## PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | Protocol for faecal microbiota transplantation in irritable bowel syndrome – the MISCEAT study: a randomised, double-blind cross-over study utilising mixed microbiota from healthy donors |
| **AUTHORS** | Hurych, Jakub; Vejmelka, Jiri; Vodolanova, Lucie; Kramna, Lenka; Larionov, Vladyslav; Kulich, Michal; Cinek, Ondrej; Kohout, Pavel |

## VERSION 1 – REVIEW

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| **REVIEWER** | zhang, faming<br>the Second Affiliated Hospital of Nanjing Medical University, Medical Center for Digestive Diseases |
| **REVIEW RETURNED** | 28-Sep-2021 |

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| **GENERAL COMMENTS** | This is a clinical trial using FMT for IBS. The study design is much different from the previous studies. The design is perfect and feasible.<br>One minor comment:<br>The results of FMT vary from different centers. The dose of the delivered microbitoa might be one of reasons. The dose of the fecal microbiota should be described exactly. This at least should be discussed as the potential limitation of the current study if researcher cannot determine the dose by the number if cells. I understand the description from Europe consensus on FMT. However, the dose description on FMT has been recommended to count the numbers of microbial cells according to the latest methodology consensus, instead of the stool weight. Please see the consensus: Fecal Microbiota Transplantation-standardization Study Group. Nanjing consensus on methodology of washed microbiota transplantation. Chin Med J (Engl). 2020 Oct 5;133(19):2330-2332. doi: 10.1097/CM9.0000000000000954. |

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| **REVIEWER** | Santos, Javier<br>Vall d'Hebron University Hospital, GASTROENTEROLOGY |
| **REVIEW RETURNED** | 20-Feb-2022 |

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| **GENERAL COMMENTS** | none |

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| **REVIEWER** | Serban, Elena Daniela<br>Iuliu Hagieganu University of Medicine and Pharmacy Faculty of Medicine, 2nd Department of Pediatrics |
| **REVIEW RETURNED** | 10-Apr-2022 |

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| **GENERAL COMMENTS** | A. General comments: This project is absolutely marvelous and of good scientific quality. The authors address an important and frequent disease, the irritable bowel syndrome (IBS), with no clear etiopathogenesis and no cure so far, which causes patients |

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| | disability and increases costs of the health care system. IBS patients reportedly represent 10–70% of the patients attending primary care and about 28% of referrals to gastroenterologists. If successful, this study would significantly improve the therapy of IBS, with high international impact. The study is well described, with attention to all details, proving that the authors have a good knowledge of the topic. I would like to emphasize that: hypotheses are clear and of good standards; methodology is very well described and of high quality - modern and adequate techniques to accomplish their objectives; primary and secondary objectives are clear and reasonable and include not only clinical and psychological and well-being effects, but also modifications of fecal microbiota (complex investigations - microbial profiles by bacteriome profiling, parasite screening and virome sequencing) on short and long-term; there is future application in real clinical practice; everything in the study schedule is pertinent; the number of selected patients is enough to ensure significance of statistical analyses; every detail is carefully addressed and analyzed. The study design is unique, innovative and original: randomized double-blind crossover study with administration of either deep-frozen stored stool microbiota (two groups, different periods), or placebo. The additional control group will address the potential issue of a carry-over effect, as well as the high inter-individual variability among subjects, and the high expected effect of placebo. Another strong point: the selection of study substances. The active substance will be identical across the whole study, and will come from mixing microbiota of healthy donors (8 people). In contrast to other studies using FMT in IBS, a defined donor stool mixture is used, selected for certain characteristics of the bacteriome, and increased in diversity. Placebo consists of the same preparation which underwent autoclaving (no live microbes). The study uses enemas, which ensure patient compliance and safety, minimize study-associated risks and discomfort, instead of colonoscopy for instillation of the study substance. The longitudinal character of this study will help to address the secondary objectives, including the long-term safety of the therapy. With the uncertainty of the onset and duration of the putative effects of FMT, the authors complement a classic cross-over design (optimal for short-term effects) with an additional control group that will enable assessing long-term effects of FMT. Strength and limitations of the study are mentioned. According to the recent literature, which reviewed studies about FMT in IBS, FMT had some good effects, but it was considered that data on FMT and IBS were too limited to draw sufficient conclusions and standardized double blinded randomized clinical trials were needed to be carried out to evaluate the effect of FMT on IBS. This is where this wonderful project comes in and fills the gap, with its unique design. I find this trial excellent and feasible and with huge potential for clinical practice.<br>B. Minor comments: Since the study is already ongoing and recruiting patients, I only suggest minor modifications (if they appear possible to be included):<br>1. The title is clear; I wonder why the abbreviation MISCEAT was considered (this is not a criticism, just a simple question). I saw that this abbreviation appears also on clinicaltrials.gov.<br>2. ABSTRACT: a. Aim: mentions effectiveness, but also safety was assessed. Maybe the authors could include also safety (if they agree). b. Methods and analysis: *Please insert “healthy” before “donors”. *Lines 72-74: “Biochemistry and haematology workup, anthropometry, bioimpedance, dietary questionnaire, and food |

