

**Official Title:**

**Research on the mechanisms of different donors in  
fecal microbiota transplantation for treating  
ulcerative colitis**

**Date: April 22, 2026**

## **Study Protocol**

### **1. Background:**

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), affects over 2 million people worldwide [1]. Although biological therapies have significantly improved the treatment outcomes for UC, nearly two-thirds of patients experience diminishing drug responses over time, making it crucial to explore novel therapeutic approaches targeting the underlying pathophysiology of UC. UC is associated with alterations in gut microbiota, reduced microbial diversity, and changes in the relative abundance of dominant bacterial populations. Specifically, UC patients exhibit a marked decrease in gut microbiota diversity at the species level, with a reduction in *Firmicutes* (e.g., *Clostridium butyricum*) and an increase in *Actinobacteria*, *Proteobacteria* (e.g., *Escherichia coli*), *Enterobacteriaceae*, *Streptococcus*, and *Bacteroides* [2]. Given the association between gut microbiota alterations and IBD activity, several studies [3-5] have proposed microbiota-based therapies, particularly fecal microbiota transplantation (FMT), as a treatment for UC.

FMT involves the infusion of fecal material from healthy donors into patients to restore gut microbiota balance. It is currently recognized as an effective treatment for recurrent or refractory *Clostridium difficile* infections [6,7]. Numerous studies suggest that FMT, as a therapeutic tool to regulate gut microbial homeostasis, holds potential in treating UC and other diseases, although the biochemical and/or immune mechanisms underlying its effects remain unclear [8,9]. Paramsothy et al. [10] demonstrated the efficacy of autologous FMT compared to placebo, utilizing a protocol involving colonoscopy-guided FMT followed by daily enemas for 5 days per week over 8 weeks. However, the high financial burden of this approach limits its broader clinical application. Another study [11] revealed that donor FMT prepared anaerobically for 1-week treatment led to a higher likelihood of remission at 8 weeks compared to autologous FMT. Further research is needed to assess its safety and maintain long-term remission rates.

Our team's high-quality research findings indicate that the gut microbiota of populations in Yunnan's ethnic minority regions exhibits significantly higher diversity and regional specificity compared to urban populations. This has potential value in enhancing FMT efficacy. Previous studies [12] revealed ethnic and regional differences in IBD prevalence in Yunnan Province, with lower rates among the Dai, Bai, and Miao ethnic groups compared to the Han population. An analysis of contributing factors highlighted the protective role of traditional ethnic diets, which increase gut microbial and viral diversity and probiotics content, thereby reducing UC prevalence. Based on this, the differences between donors in FMT may affect treatment outcomes, emphasizing the importance of identifying "high-quality" donors who maximize efficacy and minimize adverse reactions.

### **2. Objectives:**

To investigate the impact of different donors (Han and Bai ethnic donors) on the efficacy of FMT in treating UC and identify "high-quality" donors.

To isolate and culture key bacterial strains from Bai ethnic donors that contribute to their therapeutic effect.

### **3. Results:**

1.Primary Outcome: The primary outcome is a composite of steroid-free clinical remission

together with endoscopic remission or response at week 12, defined as a total Mayo score of  $\leq 2$  points with no individual sub-score  $>1$  point, and at least a 1 point reduction from baseline in the endoscopy sub-score (MES).

## 2. Secondary Outcomes:

① Steroid-free clinical remission ( defined as a total Mayo score of  $\leq 2$  points with no individual sub-score  $>1$  point )

② Steroid-free clinical response ( defined as a reduction of 3 points or more on the Mayo score, a 50% or greater reduction from baseline in combined rectal bleeding plus stool frequency Mayo sub-scores, or both )

③ Steroid-free endoscopic response ( defined as a Mayo endoscopy sub-score of 1 or less, with a reduction of at least 1 point from baseline )

④ Variations in fecal microbiota composition, function, and metabolites at weeks 0, 1, 8, and 12 within each group (Han donor group and Bai donor group)

⑤ Duration of microbiota recovery from baseline across weeks 0, 1, 8, and 12 within groups.

⑥ Intergroup differences in recipients' microbiota composition between the Han and Bai donor groups.

⑦ Proportional contributions of recipient, donor, or mixed-origin microbiota in FMT recipients.

⑧ Dominant bacterial strains in highly effective cases of FMT treatment.

## 4. Methods:

**1. Study Design:** This is a patient single-blind, single-center, randomized, parallel-group pilot clinical trial without placebo control. Patients will be randomized to either the Han donor or the Bai donor and followed up for a 12-week period. Subjects will undergo follow-up assessments for up to 12 weeks in accordance with the same schedule as the blinded phase (i.e., FMT administration at baseline, weekly telephone follow-up at week 1, 8, and on-site follow-up visits at week 12). Subjects who are randomized to the FMT arm or who do not wish to enter the open-label phase will be deemed to have completed the study, and no further follow-up will be arranged.

