

Is freezing necessary for conservation of microbiome diversity in stool samples?

ABSTRACT

A major challenge for microbiome studies is maintaining an even and accurate DNA extraction of samples with a wide range of bacterial content. Severe errors and biases are caused by sample storage and DNA extraction. Therefore, we performed a deep analysis of the gut microbiome at different sample storage conditions.

Stool samples were either frozen at -80°C or stabilized in Invitek Stool Collection Tubes with DNA Stabilizer. Microbiome composition was measured via NGS-16S typing on MiSeq (Illumina) and data analysis was done via PERMANOVA. The study shows equivalent results of microbiome composition for each donor independent from the storage condition.

METHODS FOR PRESERVATION AND STORAGE OF SAMPLES

Due to the high content of DNases/RNases and bacterial growths stool samples must be treated with care to maintain bacterial composition of donors. Different storage conditions, freezing at -80°C and sample stabilization (Fig. 1) are discussed for sample preservation.

Sample freezing at -80°C :

- Considered to be the gold standard in stool sample management
- Must be done within few hours, therefore often impractical to execute in study design with at home sampling.

Sample stabilization:

- Sample preservation at room temperature
- Samples can be stored and shipped without the need of a cooling chain which greatly facilitates study design, as subjects can conveniently take the sample at home.

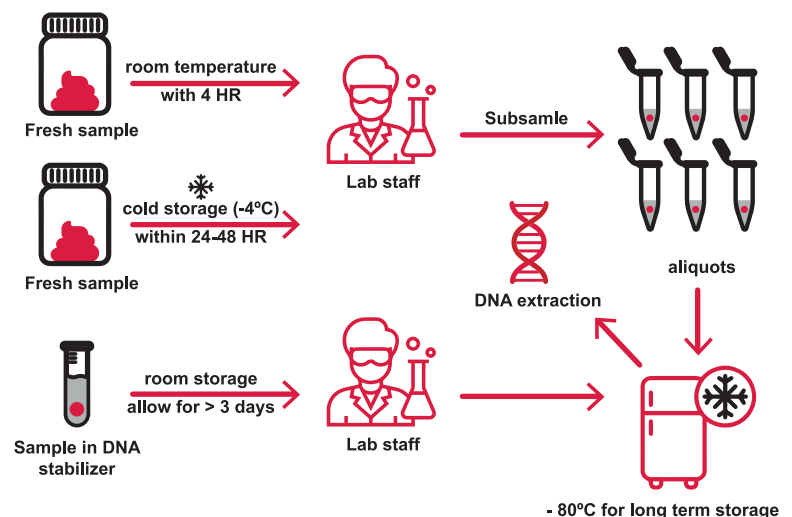


Fig. 1: Recommendation of sample collection and storage (adapted from Wu et. al. 2019, <https://doi.org/10.1016/j.jfma.2018.02.005>)

SPECIFICATIONS OF THE STOOL COLLECTION TUBE WITH DNA STABILIZER

- Integrated spoon for easy sampling
- Prefilled with DNA Stabilizer: inactivates bacteria by pre-lysis & inactivates DNases
- Preservation of microorganism titre at time point of collection
- Stabilization of samples for 3 months at RT (temp. range: $15\text{-}30^{\circ}\text{C}$)
- No need for cooling during transportation or storage
- Isolated DNA is suitable for NGS applications, microbiome diversity studies



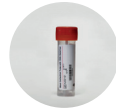
Fig. 2: Stool Collection Tube with DNA Stabilizer

Application Note

STUDY: NGS ANALYSIS OF DIFFERENT STORAGE CONDITIONS



Freezing at -80°C
Extraction after 21 days, 90 days



Invitek Stabilization, RT
Extraction after 3 days, 21 days, 90 days

Fig. 3: Storage conditions and time points for extraction in the study

To compare the effect of different storage conditions in total 55 samples from 4 different donors were analyzed. Frozen stool samples were extracted after 21 days and 90 days, samples collected with the Stool Collection Tube with DNA Stabilizer were extracted after 3 days, 21 days and 90 days (Fig.3). For control, samples were extracted shortly after collection without further storage.

Two different extraction methods were used, the PSP® Spin Stool DNA Basic Kit (Invitek) and the QIAamp DNA Stool Mini Kit (Qiagen), for the latter ones the extractions were done by the Microbiome Laboratory, University of Kiel, Germany & University Hospital Schleswig Holstein, Campus Kiel. Nucleic acids of all extractions were analysed via 16S-rRNA-Genotyping of the V1-V2 region and MiSeq-System (Illumina) by the Microbiome Laboratory in Kiel.

RESULTS SAMPLE INTEGRITY

All data obtained were subject to PERMANOVA analysis to compare the microbiota of the donors, the performance of the different extraction kits and the sample stabilization for up to 3 months. The results show that bacterial diversity is observed only between individuals but not samples (Fig.4) and both extraction kits perform equivalent (Fig. 5). Further it can be shown that sample integrity is maintained over the whole timeframe of the study (Fig. 6).

