

Enteric Pathogens Diagnostic Laboratory
University of Texas School of Public Health
EPDL-UT-SPH-FMT-001
Clinical Study Protocol: UT-SPH-FMT-0001

**Fresh, Frozen or Lyophilized Fecal Microbiota Transplantation for
Multiple Recurrent C. Difficile Associated Diarrhea**

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Clinical Study Protocol
A STUDY OF FECAL MICROBIOTA TRANSPLANTATION FOR THE TREATMENT OF RECURRENT C. DIFFICILE ASSOCIATED DIARRHEA (CDAD)

Study Number: UT-SPH-FMT-0001

Study Phase 1

Investigational Product

Fresh Healthy Donor Fecal Bacteria

Frozen Healthy Donor Fecal Bacteria

Lyophilized Healthy Donor Fecal Bacteria

Sponsor

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SYNOPSIS

TITLE	A study of fecal microbiota transplantation (FMT) for the treatment of recurrent <i>C. difficile</i> associated diarrhea (CDAD)
PROTOCOL	UT-SPH-FMT-0001
SETTINGS	Attending physicians of recipients/patients will recommend the FMT and make clinical decisions related to overall health of his/her patients. All patients undergoing therapy will be handled as outpatients at either Baylor St. Luke's Medical Center, the Digestive Disease Center at Memorial Hermann, or Kelsey-Seybold Clinic – Main Campus on Holcombe Blvd.
OBJECTIVE	To evaluate the safety of FMT (fresh, frozen or lyophilized health donor feces) for the treatment of patients with 3 or more recurrent <i>C. difficile</i> associated diarrhea (CDAD)
STUDY DESIGN	Allocation: Randomized Endpoint Classification: Safety Study Intervention Model: Parallel Assignment Masking: Open Label Primary Purpose: Treatment
INCLUSION CRITERIA - RECIPIENT	All recipients <ol style="list-style-type: none"> 1. Male and female patients \geq 18 years of age 2. Sexually active male and female patients of child-bearing potential must agree to use an effective method of birth control during the treatment and follow-up period 3. Female patients of child-bearing potential must have a negative pregnancy test in the 72 hours before the procedure 4. Required to sign an informed consent form 5. Deemed likely to survive for \geq 3 months after enrolment 6. Diagnosis of \geq 3 recurrent CDAD (RCDAD) bouts in outpatients or \geq 2 bouts of CDAD in an inpatient without other explanation for diarrhea and with \geq 2 positive fecal tests for <i>C. difficile</i> toxin 7. Referred by subjects attending physician who will provide non-transplant care for the subject 8. Received at least one course of adequate antibiotic therapy for CDAD (\geq 10 days of vancomycin at a dose of \geq125 mg four times per day, \geq 10 days of metronidazole at a dose of 500mg three times per day or fidaxomixin 200mg twice a day for 10 days) 9. Anti-CDI antibiotic treatment or probiotics stopped approximately 2 days before the transplantation
INCLUSION CRITERIA - DONOR	<ol style="list-style-type: none"> 1. Able to provide and sign informed consent. 2. Able to complete and sign the donor questionnaire 3. Able to adhere to fecal transplantation stool collection requirements
EXCLUSION CRITERIA - RECIPIENT	<ol style="list-style-type: none"> 1. Patients with neutropenia with absolute neutrophil count $<0.5 \times 10^9/L$ 2. Evidence of toxic megacolon or gastrointestinal perforation on abdominal x-ray 3. Peripheral white blood cell count $> 15.0 \times 10^9/L$ AND temperature $> 38.0^{\circ}C$ 4. Active gastroenteritis due to <i>Salmonella</i>, <i>Shigella</i>, <i>E. coli</i> 0157:H7, <i>Yersinia</i> or

	<p><i>Campylobacter, and Norovirus</i></p> <ol style="list-style-type: none"> 5. Presence of colostomy 6. Unable to tolerate HBT for any reason 7. Requiring systemic antibiotic therapy for more than 7 days 8. Actively taking <i>Saccharomyces boulardii</i> or other probiotic 9. Severe underlying disease such that the patient is not expected to survive for more than 3 months or unstable medical condition requiring daily change in treatments.
EXCLUSION CRITERIA - DONOR	<ol style="list-style-type: none"> 1. Test positive for any of variables 2. History of any type of active cancer or autoimmune disease 3. History of risk factors for acquisition of HIV, syphilis, Hepatitis B, Hepatitis C, prion or any neurological disease as determined by the donor questionnaire 4. History of gastrointestinal disorder, e.g., inflammatory bowel disease, irritable bowel syndrome, chronic constipation or diarrhea 5. Antibiotic use or any systemic immunosuppressive agents in the 3 months prior to stool donation 6. Receipt of any type of live vaccine within 3 months prior to stool donation 7. Current or previous medical or psychosocial condition 8. Body mass index over 30
SCREENING OF DONOR	<p>Stool will be tested for the following enteric pathogens:</p> <ol style="list-style-type: none"> 1. <i>Shigella</i> 2. <i>Salmonella</i> 3. <i>Campylobacter</i> 4. Shiga-toxin Producing <i>Escherichia coli</i> 5. <i>Clostridium difficile</i> Toxin A/B 6. Parasites (<i>Cryptosporidium</i>, <i>Entamoeba histolytica</i>, <i>Giardia</i>) only if the donor has travelled to developing regions in past month <p>Blood sample from donor will be tested for following items:</p> <ol style="list-style-type: none"> 1. Hepatitis B core antibody 2. Hepatitis B surface antigen 3. Hepatitis C virus antibody 4. HIV-1 and HIV-2 antibody 5. Anti-HTLV I/II 6. Serologic test for syphilis
DONOR STOOL PREPARATION	<p>Stool preparation will be started on the transplantation day:</p> <ol style="list-style-type: none"> 1. Donor stool will be used within 1 month after the screening process 2. Obtain \geq 150 grams of stool from a qualified donor requiring collection of multiple stools 3. Mix with 1500mL (1:10 dilution) sterilized 0.9% NaCl without antibacterial preservative in a large sterilized suction canister 4. Mix above solution in a homogenizer mixer with sterilized bag (Stomacher).

