VA Cooperative Studies Program #2004

Microbiota or Placebo after Antimicrobial Therapy for Recurrent *C. difficile* at Home

Protocol

Version 3.4

May 2, 2022

Principal Investigators: **Dimitri Drekonja, MD, MS**
Aasma Shaukat, MD, MPH
Biostatisticians: **Jane Zhang, Ph.D.**

< This page left intentionally blank >

Protocol Chronology

Protocol Version # / Amendment #	**Date of Protocol / Amendment**
CSSEC Protocol	10/28/15
V1.1 (After CSSEC)	8/25/16
V1.2 (Interim Draft)	7/18/17
V2.0 (Interim Draft)	4/27/18
V3.0 (Kick-off Version)	9/6/18
V3.1 (Update due to FDA Safety Memo)	7/16/19
V3.2 (Update for processing consent and randomization with VVC)	7/16/2020
V3.3 (Exclusion criteria change, AE/SAE clarification, update to ICF COVID language.	10/15/2021
V3.4 (Update for use of DocuSign as a method of signing Informed Consent form, update the modification of sample size, reduction in power, drop out rate, and enrollment time)	5/2/2022

< This page left intentionally blank >

TABLE OF CONTENTS

< This page left intentionally blank >

Letters Of Submittal

Principal Proponents

DEPARTMENT OF VETERANS AFFAIRS
Minneapolis VA Healthcare System
One Veterans Drive
Minneapolis, MN 55417

October 28, 2015

Grant Huang, M.P.H., Ph.D.
Acting Director,
VA Cooperative Studies Program (121-D)
810 Vermont Avenue, NW
Washington, DC 20420

Dear Dr. Huang:

We are submitting a proposal for the VA Cooperative Studies Program #2004: Microbiota vs. Antimicrobial Therapy for recurrent *C. difficile* at Home for review by the Cooperative Studies Scientific Evaluation Committee. The proposal assesses the efficacy of fecal microbiota transplantation (FMT) for the prevention of subsequent recurrent *Clostridium difficile* infection (CDI), when administered after successful treatment of recurrent CDI with standard antimicrobial therapy.

Clostridium difficile infection is a common nosocomial infection, and is increasingly seen in non-hospitalized patients. Although more than 90% of patients have symptom resolution with standard antimicrobial therapy, subsequent recurrence rates range from 15-30% (after the first CDI episode) to 40-50% (after two or more episodes). Fecal microbiota transplantation has shown promise as an adjunct to standard antimicrobial therapy, reducing recurrence to 15%. Recent administration of FMT via oral capsule provides an opportunity to deliver FMT via a non-invasive and convenient method. With the high burden of recurrent CDI in the Veterans Health Administration, considerable interest in FMT exists from both providers and patients. Current availability of FMT is site-dependent, with some VA facilities performing the procedure, others referring patients to community-based providers, and others having no mechanism of providing FMT. Furthermore, the lack of strong evidence for the efficacy of CDI treatment has led to regulatory uncertainty, with the Food and Drug Administration requiring an Investigational New Drug application (IND) for research involving FMT, but exercising "enforcement discretion" regarding the IND requirement for treatment of CDI with FMT.

This study is important to both our Veterans and the public. After numerous meetings and discussions, we believe that our protocol is ready for review by the CSSEC.
Thank you for your consideration.

Sincerely yours,

Dimitri Maximilian Drekonja, M.D., M.S.
Staff Physician,
Minneapolis VA Medical Center
Assistant Professor of Medicine,
University of Minnesota
Minneapolis, MN 55417

Aasma Shaukat, M.D. M.P.H
GI Section Chief,
Minneapolis VA Medical Center
Associate Professor of Medicine
University of Minnesota
Minneapolis, MN 55417

DEPARTMENT OF VETERANS AFFAIRS
VA Connecticut Healthcare System
950 Campbell Avenue
West Haven, CT 06516

COOPERATIVE STUDIES PROGRAM COORDINATING CENTER

Date: October 26, 2015 In Reply Refer To: 689/151A

From: Acting Director, CSPCC (151A)/VA CT HCS/950 Campbell Ave/West Haven, CT 06516

Subj: CSP #2004 CSSEC Submission

To: Grant D. Huang, MPH, PhD, Acting Director, CSP

1. Attached is the submission for CSSEC review of CSP #2004 "*The VA Fecal Microbiota Therapy Trial (FMT) for Recurrent Clostridium difficile Infection (MATCH)*" from the Principal Proponents, Dimitri Drekonja, MD, MS, and Aasma Shaukat, MD, MPH and the Study Biostatisticians, Jane Zhang, PhD and Tassos Kyriakides, PhD.

2. Study Design: CSP#2004 is a randomized placebo-controlled trial of fecal microbiota transplantation (FMT), a relatively new treatment strategy, for preventing recurrence of *C. difficile* infection. *C. difficile* infection is a common and often serious nosocomial complication of hospitalization, but also is occurring (or recurring) at increasing rates outside of hospital stays. It is an increasing problem in the VA Healthcare System and has been identified as one of three "urgent" threats by the US Centers for Disease Control and Prevention in its first Antimicrobial Resistance Threat Report. Recent advances in FMT as treatment for preventing *C. difficile* infection make this an important and timely study that could have a great positive impact on healthcare within the VA system.

   The study is designed to determine the efficacy of FMT to reduce the recurrence of *C. difficile* infection compared to placebo in a population of Veterans who have experienced at least one occurrence of *C. difficile* infection and have recently completed a course of standard antimicrobial therapy with resolution of symptoms.

   The target sample size is 390 participants (195 per treatment group) to be enrolled over 3 years. This sample size will provide 90% power to detect a 15% absolute reduction (from 35% to 20%) in the recurrence rate of *C. difficile* infection. While this is a large relative reduction, evidence from smaller studies and series suggest that this is realistic. The study proponents also believe that any treatment effect must be pronounced in order for FMT treatment to be accepted by clinicians and patients. Participants randomized to the study will be followed for clinical outcomes for 56 days by telephone contact with study coordinators or by an interactive voice response system as well as through the review of medical records. Safety follow-up for serious adverse events and longer-term *C. difficile* recurrence will be extended to six months post randomization.

   Stool specimens will be collected prior to treatment, during follow-up at the time of onset of symptoms of *C. difficile* and at 56 days after randomization. This will allow for the detection of C*. difficile* using PCR techniques, as well as for the assessment of the colonic microbiome before and after treatment. The latter will be an important component of the trial that will provide additional information to summarize are the effect and safety of FMT treatment as well as better understanding of how FMT changes a recipient's colonic flora.

The Principal Proponents and the Planning Committee have prepared a unique and efficient study design using regional sites for recruiting and following participants that will allow the study to complete enrollment in a shorter period of time than a conventional participating-site trial since recruitment will not be restricted to participants being seen at a limited number of funded VA medical centers. The feasibility of this design is supported by the results of a screening pilot study conducted by the Principal Proponents where cases of *C. difficile* infection were identified through medical record review and referring clinicians and patients were contacted to discuss potential participation in a trial like MATCH (see Protocol Section XI.C).

3. Analytic Plan: The primary outcome is recurrence of *C. difficile* infection (definite or probable) or death within 56 days of randomization, where the difference between definite and probable *C. difficile* infection is PCR confirmation of *C. difficile* in the stool sample. It is appropriate to include the probable cases in the primary outcome to avoid missing outcomes for symptom-confirmed cases where the stool sample could not be obtained or analyzed. The secondary analysis, including only definite C. *difficile* infection, will be performed as a supportive analysis.

   The incidence of primary and secondary outcomes occurring in the 56 day follow-up period will be analyzed using a generalized linear model with binary outcome with fixed effects (treatment assigned and previous recurrence of C. *difficile* infection [1 vs. ≥ 2 recurrences]) and planned adjustments for mixed effects, as needed. Treatment effects will be summarized by odds ratio in terms of FMT treatment relative to placebo with 95% confidence interval. All analysis will be done according to the intent-to-treat principle. Two interim analyses are planned that will allow for stopping the trial early for super efficacy or for futility or for recommending a change in the duration of the trial.

4. Budget: The total cost of the study is estimated to be $9,654,720, assuming the FMT product is provided to the study at cost (according to one vendor estimate). While this study will incur a large amount of travel costs for regional study coordinators to enroll and randomize participants, the trade off will be that there will only be study-site personnel supported at the three regional, enrolling sites.

   The Albuquerque CSP Clinical Research Pharmacy Coordinating Center (PCC) has provided a budget for receiving, storing and shipping the FMT materials as well as preparing matching (identical in appearance) placebo capsules. PCC has also researched which FMT providers may meet the manufacturing standards required for the anticipated IND. The provider and costs of the FMT product will be determined when the study is approved.

   An electronic data capture application using Medidata Rave is planned with an estimated cost $65,000 for the vendor agreement. Other application development and maintenance costs will be included in CSPCC and PCC data management and systems programming costs with annual service fees covered by a service contract managed by Perry Point CSPCC.

Respectfully submitted,

Gary R. Johnson, MS
Acting Director, CSPCC-West Haven

Department of Veterans Affairs
VA Cooperative Studies Program
Clinical Research Pharmacy Coordinating Center (151-I)
2401 Centre Avenue SE
Albuquerque, NM 87106-4180

September 8, 2015

In Reply Refer To: 501/151-I
CSP #95/ #2004
File: STD-DOC/SBF

Grant Huang, M.P.H., Ph.D.
Acting Director
VA Cooperative Studies Program (121-D)
810 Vermont Avenue, NW
Washington, DC 20420

SUBJ: Cooperative Studies Program Clinical Research Pharmacy Coordinating Center Issues Concerning CSP #2004, "MATCH: Microbiota vs. Antimicrobial Therapy for recurrent C. difficile at Home"

The following Pharmacy Coordinating Center (PCC) related issues have been identified:

Study Drug Supply

General

CSP #2004 will study a novel biological drug product which is not currently approved for commercial use in the United States. This will be the first clinical trial using a desiccated oral fecal microbiotal transplant (FMT) product which is not vulnerable to freeze-thaw cycles. Previous research in this area has evaluated various formulations of FMT material including fresh and frozen product, delivered by a variety of routes; however, the product planned for use in this study is felt to be a superior preparation that is considerably easier to handle and which can be easily adapted for use in clinical care.

As discussed below, active study drug will be manufactured by an external manufacturer and matching placebo study drug will be manufactured internally at PCC. Both drugs will be labeled, stored, and managed within PCC.

Active FMT

The PCC will purchase active study drug consisting of bottles containing 15 double-encapsulated capsules containing lyophilized, fecally-derived material. For the purposes of this study, a full treatment of FMT will consist of 12 capsules given as a single dose; three additional capsules are included so that the patient can receive the complete treatment in the unlikely event that one or more of the other capsules is damaged or destroyed. A full dose of 12 capsules contains 1×10^{12} viable (i.e., cell membranes intact) freeze-dried bacteria, so each capsule contains roughly 8.33×10^{11} viable bacteria.

The study plans to recruit 390 patients, about 195 of whom will receive active study drug. To ensure an adequate supply, PCC has assumed approximately 50% overage or 300 bottles containing one treatment each (15 capsules).

DP/tears

Each bottle of study drug will be labeled by PCC but bottles intended for patient administration will not be opened or the contents manipulated, eliminating the costs of additional processing and the need to perform stability and other analysis in-house. Instead, the manufacturer of the FMT capsules will perform the required potency and stability testing to ensure the product meets pre-determined quantitative specifications and will provide required documentation to the PCC. The manufacturer will also be responsible for ensuring that all stool donors meet eligibility requirements and that the product is free of agents known to cause infectious disease. A donor screening protocol from a potential provider of the product is included as an appendix to the protocol.

Potential providers of study drug considered were those manufacturers who indicated they were able to supply FMT oral capsules in sufficient quantities for the full three-year treatment duration of the study and who had preliminary data on stability and efficacy for the dosage form. Strong preference was given to providers who were able to meet cGMP standards for manufacturing and handling and limited preference given to products which were not adversely affected by freeze-thaw cycles. Based on these qualifying characteristics, two potential providers were identified and provided cost information for market research purposes, as detailed in the table below.

Provider	**Cost (active drug for sample size of 390 patients)**
University of Minnesota Microbiota Therapeutics Program (donation at cost)	$161,314
Crestovo/CIPAC	$506,340

Researchers at the Microbiota Therapeutics Program at the University of Minnesota (UM), a VA academic affiliate, have expressed a willingness to supply drug for this trial at cost. UM investigators developed and patented the technology to create lyophilized FMT and have been using it clinically for some months. The UM program is not currently manufacturing the medication in a cGMP manner, but anticipates that they will be able to do so in the near future using the cGMP-compliant facility maintained by the university.

In the event that the UM program is unable to provide the study drug for any reason, another option is to purchase the product from a commercial company, Crestovo (previously CIPAC), which UM has licensed to further develop lyophilized FMT. Other manufacturers could also be sought and considered in these circumstances,

Placebo FMT

To eliminate any possibility of contamination of placebo capsules with active material as well as to minimize the risk that active drug and placebo drug could be mistaken for one another during packaging at the manufacturer, PCC will manufacture and bottle placebo capsules using the same capsule shells, and packaging used for active study drug. The finished placebo product is expected to be visually indistinguishable from active drug, and other characteristics of the drug such as smell are not expected to differ significantly. We will work closely with the chosen manufacturer of active product to ensure an effective blind.

Costs of purchased drugs and supplies

All costs of purchased drugs and supplies are estimated as of the time of this submission. No assurance can be given as to the cost of these items at the time the study is initiated. If a significant length of time elapses prior to the start of the study and the actual procurement of the drugs and supplies, the risk of price changes increases.

Given that this drug will be manufactured on a small scale specifically for this study and that the University of Minnesota group has limited experience with cGMP manufacturing, there is some possibility that we would need to purchase study drug from Crestovo/CIPAC instead. This would increase the budget by about $345,000.

Storage requirements for storage at sites

Stability studies for the lyophilized formulation of the study drug which will be used in CSP #2004 are still ongoing, so limited information is available. However, preliminary results suggest that the drug is does not require continuous freezing conditions and that it can be held at room temperature during transport or short-term storage. When possible, including while the drug is stored for periods of up to six months at PCC, it will be kept at -80°C to minimize any possible degradation.

The drug will be shipped on cold packs to sites and should be maintained at the sites in a freezer at -10 to-25°C. Because similar temperature requirements exist for commonly used vaccines, we anticipate that all sites will have access to storage at these conditions. If this is not the case, it may be necessary to purchase freezers for the three participating sites.

Timing of Study Initiation

The PCC budget currently assumes a six month study start-up period; however, lead-time to study kickoff may be longer than normal for the following reasons:

- At this time, there are no formal agreements regarding intellectual property with any manufacturer. After CSSEC approval, PCC anticipates that a Cooperative Research and Development Agreement (CRADA) will need to be initiated in order to allow the study to accept the donation of study drug at cost from the University of Minnesota. Past experience with contracting and with CRADA negotiations suggests that these processes require a minimum of several months.

- To ensure that procedures employed in the manufacture of the drug are consistent with cGMP standards, PCC personnel experienced in quality assurance and regulatory requirements will visit the manufacturing facility twice. The first visit will provide information and consultation on any observed deficiencies in cGMP procedures; the second will be an audit to ensure that all requirements are met. If deficiencies are noted during the audit, the startup process could be delayed.

- As noted previously, the likely provider of the study drug is not currently capable of producing a cGMP product. If the manufacturer experience delays in initiating production, the study could also be delayed. Furthermore, the drug product likely will need to be used within a few months of production, depending on the stability results. For this reason, significant quantities cannot be held in reserve. Correspondingly, the study drug is more prone to delays in production due to technical or logistical issues with the provider.

- The University of Minnesota has filed INDs for clinical investigations of other FMT products but does not currently have an IND or Drug Master File (DMF) for the lyophilized capsules that will be used in this study. The provider has indicated that a DMF will be filed in the near future and that they are willing to provide a letter of cross reference to the FDA so that CSP #2004 can reference this information in an IND filing. However, if the DMF submission is postponed or if the FDA finds deficiencies in the information provided, this could delay approval of the IND needed to begin this study (if an IND is necessary; see next section for further details).

IND Requirement

In July, 2013, FDA issued a Guidance for Industry entitled "Enforcement Policy Regarding Investigational New Drug Requirements for Use of Fecal Microbiota for Transplantation to Treat Clostridium difficile Infection Not Responsive to Standard Therapies" which expressed an intention to exercise enforcement discretion regarding the need for an Investigational New Drug Application (IND) when using FMT to treat C. difficile infections not responding to standard therapy. A subsequent guidance issued in March, 2014, currently still in draft form, indicated that enforcement discretion would apply to FMT products "…obtained from a donor known to either the patient or the treating licensed health care provider." This study would not meet the prerequisites for the exercise of enforcement discretion stated in the draft guidance. Further, both the active and the draft Guidance state "during the period of enforcement discretion, FDA will continue to work with sponsors who intend to submit INDs for use of FMT to treat *C. difficile* infection not responding to standard therapies."

While it is unclear whether the draft Guidance will be finalized prior to the initiation of CSP #2004, PCC will act in accordance with normal procedures by contacting the FDA for formal guidance regarding whether this study qualifies for an Investigational New Drug Application (IND) exemption. Based on the requirement in 21 CFR 312.2(b) that "the drug product is lawfully marketed in the United States," CSP #2004 is likely to need an IND. Furthermore, we have had informal communications with the FDA regarding other aspects of this study, and our preliminary impression is that this study will require an IND.

We have therefore assumed for budgeting purposes that an IND will be required. After Cooperative Studies Scientific Evaluation Committee (CSSEC) approval, the PCC will submit the protocol to the FDA and obtain a definitive determination on this issue. If the study is conducted under an IND, PCC will prepare the application and CSP will be the sponsor.

Good Clinical Practices Training and Auditing/Monitoring Plan

The personnel and travel costs for kick-off meetings, training, and for monitoring/auditing of sites are in the Site Monitoring, Auditing and Resource Team (SMART) portion of the PCC budget. CSP #2004 has been tentatively assigned a "Category II" risk level, which is designated for studies requiring an IND/IDE, but not intended for NDA/PMA submission to the FDA.

During the study initiation meeting SMART will provide site coordinators and investigators with an overview of Good Clinical Practice (GCP) regulations and VA CSP policy review. The three study sites will undergo monitoring visits shortly after initial study startup and annually thereafter. Periodic and as-needed monitoring and auditing visits will also be conducted as necessary.

DP/tears

Grant Huang, M.P.H., Ph.D.

Please contact me at (505) 248-3203 if you have questions about any of these matters.

ANNE H. DAVIS-KARIM., Pharm.D.
Assistant Center Director
Pharmaceutical Management and Research

Concur/Non Concur

STUART R. WARREN, J.D, Pharm.D.
Director

Date: 9/11/15

Executive Summary/Abstract

Background:

Clostridium difficile infection (CDI) is one of the most common nosocomial infections and is increasingly seen in non-hospitalized patients. Although more than 90% of patients have symptom resolution with a course of standard antimicrobial therapy, subsequent recurrence rates range from 15-30% (after the first CDI episode) to 40-50% (after the second and subsequent episodes). Fecal microbiota transplantation (FMT) has shown promise as an adjunct to standard antimicrobial therapy, reducing recurrence among FMT recipients to 15%. The recent successful administration of FMT via oral capsule provides an opportunity to deliver FMT via a non-invasive and convenient method, which also allows for effective blinding. With the VA having a high burden of recurrent CDI, there has been considerable interest in FMT from both providers and patients. The availability of FMT is site-dependent, with some VA facilities performing the procedure, others referring patients to community-based providers, and others having no mechanism of providing FMT. Furthermore, the lack of strong evidence for the efficacy of CDI treatment has led to regulatory uncertainty, with the Food and Drug Administration requiring an Investigational New Drug application (IND) for research involving FMT, but exercising "enforcement discretion" regarding the IND requirement for treatment of CDI with FMT.

Objectives:

The primary study goal is to assess the efficacy of FMT for the prevention of subsequent recurrent CDI, when administered after successful treatment of recurrent CDI with standard antimicrobial therapy. Secondary goals are to evaluate the safety of FMT, and in the event of a positive study result, establish a mechanism for providing FMT within the VA system.

Research Plan:

This study will enroll 318 participants who have: 1) One or more episodes recurrent CDI (defined as > 3 loose/watery stools/24h for 2 consecutive days with CDI treatment, and not explained by another diagnosis PLUS laboratory confirmation of *C. difficile*; or ileus, or toxic megacolon PLUS laboratory confirmation of *C. difficile*, occurring within 90 days of a prior CDI episode), 2) Resolution or improvement of symptoms from most recent CDI episode, defined as no longer meeting the clinical definition for CDI for a 48 hour period during treatment, including not meeting the definition again after an initial improvement, 3) Within the enrollment window: from 2 days after completion of antimicrobial therapy for CDI (to allow for a washout period) to 14 days after completion of therapy or 30 days after the

onset of CDI whichever is the later, 4) Age ≥ 18 years, 5) Enrolled in a VHA facility, and 6) Able and willing to provide informed consent.

Subjects will be excluded if they have/are: 1) Unlikely to swallow capsules, 2) Pregnancy, planning to be pregnant, or breastfeeding, 3) Receipt of cytotoxic chemotherapy or immune globulin, or confirmed neutropenia (absolute neutrophil count of < 1,000 cells/mL) within the past 3 months, 4) Inflammatory bowel disease or other chronic diarrheal disease/fecal incontinence predating CDI, 5) Ongoing antibiotic use, 6) Prior FMT, 7) Life expectancy of < 8 weeks, 8) Anaphylactic food allergy, 9) Active enrollment in another research study on antibiotics, probiotics, or FMT without investigators approval, 10) Presence of an ileostomy or colostomy, 11) HIV with CD4 count < 200 cells/μL in prior 3 months, 12) Bone marrow/peripheral blood stem cell transplant in the past year, 13) Decompensated cirrhosis, or 14) Unlikely to follow study protocol.

Participants will be randomized (1:1 ratio) to FMT or placebo, stratified by number of prior recurrent CDI episodes (1 versus >1). They will be assessed for symptoms of CDI, other study outcomes, and any treatment-related adverse events at 2, 14, 28, 42, and 56 days, and month 3, 4, 5 and 6 after administration of the study treatment.

The primary outcome is recurrent CDI (definite or possible) or death within 56 days of randomization. *Definite recurrence* is defined as: new onset of more than 3 loose or watery stools in 24 hours for 2 consecutive days not explained by another diagnosis, with laboratory confirmation of *C. difficile* by enzyme-linked immunoassay (EIA) toxin test from a stool specimen; or ileus, toxic megacolon, or colectomy with laboratory confirmation.

Possible recurrence is defined identically as above but WITHOUT laboratory confirmation of *C. difficile* (not tested or negative test).

We postulate that FMT will result in a 15% absolute reduction in the recurrence rate of CDI (20% versus 35%). Using a Chi-square test with a 2-sided test of significance, $\alpha = 0.05$, a sample size of 318 participants will be required to test the primary hypothesis with 85% power, adjusted for 1% losses. The planned study duration is 6.5 years with 6 years for subject accrual and 6 months follow-up.

In order to maximize the number of potentially eligible participants and to make participation available to the largest number of Veterans, the study design is based on central enrollment, follow-up, and coordination.

