Protocol and Statistical Analysis Plan

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Title: The Use of Fecal Microbiota Transplantation in Patients with Ulcerative Colitis-associated Pouchitis

Principal Investigator: Virginia Shaffer, MD, Associate Professor of Surgery, Emory University School of Medicine

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Emory University School of Medicine
Divisions of Digestive Diseases and Infectious Diseases,
Department of Surgery

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1. SPECIFIC AIMS

The aims of this study are: 1) to determine the utility of fecal microbiota transplantation (FMT) in the treatment of patients with ulcerative colitis (UC) associated chronic antibiotic-dependent pouchitis (CADP) and chronic antibiotic refractory pouchitis (CARP) and 2) to study the changes in the microbial environment in patients with pouchitis (pre- and post-treatment) and 3) to assess the impact of therapy on the patient's perceived quality of life.

2. BACKGROUND AND RATIONALE

The spectrum of inflammatory bowel diseases (lBO) includes ulcerative colitis (UC) and Crohn's disease (CD). The etiology of these diseases is not clear but appears to involve aberrant immunological responses to intestinal bacteria in a genetically predisposed host. There is increasing evidence that the intestinal microbiota play an important role in the initiation and maintenance of lBO. Studies have demonstrated that the microbiota in patients with lBO differ from the healthy non-IBD individuals with a decrease in species such as *Clostridium leptum* and *Faecalibacterium prausnitzii.* This "dysbiosis" may result in fewer bacteria that produce short chain fatty acids which are important in protecting intestinal epithelium. The intestinal microbiota is important in maintaining homeostasis in the intestine and interacts with the host immune system to enhance immune function. Manipulation of the gut bacteria through the use of antibiotics, probiotics, and prebiotics have been shown to be beneficial in lBO. The use of antibiotics for the treatment of mild to moderate active

colonic Crohn's disease is effective in many cases. Antibiotics are often used to treat acute episodes of UC-associated pouchitis, an inflammatory condition seen in patients who undergo a restorative proctocolectomy with an ileal pouch anastomosis (IPAA). Probiotics (such as VSL-3) for the treatment of acute UC-associated pouchitis as well as for the secondary prevention of repeated episodes of pouchitis have been evaluated in several studies with positive results.

Greater than 50% of lBO patients who undergo an IPAA will develop pouchitis. Many of these patients will develop recurrent episodes of pouchitis and up to 5% will develop chronic pouchitis requiring maintenance therapy with antibiotics or other agents and may even pouch excision. The etiology of pouchitis is unknown, but theories include fecal stasis leading to bacterial overgrowth and alteration of normal commensal flora in a genetically susceptible individual. Microbiome studies in UC pouch patients have shown alterations in the fecal flora with increases in anaerobic bacteria and sulfate producing bacteria.

In a recent study, pouch microbiomal environments appeared to be distinctly unique in patients with a normal UC-associated IPAA and those with UC-associated pouchitis (Zelia GC, IBD, May 2011). Pouchitis samples showed more *Clostridium* and *Eubacterium* genera compared to healthy UC pouch patients. The current treatment approaches for pouchitis are based on small randomized controlled trials showing some efficacy with use of antibiotics and probiotics. However, the choice of antibiotics, dose, and duration of treatment are largely empiric. In addition, probiotics have limited durability after discontinuation, while the antibiotics most utilized have known side effects as well as the potential for selecting multi-drug resistant bacteria. Development and study of additional therapeutic options for pouchitis treatment are areas for further research.

Fecal microbiota transplantation (FMT) has been shown safe and effective as a method of manipulating the microbiota in patients with recurrent *Clostridium difficile* infection (van Nood et al. NEJM 2013). In the literature, there are 41 cases in 17 articles reported of fecal transplantation in the treatment of inflammatory bowel disease (IBD). The majority experienced a reduction of symptoms (19/25), cessation of IBD medications (13117), or disease remission (15/24) (Anderson, Edney et al. APT 2012). Some ofthe weaknesses ofthe available data are small number of cases, unreported or not clearly defined IBD characteristics, and lack of uniformity when measuring outcome variables. A recent prospective controlled pilot trial of use of FMT for chronic refractory pouchitis revealed negative outcomes. However, the researchers used a single dose and administered the stool preparation through a nasogastric tube. Several studies have shown lower success rates of FMT in *C. difficile* with use of nasofeeding tube versus a colonoscopy or sigmoidoscopy. In addition, a recent trial of use of FMT to treat ulcerative colitis showed improvement in clinical activity scores when five successive fecal enemas were used (Kunde 20 13). At the present, it is unclear which phenotype of IBD-ulcerative colitis, Crohn's disease, or pouchitis or what level of disease activity is best treated with fecal

microbiota transplantation.

In collaboration with sub-Investigator Colleen Kraft, MD, sixty-two (62) fecal microbiota transplants have been attempted in 56 patients at our institution for the indication of refractory *C. difficile* infection. One patient who consented for the study was found to have graft-versus-host disease was not transplanted.

To date, 61 FMT procedures have been completed in 55 patients. Data is available on 48 FMTs performed on 42 patients.

Eight FMTs have experienced relapse:

- 1 patient relapsed due to abx for UTI - resolved after receiving course of vancomycin
- 2 solid organ transplant patients with FMTs relapsed but improved after receiving a second infusion
- 1 patient required total of 3 FMTs (1 via Dobhoff, 2 colon)
- 1 patient required total of 2 FMTs via colon
- 1 patient was 95 y/o NH patient
- 1 patient had no response to 2 FMT

Three FMT subjects have experienced adverse events:

- 1 hospital admission due to abdominal pain post FMT in a patient with history of irritable bowel. Negative work-up
- 1 inpatient FMT with abdominal pain post FMT -- negative findings
- 1 subject experienced episode of diverticulitis post colon FMT[1]

Additional findings:

- 2 subjects with diagnosis of collagenous colitis
- 1 colon cancer

To date, based on available data, there has been a 95% overall response rate (primary, secondary, tertiary response rates inclusive). Secondary and tertiary response are defined as response to repeat antibiotic therapy and/or repeat FMT.

We hypothesize that the use of FMT in patients with chronic pouchitis symptoms will restore the microbial balance in these patients and help treat pouchitis. This would circumvent the need to use chronic antibiotic therapy and/or potentially salvage the pouch, and serve as a more durable form of treatment for this group of patients. In addition, we hope to show that the microbial environment of pouchitis patients will change to a more favorable one (as defined by the donor microbiota) as determined via 16S ribosomal RNA gene sequencing of the microbiota and metabolomic profiling.

3. STUDY DESIGN

The primary investigative design will be a single arm study to determine if FMT has the potential to be used in the treatment of chronic pouchitis.

3.1 ***Study Objectives:***

Primary Objective: To determine the safety and tolerability of fecal microbiota transplant (FMT) in treatment of chronic pouchitis

Secondary Objective: To study the microbial environment of IPAA/UC associated pouches in patients presenting with symptoms of pouchitis with and without endoscopy-visualized pouchitis and to assess the impact of the therapy on the patient's perceived quality of life

3.2 ***Study design Type/Phase:*** This is a two center, Phase 1 open label study designed to determine the safety, tolerability and use of fecal transplant in patients with pouchitis and to study the differences in microbiome in patients before and after treatment with FMT.

3.3 ***Sites Centers of Research*****:** This study will be conducted in the Emory Clinic- Adult Digestive Disease Clinic and Colorectal Surgery Clinic, the Emory Center Endoscopy Center, and St. Joseph's Hospital (SJH) in Atlanta, GA.

3.4 ***Measures to minimize/avoid bias***: Eligible subjects will be given options of other treatments if they choose not to participate.

3.5 ***Study duration:*** The total duration of the study for each patient will be twelve weeks.

