID medical monitor and clinical protocol manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID medical monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the investigator becomes aware of an SAE that is suspected to be related to study product, the investigator will report the event to the DMID Pharmacovigilance Group.

9.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the investigator, DMID, the Investigational New Drug (IND) sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event. DMID will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also 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. Relevant follow up information to an IND safety report will be submitted as soon as the information is available.Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All serious events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

9.3.3 Other Adverse Events

n/a

9.3.4 Reporting of Pregnancy

Pregnancies (to include if the partner of a male subject becomes pregnant) that occur during the study period will be reported via The Emmes Internet Data Entry System (IDES) on the Pregnancy Report form within five days of site awareness.

Efforts will be made to follow all pregnancies occurring through the Day 365 visit through to outcome, as described in the MOP (e.g. delivery, spontaneous abortion or therapeutic abortion).

9.4 Type and Duration of Follow-up of Subjects after Adverse Events

AEs will be followed until resolution or stability even if this extends beyond the study-reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Follow-up procedures, evaluations, and outcomes will be recorded on the subject's case report forms.

9.5 Halting Rules

Study enrollment halting rules:

Enrollment in the study will be suspended for a safety review by the DSMB if:

- New diagnosis of any infectious disease related to study product and the following agents: HIV type 1 and 2, Hepatitis A, -B, -C, *Treponema pallidum*, HTLV-1, -2, *Cyclospora*, *Salmonella*, *Shigella*, *Campylobacter*, *E. coli* 0157:H7, Shiga-toxin producing *E. coli*, ova and enteric parasites including *Isospora*, *Helicobacter pylori*, Rotavirus, Adenovirus, Norovirus, *Vibrio*, *Giardia lamblia*, *Cryptosporidium, Microsporidia,* invasive disease caused by Vancomycin-resistant *Enterococcus* (VRE), extended spectrum beta-lactamase (ESBL), carbapenemase producing gram-negative rods, or methicillin-resistant *Staphylococcus aureus* (MRSA). The subjects will not be screened for these infectious diseases prior to enrollment, but will be queried about diagnosis before enrollment and the information will be recorded on the Medical History Form. Diagnosis prior to enrollment will not preclude enrollment in the study, unless related to an exclusion criterion.
- Three or more of the randomized subjects have a Grade 3 AE of the same organ class (systemic toxicity, or clinical laboratory tests or vital signs) deemed related to the study product.
- Death of an enrolled subject.
- Any SAE, if the SAE is considered by the investigator and/or DMID Medical Monitor to be related to the study product.
- Any subject experiences laryngospasm, bronchospasm, or anaphylaxis within 24 hours after administration of study product that is considered related to study product.

Individual's halting rules:

Subjects who meet any of the following criteria must be assessed by the PI to determine if it is in the subject's best interest to stop the study product(s):

- Subject choice (Withdrawal of consent)
- Participant's non-compliance.
- Development of a significant medical condition and/or participation in the study is no longer in the best interest of the subject.

9.6 Safety Oversight

Independent Safety Monitor (ISM)

The ISM is a physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely fashion.The ISM will review SAEs in real time and other AEs as needed and provide an independent assessment to DMID. Each participating site will have an ISM with experience in infectious diseases or internal medicine, is in close proximity to the participating site, and has the authority to readily access study participant records.

Data and Safety Monitoring Board (DSMB)

Safety oversight will be under the direction of a DSMB. The DSMB is an independent group of experts that is external to DMID and advises DMID and the study investigators. The primary responsibilities of the DSMB are to 1) periodically review and evaluate the accumulated study data for subject safety, study conduct and progress, and, when appropriate, efficacy, and 2) make recommendations to DMID concerning the continuation, modification, or termination of the trial. The DSMB will be composed of at least three voting members.The membership will include a chairperson and a biostatistician who are experienced in clinical trials conduct and have prior DSMB experience. There will also be members with clinical expertise in the medical area and subject population being studied. All DSMB members will be separate and independent of study personnel participating in this trial and should not have scientific, financial or other conflict of interest related to the trial. Procedures for DSMB data reviews will be defined in the DSMB Charter that will include DSMB membership, responsibilities, and the scope and frequency of data reviews. The DSMB will have access to unblinded treatment assignments during the closed session of their meetings.

The study should be reviewed by the DSMB at least annually. The DSMB will meet by teleconference at the following time points:

- Ad hoc basis according to protocol criteria or if there are immediate concerns regarding observations during the course of the study.
- Final review meeting: 6 to 8 months after clinical database lock to review the cumulative unblinded safety and efficacy data for the study. The data will be provided in a standard summary format.The DSMB may be asked to provide recommendations in response to questions posed by DMID.

Enrollment will be monitored to ensure there is a DSMB review each year of the study during active enrollment. In the case one of the enrollment milestones has not be met and a review has not occurred within the previous 12 months, DMID may request the DSMB review the study for safety.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Site monitoring is conducted to ensure that the human subject protection, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, GCP/ICH and regulatory guidelines. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the monitoring plan. The investigator will permit authorized representatives of DMID and the respective local and national health authorities to inspect facilities and records relevant to this study, if needed.

Site visits will be made at standard intervals as defined by DMID and may be made more or less frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, informed consent forms, medical and laboratory reports, and protocol compliance. Study monitors will meet with investigators to discuss any problems and actions to be taken and document visit findings and discussions.

11 STATISTICAL CONSIDERATIONS

This is a multi-center, randomized, partially blinded trial comparing the safety and efficacy of fecal microbiota transplantation (FMT) given by enema in subjects 18 years of age or older with recurrent *Clostridium difficile*-Associated Disease (CDAD). For the most recent episode subjects will have completed a course of at least 10 days of oral vancomycin, oral/IV metronidazole, or oral fidaxomicin prior to enrollment.

11.1 Study Objective

To investigate the safety and efficacy of FMT delivered via enema after thawing of frozen, banked fecal microbiota in recurrent CDAD. The primary and secondary outcome measures are as follows.

Safety Outcome Measures

Primary Safety Outcome Measures:

- Number of subjects with an AE through 30 days after completing treatment for recurrent CDAD.
- Number of subjects with a SAE through 365 days after completing treatment for recurrent CDAD.
- Number of subjects with a new onset of related chronic medical condition through 365 days after completing treatment for recurrent CDAD.
- Number of subjects with newly acquired transmissible infectious diseases which are considered adverse events of special interest (AESI) through 365 days after completing treatment for recurrent CDAD.