< This page left intentionally blank >

Planning Committee

Dimitri Drekonja, M.D., MS.
Study Co-Chair
Minneapolis VA Healthcare System
One Veterans Drive
Minneapolis, MN 55417
Dimitri.Drekonja@va.gov

Peter Guarino, MPH, PhD.
SCHARP Director
Vaccine and Infectious Disease Division
Fred Hutchinson Cancer Research Center
1100 Fairview Avenue
Seattle, WA 98109
Pguarino@scharp.org

Aasma Shaukat, M.D., MPH.
Study Co-Chair
Minneapolis VA Healthcare System
One Veterans Drive
Minneapolis, MN 55417
Aasma.Shaukat@va.gov

Gary R. Johnson, MS.
Acting Director, WH-CSPCC (151A)
VA Connecticut Healthcare System
950 Campbell Avenue
West Haven, CT 06516
Gary.Johnson4@va.gov

Frank Lederle, M.D. (deceased)
Minneapolis VA Healthcare System
One Veterans Drive
Minneapolis, MN 55417

Anne H. Davis-Karim, Pharm.D.
Clinical Research Pharmacist
CSPCRPCC
2401 Centre Avenue SE
Albuquerque, NM 87106
Anne.Davis-Karim@va.gov

John Concato, M.D.
Director, CERC
VA Connecticut Healthcare System
950 Campbell Avenue
West Haven, CT 06516
John.Concato@va.gov

Tassos Kyriakides, Ph.D.
Study Biostatistician, CSPCC (151A)
VA Connecticut Healthcare System
950 Campbell Avenue
West Haven, CT 06516
Tassos.Kyriakides@va.gov

Jason Dominitz, M.D.
National Program Director for Gastroenterology
VA Puget Sound Healthcare System Seattle
1660 S. Columbian Way
Seattle, WA 98108
Jason.Dominitz@va.gov

Jane H. Zhang, Ph.D.
Study Biostatistician, CSPCC (151A)
VA Connecticut Healthcare System
950 Campbell Avenue
West Haven, CT 06516
Jane.Zhang@va.gov

Dale Gerding, M.D.
Chief, Medical Service
VA Chicago Health Care System
333 E. Huron
Chicago, IL 60611
Dale.Gerding2@va.gov

< This page left intentionally blank >

I. Introduction and Background

A. Background

Clostridium difficile infection (CDI) has transformed over the past 15 years from a relatively rare cause of antibiotic-associated diarrhea to the leading cause of infectious diarrhea in hospitalized patients, rivaling methicillin-resistant *Staphylococcus aureus* (MRSA) as the leading cause of all nosocomial infections [1, 2]. The impetus for this transformation was the emergence of an epidemic strain of *C. difficile* in the early 2000s [3], variously designated as the North American pulsed-field type 1 (NAP1), BI, and 027 by pulsed-field gel electrophoresis, restriction endonuclease analysis, and PCR-ribotyping, respectively. Emergence of this strain led to an increase in the incidence and severity of CDI, in addition to an increase in the mortality rate [3]. Nationwide estimates of CDI exceed 450,000, resulting in 29,000 deaths and over $4 billion in healthcare costs [1, 2, 4]. This increase in CDI has been even more striking in the Veterans Affairs (VA) Healthcare System, with rates of CDI exceeding those from non-VA facilities by 2-to-3 fold [5]. Risk factors for CDI include increasing age, hospitalization, multiple medical comorbidities, proton-pump inhibitor use, and antimicrobial use; all of which are common in the VA population.

Currently, *C. difficile* is one of three organisms to be assigned the top threat level of "urgent" by the US Centers for Disease Control and Prevention in its first Antimicrobial Resistance Threat Report, released in 2013 [6]. In contrast to the other 2 organisms receiving this designation, the inclusion of *C. difficile* was not based on limited treatment options due to antimicrobial resistance, but rather because of the high incidence rate of recurrent disease after successful treatment of CDI. Unfortunately, despite recurrence being the major challenge in treating CDI, treatment options are poorly studied, with few randomized controlled trials (RCTs) available to help inform clinicians and policy-makers.

Treatment guidelines [7] suggest that either vancomycin or fidaxomicin can be used for the treatment of the initial episode of CDI, based on recent studies suggesting that vancomycin is preferred over metronidazole for the initial episode of CDI of any severity [8], and that fidaxomicin is non-inferior to vancomycin [9, 10]. Unfortunately, although initial clinical cure is achieved in >90% of patients with each of these drugs, recurrent CDI (variously defined as an episode of CDI that occurs 8 weeks or less after a previous episode [11], or within 90 days of a previous episode [9, 12], provided that symptoms from the earlier episode resolved) occurs in 15-30% of cases [8, 9, 13]. Patients with a first recurrence have a 40%

risk of a second recurrence, after which the risk of subsequent recurrences exceeds 50%, leading to malnutrition, repeat clinic visits and hospitalizations, and decreased quality of life [14].

The optimal treatment of recurrent CDI is unknown. Guidelines from the Infectious Diseases Society of America suggest that for the first recurrence, either vancomycin in a tapered and/or pulsed dose strategy or fidaxomicin should be used, and that further episodes can be treated with a variety of strategies, including tapered vancomycin or fecal microbiota transplantation (FMT) [7]. Similarly, guidelines from the European Society of Clinical Microbiology and Infectious Diseases suggest vancomycin or fidaxomicin use for the first recurrence and FMT for multiple recurrences [15], and the American College of Gastroenterology recommends metronidazole or vancomycin in a pulsed regimen for the 1st and 2nd recurrence, with consideration of FMT after 3 recurrences [16]. The supporting evidence for these recommendations is of low quality, being largely guided by case series data and expert opinion. Comparing fidaxomicin, vancomycin, and tapered vancomycin for the treatment of recurrent CDI is the subject of CSP #596, which will provide guidance as to the optimal antimicrobial treatment for recurrent CDI. However, the role of FMT in the treatment of recurrent CDI is currently unknown.

B. Fecal Microbiota Therapy (FMT)

The use of FMT was first reported in the medical literature in 1958, when it was used to treat post-antibiotic pseudo-membranous colitis in 4 patients [17]. Since then, numerous case series have been published, with most reporting success rates in excess of 80%. FMT, also known as a "stool transplant," is thought to prevent recurrent CDI via rapid restoration of the normal diverse colonic microbiota by delivering a large inoculum of typical organisms, as opposed to allowing the gradual repopulation of the colonic microbiota through normal dietary and environmental exposure ([18]). Delivery of FMT has been performed via enema, colonoscopy, nasogastric tube, and most recently, via oral capsule [19]. In 2013, a small unblinded trial of FMT vs. 2 vancomycin-based control groups was published [20], reporting a success rate of 81% among the 16 patients randomized to FMT, vs. 23% and 31% in the 2 control groups. A recent systematic review identified a total of 520 patients with recurrent CDI treated with FMT, from 21 case series and 2 RCTs [21]. Although the overall rate of symptom resolution without recurrence was 85%, the majority of included studies were case series, and only 1 of the 2 RCTs had a non-FMT control group [20].

At the time of submitting this protocol, a 3rd randomized control trial has been presented in abstract form. This trial of FMT vs. placebo (both delivered via colonoscopy) showed similar success

rates. Specifically, FMT success was 20/22 (91%), vs. 15/24 (63%) for placebo (Oral presentation, Dr. Colleen Kelly, American College of Gastroenterology Annual Scientific Meeting, October 2015). Notably, in many of the studies it was unclear as to whether the FMT was administered as part of the initial treatment of CDI, or rather as an adjunct to standard treatment given to prevent subsequent recurrence. Accordingly, there is a need for an adequately-powered RCT designed to evaluate the efficacy of FMT for the prevention of recurrent CDI when compared to standard antimicrobial therapy.

C. Study Rationale

With more than 90% of patients having resolution of symptoms with a course of standard antimicrobial therapy, but then having recurrence rates ranging from 15-30% (after the first CDI episode) to 40-50% (after the second and subsequent episodes), adjunctive treatments to prevent recurrence after initial symptom resolution are needed. The recent successful administration of FMT via oral capsule [19] provides an opportunity to deliver FMT via a non-invasive and convenient method, which also allows the possibility of effective blinding. With the VA having a high burden of recurrent CDI, there has been considerable interest in FMT from both providers and patients. Currently the availability of FMT is site-dependent, with some VA facilities performing the procedure, others referring patients to community-based providers, and others having no mechanism of providing FMT. Furthermore, the lack of strong evidence for the efficacy of CDI treatment has led to regulatory uncertainty, with the Food and Drug Administration requiring an Investigational New Drug application (IND) for research involving FMT, but exercising "enforcement discretion" regarding the IND requirement for treatment of CDI with FMT.

With the high burden of CDI in the VA, the high recurrence rates, and the uncertainty regarding both how to treat recurrent CDI and the efficacy of FMT, a definitive RCT of FMT for the prevention of recurrent CDI is needed. A trial of capsule-administered FMT vs. placebo, both administered after successful initial treatment of recurrent CDI with standard antimicrobial therapy, for the prevention of subsequent recurrent CDI is hereby proposed.

D. Summary of Study Goals

The primary study goal is to assess the efficacy of FMT for the prevention of subsequent recurrent CDI, when administered after successful treatment of recurrent CDI with standard antimicrobial therapy. Secondary goals are to evaluate the safety of FMT, and in the event of a positive study result, establish a mechanism for providing FMT within the VA system.

< This page left intentionally blank >

II. Study Objectives and Hypotheses

A. Primary Objective

The proposed study's primary objective is to compare the incidence of recurrent CDI (definite or possible) or death within 56 days of randomization among participants receiving FMT vs. those receiving placebo capsules, after both groups have been successfully treated with one course of standard antimicrobial therapy.

Definite CDI recurrence is defined as: New onset of more than 3 loose or watery stools in 24 hours for 2 consecutive days with laboratory confirmation of *C. difficile* positive EIA toxin test from a stool specimen and absence of another diagnosis; or ileus, toxic megacolon, or colectomy with laboratory confirmation.

Possible recurrence is defined identically as above but WITHOUT laboratory confirmation of *C. difficile* (no specimen, not tested or negative test).

Our primary hypothesis is that Veterans receiving FMT after standard antimicrobial therapy will have reduced rates of recurrent CDI compared to those receiving placebo after standard antimicrobial therapy.

B. Secondary Objectives

Secondary objectives include treatment comparisons between FMT and placebo of occurrences of components of definite or possible CDI and other manifestations of CDI:

i. The incidence of recurrent CDI (definite or possible) or death within 6 months of randomization;
ii. The incidence of definite recurrent CDI within 56 days after randomization;
iii. The incidence of possible recurrent CDI within 56 days after randomization;
iv. The incidence of diarrhea that is negative for *C. difficile* by laboratory testing within 56 days after randomization;
v. Abdominal pain;
vi. Urgency;

vii. Fecal incontinence;

viii. The number of CDI recurrences within 6 months after randomization; and

ix. Self-reported quality of life within 56 days after randomization.

C. Safety Objectives

We will monitor the incidence, severity, and relatedness of serious adverse events and complications within 6 months to evaluate the safety of FMT. In the six months following study treatment, we will review electronic medical records and communicate with subjects via telephone to evaluate for possible transmission of infectious agents or development of new conditions theoretically linked to alterations in gut microbiota.

D. Exploratory Analyses

The following exploratory analyses are proposed:

i. Characterization and comparison of the colonic microbiome, before and after FMT/placebo administration;

ii. Comparison of the post-FMT colonic microbiome to the donor microbiome; and

iii. Comparison of the post-FMT microbiome of individuals who experience recurrence vs. the microbiome of those not experiencing recurrence at baseline, day 14, and day 56.

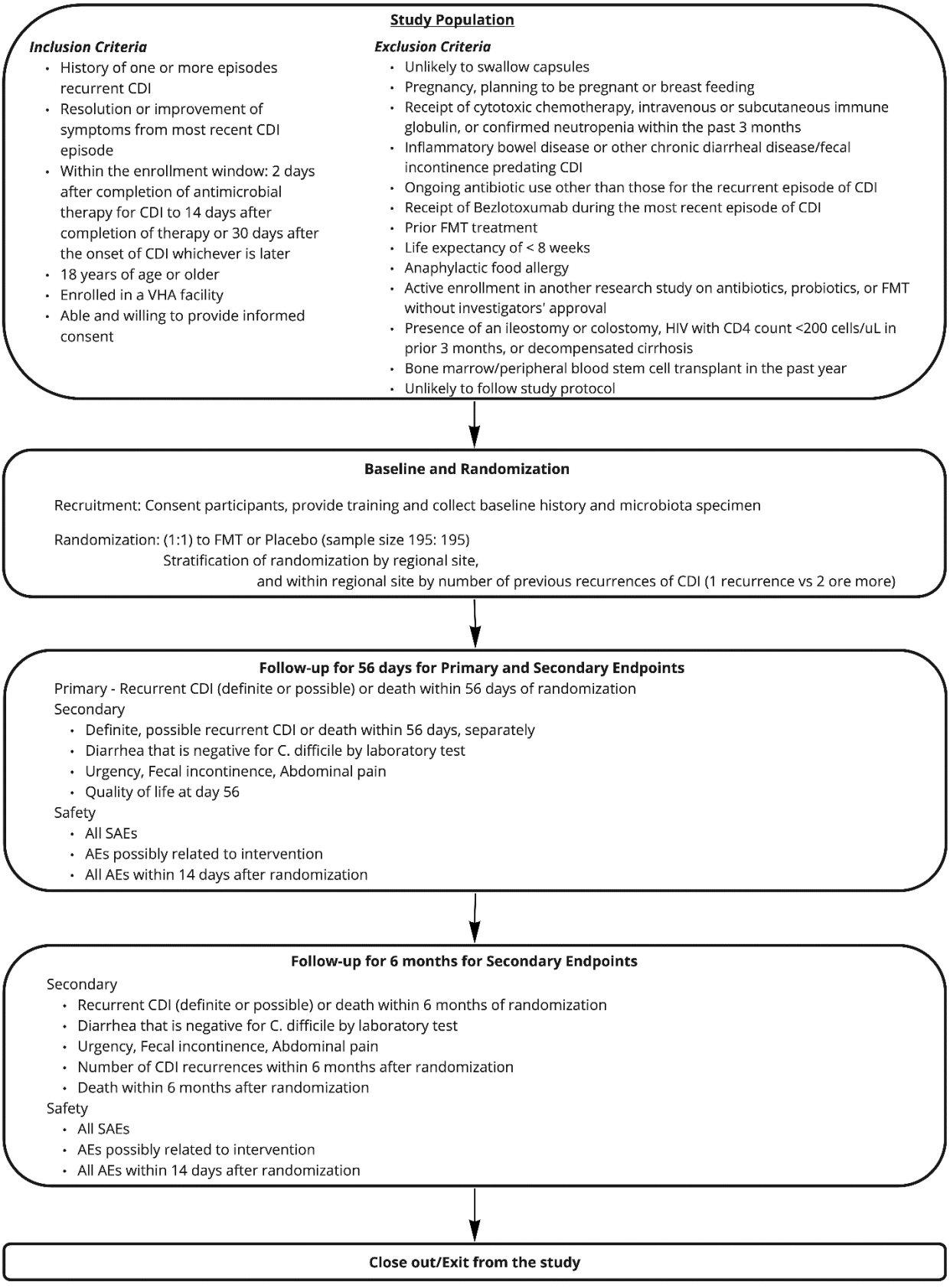
III. Summary of Study Design

A. Overview

This is a randomized, double-blind, placebo-controlled trial of capsule-delivered FMT for the prevention of recurrent CDI, administered after successful initial treatment of recurrent CDI with standard antimicrobial therapy. Initial treatment of the recurrent CDI episode will be at the discretion of the local provider, which currently could include metronidazole, vancomycin, or fidaxomicin. Patients will be contacted by Study Coordinators who will utilize direct referrals, reports of positive microbiology tests for *C. difficile*, diagnostic codes, and prescriptions to identify patients with recurrent CDI and obtain permission to contact the patient from the treating or primary care provider. Patients with CDI who have completed a standard course of antimicrobial treatment and exhibit a favorable response to treatment, who express interest in enrolling, and meet inclusion criteria will be scheduled for a visit with a Study Coordinator (SC). The SC will travel to the patient's location to enroll the patient and administer the study treatment. After obtaining informed consent and enrolling the patient, the patient will take the randomly assigned treatment capsules (FMT or placebo) under direct supervision of the Study Coordinator.

Following capsule administration, follow-up will be done via scheduled calls from either an automated Integrated Voice Response System (IVRS) or from study personnel, with ad-hoc contact triggered by either patients reporting symptoms or study personnel noting clinical manifestations of recurrence in the medical record, which will be monitored on a regular basis. The primary endpoint, first incidence of recurrent CDI (definite or possible) or death, will be assessed within 56 days of randomization. Safety outcomes and applicable secondary outcomes will be assessed until 6 months after randomization.

Figure 1. Study overview



B. Study Population and Rationale

Historically, FMT has been administered to patients with multiple recurrences of CDI. In part, this may have been due to the need for a procedure of some sort, with enema being the least-invasive method of administration, followed by nasogastric tube and colonoscopic administration. Aesthetic concerns were often invoked, with the assumption that FMT would not be acceptable to patients who would otherwise be treated with another round of antimicrobials. However, as FMT has become more common, it has been used for patients having their first recurrence of CDI [19, 20]. In the RCT comparing FMT to two vancomycin-based control groups, the number of recurrences per patient were not reported, but it is clear from the range of prior episodes that there was at least one patient with a first recurrence in each treatment group [20]. In the case series describing FMT administered via capsule, 1 of 20 patients treated had just a single recurrence of CDI [19]. Patient surveys indicate that the majority of patients are willing to undergo FMT, particularly if recommended by their provider [22]. In deciding which patients to include in this trial (1st, 2nd, or ≥ 3 episodes of recurrent CDI), consideration was given to the following:

(i) *Possible benefit of treatment:* The large effect size observed in the case series and the published RCTs is notable, especially for a condition where each subsequent episode of recurrence leads to an increased risk of still further recurrences. If FMT is shown to be as effective as suggested in these preliminary studies, reserving it for patients who are further advanced into the cycle of multiple recurrences may be counterproductive. Since FMT can now be administered via capsule, the possible harms of FMT are decreased, whereas the potential benefit remains great.

(ii) *Acceptability*: Patients with a first episode of recurrent CDI have been included in recent studies of FMT, demonstrating that at least some patients are willing to undergo this novel therapy without having gone through repeated episodes of recurrent CDI. The published experience with FMT suggests that patients tolerate the procedure very well, although having already undergone FMT, this is a unique population. In a survey of patients without CDI, 94% of respondents indicated that they would undergo FMT if recommended by their physician, although the acceptance rate may be artificially inflated by a high rate of non-responders (52%) [22]. The non-responders might be more likely to not be as accepting of FMT. Indeed, in an in-person survey of hospitalized patients with CDI who had a higher response rate of 81%, acceptance of FMT to treat their current CDI was lower (59%). However, a majority of patients (53%) indicated

that they would participate in a study of FMT vs. standard antimicrobial therapy, if such a study were available [23].

(iii) *Published experience with FMT*: Most reported cases of recurrent CDI treated with FMT are in patients with multiple recurrences. However, recent studies of FMT have included patients experiencing their first recurrence of CDI. This suggests that both patients and providers are viewing FMT as a therapy that needs not be reserved for the patient with multiple episodes of recurrence.

(iv) *Biological plausibility*: The pathogenesis of CDI begins with the elimination of the usual fecal microbiota, which normally confers some degree of colonization resistance to pathogens such as *C. difficile*. Microbiologic analysis of the diversity of the fecal microbiota shows that the diversity is markedly decreased after just one recurrence of CDI, such that it is not readily distinguishable from that of patients with multiple episodes of recurrence [24]. Accordingly, the benefits of restoring the microbiota via FMT may be as beneficial after one episode of recurrence as after multiple episodes.

(v) *Adequate number of cases*: Enrolling patients with a single episode of recurrent CDI increases the number of potentially eligible patients available for recruitment. This is illustrated in Table 1, which details the outcome of 100 patients, starting with an initial episode of CDI, and then continuing on to list the number expected to have subsequent recurrences, based on the number of patients at risk and the percentage expected to recur at each episode of recurrence.

Table 1. Number of patients expected to experience a first, second, and third recurrence, based on 100 patients with an initial CDI episode.

Episode of CDI	Number of patients expected per episode	Percent expected to recur
Initial episode	100	25%
1st recurrence	25	40%
2nd recurrence	10	50%
3rd recurrence	5	> 50%

Restricting enrollment to two or more recurrences results in a more than 50% reduction in potentially eligible patients (25 vs. 10); such a restriction will impact recruitment efforts.

(vi) *Ability to detect a treatment effect*: Enrolling patients at the first episode of recurrent CDI does affect the ability to detect a treatment effect, since those with a first recurrence are expected to have a smaller risk of subsequent recurrences than those having multiple recurrences. Accordingly, this decreases the overall number of subsequent recurrences, which affects the necessary sample size needed to detect a clinically significant treatment effect. This is further explored in the biostatistical section of the protocol (Section XII).

In summary, after considering the possible benefit of treatment, acceptability, published experience with FMT, biological plausibility, adequate number of cases, and ability to detect a treatment effect, enrollment of patients with a first recurrence of CDI is scientifically justifiable and provides an increased pool of potential study participants. Although it is anticipated that patients with multiple recurrences are more likely to enroll than patients with a first recurrence, capsule administration of FMT is the most appealing route of FMT delivery [22], and may help boost enrollment among those with a first recurrence.

C. Inclusion Criteria

In order to maximize the clinical relevance of this trial, inclusion/exclusion criteria were designed to be as inclusive as possible. The rationale for the proposed exclusion criteria are discussed below. The study's inclusion criteria are:

1) One or more episodes recurrent CDI (defined as > 3 loose/watery stools/24h for 2 consecutive days with CDI treatment, and not explained by another diagnosis PLUS laboratory confirmation of *C. difficile*; or ileus, or toxic megacolon PLUS laboratory confirmation of *C. difficile*, occurring within 90 days of a prior CDI episode with similar symptoms and laboratory confirmation);
2) Resolution or improvement of symptoms from most recent CDI episode, defined as no longer meeting the clinical definition for CDI for a 48 hour period during treatment, including not meeting the definition again after an initial improvement;
3) Within the enrollment window: 2 days after completion of antimicrobial therapy for CDI (to allow for a washout period) to 14 days after completion of therapy or 30 days after the onset of CDI whichever is later;
4) Age ≥ 18 years;
5) Enrolled in a VHA facility; and
6) Able and willing to provide informed consent.

D. Exclusion Criteria

The exclusion criteria are:

1) Unlikely to swallow capsules;
2) Pregnancy, planning to be pregnant, or breastfeeding;
3) Receipt of cytotoxic chemotherapy, intravenous or subcutaneous immune globulin, or confirmed neutropenia (absolute neutrophil count of < 1,000 cells/μL) within the past 3 months;
4) Inflammatory bowel disease or other chronic diarrheal disease/fecal incontinence predating CDI;
5) Ongoing antibiotic use other than those for the current episode of CDI;
6) Prior FMT;
7) Life expectancy of < 8 weeks;
8) Anaphylactic food allergy;
9) Active enrollment in another research study on antibiotics, probiotics, or FMT without investigators approval;
10) Presence of an ileostomy or colostomy;
11) HIV with CD4 count < 200 cells/μL in prior 3 months;
12) Decompensated cirrhosis;
13) Bone marrow/peripheral blood stem cell transplant in the past year;
14) Receipt of bezlotoxumab during the most recent episode of CDI;
15) Unlikely to follow study protocol.