3.6 ***Study Procedures***

The following protocol essentially requires two sets of study procedures. One set of study procedures outlines testing requirements for the donor of allogenic human feces (see **Table 1.** Donor Related Study Activities). The other set outlines activities and expectations of the research participant who will receive the donated stool specimen (see **Table 2**. Research Participant Study Activities). The donor set of activities will be managed by Colleen Kraft, MD, a sub-investigator and Associate Professor of Medicine within the Division of Infectious Diseases. In the past, Dr. Kraft has overseen the donor recruitment and screening within previous FMT trials via ordering the appropriate antibody screens, fecal sample preparation (e.g. use of saline, etc.) and any other necessary tests to ensure that the fecal specimen is non-infectious and safe for insertion into research participants. Dr. Kraft still oversees this process and prepares the allogenic human fecal

donation in her lab on site. However, she will not be able to provide these activities listed in **Table 1** for effort.

All activities outlined in **Table 1** would be billed to the sponsor to compensate Dr. Kraft for her time and effort to support our participants and overall project goals and needs. The donor related activities will take place before the research participant is seen for informed consent during the screening visit (see **Table 2**). This is done to ensure that the donor has been screened for infectious diseases in a timely manner and that Dr. Kraft has ample time to prepare the allogenic human fecal specimen in her lab for the transplant on the enrollment visit. Donors will be screened every four weeks which is standard protocol for Dr. Kraft's lab. More details about the donor screening process and requirements are listed in the protocol section **3.6 a) Donor Stool Collection and Processing**.

Table 1. Donor Related Study Activities

Donor Activity	Specific Test Ordered	Visit
Blood/Serology Testing	1. Hepatitis A IgM and IgG 2. Hepatitis B S Ab/Sag/Core Ab 3. Hepatitis C Ab 4. RPR 5. Human Immunodeficiency Virus (HIV) 1 and 2 Antibody	Donor Screening (every four weeks)
Stool Testing	1. *Clostridium Difficile* PCR 2. Salmonella, Shigella, and Campylobacter 3. Ova and parasites 4. *H. Pylori* antigen 5. 5.Vancomycin resistant *Enterococcus* (VRE) 6. Carbapenemase resistant *Enterobasteriacae*	
Fecal Sample Preparation for Donation[1]	N/A	

[1]The fecal sample will be prepared for the FMT in Dr. Colleen Kraft's laboratory on site. Please refer to section 3.6 a for more detailed information regarding the donor fecal sample processing activities.

Table 2 outlines a total of five complete research visits for each research participant: screening (initial), enrollment (transplant), 2 weeks post-transplant (may be a phone call at the discretion of the physician), 4 weeks post-transplant, and 3 months post-transplant. For the initial or screening visit, research participants will first complete the informed consent process with a member of the research team. Upon completion of the informed consent process, the participant will receive phlebotomy to collect blood for plasma chemistries, CBC with differential, and platelet counts.

Urine will also be collected and screened for basic urinalysis. Women of child bearing potential

(WOCBP) will be screened using urine pregnancy kits to test for beta HCG levels. These kits will be provided by the sponsor. The results of these kits will be used as the final determination of pregnancy for WOCBP. WOCBP who screen positive for beta HCG (i.e. are pregnant) will be excluded from the study for the safety of both the mother and her unborn child. Phlebotomy for plasma collection, eventual CBC with differential, and platelet count will be performed for each research visit. Urine and stool will also be collected for each study visit. In addition to phlebotomy, urine collection, and stool collection, research participants will also receive a physical exam for each research visit.

Waist and hip circumference will be measured during the screening visit only. For the second visit (i.e. enrollment), the research participant will also receive a physical exam along with a flexible sigmoidoscopy. The flex sigmoidoscopy will be performed under moderate sedation using propofol per usual anesthesia services. The flexible sigmoidoscopy will be used to gain access to the pouch and to take a mucosal tissue pouch biopsy.

Table 2. Research Participant Study Activities

Visit Procedures	**Screening**[2]	**Enrollment**	**2 Week**[3] **(+/- 3 days)**	**4 weeks (+/- 7 days)**	**3months (+/- 14 days)**
Informed Consent Process	X				
Physical Exam	X	X	X	X	X
Urine pregnancy testing for WOCBP	X				
Phlebotomy	X	X	X	X	X
<u>Labs and Specimen Processing:</u> Stool collection Plasma collection Urine collection CBC w/diff., platelet count[4]	 X X X X	 X X X X	 X X X X	 X X X X	 X X X X
Flexible Sigmoidoscopy with Moderate Sedation		X			
Mucosal Biopsy Collection		X			
Waist and hip circumference measures	X				
Fecal Microbiota Transplant		X			

Modified PDAI	X (clinical)	X	X (clinical)	X(clinical)	X (clinical)
Quality of Life Questionnaires	X			X	X
Food Recall Questionnaire		X		X	X

[2]If both screening and enrollment visits occur within 7 days, research samples may not be recollected at the enrollment visit, at the discretion of the investigator

[3]The 2-week visit may be conducted over the telephone for subjects who live a significant distance from the study site and at the discretion of the study investigator. Biological samples will not be collected on subjects who complete visit via telephone, however subject may be asked to mail in a stool sample for testing

[4] Screening labs for hemoglobin (Hgb), platelet count, and absolute neutrophil count (ANC) may be performed within six months of screening visit

3.6. *a) Donor Stool Collection and Processing*

The standard donor stool will be obtained from a donor who has undergone testing for potential transmittable infections. These include Hepatitis A, Hepatitis B, Hepatitis C, syphilis, HIV, *H pylori, Salmonella, Shigella, Campylobacter,* C. *difficile,* as well as screening for ova and parasites, and multidrug resistant organisms (specifically carbapenemase-producing *Enterobacteriaceae* and vancomycin-resistant *Enterococcus).* This testing is performed through Emory Medical Laboratories. In addition, the donor will be screened for illnesses that would exclude him from donating stool for the current study (see attached screening questionnaire). Donor screening will be completed and all results confirmed negative prior to use of donor stool for transplant. Standard donors will be re-screened every six months.

The standard donor voids the stool at home just prior to leaving for work in the morning. The stool is dropped off in the microbiology laboratory within 60 minutes of being voided. The stool is processed within 60 minutes of the drop off, and administered within 120 minutes of the processing. The time that elapses from the stool being voided by the donor and administered to the patient ranges from 60-240 minutes. Donor stool will be handled as a level 2 biohazard with appropriate universal precautions.

Appropriate Personal Protective Equipment (PPE) will be worn by personnel while handling/processing donor fecal material:

- Nitrile gloves will be worn while working with all potentially infectious agents. Gloves should be removed before touching common objects in public areas. Hand washing will be performed each time gloves are changed.
- Gloves should be removed prior to removal of other PPE. Gloves should be removed before leaving the laboratory.
- Lab coats will be worn over clothing while handling/processing stool specimen and preparing FMT solution

- Contaminated clothing will be decontaminated and laundered on-site or by a commercial laundry service.
- Protective eyewear/goggles will be used, if splash potential exists
- Laboratory access will be limited to authorized personnel only.
- Biohazard containers for disposal of contaminated materials are readily accessible in the processing area.
- Good laboratory techniques will be exercised to minimize the formation of aerosols, droplets, spatters, splashes.

The FMT processing (performed by Dr. Kraft) involves taking of the standard donor's stool which is received in the clinical microbiology laboratory along with a short questionnaire (see Appendix 2). The hood is terminally cleaned with 10% sodium hypochlorite followed by 70% ethanol. The hood area is then lined with clean lab mats. Thirty cubic centimeter (ccs) saline aliquots are poured into 50 ml centrifuge tubes and 1 cm^3 of stool is distributed in these aliquot containers. The stool is mixed gently using a tongue depressor until a smooth suspension is formed. The contents are then filtered using a standard Parapak filter. The suspension is allowed to settle in the 50-ml centrifuge tube for 10 minutes and then poured off into a sterile container. This filtrate is then drawn up in 60 cc syringes, and labeled "For Enteral Use Only". Syringes are placed into sealed biohazard labeled bags for transport.

3.6. ***b) Fecal Microbiota Transplant (FMT) Procedure***

Subjects will be asked to clean out their pouch using two over the counter enemas no longer than 3 hours before the procedure time. This is standard of care for patients undergoing routine flexible sigmoidoscopy. Subjects will also be asked to take 4 mg of loperamide (over-the-counter anti-diarrheal medication) one half hour before the FMT.