Efficacy Outcome Measures

Primary Efficacy Outcome Measures:

- Proportion of subjects with clinical response through Day 30 (±3) after randomization.
  - Clinical response is defined as those subjects who have no recurrence of CDAD.
  - CDAD is defined as bowel movements as determined by ≥3 unformed stools (soft or watery; e.g., take the shape of the container in which collected) within 24 consecutive hours with a positive PCR test for *Clostridium difficile*.

Secondary Efficacy Outcome Measures:

- Proportion of subjects with sustained clinical response through Day 60 (±5) after randomization.

Sustained clinical response is defined as those subjects who responded by Day 30 with no recurrence of CDAD through Day 60 after randomization

- Number of recurrences of CDAD through Days 30 and 60 after completing treatment for recurrent CDAD.

The date of "completing treatment for recurrent CDAD" is the date of the effective enema OR if there is no effective enema then the date of last ineffective enema. An effective enema is defined as the enema followed by no diarrhea by Day 8 OR with diarrhea at Days 5-8 but without a positive PCR test for *C. difficile*. An ineffective enema is defined as the enema followed by diarrhea at Days 5-8 but with a positive PCR test for *C. difficile*. The number of enemas allowed differs between the placebo and the FMT group as shown in the schema shown in Figure 1.

- Time (in days) from randomization until the study day when first CDAD reoccurred

Exploratory Outcome Measures:

- Changes in gut microbial diversity through 365 days after completing treatment for recurrent CDAD.
- Number of subjects with abnormal clinical laboratory tests (hematology, biochemistry) through 30 days after completing treatment for recurrent CDAD.
- Number of subjects with new onset metabolic syndrome through 365 days after completing treatment for recurrent CDAD.
- Agreement of PCR test results and toxin assay results in subjects with a clinical suspicion of CDAD

11.2 Study Hypotheses

There is a planned formal test of hypothesis comparing FMT to placebo with respect to the primary efficacy outcome measure. The hypothesis test compares the proportion of subjects in each arm with clinical response through Day 30. The null hypothesis is that there is no difference in proportions between study arms, with a two-sided alternative. A two-sided Wald test with a continuity correction for a difference in proportions will be used to test the null hypothesis that there is no difference in response rates between the FMT and placebo group.

11.3 Sample Size Considerations

Sample size considerations are based on the primary efficacy endpoint.

The goal of this trial is to establish efficacy of FMT given by enema compared to a placebo enema while limiting the number of subjects allocated to the placebo arm.

To detect a difference in clinical response of 0.25 between the treatment (FMT) and control (placebo) groups, assuming a placebo group proportion of 0.50, with 80% power using a two-sided significance level of 0.05, will require a total sample size of 147 (98 in the FMT group, 49 in the placebo group). Assuming a drop-out rate of 10% the required sample size is 162 (108 in the FMT group, 54 in the placebo group).

11.4 Final Analysis Plan

The CSR will be completed when all primary, secondary, and exploratory data are available.

A formal statistical analysis plan will be developed and finalized prior to unblinding for the final analysis.

11.4.1 Analysis Populations

The modified intent-to-treat (mITT) analysis population will include all subjects randomized into either FMT or placebo groups and received the study treatment. Subjects will be analyzed according to the treatment arm to which they were randomized. This analysis set will be used for all efficacy analyses.

The safety analysis population will include all subjects who receive the study treatment and have at least one post-treatment safety assessment. Subjects will be assigned to the group based on the actual treatment taken. This analysis set will be the primary analysis set used in all safety summaries.

11.4.2 General Analysis Methods

Descriptive statistical and graphical methods will be used to summarize the data from this study.

Descriptive statistics will include the number of subjects (n), mean, median, standard deviation, standard error of the mean (SEM), minimum, and maximum. Frequency tables (which may include the number and/or percentage of subjects or events) will be used to summarize categorical data. All summary results will be presented by treatment.

Statistical summaries (descriptive statistics and frequency tables) will be generated using SAS® Version 9.3 or higher (SAS Institute Inc., Cary, NC, USA).

11.4.3 Safety Analyses

- The observed (raw) clinical laboratory data will be summarized with descriptive statistics, as will the change values. These data also will be summarized in shift tables based on reference range status.In addition, out-of-range values will be flagged with an appended “L” (low) or “H” (high) in the data listings.
- Vital signs and physical examination findings will be summarized with descriptive statistics and/or frequency tables as appropriate. Change values also will be summarized.
- Treatment-emergent AEs, categorized by MedDRA system organ class and preferred term, will be summarized in tables. The tables will include the number of events, number of unique subjects experiencing each event, and percentage of subjects experiencing each event.
- Treatment-emergent AEs will also be tabulated in terms of the number and percentage of subjects experiencing events by intensity and relationship to study drug. In the tabulation by intensity, a subject with more than one event coded to the same preferred term will be classified according to the most severe event. Similarly, in the tabulation by relationship, a subject will be classified according to the event with the strongest relationship to study drug.The number of individual AE reports will also be tabulated by intensity and relationship.
- An overall summary of all solicited adverse events, all unsolicited adverse events, and all adverse events of special interest (AESI) will be provided. For all incidence summaries, the exact 95% confidence interval of the rate will be calculated.

The following tables and listings will be presented for unsolicited adverse events:

- Incidence of serious AEs by MedDRA SOC, preferred term, and relationship
- Incidence of AEs by MedDRA SOC, preferred term, maximum severity, and relationship
- Listing of serious AEs
- Listing of non-serious AEs of moderate or greater severity
- Listing of adverse events of special interest (AESI)

The following will be presented for solicited events:

- Summary of maximum solicited events by symptom and FMT number
- Summary of solicited events by day

11.4.4 Efficacy Analyses

Primary efficacy endpoint

The primary endpoint will be clinical response at 30 days after randomization.

The null and alternative hypotheses are:

$$H_0 : \pi_t - \pi_c = 0$$
$$H_1 : \pi_t - \pi_c \neq 0$$

Where π_c is the response rate in the control (placebo) group and π_t is the response rate in the treated (FMT) group.