Note: Single-center means all experimental activities are conducted at the same location.

## 2. Study Population:

Participants will be recruited through press conferences, advertisements, online social media platforms, health seminars, university campuses, public transportation stations, and clinics. Subordinates, students, or employees must input their respective institutional affiliations. Only participants with adequate time to complete the consent process and deliberate on participation will be enrolled. Participants voluntarily decide to participate without coercion from investigators. Non-participation will not impact employment or academic status.

### Inclusion Criteria:

(1) Age between 14 and 79 years (inclusive), any gender.

(2) Diagnosed with ulcerative colitis (UC) per established clinical, endoscopic, and histological standards, with a disease duration of over 3 months.

(3) Active mild-to-moderate UC, defined by a Mayo score of 4 – 10, including an endoscopic score  $\geq 1$  and a physician's global assessment score  $\leq 2$ .

(4) Stable baseline medication consisting of 5-aminosalicylic acid (mesalamine).

(5) Signed written informed consent.

### Exclusion Criteria:

- (1) Participants unable to provide informed consent, answer questionnaires, or supply samples.
- (2) Pregnant women or those attempting to conceive.
- (3) Participants unwilling to use effective contraception throughout the study.
- (4) Participants deemed in remission by investigators.
- (5) Evidence or history of toxic megacolon.
- (6) Isolated rectal inflammation (<5 cm in extent).
- (7) Diagnosed with Crohn's disease or indeterminate colitis.
- (8) Participants with perianal diseases (e.g., fistulae, anal fissures).
- (9) History of significant gastrointestinal surgery (e.g., colectomy) :
  - Minor surgeries will be reviewed case by case.
  - Patients with appendectomy within 3 months will be excluded.
- (10) Antibiotic use within the past 4 weeks for any reason, including for UC.
- (11) Steroid dependence requiring >20 mg prednisone or >9 mg budesonide daily at enrollment.
- (12) Recent or anticipated usage of prohibited drugs during the study period, including:
  - Rectal corticosteroids within 2 weeks prior to the first FMT.
  - Biologics (e.g., infliximab, adalimumab, vedolizumab) within 4 weeks prior to the first FMT.
  - Other major immunosuppressants (e.g., calcineurin inhibitors, antitumor drugs) within 12 weeks prior to treatment.
  - Probiotics within 4 weeks before the first FMT.
  - Experimental drugs or protocols within 12 weeks before the first FMT.
  - Anti-tuberculosis (TB or MAC) treatment within 4 weeks before the first FMT.

#### Permitted Medications:

Participants may continue using the following medications if doses are stable within specified timeframes before the first FMT:

- Oral 5-aminosalicylic acid (stable for 4 weeks).
- Azathioprine and methotrexate ( $\geq 90$  days of use with stable doses for 4 weeks).
- Oral prednisone ( $\leq 20$  mg/day, stable for 2 weeks, gradually tapered at a rate of 2.5 mg/week to discontinue by week 8).

Subjects should maintain the same doses of oral 5-aminosalicylates, thiopurines, and methotrexate during the study. For oral prednisolone, the dose had to be tapered off gradually, at a rate of 2.5mg per week, so that subjects were no longer exposed to steroids until week 8.

#### Prohibited Medications:

- Rectal corticosteroids (2 weeks before and throughout the study).
- Antibiotics, antifungals, antivirals, probiotics, or prebiotics (4 weeks before and throughout the study).
- Biologics or calcineurin inhibitors (12 weeks before and throughout the study).

Participants using prohibited medications during the study will remain enrolled, and outcomes will still be evaluated. All prohibited medication usage will be recorded.

### 3. Fecal Donor Recruitment

#### Inclusion Criteria:

- (1) Age between 12 and 45 years (inclusive), any gender.

(2) Body Mass Index (BMI) >18 and <25.

(3) Written informed consent provided.

Exclusion Criteria:

Fecal Donation:

(1) Individuals permanently disqualified from donating feces in other studies.

(2) Individuals meeting any of the following criteria.

Social Behavior:

(3) Current or past smokers.

(4) Current heavy alcohol consumers.

Diet:

(5) Currently adhering to a strict vegan diet.

(6) Use of probiotic supplements in the past two months.

Occupation:

(7) Occupations with high risk of blood-borne diseases.

Travel History:

(8) Resided for at least six months in countries at high risk for Creutzfeldt-Jakob disease between 1980 and 1996.

(9) Traveled to areas with a high incidence of infectious diseases in the past six months.

Medical History:

(10) History or presence of HIV, HTLV, hepatitis B or C, or other known infections.

