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Examining Preservation Methods for Long-Term Fecal Matter Storage

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Aims
- To analyze metabolic activity of bacteria after storage in order to determine which cryogenic technique best preserves fecal matter samples
- Personalize fecal matter transplant (FMT) process

Introduction
The human microbiota contains many diverse species of bacteria and other microorganisms. Disruption of the microbiome within the gastrointestinal tract can lead to the proliferation of harmful bacteria such as vancomycin-resistant Enterococcus (VRE) or Clostridium difficile. Clostridium difficile, or C. diff, is a common hospital-acquired pathogen which disrupts the normal function of healthy bacteria. Though typically treated with antibiotics, C. diff is often able to resurface due to antibiotic overuse.

Since antibiotics may be ineffective when treating bacterial diseases such as C. diff other treatments are needed to eliminate the threat of infection and reestablish the healthy microbiota in the gut. Fecal matter transplants (FMTs) have been used to successfully treat patients in clinical studies. However, using donor samples can put patients at risk and has resulted in at least one death reported by the U.S. Food and Drug Administration. By personalizing the donation process of fecal samples, the risk of introducing harmful microorganisms could potentially be reduced.

Future Studies
- Use DNA sequencing to determine which microorganisms are lost after storage and which metabolic activities are impacted as a result

Materials and Methods

Homogenization
- Day 0
  - Original Sample
    - 80g dog fecal sample partitioned into two parts
    - 40g mixed with 120 mL 0.85% NaCl (saline)
    - 40g mixed with 120 mL H2O (water)
  - Dilutions
    - A (10^-1), B (10^-3), C (10^-5)
    - NaCl or H2O as diluents
- Day 21
  - Aerobic and anaerobic environments created during incubation
  - Incubated at 37°C for ~36 hrs

Data Analysis
- Color change in Biolog™ plates used to compare bacterial function at Day 0 and Day 21 after -80°C storage

Results

Comparison of Percent Change Among Treatment Groups

Discussion and Conclusion
- After calculating percent change of metabolic activity among treatment groups, all treatment groups showed loss of cell function after storage
- The water glycerol treatment group had the smallest percent change out of all other treatment groups
- Treatment groups that contained glycerol and were incubated in an aerobic environment more closely resembled the metabolic activity seen in original samples
- Treatment groups with no glycerol lost the most metabolic activity after storage
- Fecal matter stored with glycerol preserves microorganism communities better than treatments with no glycerol
- This study of metabolic activity and cell viability supports earlier findings based on DNA preservation

References
3. United States Food and Drug Administration (2020). FDA In Brief: FDA warns about potential risk of serious infections caused by multi-drug resistant organisms related to the investigational use of Fecal Microbiota for Transplantation