A two-sided Wald test with a continuity correction for a difference in proportions will be used to test the null hypothesis that there is no difference in response rates between the treated and control group. A 95% confidence interval for the estimates and for the difference between treatment groups will also be calculated

The difference in proportions will be computed by subtracting the proportion for the placebo group from the proportion for the FMT group. The denominator for each group will consist of all subjects randomized to the respective group. The numerator will then be those who have a sustained clinical response at 30 days post-randomization. If a subject requires a second enema before the 30-day assessment, they will be considered a failure for this endpoint.

Secondary efficacy endpoints

The number of subjects with sustained clinical response 60 days after randomization will be described for each treatment group. The proportion in each group will be computed by the number of subjects in scenario 1, as the denominator. The numerator will be those with a sustained clinical response at 60 days post-randomization, without requiring an additional FMT. A two-sided continuity-corrected Wald test or Fisher's Exact test, as appropriate, will be used to compare the proportion of subjects with sustained clinical response between treatment groups.

The number of recurrences of CDAD at 30 and 60 days after completing treatment for recurrent CDAD will be described for each treatment group. This endpoint will be summarized for each unique scenario. By the time treatment for recurrent CDAD has been completed, treatment groups may not be comparable depending on the number of FMTs and antibiotics received for each scenario. For this reason, no formal comparisons are planned between groups.

The time (in days) until first CDAD recurrence will be analyzed using time-to-event methods. Kaplan-Meier estimates will be presented and a Log-Rank test will be used to compare treatment groups. Subjects without a recurrence will be censored at their last known contact date, or at 60 days, whichever occurs first.

11.4.5 Other

Exploratory Microbiome Analysis

A detailed statistical analysis plan that covers the planned microbiome exploratory analyses will be developed and finalized prior to database lock.

Demographic analysis

Demographic data will be summarized for the safety, mITT analysis set with descriptive statistics.

Subject disposition

Subject disposition table will present the number of subjects who were enrolled in the study, received study treatment, and completed the study by treatment group. This table will also show the number of subjects who did not complete the study, both overall and according to the reason for study termination/discontinuation.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6 GCP, Section 4.9, and regulatory and institutional requirements for the protection of confidentiality of subjects. As part of participating in a DMID-sponsored, DMID-affiliated, or manufacturer-sponsored study, each site will permit authorized representatives of the sponsor(s), DMID, and regulatory agencies to review (and, when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

Forms for use as source documents will be derived from the electronic CRFs and will be provided by the SDCC. Additional source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, laboratories, and medico-technical departments involved in the clinical trial.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the investigational site is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The PI will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The PI will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

DMID-designated clinical monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to DMID.

The SDCC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 50 and 56, and ICH E6; 62 Federal Regulations 25691 (1997), if applicable. The investigator's Institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research.

14.2 Institutional Review Board

Each participating institution will provide for the review and approval of this protocol and the associated informed consent documents, by an appropriate ethics review committee or IRB listed on the FWA. Any amendments to the protocol or consent materials must also be approved before they are placed into use unless change is for the safety of the subject. Only those IRB members who are independent of the investigators and the sponsor should provide an opinion on study related matters. Verification of IRB approval of the protocol and the written informed consent will be transmitted by the investigator or designee prior to the shipment of clinical trial material. No deviations from or changes to the protocol will be initiated without prior approval of an appropriate amendment unless change is for the safety of the subject. Each participating institution is responsible for ensuring Continuing Review at least once a year and for keeping the IRB apprised of the progress of the study and any changes to the protocol.

14.3 Informed Consent Process

The written consent document will embody the elements of informed consent as described in the Declaration of Helsinki and will adhere to the ICH Harmonised Tripartite Guideline for Good Clinical Practice. Informed consent should be implemented before any protocol-specified procedures or interventions are carried out. Informed consent will be obtained in accordance with 21 CFR 50.25 and 45 CFR 46. Information should be presented both orally and in written form.

An investigator or designee will describe the protocol to potential subjects face-to-face. The Subject Information and Consent Form may be read to the subjects, but, in any event, the investigator shall give the subjects ample opportunity to inquire about details of the study and ask any questions before the signing and dating the consent form.

Study staff must inform subjects and/or LAR that the trial involves research, and explain the purpose of the trial, those aspects of the trial that are experimental, any expected benefits, all possible risks (including a statement that the particular treatment or procedure may involve risks to the subject or to the embryo or fetus, if the subject is or may become pregnant or fathers a child, that are currently unforeseeable), the expected duration of the subject's participation in the trial, the procedures of the research study, including all invasive procedures, and the probability for random assignment to treatment groups. Subjects and/or LAR will be informed that they will be notified in a timely manner if information becomes available that may be relevant to their willingness to continue participation in the trial. They must also be informed of alternative procedures that may be available, and the important potential benefits and risks of these available alternative procedures. Subjects and/or LAR must receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they consist of, or where further information may be obtained. Subjects and/or LAR must be informed of the anticipated financial expenses, if any, to the subject for participating in the trial, as well as any anticipated prorated payments, if any, to the subject for participating in the trial. They must be informed of whom to contact (e.g., the investigator) for answers to any questions relating to the research project. Information will also include the foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated. The subjects and/or LAR must be informed that participation is voluntary and that they are free to withdraw from the study for any reason at any time without penalty or loss of benefits to which the subject is otherwise entitled.

Neither the investigator, nor the trial staff, should coerce or unduly influence a subject to participate or continue to participate in the trial. The extent of the confidentiality of the subjects' records must be defined, and subjects must be informed that applicable data protection legislation will be followed. Subjects and/or LAR must be informed that the monitor(s), auditors(s), IRB, NIAID, and regulatory authority(ies) will be granted direct access to the subject's medical records for verification of clinical trial procedures and/or data without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations, and that, by signing a written informed consent form, the subject is authorizing such access. Subjects and/or LAR must be informed that records identifying the subject will be kept confidential, and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available and, if the results of the trial are published, the subject's identity will remain confidential.