	<p>Initially, use the low setting until the sample breaks up, and then advance the speed gradually to the highest setting. Continue for 2 – 4 minutes until sample is smooth.</p> <ol style="list-style-type: none"> 5. Filter the suspension using a sterile paper coffee filter (or gauze) that has been moistened with saline and funnel (both are sterilized). Allow adequate time for slow filtration to end. NOTE: To expedite process, multiple funnel/filters can be used to combine product. 6. Repeat step 4. NOTE: it may take 2 hours to complete steps 4 and 5. 7. The resultant filtered suspension will be placed in sterilized 50 mL tubes 8. Number the samples as FMT-D-001-FRESH (5 tubes), FMT-D-001-FROZEN (5 tubes) and FMT-D-001-LYOPHILIZED (5 tubes) 9. Label the container with lot number (mm-dd-yyyy), donor ID, and date and time processed 10. Keep the samples - <ul style="list-style-type: none"> • FMT-D-001-FROZEN (5 tubes) sealed with plastic (polyethylene or similar film) at -80°C for up to 6 months in a test tube rack clearly labelled with the media lot # and expiration date • FMT-D-001-LYOPHILIZED (5 tubes) – <ul style="list-style-type: none"> ➢ Start lyophilisation within 30 minutes after completion of stool filtration ➢ Keep lyophilized samples at -80°C for up to 6 months in a test tube rack clearly labelled with the media lot # and expiration date • FMT-D-001-FRESH (5 tubes) transferred to Endoscopy procedure site immediately for the same day FMT. The fresh donor stool will be used for FMT within 8 hours after collection. 11. If necessary, repeat stool donors who are perceived to be safer than the first time donors. If the repeat donor is used within 1 month and passes the questionnaire survey, no blood or stool will be screened. If the donor has not been screened in the last month, the donor will repeat the medical history survey, and both blood and stool will be tested again for all tests listed under Screening of Donor Section above before donating again.
STUDY SCHEDULE	<p>Before FMT:</p> <ul style="list-style-type: none"> • Potential donors are screened at the CLIA certified laboratory • Eligible recipients stop other antibiotics and use oral vancomycin 125 mg four times a day for approximately 4 days before FMT which is stopped approximately 2 days before the procedure. • Informed consent (Patient Consent Form) is given to the patient along with (Patient Info Provided with Consent). • Patient completes daily diary – symptoms (Patient Data Collection Form) • Discuss and provide letter to patient (Patient Letter - Aftercare of FMT) • Send letter to patient's physician (Physician Letter - Before FMT). • Obtain stool specimen for <i>C. difficile</i> toxin test and an aliquot of stool frozen (-80°C) for future studies.

- Two donors will be contacted and informed about stool donation. We will provide all necessary supplies to the donors for stool collection the next day.
- Donor is encouraged to take one stool softener laxative the evening before the procedure and drink of plenty water.
- Recipients who will receive the FMT by colonoscopy will take a bowel lavage with 4 liters of colonoscopy prep the night before FMT (ordered by study gastroenterologist or subjects private MD)
- Recipients will complete Daily diary
- Obtain stool specimen for *C. difficile* toxin test and store an aliquot of stool at -80° C for future studies.

Day of FMT:

- Donor stool must be to the Enteric Pathogen Diagnostic Laboratory at the University of Texas School of Public Health (EPDL-UT-SPH) within two hours of passing.
- Donor's stool will be processed according to the protocol Section 5.2.3.1. The donor stool process will take 2-3 hours.
- Donor stool suspension (at least 250 mL after dilution of stool) will be delivered to the Endoscopy procedure site in a cooler with wet ice by an employee from EPDL-UT-SPH approximately thirty minutes before scheduled procedure time.
- Recipient will receive 250mL of filtered healthy donor stool (\geq 50 g stool) by colonoscopy
- Recipient completes daily diary (Patient Data Collection Form)
- After FMT procedure the recipient will be given instructions to manage mild diarrhea after FMT, what to do if diarrhea recurs, etc. (Patient letter - Aftercare of FMT)

The first week after FMT:

- Recipient will be contacted to determine health status
- Subjects will return to the clinic or be contacted via telephone/email
- Patient Completes daily diary – symptoms (Patient Data Collection Form)
- Investigator or study coordinator assesses compliance with the transplantation

Approximately seven days after transplantation (depending on their availability, weekends and holidays):

- Recipient will either return to the clinic or be contacted via telephone/email
- Daily diary will be completed by participant (Patient Data Collection Form) and returned
- Recipient will provide stool specimen for *C. difficile* toxin test and an aliquot of stool frozen (-80° C) for future studies.
- Assess compliance with study requirements

	<p>Approximately fourteen Days after transplantation (depending on their availability, weekends and holidays):</p> <ul style="list-style-type: none"> • Participant will either return to the clinic or be contacted via telephone/email • Daily diary will be completed (Patient Data Collection Form) • Recipient will provide stool specimen for <i>C. difficile</i> toxin test and an aliquot of stool frozen (-80°C) for future studies. <p>Approximately thirty days after transplantation (depending on their availability, weekends and holidays):</p> <ul style="list-style-type: none"> • Participant will either return to the clinic or be contacted via telephone/email • Daily diary will be completed (Patient Data Collection Form) • Recipient will provide stool specimen for <i>C. difficile</i> toxin test and an aliquot of stool frozen (-80°C) for future studies. • A letter to patient's physician (Physician letter – FMT Complete)
ASSESSMENTS	<p>Demographic Data/History of <i>C. difficile</i> Infection</p> <p>Demographic data (gender, race, date of birth, medical history, medication in the past 6 months, onset of diarrhea and signs or symptoms of gastrointestinal tract, any <i>C. difficile</i> toxin test performed in the past 6 months) will be recorded approximately 4 days before transplantation.</p> <p>Clinical Laboratory Tests</p> <p>Stool samples will be collected for <i>C. difficile</i> toxin tests less than 10 days before FMT and approximately 7, 14 and 30 days after FMT (depending on their availability, weekends and holidays).</p> <p>Patient Diaries</p> <p>Each recipient will receive a patient diary card at time of enrolment. Diary card will be collected at the approximate time intervals: -4 day, -1 day, day 0, day 1, day 7, day 14 and day 30 after the procedure with day 0 being the date of transplantation (depending on their availability, weekends and holidays). Recipient will record the following parameters daily:</p> <ul style="list-style-type: none"> • Date, time and consistency of each stool • Presence or absence and severity of signs or symptoms of enteric infection, including fever, gas, nausea, abdominal cramp/pain, tenesmus) in each 24 hours • Date, time and description of adverse event • Date, time, name and dose of any other medication administered
DURATION OF FOLLOWUP	<p>The duration of study participation for each patient is approximately 34 days (4 days before and 30 days after transplantation) depending on their availability, weekends and holidays. We will attempt to follow up subjects on or about 6 months after FMT in order to monitor any health effects for safety.</p>