(11) History or presence of tuberculosis.

(12) History of autoimmune diseases (e.g., connective tissue diseases, thyroid disorders, inflammatory arthritis, psoriasis, alopecia).

(13) History of or concurrent atopic diseases (e.g., asthma, atopic dermatitis, eczema, eosinophilic gastrointestinal diseases).

(14) History of cardiovascular or metabolic diseases (e.g., diabetes, hypertension, hyperlipidemia, hyperglycemia, heart disease, neurological disorders, fibromyalgia).

(15) History of or concurrent neurological diseases (e.g., multiple sclerosis, Parkinson's disease, Alzheimer's disease, dementia).

(16) History of chronic pain or chronic pain syndromes (e.g., chronic fatigue syndrome, fibromyalgia).

(17) History of or concurrent cancer.

(18) Clinically diagnosed psychiatric disorders or concurrent mental illnesses (e.g., depression, bipolar disorder, schizophrenia, eating disorders).

(19) History of congenital or chronic liver diseases.

Gastrointestinal Disorders:

(20) History of or concurrent celiac disease.

(21) History of or concurrent inflammatory bowel disease (IBD).

(22) History of or concurrent irritable bowel syndrome (IBS).

(23) History of idiopathic chronic constipation or chronic diarrhea.

(24) History of gastrointestinal malignancies or polyps.

(25) History of rectal bleeding or concurrent rectal bleeding.

Medication Use:

(26) History of using major immunosuppressants (e.g., calcineurin inhibitors, exogenous

corticosteroids, biologics, anti-tumor necrosis factor agents, systemic chemotherapy).

- (27) History of growth hormone therapy.
- (28) Use of antibiotics within the past two months.
- (29) Participation in experimental drug or vaccine trials within the past two months.
- (30) Vaccination within the past month.
- (31) Use of proton pump inhibitors (PPIs) in the past two months.

Illegal Drug Use or Substance Abuse:

- (32) Intravenous drug use without a clinical prescription.
- (33) Use of illegal drugs.
- (34) Use of recreational drugs.

Risky Sexual Behavior:

- (35) Engagement in sex work.
- (36) Sexual contact with sex workers.
- (37) Anonymous sexual encounters in the past 12 months.
- (38) Sexual contact with intravenous drug users in the past 12 months.
- (39) Sexual contact with men who have had sexual contact with other men in the past 12 months.
- (40) Male-to-male sexual behavior in the past 12 months.
- (41) History of sexually transmitted infections in the past 12 months.
- (42) Sexual contact with individuals confirmed to have HIV, HTLV, hepatitis, or syphilis in the past 12 months.

Other Behavior:

- (43) Previous imprisonment or detention in the past six months.
- (44) Tattoos or body piercings performed in the past six months.

Surgical History:

- (45) History of gastrointestinal surgery.
- (46) History of any organ transplantation (e.g., solid organ, bone marrow, cornea).

Family History:

- (47) First-degree relatives with IBD.
- (48) First-degree relatives diagnosed with colorectal cancer before age.

Screening Tests:

- (49) Abnormalities in any of the following screening tests:
  - Blood Tests: Complete blood count (CBC), biochemistry, ESR, CRP, hepatitis panel, HIV, syphilis, immunoglobulins and complement, T-SPOT, Torch 5, ANA/ANCA, EB DNA, and EB composite antibodies.
  - Stool Tests: Routine analysis, occult blood, parasites, worms (tapeworm, roundworm, hookworm, whipworm), schistosomes, Salmonella, Shigella, Clostridioides difficile (CDI), Helicobacter pylori (HP), and resistant bacteria (MDRA, MRSA, ESBL, CRE, VRE).
  - Urine Tests: Routine urinalysis and pregnancy test (if applicable for females).

Potential fecal donors will be recruited from the general population. Candidates will complete an interview and screening questionnaire, followed by laboratory testing. Fecal samples will be collected only from donors who meet all eligibility criteria.

## **5. Study Process:**

## Groups

Groups	Fresh fecal bacterial solution (Ethnic Han Donor)	Fresh fecal bacterial solution (Bai Ethnic Donor)
Planned enrollment	50 Patients	50 Patients
Planned completed 12-week follow-up	30 Patients	30 Patients

### 1.FMT:

Fresh fecal bacterial solution was obtained through the fecal bacterial transplantation isolation system (FMT-6A-50/12-AS, Nanjing), which was directly infused into the ileocecal region through the TET tube of colonoscopy within 30 minutes, 100 mL each time. The fresh fecal bacterial solution was given at an interval of one day for a total of 3 transplantations.

### 2.Mayo Score:

The Mayo score is calculated according to Table 1, with clinical remission defined as a total Mayo score  $\leq 2$  and no individual subscore  $>1$ .