Subjects will be placed in the left lateral decubitus position with elevated hips in a designated private room in clinic or endoscopy suite for 90 minutes of FMT intervention. Subjects will be asked to rotate 180 degrees slowly during a 10-minute period. Subjects will be monitored for 30 minutes after FMT for any adverse events and discharged.

Concomitant Medications: Permitted concomitant medications: Patients may remain on chronic medications during the study period.

Prohibited medications: Patients will need to be off antibiotics and probiotics for at least 48 hours prior to fecal transplant.

End of the study: The end of study for each patient is defined as the date of the last visit (i.e. 3 month visit post-transplant).

Timeline: All the proposed aims and enrollments will be carried out within 24-month period. We do anticipate a large proportion of eligible subjects may travel to Emory as the interest in this therapeutic platform is quite high with us already receiving numerous requests.

3.7 Inclusion and Exclusion criteria

The following lists outline key criteria used by the principal investigator, sub-investigators, and study team to determine who can and cannot be in this study.

Inclusion Criteria

1. Males and females between the ages of 18 and 80 (inclusive) with ulcerative colitis-associated IPAA with and without pouchitis
2. Eligible patients will be identified through the Emory Clinic (TEC), St. Joseph's Hospital (SJH), and Emory University Hospital (EUH)
3. Signed informed consent

Exclusion Criteria

1. Age <18 years or >80 years of age
2. Exposure to immunosuppressive therapy within the 4 weeks prior to enrollment or their expected use within 1 month of FMT
3. Concomitant *Clostridium difficile* infection
4. Suspected Crohn's disease
5. Documented active infection of any kind
6. Patients on anti-coagulant therapy, with platelet count less than 50,000, significant anemia with hemoglobin< 7, or those with other conditions that place them at increased risk of bleeding
7. Pregnant or breastfeeding women (pregnancy will be verified using urine pregnancy kits)
8. Need for imminent surgery
9. Absolute neutrophil count (ANC) < I 000 or history of opportunistic infection
10. Administration of any investigational drug within one month before FMT

Demographic data, including prior history of pouchitis with sub-classification into chronic antibiotic resistant (refractory) pouchitis, chronic antibiotic use as well as previous treatments will be recorded. Measures of waist and hip circumference and waist to hip ratio will be calculated and recorded. Stool samples, urine, and serum samples will be collected. Patients will be screened at entry for enteric pathogens (*C. difficile*, routine bacterial stool culture, ova and parasites) and positive results will exclude entry to the study.

Fecal samples from subjects will be analyzed for microbiome genetics, and metabolomic profiles before and four weeks after therapy. Scored symptoms according to the modified pouch disease activity index (mPDAI) (**Appendix 1**) as proposed by Shen et al. will also be

documented before pouch endoscopy. Investigation with biopsy incorporating both endoscopic and histologic criteria will be pursued. As per Shen's protocol when developing the modified PDAI (mPDAI), biopsies will be taken from the posterior wall of the pouch if the pouch has abnormal endoscopic appearance and from areas of maximal inflammation (Shen 2003). In addition to the two to four biopsies that are taken for the pathologist, additional biopsies will be taken to study the epithelial microbiome.

No more than 6 total biopsies will be taken. A single gastrointestinal pathologist, blinded to the clinical presentations and endoscopic findings, will assess the pouch biopsies for grade of inflammation. On the basis of the criteria proposed by Sanborn et al patients with a total PDAI of seven or more will be classified as having pouchitis. Symptomatic patients without endoscopic and histologic evidence of pouchitis and a PDAI less than seven points will be defined as not having pouchitis. All patients undergoing pouchoscopy will receive a universal donor fecal transplant deposited in the most proximal limit of the afferent limb after the mucosal biopsies are obtained. There will be 2 categories of patients. Patients with pouchitis symptoms with endoscopic and histologic evidence of pouchitis, and patients with pouchitis symptoms without endoscopic features, but with histologic evidence of pouchitis.

We will assess patient perceived improvement in symptoms and quality of life by asking participants to complete two surveys--Short Quality of Life Questionnaire for Inflammatory Bowel Disease and the Cleveland Global Quality of Life. Participants will be asked to complete these surveys at enrollment and again at 4 weeks and I2 weeks post-FMT procedure (**Appendix 4**).

4. BIOLOGICAL SPECIMENS TO BE ACQUIRED

1. *Fecal sample*- The patient will submit a stool specimen in a container for genomic analysis at week 0, week 2, week 4, and week 12. Prior to enrollment, the patients will undergo PCR testing for C. *difficile,* routine stool culture, and ova and parasites. Any remaining stool sample will be used for research purposes for microbiota evaluation by 16S sequencing.

2. *Mucosal Pouch Tissue* -A total of up to 6 mucosal biopsies will be taken during routine standard of care flexible sigmoidoscopy procedure in patients undergoing investigation for presumed pouchitis or for routine surveillance of the pouch in patients without pouchitis. Two to four will be sent to pathology for assessment of histology. Two to four biopsies will be obtained for research purposes. No more than a total of 6 will be taken. All non-research biopsies will be placed in saline and transported to the Pathology Department at Emory immediately. Research related

biopsies will be collected by the study team and transported to the Pathology Department at Emory University.

3. *Stool*- Additional stool will be collected after treatment 4 weeks and 3 months after fecal microbial transplant (FMT). If the first and second study visit occur within 48hours, the second collection of biologic samples will be at the discretion of the study doctor.

Samples collected through this study will be given a de-identified code that is not derived from or related to information about the individual and is not otherwise capable of being translated to identify an individual. The samples will then be barcoded and transported to a central core laboratory at Emory University managed by Dr. Colleen Kraft. Fecal and mucosal samples will be saved and stored in locked refrigerators/freezers in Dr. Kraft's laboratory in the Woodruff Memorial Building. DNA will be extracted for sequencing at this laboratory using automated extraction. Sequencing of the 16S regions will be performed in Dr. Kraft's laboratory.

Plasma and urine samples will be collected from each patient at study visits as illustrated in the study procedures table, and will be barcoded and saved in locked freezers in Dr. Kraft's laboratory in the Woodruff Memorial Building until all the samples are collected. At such time, they will be transported to Dr. Dean Jones' laboratory in the Whitehead building for metabolomic profiling.

Biological samples will not be collected on subjects who complete study visits via telephone follow-up.

5. ADDITIONAL DATA COLLECTION

Additional information will also be gathered from the patient's medical chart. This will include demographic information including age, sex and race, as well as clinical information such as
pre-existing medical conditions, allergies, medicines prescribed, diseases that run in the patient's family, any previous surgical procedures, and the results of any previous medical testing (including biopsy results).

Subjects will be asked to complete a Food Recall Questionnaire at enrollment, week 4 and week 12 **(Appendix 5).**

Any data obtained from the patient's medical record will have any and all identifiable information removed and will be kept in a password protected electronic file accessible only

by the authorized study team.

6. REQUIRED NUMBER OF PARTICIPANTS

A total of 11 patients with IPAA/UC related pouchitis will be enrolled over 12 months as part of this pilot study.

7. PATIENT SAFETY

The risks of this study are limited to those inherent with mucosal tissue biopsy and fecal microbial transplant with use of a flexible sigmoidoscopy.

The primary risk associated with a mucosal tissue biopsy is bleeding from the area of biopsy. Significant bleeding from the biopsy site is uncommon, but possible (**I** in 1,000). Even more rarely (**I** in 3,000), a perforation can be made at the site of biopsy which would require surgery to repair. To minimize these risks, standard practice is to not take a biopsy of large vessel, not to repair.

To minimize these risks, standard practice is to not take a biopsy of large vessel, not more than one biopsy from a given mucosal site and not to over distend the bowel lumen while taking biopsies. Furthermore, mucosal biopsies are not painful as the bowel lining does not have pain fibers to sense pain. In addition, all the above mentioned risks apply regardless if the biopsy is done for donating tissue to this study or for diagnostic purposes. Mucosal biopsy sampling will be performed by medical staff with expertise in performing these procedures.

Research associated peripheral blood draws may result in some discomfort or risk of bruising at the site of the needle entry. There is a remote risk of fainting from a vasovagal response or local infection. These risks will be minimized using trained phlebotomists and aseptic technique. Sample collections will be performed with the patient seated or recumbent.