E. Treatments and Rationale

Under the study protocol, individuals in both treatment arms will be randomized in a 1:1 ratio to received blinded FMT or placebo after initial treatment with antimicrobials has resulted in symptom improvement or resolution, which occurs in >90% of CDI episodes. The objective of FMT is to decrease the risk of subsequent recurrence of CDI, not treatment of CDI symptoms. As such, it can be considered as an adjunct to standard antimicrobial therapy.

A complete description of the treatment regiments is given in Section X.

F. Outcome Measures and Rationale

1. Primary Outcome

The primary outcome for this trial was selected to reflect the circumstances that currently lead clinicians and patients to consider FMT, and to provide a result that is clinically relevant. Since the great majority of patients referred for FMT are undertaking the procedure to reduce the risk of subsequent recurrent CDI, the primary outcome was chosen to provide a clear answer regarding the efficacy of FMT in preventing recurrent CDI.

The primary outcome is recurrent CDI (definite or possible) or death within 56 days of randomization.

Definite recurrence is defined as any of the following:

- The new onset of more than three loose or watery stools in 24 hours for two consecutive days, not explained by another diagnosis;
- Other clinical symptoms including ileus, toxic megacolon, or colectomy;

PLUS

- Laboratory confirmation of *C. difficile* from a stool specimen by EIA toxin test.

Possible recurrence is defined as the same clinical manifestations as above, but WITHOUT laboratory confirmation of *C. difficile* (stool test not sent, negative EIA toxin test result, or uninterpretable result).

2. Secondary Outcomes

i) The incidence of recurrent CDI (definite or possible) or death within 6 months of randomization;

ii) Definite recurrent CDI within 56 days, as defined above;

iii) Possible recurrent CDI within 56 days, as defined above;

iv) Death within 56 days since randomization;

v) Episode of diarrhea that is negative for *C. difficile* by EIA toxin test and PCR within 56 days

This is similar to a possible recurrent CDI episode, but includes only episodes of diarrhea that test negative for *C. difficile* by EIA toxin test and PCR, not episodes that are not tested or are uninterpretable;

vi) Episode of diarrhea that is negative for *C. difficile* by EIA toxin testing but positive by PCR within 56 days

This is similar to possible recurrent CDI, but includes only episodes of diarrhea that test negative for *C. difficile* by EIA toxin test, not episodes that are not tested or are uninterpretable;

vii) Abdominal pain for all possible CDI cases;

viii) Urgency for all possible CDI cases;

ix) Fecal incontinence for all possible CDI cases;

x) Number of CDI recurrences within 6 months

The number of CDI recurrences within 6 months for a patient is the count of separate CDI recurrences from randomization to 6 months after randomization; and

xi) Quality of life at day 56

We will use a brief assessment of both overall and gastrointestinal health status, using a previously validated instrument.

3. Safety Outcomes

Because of the limited information regarding long-term safety of FMT, safety outcomes will be collected until 6 months after FMT/placebo randomization. Monitoring and reporting of AEs and SAEs are further described in Section XIV.

Safety outcomes to be collected include:

i) Serious adverse events, with a focus on SAEs involving hospitalization (new or prolonged), and all-cause mortality;

ii) Adverse events which may be related to FMT treatment. This includes adverse events which Site Investigators consider related/possibly related to the study treatment and all adverse events which occur within 14 days of study treatment (since an aggregate analysis of events temporally linked to treatment could show a causal relationship when compared to placebo);

iii) Infectious transmissions which are plausibly linked to FMT treatment; and

iv) Development of new conditions theoretically linked to alterations in gut microbiota.

4. Outcome Measurements in Exploratory Analyses

We believe that evaluating microbiome is important, particularly in the context of this large trial incorporating a placebo control. Prior work demonstrating similarity between the post-transplant microbiome and the donor microbiome suggests that "engraftment" of the donor microbiome is important for clinical success. However, since these data come from uncontrolled series, this hypothesis has not been rigorously tested. The microbiome component can give us valuable insight in the event the study has positive or negative results. If a clear correlation between recurrent CDI and failure to engraft is demonstrated, this would clarify the mechanism whereby FMT confers its benefit. Conversely, if subjects with engraftment of the donor microbiome recur at a similar rate as do those without engraftment, this would suggest that other factors may be contributing to recurrence.

A stool sample for the assessment of the colonic microbiome will be obtained at the time of randomization, at 14 days after randomization, and 56 days after administration of FMT/placebo. The first sample will be transported to the central laboratory performing the microbiome characterization by the Study Coordinator, whereas the second sample and last sample will be mailed to the same laboratory by the participant via a pre-paid mailer.

Shannon index (diversity) and the abundance-based coverage estimate (ACE) (richness) will be the outcome measurements to evaluate microbiome.

The following exploratory analyses are proposed:

i. Characterization and comparison of the colonic microbiome of individuals with CDI randomized to FMT and placebo, before and after FMT/placebo administration;
ii. Comparison of the post-FMT colonic microbiome of individuals randomized to FMT and placebo to the donor microbiome; and
iii. Comparison of the post-FMT microbiome of individuals that experience a recurrence to the microbiome of those not experiencing recurrence.

G. Sample Size

The target sample size will be 318 participants. We anticipate the rate of recurrent CDI following standard antimicrobial therapy is 35%; the study is powered to detect a clinically significant absolute difference of 15%. This difference is chosen based on the non-invasive nature of the intervention, as well as favorable safety data from the > 500 cases of FMT reported in the literature. Using the 318 participants

and above assumptions, there will be 85% power to detect a 15% absolute difference (20% vs. 35%) with a two-sided significant threshold of 0.05, and a 1% drop-out rate. Complete details of the sample size calculation are described in Section XII.

H. Data Collection and Follow-up

Baseline data will be collected at the time of enrollment, which occurs when the patient signs the informed consent form in the presence of a Study Coordinator (SC). The SC will have reviewed the patient's medical record as part of the screening process, and been in telephone contact to verify interest in participation, assess inclusion/exclusion criteria, and resolution/improvement of CDI symptoms while on standard antimicrobial therapy. However, baseline data will only be recorded after enrollment. As part of the baseline data, a quality of life questionnaire will be administered [25], assessing overall and gastrointestinal quality of life.

At the time of capsule administration, participants will be asked to provide an optional stool sample for characterization of the microbiome. Participants will be provided with: a diary to record symptoms and details of their bowel patterns in case they develop diarrhea, a copy of the Bristol stool chart to help them characterize their stool in a consistent fashion, and stool collection/mailing kits. The mailing kits will be used to collect stool samples in the event of new diarrhea occurring after the study visit, including collection of a sample for characterization of the colonic microbiome at day 14 and at approximately day 56. Additionally, participants will be provided with a pamphlet describing the study, instructions on what to do in the event of recurrent diarrhea or other symptoms of CDI, a study participant card to inform healthcare providers about their participation, and contact information for the Study Coordinator. Following capsule administration, the remainder of the study activities will occur via scheduled telephone calls.

Participants will be assessed for symptoms of CDI and adverse events with a call from study personnel at days 2, 14, and 56 after capsule administration. The day 56 (8 week) call will be used to assess the primary endpoint, confirmed or possible recurrent CDI, as well as the secondary endpoints, including the quality-of-life questionnaire. Interspersed amid these calls will be bi-weekly calls (on day 28 and 42) from an automated system or study personnel that will prompt participants to answer questions regarding any new diarrhea, abdominal pain, new antibiotic use, AE/SAE, or concerns that they are experiencing an onset of CDI. Affirmative answers will be followed up by a call from the Study Coordinator, who will ask further details regarding the positive response. Participants experiencing new

diarrhea, in addition to seeking clinical care at their local facility, will be further contacted by the Study Coordinator, collect a stool sample using a collection kit provided by the SC, and mail in a pre-paid mailer to the central laboratory, where it will be tested for *C. difficile* toxin B by PCR and for existing toxin by EIA. For the purposes of differentiating confirmed vs. possible CDI recurrence, we will use the EIA toxin test.

After randomization, from week 8 to month 6, the automated calls or study initiated contacts will decrease in frequency to once a month, with the same triggers for a follow-up call from a Study Coordinator. At month 6 a final call by the SC will occur to assess any new symptoms of recurrent CDI, serious adverse events and possibly related non-serious adverse events, and any other reported symptoms. A schematic of the study timeline for an individual participant is displayed in Table 2.

Table 2. Study Timeline

Time	Initial episode of CDI	Recurrent CDI within 90 days of the initial one	Washout 2-14 days after antibiotic treatment	**Day 0 Officially in the study**	Day 1-56	Day 57-180
Patient events	Acute CDI episode	Recurrent CDI episode	Antibiotic washout	**Receive study treatment FMT or placebo**	-Monitor symptoms -Report new diarrhea, AE/SAE	-Monitor symptoms -Report new diarrhea, AE/SAE
Study activities	-Note episode -Monitor for recurrence in next 90 days	-Note episode -Assess eligibility -Ask provider to ask patient if can contact -Assess interest -Schedule study visit	-Ensure still without symptoms -Verify interest in study -Travel to patient	**-Verify eligibility** **-Obtain consent** **-Randomize** **-Administer capsules** **-Collect stool sample (optional)** **-QoL assessment**	-Scheduled calls days 2, 14, 28, 56 -Stool sample day 14, day 56 (optional) and when a possible CDI occurs (mailed) -QoL assessment at day 56	-Monthly calls -Study complete at day 180

*Note that these pre-enrollment days overlap, as some patients will receive longer-duration treatment for their episode of recurrent CDI, or a longer washout period based on ability to schedule a study visit.

I. Alternate Design Considerations

Adding a second route of FMT delivery was considered, with the possibility of using 2 randomizations (first randomizing to 1 of 2 routes of delivery, then randomizing to FMT vs. placebo). This would allow an overall comparison of FMT (via any route) vs. placebo, and a second comparison of FMT by route of delivery. The increased sample size, cost, and complexity of this approach were deemed to be prohibitive, and this approach was discarded. An uncontrolled observational study was considered to be largely confirmatory, since the numerous published case series provide similar data. Furthermore, the consensus of the Planning Committee members was that a higher-quality study was needed to help

definitively answer the question of whether FMT should be widely offered within the VA system, and if so, how it could be delivered in a safe and accessible manner.

< This page left intentionally blank >

IV. Importance to the Veterans Administration

During the past decade, the incidence rate of CDI has increased dramatically in North America and Europe, with a corresponding increase in disease severity [3, 26, 27]. A recent publication based on surveillance from multiple US sites indicates that the number of yearly CDI cases in the US exceeds 450,000 [2]. Increases have occurred in all age groups, but the elderly have been disproportionally affected. Mortality attributable to CDI has also increased dramatically, with recent estimates from the CDC of 29,000 deaths annually in the US [2]. These marked changes in the CDI incidence and mortality have coincided with the emergence and spread of an epidemic strain of *C. difficile* variously designated as BI/NAP1/027 by different typing methods [3]. Currently, *C. difficile* is the most prevalent cause of healthcare-associated enteric infections in the US, rivaling MRSA as the leading cause of all nosocomial infections [1].

CDI has been a particularly important problem in VA hospitals since the risk factors for CDI are common in Veteran patients, i.e. elderly, with multiple medical comorbidities, prolonged hospital stays, and frequently exposed to antibiotics. CDI trends in VA hospitals have mirrored or exceeded those seen in non-VA hospitals. The incidence of CDI among Veterans has recently been reported, based on systematic surveillance by Multidrug-resistant Organism prevention coordinators at each VA facility [5]. From October 2010 through June 2012, there were a total of nearly 10,000 CDI cases recorded, with 0.66 cases per 100 admissions [5]. Viewed as rates of hospital-onset, healthcare facility associated CDI (the most common classification of CDI), VA rates were 9.3/10,000 patient days. This compares to a reported incidence of 6.4 to 7.9 cases/10,000 patient-days in non-VA hospitals and nursing homes in 2006 [28], and 2.8 cases/10,000 patient-days in a national survey of community hospitals (1). Prior studies suggest that 1% of all patients hospitalized in the VA were diagnosed with CDI [29]. According to VA administrative data sources, there were approximately 624,858 hospitalizations in VA facilities in FY 2012, meaning that a 1% incidence of CDI resulted in 6,249 cases among inpatients alone. With VA-based studies reporting recurrence rates of 22-30%, this translates to estimated 1,375 – 1,875 cases of recurrent CDI annually. These estimates are consistent with FY 2012 data regarding CDI diagnoses (available in the inpatient and outpatient treatment files), which report 6,046 cases of CDI and 1,517 cases of recurrent CDI. When outpatient CDI diagnoses were included, the total number of initial CDI cases rose from 6,046 to 8,878.

The economic burden of recurrent CDI is substantial, with estimates ranging from $4,000 to $10,000 per episode. Application of this range to the approximately 1,500 annual cases of recurrent CDI in hospitalized Veterans yields an estimated total annual cost for recurrent CDI of $6 million to $15 million. As demonstrated by the nearly 3,000 outpatient CDI diagnoses in the VA FY 2012 data, CDI has increasingly been reported in non-hospitalized patients, implying that studies of hospitalized patients likely underestimate the financial burden of CDI in the VA. Finally, CDI is also associated with substantial mortality. CDI-attributable mortality rose to 48 per 1 million person years in 2006-7, a 5-fold increase from 1999-2000 [30]. A more recent publication estimates the US CDI annual mortality at 29,000 [2].

Recurrent CDI is perhaps the most challenging dilemma facing VA clinicians who treat this disease. Although effective treatments are available for CDI, 15 to 30% of patients who respond to initial courses of treatment develop recurrent CDI, usually within 1-4 weeks of completing treatment with either vancomycin or metronidazole [14]. The risk of recurrent CDI is even greater in patients who have already had one or more recurrences, and many patients develop repeated episodes that may continue to occur over a period of months or years. The GI Field Advisory Committee receives numerous inquiries every month about stool transplants for Veterans with recurrent CDI (personal communication, Jason Dominitz). Currently there are no VHA guidelines for FMT. Treatment of recurrent CDI is a priority research area for the VA and there is already one CSP study (CSP #596) underway comparing three antimicrobial regimens for the treatment of recurrent CDI. However, a study to determine whether FMT adds clinical benefit when administered after standard antimicrobial therapy is urgently needed.

V. Study Population

A. Inclusion Criteria

In order to maximize the clinical relevance of this trial, inclusion/exclusion criteria were designed to be as inclusive as possible. The rationale for the proposed exclusion criteria are discussed below. The study's inclusion criteria are:

1) One or more episodes recurrent CDI (defined as > 3 loose/watery stools/24h for 2 consecutive days with CDI treatment, and not explained by another diagnosis PLUS laboratory confirmation of *C. difficile*; or ileus or toxic megacolon PLUS laboratory confirmation of *C. difficile*, occurring within 90 days of a prior CDI episode with similar symptoms and laboratory confirmation);
2) Resolution or improvement of symptoms from most recent CDI episode, defined as no longer meeting the clinical definition for CDI for a 48 hour period during antimicrobial treatment, including not meeting the definition again after an initial improvement;
3) Within the enrollment window: 2 days after completion of antimicrobial therapy for CDI (to allow for a washout period) to 14 days after completion of therapy or 30 days after the onset of CDI whichever is later;
4) Age ≥ 18 years;
5) Enrolled in a VHA facility; and
6) Able and willing to provide informed consent.

B. Exclusion Criteria

The exclusion criteria are:

1) Unlikely to swallow capsules;
2) Pregnancy, planning to be pregnant, or breastfeeding;
3) Receipt of cytotoxic chemotherapy, intravenous or subcutaneous immune globulin, or confirmed neutropenia (absolute neutrophil count of < 1,000 cells/μL) within the past 3 months;
4) Inflammatory bowel disease or other chronic diarrheal disease/fecal incontinence predating CDI;

5) Ongoing antibiotic use other than for treatment current CDI episode or long-term antibiotic use;
6) Prior FMT;
7) Life expectancy of < 8 weeks;
8) Anaphylactic food allergy;
9) Active enrollment in another research study on antibiotics, probiotics, or FMT without investigators approval;
10) Presence of an ileostomy or colostomy;
11) HIV with CD4 count < 200 cells/μL in prior 3 months;
12) Bone marrow/peripheral blood stem cell transplant in the past year;
13) Decompensated cirrhosis;
14) Receipt of bezlotoxumab during the most recent episode of CDI;
15) Unlikely to follow study protocol.

Rationale for each exclusion criterion

Unlikely to swallow capsules:

With FMT delivery via capsule, the ability to safely swallow capsules is a basic requirement. In previous studies where FMT was delivered in capsule-form, the size of the capsules was "00"- which is approximately the size of a large multivitamin pill; capsules are expected to be the same size in this study. All patients, including an 11 year old, were able to swallow the capsules without difficulty [19]. Patients needing liquid formulations of medications or those having medications administered through a feeding tube would be considered ineligible due to a higher risk of aspiration or airway obstruction.

Pregnancy:

Data supporting FMT safety during pregnancy is not available. Based on the absence of such data and the heightened safety concerns when dealing with a vulnerable population, pregnancy is an exclusion criterion. Women of child-bearing potential (those who are not permanently sterilized or post-menopausal for at least 12 mos.) will undergo a urine pregnancy test on the day of FMT/Placebo prior to enrollment. Women who are breastfeeding are excluded for similar heightened safety concerns regarding the effects on the infant.

Specific immune-compromising states:

The immune-compromising conditions that preclude study participation are based on a case series of immune-compromised patients, who reported a favorable response to FMT with few adverse events [31]. This study included patients with the Acquired Immune Deficiency Syndrome, solid organ transplants, oncologic conditions, immunosuppressive therapy for inflammatory bowel disease, and others. Cytotoxic immunotherapy is an exclusion criterion because it induces significant disruption of the intestinal mucosa, increasing the risk of bacterial translocation and subsequent sepsis. Similarly, neutrophils are the main defense against bacterial translocation, and thus a known deficiency in this defense is an exclusion criterion. Patients with a recent history of low ANC (<1,000 cells/μL) will be retested before enrollment in the study. Immune globulin therapy is an exclusion based on anecdotal reports of using immune globulin to treat CDI, and because patients with common-variable immune deficiency (a common reason for immune globulin therapy) often have diarrhea as a manifestation of their underlying disease. Advanced HIV (defined as CD4 count <200), bone marrow/peripheral stem cell transplant recipients, and those with decompensated cirrhosis will also be excluded based on theoretical increased risk of infectious complications.

Inflammatory bowel disease or other chronic diarrheal disease/fecal incontinence predating CDI:

Since diarrhea will result in meeting the study's primary outcome, it is critical that patients experiencing chronic diarrhea or fecal incontinence are not included, as a continuation of this chronic condition will result in treatment failure. However, if the diarrhea and incontinence symptoms have developed only after the patient had a CDI episode, the patient should not be excluded, as this is precisely the type of chronic morbidity that FMT is purported to improve.

Long-term antibiotic use or use of antibiotic other than for treatment of CDI or prior FMT:

Since the mechanism of FMT for the prevention of CDI is the restoration of the normal fecal microbiome, continued use of antibiotics, or concurrent use of an antibiotic other than that for current CDI episode (which disrupt the microbiome) will likely lead to treatment failure, and as such is an exclusion criterion. Failure of a prior FMT is also an exclusion criterion since such patients would be expected to have a higher failure rate after a subsequent FMT.

Life expectancy of < 8 weeks:

Although it is recognized that physician estimates of life expectancy may be inaccurate, patients who are unlikely to survive until the 8 week primary outcome assessment will not be eligible for enrollment.

Anaphylactic food allergy:

Although anaphylaxis due to food antigens present in donor stool has not been reported. We have opted to exclude patients with documented anaphylaxis to food since we cannot guarantee that the donor stool will be free of the particular antigen. This is not anticipated to be a common occurrence.

Active enrollment in another research study on antibiotics, probiotics, or FMT without investigators approval:

Use of antibiotics or probiotics may affect the CDI recurrence rate, and as such, participation in such studies and CSP #2004 will not be allowed. A notable exception to this is that patients who experience recurrent CDI while in CSP #596 (fidaxomicin vs. vancomycin vs. tapered vancomycin) will be offered enrollment in the proposed study. The co-chair of CSP #596 is a member of the CSP #2004 planning committee, and has indicated that once study participants have met the primary endpoint (recurrent CDI) in that trial, there is no objection to them enrolling in the proposed study. Patients with recurrence after a prior FMT will be excluded as they will have already received the study intervention, and still recurred. Thus, they are likely to reflect a subgroup of patients at extreme risk of recurrence.

Presence of an ileostomy or colostomy:

Although CDI has been reported in patients with surgical removal of the colon and a resulting ileostomy or colostomy, this is a rare scenario. The normal flora of the colon has been reasonably well characterized, whereas the flora of the distal small bowel in patients with an ileostomy or colostomy is unknown. It is also difficult to assess the ostomy output for study inclusion and end points. Whether they would derive the same benefit from administration of FMT is uncertain; and due to the difficulty of assessing eligibility and outcomes, such patients will be excluded.

Unlikely to follow study protocol:

If a patient is deemed unable to follow the study protocol after screening by the Study Coordinators (SC) or Site Investigators (SIs), the patient will be excluded. This allows SCs (in conjunction with the SIs) flexibility in dealing with more unusual circumstances that may preclude successful participation, but which are not anticipated ahead of time. Each such exclusion will be discussed with the SI, and a reason will be recorded. Possible reasons include: inability to consistently reach patient via telephone, documented history of medical non-compliance, unwillingness to meet with SCs, inability to identify another individual who will know the patient's whereabouts. Patients who are currently inpatient at a VA facility will also be considered unable to follow study protocol since VA regulations would not permit study drug to be administered under those circumstances. At the discretion

of the SC and SI, this may also include individuals enrolled in another interventional trial which may interfere with the ability to participate in MATCH.

Bezlotoxumab is a relatively new drug indicated for prevention of CDI recurrence which is used rarely in the VA (13 times in FY21 per VA PBM). While its use is uncommon, including a drug approved for the same indication as we are studying could decrease recurrence rates and potentially introduce bias. Thus, use of this medication will be added as a study exclusion criterion.

C. Inclusion of Women and Minorities

Because female Veterans comprise such a small proportion of the VA population (approximately 5%), the study recruitment team will make a special effort to recruit female Veterans. No special recruitment effort of minorities is planned because they are well represented among the VA population. Demographics, including race/ethnicity and gender, will be monitored in randomized as well as excluded patients. No patient will be excluded on the basis of gender or race.

< This page left intentionally blank >

VI. Human Rights Issues and Informed Consent Procedures

A. Risks and Benefits

The risks of FMT have thus far been largely procedural, including an episode of aspiration that was attributed to the sedation used for colonoscopic administration of FMT, and peritonitis that occurred after administering FMT via an indwelling gastric tube [32]. A patient who was given FMT via colonoscopy for recurrent CDI experienced diverticulitis with symptoms beginning a few hours after the treatment; it was unclear whether the event was due to the FMT treatment itself or whether it resulted from the colonoscopy [33]. Infectious complications have been rare and mostly mild; a single case of norovirus was reported shortly after FMT in one patient, but this occurred in the setting of a healthcare worker who cared for the patient also having an acute norovirus-like illness [34]. An episode of zoster was also reported post-FMT; the relatedness of the 2 events is unknown [35]. Serious systemic infections have also been rarely reported. The FDA noted two cases, one fatal, of invasive infections with extended-spectrum beta-lactamase (ESBL)-producing E. coli in two immunocompromised adults. [54] The fecal microbiota transplant used to treat these individuals came from a single donor and was not tested for ESBL organisms prior to use. Following notification of this issue on June 13, 2019, the manufacturer of the study drug (University of Minnesota) began testing all donor stool for multi-drug resistant organisms (MDROs) as required by the FDA, in addition to continuing donor screening to exclude potential donors at high risk of MDRO colonization. Testing was also performed to rule out common MDROs in all lots of study drug taken by participants in CSP #2004.