Consent forms must be in a language fully comprehensible to the prospective subjects. Informed consent shall be documented by the use of a written consent form approved by the IRB and signed and dated by the subject and the person who conducted the informed consent discussion. The signature confirms that the consent is based on information that has been provided and all questions have been answered to the prospective subject's satisfaction. Each subject's signed

informed consent form must be kept on file by the investigator for possible inspection by Regulatory Authorities and/or the sponsor and Regulatory Compliance persons.The subject should receive a copy of the signed and dated written informed consent form and any other written information provided to the subjects, and should receive copies of any signed and dated consent form updates and any amendments to the written information provided to subjects.

14.4 Informed Consent/Assent Process (in Case of a Minor)

n/a

14.5 Exclusion of Women, Minorities, and Children (Special Populations)

Children are excluded for safety reasons.

14.6 Subject Confidentiality

Subject confidentiality is held strictly in trust by the participating investigators, their staff, and the sponsor and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval from the sponsor.

The study monitor or other authorized representatives of the sponsor and FDA may inspect all documents and records required to be maintained by the Investigator, including, but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

To protect privacy, we have received a Certificate of Confidentiality. With this Certificate, the researchers cannot be forced to release information that may identify the research subject, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify the subject, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects, like this study, or for information that must be released in order to meet the requirements of the Federal Food and Drug Administration (FDA).

A Certificate of Confidentiality does not prevent the subject from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate of Confidentiality does not prevent the researchers from reporting without the subject's consent, information that would identify the subject as a participant in the research project regarding matters that must be legally reported including: child and elder abuse, sexual abuse, or wanting to harm themselves or others.

14.7 Study Discontinuation

The NIAID/DMID has the right to terminate this study or an individual site's participation at any time. Reasons for terminating the study may include, but are not limited to, the following:

- Incidence or severity of adverse events indicates a potential health hazard;
- Data recording is inaccurate or incomplete;
- Investigator does not adhere to the protocol or applicable regulatory guidelines in conducting the study.

14.8 Future Use of Stored Specimens

Future use specimens along with any leftover blood and stool specimens will be stored and may be used for future research under a future protocol. These specimens may enable us to learn more about fecal transplant and recurrent CDAD patient's pro-inflammatory response. These specimens will be stored indefinitely at the DMID repository after the study is completed. In the informed consent document, subjects will be given an opportunity to choose whether or not their de-identified barcoded specimens are stored for future use. For subjects who choose not to allow storage of their samples for future use, these samples will be destroyed at the end of the study. All proposed research projects will be subject to approval by DMID and approval by an IRB prior to release of any specimens. No human genetic tests will be performed on specimens.

There are no benefits to subjects in the collection, storage and subsequent research use of specimens. Reports about future research done with subject's samples will NOT be kept in their health records, but subject's samples may be kept with the study records or in other secure areas. Subjects can decide if they want their samples to be used for future research or have their samples destroyed at the end of the study. A subject's decision can be changed at any time before the end of the study by notifying the study doctors or nurses in writing. However, if a subject consents to future use and some of their blood and stool has already been used for research purposes, the information from that research may still be used.

Samples may be shared with other investigators at other institutions. The samples will not be sold or used directly for production of any commercial product.Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect subject's confidentiality.

Research using stored specimens may be conducted by other institutions. Emmes will provide the DMID repository with a list of specimens labeled by barcodes, after DMID has authorized the use of these specimens for the future studies. Any specimens and data provided to the receiving-institution will be coded. Unequivocally, neither individual personal identifiers nor the key linking coded data to individuals will be released to the receiving-institution.

The use of any of these specimens for any future studies will only be performed after DMID has authorized the use of these specimens and appropriate institutional approvals are obtained.

15 DATA HANDLING AND RECORD KEEPING

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All data collection forms should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Copies of the electronic CRF (eCRF) will be provided for use as source documents and maintained for recording data for each subject enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained.

DMID and/or its designee will provide guidance to investigators on making corrections to the data collection forms/source documents and eCRFs.

15.1 Data Management Responsibilities

All source documents and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse Events must be graded, assessed for severity and causality, and reviewed by the site Principal Investigator or designee.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site Principal Investigator.During the study, the investigator must maintain complete and accurate documentation for the study.

The Emmes Corporation will serve as the Statistical and Data Coordinating Center for this study, and will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2 Data Capture Methods

Clinical data (including AEs, concomitant medications, and solicited events data) and clinical laboratory data will be entered into a 21CFR11-compliant Internet Data Entry System provided by The Emmes Corporation. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

15.3 Types of Data

Data for this study will include safety and efficacy and microbiological outcome measures.

15.4 Timing/Reports

Interim statistical reports may be generated as deemed necessary and appropriate by DMID. Other safety summary reports may be generated for the DSMB. A final report will be prepared when all primary and secondary safety and efficacy endpoint data are available .

15.5 Study Records Retention

Study files (except for future use consent forms) must be maintained for a minimum of two years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in and ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by local regulations. NO records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained. Consent forms for future use will be maintained as long as the sample exists.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or protocol-specific MOP requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1, and 5.20.2.

It is the responsibility of the site PI/study staff to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five

working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID, via the Emmes IDES.

All protocol deviations, as defined above, must be addressed in study subject source documents. A completed copy of the DMID Protocol Deviation Form must be maintained in the Regulatory File, as well as in the subject's source document. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.

16 PUBLICATION POLICY

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

- NIH Public Access Policy, http://publicaccess.nih.gov/
- NIH Office of Extramural Research (OER) Grants and Funding, http://grants.nih.gov/grants/oer.htm

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov, no later than 21 days after the enrollment of the first subject. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting.

As part of the result posting a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan will be posted on ClincialTrials.gov.

For this trial the responsible party is DMID which will register the trial and post results.

The responsible party [does not plan to request certification of delayed posting.