STUDY POPULATION	<p><u>Recipient:</u> 100 adult male or female patients with ≥ 3 RCDAD as outpatients or ≥ 2 RCDAD while in the hospital having completed a course of vancomycin or fidaxomicin.</p> <p><u>Donor:</u> 20 potential donors will be identified to provide stools of ≥ 50 g each (total ≥ 150 g).</p>
STUDY AGENT, DOSE & MODE OF ADMINISTRATION	Filtered feces (250mL, either Fresh or Frozen or Lyophilized sample) from a screened healthy donor will be administered by colonoscopy
EFFICACY EVALUATION	<ul style="list-style-type: none">Primary Endpoint: Safety of fecal transplantation (Fresh or Frozen or Lyophilized)Secondary Endpoint: Failure to have a subsequent bout of CDAD in 30 days post FMT
SAFETY EVALUATION	Safety will be assessed by evaluating any unfavorable or unintended sign, symptom, or disease temporally associated with FMT procedure will be recorded as adverse experience. We will also collect information about the frequency and/or severity of the signs or symptoms of enteric infection (defined as fever, moderate to severe gas/flatulence, nausea, vomiting, abdominal cramps or pain, rectal tenesmus, or defecation urgency). We will attempt to follow subjects on or about 6 months after FMT to obtain information about recurrence or any adverse effects.

1. ETHICS

The study is reviewed, approved and conducted in accordance with regulations mandated by the University of Texas Health Science Center at Houston institutional review board. In addition, the study will be conducted with the College of American Pathologists standards that meet regulations relating to Good Clinical Practice (GCP). These standards respect the following guidelines:

1. GCP: Consolidated Guideline (International Conference on Harmonization [ICH] of Technical Requirements for the Registration of Pharmaceuticals for Human Use, May 1996).
2. United States (US) Code of Federal Regulations (CFR) dealing with clinical studies (21 CFR Parts 50, 54, 56, 312, 314, and 511) [1].
3. Declaration of Helsinki, concerning medical research in humans ("Recommendations Guiding Physicians in Biomedical Research Involving Human Patients," Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989 and revised version of Somerset West, Republic of South Africa, October, 1996, Note of Clarification added by the WMA [World Medical Association] General Assembly, Washington 2002, Note of Clarification added by the WMA General Assembly, Tokyo 2004).

2. INTRODUCTION

2.1 Background

In the United States and Canada there has been an alarming increase in the incidence and severity of CDAD in the last decade. It is now estimated that between 500,000 and 700,000 cases of CDAD occur in U.S hospital annually with an estimated hospital excess cost of care of about 3.2 billion dollars [2, 3]. With the increased risk of developing CDAD, high rates of disease recurrence are being seen without the availability of adequate treatment [4, 5]. Inflammatory Bowel Disease refractory to conventional treatment remains a major clinical problem [6, 7].

It is now accepted that the disruption of the normal balance of colonic microbiota secondary to antibiotic use facilitates the development of CDAD and improvements in the quality and quantity of flora is associated with recovery of infection and disease recurrence. Studies have shown that patients with recurrent CDAD have decreased anaerobic *bacteroidetes* and alteration of counts of *firmicutes* in their stool compared to patients recovering from single episodes of CDAD [8]. Numerous case reports and retrospective case series have demonstrated the benefit of fecal microbiota transplantation (FMT) in patients with severe or recurrent CDAD with cure rates over 90% [9-13]. FMT involves administration of a suspension of feces obtained from a healthy individual into the colon of a patient with recurrent CDAD to promote normalization of flora and inhibition of the infecting *C. difficile* [10]. Probiotics have been used widely for patients with CDAD in efforts to repair the disruption of microbiota leading to prevention and control of CDAD [14]; however, the available probiotics have been shown to have a limited effect failing to reach the efficacy of fecal transplantation from a healthy volunteer.

2.2 Current Treatment of *C. difficile* Associated Diarrhea (CDAD) and Recurrent CDAD (RCDAD)

The administration of vancomycin, metronidazole or fidaxomycin is the first choice for antibiotic therapy for CDAD especially for severe clinical illness defined as the presence of important comorbidity, altered sensorium, unstable clinical course, confinement in an intensive care unit, renal

failure (creatinine level \geq 1.5 times the upper limit of normal), reduced serum albumin level (<2.5 g/dL), hypotension, or leukocytosis with a white blood cell count greater than $15,000/\text{mm}^3$ [19].

A unique feature of CDAD is the very high recurrence rate seen in 25% of patients treated with equivalent frequency with either metronidazole or vancomycin [20, 21], typically beginning 7 to 14 days after therapy is completed for the first bout. Primary treatment of CDI with fidaxomicin reduces the rate of recurrence to 15% for infection caused by non-NAP1 strains [22]. A meta-analysis of 39 studies, including 11 prospective, 21 retrospective and 7 randomized clinical trials showed that there was 22% treatment failure for metronidazole versus 14% for vancomycin. Recurrence rates were 27% for metronidazole and 24% for vancomycin [23]. In smaller case reports, tigecycline [24], rifaximin [25], and nitazoxanide [26] showed response rates of 86%, 79% and 74% respectively for refractory CDAD. Fidaxomixin has been showed to be the same value as vancomycin with regard to cure rate but was associated with a significantly lower recurrence rate [22].