Other possible complications relate to the possible role of the fecal microbiome in autoimmune disease and metabolism. A patient with quiescent inflammatory bowel disease was reported to have a flare of his disease after FMT; again, it is uncertain as to whether this was related to the CDI, the FMT, or coincidental [36]. A case of excessive weight gain post-FMT was recently reported in a patient who received stool from an obese donor [37]. Two cases of peripheral neuropathy after FMT have also been reported in the literature. A relationship with FMT has been posited but is uncertain [38].

These risks will be clearly presented in the informed consent form, and patients will have ample opportunity to ask questions in the time between the first contact with the study team and enrollment (expected to be 7-28 days).

The possible benefits of FMT are: a decreased risk of recurrent CDI, avoidance of prolonged courses of antimicrobials and healthcare visits, and improved quality of life. The informed consent process will review the preliminary efficacy data for FMT and standard antimicrobial therapy, and present this information using simple and straightforward language.

B. Informed Consent Process

The informed consent process will be initiated as soon as a potentially eligible patient indicates interest in participation. A waiver of informed consent will be sought for recruiting purposes only, which will allow the Study Coordinators (SCs) to identify and review the medical records of patients with recurrent CDI. Once the SCs establish that the patient is willing to be called about the study (which will be facilitated by communicating with the treating provider or other responsible provider), the patient will be contacted and information about the study will be provided. If the patient remains interested and meets eligibility criteria, an informed consent form and information about the study will be mailed to the patient to ensure that there is sufficient time to review the information prior to enrollment. In the event that time does not permit mailing the material for the patient to review, study staff may review the material via telephone. Prior to enrollment, the SC will call the potential participant to go over study materials and informed consent and answer any questions.

The study's informed consent can be signed either by wet signature or electronic/digital signature. When an electronic document with a digital signature is used for the documentation of consent, this study will use the DocuSign platform which is 21 CFR, Part 11 complaint to obtain the required signatures. A script to be used with DocuSign will be included in the Study's Operations Manual.

The SC will also confirm that the potential participant is willing to proceed with the visit, including the need to drink only clear liquids for 4 hours prior to the study visit and for 2 hours after swallowing the study treatment. At enrollment, the SC(s) will review the informed consent form with the participant, answer any questions, ensure eligibility criteria are met, and obtain the participant's signature on the informed consent form. As indicated by the inclusion criteria, only participants capable of providing informed consent will be enrolled (i.e., informed consent from a surrogate decision maker will not be considered).

With the emergence of the novel coronavirus that causes COVID-19, the possibility of transmission of COVID-19 through travel or fact-to-face meeting needs to be considered. There may be

some condition when travel or face-to-face meeting is not feasible. Due to COVID-19 or for other reasons, the study may process consent with the VA Video Connect (VVC). VVC is an approved VA Mobile app which meets VA mobile application compliance/data security control requirements (encryption in transit, etc.) and secure software security standards. VVC is based on the use of the mobile application used within Telehealth. Potential participants will need a device with access to this VA Mobile app. After confirming a potential participant is willing to process consent through VVC, two copies of ICFs will be mailed to the participant and a VVC meeting will be scheduled.

During the VVC call, SC explains the consent form line by line to the participant and answers all questions. Participant signs two sets of consent forms while meeting with and /or SC over VVC. Veteran keeps one copy of the ICF for reference until the signed forms are received. Participant mails one signed consent back to Minneapolis. After SC receives the signed consent, the consent process with VVC is completed and randomization visit via VVC will be arranged.

Given the use of VA Video Connect (VVC) to effect remote participant consent and randomization, there could be a time lag between the day of signing of the informed consent form (ICF) to the day of randomization/administration of study medication. If this time lag is more than 60 days, a re-consent is required.

C. Withdrawal

After randomization, study participation consists of recording symptoms, reporting them via the scheduled and as-needed phone calls, submitting a stool sample in the event of an episode of diarrhea, and submitting an optional day 14 and day 56 stool samples for microbiota characterization. Accordingly, attempts to minimize withdrawal will be made by discussing, prior to enrollment, the importance of participating in the study. Participants will be asked to provide several means of contacting them, including a home phone, cell phone, mailing address, and the contact information of a person who would be able to reach them. If participants opt to withdraw from the study and not participate in further symptom reporting, two types of withdrawal will be available to the participant:

1) No active follow-up, but allow study staff to assess for outcomes such as recurrent CDI and mortality via electronic record review; or
2) Complete withdrawal, with no active or electronic follow-up beyond the time of withdrawal.

Participants opting for complete withdrawal prior to the primary outcome assessment will have their status at the time of withdrawal carried forward for the primary outcome assessment (recurrent CDI or no recurrent CDI).

VII. Outcome Measures and Rationale

A. Primary Outcome

The primary outcome for this trial was selected to reflect the circumstances that currently lead clinicians and patients to consider FMT, and to provide a result that is clinically relevant. Since the great majority of patients referred for FMT are undertaking the procedure to reduce the risk of subsequent recurrent CDI, the primary outcome was chosen to provide a clear answer regarding the efficacy of FMT in preventing recurrent CDI.

The primary outcome is recurrent CDI (definite or possible) or death within 56 days of randomization.

Definite recurrence is defined as any of the following:
-the new onset of more than three loose or watery stools in 24 hours for two consecutive days not explained by another diagnosis
-other clinical symptoms including ileus, toxic megacolon, and colectomy
PLUS
-laboratory confirmation of *C. difficile* from a stool specimen by toxin testing.

Possible recurrence is defined as the same clinical manifestations as above, but WITHOUT laboratory confirmation of *C. difficile* (stool test not sent, negative result for EIA toxin test, or uninterpretable result). Site Investigators (SIs) will be asked to review cases where it is unclear to the Study Coordinator as to whether the outcome has been met; SIs will also evaluate the relatedness of the intervention to any reported adverse events (AEs) or serious adverse events (SAEs).

Using recurrent CDI as the primary outcome reflects the reality that preventing recurrence drives most patients to consider and providers to offer FMT. In reviewing the literature on FMT, many studies report resolution of CDI symptoms and recurrent CDI as outcomes, administered after a course of antimicrobial therapy, and many specifically state that FMT was administered after patients became "asymptomatic" or had "a reduction of symptoms" [21]. Preventing recurrence (vs. treatment of an active episode of CDI) also comports with how recent reviews recommend utilizing FMT, and with our clinical experience in referring patients to undergo FMT. Accordingly, the prevention of recurrent CDI among

patients who have had a favorable response to standard antimicrobial therapy is the appropriate primary outcome for this trial.

The definition of recurrent CDI (both definite and possible) reflects the importance of symptoms in diagnosing and managing recurrent CDI. It also highlights an area of uncertainty which any study of recurrent CDI encounters, namely that not every episode of diarrhea occurring after a prior episode of CDI is recurrent CDI, regardless of stool test results and response to empiric treatment. Discussing this difficulty requires some baseline definitions: Recurrent CDI is defined as an episode of CDI occurring within 56 days of a prior CDI episode [11], presuming the symptoms from the prior episode resolved. The definition for CDI includes symptoms (typically diarrhea, but rarely ileus, toxic megacolon, or colectomy), plus a stool laboratory test indicating presence of *C. difficile*, its toxins, or the genetic elements encoding the toxins. Unfortunately, studies have demonstrated that asymptomatic patients with a recent episode of CDI test positive for a prolonged time post-infection, regardless of test used [39, 40]. Accordingly, guidelines for CDI discourage using a microbiological test-of-cure, but rather recommend symptom assessment.

For this trial, universal stool testing for any subject experiencing diarrhea during follow-up will be attempted. This will be accomplished by the Study Coordinator educating the participant, both at enrollment and during subsequent contacts, stressing the need to be evaluated for recurrent CDI should diarrhea or other CDI symptoms recur. Subjects will also be provided with a home collection kit with a postage-paid package whereby they can submit a stool sample to a central laboratory for *C. difficile* testing by EIA and PCR, which will be useful in the event that their local provider opts to empirically treat for CDI without laboratory confirmation, and provides consistent testing with the same testing method. Subjects with symptoms plus a positive EIA toxin stool test (from either the central or a local laboratory) will be classified as definite recurrent CDI. Subjects with symptoms not explained by another diagnosis but without laboratory confirmation (negative or no testing) or with laboratory confirmation other than EIA toxin will be classified as possible recurrent CDI. Definite and possible cases will be combined as recurrent CDI for the purposes of the primary outcome. Finally, death will also be considered as meeting the primary outcome, since death eliminates the possibility of recurrent CDI.

Justification of the primary end point assessment at day 56:

Using day 56 as the primary endpoint for recurrence (vs. a longer period) was carefully considered. Assessing the primary outcome at day 56 is important from the scientific, clinical, and regulatory standpoint. Clinically, recurrence generally occurs within 7 to 14 days after cessation of

antimicrobial therapy. However, non-CDI diarrheal illness is very common in the population (0.72 episodes/person-year). The combination of the main manifestation of CDI (diarrhea) being non-specific and common, plus a test that can remain persistently positive regardless of symptoms, makes it difficult to distinguish recurrent CDI from diarrhea caused by either another pathogen or non-infectious diarrhea, including a post-infectious motility disorder. There is currently no standardized approach to differentiating recurrent CDI from diarrhea of another cause. Since any episode of diarrhea could count as an outcome for recurrent CDI in our study, we would be at risk of counting many episodes of non-CDI diarrhea as our endpoint beyond 56 days.

From the regulatory perspective, recurrence at 56 days meets the FDA standards, as seen in the recent approval process of fidaxomicin. Day 56 endpoint also allows comparison with other pivotal studies evaluating therapies for recurrent CDI. Prior clinical trials evaluating FMT for recurrent CDI have used time from treatment to endpoint of 56 days (Youngster 2014; Kelly 2015) and 70 days (Van Nood) respectively, while the recently funded CSP #596 is using day 59 as its endpoint. In this study, the primary endpoint assessment is at day 56 from randomization and day from 58-90 from end of treatment for prior episode. Using the same definition allows for comparison across trials, and provides clinicians with a uniform outcome to assess when selecting treatment.

From the study design perspective, using a 6 month endpoint would have the following challenges: drop outs and loss of follow-up, dilution of endpoint with non-CDI related GI symptoms or other etiologies of diarrhea and difficulty in power and sample size calculations since recurrence rates are largely unknown at 6 months. In summary, we chose to apply 56 days as the primary endpoint and use recurrence at 6 months as secondary endpoint in order to reduce complexity to the study.

B. Secondary Outcomes

We realize that the durability of response after FMT is unknown, and is an important question. Therefore, we propose to collect recurrence of CDI or death at 6 months as the first secondary endpoint and other secondary endpoints.

i) Recurrent CDI (definite or possible) or death within 6 months of randomization;
ii) Definite recurrent CDI within 56 days, as defined above;
iii) Possible recurrent CDI within 56 days, as defined above;
iv) Death within 56 days;

v) Episode of diarrhea that is negative for *C. difficile* by EIA toxin testing and PCR within 56 days

This is same as a possible recurrent CDI, but includes only episodes of diarrhea that test negative for *C. difficile* by EIA and PCR, not episodes that are not tested or are uninterpretable;

vi) Episode of diarrhea that is negative for *C. difficile* by EIA toxin testing but positive by PCR within 56 days

This is similar to a possible recurrent CDI, but includes only episodes of diarrhea that test negative for *C. difficile* by EIA toxin testing, not episodes that are not tested or are uninterpretable;

vii) Abdominal pain among all possible CDI cases;

viii) Urgency among all possible CDI cases;

ix) Fecal incontinence among all possible CDI cases;

x) Number of CDI recurrences within 6 months

The number of CDI recurrences within 6 months for a patient is the count of separate CDI recurrences from randomization to 6 months after randomization; and

xi) Quality of life at day 56

We will use a brief assessment of both overall and gastrointestinal health status, using a previously validated instrument.

Secondary outcomes include the individual components of the primary outcome (definite recurrence, possible recurrence, and death), as well as PCR negative diarrhea, EIA toxin negative diarrhea, number of CDI recurrences, and self-reported quality of life. Additionally, an assessment for non-diarrheal manifestations of CDI such as abdominal pain, urgency, and fecal incontinence will be performed. A brief assessment of both overall and gastrointestinal health status, using a previously validated instrument, will be performed [25].

Because abdominal pain is common and has a wide variety of causes beyond CDI, the assessment of abdominal pain will be standardized in the operations manual. In brief, assessment will begin with asking participants about any abdominal pain during the assessed time period, whether any pain present is new or worsened since the study intervention, their rating of the severity on a standardized pain scale of 0 to 10, and whether they sought medical care for the pain. If medical care was sought, records regarding

that care will be requested by study personnel, and the pain will be placed into 3 distinct categories by Study Coordinators and SIs:

1) Abdominal pain with non-CDI cause (e.g., documented renal calculus or appendicitis);
2) Abdominal pain without clear non-CDI cause; or
3) Abdominal pain occurring in conjunction with criteria meeting primary endpoint (e.g., diarrhea, ileus, toxic megacolon).

Urgency will be assessed based on a modified 0 to 3 scale addressing frequency, intensity, and impact on social performance, with 0 representing normal control, 1 representing occasional feelings of urgent need for defecation, 2 representing frequent feelings of urgent need for defecation with sudden need for a toilet interfering with social functions, and 3 being inability to control defecation.

Fecal incontinence will be assessed via two questions based on modification of several scales: 1). Have you had new or worsening symptoms of unintentionally passing liquids or solids from your rectum more than once per week for at least two weeks; and 2). Have you required use of pads or had to alter your lifestyle based on these or similar symptoms? If either is answered affirmatively, the definition of fecal incontinence will be met.

The inclusion of PCR negative diarrhea and EIA toxin negative diarrhea as separate entities is to assess the proportion of possible recurrent CDI or subjects with recurrent CDI that tests negative for CDI using different testing methods, and whether this proportion is affected by FMT. The use of a direct EIA test for preformed toxin was selected based on FDA input, and recent data suggesting that PCR may be an overly-sensitive test, detecting *C. difficile* genetic material that is of little clinical relevance.([41]) Additionally, this also accounts for all possible combinations of symptoms plus CDI test status (Table 3).

Table 3. Possible combinations of symptoms and test status and subsequent outcome classification

CDI symptom status*	CDI test status**	Primary outcome (definite or possible recurrent CDI)
Absent	Negative	Not met
Absent	Positive (if ordered off protocol)	Not met
Present	Positive toxin test (regardless of PCR test)	Met (definite recurrent CDI)
Present	Negative toxin and negative PCR test	Met (possible recurrent CDI)
Present	Negative toxin and positive PCR test	Met (possible recurrent CDI)
Present	Toxin testing not performed but positive PCR test	Met (possible recurrent CDI)
Present	Not sent or no conclusive test result for both toxin test and PCR	Met (possible recurrent CDI)

* CDI symptoms include: diarrhea, toxic megacolon, ileus, and not explained by other diagnosis

**In the case of discordant results for a given episode between two sources, such as outside, local or central laboratories, any positive test for toxin by EIA will be used for outcome classification.

C. Safety Outcomes

Because of the limited information regarding long-term safety of FMT, safety outcomes will be collected until 6 months after FMT/placebo randomization. Monitoring and reporting of AEs and SAEs are further described in Section XIV.

Safety outcomes to be collected include:

i) Serious adverse events, with a focus on SAEs involving hospitalization (new or prolonged), and all-cause mortality;
ii) Adverse events which may be related to FMT treatment. This includes adverse events which Site Investigators consider related/possibly related to the study treatment and all adverse events which occur within 14 days of study treatment (since an aggregate analysis of events temporally linked to treatment could show a causal relationship when compared to placebo);
iii) Infectious transmissions which are plausibly linked to FMT treatment; and
iv) Development of new conditions theoretically linked to alterations in gut microbiota.

D. Outcome Measurements in Exploratory Analyses

An optional stool sample for the assessment of the colonic microbiome will be obtained at the time of randomization, at day 14, and at 56 days after administration of FMT/placebo. The first sample will be sent to the designated storage facility by the Study Coordinator, whereas subsequent samples will be mailed to the same facility by the participant via a pre-paid mailer.

Shannon index (diversity) and the abundance-based coverage estimate (ACE) (richness) will be the outcome measurements to evaluate microbiome.

The following exploratory analyses are proposed:

i. Characterization and comparison of the colonic microbiome of individuals with CDI randomized to FMT and placebo, before and after FMT/placebo administration;
ii. Comparison of the post-FMT colonic microbiome of individuals randomized to FMT and placebo to the donor microbiome;
iii. Comparison of the post-FMT microbiome of individuals that experience a recurrence (at day 14 and at day 56) to the microbiome of those not experiencing recurrence (at day 56);
iv. Comparison of the number of CDI episode within six months after randomization between two treatment arms.

    The number of CDI episodes within 6 months for a patient is the count of separate CDI episode from randomization to day 180 after randomization.

FMT is a promising therapy for recurrent CDI, with high level of public interest and demand, which has outpaced the scientific evidence. However, there is paucity of data on the mechanistic and translational aspect of how FMT may work. Prior work demonstrating similarity between the post-transplant microbiome and the donor microbiome suggests that "engraftment" of the donor microbiome is important for clinical success. However, since these data come from uncontrolled series, this hypothesis has not been rigorously tested. Advances in DNA sequencing technologies have created a new field of research, called metagenomics, allowing comprehensive examination of microbial communities. The current study provides a unique opportunity to characterize the gut microbiota and make meaningful comparisons between microbial composition of individuals pre-post FMT vs. placebo, and between donor and recipient, regardless of whether the study has positive or negative results. If a clear correlation between recurrent CDI and failure to engraft is demonstrated, this would clarify the mechanism whereby FMT confers its benefit. Conversely, if subjects with engraftment of the donor microbiome recur at a

similar rate as do those without engraftment, this would suggest that other factors may be contributing to recurrence. Collecting samples is not a barrier to enrollment, since participants can opt out of this portion of the study and these comparisons would allow us to confirm the hypothesis that recurrent CDI is associated with reduced diversity and compositional changes in the fecal microbiota, and characterize post-FMT microbiota profiles that may serve as a benchmark for future studies.

The development of mechanistic models for microbial engraftment and clinical course is of great interest to the international scientific community. At the federal level, the NIH Human Microbiome Project is one example of similar efforts designed to take advantage of large scale, high through 'omics' analyses to study the microbiome in human health. Adding these exploratory aims pose minimum burden on the study and enable us to contribute to the ongoing efforts to understand the human microbiome.

VIII. Baseline Assessments and Procedures

A. Recruitment and Screening

A relatively novel method of enrollment will be used in this study, with central Study Coordinators identifying eligible patients in the entire VA system. The identification of eligible patients will be accomplished using multiple strategies, as outlined below, which include electronic case finding and direct referrals from clinical providers.

Our main strategy for recruitment is through electronic case finding of all new diagnoses of CDI across the VA using Corporate Data Warehouse (CDW) support through VA Informatics and Computing Infrastructure (VINCI), which is updated daily. An incident case of CDI will be identified through electronic monitoring of orders for *C. difficile* testing, lab results, diagnostic codes for outpatient and inpatient visits, and prescriptions. A pilot screening study has been completed at the Minneapolis VA Healthcare System using the above case-finding system, which was developed with support from the Centers for Chronic Diseases and Outcomes Research at the Minneapolis VA. Once the system has been successfully refined, it will be transitioned to an automated system. The Study Coordinators will screen cases found through this automated system, and will then utilize the patient's electronic medical record to assess for inclusion/exclusion criteria using national Computerized Patient Record System (CPRS) access via Compensation and Pension Records Interchange (CAPRI).

If the patient appears eligible at initial screening, a Study Coordinator (SC) will contact the patient's provider and ask the provider or a member of their care team to obtain permission for the SC to initiate contact via telephone. If the patient agrees to be contacted, the SC will contact the patient, provide information about the study and, if the patient is interested in participating, mail an informed consent form for the patient to review. At this time, study personnel will make travel arrangements to meet the potential participant at their home for enrollment and treatment administration, which will take place 2-14 days after stopping standard antimicrobial therapy. Study personnel will review the medical records for any indications that a home visit places the visiting Study Coordinator at a safety risk, utilizing methods that VA homecare providers routinely use prior to all new homecare visits. If there is a concern for safety, two SCs will travel to that individual's residence. Based on the experience of the Minneapolis VA Health Care System Homecare services, which provided approximately 8,500 homecare visits over the past 12 months, this is extremely unlikely. In their experience providing healthcare to diverse patient groups in

numerous urban and rural areas of Minnesota and Wisconsin, there have been no incidents where home healthcare was not feasible.

Prior to committing to travel, the Study Coordinator (SC) will contact the potential participant to verify that they have completed their standard antimicrobial therapy, that they are still planning on enrolling, and that they have met the criteria of having resolved/improved symptoms of CDI (3 or fewer stools per day for at least 2 days, without subsequent worsening, and no other manifestations of CDI such as ileus or toxic megacolon). If all these conditions are met, a Study Coordinator will arrange travel to the potential participant's home at a mutually agreed-upon date and time, with the randomly allocated treatment detailed in the randomization scheme. Once at the participant's home, the SC will review the study information with the participant, answer any remaining questions, obtain written informed consent, and then proceed with data collection and treatment administration.

With the emergence of the novel coronavirus that causes COVID-19, the possibility of transmission of COVID-19 through travel or face-to-face meeting needs to be considered. For safety or other reasons, the study may conduct consent and randomization remotely using VVC. After a potential participant signs a consent with VVC, a second VVC will be scheduled for administration of study therapy. Prior to the 2nd VVC, the Study Coordinator (SC) will mail the study therapy to the participant along with other study supplies. During the 2nd VVC, the SC will confirm that they are still planning on enrolling and that they have met the criteria of having resolved/improved symptoms of CDI (3 or fewer stools per day for at least 2 days, without subsequent worsening, and no other manifestations of CDI such as ileus or toxic megacolon). If all these conditions are met, proceed with data collection and instruct the participant take the study therapy, instruct them collect study specimen, and show them how to collect, package and handle future study specimen. The SC will encourage family/friend to be available if they are part of the veteran's household. The second VVC meeting should last an hour after swallowing study medication to make sure patient is tolerating study medication.

To ensure safety during the VVC the following steps will be taken:

a) We will be screening very carefully for swallowing difficulty, both on chart review and on participant interviews and exclude anyone with any current or past history of swallowing difficulties
b) We will recommend that a second person be present with the participant at the time of swallowing pills. We will be flexible at timing the visit when a second person may be available

In case of a choking emergency, we will use the standard 1-911 feature built into VVC for emergency services. Currently this is available for all VVC visits, and dials the local emergency service listed in the medical record for the participant. At the beginning of the visit, we will clarify this information with the participant.