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| | records data will be obtained at study visits during the follow-up period" Here, I suggest to start with food records, dietary questionnaire, then to mention clinical data (anthropometry), and then investigations (biochemistry and haematology workup, bioimpedance). *Line 76: "Secondary outcomes are IBS-SSS at two and 32 weeks compared to placebo", however the authors mentioned above that "The irritable bowel syndrome severity symptom score (IBS-SSS) questionnaire scores will be collected at baseline (week -1), and then at weeks 3"; therefore, there is no IBS-SSS questionnaire previewed for week 2. Please revise. From the main text, it appears that, in fact, it is two weeks after the intervention, which is correct. Please write then "Secondary outcomes are IBS-SSS at two weeks after the intervention and 32 weeks compared to placebo". *Lines 76-78: I would suggest to mention initially clinical data (e.g. changes in urgent defecations frequency, Bristol stool scale, abdominal pain, bloating and anthropometric parameters) and then changes in the gut microbiome.*I suggest to include in the abstract, among secondary outcomes, also "the psychological and well-being effects of the therapy scored by IBS-QoL questionnaires", as it appears from the main text, since they are important.<br>3. INTRODUCTION: a. References are a bit old for 2022; however, the study protocol was approved in 2018. Maybe the authors would consider to insert some recent ones (if possible and not altering he protocol). I suggest to include here some of the findings of some published systematic reviews and meta-analyses. Some examples are listed below:<br>1. Xu D, et al. Efficacy of Fecal Microbiota Transplantation in Irritable Bowel Syndrome: A Systematic Review and Meta-Analysis. Am J Gastroenterol. 2019 Jul;114(7):1043-1050.<br>2. Ianiro G, et al. Systematic review with meta-analysis: efficacy of faecal microbiota transplantation for the treatment of irritable bowel syndrome. Aliment Pharmacol Ther. 2019 Aug;50(3):240-248.<br>3. Myneedu K, et al. Fecal microbiota transplantation in irritable bowel syndrome: A systematic review and meta-analysis. United European Gastroenterol J. 2019 Oct;7(8):1033-1041.<br>4. Wu J, et al. Efficacy of Fecal Microbiota Transplantation in Irritable Bowel Syndrome: A Meta-Analysis of Randomized Controlled Trials. Front Cell Infect Microbiol. 2022 Feb 28;12:827395. This one searched MEDLINE, EMBASE, and the Cochrane Central Register through September 2021. I suggest the authors to include some of the findings of this manuscript.<br>5. Elhusein AM, Fadlalmola HA. Efficacy of Fecal Microbiota Transplantation in Irritable Bowel Syndrome Patients: An Updated Systematic Review and Meta-Analysis. Gastroenterol Nurs. 2022 Jan-Feb 01;45(1):11-20. The authors searched Scopus, PubMed, Cochrane, and Web of Science databases through June 2021. Again, some results of this manuscript could be commented.<br>The authors could also conclude, having all these papers, that the efficacy of FMT in IBS was not remarkable (sure, depending on the design of the study, the methods and so on). We et al concluded that "The GRADE quality evidence to support recommending FMT in IBS was very low.".<br>b. Lines 125-128 – Please again mention clinical data and then gut microbiome composition.<br>4. METHODS AND ANALYSIS: Line 315 - Please define abbreviation "GMT" (… it is not "Greenwich Mean Time"); probably, the authors refer to gamma-glutamyl transpeptidase (gamma-glutamyl transferase). Its usual abbreviation is GGT. |