2.3 Summary of Fecal Microbiota Transplantation

Given the unacceptable failure rate of antibiotic treatment and in particular recurrence with standard therapy, alternative treatment approaches have been explored for those who have failed standard antibiotic therapy. Once the balanced gastrointestinal bacteria are disturbed by antibiotics, *C. difficile* has the opportunity to grow due to the loss of the normal bacterial population. Fecal transplantation is a strategy that restores the diversity of the gut microflora, which may confer protection against toxigenic *C. difficile* [8, 10]. FMT recreates the normal fecal microbiota, allowing the inhibition of *C. difficile* [32]. In addition, there is a possibility that the transplantation of healthy flora results in an immunological response, facilitating the elimination of *C. difficile*. Fecal transplantation treatment was introduced with the first reported case in 1958 by Eiseman *et al.* [33]. Over the subsequent 55 years, promising reports of FMT have suggested a $\sim 90\%$ clinical cure rate for recurrent CDAD [34]. More recent studies in 2012 confirmed these numbers with cure rates of 86% to 94% [35-38]. These results indicate that fecal transplantation for RCDAD is significantly superior to those with conventional antibiotic therapy. However, using fresh donor stool has its practical barriers, such as difficulty in stool preparation, and standardization of donor stool. It has been proposed to use frozen fecal extracts in the transplantation [36]. FMT needs to be a standardized and simplified to optimize the process and make it more efficient.

A description of the Fecal Microbial Transplantation (FMT) is available to physicians and patients (Introduction to FMT)

3. OBJECTIVE

We will generate a frozen, lyophilized or fresh FMT inoculum from well-screened healthy volunteer donors of ≥ 150 gram/sample. Delivery of FMT will be performed colonoscopically. This study will evaluate the safety of a frozen or lyophilized or fresh inoculum administered by colonoscopy. Fecal samples from donors and recipients will be saved for future studies.

4. STUDY DESIGN

This is a single center, randomized, parallel assignment, open label safety study conducted in patients with RCDAD. Approximately 100 patients will be enrolled in the study and randomized at 1:1 ratio to receive fresh or frozen or lyophilized donor stool.

4.1 Study Structure

Donors will be enrolled and screened at the Center for Infectious Disease laboratory for enteric pathogens at University of Texas School of Public Health (EPDL-UT-SPH) which is CAP and CLIA certified laboratory. The donors will come from a variety of places, including the University of Texas School of Public Health.

Recipients All patients to be treated must have a physician who is willing to care from them after discharge from our facility. Recipients will need to have insurance or be willing to self-pay for this therapy. Attending physician of recipients/patients will order the FMT and make clinical decisions related to overall health for his/her patients. All patients undergoing therapy will be handled as outpatients at either Baylor St. Luke's Medical Center in the Texas Medical Center, the Digestive Disease Center at Memorial Hermann in the Texas Medical Center, or Kelsey-Seybold Clinic – Main Campus on Holcombe Blvd. Once the procedure is completed, the recipient will be transferred back to their original medical treatment facility or be released to home care under the care of their physician. A gastroenterologist with privileges at Endoscopy procedure site will perform the FMT via colonoscopy. The Center for Infectious Disease enteric pathogens laboratory at the University of Texas School of Public Health will prepare donor stool for administration via a colonoscopy.

Evaluation of the safety and recurrence clinical symptoms of FMT will be performed by the primary physicians and research coordinators from the EPDL-UT-SPH. The primary endpoint is to evaluate the safety of the FMT (fresh or frozen or lyophilized health donor feces) for the treatment of patients with 3 or more RCDAD. A second efficacy endpoint is to evaluate the recurrence of clinical symptoms after initial therapy. In order to monitor any health effects for safety, participants will be contacted on or about 6 months following FMT procedure.

4.2 Study Population

Our recent study demonstrated that a majority of Houston gastroenterologists and infectious diseases specialists supported the creation of a fecal microbiota transplantation center in Houston [39]. The recipients with RCDAD will be referred generally by gastroenterologists and infectious diseases specialists or other physicians. We will screen at least 120 recipients and 70 donors. One Hundred recipients with RCDAD will be included in the study using the following entry criteria.

Subject will be referred by his or her attending physician who will provide non-transplant care for the subject.

Volunteer donors from the University of Texas School of Public Health will provide stools for use for the 100 patients. The donors will not be compensated. Volunteers may provide stools for more than one patient. The donors must not be students or lab technicians working at EPDL-UT-SPH. The donors will be screened and surveyed for health and medication use, sexual behavior, travel, and other risk factors for infectious diseases during a private and confidential interview (Donor – Prescreen Form). All fecal material from donors will be tested/ screened for enteric pathogens mentioned in protocol section 4.3.3. Donors will be notified by an email in the event an infection is identified.

4.3 Study Procedures

4.3.1 Informed Consent

Written informed consent must be obtained from each recipient (Patient Consent Form) or donor (Donor Consent Form) before he/she undergoes any procedures required by this protocol, including screening procedures. Informed consent forms may be emailed to recipients and returned by E-mail or fax.

4.3.2 Identification Number

Recipient: Each patient who signs an informed consent form will be assigned a study number at the clinic. The study number will consist of the letter „FMT-R-XXX” followed by a 3 digit identification number (e.g., FMT-R-001). Recipients need to meet the inclusion and exclusion criteria (listed below in table).

Donor: Every donor who signs an informed consent form will be assigned a screening number at the EPDL-UT-SPH. Subjects must be screened before they can become a qualified donor. The screening number will consist of the letter „FMT-D-” followed by a 3 digit identification number (e.g., FMT-D-001). This will be the only ID for this donor. Donors will provide stool volume within 2 hours to EPDL on the FMT day. The stools will be accepted if they are ≥ 150 g. Stools of lower weight (≤ 150 g but ≥ 50 g) will be used as fresh product only, no frozen and lyophilized transplantation product will be saved. We will collect an additional stool from the same donor within one month screened for frozen and lyophilized transplantation products.