A second recruitment strategy is that patients identified through the central electronic case finding described above will be sent a letter of invitation with information about the study. Interested individuals that contact the study team will be screened by Study Coordinators for eligibility. Those individuals that do not respond (an opt-out number will be provided for those NOT wishing to be contacted) will be contacted by the SCs after 1 week to provide information about the study and invite them to participate. Patients that are contacted outside the window of recruitment will be tracked separately and encouraged to contact the study team if future CDI episodes occur.

A third recruitment strategy will be to disseminate information about the trial to VHA Infectious Disease (ID) and Gastrointestinal (GI) physicians. The bulk of recurrent CDI patients are seen by either ID or GI providers in the VHA. Dr. Shaukat is a member of the GI field advisory committee, and has access to the VHA Gastroenterologists email list. Similarly, Dr. Drekonja has access to the VHA ID providers email list. Both physicians already receive regular inquires by colleagues about FMT for CDI treatment.

A fourth recruitment strategy would be to screen and enroll individuals that are not eligible to participate in CSP #596. Additional details are provided in Section XII B.

A final strategy will be to disseminate information about the study to Veterans through advertisement on newsletters, flyers in the VA Medical Centers, and contacting community Veteran groups.

B. Baseline Data Collection

The following baseline data will be collected at the time of enrollment:

1) Demographics (gender, age, race, ethnic status, marital status, education level, service history, height, weight);
2) Contact information for participant and two (2) additional contacts;
3) Past medical history

a) Surgical history
b) Medical history
c) Prior CDI history (number of prior episodes and dates, previous treatments, concomitant non-CDI antibiotic use during current CDI episode)
d) Medication use (prescription, over-the-counter, supplements, including probiotics)
e) Laboratory results from the time of the most recent CDI diagnosis, if available (including complete blood count, serum creatinine, and albumin); and

4) Gastrointestinal Quality of Life Index (QoLI).

C. Treatment Administration

The assigned treatment will be administered to the participant by the Study Coordinator traveling to conduct the study visit, who will observe the participant ingest the FMT capsules. The capsules will be administered after 4 hours of clear liquids only, to ensure an empty stomach. No acid-suppressive medications are needed, and the participant can drink clear liquids to help facilitate in swallowing the capsules. After treatment administration, participants will be asked to ingest only clear liquids for 2 hours, and refrain from lying supine to minimize any chance of reflux. Details of capsule administration will be based on the manufacturer's recommendation based on past clinical use, but is currently planned for a total of 5 capsules.

After the participant has successfully ingested the capsules, the Study Coordinator will ensure that the participant has all the study supplies, knows how and when to contact the study team, and completes the in-person study procedures. Within a week of enrollment, the participant will be entered in the local CPRS (Minneapolis or a regional site) where study enrollment and other notes will be posted. The participant's primary/referring provider will be notified of the participant's enrollment in the study, and where to find the research notes in their medical record. The remainder of the study follow-up will be completed via telephone, as described in Section III.H.

D. Stool Specimens

Two types of stool testing will be collected during study participation: (i) testing at the time of a new diarrhea episode, and (ii) optional collection for characterization of the colonic microbiota after randomization, before study drug administration, at day 14, and at the end of the study (day 56).

The testing of diarrheal stools will be done by EIA toxin test and by PCR detection of the toxin B gene. Based on emerging data that PCR testing may lead to over-diagnosis, the EIA toxin test will be the test used to differentiate definite vs. possible CDI. Both will be performed at a central study laboratory. Samples will be collected by the participant using collection kits that are routinely used for home stool collection, and sent to the central laboratory in pre-paid mailers. Both the collection kit and the mailer will be provided by the Study Coordinator (SC) at the time of enrollment.

The optional collection of stool for characterization of the microbiota will be done at the time of enrollment, at day 14 and at day 56. These samples will be stored at a central location until microbiome sequencing is performed to characterize the diversity and relative abundance of bacterial species. Collection will be done at the day of randomization, prior to administration of study drug and by the participant, with guidance from the SC, at day 14, and at day 56. The participant will be asked to provide a stool sample using the collection kit that the Study Coordinator will provide; the participant will then obtain a stool sample using a collection kit. If this cannot be performed by the participant, the participant can opt to have the SC assist in collection, or forego the stool collection step but still participate in all other study procedures.

< This page left intentionally blank >

IX. Stratification and Randomization

A. Stratification of Randomization

All eligible participants will be randomized in a 1:1 ratio to the two treatment arms, FMT or placebo. Randomization will be stratified by regional site, and within regional sites by number of previous recurrences (1 vs. 2 or more). Randomization will be performed by an automated randomization system located at the study web site using a computer generated randomization scheme produced by the West Haven Cooperative Studies Program Coordinating Center (WH-CSPCC).

Stratification on the basis of previous recurrences (1 vs. 2 or more) is necessary for several reasons. Since the recurrent CDI rate among patients with one recurrence is lower than those with two or more recurrences (Table 1), stratification on previous recurrences will permit a balanced distribution of the number of prior recurrence between two treatment arms, and then maintain the statistical power to detect the designed treatment effect. Additionally, the stratification provides an opportunity to evaluate any potential different treatment effects between patients with one previous recurrence and patients with more than one recurrence.

B. Randomization Procedure

Prior to traveling to a potential participant, the Study Coordinator (SC) will log in to the study web site, obtain the next available study treatment serial number for that coming enrollment/ randomization, obtain the study treatment identified by the serial number, and travel to the potential participant with the assigned treatment. Following enrollment, the participant will swallow the assigned treatment capsules under direct observation of the SC, which will complete administration of the study treatment. After an enrollment trip, the SC will log in to the randomization web site again and provide feedback about the success of the randomization. Detailed instructions are documented in the Operations Manual.

C. Blinding

The study subjects, personnel, Study Co-Chairs' office, and the team members (except Biostatisticians at the WH-CSPCC and the clinical research pharmacist in Albuquerque, NM) will be blinded to the treatment assignment. Placebo treatment will consist of identical-appearing placebo

capsules using inert filler materials. The capsules used are opaque, making the FMT and placebo capsules indistinguishable to external inspection. The bottles in which the study medication will be provided will also be opaque and will remain sealed until the time the treatment is administered to the participant. The Study Coordinators will observe the participant during administration, ensuring that the capsules are not opened or otherwise manipulated in an effort to unblind the treatment.

X. Treatment Regimens

A. Overview

Under the study protocol, FMT or placebo will be administered after initial treatment with antimicrobials has resulted in symptom improvement or resolution, which occurs in >90% of CDI episodes. The objective of FMT is to decrease the risk of subsequent recurrence of CDI, not treatment of CDI symptoms. As such, it can be considered as an adjunct to standard antimicrobial therapy. Recurrence of CDI has been variably defined in the literature, and in recent trials has anywhere from 56 to 90 days after the initial CDI episode. The various times between the initial and recurrent episodes of CDI in key studies are presented in Table 4.

Table 4. Time between prior and current CDI episodes and time from treatment until primary outcome assessment, for various studies.

Study author, year (description)	Time between prior and current CDI episodes	Time from treatment to primary outcome assessment	Comments
Youngster 2014 (encapsulated FMT series)	Not defined	56 days	Time also not defined in online appendix
Van Nood, 2013 (NEJM FMT trial)	56-70 days after cessation of antibiotic therapy	70 days	Time between episodes from prior methods paper
Cornely 2012 (Clin Inf Disease, Fidaxomicin vs. vancomycin for recurrent CDI)	3 months	28 days	Not clear if 3 months from diagnosis or end of therapy
Louie 2011 (NEJM Fidaxomicin vs. vancomycin trial)	3 months	28 days	Not clear if 3 months from diagnosis or end of therapy
Johnson 2007 (Rifaximin for recurrent CDI)	Not stated	Not stated	
Pepin 2006 (Metronidazole vs. vancomycin for recurrent CDI)	60 days	60 days	Both from time of diagnosis
McFarland 2002 (Vancomycin taper for recurrent CDI)	1 year	2 months	
Surawicz 2000 (Vancomycin vs. metronidazole for recurrent CDI)	1 year	2 months	
CSP 596 (Vancomycin vs. vancomycin taper vs. fidaxomicin)	56 days	59 days	28 days after vancomycin taper; 49 days after vancomycin and fidaxomicin standard therapy

Initial treatment of the recurrent CDI episode

The optimal treatment of recurrent CDI is unknown. Guidelines from the Infectious Diseases Society of America suggest that for the first recurrence, either vancomycin in a tapered and/or pulsed dose strategy or fidaxomicin should be used, and that further episodes can be treated with a variety of approaches, including FMT [7]. Similarly, guidelines from the European Society of Clinical Microbiology and Infectious Diseases suggest vancomycin or fidaxomicin for the first recurrence, and FMT for multiple recurrences [15], and the American College of Gastroenterology recommends metronidazole or vancomycin in a pulsed regimen for the first and second recurrence, with consideration of FMT after 3 recurrences [16]. The supporting evidence for these recommendations is of low quality, being largely guided by case series data and expert opinion.

For this trial, the choice of standard antimicrobial therapy will be at the discretion of the treating provider. Prior guidelines suggested metronidazole or vancomycin for the treatment of recurrent CDI, although vancomycin use has been increasing [42], and will likely continue to increase based on updated guidelines and a recent study demonstrating significantly improved initial cure rates vs. metronidazole [8], although recurrence rates are statistically not different between the two drugs. Fidaxomicin has been newly recommended as standard therapy for recurrent CDI by the updated Infectious Diseases Society of America guidelines , and was already suggested by European guidelines. Currently, its use in the VA is restricted to patients with three or more recurrences; however, future use may change pending the updated guidelines and formulary decisions.

The decision to enroll patients after any standard antimicrobial therapy vs. dictating a specific agent was carefully considered. Few RCTs of recurrent CDI are available to guide drug selection, and those that are available have not identified an agent as being clearly superior [12, 43] to others. One study combined a subgroup of patients with recurrent CDI from two trials comparing vancomycin to fidaxomicin, which showed a reduction in recurrence with the use of fidaxomicin (20% vs. 36%: P = .045) [12]. However, this finding requires confirmation, which is a goal of CSP #596 (standard dose vancomycin vs. tapered vancomycin vs. fidaxomicin). In the absence of data demonstrating a clear benefit for one agent, mandating use of a certain drug for the initial treatment of CDI is not justifiable. Furthermore, this imposes an artificial scenario, which in turn could render study findings out of sync with real-world practice. Requiring treatment with a specific drug would also limit the available patient pool, since antibiotic treatment may be initiated prior to any consideration of entry into the study.

Finally, dictating use of one drug raises the risk of serious problems should that agent be withdrawn from the market or found to be inferior to another agent during the trial. Accordingly, initial treatment will be allowed to utilize any standard antimicrobial that is recommended for recurrent CDI by a major clinical guideline (Infectious Diseases Society of America, American College of Gastroenterology, or European Society of Clinical Microbiology and Infectious Diseases); currently such guidelines include metronidazole, vancomycin, rifaximin, or fidaxomicin [7, 15, 16]. If a new agent is released and approved for use during the trial, demonstration of non-inferiority or superiority to a guideline-recommended agent would allow classification as a standard antimicrobial treatment.

In order to be eligible to enroll and be randomized in the trial, patients must demonstrate symptom improvement or resolution, irrespective of which standard antimicrobial treatment they have been prescribed. Symptom improvement or resolution is defined as no longer meeting the clinical definition of acute CDI for a consecutive 48 hour period during treatment, and not again meeting the definition after an initial improvement. The clinical criteria for CDI are more than three loose or watery stools/24 hours, or other uncommon manifestations such as ileus, or toxic megacolon. Thus, to be eligible for study enrollment, patients will need to have three or fewer non-watery stools/24 hours and be without any of the more rare but severe manifestations of CDI for a 48 hour period, and not have subsequent worsening of symptoms. Window for enrollment will be from 2 days after completion of antimicrobial therapy for CDI (to allow for a washout period) to 14 days after completion of therapy or 30 days after the onset of CDI whichever is later, with optimal goal of enrollment between days 2 and 7 after antimicrobial therapy.

B. Fecal Microbiota Treatment (FMT)

Key issues regarding FMT include the source and route of delivery. Summary information is included below; for more information, please refer to the Investigator's Brochure/Drug Information report.

1. Source:

Published studies have used locally-procured stool from either related or anonymous donors, while others have utilized a group of dedicated donors for their FMT program. Using a central pool of donors has several advantages over identifying a new donor for each patient undergoing FMT. These include eliminating the need to identify a suitable donor for each new patient, reducing the number of donors undergoing the cost and process of extensive stool and serological testing prior to stool donation,

and thus reducing the risk of a false-negative test exposing a recipient to a possible infectious complication. Additionally, utilizing a stable and clearly defined cohort of donors will allow for stringent quality control and inspection of the donation and preparation process. The option of creating a system of donor identification, screening, and testing, as well as the development of the capacity to process the stool into a form suitable for administration were considered. However, there are several institutions with already-established programs and capacity, and it would be much more time and cost-effective to partner with one of these programs to provide the donor material and processing capacity needed for this trial.

In collaboration with the Albuquerque Cooperative Studies Program Clinical Research Pharmacy Coordinating Center (CSPCRPCC), a list of essential criteria for such a partner were developed: (i) an existing or pending Investigational New Drug (IND) application and/or Biologic Master File (BMF) for the FMT product, (ii) a published protocol for donor screening, testing, and material preparation, (iii) safety record, (iv) efficacy record, and (v) the ability to provide capsules manufactured under cGMP conditions. Additional desirable features include preliminary efficacy data for FMT delivered via capsule form, dose standardization, and ease of handling (i.e., no need for freezer storage and ease of transport).

2. Route of delivery:

The original letter-of-intent for this study had proposed to use an enema rather than colonoscopy or naso-jejunal tube delivery, which at the time were the three routes of delivery with published case series. This was based on the principal that an enema is less invasive and easier to blind vs. colonoscopy and naso-jejunal tube. However, a recent publication of a small case series demonstrating that FMT could be delivered orally led to a renewed consideration of this approach. Capsule-delivered FMT offers a further improvement in tolerability, ease of administration, and ease of blinding when compared to alternate routes considered: enema, naso-duodenal or naso-jejunal, or colonoscopy administration. It also eliminates the need for more advanced care such as radiology services to verify naso-jejunal tube placement, or an endoscopist to perform the colonoscopy.

Efficacy of FMT via enema, colonoscopy, or naso-jejunal tube is similar, with no significant differences in prevention of recurrence (albeit with few studies that directly compared methods) [44, 45]. Aggregated success rates for FMT via enema, colonoscopy, and naso-jejunal administration are 78%, 90%, and 77%, respectively [21]. A study of 116 patients randomized to FMT via colonoscopy versus frozen capsule found that both treatments prevented recurrence in 96.2% of treated patients [19, 45]. Mechanistically, delivery via capsule is analogous to delivery via naso-jejunal tube, especially since the

capsules used typically dissolve after 2-3 hours, by which time they are expected to have entered the small intestine. With initial efficacy data comparable to that seen with other routes of delivery, and the much more favorable profile regarding tolerability, logistics, and safety, FMT via capsule is believed to be the best delivery modality for this trial.

3. Dose:

The optimal dose for FMT is unknown. Significant variability has been noted between different publications, both regarding the initial amount of stool used, the various volumes of diluents used, and the use of centrifugation to concentrate the stool organisms. The dose used for the recent case series of FMT via capsule was chosen arbitrarily (personal communication, Ilan Youngster). The FMT dose used in this study will be based on the dose recommended for the specific product when used in clinical care. Detailed information about the dose and number of capsules each patient will consume will be provided in the Operations Manual.

In summary, Veteran patients who have received standard antimicrobial therapy for CDI as prescribed by their treating clinician, have resolution of CDI or have maintained improvement of symptoms, and who consent to participate in the study, will receive FMT or placebo via oral administration of FMT or placebo capsules.

XI. Follow-up

A. Time Table

The study time-table for study activities and data collection is laid out below (Table 5):

Table 5. Study activities and data collection

Time	Prior to Rand.	Rand. Day 0	48 hours	Week 2	Weeks 4, 6	Weeks 8	Subject contact the study	Month 3, 4, 5	Month 6
Form1 /screening log	X								
Coordinator visit		X							
Coordinator call			X	X		X			X
AVRS call/Coordinator call (Form 17)					X			X	
Stool sample (optional)		X		X		X			
Forms 2-8 (Baseline)		X							
Form 10/11 (Safety follow-up)			X	X					
Form 12/13 (Evaluation)						X			X
Form 18 (Exit)									X
Form 14/15(AE/SAE)		@	@	@	@	@	@	@	@
Form 16 Unscheduled Contact			@	@	@		@	@	
Form 9 (CDI Evaluation) & diagnosis stool sample			@	@	@	@	@		
Form 7 (QoL)		X				X			

@ Fill out an AE or SAE form when an AE or SAE occurs or study team becomes aware of event (see Section XIV); fill out a form 9 when a potential episode of recurrent CDI is notified.

Overall, this is a short-duration study, with patients becoming potentially eligible by having an episode of recurrent CDI, responding to standard antimicrobial treatment, enrolling, and being randomized to FMT vs. placebo capsules. Follow-up lasts for 6 months, after which study participation is completed.

B. Follow-up Procedures

Study follow-up will be done by telephone, using a combination of scheduled calls by an automated system, triggered calls based on responses to the automated calls, and scheduled calls from Study Coordinators (SCs).

Participants will be assessed for symptoms of CDI and any treatment-related adverse events with a call from study personnel at days 2, 14, and 56 after capsule administration. The day 56 (8 week) call will be used to assess the primary endpoint, confirmed or possible recurrent CDI, as well as the secondary endpoints, including the quality-of-life questionnaire. Interspersed amid these in-person calls will be bi-weekly calls (on day 28 and 42) from an automated system or study personal that will prompt participants to answer general questions regarding any new diarrhea, abdominal pain, new antibiotic use, or concerns that they are experiencing a recurrence of CDI. Affirmative answers will be followed up by a call from the SC, who will ask further details regarding the positive response. Participants experiencing new diarrhea, in addition to seeking clinical care at their local facility, will be instructed to contact the study coordinator, collect a stool sample using a collection kit provided by the SC, and mail in a pre-paid mailer to the central laboratory, where it will be tested for *C. difficile* toxin by EIA, and the presence of the toxin B gene by PCR. If an automated system call fails to reach a participant within a six day time window, a SC will follow-up with the participant with at least three phone contacts and carefully document these attempts. Study Coordinators will continue to assess for study endpoints via medical record review, even in the unlikely event where no phone contact can be made.

After randomization, from week 8 to month 6, the automated calls or study personal contact will decrease in frequency to once a month, with the same triggers for a follow-up call from a Study Coordinator. At month 6 a final call by the SC will occur to assess any new symptoms of recurrent CDI, serious adverse events, hospitalization, and any other reported symptoms. A schematic of the study timeline for an individual participant is displayed above (Table 5).

Symptoms of recurrent CDI will be managed clinically by the local providers, with results monitored remotely by SCs. Participants will be asked to collect and mail a stool specimen to the central laboratory for *C. difficile* testing if they experience symptoms of recurrence.

C. Study Withdrawal and Crossover

We hope to limit study withdrawal by carefully educating potential participants regarding study expectations and procedures prior to enrollment. Furthermore, the fact that follow-up is via telephone should limit the demands on a participant's time and expenses. Cross-over is not planned in this study; FMT will not be provided to those experiencing recurrence. Patients experiencing recurrence will be asked whether they have undergone FMT outside of the study protocol, and if so, to provide details regarding administration (route, setting, supervision, etc.).

D. Study Termination and Closeout

At the 6 month follow-up visit, participation in the trial is concluded. A note indicating study completion will be placed into the Computerized Patient Record System (CPRS), and the provider(s) caring for the participant will be notified.

< This page left intentionally blank >

XII. Biostatistical Considerations

A. Hypothesized Event Rates, Treatment Effect Size, and Sample Size

1. Effect size

Sample size calculations were initiated with a determination of what improvement in the rate of recurrence would be sufficiently large to be considered clinically significant. For a high-risk procedure, the benefit must be substantial enough to outweigh the potential risks. In the case of FMT, approximately 500 cases have been reported in the medical literature, with few reports of harm [21]. Those that were reported were largely related to the route of delivery—including a fatal aspiration event associated with colonoscopy, and peritonitis associated with administration of FMT via an indwelling gastric tube. Since the protocol proposal is FMT administration via capsules, which confers substantially less risk, it is appropriate to consider a 15%-20% reduction in recurrent CDI rates as clinically significant. An absolute improvement (reduction) of 15% was selected as the threshold for clinical significance, meaning that if the placebo group had a recurrence rate of 35%, the FMT group would have a rate of 20% to be considered a clinically significant difference.

2. Expected Recurrence Rate after Standard Antimicrobial Therapy

Recurrence rates for CDI after antimicrobial treatment are well-described for the initial episode; unfortunately, subsequent rates of recurrence are less well known. Data regarding subsequent rates of recurrence derive from a few RCTs that included subjects with a previous episode of CDI, and several observational studies. Two RCTs comparing fidaxomicin to vancomycin for the treatment of CDI have been reported [9, 10], each containing a subgroup of patients with a prior episode of CDI (the rest experiencing their first episode). A subsequent publication assessed the difference in recurrence by treatment arm in those with prior CDI [12]. The overall recurrence rate for those with prior CDI was 27% (36% vancomycin vs. 20% fidaxomicin: P = .045). Another trial compared high-dose vancomycin vs. low-dose vancomycin vs. metronidazole for the treatment of recurrent CDI, each with the addition of a probiotic (*Saccharomyces boulardii*) vs. placebo [43]. In this study, the overall recurrence rate was 45%. Among the six different treatment arms, in five treatment arms recurrence rates were between 45% and 51%; the combination of *S. boulardii* and high-dose vancomycin had a recurrence rate of 17% (3/18). Finally, in the trial of FMT vs. two vancomycin-based control groups, the combined recurrence rate in the vancomycin groups was 73% (19/26) [20].

Observational data regarding subsequent recurrence after treatment of recurrent CDI is variable, both in terms of results and the population studied. In contrast to the RCTs which predominantly studied patients being treated for their first episode of recurrence, many of the observational studies report on patients with multiple episodes of recurrence. Reporting and publication bias is a significant concern, since successful experiences may be more likely to be submitted and accepted for publication. Those caveats aside, there is still valuable information regarding recurrence rates to be gained from this literature. An older case series describes no recurrence (after a mean of 6 months follow-up) among 22 patients treated with vancomycin using a tapered/pulsed dosing strategy [46], whereas another study evaluating tapered or pulsed dose vancomycin reported a 38% recurrence rate (10/26) [47]. Finally, a small case series described successful treatment without recurrence in 7 of 8 patients using vancomycin followed by a course of rifaximin [48]. Details of these studies are summarized in Table 6.

