**The original reviewer's comments are in *greyed italics* with our responses in normal type face.**

To Reviewer #2,

*Manuscript Summary:*

*Francis et al described the protocol of oral administration pf probiotics to neonatal mice using gavage. This method is of great interest in current field of probiotics and microbiome research, and will benefit several scientific groups. The paper is written well to follow, however this reviewer find few minor comments, that may help to increase clarity for readers.*

*Minor Concerns:*

*1. Is there any recommendation for optimizing probiotics content? The maximum number of bacteria to be given for neonates.*

We thank the reviewer for the thoughtful and encouraging comments on the manuscript. We are excited to see that the reviewer finds our work as a significant contribution to the field. The suggestions have been taken into consideration in the revision of our manuscript. Specifically, the optimization of the probiotic usually is built off its previously known effectiveness. A dose titration can be done to determine the safe zone for the administration of the probiotics. If the probiotics are administered in combination with the prebiotics (a synbiotic formula), then the viscosity of solution creates a challenge for gavage and dosing. In this case the limits on the doses are due to the physical properties of the gavaged liquid.

*2. Page 4, line 160, what is the suggested solvent in dissolving the mixture? I believe it 5% dextrose solution, if so mentioning here in bracket may help for readers.*

The solvent for dissolution has now been named as suggested (page 4). We agree that it is best to be explicit in a protocol, so it is presented as a ready-to-use format for the end-user.

*3. Page 6, line 222, does the description "by moving it away from you" mean "by moving it away from your hand"?, making more specific may help to readers.*

The suggestion for page 6 regarding the calibrated movement of the syringe while holding the pup has also been addressed and elaborated in a way to create better imagery of the small movements needed do undertake this procedure.

*4. For the fecal samples collection on page 7, was there any optimization was suggested, how long it will take for the gavaging mixture to go through the intestine?*

The comment regarding how long the gavage mixture will take to pass through the intestine is addressed in protocol step 5.5.

*5. Since different doses of LP copies were used in the two gavaging schedules, were the values/data were comparable?*

We apologise for the unclear representation of the dosage; in both gavage schedules, the dosage was the same ($10^6$ CFU) per gavage but the frequency of the procedure was different – once every 24 hours versus once every 48 hours. This setup made the values and data comparable.

We thank the reviewer again for the sincere and thoughtful comments and we hope we have adequately addressed the concerns and have improved the quality of the manuscript.