There is not significant risk to urine donation. There is not significant risk to stool donation.

The primary risks of fecal microbiota transplantation are transmission of infection and potential immune stimulation. We will be using a standard donor who will be screened for transmittable infections through serologic and stool testing. In addition, the donor will be screened clinically for illness **in** which there is potential for disruption of normal gastrointestinal flora such as irritable bowel syndrome. The donor will be excluded from the study if he is found to have such an illness. The risk associated with placement of the transplant enema through flexible sigmoidoscopy is discomfort. Small volume (100 mL-200mL) enemas will be used to minimize this risk.

7.1 Women of Childbearing Potential (WOCBP)

Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive serum human chorionic gonadotrophin laboratory test (>5 miU/mL) will be excluded from participation in this study.

Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, including women whose career, lifestyle, or sexual orientation precludes intercourse with a male partner and women whose partners have been sterilized by vasectomy or other means must agree to use two birth control methods throughout their participation in the study. The two methods can be a double barrier method or a barrier method plus a hormonal method.

Subjects will be counseled by the study team regarding adequate forms of contraception.

- Adequate barrier methods of contraception include: diaphragm, condom (by the partner), intrauterine device (copper or hormonal), sponge or spermicide. Hormonal contraceptives include any marketed contraceptive agent that includes an estrogen and/or a progestational agent.
- Reliable contraception should be maintained throughout the study.

  Women are considered post-menopausal and not of child bearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or six months of spontaneous amenorrhea with serum FSH levels > 40 mlU/mL and estradiol < 20 pg/mL] or have had surgical bilateral oophorectomy (with or without hysterectomy) at least six weeks prior to study enrollment. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment is she considered not of child bearing potential.

8. ANTICIPATED BENEFITS

Given that over 50% of patients will have a recurrent episode of pouchitis after an initial episode. Fecal microbiota transplant has the potential for preventing further episodes and obviate the need for repeated course of antibiotics if it proves to be effective.

9. DATA ANALYSIS

Patients will be identified by physicians on this protocol in The Emory Clinic or Emory

University Hospital. Patients will be consented using an IRB approved informed consent and undergo the procedure on their follow up visit.

Since this is a pilot study, no formal power calculation is provided. Descriptive statistics and ANOVA will be used to detect differences between groups. One-sample t-test or Wilcoxon's signed rank-tests will be used to see if there were any significant changes in PDAI and QOL scores before and after transplant. A Simon's two stage design will be used to decide whether to enroll more patients in the future.

Data relating to laboratory results will be obtained through the EMR computerized patient information systems EML microbiological reports. A dataset will be created in a secure file, with access given only to authorized study staff.

A list of possible cases that have been reviewed for inclusion will be secured by study staff. This list will contain subject name, birth date, medical record number, and the number of the corresponding data abstraction sheet. Such a list will be maintained so as not to duplicate the abstraction of a single subject. Once the data from all included subjects is entered into a database, the list of identifiable patient information will be destroyed.

Data analysis will be performed with Microsoft Excel, SAS Institute software, and SPSS software.

10. END POINTS

The primary endpoint of this study will be resolution of clinical pouchitis symptoms using the clinical component of the modified pouchitis disease activity index without relapse for 3 months. Secondary endpoints include 16S ribosomal gene sequencing and metabolomic profiles that are more favorable (as defined by the donor microbiota). An additional secondary endpoint will be improvement of quality of life as determined by QOL questionnaires.

11. DATA SAFETY MONITORING PLAN

A committee consisting of two physicians, Dr Jay Varkey (Assistant Professor, Division of Infectious Diseases) and Dr Jenny Han MD, (Assistant Professor, Division of Pulmonary and Critical Care) has been created. Dr. Varkey and Dr. Han have agreed to be part of the committee and will not have any direct participation or interests in the study. The committee will meet emergently in the event of the reported SAE and consider the future course based on the outcome of the event. If no SAE is reported, the committee will meet every 6 months to assess the safety and review the AEs.

12. CLINICAL ADVERSE EVENTS

An adverse event is any untoward medical occurrence in a subject administered the study procedure and which does not necessarily have a causal relationship with the study procedure. An adverse event can thus be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of the study drug, whether or not considered related to the study drug. Pre-existing conditions that worsen during the study are considered adverse events. For the purposes of this study, AE's related to study procedures will be collected.

12.1 Severity

Severity of the adverse event will be defined as follows:
Mild – Discomfort noticed but no disruption of normal daily activity Moderate – Discomfort sufficient to reduce or affect daily activity Severe – Inability to work or perform normal daily activity Life Threatening – Represents an immediate threat to life.

12.2 Determination of Relationship of Adverse Event to FMT

There will be four categories of possible relationship between the adverse event and FMT. Determination of drug-relatedness of the adverse event to FMT will be determined by the investigator.

Category 1: Probable (must have first three)
A probable relationship will be assigned to an adverse event that is considered, with a high degree of certainty, to be related to FMT. An adverse event may be considered probable if:

1. It follows a reasonable temporal sequence from administration of the FMT.
2. It cannot be reasonable explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
4. It follows a known pattern of response to FMT

Category 2: Possible (must have first two)
A possible relationship will be assigned to an adverse event when the connection with FMT administration appears unlikely but cannot be ruled out with certainty. An adverse event will be considered possible if:

1. It follows a reasonable temporal sequence from administration of FMT
2. It may have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject
3. It follows a known pattern of response to FMT

Category 3: Remote (must have first two)

An adverse event will be considered remote if:

1. It does not follow a reasonable temporal sequence from administration of FMT
2. It may readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
3. It does not follow a known pattern of response to FMT

Category 4: Unrelated

An adverse event will be considered unrelated if it is judged to be clearly and incontrovertibly due only to extraneous causes such as disease, environment, etc. while not meeting the criteria for drug relationship as listed above for remote, possible, or probable.

12.3 Serious Adverse Event

A serious adverse event is any experience that suggests a significant hazard, contraindication, side effect or precaution and meets at least one of the following criteria:

- Is fatal (results in death);
- Is life-threatening;
- Requires in-patient hospitalization or prolongs existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Results in a congenital anomaly/birth defect;
- Is medically significant or requires intervention to prevent one or other of the outcomes listed above.

The clinical judgment of the investigator shall be used in deciding whether a certain situation may warrant consideration as a serious adverse event but may not meet the above criteria. This medical event may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the outcomes listed in the definitions above.

12.4 Reporting of Serious Adverse Events

All adverse events considered serious and unexpected shall be reported to

- FDA (via form MedWatch 3500-see Appendix 3) within 10 calendar days (by fax)
- Emory University IRB within 10 business days
- DSMB within 7 days

13. CONFIDENTIALITY

The Investigator will take the following precautionary measures to protect the privacy and

confidentiality of the subject's research and/or medical records.

- The donated blood and mucosal tissue collected through this study will be given a code (or number) that is not derived from or related to information about the individual and is not otherwise capable of being translated to identify an individual.
- Access to medical and research records will be limited to clinical and research personnel assigned to data collection and monitoring for this study. Only those medical records pertaining to the study will be reviewed.

- Research records will be de-identified and maintained in a secured location.
- Dates recorded on research records will be limited to those essential to completion of the study.
- No individual identifiers will be used in any publications or reports resulting from the study.

PI and authorized study staff will have access to a secure file containing linkages of biological samples to subject identities.
A copy of the consent form will be included in the subject's medical research record.

13.1 Compliance Statement

The study will be conducted in accordance with the protocol, Good Clinical Practices, the relevant ICH guidelines, the applicable regulatory requirements, and the ethical principles that have their origins in the Declaration of Helsinki. As required by United States Food and Drug Administration (FDA) Code of Federal Regulations (CFR) (21 CFR 56) and the Declaration of Helsinki, the study protocol, amendments, and Informed Consent form will be reviewed and approved, according to 21 CFR §50 and §56, respectively, by IRB.

13.2 Subject Information and Consent

The study will be explained to each subject, they will have the opportunity to read the informed consent document, ask questions and have their questions answered to their satisfaction. Alternatives to participation in this study will be explained. Patients will be informed that their participation in this study is voluntary and that their decision regarding participation will not affect their ability to receive care. Prior to any study procedures, the IRB approved Informed Consent form will be signed by each subject. A copy of the consent form will be given to each patient approached for participation in the study.