Refer to:

- Public Law 110-85, Section 801, Clinical Trial Databases
- 42CFR11
- NIH NOT-OD-16-149

17 LITERATURE REFERENCES

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18 SUPPLEMENTS/APPENDICES

APPENDIX A-1: SCHEDULE OF EVENTS OF ENEMA #1

Table 1: Scenario 1 - Receipt of placebo or FMPE with no diarrhea or diarrhea with negative *C. difficile* testing at study Days 5-8 after randomization

	Screening Visit	Enrollment Visit (1st Enema)	Day 4[a]	Day 8	Day 9 post Enema	Day 15[a] Day 22[a]	Day 30 Visit	Day 60 Visit	Day 120[a] Day 180[a] Day 240[a] Day 300[a]	Day 365 Visit	Unscheduled Visit for Recurrence
Visit	A00	A01	A02	A03	A04	A05, A06	A07	A08	A09, A10, A11, A12	A13	supplemental
Window	(-21 to -1)	0	-1	-3	+3	±2	±3	±5	±7	±14	n/a
Informed Consent	X										
Inclusion/ Exclusion	X	X		X[m]							
Test of Comprehension	X										
Medical history	X	X[k]		X[k]	X[k]		X[k]	X[k]		X[k]	X[k]
HIV screen if no known history of HIV + within the last 6 months	X										
Randomization		X									
Physical exam	X	X[i]		X[i]	X[i]		X[i]	X[i]		X[i]	X[i]
Vital signs [b]	X	X		X	X		X	X		X	X
Measuring waist circumference		X						X		X	
Clinical lab tests [c]	X	X			X		X	X		X	X[c]

	Screening Visit	Enrollment Visit (1st Enema)	Day 4[a]	Day 8	Day 9 post Enema	Day 15[a] Day 22[a]	Day 30 Visit	Day 60 Visit	Day 120[a] Day 180[a] Day 240[a] Day 300[a]	Day 365 Visit	Unscheduled Visit for Recurrence
Visit	A00	A01	A02	A03	A04	A05, A06	A07	A08	A09, A10, A11, A12	A13	supplemental
Window	(-21 to -1)	0	-1	-3	+3	±2	±3	±5	±7	±14	n/a
Blood samples [d]		X			X		X	X		X	
Stool sample [e]	X	X		X	X		X	X		X	X
AEs/SAEs/ AESIs/Chronic Med. Conditions [f]		X	X	X	X	X	X	X	X	X	X
Bowel preparation and antiperistaltic Instructions	X			X							
Distribution of memory aid		X									
Review memory aid			X	X	X	X	X				[X][j]
Prior and/or concomitant medications [g]	X	X	X	X	X	X	X	X	X	X	X
Serum/Urine pregnancy test	X[1]	X		X							
Evaluation of signs and symptoms of CDAD [h]	X	X	X	X	X	X	X	X			X

	Screening Visit	Enrollment Visit (1st Enema)	Day 4[a]	Day 8	Day 9 post Enema	Day 15[a] Day 22[a]	Day 30 Visit	Day 60 Visit	Day 120[a] Day 180[a] Day 240[a] Day 300[a]	Day 365 Visit	Unscheduled Visit for Recurrence
Visit	A00	A01	A02	A03	A04	A05, A06	A07	A08	A09, A10, A11, A12	A13	supplemental
Window	(-21 to -1)	0	-1	-3	+3	±2	±3	±5	±7	±14	n/a
Investigator Determination of clinical response			X	X	X	X	X	X			X
Enema administration		X									
Inquiry about interim diagnosis of CDAD									X	X	
Provide oral vancomycin				X[m]							

[a] Subject interviews may be conducted by telephone. Subjects will also be contacted at Day 4 after enema treatment to confirm clinical response and then weekly thereafter until recurrence or the 60-day post-treatment visit whichever comes first, to evaluate possible CDAD recurrence. Staff will evaluate for symptoms of CDAD during phone visit and consult with clinician if needed. Subject interviews will be supplemented by review of the subject's personal records (if hospitalized) and memory aid.

[b] Includes blood pressure, pulse, weight and body temperature. Height will be collected at screening visit only.

[c] Clinical labs include potassium, creatinine, liver function tests (ALT), hemoglobin, WBC, neutrophil count, and platelets.

[d] Metabolic Syndrome markers including triglyceride level, HDL, LDL, HDL/LDL ratio, fasting glucose (Days 1, 60 and 365), future use (Day 1, 9, 30, 60 and 365), storage of sera and plasma to look at infectious diseases potentially transmitted by FMT (Day 1) .

[e] Stool sample will be split into different aliquots; (1) for *C. difficile* PCR, (1) for *C difficile* toxin assay, (at screeing and when signs and symptoms of CDAD are present), (1) for microbiome determination, (1) for safety assessment following AESI related to newly acquired transmissible infectious agents (Appendix E)

[f] AEs will be collected from the time of informed consent through Day 30 post completion of treatment. SAEs will continue to be collected and assessed through the Day 365. AESIs will be collected through 365 days after completing treatment for recurrent CDAD (Appendix E). New onset related chronic medical conditions will be collected from the time the first enema is administered through Day 365.

[g] The following concomitant medications will be recorded for 90 days prior to screening and throughout Day 365: anti-infectives, probiotics, any medications used in the treatment of CDAD, any medications that could affect peristalsis, any drugs with antacid properties, any NSAID, any antihistamines, any lipid lowering agents, antineoplastic agents.

[h] Clinician will evaluate for signs and symptoms of CDAD during clinic visit. Intercurrent diarrhea status is to be assessed during subject interviews, supplemented by review of the subject's personal records (if hospitalized), memory aid.

[i] A history driven physical exam performed depending on interim medical history

[j] Review of memory aid performed only if unscheduled visit occurs prior to Day 30.

[k] Interim medical history

[l] At screening only blood pregnancy test should be performed

[m] If the clinical suspicion is high vancomycin should be dispensed and started immediately however this scenario is based on the fact that the PCR will come back negative, but if the clinical suspicion is low, vancomycin will be dispensed.