Table 1 Mayo Score

Item	Score	Evaluation
1. Stool frequency <sup>a</sup>	0	Normal number of stools
	1	1-2 more than normal
	2	3-4 more than normal
	3	$\geq 5$ more than normal
2. Rectal bleeding <sup>b</sup>	0	No blood seen
	1	Streaks of blood with stool in in less than half of the cases
	2	Obvious blood with stools in most of the time
	3	Blood alone passed
3. Endoscopic findings	0	Normal mucosa or inactive disease
	1	Mild activity (erythema, decreased vascular pattern, mild friability)
	2	Moderate activity (marked erythema, lack of vascular pattern, friability, erosions)
	3	Severe activity (spontaneous bleeding, large ulcerations)
4. Physician's global assessment	0	Normal <sup>c</sup>
	1	Mild disease <sup>d</sup>
	2	Moderate disease <sup>e</sup>
	3	Severe disease <sup>f</sup>

a Each patient serves as their own control to establish the degree of abnormal stool frequency.

b The daily rectal bleeding score represents the most severe bleeding observed on a single day.

c No symptoms of colitis; the patient feels well, with a flexible sigmoidoscopy score of 0.

d Mild symptoms with mild abnormalities observed on sigmoidoscopy; primarily scored as 1.

e More severe abnormalities, with sigmoidoscopy and symptom scores between 1 and 2.

f Sigmoidoscopy and symptom scores of 2 to 3, where the patient may require corticosteroid treatment and hospitalization.

Note: A score of  $\leq 2$ , with no individual subscore  $>1$ , indicates clinical remission. A score of 3 – 5 indicates mild activity, 6 – 10 indicates moderate activity, and 11 – 12 indicates severe activity.

### **3. Clinical Laboratory Tests**

Blood samples will be collected for testing, including complete blood count (CBC), blood biochemistry, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR).

### **4. Histological Assessment**

Colon biopsies will be performed during colonoscopy for histological evaluation.

## **5. Assessment Schedule (Table 2)**

### **1. Screening and Group Assignment Visit (-21 Days to Day 0)**

For all consenting patients, baseline demographic and clinical information was collected prior to group assignment, including: Age, gender, and smoking status (current smoker, former smoker, or non-smoker).

- (1) Use of nonsteroidal anti-inflammatory drugs (NSAIDs) or other anticoagulants.
- (2) Use of proton pump inhibitors (PPIs), H2 receptor blockers (H2RB), or other antacids.
- (3) Presence of primary sclerosing cholangitis (PSC).
- (4) History of appendectomy.
- (5) Duration and severity of ulcerative colitis (UC).
- (6) Current treatments and medications.
- (7) Self-reported medication adherence.

Eligible participants were then randomly allocated in a 1:1 ratio to two parallel groups to receive FMT from either a Han donor or a Bai donor.

### **2. Treatment and Follow-Up (Week 0 to Week 12)**

Participants will undergo two colonoscopies with biopsies performed at two time points: before treatment, at 8 – 12 weeks post-FMT

Stool samples will be collected at the following times: before treatment, at the end of FMT treatment, 8 weeks post-FMT, 12 weeks post-FMT.

During each visit, Mayo scores (including endoscopic subscoring) were evaluated by blinded outcome assessors. Biopsies were performed for histological assessments, and histological scoring was conducted by a blind pathologist. A dedicated telephone line was available for patients to report any adverse events or worsening of symptoms between scheduled visits. All investigators were aware of group allocation, while endoscopists, pathologists, and outcome assessors remained fully blinded to group assignments to minimize assessment bias.

## Sample collection plan

Study visit (day/week)	Screening & Vist (Day)	Treatment & Follow &Up (Week)	Treatment & Follow &Up (Week)	Treatment & Follow &Up (Week)
	-21 to 0	1	8	12
Window period (day)	0	0		
Informed consent	x			
Inclusion and exclusion criteria	x			
Demographics and medical history	x			
Pregnancy test (if applicable)	x	x		
Infusion of FMT		Xxx (The Bacterial solution was given at an interval of one day, and a total of 100 mL was transplanted 3 times)		
Mayo score (including MES)	x		x	
Clinical laboratory test	x	x	x	
Fecal calprotectin	x	x	x	x
Adverse.events/serious adverse events		x	x	x

Follow-up Time Points

Groups  (40 Patients/Group)	Samples	Before FMT ( Day )	Follow Up ( Week )	Follow Up ( Week )	Follow Up ( Week )
		Baseline (0)	1	8	12
Fresh fecal bacterial solution  (Normal /Ethnic Han Donor)	Stool	✓	✓	✓	✓
	Biopsies	✓			✓
	Food Record Questionnaire	✓	✓	✓	✓
Fresh fecal bacterial solution  ( Bai Ethnic Donor)	Stool	✓	✓	✓	✓
	Biopsies	✓			✓
	Food Record Questionnaire	✓	✓	✓	✓

Note: (The procedures of sample collections and subpackaging are as follows)

- ① Study stool: The stool of patients are collected and packed into 2ml cryogenic vials.
- ② Study biopsies: One piece was selected from the ileocecal region and two pieces were taken from the most severe lesion of the rectum (10cm from the anus) and placed in a 2ml cryopreserved tube.