The Emory University IRB will review the written Informed Consent Form. These documents will meet requirements for subject information, as outlined in FDA regulations (21 CFR 50), ICH Guideline E6, and the Declaration of Helsinki.

14. COSTS

14.1 Donor Activities Related Costs

The cost of all donor serology and stool testing will be covered by the sponsor. Fecal sample preparation will also be covered by the sponsor.

14.2. Research Participant Activities Related Costs

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All physical exams will be covered by principal investigator and/or sub-investigator effort. Waist to hip circumference will be taken during the physical exam for each visit, therefore, they will also be considered as physician effort. Urine pregnancy kits used during for screening WOCBP will be covered by sponsor funds. All associated labs and specimen processing (i.e. stool collection, plasma collection, urine collection, and CBCs with differential and platelet count will also be covered. The flexible sigmoidoscopy with moderate sedation will be covered by the sponsor. Eligible research participants who enroll in the study will not be paid for their participation.

15. REFERENCES

1. Zelia GC, Hait EJ, Glavan T, Gevers D, Ward DV, Kitts CL, Korzenik JR; Distinct microbiome in pouchitis compared to healthy pouches in ulcerative colitis and familial adenomatous polyposis. lntlamm Bowel Dis. 2011 May; 17(5):1092-100

2. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JF, Tijssen JG, Speelman P, Dijkgraaf, MG, Keller JJ; Duodenal infusion of donor feces for recurrent *Clostridium difficile.* N. Engl J Med.2013 Jan 31;368(5):407-15.

3. Anderson JL, Edney RJ, Whelan K; Systematic review: faecal microbiota transplantation in the management of inflammatory bowel disease. Aliment Pharmacol Ther. 2012 Sep;36(6):503-16.

4. Kunde S1, Pham A, Bonczyk S, Crumb T, Duba M, Conrad H Jr, Cloney D, Kugathasan S.;Safety, tolerability, and clinical response after fecal transplantation in children and young adults with ulcerative colitis.J Pediatr Gastroenterol Nutr. 2013 Jun;56(6):597-601. doi: 10.1097/MPG.ObO l3e318292fa0d.

5. Shen B, Achkar JP, Connor JT, Ormsby AH, Remzi FH, Bevins CL, Brzezinski A, Bambrick ML, Fazio VW, Lashner BA.Modified pouchitis disease activity index: a simplified approach to the diagnosis of pouchitis. Diseases of Colon and Rectum.

2003 Jume 46(6): 748-53.

6. Khalili H1, Ananthakrishnan AN, Konijeti GG, Higuchi LM, Fuchs CS, Richter JM, Chan AT. Measures of obesity and risk of Crohn's disease and ulcerative colitis. Inflamm Bowel Dis. 2015 Feb;21(2):361-8.

APPENDIX 1:
MODIFIED POUCH DISEASE ACTIVITY INDEX (MPDAI)

Appendix 1: Modified PDAI

Table 1.
The Pouchitis Disease Activity Index[4]

Criteria	Score
Clinical	
Stool frequency	
Usual postoperative stool frequency	0
1–2 stools/day :- postoperative usual	1
3 or more stools/day :- postoperative usual	2
Rectal bleeding	
None or rare	0
Present daily	1
Fecal urgency or abdominal cramps	
None	0
Occasional	1
Usual	2
Fever (temperature :- 37.8° C)	
Absent	0
Present	1
Endoscopic inflammation	
Edema	1
Granularity	1
Friability	1
Loss of vascular pattern	1
Mucous exudates	1
Ulceration	1
Acute histologic inflammation	
Polymorphic nuclear leukocyte infiltration	
Mild	1
Moderate + crypt abscess	2
Severe + crypt abscess	3
Ulceration per low-power field (mean)	
:-25%	1
25–50%	2
:-50%	3

APPENDIX 2:
FECAL DONOR QUESTIONNAIRE

Appendix 2: Fecal donor questionnaire

Fecal Transplant Donor History Questionnaire

Donor Name_____

Patient Name_____

Date_____

Please Circle Yes or No

*Answers will be kept strictly confidential, please answer honestly

Are you

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

Have you

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

In the past 8 weeks have you

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

In the past 12 months have you

7. Had a blood transfusion?	Yes	No

FMT in UC-associated Pouchitis
v.date 10.30.2015

8. Had a transplant (organ, tissue, bone marrow)? Yes No

9. Had a skin or bone graft? Yes No

10. Come into contact with someone else's blood? Yes No

11. Had an accidental needle stick? Yes No

12. Had sexual contact with anyone who has HIV/AIDS? Yes No

13. Had sexual contact with a prostitute or anyone else who takes money or drugs as payment for sex? Yes No

14. Had sexual contact with anyone who has ever used needles to take drugs or steroids, or anything NOT prescribed by their doctor? Yes No

15. Had sexual contact with anyone who has hemophilia or has used clotting factor concentrates? Yes No

16. *Female donors*: Had sexual contact with a male who has ever had sexual contact with another male (male donors circle "I am male)? Yes No I am male

17. Had sexual contact with a person who has hepatitis? Yes No

18. Lived with a person who has hepatitis? Yes No

19. Had a tattoo? Yes No

20. Had an ear or body piercing? Yes No

21. Been treated for syphilis or gonorrhea? Yes No

22. Been in lockup, jail or prison for >72 hours? Yes No

In the past three years have you

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

From 1980 through 1996

24. Did you spend time that adds up to three (3) months Yes No

or more in the United Kingdom?

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

From 1980 to the present, did you

26. Spend time that adds up to five (5) or more years in Europe? Yes No
27. Receive a blood transfusion in the United Kingdom or France? Yes No

From 1977 to the present, have you

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

Have you EVER

30. tested positive for HIV/AIDS virus? Yes No
31. used needles to take drugs or steroids or anything NOT prescribed by your doctor? Yes No
32. used clotting factor concentrates? Yes No
33. had viral hepatitis? Yes No
34. had any type of cancer (including leukemia)? Yes No
35. had sexual contact with anyone who was born or lived in Africa? Yes No
36. been in Africa? Yes No
37. had sex for drugs or money? Yes No

38. had any of the following gastrointestinal diseases or other medical problems?
    - Irritable bowel syndrome? Yes No
    - Crohn's disease? Yes No
    - Ulcerative Colitis? Yes No
    - Chronic diarrhea? Yes No

- Gastrointestinal cancers? Yes No
- Celiac disease? Yes No
- Morbid obesity? Yes No
- Metabolic syndrome Yes No
- Colon polyps Yes No
- Chronic fatigue Yes No
- Suppressed immune system? Yes No
- Allergic disorder? Yes No

39. received a dura mater (brain covering) graft? Yes No

40. received growth hormone made from human Pituitary glands? Yes No

41. Have any of your relatives had Creutzfeldt-Jakob disease? Yes No

General Medical History

42. Have you had any gastrointestinal surgery (for example: appendectomy, gallbladder surgery, gastric bypass) Yes No
If yes, please list: _____

43. Do you have any autoimmune diseases (for example: Rheumatoid arthritis, Multiple Sclerosis, Lupus) Yes No

If yes, please list:_____

44. Have you ever been treated for any cancer or malignancy? Yes No

45. What is your weight? _____ What is your height? _____

APPENDIX 3:
MEDWATCH FORM

Please see attached US Food and Drug Administration Document entitled FDA 3500.

APPENDIX 4:
QUALITY OF LIFE SURVEY TOOLS

Appendix 4: Quality of Life Survey tools

Cleveland Global Quality of Life Score	
Please rate the following on a scale of 0-10 (where 10 is the best)	
Current Quality of Life	
Current Quality of Health	
Current Energy Level	

Short Quality of Life Questionnaire for Inflammatory Bowel Disease

Name _____ Date_____

This questionnaire is designed to find out how you have been feeling during the last 2 weeks. You will be asked about symptoms you have been having as a result of your inflammatory bowel disease, the way you have been feeling in general, and how your mood has been. Please circle the number of your choice below each question.