**VERSION 1 – AUTHOR RESPONSE**

Response to reviewer #1
- Comment: One minor comment: The results of FMT vary from different centers. The dose of the delivered microbitoa might be one of reasons. The dose of the fecal microbiota should be described exactly. This at least should be discussed as the potential limitation of the current study if researcher cannot determine the dose by the number if cells. I understand the description from Europe consensus on FMT. However, the dose description on FMT has been recommended to count the numbers of microbial cells according to the latest methodology consensus, instead of the stool weight. Please see the consensus: Fecal Microbiota Transplantation-standardization Study Group. Nanjing consensus on methodology of washed microbiota transplantation. Chin Med J (Engl). 2020 Oct 5;133(19):2330-2332. doi: 10.1097/CM9.0000000000000954.

- Response: In accord with this comment, we have added quantification of the cell content: this was performed using real-time PCR relative to a standard curve derived from a bacterial culture, as well as to previously used stool transplants from another FMT centre. The result is now presented, referring to the recommendation by the Nanjing consensus (line 218 and onwards in the clean copy, respectively line 221 and onwards in the document with changes highlighted). We would like to note that the published consensus lacks reference to the cell count quantification method, so a direct comparison is impossible.
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Response to reviewer #2
- Comment: None
o Response: not required.

Response to reviewer #3
- Minor comments: Since the study is already ongoing and recruiting patients, I only suggest minor modifications (if they appear possible to be included):

- 1. The title is clear; I wonder why the abbreviation MISCEAT was considered (this is not a criticism, just a simple question). I saw that this abbreviation appears also on clinicaltrials.gov.
o Response: The abbreviation comes from a latin word misceatur, which means “to be mixed”. It is also an acronym of the study (the letters form it are bolded): Faecal Microbiota transplantation in Irritable bowel Syndrome: a randomised, double-blind Cross-over study utilising mixEd microbiota from heAlThy donors.
- 2. ABSTRACT: a. Aim: mentions effectiveness, but also safety was assessed. Maybe the authors could include also safety (if they agree). b. Methods and analysis:
o *Please insert “healthy” before “donors”.
o *Lines 72-74: “Biochemistry and haematology workup, anthropometry, bioimpedance, dietary questionnaire, and food records data will be obtained at study visits during the follow-up period” Here, I suggest to start with food records, dietary questionnaire, then to mention clinical data (anthropometry), and then investigations (biochemistry and haematology workup, bioimpedance).
o *Line 76: “Secondary outcomes are IBS-SSS at two and 32 weeks compared to placebo”, however the authors mentioned above that “The irritable bowel syndrome severity symptom score (IBS-SSS) questionnaire scores will be collected at baseline (week -1), and then at weeks 3”; therefore, there is no IBS-SSS questionnaire previewed for week 2. Please revise. From the main text, it appears that, in fact, it is two weeks after the intervention, which is correct. Please write then “Secondary outcomes are IBS-SSS at two weeks after the intervention and 32 weeks compared to placebo”.
o *Lines 76-78: I would suggest to mention initially clinical data (e.g. changes in urgent defecations

frequency, Bristol stool scale, abdominal pain, bloating and anthropometric parameters) and then changes in the gut microbiome.
o *I suggest to include in the abstract, among secondary outcomes, also “the psychological and well-being effects of the therapy scored by IBS-QoL questionnaires”, as it appears from the main text, since they are important.
o Response: All corrected accordingly.