Table 6. Studies reporting antimicrobial treatment of recurrent CDI and subsequentrecurrence rates

Study	Study type	N	Population	Treatment	Recurrence rate
Cornely 2012 [12]	RCT	128	Patients with 1st recurrence of CDI	Vancomycin or fidaxomicin	27%
Surawicz 2000 [43]	RCT	170	Patients with 1st recurrence of CDI	High-dose vancomycin, low-dose-vancomycin, or metronidazole, all with *S. boulardii* vs. placebo	45%
van Nood 2013[20]	RCT	26	Patients with recurrent CDI (1-9 episodes)	Vancomycin or vancomycin plus bowel lavage	73%
Pepin 2006 [49]	Retrospective cohort	463	Patients with 1st recurrence of CDI	Vancomycin or metronidazole	33%
McFarland 2002 [47]	Retrospective cohort	26	Patients with recurrent CDI (1-14 episodes)	Vancomycin taper/pulsed dose	38%
Tedesco 1985 [46]	Case series	22	Patients with multiple relapses	Vancomycin taper/pulsed dose	0%

Johnson 2007 [48]	Case series	8	Patients with recurrent CDI (4-8 episodes)	Vancomycin followed by rifaximin	13%

The three largest studies report recurrence rates of 27%, 33%, and 45%. All of these included only patients with a first recurrence of CDI; subsequent recurrence rates are typically higher. Importantly, the placebo arm of the recently presented RCT (Kelly, oral presentation) had a recurrence rate of 37%, although the precision of this estimate is limited by the sample size of 24. **Accordingly, we believe that 35% is a conservative estimate for an expected recurrence rate after treatment with standard antimicrobial therapy**. If enrolled patients have a history of multiple recurrences, the recurrence rate is likely to be in the 40%-50% range [14].

Expected recurrence rates after FMT have recently been systematically reviewed and summarized. The overall recurrence rate, including case series and RCTs of all FMT delivery methods, is 15% [21]. The single published series regarding capsule delivery of FMT reported a recurrence rate of 30% (6/20) [19], with another series (presented in abstract form only) reporting no recurrences in 29 patients (Abstract 89. Fecal microbiome transplantation (FMT) via oral fecal microbial capsules for recurrent *Clostridium difficile* infection (rCDI) Session: Oral Abstract Session: New Considerations in *C. difficile* Prevention and Treatment/ October 2, 2013, IDWeek, San Francisco). Given the overall recurrence rate for FMT of 15%, the data from the published study of capsule-delivered FMT indicating a recurrence rate of 30%, and taking into account the unpublished study, **we believe that anticipating a 20% recurrence rate after standard antimicrobial therapy followed by FMT is appropriate.**

3. Sample size of the primary outcome

Accordingly, sample size was estimated using the following assumptions:

1) Recurrence rate of standard antimicrobial therapy followed by placebo of 35%;
2) Recurrence rate of standard antimicrobial therapy followed by FMT of 20%;
3) Two-sided significance level of 0.05; and
4) Overall drop-out rate of 1%.

Using the method described in Fleiss [50] for comparing two proportions, a total sample size of 318 provides 85% power to find an absolute difference of 15% in the recurrence rate with the addition of FMT to standard antimicrobial therapy for recurrent CDI.

Table 7. Sample Size Estimation Table

Recurrent CDI or All-Cause Mortality by Day 56			Power		
Placebo (Control Arm)	FMT (Experimental Arm)	Absolute Reduction	0.80	0.85	0.90
45%	35%	10%	758	866	1014
	30%	15%	327	374	436
	25%	20%	177	202	236
40%	30%	10%	717	820	961
	25%	15%	305	349	408
	20%	20%	164	181	218
35%	23%	12%	450	515	602
	20%	15%	276	318	372
	17%	18%	186	212	247
30%	22%	8%	950	1086	1270
	15%	15%	243	277	323
	12%	18%	159	182	212

Using the assumption in the above target sample size of 318, the following scenarios were considered as sensitivity power analyses:

- If the recruitment rate is less than anticipated, the study will still maintain 82% power to detect an absolute reduction of 15% with a sample size of 300 (83% of the target sample size).
- If the control group event rate was 30%, lower than anticipated, the study would still maintain 90% power to detect an absolute reduction of 14%.
- If the effect size is smaller than hypothesized, the study would still maintain 75% power to detect an absolute reduction of 13% while the control event rate maintains as 35%.

In summary, the anticipated rate of recurrent CDI following standard antimicrobial therapy is 35% (see Table 6); the study is powered to detect a clinically significant difference of 15%. As previously discussed, this difference is chosen based on the non-invasive nature of the intervention, as well as favorable safety data from the > 500 cases of FMT reported in the literature. The rate of recurrence will be affected by the mixture of patients enrolling; if most enroll after their first recurrence, the rate of subsequent recurrence may be slightly lower, whereas if most enroll after multiple recurrences, 35% is likely an underestimate. Using the above assumptions, the previously specified 85% power with a two-sided significant threshold of 0.05, and a 1% drop-out rate, the total sample size is 318 participants.

B. Duration of Study/Feasibility/Number of Participating Sites

It is estimated (see Section XII about the feasibility details) that there will be approximately 1,600 unique Veterans eligible for the trial overall in a year, per inclusion criteria. Additionally, we have calculated that 44% of cases/patients are from the top 26 sites in terms of recurrent CDI cases, where CSP #596 will recruit from. These 704 patients will not be eligible for enrollment in CSP #2004 initially, because enrolling from those sites would decrease eligible subjects for CSP #596, adversely affecting that study. However, those ineligible for or experiencing recurrences in CSP #596 will be offered participation in our study, a situation which we hope to encourage by maintaining a strong collaboration between the chairs of CSP #596 and #2004. The anticipated recurrence rate in CSP #596 is 47%; accordingly, approximately 330 patients initially in CSP #596 may be offered enrollment in CSP #2004.

In the spring of 2021, the study fell behind in recruitment. The MATCH study Executive Committee believe the following four reasons caused the recruitment to fall below target:

- There was a three-month initial delay in recruitment until the study therapy became available.
- In July 2019, the FDA placed a clinical hold on all FMT studies while it was investigating a serious adverse event (death) caused by a multi-drug resistant organism (MDRO) infection, acquired through a fecal microbiota transplant (note: this event was not in CSP #2004). Enrollment was reopened 1.5 months later, after CSP #2004 provided satisfactory measures to mitigate the MDRO infection risk.
- As a result of the COVID-19 pandemic, study recruitment was put on hold for five months (March 18, 2020 – August 28, 2020) to minimize exposure risk for potential participants and research personnel.
- Even after the study resumed in August 2020, the ongoing pandemic and associated exposure risk have caused patients/potential study participants to be reluctant to seek care and therefore unable to provide a stool sample for diagnostic testing. As a result, providers at some VAMCs could not order a confirmatory stool test for CDI and instead diagnosed recurrent CDI using clinical criteria only. Hence, the study's database screening algorithm could not identify patients who might have been eligible for the study.

Due to the above reasons, the study Executive Committee decided to modify the sample size from 390 to 318, reduce power from 90% to 85% and increase enrollment time from 3-years to 6-years. Our original randomization target and power were calculated based on a 15% absolute reduction in the cumulative event rate for the primary endpoint for FMT and placebo. In the modified sample size calculation, assuming a 1% dropout rate, a sample size of 318 participants (159 in each treatment arm) is required to detect this difference using a 2-sided test of significance with $\alpha = 0.05$ and $\beta = 0.15$ (power of 85%).

The Study Leadership, with endorsement of the study's DMC, requested a 36-month extension of recruitment. This, in combination with a modified sample size, would maintain a high statistical power (at least 85%) to test the primary hypothesis.

C. Interim Monitoring and Analysis

Interim monitoring will be performed by the WH-CSPCC and will focus on recruitment, baseline comparability of treatment groups, protocol adherence, completeness of data, accrual of primary endpoint events (i.e., information accrual), safety, and treatment efficacy. The WH-CSPCC will provide interim reports to the Data Monitoring Committee (DMC), which will meet for two scheduled interim analyses.

1. Efficacy and Futility Analyses

Two interim analyses of the primary outcome (recurrent CDI or death) are planned, after which the DMC will decide whether to continue the trial or not. The DMC will have discretion to request additional or differently scheduled interim analyses. The first interim analysis will be done when at least 159 subjects have been enrolled and followed for more than 56 days (50% accrual of primary outcome information), and the second when at least 239 subjects have been enrolled and followed for more than 56 days (75% accrual of primary outcome information). For both interim analyses, a stopping rule with a wide boundary such as the Haybittle and Peto method [51, 52] will be used. It is suggested that the significance level for the interim analyses will be 0.001 and the two-sided significance level for the final analysis will be 0.05. The inflation of the overall type I error will be negligible. At the first interim analysis, there will be approximately 80% power to detect an 18.5% absolute reduction in the primary outcome relative to the placebo arm (based on a placebo rate of 35%). At the second interim analysis, there will be approximately 82% power to detect a 16% absolute reduction in the primary outcome relative to the placebo arm (again assuming a 35% primary outcome rate in the placebo arm).

At the time of the two interim analyses, a futility analysis will be performed to assess the likelihood of eventual success based on the observed data. The conditional power [53] to fulfill the study will be provided to the DMC as either an exact value of power or through a Yes/No question. If the conditional power is low, the DMC may recommend stopping the study or continuing with an adjustment to the sample size and extension of the trial. Definite recurrent CDI, a secondary endpoint, is as important as possible recurrent CDI, which is a symptom-based component of the primary endpoint. Accordingly, we will process the futility analysis to both primary endpoint as well as definite recurrent CDI, one of the key secondary endpoints.

2. Interim Safety Monitoring

Safety will be monitored by the WH-CSPCC, the CSPCRPCC, and the Study Co-Chairs' Office throughout the study. Safety reports will be submitted to the DMC approximately every 6 months after enrollment begins, or more frequently, if requested by the DMC. For reports to the DMC closed session, serious adverse events (SAEs) will be summarized by treatment groups and relatedness to the assigned interventions. The proportion of participants experiencing an SAE in each treatment group will be calculated and analyzed using the chi-square test or Fisher's exact test, as appropriate. In the event that SAEs or mortality are noted to be excessive in either study arm, the DMC may consider recommending that the trial be stopped or that the protocol be modified.

D. Final Statistical Analysis of the Data

All primary analyses will be according to the principle of intent-to-treat; i.e., subjects will be analyzed according to their original treatment assignment regardless of protocol adherence.

1. Baseline Comparability

In order to assess the adequacy of randomization, the baseline characteristics to be compared and summarized include: age, gender, ethnicity, medical comorbidities, and number of prior CDI episodes experienced. The distribution of baseline patient characteristics between groups will be evaluated using descriptive statistics. *A priori* baseline variables which will be used for covariate adjustment include age, gender, number of prior CDI episodes, and immunodeficiency.

2. Analysis of Primary Outcome Measure

The primary outcome (recurrent CDI or death within 56 days) will be compared between the FMT arm and the placebo arm, using a p-value of 0.05 (two-sided). A generalized linear model will be used for the analysis since it is appropriate for a binary endpoint and can be adjusted for covariates and tested for interactions. Based on the study, design treatment arm, as well as a previous recurrence of CDI (1 vs. =>2) will be considered as fixed effects, and the status of having a recurrence of CDI or death will serve as the outcome. The number of primary outcomes as well as the percentage of primary outcomes will be summarized by treatment. The odds ratio of having a primary outcome in terms of FMT treatment to the placebo with 95% confidence interval will also be provided. The primary hypothesis will be tested by the above generalized linear model with treatment adjusted for study design. Treatment by number of prior CDI episodes interactions will be examined in exploratory analyses. A p-value of 0.05 will be used for all sub-group analyses of the primary outcome.

As another exploratory analysis of the primary outcome, we will test the treatment effect adjusted for study design as well as a set of pre-specified baseline covariates to examine their influence on the treatment comparison. Treatment by covariate interactions will be examined for the following baseline covariates: gender, age, number of prior CDI episodes, and immunosuppression. A p-value of 0.05 will be used for all sub-group analyses of the primary outcome.

Survival status on participants who cannot be contacted by day 56 will be obtained using the VA Beneficiary Identification and Records Locator System (BIRLS), the National Center for Health Statistics' National Death Index database, and the Social Security Administration's Death Master File. Participants who completely withdraw from the study will have their last assessment (recurrent CDI or not) carried forward, whereas those partially withdrawing (i.e., allowing medical record review) will have their CDI status updated based on information in the medical record.

3. Analysis of Secondary Outcomes

The first secondary outcome (recurrent CDI or death within 6 months) will be summarized by incidence between the FMT arm and the placebo arm. Treatment group comparisons will be based on generalized linear model. The odds ratio of having a recurrent CDI or death within 6 months in terms of FMT treatment to the placebo tested and the estimator with 95% confidence interval will also be provided. Kaplan-Meier survival curves, adjusted for censoring due to loss to follow-up, will also be used to evaluate treatment effects for 6-month recurrent CDI. The Cox proportional hazards model will also be

used to test the effect of treatment adjusted for the study design. The proportional hazard assumption will be tested using log (log) plots with visual examination, in order to assure the validity of this analysis. If the assumption is not valid, appropriate adjustments will be made, such as adding time by covariate interaction terms or use of stratification.

Analyses of secondary outcomes (definite recurrent CDI, possible recurrent CDI, death, urgency, fecal incontinence, diarrhea that is negative for *C. difficile* by PCR, number of CDI recurrences within 6 months, and quality of life at day 56) between treatment groups will be assessed using a p-value of 0.05, without adjusting for multiple comparisons.

The same method of analysis that was applied for primary outcomes will be used for the secondary outcomes of: definite or possible recurrent CDI by 6 month, definite recurrent CDI by day 56, possible recurrent CDI by day 56, death by day 56.

The secondary endpoints of abdominal pain, urgency, fecal incontinence, and diarrhea that is negative for *C. difficile* by PCR are among the secondary analyses. They will be performed among both possible recurrent CDI cases as well as definite recurrent CDI cases. Similar method of analysis that was applied to the primary outcome will be used for these secondary endpoints.

The number of definite or possible CDI recurrences by 6 months will be summarized as mean with standard deviation by treatment group and be analyzed by an ANOVA model. We will also categorize them into three levels of discrete ordinal measurement – none, one, and more than one. A generalized linear model with a cumulative logit link function will be used to investigate the effects of treatment on a number of CDI recurrences. Otherwise, a weighted-least-squares analysis will be used to assess the mean score of number of CDI recurrences for each treatment arm. As for the primary outcome, treatment comparisons will be adjusted for both the study design and the pre-specified set of baseline covariates.

Kaplan-Meier survival curves, adjusted for censoring due to loss to follow-up, will be used to evaluate treatment effects for 6-month all-cause mortality. Treatment group comparisons will be based on the log-rank test. The Cox proportional hazards model will also be used to test the effect of treatment adjusted for the study design. The proportional hazard assumption will be tested using log (log) plots with visual examination, in order to assure the validity of this analysis. If the assumption is not valid,

appropriate adjustments will be made, such as adding time by covariate interaction terms or use of stratification.

Quality of life (a continuous measurement) differences between the two treatment arms will be analyzed using a linear model. Since some of the censoring, such as death, may be informative, we will conduct the analyses both with and without adjusting for the possibility of informative censoring. First, the analysis will be done on the observed quality of life, regardless of whether the censoring is informative or not. Then, we will assign a lower score to those missing due to death and analyze the data adjusted for the informative censoring as a sensitivity analysis.

4. Analysis of Safety Outcomes

Kaplan-Meier survival curves, adjusted for censoring due to loss to follow-up, will be used to evaluate treatment effects for 6-month all-cause mortality. Treatment group comparisons will be based on the log-rank test. The Cox proportional hazards model will also be used to test the effect of treatment adjusted for the study design. The proportional hazard assumption will be tested using log (log) plots with visual examination, in order to assure the validity of this analysis. If the assumption is not valid, appropriate adjustments will be made, such as adding time by covariate interaction terms or use of stratification.

The number of SAEs and proportion of participants experiencing an SAE up to six months in each treatment group will be summarized by treatment arm, and MedDRA System Organ Class. The same summary will be done on all protocol specified AEs.

5. Analysis of Exploratory Outcomes

The Shannon index (diversity) and the abundance-based coverage estimate (ACE) (richness) will be used as endpoints to perform the following exploratory analyses in order to evaluating the microbiome of patients pre- and post-treatment:

i. Characterization and comparison of the colonic microbiome of individuals with CDI randomized to FMT and placebo, before and after FMT/placebo administration;
ii. Comparison of the post-FMT colonic microbiome of individuals randomized to FMT and placebo to the donor microbiome; and

iii. Comparison of the post-FMT microbiome of individuals that experience a recurrence to the microbiome of those not experiencing recurrence.

Using collected stool samples, we will extract DNA, quantify and amplify microbial communities, and use 16S rRNA gene profiling using V5 + V6 hypervariable regions sequencing on the Illumina MiSeq platform to characterize the microbial community structure. Taxonomic classification will be performed against the version 14 data release from the Ribosomal Database Project. Specifically, we will be quantifying microbial diversity using two validated indices to characterize microbial diversity and richness with the Shannon index (diversity) and the abundance-based coverage estimate (ACE) (richness) respectively. These two indices are calculated to assess parametric and non-parametric diversity. The Shannon index is scored between 0 and 7 while the ACE ranges from 0 to 8000.

Student t-test (paired and unpaired), Pearson and Spearman correlations will be used to compare scores pre-post FMT and donor-recipient. Our hypothesis is that post- FMT samples will show increased diversity and richness compared to pre-FMT, and that post-FMT changes will resembles the microbiota composition of donor specimens, and that post-FMT changes will be sustained over 6 months.

Prior estimates from the literature suggest that the mean Shannon index for pre and post FMT will be 1.68+0.75 and 3.37 $\pm$0.46 respectively. Assuming half the study participants agree to provide an additional sample for characterization of stool microbiome (n=200) we would have >95% power to estimate differences pre and post FMT.

Additional analyses may include: differences in beta diversity (community composition) using analysis of similarity (ANOSIM), other diversity indices [number of operational taxonomic unit (OTU) sequences (S obs), sequencing coverage estimation, UniFrac analysis, ANOSIM analysis, principal coordinate analysis (PCoA), Mantel tests, Kruskal – Wallis analysis, and analysis of molecular variance (AMOVA) will be performed.

< This page left intentionally blank >

XIII. Feasibility

A. Estimating the Patient Population

An assessment of study participant availability was performed by searching national VA data from the Cooperate Data Warehouse (CDW) to identify recurrent CDI episodes in fiscal year 2014. We queried the laboratory test name file to identify all diagnostic tests associated with CDI. These included nucleic acid amplification tests (NAAT), EIA toxin test, or other tests (glutamate dehydrogenase [GDH] plus NAAT, GDH plus toxin A/B EIA, cell culture cytotoxin assay, or anaerobic toxigenic culture).

A total of 108,664 CDI test result records were extracted from patients identified in the last 2 quarters of fiscal year 2013 through fiscal year 2014, of which 15.6% (n = 16,952) of tests were positive using data from the CDW laboratory chemistry data domain. We applied a definition of recurrent CDI episode as follows: the number of days from a previous positive CDI lab result to the current positive lab result was more than or equal to 10 and less than or equal to 90 days. We identified a total of 11,242 positive CDI results in fiscal year 2014 on 8,726 unique Veteran patients. Of these, 1,370 (15.7%) had a laboratory-confirmed episode of recurrent CDI. In addition to the laboratory confirmed cases, we identified 1,476 (16.9%) cases of recurrent CDI based on inpatient or outpatient diagnoses (but without laboratory confirmation in CDW data).

A pilot study at the Minneapolis VA was performed to determine what percentage of these cases lacking lab confirmation have lab confirmation documented in the electronic medical record (either by a non-VA laboratory result, or a VA result not captured by our search of CDW data). The table below displays the laboratory confirmed cases of recurrent CDI, and the cases lacking laboratory confirmation.

Table 8. Cases of recurrent *Clostridium difficile* infection (CDI) identified using VA data from Corporate Data Warehouse, including laboratory-confirmed cases and cases lacking laboratory confirmation.

	Number of individuals
Unique Veterans with laboratory confirmed CDI in year 2014	8,726
Unique Veterans with laboratory confirmed *recurrent* CDI in year 2014	1,370 (15.7%)
Unique Veterans with a *recurrent* CDI diagnosis WITHOUT laboratory confirmation in year 2014	1,476 (16.9%)

CSP #596, Optimal Treatment for Recurrent *Clostridium difficile* Infection (CDI), is another ongoing, randomization study in 26 VA sites. The patient population of CSP #596 is also recurrent CDI patients. The primary objective of CSP #596 is to determine whether 1) standard fidaxomicin treatment and 2) standard vancomycin treatment followed by taper and pulse vancomycin treatment are superior to standard vancomycin treatment alone for sustained clinical response at day 59 for all treatments, for participants with either their first or second recurrence of CDI. This study, CSP #2004, is mainly enrolling from sites that were not selected by CSP #596, although subjects not eligible for or experiencing recurrence in CSP #596 are eligible to enroll in CSP #2004. Accordingly, we assume that CSP #596 will be recruiting from most of the top 26 sites in terms of recurrent CDI cases, although as discussed above, the anticipated 47% recurrence rate in CSP #596 may result in approximately 330 patients who may be offered participation in CSP #2004. This is particularly important as these patients have already demonstrated a willingness to enroll in a CDI clinical trial and participate in the research process.

Using this data it is estimated that there will be approximately 1,600 unique Veterans eligible for the trial in a year (assuming that ~15% of the approximately 1,500 patients with a CDI diagnosis but no lab confirmation in CDW will have laboratory confirmation from a non-VA laboratory or from a VA laboratory not captured by CDW). Additionally, we have calculated that 44% of the cases are from the top 26 sites in terms of recurrent CDI cases, where CSP #596 will recruit from. Considering the 97 sites that are not involved in CSP #596, there will be approximately 896 unique Veterans eligible for the CSP #2004 study. It is anticipated that a total of 130 potential participants would have to be enrolled per year (14.5% of eligible) in order to accrue 390 participants in 3 years.

In summary, we plan a 3-year recruitment to enroll a total 390 participants (130/year) with an annual enrollment rate of 14.5% among eligible participants. In the Fall 2021, the study was approved to modify a total 3-year recruitment into a total 6-year recruitment to enroll a total 318 participants (60/year).

B. Centralized and Regional Enrollment and Follow-up

The study is expected to complete enrollment of at least 318 participants in 6 years, with a minimum of 56 days of follow-up for study outcomes for all randomized participants. The study design is based on conducting the trial with VA-system-wide central enrollment, central follow-up and coordination, as described in section A of Section VIII. Running the study centrally includes centralized screening, recruitment, and follow-up for participants' safety as well as outcome data collection. Central enrollment is selected for two main reasons:

a. Feasibility: The estimated 1,600 eligible patients (per year) are distributed among 120+ VA sites. In order to reach the target of 60 participants per year, all non-CSP #596 sites would have to participate. Given the small number of cases at any given facility (15-25/year), the resources, time and effort required for a traditional participating-site design would not be practical. In order to maximize efficiency in enrolling eligible participants central enrollment is a pragmatic and resource effective approach.

b. Patient centered approach: Central enrollment provides the opportunity to reach geographically diverse patients across all VA facilities nationally, without restriction of site and status of the facility. A critical design factor is the timing between episodes of CDI, initiation of treatment and window available for enrollment. A central enrollment strategy would allow identification of recurrent cases in real-time, and being able to reach the patient in a timely manner. This also avoids the burden and inconvenience for the patient to come to a VA facility.

In addition, participants' safety records such as SAEs will be reported by SCs and SIs using CDW data and medical record review. This allows for morbidity and mortality events to be captured in a uniform and comprehensive fashion compared to a usual trial setting.