## **7. Randomization, Blinding, and Treatment Protocols:**

### **1. Randomization:**

Randomization codes will be computer-generated. Subjects will be randomized according to the sequence of these codes.

### **2. Blinding Maintenance:**

This trial adopts a single-blind design, where participants will be blinded to the identity of the FMT donor, while investigators will be aware of the donor identity. The donor's identity will only be accessible to a limited number of staff responsible for preparing the FMT solutions.

### **3. Treatment Protocol in Emergency Situations:**

In cases of medical emergencies requiring unblinding for patient care, investigators will be allowed access to the donor allocation. Donor allocation should remain blinded unless necessary to ensure the participant's urgent medical care. The rationale for unblinding, along with the date and details of the unblinding, must be clearly documented. All participants will undergo follow-up by week 6 unless they explicitly decline.

## **8. Research Samples**

### **1. Sample Collection and Storage**

All samples will be stored at -80 °C.

### **2. Sample Analysis**

#### **Fecal Calprotectin**

Once sufficient fecal samples are collected, QUANTA Lite calprotectin ELISA will be used to measure fecal calprotectin concentrations in batches. This is an enzyme-linked immunosorbent assay (ELISA) system based on a colorimetric detection of polyclonal antibodies to calprotectin. The ELISA range is 10 – 1800 mg/g, requiring a sample volume of 50 – 150 mL. The assay sensitivity is less than 10 mg/g. Analysis of the treatment arms will be performed in a blinded manner.

#### **Microbial Analysis**

##### **(1) Nucleic Acid Extraction**

According to the protocol, DNA will be extracted from the study samples using the Maxwell® RSC PureFood GMO and Authentication Kit. Fecal pellets will first be mixed with CTAB buffer and vortexed, followed by heating for 5 minutes. Proteinase K and RNase A will then be added to the sample, which will be incubated at 70 °C. After centrifugation, the supernatant will be collected and transferred to the Maxwell® RSC instrument for further processing.

##### **(2) Microbiome and Metabolomics Analysis**

Collected specimens will undergo laboratory microbial analysis, including the identification of bacterial, viral, and fungal components in the human microbiome. DNA will be enriched and extracted using different kits, such as the Qiagen QIAamp® DNA Mini Kit, Qiagen QIAamp DNA Stool Mini Kit, Mobio UltraClean® Tissue & Cells DNA Isolation Kit, and Maxwell® RSC PureFood GMO and Authentication Kit. Subsequent metagenomic sequencing will be performed

using the Novoseq 6000 system (2x300 bp paired-end reads). Bacterial, fungal, and viral analyses will be conducted using methplan2, UNITE2-Bowtie2, and Kraken2, respectively. To compare differences in microbiome, metabolome, and meta transcriptome configurations among treatment groups, donors, and specimens at different time points, statistical tools such as DESeq, Random Forest, LEfSe linear discriminant analysis and MasSlin2 will be used. DESeq and Random Forest will be executed in R, while Lefse analysis will be performed on the Huttenhower Lab Galaxy server (<http://huttenhower.sph.harvard.edu/>). DB-RDA analysis in R will describe the effects of clinical factors on multivariate datasets.

#### Animal Studies

Biological samples collected in this study can be used for animal research. For example, fecal samples from FMT donors and recipients can be orally administered to animal models to study microbiome effects.

### 3. Subsequent Handling of Specimens

Original specimens and derivative materials will be stored for up to 10 years. Specimens may be shipped abroad for analysis or used for research on the gut microbiome. Data generated from this project, including genetic, metagenomic, metabolomic, microbiome, and clinical information, will have all personal identifiers removed (de-identified) and stored in a secure electronic database, such as the Genotype and Phenotype (dbGaP) database managed by the National Institutes of Health (NIH), the Sequence Read Archive (SRA) at NIH, or academic institutions like the Li Ka Shing Health Sciences Research Institute at The Chinese University of Hong Kong or the Wellcome Sanger Institute. Only qualified researchers with institutional or employer support will have access to these data. These researchers will be required to agree not to attempt re-identification of the donors from genetic information.