- 3. INTRODUCTION:
o a. References are a bit old for 2022; however, the study protocol was approved in 2018. Maybe the authors would consider to insert some recent ones (if possible and not altering he protocol). I suggest to include here some of the findings of some published systematic reviews and meta-analyses. Some examples are listed below:
1. Xu D, et al. Efficacy of Fecal Microbiota Transplantation in Irritable Bowel Syndrome: A Systematic Review and Meta-Analysis. Am J Gastroenterol. 2019 Jul;114(7):1043-1050.
2. Ianiro G, et al. Systematic review with meta-analysis: efficacy of faecal microbiota transplantation for the treatment of irritable bowel syndrome. Aliment Pharmacol Ther. 2019 Aug;50(3):240-248.
3. Myneedu K, et al. Fecal microbiota transplantation in irritable bowel syndrome: A systematic review and meta-analysis. United European Gastroenterol J. 2019 Oct;7(8):1033-1041.
4. Wu J, et al. . Front Cell Infect Microbiol. 2022 Feb 28;12:827395. This one searched MEDLINE, EMBASE, and the Cochrane Central Register through September 2021. I suggest the authors to include some of the findings of this manuscript.
5. Elhusein AM, Fadlalmola HA. Efficacy of Fecal Microbiota Transplantation in Irritable Bowel Syndrome Patients: An Updated Systematic Review and Meta-Analysis. Gastroenterol Nurs. 2022 Jan-Feb 01;45(1):11-20. The authors searched Scopus, PubMed, Cochrane, and Web of Science databases through June 2021. Again, some results of this manuscript could be commented.
The authors could also conclude, having all these papers, that the efficacy of FMT in IBS was not remarkable (sure, depending on the design of the study, the methods and so on). We et al. concluded that “The GRADE quality evidence to support recommending FMT in IBS was very low.”
o b. Lines 125-128 – Please again mention clinical data and then gut microbiome composition.
o Response: All corrected accordingly. On lines 114-115 of the clean copy (117-118 of the marked copy, respectively), a sentence pointing to the start of the trial in 2018 was added to underline the time difference between the start of the trial and publishing the protocol. Most importantly to the reviewer’s comment, an extension of RCTs in IBS was added on lines 121-125 of the clean copy (124-129 in the marked copy, respectively) to describe the current evidence since starting the trial more precisely.

- 4. METHODS AND ANALYSIS:
o Line 315 - Please define the abbreviation “GMT” (… it is not “Greenwich Mean Time”); probably, the authors refer to gamma-glutamyl transpeptidase (gamma-glutamyl transferase). Its usual abbreviation is GGT.
o Response: Corrected accordingly. We agree that GGT is internationally recognised.

As requested, all adjustments in the revised version of the manuscript have been bolded to make them easier to recognise

We hope that the manuscript is now suitable for publication, and we are looking forward to hearing from you soon.

**VERSION 2 – REVIEW**

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| **REVIEWER** | zhang, faming |

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| | the Second Affiliated Hospital of Nanjing Medical University, Medical Center for Digestive Diseases |
| **REVIEW RETURNED** | 10-May-2022 |

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| **GENERAL COMMENTS** | The revision is fine now. |

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| **REVIEWER** | Serban, Elena Daniela<br>Iuliu Hagieganu University of Medicine and Pharmacy Faculty of Medicine, 2nd Department of Pediatrics |
| **REVIEW RETURNED** | 10-May-2022 |

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| **GENERAL COMMENTS** | Congratulations to the Authors! The manuscript appears clear and professional now. I fully support its publications. |