Appendix A-2: Schedule of Events of Enema #2

Table 2: Scenario 2 - Receipt of placebo with diarrhea and positive *C. difficile* testing Days 5-8 after randomization followed by no diarrhea or diarrhea with negative *C. difficile* testing at study days 5-8 after FMT or

Scenario 4 - Receipt of FMT with diarrhea and positive *C. difficile* testing Days 5-8 after randomization

	Screening Visit	Enrollment Visit (1st Enema)	Day 4[a]	Day 8	FMT visit	Day 4[a]	Day 8	Day 9 Visit	Day 15[a] Day 22[a]	Day 30 Visit	Day 60 Visit	Day 120[a] Day180[a] Day 240[a] Day 300[a]	Day 365 Visit	Unscheduled Visit for Recurrence
	Occurred under Scenario 1					***Post Last Enema***								
Visit	A00	A01	A02	A03	B04	B05	B06	B07	B08, B09	B10	B11	B12, B13, B14, B15	B16	Supplemental
Window			-1	-3	0	-1	-3	+3	±2	±3	±5	±7	±14	n/a
Informed Consent	X													
Inclusion/ Exclusion	X	X		X[m]	X		X							
Test of Comprehension	X													
Medical history	X	X[k]		X[k]	X[k]		X	X[k]		X[k]	X[k]		X[k]	X[k]
HIV screen if no known history of HIV + within the last 6 months	X													
Randomization		X												
Physical exam	X	X[i]		X[i]	X[i]		X[i]	X[i]		X[i]	X[i]		X[i]	X[i]

	Screening Visit	Enrollment Visit (1st Enema)	Day 4[a]	Day 8	FMT visit	Day 4[a]	Day 8	Day 9 Visit	Day 15[a] Day 22[a]	Day 30 Visit	Day 60 Visit	Day 120[a] Day180[a] Day 240[a] Day 300[a]	Day 365 Visit	Unscheduled Visit for Recurrence
	Occurred under Scenario 1					***Post Last Enema***								
Visit	A00	A01	A02	A03	B04	B05	B06	B07	B08, B09	B10	B11	B12, B13, B14, B15	B16	Supplemental
Window			-1	-3	0	-1	-3	+3	±2	±3	±5	±7	±14	n/a
Vital signs [b]	X	X		X	X		X	X		X	X		X	X
Measuring waist circumference		X			X						X		X	
Clinical lab tests [c]	X	X			X			X		X	X		X	X[c]
Blood samples [d]		X			X			X		X	X		X	
Stool sample [e]	X	X		X	X		X	X		X	X		X	X
AEs/SAEs/AESIs/Chronic Med. Conditions [f]		X	X	X	X	X	X	X	X	X	X	X	X	X
Bowel preparation and antiperistaltic Instructions	X			X[m]			X							
Distribution of memory aid		X			X									
Review memory aid			X	X		X	X	X	X	X				

	Screening Visit	Enrollment Visit (1st Enema)	Day 4[a]	Day 8	FMT visit	Day 4[a]	Day 8	Day 9 Visit	Day 15[a] Day 22[a]	Day 30 Visit	Day 60 Visit	Day 120[a] Day180[a] Day 240[a] Day 300[a]	Day 365 Visit	Unscheduled Visit for Recurrence
	Occurred under Scenario 1					***Post Last Enema***								
Visit	A00	A01	A02	A03	B04	B05	B06	B07	B08, B09	B10	B11	B12, B13, B14, B15	B16	Supplemental
Window			-1	-3	0	-1	-3	+3	±2	±3	±5	±7	±14	n/a
														[X][j]
Prior and/or concomitant medications [g]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine/Serum pregnancy test	X[l]	X		X	X		X							
Evaluation of signs and symptoms of CDAD [h]	X	X	X	X	X	X	X	X	X	X	X			X
Investigator Determination of clinical response			X	X		X		X	X	X	X			X
Provide oral vancomycin				X[m]			X[n]							
Enema administration		X			X									
Inquiry about interim diagnosis of CDAD												X	X	

[a] Subject interviews may be conducted by telephone. Subjects will also be contacted at Day 4 after enema treatment to confirm clinical response and then weekly thereafter until recurrence or the 60-day post-treatment Visit ,whichever comes first to evaluate possible CDAD recurrence. Staff will evaluate for symptoms of CDAD during phone visit and consult with clinician if needed. Subject interviews will be supplemented by review of the subject's personal records (if hospitalized) and memory aid.

[b] Includes blood pressure, pulse, weight and body temperature. Height will be collected at screening visit only.

[c] Clinical labs include potassium, creatinine, liver function tests (ALT), hemoglobin, WBC, neutrophil count, and platelets.

[d] Metabolic Syndrome markers including triglyceride level, HDL, LDL, HDL/LDL ratio, fasting glucose (FMT visit, Day 60 and Day 365), future use (FMT visit, Day 9, 30, 60 and 365), storage of sera and plasma to look at infectious diseases potentially transmitted by FMT (FMT visit).

[e] Stool sample will be split into different aliquots; (1) for *C. difficile* PCR, (1) for *C. difficile* toxin assay, (at screening and when signs and symptoms of CDAD are present), (1) for microbiome determination, (1) for safety assessment following AESI related to newly acquired transmissible infectious agents (Appendix E), and (1) for future use.

[f] AEs will be collected from the time of informed consent through Day 30 post-FMT treatment. SAEs will continue to be collected and assessed through Day 365. AESIs will be collected through 365 days after completing treatment for recurrent CDAD (Appendix E). New onset related chronic medical conditions will be collected from the time the first enema is administered through Day 365.

[g] The following concomitant medications will be recorded for 90 days prior to screening and throughout Day 365 post FMT: anti-infectives, probiotics, any medications used in the treatment of CDAD, any medications that could affect peristalsis, any drugs with antacid properties, any NSAID, any antihistamines, any lipid lowering agents, antineoplastic agents.

[h] Clinician will evaluate for signs and symptoms of CDAD during clinic visit. Intercurrent diarrhea status is to be assessed during subject interviews, supplemented by review of the subject's personal records (if hospitalized), memory aid.

[i] A history driven physical exam performed depending on interim medical history

[j] Review of memory aid performed only if unscheduled visit occurs prior to Day 30.

[k] Interim medical history

[l] At screening only blood pregnancy test should be performed

[m] If the clinical suspicion is high vancomycin should be dispensed and started immediately and if PCR test is positive the drug should be continued, but if the clinical suspicion is low, vancomycin will be dispensed but won't be started until the PCR results are back and subject is notified. For the GoLYTELY and loperamide - the subject will come and pick them from the clinic at least one day prior to the enema procedure.

[n] If the clinical suspicion is high vancomycin should be dispensed and started immediately however these scenarios are based on the fact that the PCR will come back negative at this timepoint or there is no more FMT to be offered, but if the clinical suspicion is low, vancomycin will be dispensed.