1. How often has the feeling of fatigue or being tired and worn out been a problem for you during the past 2 weeks?
   1. All of the time
   2. Most of the time
   3. A good bit of the time
   4. Some of the time
   5. A little of the time
   6. Hardly any of the time
   7. None of the time

2. How often during the last 2 weeks have you delayed or canceled a social engagement because of your bowel problem?
   1. All of the time
   2. Most of the time
   3. A good bit of the time
   4. Some of the time
   5. A little of the time
   6. Hardly any of the time
   7. None of the time

3. As a result of your bowel problems, how much difficulty did you experience doing leisure or sports activities you would liked to have done during the past 2 weeks?
   1. A great deal of difficulty; activities made impossible
   2. A lot of difficulty
   3. A fair bit of difficulty
   4. Some difficulty
   5. A little difficulty
   6. Hardly any difficulty
   7. No difficulty; the bowel problem did not limit sports or leisure activities

4. How often during the past 2 weeks have you been troubled by pain in the abdomen?
   1. All of the time
   2. Most of the time
   3. A good bit of the time
   4. Some of the time
   5. A little of the time
   6. Hardly any of the time
   7. None of the time

Irvine, E. J., et. al. The short inflammatory bowel disease questionnaire: A quality of life instrument for community physicians managing inflammatory bowel disease. Am J Gastroenterology. 1996; 91(8):1571-8 Aug.

5. How often during the past 2 weeks have you felt depressed or discouraged?
   1. All of the time
   2. Most of the time
   3. A good bit of the time
   4. Some of the time
   5. A little of the time
   6. Hardly any of the time
   7. None of the time

6. Overall, in the past 2 weeks, how much of a problem have you had with passing large amounts of gas?
   1. A major problem
   2. A big problem
   3. A significant problem
   4. Some problem
   5. A little trouble
   6. Hardly any trouble
   7. No trouble

7. Overall, in the past 2 weeks, how much of a problem have you had maintaining or getting to the weight you would like to be?
   1. A major problem
   2. A big problem
   3. A significant problem
   4. Some problem
   5. A little trouble
   6. Hardly any trouble
   7. No trouble

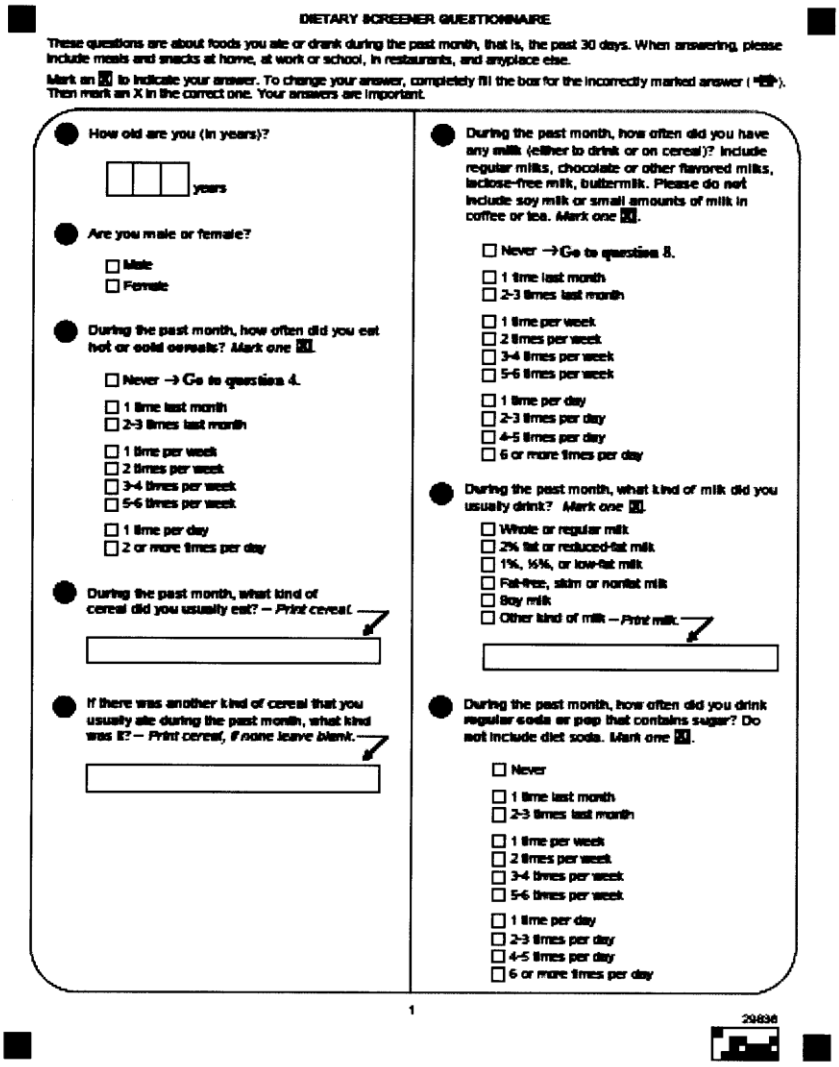
8. How often during the past 2 weeks have you felt relaxed and free of tension?
   1. All of the time
   2. Most of the time
   3. A good bit of the time
   4. Some of the time
   5. A little of the time
   6. Hardly any of the time
   7. None of the time

9. How much of the time during the past 2 weeks have you been troubled by a feeling of having to go to the bathroom even though your bowels were empty?
   1. All of the time
   2. Most of the time
   3. A good bit of the time
   4. Some of the time
   5. A little of the time
   6. Hardly any of the time
   7. None of the time

10. How often during the past 2 weeks have you felt angry as a result of your bowel problem?
    1. All of the time
    2. Most of the time
    3. A good bit of the time
    4. Some of the time
    5. A little of the time
    6. Hardly any of the time
    7. None of the time

Irvine, E. J., et. al. The short inflammatory bowel disease questionnaire: A quality of life instrument for community physicians managing inflammatory bowel disease. Am J Gastroenterology. 1996; 91(8):1571-8 Aug.

APPENDIX 5:
FOOD RECALL QUESTIONNAIRE



DIETARY SCREENER QUESTIONNAIRE

These questions are about foods you ate or drank during the past month, that is, the past 30 days. When answering, please include meals and snacks at home, at work or school, in restaurants, and anyplace else.

Mark an ☒ to indicate your answer. To change your answer, completely fill the box for the incorrectly marked answer (■). Then mark an X in the correct one. Your answers are important.

1. How old are you (in years)?

   ☐☐☐ years

2. Are you male or female?

   ☐ Male
   ☐ Female

3. During the past month, how often did you eat hot or cold cereals? *Mark one* ☒.

   ☐ Never → Go to question 4.

   ☐ 1 time last month
   ☐ 2-3 times last month

   ☐ 1 time per week
   ☐ 2 times per week
   ☐ 3-4 times per week
   ☐ 5-6 times per week

   ☐ 1 time per day
   ☐ 2 or more times per day

4. During the past month, what kind of cereal did you usually eat? — *Print cereal.*

   ☐

5. If there was another kind of cereal that you usually ate during the past month, what kind was it? — *Print cereal, if none leave blank.*

   ☐

6. During the past month, how often did you have any milk (either to drink or on cereal)? Include regular milks, chocolate or other flavored milks, lactose-free milk, buttermilk. Please do not include soy milk or small amounts of milk in coffee or tea. *Mark one* ☒.

   ☐ Never → Go to question 8.

   ☐ 1 time last month
   ☐ 2-3 times last month

   ☐ 1 time per week
   ☐ 2 times per week
   ☐ 3-4 times per week
   ☐ 5-6 times per week

   ☐ 1 time per day
   ☐ 2-3 times per day
   ☐ 4-5 times per day
   ☐ 6 or more times per day

7. During the past month, what kind of milk did you usually drink? *Mark one* ☒.

   ☐ Whole or regular milk
   ☐ 2% fat or reduced-fat milk
   ☐ 1%, ½%, or low-fat milk
   ☐ Fat-free, skim or nonfat milk
   ☐ Soy milk
   ☐ Other kind of milk — *Print milk.*

   ☐

8. During the past month, how often did you drink regular soda or pop that contains sugar? Do not include diet soda. *Mark one* ☒.