4.3.3 Inclusion and Exclusion Criteria

	Inclusion Criteria	Exclusion Criteria
Recipients	<ol style="list-style-type: none">1. Male and female patients ≥ 18 years of age2. Sexually active male and female patients of child-bearing potential must agree to use an effective method of birth control during the treatment and follow-up period3. Female patients of child-bearing potential must have a negative pregnancy test in the 72 hours before the procedure4. Required to sign an informed consent form5. Deemed likely to survive for ≥ 3 months after enrolment6. Diagnosis of ≥ 3 recurrent CDAD (RCDAD) bouts in outpatients or ≥ 2 bouts of CDAD in an inpatient without other explanation for diarrhea and with ≥ 2 positive fecal tests for <i>C. difficile</i> toxin7. Referred by subjects attending physician who will provide non-transplant care for the subject8. Received at least one course of adequate	<ol style="list-style-type: none">1. Patients with neutropenia with absolute neutrophil count $<0.5 \times 10^9/L$2. Evidence of toxic megacolon or gastrointestinal perforation on abdominal x-ray3. Peripheral white blood cell count $> 15.0 \times 10^9/L$ AND temperature > 38.0 °C4. Active gastroenteritis due to <i>Salmonella</i>, <i>Shigella</i>, <i>E. coli</i> 0157:H7, <i>Yersinia</i> or <i>Campylobacter</i>, and <i>Norovirus</i>5. Presence of colostomy6. Unable to tolerate HBT for any reason7. Requiring systemic antibiotic therapy for more than 7 days8. Actively taking <i>Saccharomyces</i>

	<p>antibiotic therapy for CDAD (≥ 10 days of vancomycin at a dose of ≥ 125 mg four times per day, ≥ 10 days of metronidazole at a dose of 500mg three times per day or fidaxomixin 200mg twice a day for 10 days</p> <p>9. Anti-CDI antibiotic treatment or probiotics stopped 2-4 days before the transplantation</p>	<p><i>boulardii</i> or other probiotic</p> <p>9. Severe underlying disease such that the patient is not expected to survive for more than 3 months or unstable medical condition requiring daily change in treatments</p>
Donors	<p>1. Able to provide and sign informed consent</p> <p>2. Able to complete and sign the donor questionnaire</p> <p>3. Able to adhere to fecal transplantation stool collection requirements</p>	<p>1. Test positive for any of variables</p> <p>2. History of any type of active cancer or autoimmune disease</p> <p>3. History of risk factors for acquisition of HIV, syphilis, Hepatitis B, Hepatitis C, prion or any neurological disease as determined by the donor questionnaire</p> <p>4. History of gastrointestinal disorder, e.g., inflammatory bowel disease, irritable bowel syndrome, chronic constipation or diarrhea</p> <p>5. Antibiotic use or any systemic immunosuppressive agents in the 3 months prior to stool donation</p> <p>6. Receipt of any type of live vaccine within 3 months prior to stool donation</p> <p>7. Current or previous medical or psychosocial condition</p> <p>8. Body mass index over 30</p>

5. METHODOLOGIES

5.1 Recipient

5.1.1 Pre-screening

Prior to procedure recipient will be screened with a screening log that is compliant with local regulations regarding patient confidentiality. The screening log will be designed to document the rationale for screening the patient (inclusion criteria) and not enrolling the patient (exclusion criteria, patient declined to participate). The information will be obtained from the Pre-screening (Recipient Prescreen Form) and then further evaluated if pre-qualified obtaining more detailed medical History (Recent Medical History Form). The investigator will record all patients screened for the study in the screening log, even if they do not sign an informed consent form. However, patients must sign an informed consent form before any study procedures are performed on the patient. Following documents will be obtained:

- Informed consent
- Patient demographics and medical history

Letters to be sent:

- Patient letter – Aftercare of FMT
- Physician letter – Physician Letter - Before FMT

CDAD is defined as ≥ 3 loose or watery stools per day for at least 2 consecutive days or ≥ 8 loose or watery stools within 48 hours and with positive *C. difficile* toxin. Recurrent CDAD (RCDAD) is defined as the reappearance of either a symptom or a positive toxin assay within 60 days of the treatment. Antimicrobial agents will be discontinued approximately 2 days before the treatment. Recipient will stop any other antibiotics. A fresh stool sample will be collected from a recipient, tested for *C. difficile* toxins and an aliquot (2 mL) saved at -80°C for future studies before FMT.

If appropriate subjects will visit a study gastroenterologist for evaluation before planned colonoscopy or duodenal intubation.

5.1.2 One day before fecal transplantation

For FMT by colonoscopy bowel Preparation will take place 24 hours before FMT as prescribed by gastroenterologist.

A fresh stool sample will be collected from a recipient, tested for *C. difficile* toxins and an aliquot (2 mL) saved at -80°C for future studies.

5.1.3 Day of Fecal transplantation

Recipient cannot consume any solid food, alcohol or any liquids on the day of FMT. Each patient should have completed the informed consent (Patient - Consent Form). The gastroenterologist will insert 250mL of filtered stool into the colon by colonoscopy.

A fresh stool sample will be collected from a recipient before FMT, and approximately 7, 14, and 30 days after FMT, tested for *C. difficile* toxins and an aliquot (2 mL) stored at -80°C for future studies. (Patient Letter – Aftercare of FMT) A letter will be provided to the recipient indicating the need to avoid antibiotics in the next few months, to take a probiotic (*Saccharomyces boulardii*) if an antibiotic is needed and to consume foods that feed the intestinal bacteria such as bananas, nuts, oat or bran, root vegetables and flaxseeds and what is recommended if their diarrhea recurs.