Appendix A-3: Schedule of Events of Enema #3

Table 3: Scenario 3 - Receipt of placebo with diarrhea and positive *C. difficile* testing Days 5-8 after randomization followed by diarrhea and positive *C. difficile* testing 5-8 days after first FMT

	Screening Visit	Enrollment Visit	Day 4[a]	Day 8	First FMT Visit	Day 4[a]	Day 8	2nd FMT Visit	Day 4[a]	Day 8 Visit	Day 9	Day 15[a] Day 22[a]	Day 30 Visit	Day 60 Visit	Day 120[a] Day180[a] Day 240[a] Day 300[a]	Day 365 Visit	Unscheduled Visit for Recurrence
		Occurred under 2							***Post Last Enema***								
Visit	A00	A01	A02	A03	B04	B05	B06	C07	C08	C09	C10	C11, C12	C13	C14	C15, C16, C17, C18	C19	Supplemental
Window			-1	-3		-1	-3		-1	-3	+3	±2	±3	±5	±7	±14	n/a
Informed Consent	X																
Inclusion/ Exclusion	X	X		X[m]	X		X	X									
Test of Comprehension	X																
Medical history	X	X[k]		X[k]	X[k]		X[k]	X[k]		X[k]	X[k]		X[k]	X[k]		X[k]	X[k]
HIV screen if no known history of HIV	X																

	Screening Visit	Enrollment Visit	Day 4[a]	Day 8	First FMT Visit	Day 4[a]	Day 8	2nd FMT Visit	Day 4[a]	Day 8 Visit	Day 9	Day 15[a] Day 22[a]	Day 30 Visit	Day 60 Visit	Day 120[a] Day180[a] Day 240[a] Day 300[a]	Day 365 Visit	Unscheduled Visit for Recurrence
		Occurred under 2							***Post Last Enema***								
Visit	A00	A01	A02	A03	B04	B05	B06	C07	C08	C09	C10	C11, C12	C13	C14	C15, C16, C17, C18	C19	Supplemental
+ within the last 6 months																	
Randomization		X															
Physical exam	X	X[i]		X[i]	X[i]		X[i]	X[i]		X[i]	X[i]		X[i]	X[i]		X[i]	X[i]
Vital signs[b]	X	X		X	X		X	X		X	X		X	X		X	X
Measuring waist circumference		X			X			X						X		X	
Clinical lab tests[c]	X	X			X			X			X		X	X		X	X[c]
Blood samples[d]		X			X			X			X		X	X		X	
Stool sample[e]	X	X		X	X		X	X		X	X		X	X		X	X

	Screening Visit	Enrollment Visit	Day 4[a]	Day 8	First FMT Visit	Day 4[a]	Day 8	2nd FMT Visit	Day 4[a]	Day 8 Visit	Day 9	Day 15[a] Day 22[a]	Day 30 Visit	Day 60 Visit	Day 120[a] Day180[a] Day 240[a] Day 300[a]	Day 365 Visit	Unscheduled Visit for Recurrence
		Occurred under 2							***Post Last Enema***								
Visit	A00	A01	A02	A03	B04	B05	B06	C07	C08	C09	C10	C11, C12	C13	C14	C15, C16, C17, C18	C19	Supplemental
AEs/SAEs/AESIs/Chronic Med. Conditions[f]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Bowel preparation and antiperistaltic Instructions	X			X[m]			X[m]										
Distribution of memory aid		X			X			X									
Review memory aid			X	X		X	X		X	X	X	X	X				[X][j]
Prior and/or concomitant medications[g]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

	Screening Visit	Enrollment Visit	Day 4[a]	Day 8	First FMT Visit	Day 4[a]	Day 8	2nd FMT Visit	Day 4[a]	Day 8 Visit	Day 9	Day 15[a] Day 22[a]	Day 30 Visit	Day 60 Visit	Day 120[a] Day180[a] Day 240[a] Day 300[a]	Day 365 Visit	Unscheduled Visit for Recurrence
		Occurred under 2							***Post Last Enema***								
Visit	A00	A01	A02	A03	B04	B05	B06	C07	C08	C09	C10	C11, C12	C13	C14	C15, C16, C17, C18	C19	Supplemental
Urine/Serum pregnancy test	X[l]	X		X	X		X	X									
Evaluation of signs and symptoms of CDAD[h]	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
Investigator Determination of clinical response			X	X		X	X		X	X	X	X	X	X			X
Provide oral vancomycin				X[m]			X[m]										
Enema administration		X			X			X									
Inquiry about interim diagnosis of CDAD															X	X	

[a] Subject interviews may be conducted by telephone. Subjects will also be contacted at Day 4 after enema treatment to confirm clinical response and then weekly thereafter until recurrence or the 60-day post-treatment Visit , whichever comes first, to evaluate possible CDAD recurrence. Staff will evaluate for symptoms of CDAD during phone visit and consult with clinician if needed. Subject interviews will be supplemented by review of the subject's personal records (if hospitalized) and memory aid.

[b] Includes blood pressure, pulse, weight and body temperature. Height will be collected at screening visit only.

[c] Clinical labs include creatinine, potassium, liver function tests (ALT), hemoglobin, WBC, neutrophil count, and platelets.

[d] Metabolic Syndrome markers, including triglyceride level, HDL, LDL, HDL/LDL ratio, fasting glucose (FMT visit, Day 60 and Day 365), future use (FMT visit, Day 9, 30, 60 and 365, storage of sera and plasma to look at infectious diseases potentially transmitted by FMT (FMT visit).

[e] Stool sample will be split into different aliquots; (1) for *C. difficile* PCR, (1) for *C. difficile* toxin assay, (at screening and when symptoms of CDAD are present), (1) for microbiome determination, (1) for safety assessment following AESI related to newly acquired transmissible infectious agents (Appendix E), (1) for future use.

[f] AEs will be collected from the time of informed consent through Day 30 post-FMT treatment. SAEs will continue to be collected and assessed through Day 365. AESIs will be collected through 365 days after completing treatment for recurrent CDAD (Appendix E). New onset related chronic medical conditions will be collected from the time the first enema is administered through Day 365.

[g] The following concomitant medications will be recorded for 90 days prior to screening and throughout Day 365 post FMT: anti-infectives, probiotics, any medications used in the treatment of CDAD, any medications that could affect peristalsis, any drugs with antacid properties, any NSAID, any antihistamines, any lipid lowering agents, antineoplastic agents.

[h] Clinician will evaluate for signs and symptoms of CDAD during clinic visit. Staff will evaluate for symptoms of CDAD during phone visit and consult with clinician if needed. [i] A history driven physical exam performed depending on interim medical history.