   ☐ Never

   ☐ 1 time last month
   ☐ 2-3 times last month

   ☐ 1 time per week
   ☐ 2 times per week
   ☐ 3-4 times per week
   ☐ 5-6 times per week

   ☐ 1 time per day
   ☐ 2-3 times per day
   ☐ 4-5 times per day
   ☐ 6 or more times per day

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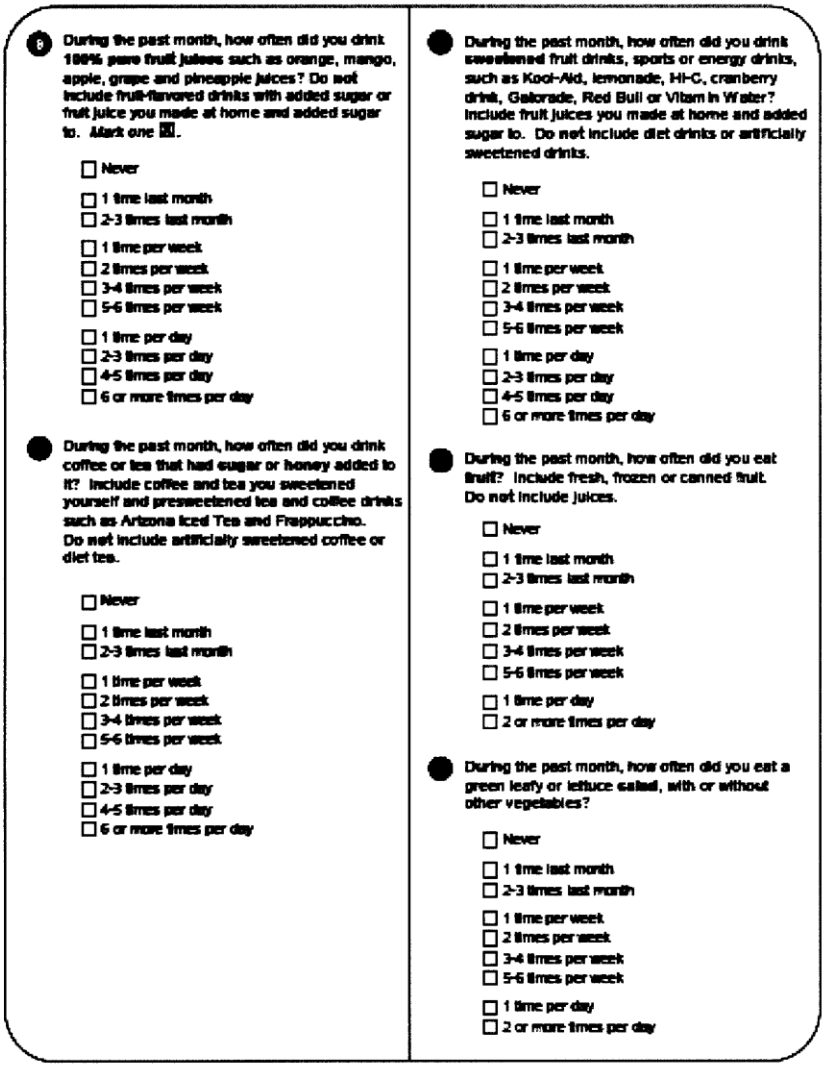

During the past month, how often did you drink 100% pure fruit juices such as orange, mango, apple, grape and pineapple juices? Do not include fruit-flavored drinks with added sugar or fruit juice you made at home and added sugar to. *Mark one* ☒.

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2-3 times per day
- ☐ 4-5 times per day
- ☐ 6 or more times per day

During the past month, how often did you drink coffee or tea that had sugar or honey added to it? Include coffee and tea you sweetened yourself and presweetened tea and coffee drinks such as Arizona Iced Tea and Frappuccino. Do not include artificially sweetened coffee or diet tea.

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2-3 times per day
- ☐ 4-5 times per day
- ☐ 6 or more times per day

During the past month, how often did you drink sweetened fruit drinks, sports or energy drinks, such as Kool-Aid, lemonade, Hi-C, cranberry drink, Gatorade, Red Bull or Vitamin Water? Include fruit juices you made at home and added sugar to. Do not include diet drinks or artificially sweetened drinks.

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2-3 times per day
- ☐ 4-5 times per day
- ☐ 6 or more times per day

During the past month, how often did you eat fruit? Include fresh, frozen or canned fruit. Do not include juices.

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

During the past month, how often did you eat a green leafy or lettuce salad, with or without other vegetables?

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

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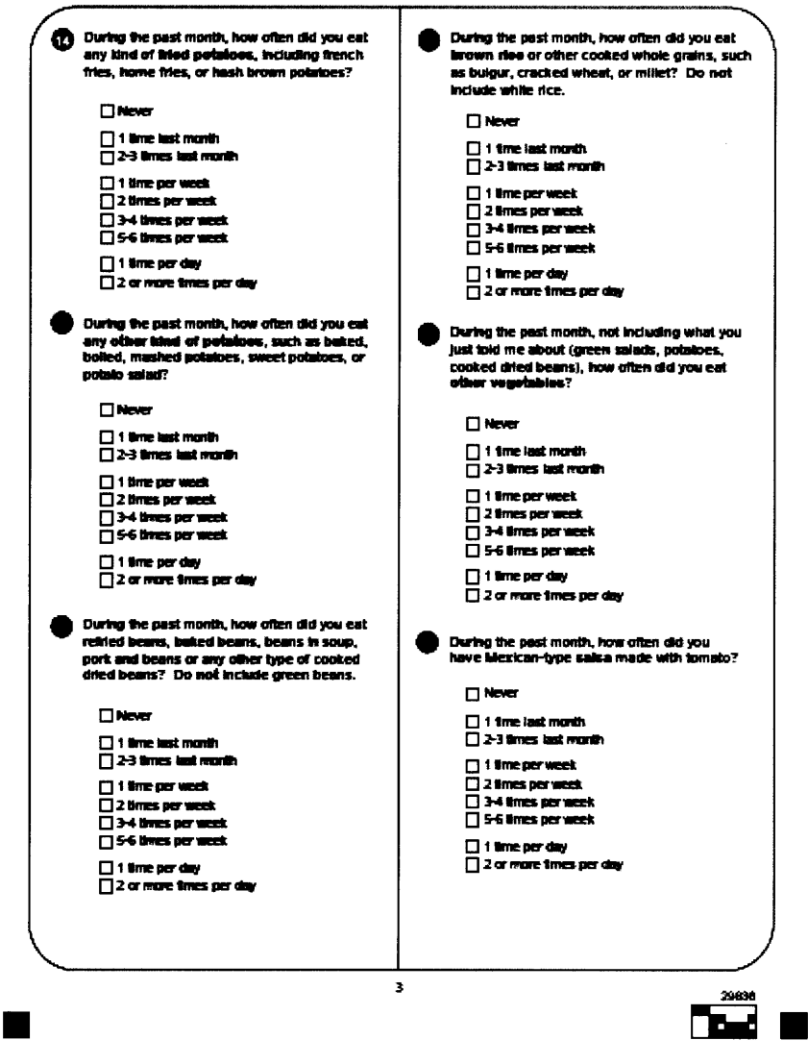

14 During the past month, how often did you eat any kind of fried potatoes, including french fries, home fries, or hash brown potatoes?

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

During the past month, how often did you eat any other kind of potatoes, such as baked, boiled, mashed potatoes, sweet potatoes, or potato salad?

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

During the past month, how often did you eat refried beans, baked beans, beans in soup, pork and beans or any other type of cooked dried beans? Do not include green beans.

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

During the past month, how often did you eat brown rice or other cooked whole grains, such as bulgur, cracked wheat, or millet? Do not include white rice.

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

During the past month, not including what you just told me about (green salads, potatoes, cooked dried beans), how often did you eat other vegetables?

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

During the past month, how often did you have Mexican-type salsa made with tomato?