5.1.4 During the first week after fecal transplantation

The recipient will be contacted by the investigator approximately 24 hours after the procedure is performed. The following procedures will be completed:

Review recipient diary with the recipient to ensure that the following information is recorded correctly and collect the completed pages:

- Date and time of the procedure completed
- Date, time and any adverse experience and/or name of dosage of any medication
- Presence or absence and severity of signs or symptoms of any adverse experience

5.1.5 Approximately Seven days after fecal transplantation (depending on their availability, weekends and holidays)

The recipient will be contacted by the investigator approximately 7 days after the procedure is performed. The following procedures will be completed:

- Review recipient diary with the recipient to ensure that the following information is recorded correctly and collect the completed pages:
 - Date and time of the procedure completed
 - Date, time, and any adverse experience and/or name of dosage of any medication
 - Presence or absence and severity of signs or symptoms of any adverse experience
 - A fresh stool sample will be collected from recipient, tested for *C. difficile* toxins and an aliquot (2 mL) stored at -80°C for future studies.

5.1.6 Approximately Fourteen days after fecal transplantation (depending on their availability, weekends and holidays)

The recipient will be contacted by the investigator approximately 14 days after the procedure is performed. The following procedures will be completed:

- Review recipient diary with the recipient to ensure that the following information is recorded correctly and collect the completed pages:
 - Date and time of the procedure completed
 - Date, time, and any adverse experience and/or name of dosage of any medication
 - Presence or absence and severity of signs or symptoms of any adverse experience
 - A fresh stool sample will be collected from recipient, tested for *C. difficile* toxins and an aliquot (2 mL) stored at -80°C for future studies.

5.1.7 Approximately Thirty days after fecal transplantation (depending on their availability, weekends and holidays)

The recipient will be contacted by the investigator approximately 30 days after the procedure is performed. The following procedures will be completed:

- Review recipient diary with the recipient to ensure that the following information is recorded correctly and collect the completed pages:
 - Date and time of the procedure completed
 - Date, time, and any adverse experience and/or name of dosage of any medication

- Presence or absence and severity of signs or symptoms of any adverse experience
- A fresh stool sample will be collected from recipient, tested for *C. difficile* toxins and an aliquot (2 mL) stored at -80°C for future studies.
- After 30 days a letter will be sent to you indicating that you have successfully completed the study and that all future medical issues should be handled by your non-FMT physician.

5.1.8 Safety data

We will attempt to contact recipients on or about 6 months following FMT in order to monitor any health effects for safety.

5.1.9 Treatment Failure

Treatment failure occurs when a new episode of *C. difficile* occurs which includes > three plus watery diarrhea episodes per day and a positive *C. difficile* toxin test. A letter will be sent to the patient's physician relaying this information (Physician Letter – Treatment Failure)

5.2 Donor

Project investigator/coordinator from the University of Texas School of Public Health will be responsible for donor positive identification. They will walk the donor with a FMT number and date of birth to the blood center at CHI St. Luke's Medical Center. An employee from blood center will draw the donor in the presences of the University of Texas School of Public Health investigator/coordinator and label tubes with FMT # and date of birth provided.

5.2.1 Pre-screening

Approximately 70 potential donors may need to be pre-screened to allow recruitment of at least 20 qualified donors (Donor – Prescreen Form). Identified donors will be found from various employees without direct patient contact, except students and lab technicians in EPDL-UT-SPH. Before a stool is used for transplantation, the donor will be screened for infectious diseases using the following tests.

Stool will be collected in a semi-sterilized container. Stool needs to be delivered to the Laboratory for EPDL-UT-SPH in room █ within 8 hours after collection. Every fecal donation will be tested for enteric pathogens, including *Shigella*, *Salmonella*, *Campylobacter*, Shiga-toxin Producing *Escherichia coli*, *Clostridium difficile* Toxin A/B.

Blood samples from the donor will be collected with a sterilized needle (25mL blood will be collected) at the St. Luke's Medical Center Blood Bank and tested for conditions screened for blood transfusions including Hepatitis B core Antibody, Hepatitis B Surface Antigen, Hepatitis C virus Antibody, HIV-1 and HIV-2 Antibody, Anti-HTLV I/II, Serologic Test for Syphilis

5.2.2 One day before transplantation

- Investigator will contact qualified donors to have at least 2 donors who would be able to provide \geq 50 grams of stool on the day of FMT for fresh FMT.
- Donor are encouraged to take one stool softener laxative the evening before the procedure and drink of plenty water.
- We will provide the supplies and instructions for sample collection.

5.2.3 Day of Fecal transplantation

5.2.3.1 Donor Stool Preparation, Storage and Shelf Life

1. Obtain \geq 150g of stool per transplant (multiple stools will be required to obtain \geq 150 grams). When the randomization calls for a fresh sample \geq 50 g of stool will be accepted and used in totality not more than 4 hours before procedures
2. Stools of lower weight (\leq 150g but \geq 50g) will be used as fresh FMT only, no frozen and lyophilized transplantation product will be saved. We will collect additional stool from the same donor during the month screened for frozen and lyophilized transplantation products.
3. Donor stool is mixed with 1500mL (1:10 dilution) sterilized 0.9% NaCl without antibacterial preservative in a large sterilized suction canister
4. The solution is mixed in a homogenizer mixer with sterilized bag (Stomacher). Initially, use the low setting until the sample breaks up, and then advance the speed gradually to the highest setting. Continue for 2 – 4 minutes until sample is smooth.
5. The suspension is filtered using a sterile paper coffee filter (or gauze) that has been moistened with saline and funnel (both are sterilized). Allow adequate time for slow filtration to end. NOTE: To expedite process, multiple funnel/filters can be used to combine product.
6. Repeat step 4. NOTE: it may take 2 hours to complete steps 4 and 5.
7. Place filtered suspension in 15 sterilized 50 mL tubes
8. Number the samples as FMT-D-001-FRESH (5 tubes), FMT-D-001-FROZEN (5 tubes) and FMT-D-001-LYOPHILIZED (5 tubes) (Form 129-12-03)
9. Label the tubes with lot number (mm-dd-yyyy) and date and time processed
10. Keep the samples –
 - FMT-D-001-FROZEN (5 tubes) sealed with plastic (polyethylene or similar film) at -80°C in room [REDACTED] for up to 6 months in a test tube rack clearly labelled with the media lot # and expiration date (6 months after preparation date)
 - FMT-D-001-LYOPHILIZED (5 tubes) –
 - Start lyophilisation within 30 minutes after completion of stool filtration
 - Keep lyophilized samples at -80°C in room [REDACTED] for up to 6 months in a test tube rack clearly labelled with the media lot # and expiration date (6 months after preparation date)

- FMT-D-001-FRESH (5 tubes) transferred to SLMC Endoscopy Unit immediately for the same day FMT. The fresh donor stool will be used for FMT within 8 hours after collection.

