# Research Plan

## A clinical trial study on the effects of PEG laxatives on gut microbiota in patients undergoing appendectomy

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# Ⅰ、 Research Background

Colonoscopy is a must-have item in colorectal cancer check-ups, and for high-risk groups, annual check-ups are the most effective way of early prevention; A large amount of polyethylene glycol (PEG2000-4000) is used as a laxative and lubricant in colonoscopy at present; The extensive use of PEG can cause long-term and irreversible damage to the gut microbiota, posing a great threat[1] to health.

The appendix, located at the base of the cecum, is a slender tubular structure that protrudes from the posterior wall inside the cecum and was once thought to be part of an evolutionary redundancy. Appendicitis has long been the most common surgical acute abdominal disease, and once acute appendicitis occurs, traditional treatments often opt for the removal of the appendix. In recent years, researchers have revealed that appendectomy is associated[2] with mental disorders, colorectal cancer, cardiovascular diseases, etc. Besides being an important organ involved in immunity, the appendix also has the function of storing and protecting intestinal microbiota. When there is a disruption of the gut microbiota in the human body, the microorganisms retained in the appendix are released into the gut to reduce the impact[3] of the disruption. Previous researchers' metagenomic sequencing of gut microbiota in patients who have undergone appendectomy shows significant changes in their gut microbiota compared to the normal population, which in turn affects central nervous system function through the microbiota-gut-brain axis and poses potential risks to host health and behavior, such as Parkinson's disease[4-7]. Therefore, appendectomy may have potential adverse effects on the human body, such as significantly altering the composition of the gut microbiota, reducing the ability to restore intestinal homeostasis, and lowering[8, 9] the production of short-chain fatty acids (SCFAs).

This study will observe the effects of PEG on the gut microbiota, and by analyzing the changes in the gut microecology before and after colonoscopy, design better intestinal cleansing drugs and intestinal repair probiotics to further reduce the adverse effects of colonoscopy on patients. At the same time, explore the interaction between the appendix and the gut microbiota and its significance.

## 2. Objectives of the study

The study intends to recruit a group of people who have undergone appendectomy for colonoscopy, (1) to observe changes in gut microbiota before and after colonoscopy in the appendectomy population; (2) To analyze whether the changes in gut microbiota before and after colonoscopy in the appendectomy population were different from the recovery of the normal population.

## Iii. Study Design

This study uses a parallel controlled monitoring clinical trial approach to investigate the short-term and long-term effects of PEG use on the gut microbiota of patients, including tracking analysis of the recovery and impairment of the gut microbiota, and comparing the recovery and impairment of the microbiota with those who did not undergo appendectomy to design corresponding microecological restoration probiotics. This study used bioinformatics methods to statistically analyze the data information of the samples.

## 4. Selection of subjects

### 1. Inclusion criteria

The subjects were mainly recruited who met the following conditions: 1) Had undergone appendectomy or appendectomy for right-sided colon cancer; 2) were aged between 18 and 75 years old. 3) Prepare for colonoscopy; 4) Be in good health and have no history of major organ disease.

Control subjects were recruited to meet the following conditions: 1) age 18-75 years; 2) No appendectomy or right colon cancer appendectomy; 3) Good health, no history of major organ disease.

### 2. Exclusion criteria

Subjects excluded the following conditions: (1) contraindications for colonoscopy: such as cardiopulmonary insufficiency, etc. (2) Intolerance to PEG laxatives; (3) Pregnant women; (4) Mental disorders. Among them, the colonoscopy population without appendectomy was used as the control; 4) Use of antibiotics within half a year.

Control subjects excluded the following conditions: (1) presence of organic disease; Such as cardiopulmonary insufficiency, etc. (2) Pregnant women; (3) Mental disorders; 4) Use of antibiotics