#### 9. Withdrawal Procedures

Participants may withdraw from study at any time without affecting their access to standard medical care. No further data or samples will be collected after withdrawal. However, any previously collected samples or data that have been processed or uploaded to the databases cannot be deleted, and data already downloaded by qualified researchers cannot be retrieved.

#### 10. Sample Size

The study includes 50 UC recipients for FMT from Bai donors and 50 UC recipients for FMT from Han donors, totaling 100 participants.

#### 11. Statistical Analysis

Continuous variables will be expressed as means (SD) and compared between groups using unpaired t-tests. Categorical variables will be compared using the chi-square test or Fisher's exact test. A modified intent-to-treat analysis will include all participants who have received at least one FMT treatment. Participants requiring additional treatment, violating the study protocol, failing to discontinue corticosteroids by week 12, or discontinuing the study for any reason will be considered treatment failures. Per-protocol analysis will include participants who completed the 12-week blind treatment course without protocol violations. Missing data were handled using complete-case analysis in the primary analysis. A two-sided p-value of <0.05 will be considered statistically significant. Safety data will be summarized using descriptive statistics. All statistical analyses will be performed using R (4.0.5).

#### 12. Potential Risks

There is a potential risk of perforation during colonoscopy, but the overall incidence of

complications is less than 1%. The risk of infectious diseases during fecal microbiota transplantation (FMT) is minimal due to rigorous donor screening prior to transplantation. To date, FMT appears to be safe. Over 200 FMT procedures have been conducted at the Academic Medical Center in Amsterdam and more than 3,000 at the Digestive Disease Center in Sydney, Australia, without any reports of serious adverse events. Mild adverse reactions such as bloating, flatulence, borborygmi, vomiting, and abdominal discomfort may occasionally occur. Only qualified researchers have access to de-identified data in the database. Furthermore, they agree not to use this information to attempt to identify the data providers, ensuring participant confidentiality. However, given the availability of current public-domain technologies and expected advancements in the coming years, identifying specific individuals from genetic or other information may become feasible and increasingly straightforward. Participants and their family members may face potential risks (e.g., those related to insurance, employment, cybersecurity breaches, and law enforcement) due to shared genetic characteristics.

### 13. Pregnancy

All women of childbearing potential, except postmenopausal or sterilized women, are required to undergo pregnancy testing. Women of childbearing potential must use contraception throughout the study. If a female participant becomes pregnant during the study, she must immediately notify the research team. In such cases, the participant will be withdrawn from the study. If feasible, an early termination visit will be scheduled after pregnancy confirmation.

### 14. Non-return of Research Results

The results of microbiome analyses will not be returned to participants. In other words, this data will not be used to influence the clinical care received by participants or their genetically related family members. The potential benefit of this study lies in improving care and management in the future.

### 15. Costs, Expenses, and Compensation

To assist participants with costs related to research participation, including travel and other expenses, fecal microbiota transplantation (FMT) will be provided free of charge during each required study visit. The total cost of the three FMT procedures is estimated to be 18,000 RMB.

### 16. Consent and Withdrawal

Participants may withdraw from the study at any time without affecting their standard medical care. Any samples collected from the participant may be destroyed upon request. However, de-identified data already entered the database cannot be deleted, nor can data already downloaded by authorized researchers be retrieved. Withdrawal from the study will not in any way affect the participant's access to medical care, and they will continue to receive healthcare services from our hospital.

### 17. Adverse Events (AEs)

#### 1. Severity Levels

The severity of adverse events (AEs) will be graded on a scale of 1 to 5.

#### 2. Collection and Reporting

The collection of AEs, including serious adverse events (SAEs), will begin from the first administration of FMT. Routine AE collection will continue until week 1. Since participants will transition to standard care after week 1, AEs occurring beyond this period will not be collected.

#### Anticipated Clinical Events Following FMT

FMT is known to be associated with certain characteristic signs and symptoms (listed below). These

symptoms are typically mild and resolve within three days post-FMT. Unless symptoms persist for more than three days or become more severe, they will not be recorded as AEs.

- |                                |                        |                        |
|--------------------------------|------------------------|------------------------|
| ▪ Sore throat                  | ▪ Abdominal distention | ▪ Vomiting             |
| ▪ Fever                        | ▪ Abdominal pain       | ▪ Excessive flatulence |
| ▪ Increase of CRP              | ▪ Abdominal fullness   | ▪ Constipation         |
| ▪ Diarrhea                     | ▪ Bloating             | ▪ Decreased appetite   |
| ▪ Increased in stool frequency | ▪ Belching             | ▪ Headache             |
| ▪ Loose stool                  | ▪ Cramping             | ▪ Nausea               |
| ▪ Abdominal discomfort         | ▪ Gassiness            | ▪ Borborygmus -----    |
|                                | ▪ Fatigue              |                        |

UC is associated with certain characteristic signs and symptoms, including diarrhea, rectal bleeding, and abdominal pain. These signs and symptoms may be present at baseline and can persist or fluctuate throughout the study based on the subject's medical history. These signs and symptoms will not be collected as AEs. However, an exacerbation of disease activity (e.g., an increase in daily rectal bleeding or abdominal pain beyond the subject's normal fluctuations, or the emergence of new signs and symptoms of UC) will be collected as AEs and reported in accordance with regulatory requirements. Extraintestinal manifestations of UC (e.g., joint pain, arthritis, uveitis) that develop or worsen during the study will also be considered AEs.