11. Upon completion of the process, stool samples must be recorded into the Donor Specimens Log (Form #131-12-05)

12. Repeat stool donors will be used for three or more recipients. We consider donors having safely provided fecal bacteria to other recipients safer than first time donors. If the repeat donor stool is used within 1 month of passing the questionnaire survey, no blood and stool will be screened. If the donation is over 1 month of the before mentioned process, then the medical history must filled out again and both blood and stool will be screened/tested as listed in Screening Of Donor section of protocol.

5.2.3.2 Prepare for Transplantation

1. Center for Fecal Transplantation will notify EPDL-UT-SPH 10 days before the procedure about potential patients by Email (Zhi-Dong.Jiang@uth.tmc.edu).
2. Center for Fecal Transplantation will confirm the details about the procedure 24 hours before the transplantation with EPDL-UT-SPH Email (Zhi-Dong.Jiang@uth.tmc.edu).
3. On the procedure day, $\geq 250\text{mL}$ of suspension will be delivered to the hospital Micro-Laboratory in a cooler with wet ice by an employee from EPDL-UT-SPH Email (Zhi-Dong.Jiang@uth.tmc.edu).

6 EVALUATION OF SAFETY

6.1 Primary Safety Endpoints

The following safety endpoints will be evaluated in this study.

6.1.1 Medical conditions

Clinical improvement is monitored by telephone and or E-mail during 30 days after FMT. Minor clinical management of the subject with over the counter medication (e.g. loperamide, acetaminophen and *Saccharomyces boulardii* [probiotic]) will be performed in the case of mild diarrhea and abdominal pain.

All subjects will be followed up by phone the day after FMT to assess health status during week one. Study subjects will be monitored on approximately days 7, 14 and 30 after FMT, at which time we will review the recipient diary with the recipient to review any adverse experience or medication taken since the medical history obtained at FMT. In order to monitor any health effects for safety, participants will be contacted on or about 6 months following FMT.

Investigators should determine if any adverse experience or medication need to be further studied. Donor will be contacted and tested if it is necessary. All actions will be

recorded on with medical condition, dates of adverse experience and medication taken, indication for new medication taken, and total daily dose (Patient Data Collection Form).

Recipients should be instructed at FMT to contact the Investigator if they have any questions regarding adverse experience or the appropriateness of a medication after FMT.

6.1.2 Monitoring for safety

The following definitions of terms are guided by the International Conference of Harmonization and US Code of Federal Regulations (21 CFR 312.32).

An adverse experience is any unfavorable or unintended sign, symptom, or disease temporally associated with FMT procedure, whether or not considered related to the procedure, including, but not limited to:

- Any symptom not previously reported by the recipients (Recent Medical History)
- An exacerbation of a pre-existing illness, increases in the frequency and/or severity of the signs or symptoms of CDAD (defined as positive *C. difficile* toxin test and enteric symptoms)
- A significant increase in frequency or intensity of a pre-existing episodic event or condition
- A condition first detected or diagnosed after study drug administration even though the condition may have been present before the procedure

Details of all adverse experiences that occur after FMT through approximately day 30 visit will be collected as indicated above.

Serious adverse experience is any adverse experience that:

- Results in death
- Is life threatening (at immediate risk of death from the procedure as it occurred)
- Requires inpatient hospitalization (overnight stay) or prolongs a current hospitalization
- Causes a persistent or significant disability/incapacity
- Medical important (any event that requires medical or surgical intervention to prevent one of the outcomes listed above)

The investigator will exercise medical and scientific judgment when deciding whether expeditious reporting is appropriate in other situations not strictly meeting the listed criteria above. The investigators will meet/discuss with experts in the field if there is a question of whether the adverse experience would be considered serious.

Severity – The adverse experience will be documented on the appropriate page in the Patient Diary according to the following descriptors:

- Mild: associated with no limitation of usual activities or only slight discomfort
- Moderate: associated with limitation of usual activities or significant discomfort

- Severe: associated with inability to carry out usual activities or very marked discomfort

Relationship – the relationship of adverse experience to FMT will be assigned by the Investigator according to the following definitions:

- Probable: a reaction that follows a reasonable temporal sequence from the procedure that follows a known or expected response pattern to the suspected procedure and that could not be reasonably explained by the known characteristics of that patient's clinical state
- Possible: a reaction that follows a reasonable temporal sequence from the procedure that follows a known or expected response pattern to the procedure but could readily have been produced by a number of other factors
- Unlikely: a reaction that does not follow a reasonable temporal sequence from the procedure but for which causality from FMT cannot be ruled out.
- Not related: a reaction for which sufficient data exist to indicate that the etiology is unrelated to the procedure

6.1.4 Pre-existing signs and symptoms and medical conditions

Medical conditions that are present at or before the procedure that manifest with the same severity or frequency will not be recorded as adverse experience. Similarly, signs or symptoms related to a pre-existing disease will not be recorded as adverse experience unless there is an increase in the severity or frequency of the signs or symptoms. These pre-existing conditions, signs, or symptoms will be recorded on the Recent Medical History Form.

6.1.5 Progression of underlying conditions as an adverse experience

If the progression of the underlying condition might be reasonably anticipated given the nature and severity of the underlying condition, then the progression of the underlying condition *per se* will not constitute an adverse experience. However, if the progression of the underlying condition is fatal, then the progression of the underlying condition should be reported as an adverse experience.