[j] Review of memory aid performed only if unscheduled visit occurs prior to Day 30.

[k] Interim medical history

[l] At screening only blood pregnancy test should be performed

[m] If the clinical suspicion is high, vancomycin should be dispensed and started immediately and if PCR test is positive the drug should be continued, but if the clinical suspicion is low, vancomycin will be dispensed but won't be started until the PCR results are back and subject is notified. For the GoLYTELY and loperamide - the subject will come and pick them from the clinic at least one day prior to the enema procedure.

Appendix B: Laboratory Adverse Event Grading Scale**

The clinical adverse event grading scale below is based on the FDA Guidance for Industry dated September 2007.

Table 4: Adverse Event Grading

Laboratory	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Potassium, high, mEq/L	5.2	5.3-5.4	≥ 5.5
Potassium, low, mEq/L	3.1	3.0	<3.0
Creatinine mg/dL	1.5-1.7	1.8-2.0	> 2.0
Liver Function Tests (ALT) increase by factor	1.1-2.5 x ULN*	>2.5-5.0 x ULN*	>5.0 x ULN*
Hgb (female), g/dL	11.0-11.3	9.5–10.9	<9.5
Hgb (male), g/dL	12.5-12.8	10.5- 12.-4	<10.5
WBC, increase, cells, x 10^3u/L	10.8-15.0	15.1 - 20.0	>20.0
WBC, decrease, cells, x 10^3u/L	2.5-3.1	1.5-2.4	<1.5
Absolute Neutrophil Count (female), x 10^3u/L	0.800-0.909	0.600-0.799	≤0.599
Absolute Neutrophil Count (male), x 10^3u/L	0.650-0.669	0.600-0.649	≤0.599
Platelets, decrease, cells/mm^3	125,000-134,000	100,000- <125,000	<100,000

* ULN is upper limit of normal in lab.

** Any clinical lab value Grade 3 (unless present at baseline) will be reported as an AE

Appendix C: Vital Signs Adverse Event Grading Scale

Table 5: Vital Signs Adverse Events Grading Scale**

Adverse Event	Severity	Parameter
Fever	Mild (Grade 1)	38.0 – 38.4°C 100.4 – 101.2°F
	Moderate (Grade 2)	38.5 – 38.9°C 101.3 – 102.0°F
	Severe (Grade 3)	≥ 39.0°C >102.0°F or ER visit or hospitalization
Hypertension (systolic)	Mild (Grade 1)	141-150 mm Hg
	Moderate (Grade 2)	151-155 mm Hg
	Severe (Grade 3)	>155 mm Hg or ER visit or hospitalization for malignant hypertension
Hypertension (diastolic)	Mild (Grade 1)	91-95
	Moderate (Grade 2)	96-100
	Severe (Grade 3)	>100 or ER visit or hospitalization for malignant hypertension
Hypotension (systolic)	Mild (Grade 1)	85-89 mm Hg
	Moderate (Grade 2)	80-84 mm Hg
	Severe (Grade 3)	<80 mm Hg or ER visit or hospitalization for hypotensive shock
Bradycardia *	Mild (Grade 1)	50-54 bpm*
	Moderate (Grade 2)	45-49 bpm
	Severe (Grade 3)	<45 bpm or ER visit or hospitalization for arrhythmia
Tachycardia	Mild (Grade 1)	101-115 bpm
	Moderate (Grade 2)	116-130 bpm
	Severe (Grade 3)	>130 bpm or ER visit or hospitalization for arrhythmia

*If subject baseline heart rate is <55 beats per minute and the investigator determines that this is not clinically significant (e.g., athletes) and heart rate increases > 55 beats per minute on moderate exercise (two flights of stairs), this will not be considered a Grade 1 adverse event.
**A change in vital signs is not considered an adverse event unless there is a severity grade increase of 1 or more from baseline.

Appendix D: Solicited Adverse Event Grading Scale

Table 6: Solicited Adverse Events Grading Scale

Clinical Feature	Grade 1	Grade 2	Grade 3
Fever	38.0 – 38.4°C 100.4 – 101.2°F	38.5 – 38.9°C 101.3 – 102.0°F	≥ 39.0°C >102.0°F or ER visit or hospitalization
Chills	Mild	Moderate	Severe or ER visit or hospitalization
Nausea	Mild	Moderate	Severe or ER visit or hospitalization
Diarrhea	≥3 loose or liquid stools or ≥300-599 gm of loose or liquid stool /24 consecutive hours	4-5 loose or liquid stools or ≥600-800 gm of loose or liquid stool /24 consecutive hours	≥6 liquid stools or >800 gm of loose or liquid stool / 24 hours or ER visit or hospitalization or requires outpatient IV hydration
Vomiting	1-2 episodes in 24 hours	3-5 episodes in 24 hours	>5 episodes in 24 hours or ER visit or hospitalization or requires outpatient IV hydration
Constipation	Occasional or intermittent symptoms; occasional use of stool softeners, laxatives, dietary modification, or enema	Persistent symptoms with regular use of laxatives or enemas; limiting instrumental activities of daily living	Obstipation with manual evacuation indicated; limiting self-care activities of daily living
Abdominal Cramps	Mild	Moderate	Severe or ER visit or hospitalization
Abdominal bloating	Mild	Moderate	Severe or ER visit or hospitalization
Flatulence	Mild	Moderate	Severe or ER visit or hospitalization
Malaise, fatigue	Mild	Moderate	Severe or ER visit or hospitalization

Loss of appetite	Mild	Moderate	Severe or ER visit or hospitalization

Scoring guidelines:

- Mild: events require minimal or no treatment and do not interfere with the patient's daily activities.
- Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe: events interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Note: Definitions in the table will take precedence over the footnotes for diarrhea, vomiting, and constipation

Appendix E: Algorithm for Adverse Events of Special Interest

Figure 2: Algorithm for Adverse Events of Special Interest

*High risk patients include immunocompromised OR 70 years and above OR multiple comorbidities as determined by the investigator
** Ova and enteric parasites include Cyclospora, Isospora, *Giardia lamblia*, *Cryptosporidium* and *Microsporidia*.
*** Viruses include Rotavirus, Adenovirus and Norovirus