In summary, centralized recruitment and follow-up is necessary for the purpose of conducting CSP #2004 efficiently. The centralized recruitment in this trial will be done ethically with protection of

the patients' rights and privacy. Patients' safety will be monitored through patient contacts and medical record review as would be done in a traditional participating-site trial setting. This approach allows for collection of high quality study data, similar to a standard design. Prior CSP studies (such as CSP#566) have also used this strategy successfully.

C. A Pilot Study to Test Recruitment Process

To assess feasibility and test the proposed enrollment methods, a pilot study was conducted at the Minneapolis VA Medical Center. A list of 100 CDI cases within VISN 23 in the last year was obtained based on the following criteria: ICD-9 diagnosis OR Positive PCR (the lab test used currently at this facility) and treatment for CDI for index and prior episode. These criteria are different from what we plan to use in the trial, because we wanted to assess the specificity of our search algorithm for finding a positive PCR, i.e. for charts where only an ICD-9 is found, and how many may have a PCR that our search was missing.

We were able to review charts for 69 patients (31 were not viewable with our current level of authorization; a request for CAPRI access to view these records is pending). Among the 69 viewable records, 31 were identified as having an ICD-9 code for CDI but no PCR result. Of these 31 charts, there were no cases where chart review identified a positive PCR that was missed by electronic screening (100% specificity). Of the remaining 38 records, 10 were not eligible per study criteria (main reasons were patient comorbidity such as terminal cancer, inflammatory bowel disease or dementia, and living in a nursing home). Of the 28 that were eligible, we were not able to reach the primary care physician (PCP) or treating provider in 4 cases (no listed phone numbers or incorrect numbers, or did not return phone calls or emails in a week). Of the 24 patients whose PCP's we were able to reach, 100% reported having heard about FMT as a therapy for preventing recurrent CDI. Most PCPs were reached successfully within one working day. Twenty-three of the 24 (95%) said they would be happy to contact their patient about the study and call us back. Two PCPs gave us additional patient names that would be potentially eligible. A summary is provided in Table 9.

Table 9. Summary of Pilot Study at the Minneapolis VA Medical Center

List of potentially eligible in VISN 23	100
Patients charts we had access to	69
Chart with +PCR (that would be study eligible)	38
Eligible to enroll by study criteria	28 (74% of the 38)
Unable to contact PCP	4(14% of the 28)
Contacted PCP	24
Average time to make contact	8 hours (many same day)
PCP aware of FMT as an option	24 (100% of all contacted PCP)
PCP agreed to call patient and call us back	23 (95% of all contacted PCP)

An additional 10 patients with a recent positive PCR (within 2 weeks) were subsequently used to assess the timing and logistics of contacting patients who had been recently diagnosed and treated for recurrent CDI; 2 such patients were identified. In both cases the provider was contacted, was willing to contact the patient, and was able to do so on the same day. Both patients agreed to be contacted and discuss the details of their recent CDI episode. Both had substantially improved on their current CDI therapy, such that they would meet CSP #2004 eligibility criteria. Both met eligibility criteria with at least 7 days of antimicrobial therapy remaining (7 and 13 days, respectively). Thus, if we were currently enrolling, the Study Coordinators would have at least 9 days to organize travel and administer the study intervention, assuming a 2-day washout period after completing antimicrobial therapy. Since the allowed washout period is 2-7 days, the time period available to arrange travel would be 9-14 days and 15-20 days, respectively.

Limitations: Since most of the providers contacted were in Minneapolis or the surrounding area, the rate of potential participation may be higher than in the actual study.

< This page left intentionally blank >

XIV. Adverse Event Assessment and Reporting

A. Definitions

Adverse events and SAEs will be collected according to the ICH Guideline for Clinical Safety Data Management (ICH-E2A) and CSP Global SOP 3.6 definitions.

1. Adverse Event (AE)

An AE is any untoward physical or psychological occurrence in a human participating in research. An AE can be any unfavorable and unintended event, including an abnormal laboratory finding, symptom, or disease associated with the research or the use of a medical investigational test article. An AE does not necessarily have to have a causal relationship with the research. (see VHA Handbook 1058.01, 21 CFR 312.32 and CSP Global SOP 3.6)

2. Serious Adverse Events (SAEs)

An SAE is an adverse event (as defined above) that, in the view of either the Site Investigator or sponsor:

- Results in death;
- Is a life-threatening experience;
- Requires inpatient hospitalization (≥24 hours) or prolongation of existing hospitalization;
- Results in persistent or significant disability or incapacity;
- Results in a congenital anomaly/birth defect; or
- Any other condition that, based upon medical judgment, may jeopardize the participant and require medical, surgical, behavioral, social or other intervention to prevent one of the above outcomes.

3. Relatedness

Relatedness involves an assessment of the degree of causality (attributability) between the study intervention and the event. The assessment of relatedness provided by the Site Investigator is part of the information used by the sponsor to determine if the adverse event presents a patient safety concern or requires regulatory reporting. Pursuant to CSP Global SOP 3.6, an AE or SAE is deemed to be attributable to the use of a study drug/device if "there is a reasonable possibility that the experience may have been caused by the drug/device or by participation in the trial." Thus, all adverse events with a reasonable causal relationship to the investigational treatment should be considered "possibly related" or

"related." A definite relationship does not need to be established, but there should be some evidence to suggest a causal relationship between the investigational treatment and the adverse event (21 CFR 312.32).

4. Unanticipated or unexpected

VHA policy (handbooks 1200.05 and 1058.01), state that "unanticipated and unexpected refer to an event or problem in human research that is new or greater than previously known in terms of nature, severity, or frequency, given the procedures described in protocol-related documents and the characteristics of the study population." Thus, whether an event is anticipated or unanticipated is determined based on pre-specified characteristics of the study population and treatment. In addition to study outcomes including recurrent CDI and certain complications thereof, events which are expected in this study are diarrhea, abdominal pain, vomiting and nausea. Other symptoms or diagnoses not listed here may also be judged expected on the basis of published information on this population and study treatment.

5. Severity

NIH Division of AIDS grading scale (see the appendix in the Operations Manual) for both solicited and non-solicited adverse events will be used to classify the severity of adverse events and serious adverse events.

B. Reporting of AEs and SAEs in CSP #2004

The following adverse events will be collected and reported to the sponsor on study forms:

- All serious adverse events, beginning at **randomization**/administration of study medication (defined as the opening of the study drug bottle) and continuing until the six-month visit;
- All adverse events which are believed to be related or possibly related to the study treatment continuing until the six month visit; and
- All adverse events regardless of seriousness or relatedness for the first 14 days after study treatment.

Site staff will assess participants for AE/SAEs during all follow up visits/phone calls using open-ended questions concerning any new symptoms or side effects. All participants will be monitored for adverse events from the time of randomization/ administration of study medication to the study exit time (typically at 6 months after study treatment). The SI will evaluate whether each AE or SAE is potentially related or unrelated to the study intervention; an adjudication committee will also be utilized in

ambiguous cases. The SI should also continue with follow-up assessments through monthly contact until resolution of the adverse event is achieved, or the adverse event is stabilized with no further change expected.

C. Role of the Site Investigator in Event Monitoring

The Site Investigators as well as Study Co-Chairs will be responsible for the following adverse medical event reporting requirements:

- Reviewing the accuracy and completeness of all adverse events reported;
- Complying with study policies as well as IRB policies for reporting adverse events, serious adverse events, and unanticipated problems involving risks to participants or others;
- Reporting to CSP all SAEs within 72 hours of becoming aware of the event;
- Reporting to the IRB safety issues reported to the site by the Sponsor; and
- Closely monitoring research subjects for any new AEs and SAEs.

D. AE and SAE Monitoring and Reporting

AEs and SAEs will be monitored at the study sites throughout the period of the study, beginning at randomization/ administration of study medication and continuing through end-of-study for each participant. The study will report all SAEs and those non-serious AEs determined to be possibly related, or related to the intervention or study. Reportable AEs will be collected and recorded on the appropriate case report form. All SAEs, including both those related to the study intervention and those not related to the study intervention will be collected and recorded on the appropriate case report form.

All SAEs require expedited reporting. An SAE case report form will be completed and submitted via the data capture system within 72 hours of the Site Investigator/ Study Coordinator initially becoming aware of the event. The Study Pharmacist at CSPCPRCC will be responsible for evaluating all SAEs for participant safety concerns in a timely manner.

SAEs that suggest evidence of a causal relationship between one of the study interventions and the adverse medical event and which are unexpected will be reviewed by the Study Leadership Team (i.e., Study Chairpersons, Study Biostatistician, Study Project Manager, and the Study Pharmacist) prior to notifying VA Central Office whenever feasible.

The CSP #2004 Biostatistician(s) and the Clinical Research Pharmacist will tabulate and summarize all AEs and SAEs for the DMC on a schedule set by the DMC, but no less than annually. The DMC will also determine when the committee should be unblinded to treatment assignment in reviewing AE/SAE data. The DMC will advise the CSP Director whether the study should continue or be stopped for safety reasons. Any SAE which is assessed as at least possible related to FMT and any suspected or proven transmission of infection from the FMT product to a participant will be reported to the DMC within 7 calendar days. FDA safety reporting will adhere to the FDA's Guidance for Industry and Investigators: Safety Reporting Requirements for INDs and BA/BE studies.

E. Authorization to Break the Blind

Under unusual circumstances, chiefly related to participant safety, unblinding may be necessary. This is usually done after consultation with the Study Co-Chairs (Dimitri Drekonja, MD or Aasma Shaukat, MD). If Drs. Drekonja and Shaukat are unavailable, the CSPCRPCC Clinical Research Pharmacist (Annie Davis-Karim or another Clinical Research Pharmacist) or the Study Biostatistician (Jane Zhang) should be contacted.

If knowledge of treatment assignment is required to treat an emergency, the treating physician may contact the CSPCRPCC through the 24-hour emergency call service at (505) 248-3203 to obtain study drug assignment information. The CSPCRPCC will notify the Study Co-Chair's Office and the WH-CSPCC by telephone as soon as possible after an unblinding has occurred.

XV. Study Monitoring and Quality Control

A. Procedures Overview

The VA Cooperative Studies Program establishes overall policies and procedures that are applied to all VA cooperative studies through the Study Co-Chairs' office at the Minneapolis VA Healthcare System, and the WH-CSPCC. The Cooperative Studies Scientific Evaluation Committee (CSSEC) reviews the scientific merit of all new cooperative study proposals. The CSSEC is composed of both VA and non-VA clinical research scientists and biostatisticians. The organizational and administrative structure of this cooperative study is similar to others in the Cooperative Studies Program and includes the components described below.

B. Monitoring Bodies and CSP Monitoring

The Executive Committee is chaired by the Study Co-Chairs and consists of the Study Biostatisticians, Study Research Pharmacist, selected participating investigators, and expert consultants. The Executive Committee is concerned with overall study management and is the decision-making body for the operational aspects of the study. The Executive Committee monitors the performance of participating medical centers and the quality of data collected, and plans and oversees the publication and presentation of all data from the study. The Executive Committee must grant permission before any study data may be used for presentation or publication. This committee will hold conference calls on a monthly basis to review the study progress, and meet every 12 months to review blinded study data and decide upon any study changes.

The WH-CSPCC and the Study Co-Chairs will administer the trial, oversee its organization, and perform the day-to-day scientific and administrative coordination of the study. This includes development of the protocol, operations manual, and case report forms; ensuring appropriate support for the participating centers; scheduling meetings and conference calls; responding to site queries about the protocol; conducting site visits; preparing interim and final progress reports; and archiving study data. Participant accrual and data quality are monitored closely to ensure that the study is progressing satisfactorily.

The Minneapolis VA Health Care System IRB will serve as the oversight body for the protection of human subjects. Any additional sites (two are planned) will also submit the protocol to their IRB of record for the oversight of the research activities at those sites.

The Albuquerque CSPCRPCC will be responsible for acquiring, storing, and providing study medication to the Study Coordinators who will oversee treatment administration. Additionally, the CSPCRPCC will be responsible for monitoring the safety of trial participants through the review, assessment, and communication of adverse events (AEs) and SAEs reported by study personnel. The CSPCRPCC will maintain ongoing communication with the Study Co-Chairs, the Executive Committee, the WH-CSPCC, and CSP Central Office. The CSPCRPCC will serve as liaison to the Food and Drug Administration (FDA), including filing and maintaining the Investigational New Drug application (IND) and reporting certain SAEs as required by Federal regulations. In conjunction with the WH-CSPCC, the CSPCRPCC will prepare safety data reports for various committees including the DMC, IRB, Executive Committee, and the study group.

The DMC will provide interim reviews of the study's ongoing progress. The DMC is composed of clinical and statistical members with expertise in clinical trials and/or the study subject area. These experts are not participants in the trial and have not participated in the planning of the protocol. The Study Co-Chairs, Study Biostatisticians, Clinical Research Pharmacist, and the Director of the WH-CSPCC are *ex officio* (liaison, non-voting) members of the DMC. The DMC will meet at least annually to review the progress of the study and monitor participant intake, outcomes, adverse events, serious adverse events, and other issues related to participant safety. The DMC's primary responsibilities will be to review safety and the progress of the study and to decide whether or not the study should continue. To help the DMC make its assessment, the Study Co-Chairs and Study Biostatisticians will provide the DMC with appropriate monitoring data before each meeting. The DMC makes recommendations to the Director, VA CSR&D about whether the study should continue or be stopped.

A member of the Human Rights Committee (HRC) at the WH-CSPCC may conduct a site visit to at least one participating center during the course of the study to determine if participants' rights and safety are being properly protected. The HRC member may interview study participants during the site visit.

The Outcome Adjudication Committee will consist of 3 members nominated by the Executive Committee and chosen for their clinical and technical expertise. Since recurrent CDI is a clinical diagnosis, and motility disorders can cause symptoms similar to recurrent CDI, committee members will review all cases of possible recurrent CDI. In conjunction with the Study Co-Chairs, they will establish a standard adjudication procedure to categorize all possible recurrences, and whether they meet the primary endpoint of definite or possible CDI.

There will be two affiliated sites for CSP #2004, selected to facilitate Study Coordinator travel throughout the United States. The Site Investigator (SI) at each center is responsible for the conduct of the study at the center. The SI is expected to attend all annual Study Group meetings, as well as to hire and supervise local study personnel. By agreeing to participate in the study, the affiliated site delegates responsibility for global monitoring of the ongoing study to the DMC, HRC, and CSPCRPCC. However, the IRB of the medical center will have oversight of the research activities conducted at each affiliated site.

The Study Group will consist of the Study Co-Chairs, CSP staff (Biostatisticians, Project Manager, Clinical Research Pharmacist, and others), Site Investigators, and Study Coordinators. The Study Co-Chairs will lead the Study Group, which typically meets monthly by teleconference and once per year in a face-to-face meeting to discuss the progress of the study, any problems encountered, and any suggestions for improving the study.

The CSP Site Monitoring, Auditing, and Resource Team (SMART), located at the CSPCRPCC in Albuquerque, will monitor the trial for compliance with Good Clinical Practices. GCP monitors from SMART will visit participating sites shortly after sites randomize their first few participants and yearly thereafter to monitor investigator regulatory compliance, protocol adherence, and overall research practices. Additional monitoring visits may be conducted as deemed necessary by study leadership or SMART. SMART will provide an orientation to GCP at the study kick-off meeting and provide GCP tools to enhance compliance.

The SMART reviewer examines participant study files, including source documents held electronically, in clinic files and participants' official VA medical records and reviews regulatory essential documents, such as IRB correspondence. Areas of particular concern are informed consent issues, protocol adherence, safety monitoring, IRB reviews and approvals, regulatory documents, participant records, drug accountability, site operations, and investigator involvement in the trial. Monitoring may include but is not limited to the informed consent process, data validation, source verification, and safety reporting. Additionally, SMART will conduct periodic routine audits throughout the course of the study and for-cause audits of participating sites only as requested by study leadership or CSPCO. Audits can be announced or unannounced.

In addition to SMART visits, WH-CSPCC, the Executive Committee, and the DMC will monitor protocol adherence centrally through periodic reports, data queries and SC/SI conference calls. Additional, site-specific monitoring may be conducted if triggered by study performance metrics.

The site is required to document protocol breaches and any medical center with repeated protocol violations is reported to the Executive Committee and the Director, VA CSR&D. If a Site Investigator feels that adherence to the protocol will be detrimental to a particular participant's health or well-being, the interest of the participant must take precedence.

C. Monitoring Participant Intake

The Study's Executive Committee, Study Co-Chairs, and the Study Biostatisticians will monitor the intake rate and operational aspects of the study. The target enrollment for the study is 318Veterans. If recruitment is not proceeding at an appropriate rate, the Study Co-Chairs and the Study Biostatisticians will scrutinize the reasons for participant exclusions and other barriers to recruitment. Based on this information, the Executive Committee may choose, with the approval of the Director, VA CSR&D, to make modifications to the inclusion/exclusion criteria, or extend the recruitment period and/or to extend the total length of the study.

Medical centers will only be allowed to continue in the study if adequate participant intake is maintained. The target recruitment for the study is 5 participants per month. Because there is usually a ramp-up in recruitment early on, if the Minneapolis site does not enroll at least 32 participants during the first six months after being fully staffed, or 65 participants within the first year, it will be considered for probation or reduction in funding. If the Minneapolis site does fall behind in enrollment as described above, we will consider opening one or two regional sites. For all regional sites, the expected enrollment is 4 participants per month. A site that does not enroll at least 12 participants during the first six months, or 24 participants within the first year, will be considered for probation or reduction in funding. If a site is placed on probation, the Study Co-Chairs and Study Biostatistician will confer with the site personnel and, if necessary, visit the site to help improve the rate of recruitment. If there is no improvement in accrual after the probation period, the site may be subject to reduced funding or possible termination as a study site. The Executive Committee only takes actions leading to discontinuation of a site with the concurrence of the Director, VA CSR&D. If a site is terminated from the trial, resources are reallocated to other sites or used to start up a backup site.

D. Monitoring Medical Center (Site) Performance

Strict adherence to the protocol is expected of all centers, and is monitored by the DMC, the Executive Committee and the Study Group. Data quality and the completeness of data retrieval are closely monitored on an ongoing basis by the WH-CSPCC. The Study Biostatistician presents interim monitoring reports, overall and by site, to the Executive Committee and the DMC that include the following types of information: recruitment of participants, characteristics of the population, completeness of data retrieval, and data quality. If an item is identified as an outlier in terms of data quality, a site conference call or site visit is initiated to assess the reasons that problems are occurring and how they can be corrected.

E. Monitoring Participant Safety

The Study Co-Chairs and/or Site Investigators are responsible for following AE reporting requirements. These responsibilities include: 1) reviewing the accuracy and completeness of all AEs reported, 2) compliance with CSP, IRB, and local policies for reporting AEs and/or SAEs, and 3) closely monitoring research participants for any new AEs or SAEs. Study participants are monitored remotely using a combination of in-person and automated telephone calls, and via periodic monitoring of the medical record during the 6 month follow-up period. Participants will also be asked to contact study personnel regarding all SAEs and any non-serious AEs which occur within 14 days of or are potentially related to study treatment.

F. Minimizing Attrition

As a primary way to minimize withdrawal from the study, study personnel provide thorough pre-enrollment education for all prospective participants about the study objectives and procedures in order to assess and confirm the participants' commitment to and feasibility for long-term follow-up. Study personnel also provide ongoing education and feedback during the study to reinforce the participants' commitment to long-term follow-up. If participants do opt to withdraw, partial (allowing medical record review) or full withdrawal will be discussed.

To compensate participants for the time and effort required to conduct follow-up calls, compensation will be provided using the following schedule: $100 at enrollment, $50 at day 56, and $100 at 6 months (study completion). All follow-up will be conducted centrally, via telephone, minimizing participant burden. All participants will be compensated for the completion of 1st study visit regardless of

whether they continued in the study. A relatively modest payment was selected to minimize the risk of financial coercion.

XVI. Data Management Procedures

A. Data Collection Methods

1. Data Capture

Data for CSP #2004 will be managed by the West Haven Cooperative Studies Program Coordinating Center (WH-CSPCC) and will utilize several technologies to manage data collection. The first is iDatafax, a web-based Electronic Data Capture (eDC) system supports both faxing and eDC components for data collection. The system uses a data entry component eDC and intelligent character recognition (ICR) for faxes. The iDataFax application also provides a data management structure where source documents associated with CRFs can be maintained for each participant within the Participant Study folder. All the CRFs (1, 2, 4 - 18) utilize iDataFax version 2016 eDC application.

The second is Teleform, a paper-based forms processing application utilizing optical character recognition (OCR) for verifying Case Report Forms (CRFs). Teleform version 11.2 design capabilities are used for the development of Case Report Forms (CRFs) as well as used for capturing personal health information (PHI) data (Informed Consent, HIPAA, Participant Contact Information-Form 3). Teleform application will be used for data capture of (PHI) which will be faxed to the WH-CSPCC fax server as soon as it is completed. Once the CRFs are received at the WH-CSPCC, they will be processed through the Teleform reader and verifier software where a Research Associate will review the CRFs for consistency and completeness. CRFs processed by way of Teleform verifier will be exported to an image file folder, and the extracted data items will be exported to a comma delimited file in a data capture folder, both on a secure WH-CSPCC file server. All changes or corrections to data entered on paper CRFs will be dated and initialed by site personnel on the original CRF and the associated data edit sheet if applicable.

All CRFs (not containing PHI) will either be entered into eDC component or faxed to iDataFax system. When data is entered into the eDC and saved, the system records the information into the study database. Through iDataFax, paper CRFs can also be faxed from study personnel. The faxed information will be delivered to the WH-CSPCC as data images directly into a folder system where the images are stored as fax files. Once the faxed CRF is received at the WH-CSPCC, the data image created by the iDataFax software is verified with the data recorded on the CRF and is saved into the study database.

iDataFax will store, display, and be used to manage CRF data collected via paper as well as data collected via eDC. iDataFax will be set up to provide direct feedback on the completeness and accuracy of data as it is being entered. Range checks for all data fields will identify invalid and extreme outlier values. Missing data or data values that exceed the range checks will be flagged when the data is entered into the system. If the data are corrected and the value appears within the acceptable range, then the error flag will disappear. Other integrity checks will be conducted to identify form completion errors and logically inconsistent data values within forms or between forms. Most of these checks will be programmed into the iDataFax system to automatically fire when data is entered, but others will be added manually into the system by the study programmer at the WH-CSPCC. If data are not corrected prior to CRF submission, the flag will turn into an automatic data query and will remain outstanding until addressed by personnel and/or the study team. Queries should be handled promptly with careful attention given to resolving all outstanding outlier, missing, or inconsistent data values. Reasons for any temporarily or permanently missing data should be noted in the appropriate query reply field.

CRF data collection within iDatafax application will be saved on a server in CSP Palo Alto center. Databases on this server are considered the official database of record and should maintain tight access control. Accessing the system requires a VA intranet connection and a browser; the eDC system is accessible via each site's study sub-site on SharePoint. The CRF data will be exported from iDatafax server in Palo Alto to SQL database server located in Martinsburg, VA. The data stored within the SQL database is parsed into SQL views. The WH-CSPCC will be able to access each CRF data within the SQL database by way of SQL view. Data will be extracted from the views and transferred into SAS datasets downloaded to WH-CSPCC's UNIX server as the final study database.

Data summaries will be prepared by the WH-CSPCC and forwarded to the Study Co-Chairs and site personnel on a monthly basis. For monitoring purposes, reports of site performance in recruitment, treatment compliance, follow-up, and risk based monitoring (RBM) tables will be generated monthly.

