To:

Editor-in-chief

Biomolecules

Re: Second revision of the manuscript Rehorova et al. Multi-donor Faecal Microbial Transplantation for Critically Ill Patients: Rationale and Standard Operating Procedure

Dear Editor, Dear Reviewers,

Thank you for insightful review of our above-named manuscript. In this letter, we describe the changes we have made to our paper and explain them. The revised manuscript in "track changes" format is attached as well as the final revised version.

REVIEWER #1

R1Q1 The authors have improved the manuscript, in advance. Although, from my point of view, the relevance of the manuscript and the included SOP is still lacking in terms of a view outside of the Czech Republic. The usefulness as template for comparable efforts within other and mostly higher legislative and methodological requirements (in other western countries), is limited.

We are very sorry to hear that Reviewer #1 considers the Czech Republic a country with lower legislative and methodological standards. As a member state of the EU, the drug trial regulations and legislature are compatible of EU standards and laws. Our SOPs are fully compliant with *European consensus conference on faecal microbiota transplantation in clinical practice,* as well as joint British Society of Gastroenterology and Healthcare Infection Society guidelines and OpenBiome stool bank instructions. We admit local specifics do exist and that the methodology of FMT procedure can mature over time (see R2Q2), but still believe our SOPs having broader applicability beyond the borders of our beloved country in the heart of Europe.

R1Q2 In its current form, the manuscript still has relevant issues regarding English grammar and style. It must be thoroughly revised by an English-native speaking person.

This has been done and multiple typos were corrected.

REVIEWER #2

R1 Q1 The author didn't revise the manuscript as I mentioned last time, especially in point 4:the name of the genus Clostridium has been updated. Please update the new name of this bacteria.

We apologize for omitting this update and corrected the name to *Clostridioides difficile* thorough the text.

The English writing style is still need to be improved. Spelling mistakes are universe through the whole paper: line 83, 90,122 etc.

Please see answer R1Q2

R2Q2 The FMT in ICU patients is not suitable for wide application due to its safety. The transplantation of functional molecues, certain mixed probiotics or benefical metabolites is considered to be much safer and more effective.

Firstly, it should be stressed that FMT for critically ill patients cannot be used outside the context of clinical trials. We stated this in the original manuscript, but we have altered the Discussion so it is even clearer that this is the case. We agree that functional metabolites could be more easily standardized in clinical trials as compared to FMT, but they are not without risk either (doi:10.3390/biom11101459). Although the risks of FMT are not to be underestimated, therapy does not have to be perfect to be useful (doi: 10.1097/MCC.0000000000000489 , 10.1038/s41575-019-0254-3, 10.1016/j.cell.2018.08.047 , 10.1186/s13054-019-2604-5) and we believe that FMT is simply understudied and more work needs to be done before definitive conclusion could be made.

R2Q3 *The reference is relative out of date for this paper. FMT application has many new progress and safety issues these 3 years. However, these reference are absent.*

We have added a recent study on FMT, which mentions death due to MDRO bacteremia after FMT (doi:10.1056/NEJMoa1910437) as well as a current review from our group. (doi: 10.3390/biom11101459).

REVIEWER #3

R3Q1 *Řehořová, Cibulková and colleagues have adequately responded to most of my earlier questions.*

Thank you.

R3Q2 *It remains unclear to me how externally valid this SOP will be. While the exclusion criterion of being at least 48 hours off antibiotics is now explicitly stated, how many ICU patients will be eligible to be included in any FMT trial with this criterion as stated. Also, as stated this criterion is overly broad. A patient on IV vancomycin monotherapy for MRSA bacteremia, for example, likely has little to no ongoing effect on the colonic microflora, even in an end stage renal disease patient who could have detectable serum levels for weeks. In contrast, patients treated with enteral vancomycin have had detectable stool vancomycin levels for (PMID: 24098459) up to 8 days after discontinuation. There is no comparable stool PK data for most other antibiotics, particularly for fidaxomicin. The authors should very carefully consider this issue, as FMT for C. difficile infection will likely be futile due to engraftment issues if it is ignored yet likely to involve withholding standard-of-care treatment if use of FMT to treat CDI in the ICU setting is really to be attempted.*

This is an important comment. We should have stressed that this SOPs were designed to treat antibiotic-associated diarrhea, with the treatment initiated BEFORE C. dif. result is known in most cases. Concern about non-absorbable antibiotics is relevant and we adopted the thext to reflect it. In addition, modern faecal derivation systems allowing continuous drainage of the liquid stool are perfect opportunity to study “enteral kinetics” on non-absorbable antibiotics.

R3Q3 *The donor exclusion criteria are now clearer, but it is clear that residual risks of donor-derived infection remains, and these should be communicated in informed consent documents, particularly since the authors feel it is necessary to include critically ill patients for whom only proxy consent will be obtainable. I would include among these risks:*

HPV infection, as there is no donor screening for this

STI, as there is no rectal screening for chlamydia, gonorrhea, or HSV outlined and all sexual risks are self-reported

GI pathogens currently unknown to medical science

While some of the above risks are likely irrelevant for older individuals, they may have considerable significance for younger patients.

We agree with this comment and add this to the Discussion. Please note that these SOPs are in accordance with other guidelines including OpenBiom and dictate minimum standards. The ultimate responsibility to accept or reject donor is with the physician examining donors. Indeed, if there are any concerns, the battery of tests can be extended to include STDs.

R3Q4 *My concern in reviewing the supplementary material and the main document is that there still aren't written donor deferral criteria, and the authors outline a large number of screening tests that may disqualify donors, some needlessly. If a patient has an serum alkaline phosphatase that is below reference range on hepatic function testing, for example, it could be because the donor has unrecognized hypophosphatasia or, more likely, because they are in the lower bound of normal range for this test. Some investigator discretion/evaluation process should be outlined here to account for this whereas some tests (HIV, hepatitis B S Ag) should be hard stops.*

We agree that clinical judgement is always needed and Reviewer #3 describes exactly that. We have reformulated the criteria to put more emphasis on the final decision made by physician examining donors (See also R3Q3).

R3Q5 *The authors should expand the recipient exclusion criteria now outlined on p.6 line 288-289 into a bulleted list. "Severe neutropenia" should be defined, as should "recent colorectal surgery," i.e. how recent is "recent" and does a percutaneous endoscopic gastrostomy tube count or not? It is not technically a "colorectal" surgery and probably the most common GI procedure ICU patients undergo.*

We have defined the terms “severe neutropenia” and “recent colorectal surgery” more specifically in the revised text. The percutaneous gastrostomy is not a colorectal procedure and does not represent a contraindication.

We thank all reviewers for their ongoing efforts to improve the manuscript. We hope the revised form will be acceptable for publication in Biomolecules.

Best regards

Frantisek Duska (on behalf of the authors)