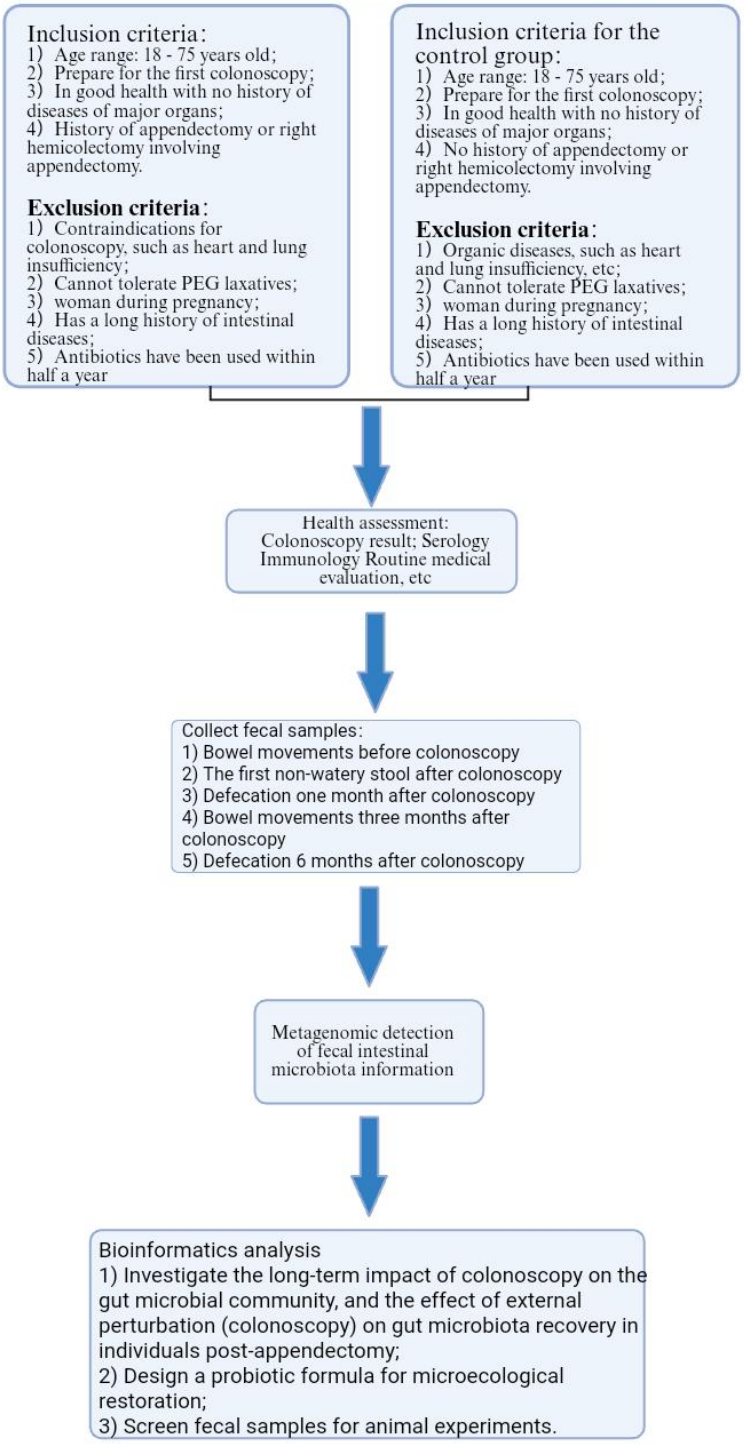
within half a year.

## 5. Estimation of sample size

About 10 people were included in this study.

## Vi. Study Process

The technical route of this study is as follows:



## Vii. Possible risks and Preventive measures

The possible discomforts ofthis study include: psychological and sensory discomforts caused by fecal samples being mainly taken from the subjects' homes, including nausea and vomiting. The fecal sampling box comes with a pair of gloves and two sheets of toilet paper.

## VIII、Data collection and statistical analysis

Fecal samples of patients were sent to the company for metagenomic sequencing to obtain metagenomic data of microorganisms in the fecal samples.

The raw files obtained from the DNBSEQ-T7 platform were converted into short reads (raw data) via base call, and these short reads were recorded in FASTQ format [30] containing sequence information and corresponding sequencing quality information.

Sequence artifacts, including reads containing adapter contamination, low-quality nucleotides and unidentifiable nucleotides (N), undoubtedly set obstacles for subsequent reliable bioinformatics analysis. Therefore, quality control is a necessary step to ensure meaningful downstream analysis.

Fastp(version 0.23.1)[31] was used to perform basic statistics on the quality of the original reads.

The data processing steps are as follows:

(1) Discard the paired reads if one of the reads contains adapter contamination;

(2) Abandon paired reads if the number of uncertain bases exceeds 10% in each read;

(3) Discard pairs of reads if the proportion of low-quality bases (Phred quality <5) in any read exceeds 50%.

After passing the quality inspection, a total of 0.2g of DNA is required for each sample as the input material for DNA library preparation. First, genomic DNA samples are ultrasonically crushed by a Covaris ultrasonic disruptor to meet a size requirement of 350bp. After terminal repair, adding A tail, adding sequencing headers, fragment screening, PCR amplification, and then using the AMPure XP system (Beverly, USA) to purify the product, the library quality was evaluated and quantified on the Agilent 5400 system (Agilent, USA), Quantification was performed using QPCR (1.5 nM). The 5 'ends of each library were phosphorylated and cyclized. Then cyclic amplification is carried out to produce DNA nanospheres. Finally, the DNA nanospheres were loaded into flow cells for DNBSEQ-T7 sequencing at Novogene Bioinformatics Technology Co., LTD. (Beijing, China). The entire library preparation process is completed, resulting in the final DNA library.

Additionally, calculate the Bray-Curtis distance using the vegan package for principal coordinate analysis (PCoA). Linear discriminant analysis effect size (LEfSe) analysis was used for the Kruskal-Wallis test and the Wilcoxon rank sum test, and a $p < 0.05$ was considered statistically significant. The LDA score was set to 2.0 to identify the characteristics with significant differences between groups. This study retained taxa with an average relative abundance greater than 0.0001(0.01%). The bar charts were visualized using the "ggplot2" package in R software (version 4.0.5)(https://www.r-project.org/).

All data were expressed as mean ±standard error (SEM). The differences between groups were analyzed by one-way ANOVA using GraphPad Prism 7.0 software, and the results of the analysis were obtained by Tukey Test.

## Ix. References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