Effect of Stool Transportation Conditions on Microbiota Diversity

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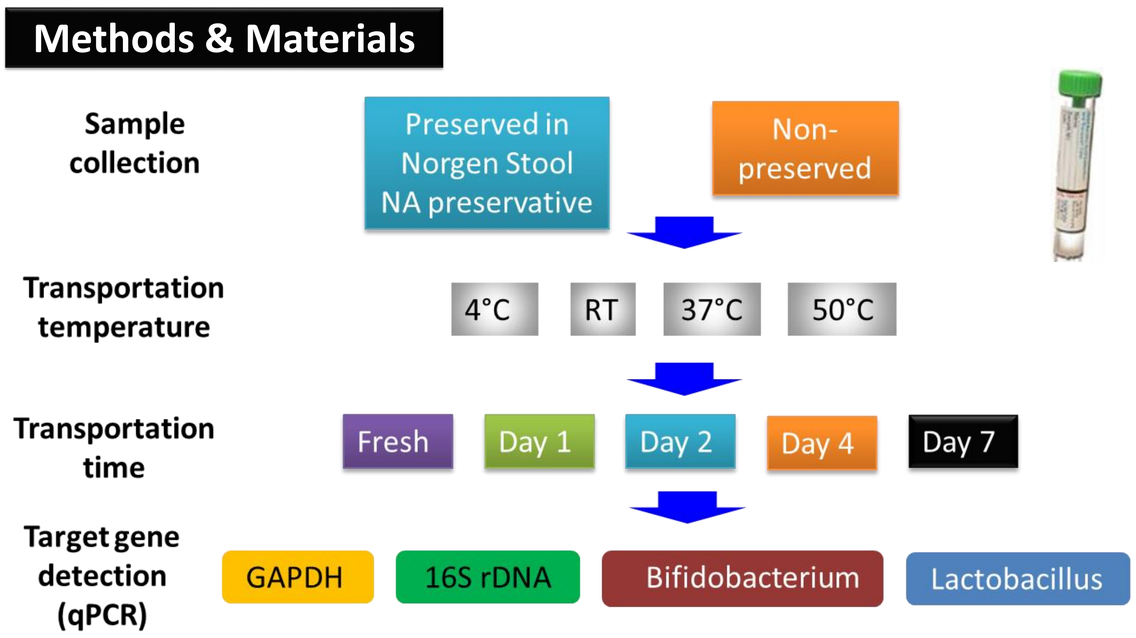
Abstract

Nucleic acid (NA) based applications are a powerful tool driving research in the area of human cancer marker discovery, pathogen diagnosis and genetic tests. These nucleic acids are isolated from many different human specimens, and it is known that the method of sample transportation prior to nucleic acid purification can influences the sample's stability, greatly affecting the NA contents and therefore resulting in data variability. Despite this knowledge, however, the importance of sample preservation and transportation has not been extensively studied before. Therefore, this study focuses on the effect of transportation conditions, particularly temperature fluctuation, on microbiota diversity in human stool. A number of different microbial targets were monitored by qPCR from stool samples exposed to 5 different temperatures, with and without the addition of a stool NA preservative (Norgen Biotek Corp.). The results indicate an intriguing observation in that the population of a certain microorganism was affected by the temperature and the incubation time, resulting in variability of the microbial population analysis as demonstrated by qPCR.

Methods & Materials

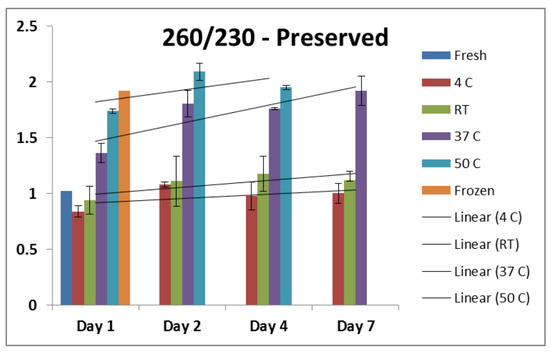


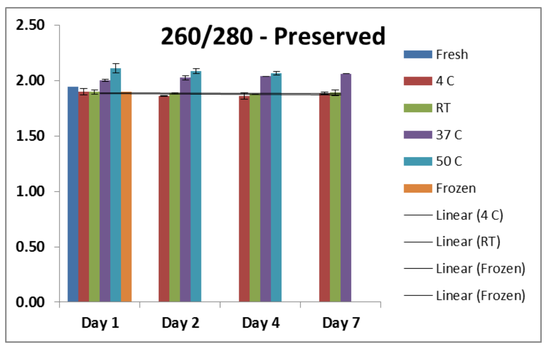
Outline of the experiment set up . Samples were collected from healthy donors and split into the 4 collection tubes with or without a Norgen stool nucleic acid (NA) preservative. Each tube was incubated at a different temperature to simulate the various transportation conditions. Total DNA then was isolated at different time points, and the DNA was directly used for real-time PCR (qPCR) for the target gene monitoring.