B. Data Quality Control

During study start-up, the CRFs will be field-tested using eDC data enter, data capture and fax-based system. Communication between WH-CSPCC, CSPCRPCC, the Study sites, and the central database will be tested.

On a weekly basis, or more frequently, programmers at WH-CSPCC will transfer the cumulative data in the central database to SAS datasets on the WH-CSPCC UNIX server. SAS programs will be run to generate reports that summarize the accumulated study data and data exceptions. These notices may request further completion, correction, or verification of specific data items. A computerized record will be kept of the number and types of errors to ensure a high level of data integrity. Interim progress reports of cumulative errors and overall data quality will be sent to the Site Investigators, the Executive Committee, and the DMC. Unresolved data queries will be included in the datasets that will be used in interim reports. However, every effort will be made to resolve all outstanding Data Correction Forms (DCFs) prior to a DMC report.

Data files on the central study database containing the accumulated participant information will be examined for completeness and consistency at regular intervals. Tested and validated computer programs will check newly entered forms for missing or out-of-range values. Computer-generated notices will be mailed to participating investigators requesting completion of forms and follow-up on DCFs for correction or verification of specific data items. A computerized record of the types of errors will be kept in order to ensure a high level of data integrity.

At periodic intervals, a cumulative record of errors and data quality progress reports will be sent to Site Investigators and the Study Co-Chairs. Data edits and removal of duplicate records will be applied to the data files on a regular basis, and cleaned (final) files through the time of the most recent running of data edits will be created. These final files will be used to run monitoring reports on a regular basis. The progress of data collection will be monitored with computerized data form inventory programs that will produce a profile of all forms expected and received for each study participant. Missing-form reports will be generated and sent to the sites periodically during the enrollment phase of the study.

Data quality will be monitored on an ongoing basis by the WH-CSPCC. The Study Biostatistician will present interim monitoring reports to the Executive Committee at least monthly and to the DMC at least annually. Interim reports will include recruitment of participants, characteristics of the population, completeness of data retrieval, and data quality.

C. Electronic Study File

CSP has established a Clinical Trials Management System (CTMS). This system is hosted on a server at a secure VA regional data facility and is based in a MS SharePoint platform. An MS SharePoint

site or similar CTMS will be used for maintaining an electronic version of the Central Study File for this study. Participating medical centers will be able to access current and past versions of the Study Protocol, Operations Manual, Operations Memoranda and other work instructions, CRFs, and other study-related documentation, as well as meeting announcements, conference call notices, and study newsletters using the CTMS.

D. Quality Control of the Process

The Study Co-Chairs and the WH-CSPCC will prepare an Operations Manual that will be provided to the Site Investigators as a guide to the operation and management of the study as well as a technical reference manual. A training session will be held at the study kick-off meeting for all study personnel in order to: (1) assure uniformity in participant management and data collection procedures, and (2) train the personnel in study procedures and criteria.

Study procedures will be reinforced by the use of regular conference calls, particularly in the first few months of the study. All study personnel will attend group meetings during the enrollment period when study procedures again will be discussed in detail. The Study Co-Chairs' Office and the WH-CSPCC study personnel will be available to clarify study procedures by telephone, fax and e-mail.

If the Executive Committee determines that a procedure must be changed, the participating sites will be informed by conference call and an operations memo, and an updated section of the Operations Manual pertinent to the changed procedure will be provided to all sites.

E. Data Security

CSP has a commitment to maintaining data security and patient privacy. Standard practices and policies as part of the responsible conduct of clinical research studies are implemented and reviewed periodically. CSP Center Directors are responsible for ensuring that all CSP Data Security Policies are enforced within their Centers. All study data collected will be handled, maintained and stored according to CSP standard practices and policies. This includes but is not limited to the following:

- Protected Health Information (PHI) as defined by HIPAA will not be used for any purpose that is not related to the activities of this study
- Records are identified only by a participant identification number
    - Patient identification numbers are not derived from or related to information about an individual

    - All electronic PHIs are stored on secure servers and may not be moved to a PC or other external device
    - Paper CRFs, if any, are stored in locked file cabinets and rooms
- When necessary, PHI may be transported between secure servers. PHI must be encrypted and password protected while being transferred using a FIPS 140-2 certified program. Any removable storage device used to transfer PHI (e.g., hard-copy printouts, data tapes, encrypted CDs, encrypted USB drives, etc.) should either be destroyed after transfer is complete or given to the Data Security Administrator to be secured in a secure, fireproof safe. A trackable mail system must be used for the physical data transfers
- Data from studies are utilized at WH-CSPCC and are not removed from the Center
- No PHI may be sent via MS Outlook or Exchange unless the message is secured utilizing encryption and VA-authorized security protocol
- Documents sent for medical evaluation purposes (e.g., endpoint adjudication) are sent via trackable express mail or password protected email. Personal information is redacted by the VAMC or WH-CSPCC if not determined to be necessary for completing the evaluation
- Only VA-owned equipment or equipment configured to VA security standards is permitted to directly connect to the CSP networks in accordance with VA Directive 6504
- Training, reminders, and signed data security statements are used to ensure CSP personnel understand VA policies
- Sharing of CSP study data outside of CSP requires the approval of the Director, CSR&D, and data use agreements. In addition, sharing of data outside of the VA requires local ISO, PO, and ACOS-R approvals.

Any data capture system used for data collection in this study will be fully compliant with US Federal regulations regarding electronic web-based data capture systems established by the FDA under 21 CFR 11. Data entered directly into the central database provides the official clinical record for data collection. Source documentation is handled in the same manner as a paper based system. All paper-based records will be kept under lock and key.

The electronic data capture system will utilize state-of-the-art technologies that meet or exceed the current VA standards for transport in order to protect the data during transmission. In brief, electronic systems will employ secure socket layer technology and FIPS 140-2 compliant encryption algorithms to ensure that data is not vulnerable during transport. Hard copy data will be sent via a traceable mail system (i.e., UPS), via a courier, or via secure fax.

Access to the study data will be afforded the same level of security as all forms of VA protected and/or highly sensitive information. Access is heavily restricted to individuals with CSP approval to access the data. Individuals must be properly credentialed research staff and must be compliant with VA security trainings (i.e., Research Data Security, HIPAA and VA Privacy Training, Cyber-Security, and Good Clinical Practices). In addition, research data will be stored on VA secure servers with restricted permissions for copying and exporting data. Only properly approved Coordinating Center personnel will have the ability to copy and export data. These individuals have received training on the local SOPs governing their permissions and will not access or export data without written approval from the Coordinating Center Director. Furthermore, the permissions of the electronic systems are structured such that individual sites can only see the data for their study participants, and they cannot see or access the data for another clinical site or for another participant.

Backup copies of the database will be transferred to the WH-CSPCC behind the VA firewall on a frequent basis depending on the study need. These backup copies will be transferred and stored across secure connections according to VA regulations and WH-CSPCC operating procedures. Periodic off-site back-ups will be made as part of a comprehensive disaster recovery plan.

F. Data Sharing

Upon final analyses of the stated objectives in this proposal, the study plans to submit results for publication in scientific peer-reviewed journals and provide summary results on clinicaltrials.gov. After acceptance of the primary and other stated analyses by a journal, CSP will make these publication(s) available via the National Library of Medicine's PubMed Central within a year of the date of publication.

Digital data underlying primary scientific publications from this study will be held as part of a data sharing resource maintained by the Cooperative Studies Program (CSP). Study data held for this purpose may include data, data content, format, and organization. The data may contain but are not limited to individually identifiable information, other protected health information, and study codes. The data may be available to the public and other VA and non-VA researchers under certain conditions and consistent with the informed consent and CSP policy which prioritize protecting subjects' privacy and confidentiality to the fullest extent possible. A detailed plan for data sharing will be developed in accordance with current technology, infrastructure, best practices, and policies and procedures in place at the time of oversight committee reviews (e.g. Privacy Board, IRB, Information Security and IT standards). The plan will include how data will be discovered, retrieved, and analyzed, managed and will

note the materials that are available in machine readable formats. This plan may be revised to ensure consistency with VA, including CSP policies and standards for overall management and sharing.

< This page left intentionally blank >

XVII. Good Clinical Practices

A. Role of Good Clinical Practices

This trial will be conducted in compliance with Good Clinical Practice (GCP) regulations. The intent of these regulations is to safeguard particpants' welfare and assure the validity of data resulting from the clinical research. The VA Cooperative Studies Program will assist Site Investigators (SIs) in complying with GCP requirements through its Site Monitoring, Auditing, and Resource Team (SMART) based in Albuquerque, NM. SMART serves as the Quality Assurance arm of CSP for GCP compliance. Study site personnel will receive GCP orientation at the study organizational meeting. SMART will provide training, manuals and materials to assist study personnel in organizing study files, and will be available throughout the trial to advise and assist SIs regarding GCP issues.

B. Summary of Monitoring and Auditing Plans

Monitoring Visits

- Initiation visits at each site soon after sites randomize their first few participants.
- Yearly routine monitoring visits to each site.
- Final monitoring visit to each site during the last year of the study.
- Additional monitoring visits may be conducted as deemed necessary by study leadership or SMART

Audits

- Routine audits – independent site visits to one or more sites per year as determined by SMART.
- For-cause audits – independent audit of a site as requested by study leadership or CSPCO.
- Audits may be scheduled or unannounced.

< This page left intentionally blank >

XVIII. Publications

A. Publication Policy

According to the policy of the Cooperative Studies Program, outcome data will not be revealed to the Study Co-Chairs or Site Investigators until data collection is completed and the study database is locked. This policy safeguards against possible biases affecting the data collection.

All presentations and publications from this study will follow CSP policy as stated in the CSP Guidelines. The presentation or publication of any or all data collected by Site Investigators on participants of CSP #2004 is under the direct control of the Executive Committee. No individual Site Investigator has the right to perform analyses, make interpretations, make public presentations, or seek publication of any or all of the data without the approval of the Executive Committee. This is true whether the publication or presentation is concerned with the results of the principal undertaking or is associated with the study in some other way.

The Executive Committee has the authority to establish one or more publication committees (usually comprised of subgroups of Site Investigators and some members of the Executive Committee) for the purpose of producing manuscripts for presentation and publication. A presentation or publication, formulated by the Executive Committee or its authorized representatives, should be circulated to all members of the Executive Committee for review, comments, and suggestions prior to submission of the manuscript to the presenting or publishing body.

All publications must give proper recognition to the Department of Veterans Affairs Cooperative Studies Program and should list or reference all principal and Site Investigators in the study. Any manuscript, abstract, or letter to the editor submitted for publication or presentation must be sent to the CSP Director for approval prior to submission for publication. Primary manuscripts will also be presented to the members of the study's DMC for information purposes.

B. Planned Publications

An intended plan of the main publications is given below:

Table 10. Intended study publication plan

Manuscript	Projected time of submission
Study design and baseline patient characteristics	6 months after enrollment is completed
Primary efficacy analysis	6-12 months after end of study
Secondary and exploratory outcomes, including safety outcomes	12-18 months after end of study

XIX. REFERENCES

1. Miller BA, Chen LF, Sexton DJ, Anderson DJ**.** Comparison of the burdens of hospital-onset, healthcare facility-associated *Clostridium difficile* Infection and of healthcare-associated infection due to methicillin-resistant Staphylococcus aureus in community hospitals. Infect Control Hosp Epidemiol. 2011;32(4):387-90.
2. Lessa FC, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, Dunn JR, et al. Burden of *Clostridium difficile* Infection in the United States. N Engl J Med. 2015;372(9):825-34.
3. Loo VG, Poirier L, Miller MA, Oughton M, Libman MD, Michaud S, et al. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. N Engl J Med. 2005;353(23):2442-9.
4. Dubberke ER, Olsen MA**.** Burden of *Clostridium difficile* on the healthcare system. Clin Infect Dis. 2012;55 Suppl 2:S88-92.
5. Evans ME, Simbartl LA, Kralovic SM, Jain R, Roselle GA**.** *Clostridium difficile* infections in Veterans Health Administration acute care facilities. Infect Control Hosp Epidemiol. 2014;35(8):1037-42.
6. Cameron RG, Binmoeller KF, Shah JN, Hashimoto Y, Kane S, Bhat YM**.** Efficacy of eradication of non-nodular barrett's after mucosectomy in patients with high-grade dysplasia using a CO2-based cryoablation system: An initial experience in a us patient cohort. Gastrointestinal Endoscopy. 2012;75(4):AB466-AB7.
7. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, et al. Clinical Practice Guidelines for *Clostridium difficile* Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis. 2018;66(7):e1-e48.
8. Johnson S, Louie TJ, Gerding DN, Cornely OA, Chasan-Taber S, Fitts D, et al. Vancomycin, metronidazole, or tolevamer for *Clostridium difficile* infection: results from two multinational, randomized, controlled trials. Clin Infect Dis. 2014;59(3):345-54.
9. Louie TJ, Miller MA, Mullane KM, Weiss K, Lentnek A, Golan Y, et al. Fidaxomicin versus Vancomycin for *Clostridium difficile* Infection. N Engl J Med. 2011.
10. Cornely OA, Crook DW, Esposito R, Poirier A, Somero MS, Weiss K, et al. Fidaxomicin versus vancomycin for infection with *Clostridium difficile* in Europe, Canada, and the USA: a double-blind, non-inferiority, randomised controlled trial. Lancet Infect Dis. 2012;12(4):281-9.

11. McDonald LC, Coignard B, Dubberke E, Song X, Horan T, Kutty PK**.** Recommendations for surveillance of *Clostridium difficile*-associated disease. Infect Control Hosp Epidemiol. 2007;28(2):140-5.
12. Cornely OA, Miller MA, Louie TJ, Crook DW, Gorbach SL**.** Treatment of first recurrence of *Clostridium difficile* infection: fidaxomicin versus vancomycin. Clin Infect Dis. 2012;55 Suppl 2:S154-61.
13. Zar FA, Bakkanagari SR, Moorthi KM, Davis MB**.** A comparison of vancomycin and metronidazole for the treatment of *Clostridium difficile*-associated diarrhea, stratified by disease severity. Clin Infect Dis. 2007;45(3):302-7.
14. Johnson S**.** Recurrent *Clostridium difficile* infection: a review of risk factors, treatments, and outcomes. J Infect. 2009;58(6):403-10.
15. Debast SB, Bauer MP, Kuijper EJ**.** European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for *Clostridium difficile* infection. Clin Microbiol Infect. 2014;20 Suppl 2:1-26.
16. Surawicz CM, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, et al. Guidelines for diagnosis, treatment, and prevention of *Clostridium difficile* infections. Am J Gastroenterol. 2013;108(4):478-98; quiz 99.
17. Eiseman B, Silen W, Bascom GS, Kauvar AJ**.** Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. Surgery. 1958;44(5):854-9.
18. Bakken JS, Borody T, Brandt LJ, Brill JV, Demarco DC, Franzos MA, et al. Treating *Clostridium difficile* infection with fecal microbiota transplantation. Clin Gastroenterol Hepatol. 2011;9(12):1044-9.
19. Youngster I, Russell GH, Pindar C, Ziv-Baran T, Sauk J, Hohmann EL**.** Oral, capsulized, frozen fecal microbiota transplantation for relapsing *Clostridium difficile* infection. JAMA. 2014;312(17):1772-8.
20. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. N Engl J Med. 2013;368(5):407-15.
21. Drekonja DM, Reich J, Gezahegn S, Greer N, Shaukat A, MacDonald R, et al. Fecal Microbiota Transplantation for *Clostridium difficile* Infection – A Systematic Review. Ann Intern Med. 2015;In press.
22. Zipursky JS, Sidorsky TI, Freedman CA, Sidorsky MN, Kirkland KB**.** Patient Attitudes Toward the Use of Fecal Microbiota Transplantation in the Treatment of Recurrent *Clostridium difficile* Infection. Clin Infect Dis. 2012;55(12):1652-8.

23. Goodman C, O'Rourke N, Amundson C, Drekonja D**.** Patient Knowledge and Attitudes About Fecal Microbiota Therapy for *Clostridium difficile* Infection. Federal Practitioner. 2017;34(1):17-21.
24. Chang JY, Antonopoulos DA, Kalra A, Tonelli A, Khalife WT, Schmidt TM, et al. Decreased diversity of the fecal Microbiome in recurrent *Clostridium difficile*-associated diarrhea. J Infect Dis. 2008;197(3):435-8.
25. Eypasch E, Williams JI, Wood-Dauphinee S, Ure BM, Schmulling C, Neugebauer E, et al. Gastrointestinal Quality of Life Index: development, validation and application of a new instrument. Br J Surg. 1995;82(2):216-22.
26. McDonald LC, Killgore GE, Thompson A, Owens RC, Jr., Kazakova SV, Sambol SP, et al. An epidemic, toxin gene-variant strain of *Clostridium difficile*. N Engl J Med. 2005;353(23):2433-41.
27. Muto CA, Pokrywka M, Shutt K, Mendelsohn AB, Nouri K, Posey K, et al. A large outbreak of *Clostridium difficile*-associated disease with an unexpected proportion of deaths and colectomies at a teaching hospital following increased fluoroquinolone use. Infect Control Hosp Epidemiol. 2005;26(3):273-80.
28. Campbell RJ, Giljahn L, Machesky K, Cibulskas-White K, Lane LM, Porter K, et al. *Clostridium difficile* infection in Ohio hospitals and nursing homes during 2006. Infect Control Hosp Epidemiol. 2009;30(6):526-33.
29. Buchner AM, Sonnenberg A**.** Epidemiology of *Clostridium difficile* infection in a large population of hospitalized US military veterans. Dig Dis Sci. 2002;47(1):201-7.
30. Hall AJ, Curns AT, McDonald LC, Parashar UD, Lopman BA**.** The roles of *Clostridium difficile* and norovirus among gastroenteritis-associated deaths in the United States, 1999-2007. Clin Infect Dis. 2012;55(2):216-23.
31. Kelly CR, Ihunnah C, Fischer M, Khoruts A, Surawicz C, Afzali A, et al. Fecal microbiota transplant for treatment of *Clostridium difficile* infection in immunocompromised patients. Am J Gastroenterol. 2014;109(7):1065-71.
32. Solari PR, Fairchild PG, Noa LJ, Wallace MR**.** Tempered enthusiasm for fecal transplant. Clin Infect Dis. 2014;59(2):319.
33. Mandalia A, Kraft CS, Dhere T**.** Diverticulitis after fecal microbiota transplant for *C. difficile* infection. Am J Gastroenterol. 2014;109(12):1956-7.
34. Schwartz M, Gluck M, Koon S**.** Norovirus gastroenteritis after fecal microbiota transplantation for treatment of *Clostridium difficile* infection despite asymptomatic donors and lack of sick contacts. Am J Gastroenterol. 2013;108(8):1367.

35. Kleger A, Schnell J, Essig A, Wagner M, Bommer M, Seufferlein T, et al. Fecal transplant in refractory *Clostridium difficile* colitis. Dtsch Arztebl Int. 2013;110(7):108-15.
36. De Leon LM, Watson JB, Kelly CR**.** Transient flare of ulcerative colitis after fecal microbiota transplantation for recurrent *Clostridium difficile* infection. Clin Gastroenterol Hepatol. 2013;11(8):1036-8.
37. Alang N, Kelly CR**.** Weight gain after fecal microbiota transplantation. Open Forum Infect Dis. 2015;2(1):ofv004.
38. Didesch MM, Averill A, Oh-Park M**.** Peripheral Neuropathy after fecalmicrobiota transplatation for *Clostridium difficle* infection: a case report. P M&R. 2016;doi: 10.1016/j.pmrj.2016.01.009.
39. Sethi AK, Al-Nassir WN, Nerandzic MM, Bobulsky GS, Donskey CJ**.** Persistence of skin contamination and environmental shedding of *Clostridium difficile* during and after treatment of *C. difficile* infection. Infect Control Hosp Epidemiol. 2010;31(1):21-7.
40. Jinno S, Kundrapu S, Guerrero DM, Jury LA, Nerandzic MM, Donskey CJ**.** Potential for transmission of *Clostridium difficile* by asymptomatic acute care patients and long-term care facility residents with prior *C. difficile* infection. Infect Control Hosp Epidemiol. 2012;33(6):638-9.
41. Polage CR, Gyorke CE, Kennedy MA, Leslie JL, Chin DL, Wang S, et al. Overdiagnosis of *Clostridium difficile* Infection in the Molecular Test Era. JAMA Intern Med. 2015;175(11):1792-801.
42. Pakyz AL, Carroll NV, Harpe SE, Oinonen M, Polk RE**.** Increase in use of vancomycin for *Clostridium difficile* infection in US hospitals. Infect Control Hosp Epidemiol. 2010;31(8):867-8.
43. Surawicz CM, McFarland LV, Greenberg RN, Rubin M, Fekety R, Mulligan ME, et al. The search for a better treatment for recurrent *Clostridium difficile* disease: use of high-dose vancomycin combined with Saccharomyces boulardii. Clin Infect Dis. 2000;31(4):1012-7.
44. Youngster I, Sauk J, Pindar C, Wilson RG, Kaplan JL, Smith MB, et al. Fecal Microbiota Transplant for Relapsing *Clostridium difficile* Infection Using a Frozen Inoculum From Unrelated Donors: A Randomized, Open-Label, Controlled Pilot Study. Clin Infect Dis. 2014;58(11):1515-22.
45. Kao D, Roach B, Silva M, Beck P, Rioux K, Kaplan GG, et al. Effect of Oral Capsule- vs Colonoscopy-Delivered Fecal Microbiota Transplantation on Recurrent *Clostridium difficile* Infection: A Randomized Clinical Trial. JAMA. 2017;318(20):1985-93.
46. Tedesco FJ, Gordon D, Fortson WC**.** Approach to patients with multiple relapses of antibiotic-associated pseudomembranous colitis. Am J Gastroenterol. 1985;80(11):867-8.

47. McFarland LV, Elmer GW, Surawicz CM**.** Breaking the cycle: treatment strategies for 163 cases of recurrent *Clostridium difficile* disease. Am J Gastroenterol. 2002;97(7):1769-75.
48. Johnson S, Schriever C, Galang M, Kelly CP, Gerding DN**.** Interruption of recurrent *Clostridium difficile*-associated diarrhea episodes by serial therapy with vancomycin and rifaximin. Clin Infect Dis. 2007;44(6):846-8.
49. Pepin J, Routhier S, Gagnon S, Brazeau I**.** Management and outcomes of a first recurrence of *Clostridium difficile*-associated disease in Quebec, Canada. Clin Infect Dis. 2006;42(6):758-64.
50. Fleiss J**.** Statistical Methids for Rates and Proportions. New York: John Wiley & Sons, inc.; 1981.
51. Haybittle JL**.** Repeated assessment of results in clinical trials of cancer treatment. Br J Radiol. 1971;44(526):793-7.
52. Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. I. Introduction and design. Br J Cancer. 1976;34(6):585-612.
53. Proschan M, KK L, eds. Statistical Monitoring of Clinical Trails. A Unified Approach. New York: Springer-Verlag.
54. Important Safety Alert Regarding Use of Fecal Microbiota for Transplantation and Risk of Serious Adverse Reactions Due to Transmission of Multi-Drug Resistant Organisms. Reported 6/13/19 by the US Food and Drug Administration. https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/important-safety-alert-regarding-use-fecal-microbiota-transplantation-and-risk-serious-adverse

< This page left intentionally blank >