Effect of processing conditions on microbial viability and survival in fecal microbiota transplant

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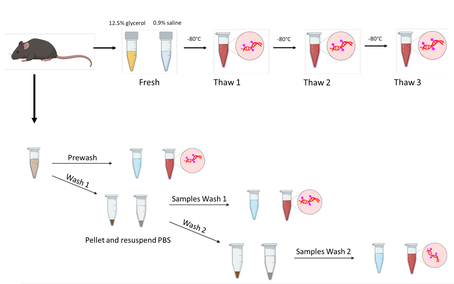
Introduction

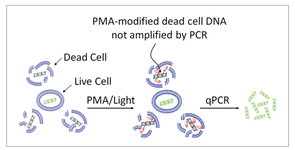
- Fecal microbiota transplantation (FMT) is the administration of microbes from healthy donor stool in order to modify the recipient's microbiome for the purpose of treatment or prevention of a medical condition
- Donor microbiota are exposed to a number of different environmental conditions throughout manufacturing and storage of FMT products, some of which may reduce the viability of microbes received by patients
- The therapeutic effects of FMT are generally believed to come largely from engraftment of donor microbes within the recipient, thus viability loss may have understudied impacts on clinical efficacy of FMT
- Knowledge of the conditions which decrease or maintain microbial survival can inform better protocol development and standardization

Objectives

- Quantify changes in microbial viability and community structure associated with different stool processing and storage conditions
- To identify manufacturing conditions that might affect the outcome of an FMT and determine what type of preparation of FMT will best preserve microbial viability and engraftment
- Compare microbial engraftment between multi-donor FMT and single-donor FMT to better inform risk-benefit analyses between single donor and multi-donor FMT

Materials and Methods





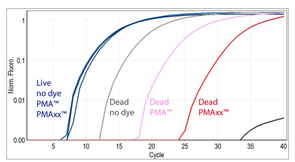


Image source: https://biotium.com/technology/pma-for-viability-pcr/

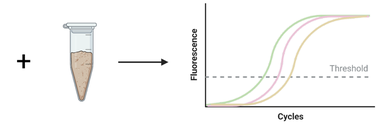
Experimental treatments

- Effect of oxygen on bacterial viability investigated by repeated pellet and wash of FMT material under aerobic or anaerobic conditions.
- Effect of cryoprotection investigated with repeated freeze-thaw cycles at -80°C using FMT material with or without glycerol (12.5% v/v) in normal saline.
- Bacterial quantification qPCR results were obtained to determine the number of viability of microbiota between different preparation conditions.

Verification of PMA treatments in stool matrix

- Defined bacterial consortia spiked into autoclave-sterilized human stool to verify PMA treatments in stool matrix and investigate effects on viability.

Strain	Target OD600
B. finegoldii	0.14
B. stercoris	0.06
Bif. catenulatum	0.08
L. ruminis	0.33



Mock microbial community + *Human stool matrix* → *qPCR testing*

Estimating total bacterial viability

- Propidium monoazide (PMA) selectively enters and binds DNA of non-viable cells with compromised membrane integrity.
- FMT materials made with freshly collected mouse feces were suspended in sterile saline or PBS (1:100 w/v) and filtered to 100 microns prior to PMA treatment.
- UV light exposure (30-minutes) covalently links PMA with non-viable DNA, preventing PCR amplification.
- qPCR targeting universal bacterial 16s gene used to estimate bacterial abundance with and without PMA treatment and quantify changes in total viability.

Results and Discussion

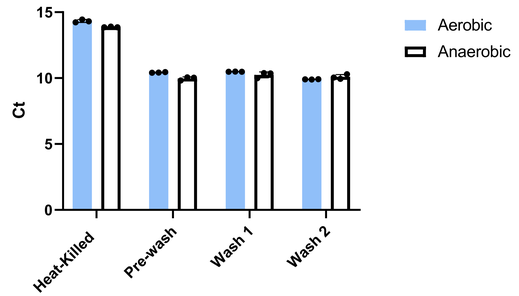


Figure 1. Minimal impact from two washes performed either aerobically and anaerobically. Little change in microbial viability seen throughout either the aerobic or anaerobic wash steps.