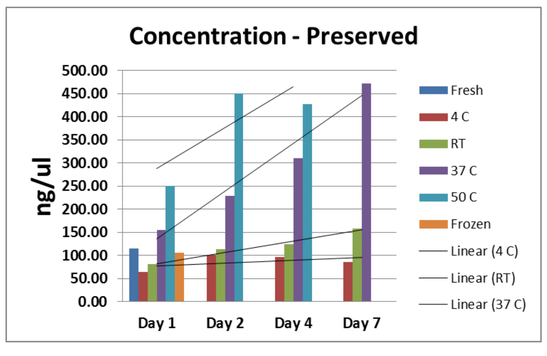
Results

- **Preservative affects stool DNA quality (A 260/280 and A 230/280) and quantity (concentration) for the variable sample transportation conditions.**

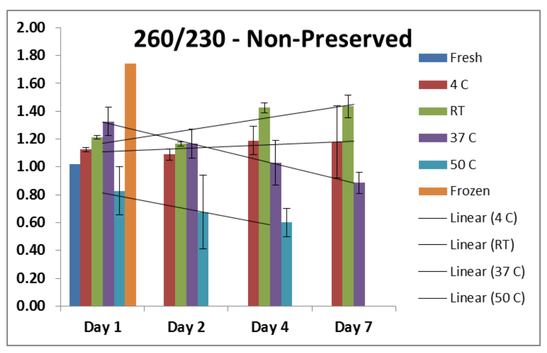
Preserved

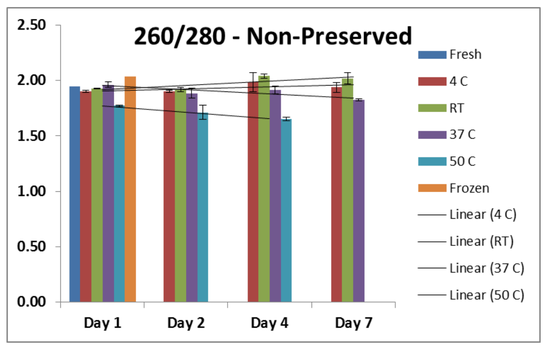


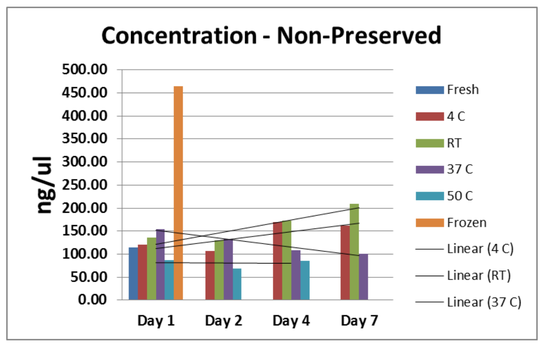




Non-Preserved

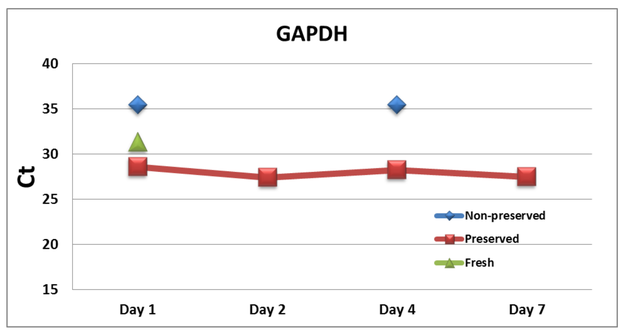


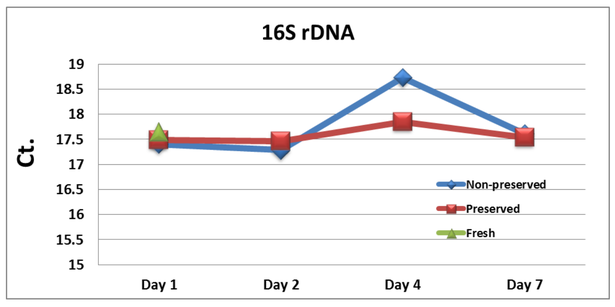


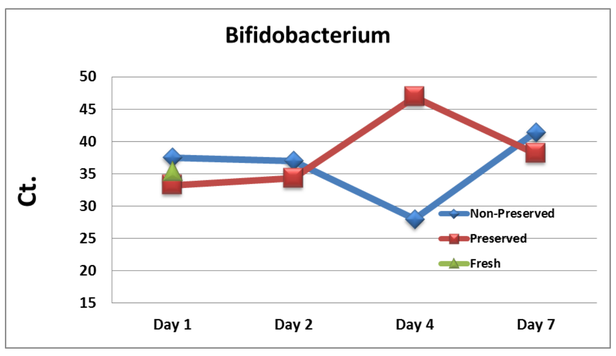


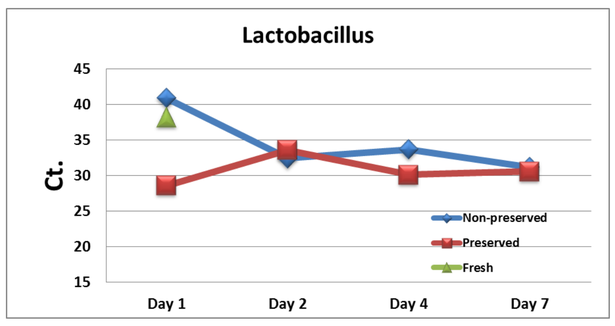
- **Preservative was able to provide more reliable gene stability, as indicated by real-time PCR (SYBR Green).** Human (GAPDH), gram negative (16S rDNA) and two gastrointestinal gram positive bacteria were monitored from the stool samples stored at ambient or 50 °C temperature with/without Norgen's stool nucleic acid (NA) preservative.