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

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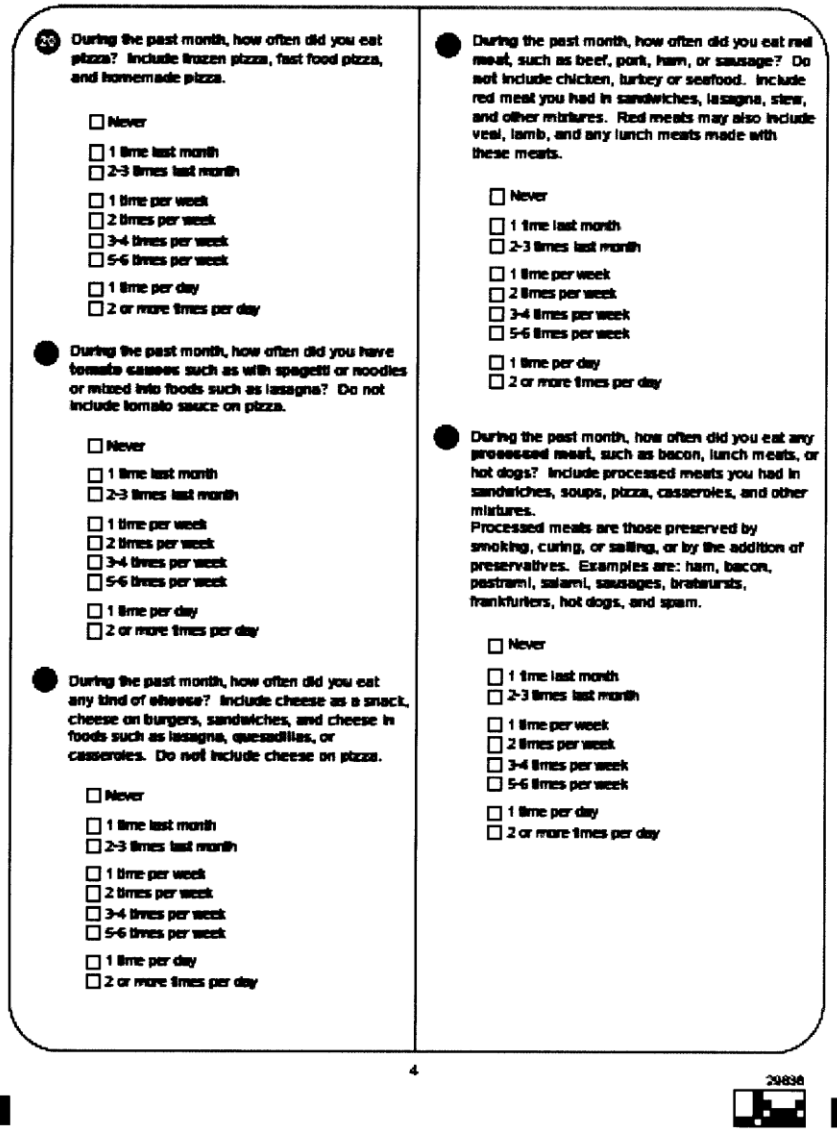

29. During the past month, how often did you eat pizza? Include frozen pizza, fast food pizza, and homemade pizza.

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

30. During the past month, how often did you have tomato sauces such as with spagetti or noodles or mixed into foods such as lasagna? Do not include tomato sauce on pizza.

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

31. During the past month, how often did you eat any kind of cheese? Include cheese as a snack, cheese on burgers, sandwiches, and cheese in foods such as lasagna, quesadillas, or casseroles. Do not include cheese on pizza.

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

32. During the past month, how often did you eat red meat, such as beef, pork, ham, or sausage? Do not include chicken, turkey or seafood. Include red meat you had in sandwiches, lasagna, stew, and other mixtures. Red meats may also include veal, lamb, and any lunch meats made with these meats.

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

33. During the past month, how often did you eat any processed meat, such as bacon, lunch meats, or hot dogs? Include processed meats you had in sandwiches, soups, pizza, casseroles, and other mixtures.
Processed meats are those preserved by smoking, curing, or salting, or by the addition of preservatives. Examples are: ham, bacon, pastrami, salami, sausages, bratwursts, frankfurters, hot dogs, and spam.

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

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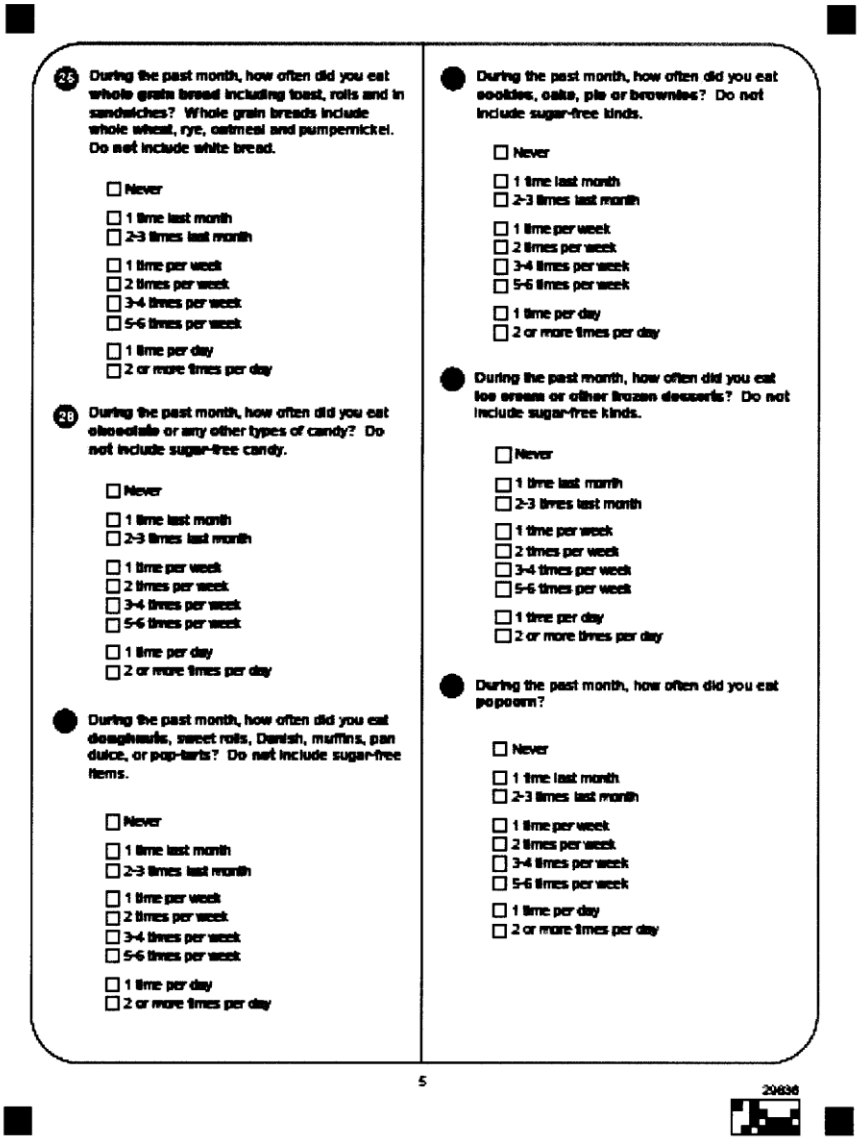

25 During the past month, how often did you eat whole grain bread including toast, rolls and in sandwiches? Whole grain breads include whole wheat, rye, oatmeal and pumpernickel. Do not include white bread.

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

26 During the past month, how often did you eat chocolate or any other types of candy? Do not include sugar-free candy.

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

27 During the past month, how often did you eat doughnuts, sweet rolls, Danish, muffins, pan dulce, or pop-tarts? Do not include sugar-free items.

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

28 During the past month, how often did you eat cookies, cake, pie or brownies? Do not include sugar-free kinds.

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

29 During the past month, how often did you eat ice cream or other frozen desserts? Do not include sugar-free kinds.

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

30 During the past month, how often did you eat popcorn?

- ☐ Never
- ☐ 1 time last month
- ☐ 2-3 times last month
- ☐ 1 time per week
- ☐ 2 times per week
- ☐ 3-4 times per week
- ☐ 5-6 times per week
- ☐ 1 time per day
- ☐ 2 or more times per day

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