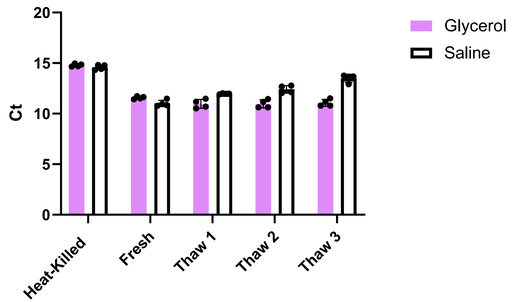


Figure 2. Impact of multiple freeze-thaws on viability of stool resuspended either with or without glycerol. Throughout the freeze-thaws saline decreases in microbial viability while microbial viability remained relatively unchanged when resuspended in glycerol, implying glycerol aids in survival.

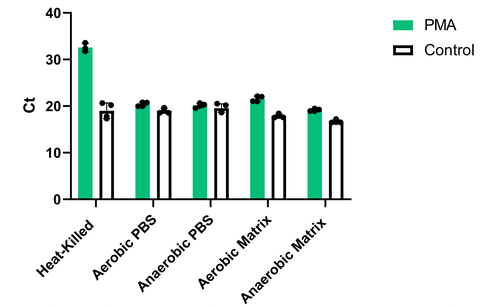


Figure 3. Viability loss in a 4-strain consortium suspended in either sterile PBS or autoclaved stool matrix, under aerobic and anaerobic atmospheres. Control groups show samples without PMA. Oxygen had minimal effect on consortium viability when resuspended in PBS, but had greater impact when consortium spiked into stool matrix, suggesting the stool matrix has oxygen-dependent influence on bacterial survival.

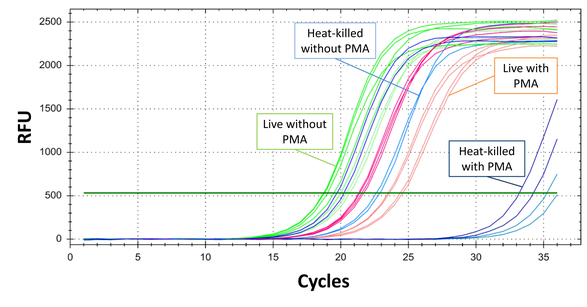
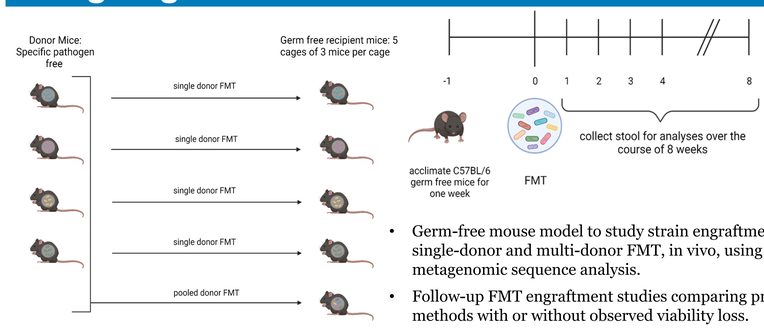


Figure 4. Amplification curve of the qPCR output of consortia with stool matrix. Heat-killed samples with PMA shown with late amplification after 30, whereas live samples with no PMA treatment are seen to be the first amplifying.

Ongoing Studies and Future Directions



- Germ-free mouse model to study strain engraftment from single-donor and multi-donor FMT, in vivo, using metagenomic sequence analysis.
- Follow-up FMT engraftment studies comparing preparation methods with or without observed viability loss.

Conclusions

- Preliminary studies found little evidence of viability loss from repeated pelleting and washing of mouse microbiota, regardless of oxygen in wash media.
- Successive freeze-thaw cycles caused progressive decreases in total viability of microbiota suspended in saline, but the addition of 12.5% glycerol to suspension mitigated the effects.
- Autoclaved stool matrix had unexpected impacts on PCR outcomes when spiked with defined bacterial consortia. Follow-up needed to better understand relationships between of stool matrix, oxygen, and PMA treatment/bacterial viability.

Acknowledgements

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