Ambient temperature

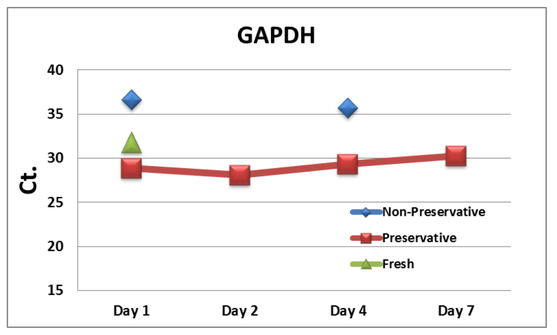


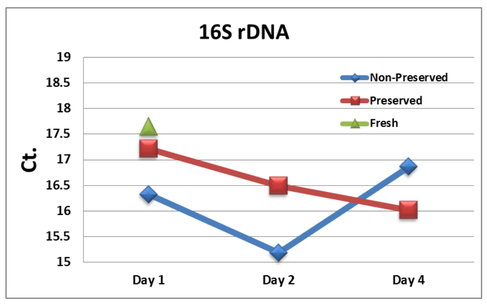


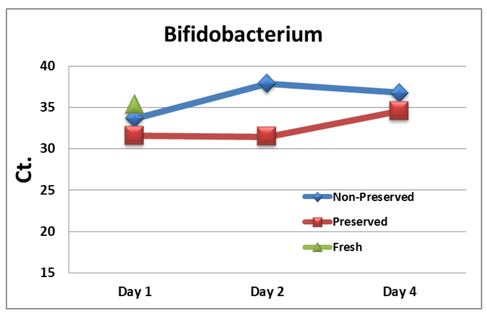


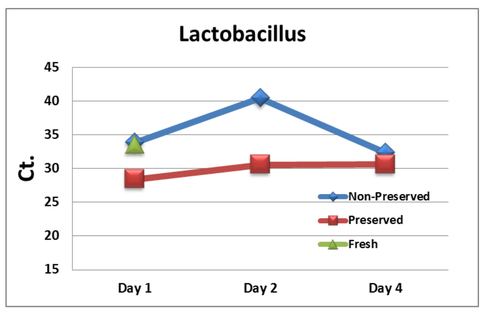


50 °C









Summary

- Preservation of biological information at the site of sample collection, without altering the original nature of the specimens, is critical.
- Stool preservative improves the DNA quality (A 260/230) and yield from the samples being transported at RT, warm (37°C) or hot (50°C) temperature conditions.
- The human gene GAPDH was well stabilized in preserved stool samples, even with a temperature shift (RT to 50°C)
- The population of gram negative bacteria (indicated by 16s DNA gene) was found to change dramatically without the preservative during the simulated transportation period.

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