#### 18. References



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**19. Clinical study approval document issued by the Ethics Committee of the First Affiliated Hospital of Kunming Medical University**

**Clinical study approval document issued by the Ethics Committee of the First Affiliated Hospital of Kunming Medical University**

(2025) Lun L No.111

Project Name	Research on the mechanisms of different donors in fecal microbiota transplantation for treating ulcerative colitis (Mechanism and clinical study of fecal bacteria transplantation for inflammatory bowel disease)				
Application Authority(person)	The First Affiliated Hospital of Kunming Medical University				
Undertake the Department	Department of gastroenterology	Principal investigator	According to should ray Yang Sun	Professional ranks and titles	Associate Chief Physician
Source of research funds	<input type="checkbox"/> Government <input type="checkbox"/> Foundation <input type="checkbox"/> Company <input type="checkbox"/> International Co-operation <input checked="" type="checkbox"/> Others(please indicate)   Yunnan Clinical Research Center for Digestive System Diseases				
Send review documents	1.Study protocol(Versio No:1.0. Date:March 13,2025) 2.Informed Consent Form(Versio No:1.0. Date:March 13,2025) 3.Case Report Form(Versio No:1.0. Date:March 13,2025) 4.CV of investigator and GCP certificate				
Ethical review methods	<input type="checkbox"/> Conference Review <input checked="" type="checkbox"/> Quick Review				
juror	Hao Qinglin and Zhang Ling				
Review comments form the Ethics Committee	Agreed to proceed the center				
The responsibilities, composition, operating procedures, and records of this Ethics Committee comply with ICH-GCP and relevant laws and regulations of China.					
Matter need attention:  1. This clinical trial shall be commenced within 1 year from the date of approval granted by the Ethics Committee. Failure to initiate the trial within this time limit shall result in automatic expiration of this Approval Letter.  2. The research shall be conducted in accordance with the protocol approved by this Ethics Committee, and shall comply with the principles of CFDA/GCP and the Declaration of Helsinki.  3. From the date of approval, periodic continuing review shall be conducted every 12 months (the review frequency may be adjusted based on actual progress): Please submit the Continuing Review Form to the Ethics Committee 1 month prior to the scheduled review date.  4. During the course of the research, any modifications to the study protocol, informed consent form, or other related documents must be submitted with the <i>Amendment Application Form</i> and the relevant materials specified in the “Checklist of Submission Documents.”Such amendments may only be implemented after review and approval by the Ethics Committee.  5. In the event of a serious adverse event (SAE) or an unexpected adverse event affecting the risk-benefit ratio of the study, written notification must be submitted to the Ethics Committee concurrently with the report to the CFDA. The CFDA <i>Serious Adverse Event Report Form</i> , or the <i>Serious Adverse Event/Unexpected Adverse Event Report Form</i> published by this Ethics Committee, or other equivalent reporting forms with relevant content may be used; however, reports in foreign languages must be accompanied by a Chinese summary. The Ethics Committee reserves the right to make new decisions based on its assessment.  6. Instances of non-compliance or protocol violations must be promptly reported using the <i>Non-compliance/Protocol Deviation Report Form</i> .  7. Premature termination of the study must be promptly reported by submitting the <i>Study Termination Report Form</i> .  8. Upon completion of the study, the <i>Study Closure/Completion Report Form</i> must be submitted.  9. Prompt written notification of significant decisions made by other Ethics Committees is required.					
Signature of Chairperson or Vice-Chairperson:  Ethics Committee of the First Affiliated Hospital of Kunming Medical University (Seal) <div style="text-align: right;">2025, August 25 </div>					

## The First Affiliated Hospital of Kunming Medical University

### Informed consent form for microflora transplantation

#### (FMT/miniFMT)

surname and personal name: \_\_\_\_\_ administrative or technical offices: \_\_\_\_\_  
Bed number: \_\_\_\_\_ Bed Hospitalization Number: \_\_\_\_\_ Date: \_\_\_\_\_

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#### Disease conditions and treatment recommendations:

##### 1. Basic information of the patients:

diagnose: \_\_\_\_\_

age: \_\_\_\_\_ Gender: \_\_\_\_\_ Allergic history: \_\_\_\_\_

Pregnancy history: ☐ with ☐ with no

Number of previous FMT treatments: \_\_\_\_\_ (times), with or without adverse effects: ☐ have ☐ have no

FMT pre-treatment examination:

ALT \_\_\_\_\_ U/L; AST \_\_\_\_\_ U/L; CRP \_\_\_\_\_ mg/L; ESR \_\_\_\_\_ mm;

EB-DNA \_\_\_\_\_ Copy/ml; HCMV-DNA \_\_\_\_\_ Copy/ml;

Stool routine + occult blood: [ ];

HbsAg [ ]; Anti-HBs [ ]; HbeAg [ ]; Anti-Hbe [ ]; Anti-Hbc [ ];  
Anti-HCV [ ]; Anti-HIV1/2 [ ]; Syphilis: [ ]; Others: [ ]

##### 2. Treatment recommendations

I have obtained the following explanations: microflora transplantation includes fecal bacteria transplantation (FMT) and formula microflora transplantation (mini FMT or SMT), the indications for microflora transplantation, foreign and domestic applications and research status, the benefits for the diagnosis and treatment of diseases, the chances of surgical (or regimen) treatment success,

Including: the benefits of functional intestinal flora reconstruction, the recent improvement or cure probability of the disease, the long-term evolution of the disease, different diseases and no differences in efficacy of the same subcategory of diseases, odds of success during treatment, and changes in efficacy during follow-up. The doctor has told me that I have one \_\_\_\_\_,

Depending on the condition, faecal bacteria transplantation (FMT / mini FMT) treatment can be performed.

#### Our hospital has clearly informed the patient(close relatives/guardians) of the following contents:

- (1) Although doctors have tried to eliminate possible risks, there may still be the risk of disease infection, "window period" problems that cannot be detected, wrong test results, and unpredictable risks; formula flora transplantation may also have unpredictable risks.
- (2) Possible main risks and complications in the process of microflora transplantation

and related diagnosis and treatment, including failure of operation, perforation, bleeding, abdominal infection, etc. In case of occurrence, agree to the doctor to give the corresponding diagnosis and treatment disposal.

(3) After the microflora transplant, my disease may be cured, partially effective, recurrent, or recurrent. Repeat implementation of microflora transplantation therapy may be required. I can choose to abandon this treatment strategy at any stage.

(4) For the diagnosis and treatment needs of the condition, the members of the diagnosis and treatment team can implement relevant alternative diagnosis and treatment measures.

**patients(close relatives/guardians) include the following contents:**

(1) I agree to and authorize it \_\_\_\_\_ (Physician signature) And its diagnosis and treatment team to implement the following possible options: via colonoscopy, on Gastrointestinal endoscopy, middle gastrointestinal endoscopy, gastric tube, nasojejunal tube, enema, oral capsule containing intestinal bacterial fluid, fistula tube, rectal graft tube (TET) (the involved endoscopy and anesthesia informed consent were signed separately).

(2) The type of transplanted bacteria I received was: ☐ fresh fecal bacteria ☐ frozen fecal bacteria ☐ formula bacteria.

(3) For faecal bacteria transplantation, I agree that the source of faecal bacteria is: ☐ relatives ☐ friends ☐ Other healthy people, as suggested by the doctor. For formula flora transplantation, I knew that the group selection was determined by the doctor.

(4) I agree that the hospital will preserve and use my fecal bacteria, body fluids and tissue specimens for teaching and medical research without involving personal identifiable information and privacy.

(5) Each doctor and patient s hall hold one copy of this informed consent. I have been told that the property right of this document belongs to the institute and shall not be issued and distributed without permission.

(6) After full notification, I have read or passed my relatives have read and explained the above content, and I hereby sign the informed consent form.

**patients(close relatives/guardians)carefully promisethat:**

Patient (close relative / guardian) signature: Relationship: Time: \_\_year\_\_moon\_\_sun

Doctor Signature: Time: \_\_year\_\_moon\_\_sun

**The First Affiliated Hospital of Kunming Medical University**

**Informed consent form for microflora transplantation**

**(FMT/miniFMT)**

surname and personal name:                      administrative or technical offices:  
Bed number:                      Bed Hospitalization Number:                      Date:

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**Commitment content**

(I) My name is XXX, and I have understood the process and related risks of fecal transplantation. I strongly require fecal transplantation treatment, and I am willing to take all the risks.

(Family member) I am XXX from XXX. I have understood the process and related risks of fecal transplantation, so I strongly demand the treatment of fecal transplantation, and I am willing to take all the risks.