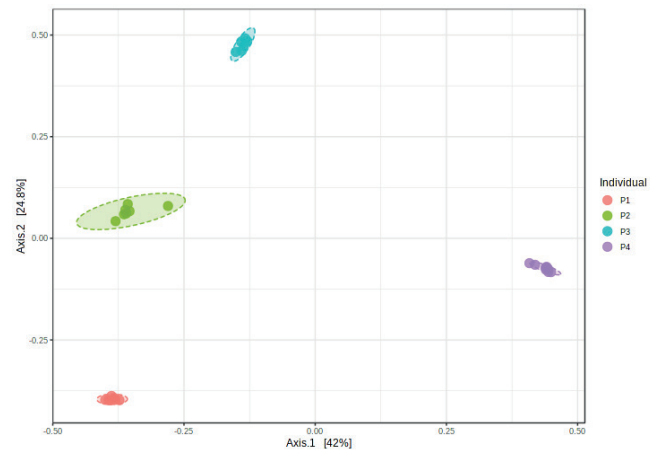


Fig. 4: Analysis of the bacterial populations of all four individuals in the study

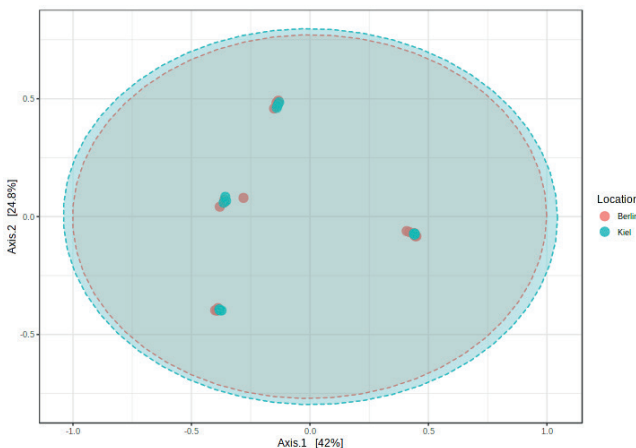


Fig. 5: Analysis of the different extraction methods. PSP® Spin Stool DNA Basic Kit (Berlin) and QIAamp DNA Stool Mini Kit (Kiel)

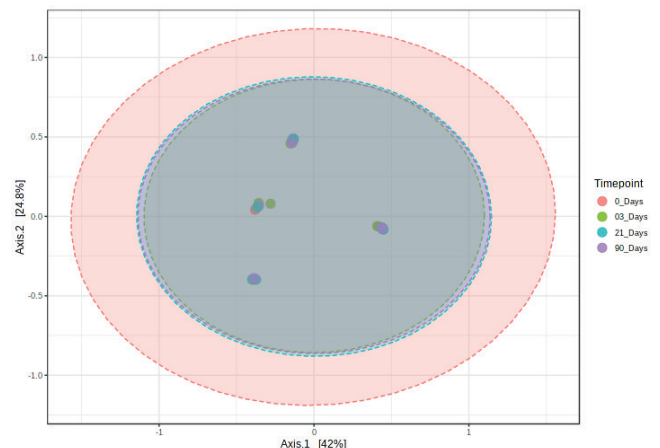


Fig. 6: Analysis of the bacterial composition of stabilized samples after 0, 3, 21 and 90 days.

Application Note

FREEZING VS. STABILIZATION

To investigate the effect of freezing vs. stabilization, the percentage composition of the 17 most common bacterial families at different storage conditions and times was examined, Fig. 7 shows the result for one donor. Additionally, a calculation via a modified Chi-Square was done (not shown) to prove that the bacterial composition and abundance is comparable at all time points. Overall, the result shows that there is no significant difference between the different storage conditions, freezing or stabilization.

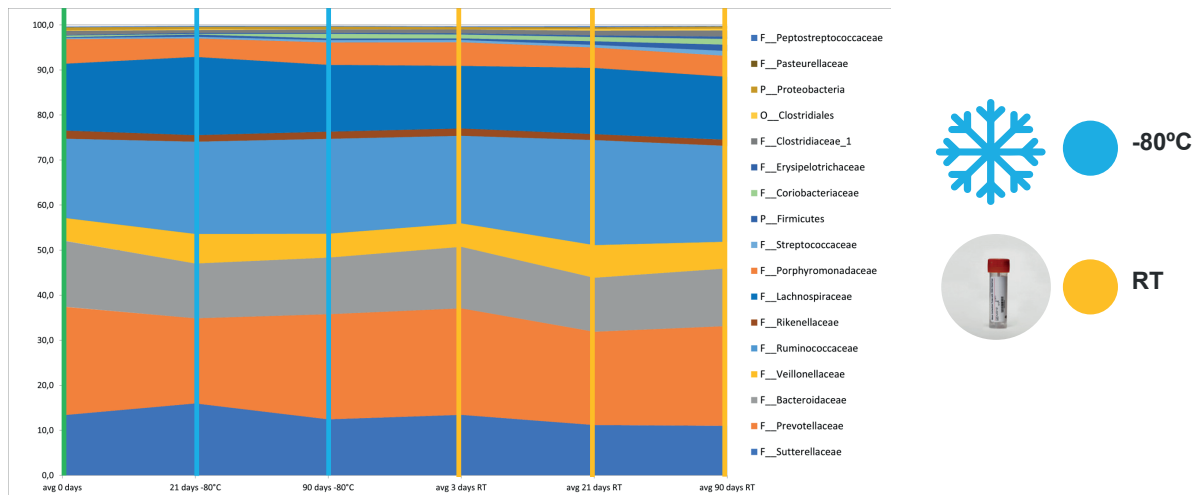


Fig. 7: Composition of bacterial families for one donor at different time points and storage conditions.

CONCLUSIONS

- Samples stored in the Stool Collection Tubes with DNA stabilizer are efficiently preserved over the whole timeframe of 3 months analyzed.
- The different storage conditions, freezing at -80°C and stabilization at RT do not show any significant difference in bacterial composition or abundance.
- With the Stool Collection Tubes with DNA Stabilizer samples have high integrity, also with third party extraction kits, e.g. Qiagen*
- Sample stabilization in Stool Collection Tubes with DNA Stabilizer is a suitable and reliable alternative to freezing samples, which greatly facilitates sample collection, transport, and storage.

* for further information on compatibility with other extraction kits you may reach out to techsupport@invitek.com

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