6.1.6 Recording and documenting adverse experience

The Investigator must completely and promptly record each new adverse experience and serious adverse experience, even if the relationship of adverse experience to the procedure is assessed by the Investigator to be "unlikely" or "not related". In addition, the investigator must document and follow serious adverse experiences that occur from the procedure through 30 days after the FMT. The Investigator should attempt, if possible, to establish a diagnosis based on the presenting signs and symptoms. If an adverse experience meets the definition of a serious adverse experience then the Investigator must also complete the serious adverse experience, and also send any supporting

source documents directly to the University of Texas Health Science Center IRB as soon as the event is discovered. At each visit, after the patient has had an opportunity to mention any problems spontaneously, the Investigator (or designee) will inquire about adverse experience by asking the standard questions listed in, such as:

- Have you had any medical problems since your last visit?
- Have any medical problems present at your last visit changed, i.e., stopped, worsened, or improved?
- Have you taken any new medicines, other than study drug, since your last visit?

Any spontaneous adverse experience information provided by the patient will be reported. If an adverse experience has not resolved at the time of the Final Visit (day 30 Visit), the Investigator should evaluate the status of the adverse experience at the Follow-Up telephone/email contact and update to reflect the status of the adverse experience (e.g. ongoing or resolved).

6.1.7 Investigator reporting of serious adverse experience

All serious adverse experience must be reported to the University of Texas Health Science IRB using the serious adverse experience facsimile or email or by telephone/email as soon as the serious adverse experience is discovered, and within 24 hours after the Investigator recognizes or classifies the event as a serious adverse experience. A brief description of the event must be provided at the time of the initial serious adverse experience report. The initial serious adverse experience report should be followed up by additional information using the serious adverse experience within 48 hours. The reports should identify the patient by their unique patient number instead of names. The completed serious adverse experience Form will be used by the investigators in regulatory filings. The investigator is responsible for continuing to report to the University of Texas Health Science IRB any new or relevant follow-up information obtained concerning the serious adverse experience. The results of any additional assessments conducted must be also reported to the University of Texas Health Science IRB.

6.1.8 Notification of post-study serious adverse experience

Investigators are not obligated to actively seek follow-up information for patients with adverse experience after the conclusion of the study (i.e., > 30 days after the FMT procedure). However, if the investigator becomes aware of an adverse experience that occurs after the patient completes and the adverse experience is considered by the Investigator to be at least possibly related to study procedure, the investigator must notify the University of Texas Health Science Center IRB.

6.2 Secondary efficacy endpoint

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The secondary endpoint is failure to have a subsequent bout of CDAD in 30 days post FMT. It is defined as recipient has had another episode of diarrhea (≤ 3 unformed stools in 24 hours) with

positive *C. difficile* toxin A/B test. An unformed stool is defined as either a soft or watery stool. Data will be recorded in the CRFs. Patients will be asked to seek further medical treatment with their primary physicians. No further FMT will be performed.

8 DATA COLLECTION AND ANALYSIS

7.1 Recording of data

The Investigator must maintain adequate and accurate source documents. The source documents will be reviewed and signed at the investigative site by the investigators periodically.

7.2 Statistical Methods

7.2.1 General considerations

All analyses will be performed and all tables, figures, and data listings will be prepared using SAS (Cary, NC). Summary statistics for continuous variables will include the mean, standard deviation, median, minimum, and maximum value; categorical variables will be presented as counts and percentages. For all analyses, baseline is defined as the date of fecal transplantation.

7.2.2 Power calculations

No power calculation was performed since this is a pilot study. We will evaluate the program/center after enrollment of 100 recipients with RCDAD. Thirty patients (33 in each group, receive either fresh or frozen or lyophilized stools from healthy donors) with CDAD will be included in the study using the entry criteria in section 4.3.3.

7.2.3 Analysis Populations

All analyses will be conducted using the intent-to-treat population. Intent-to-treat is defined as all recipients who have started the procedure.

Listings and summary tables with descriptive statistics will be prepared for demographic data (gender, date of birth, race/ethnicity), and medical history data (including the date and time of onset of CDAD and signs or symptoms of enteric infection, episodes of RCDAD).

7.2.4 Randomization procedures

An independent statistician will develop a randomization list using SAS. Randomization in permuted blocks will be used to achieve balance across treatment groups. The randomization scheme consists of a sequence of blocks such that each block contains a pre-specified number of treatment assignments in random order. The purpose of this is to balance the randomization scheme at the completion of each block. The target sample size is 100 recipients (33 receive fresh, 33 receive frozen and 33 receive lyophilized stool), blocks of size 3 are natural. Subject study identification assignment log will be completed and kept in the study binder.

7.2.5 Analyses of safety

All adverse experience and serious adverse experience will be coded using the Medical Dictionary for Regulatory Activities Version 12.0 which is a registered trademark of the International Federation of Pharmaceutical Manufacturers and Associations. The number and percentage of patients reporting at least one occurrence of adverse experience and/or serious adverse experience for each system organ class and preferred term will be tabulated. Term will also be tabulated by severity and by the relationship to the procedure. For multiple occurrences of the same adverse experience with different severities, the adverse experience with the highest severity will be tabulated. For multiple occurrences of the same adverse experience with different relationships to the procedure (related and not related); the adverse experience will be tabulated as related. All adverse experience and or serious adverse experience for all patients will be presented in data listings. Narratives will also be prepared for each serious adverse experience.

Physical examination findings will be presented in data listings. Listings and summary tables with descriptive statistics will be prepared for each vital sign parameter (diastolic and systolic blood pressure, heart rate, oral temperature, respiratory rate). In addition, the percent change from baseline will be calculated for each continuous variable.

7.2.6 Secondary efficacy analyses

The proportion of patients in each group (fresh, frozen and achieving Clinical Cure will be compared using the Chi-square test. The proportion of recipients with a subsequent bout of CDAD in 60 days post FMT in each treatment group will be compared using the Chi-square test.

8 PROTECTING PRIVACY

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

9 CONTACTS

- Dr. Herbert L. DuPont at 832-355-4122.
- The University of Texas Health Science Center Committee for the Protection of Human Subjects at (713) 500-7942.
- The CHI St. Luke's Medical Center Institutional Review Board at (832) 355-3347

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