

Microbiomics

APPLICATION NOTE 4

DANASTOOL Sample Collection MICROBIOME Kit

Detection of SARS-CoV-2, Human Genomic DNA and Microbiome Analysis from preserved Stool Samples in DANASTOOL Sample Collection MICROBIOME Kit

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Introduction

The use of stool as a biological sample for nucleic acid isolation and subsequent biomarker or viral nucleic acid detection has only recently been recognized.

Appropriate preservation and storage of stool samples is crucial in maintaining DNA integrity, viral nucleic acids and microbial community composition for downstream applications and analysis.

DANAGEN-BIOTED S.L has developed DANASTOOL Sample Collection MICROBIOME Kit containing a liquid solution that allows for the long-term preservation of stool samples at ambient room temperatures.

DANASTOOL Sample Collection MICROBIOME Kit is designed for the collection, storage and stabilization of stool samples. It comes in a tube with spoon and liquid stabilization solution that preserves the MICROBIOME profiling.

This application note investigates the ability of our stool preservative system to maintain the integrity of human DNA and microbial DNA, as well as viral nucleic acids for long-term times and its direct use in sensitive downstream diagnostics, including qPCR, Next-generation sequencing (NGS) and Microbiome characterization using different DANAGEN isolation kits.

Material and Methods

Stool sample collection

A single stool sample of 800 mg was collected from healthy donor using our DANASTOOL Sample Collection MICROBIOME Kit and stored at room temperature.

DNA isolation

Human genomic DNA was isolated from 1000 μ l of preserved stool sample. Isolation was carried out on day 1, day 8 and day 15 and processed following the recommended protocol of our DANAGENE MICROBIOME FECAL DNA Kit. All samples were done in duplicate.

Viral RNA isolation

Preserved stool samples were spiked with a positive sample for COVID. Isolation was carried out on day 1, day 8 and day 15 and processed following the recommended protocol modification of our DANAGENE MICROBIOME RNA Kit. All samples were done in duplicate.

Quality and Quantification of extracted DNA

For DNA quantification, DNA concentration was determined fluorometrically on the Qubit 4.0 fluorometer (Thermo Fisher Scientific, USA) using the QUBIT dsDNA BR Assay Kit.

For DNA quality, DNA purity was determined via 260/280 and 260/230 ratios measured on the NanoDrop (Thermo Fisher Scientific, USA).

Gel Electrophoresis

For visual analysis of Human DNA and Microbial DNA size and integrity, 20 μ l of DNA from the final elution were loaded onto a 1% agarose TAE gel and run for 30 minutes at 125 V.

Real-Time PCR analysis

The purified DNA was used as a template in a Real-Time PCR reaction for the detection of human genomic DNA using:

1. GAPDH primers following the protocol of qMAXSen Green qPCR MasterMix (2x) (Canvax Biotech, Spain).
2. The cfhDNA MONODOSE dtec-qPCR (Genetic PCR SolutionsTM, Spain) for the detection of Cell-free human DNA. The target is a multiple-copy gene, 200 copies per genome, with a slow evolutionary rate.

For the detection of SARS-CoV-2, One-Step RT-PCR was performed following the protocol of the CoVID-19 dtec-RT-qPCR (Genetic PCR SolutionsTM, Spain).

All qPCR analysis were performed in duplicate.

16S rRNA Gene Sequencing

Genomic DNA amplification was conducted using the 16S Barcoding Kit (SQK-RAB204; Oxford Nanopore Technologies, Oxford, UK) with the following PCR conditions:

Initial denaturation at 95°C for 1 minute, 25 cycles of 95°C for 20s, 55°C for 30s, and 65°C for 2 minutes, followed by a final extension at 65°C for 5 minutes.

Amplifies were purified using Agencourt AMPure XP beads (Beckman Coulter) and quantified by fluorometric quantification with Qubit (Thermo Fisher Scientific).

A total of 100 ng of DNA was used for library preparation and sequenced in MiniON flow cells (FLO-FLG001; Oxford Nanopore Technologies) according to the manufacturer's protocol.

Results and Discussion

When we developed our DANASTOOL Sample Collection Microbiome Kit, we established that the microbial composition of preserved stool samples is unchanged for at least 1-2 months. This application note illustrates that also our stabilization solution allows stabilization of the Microbial DNA and Host Human DNA, as well as the presence of viruses such as the SARS-CoV-2 for 15 days without any alterations in the quality.

Detection of Human Genomic DNA

DNA quantity was assessed using both gel electrophoresis (**Figure 1**) and spectrophotometry (Qubit) (**Figure 2**). We can observe how the DNA concentration ranges between 7.0 to 15.0 μg from 1000 μl of preserved stool sample (total input 8.0 ml) and it remains stable over time.

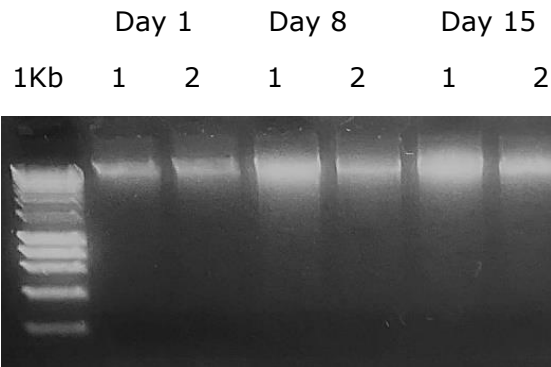


Figure 1. Gel electrophoresis of microbial and host DNA purified from preserved stool samples

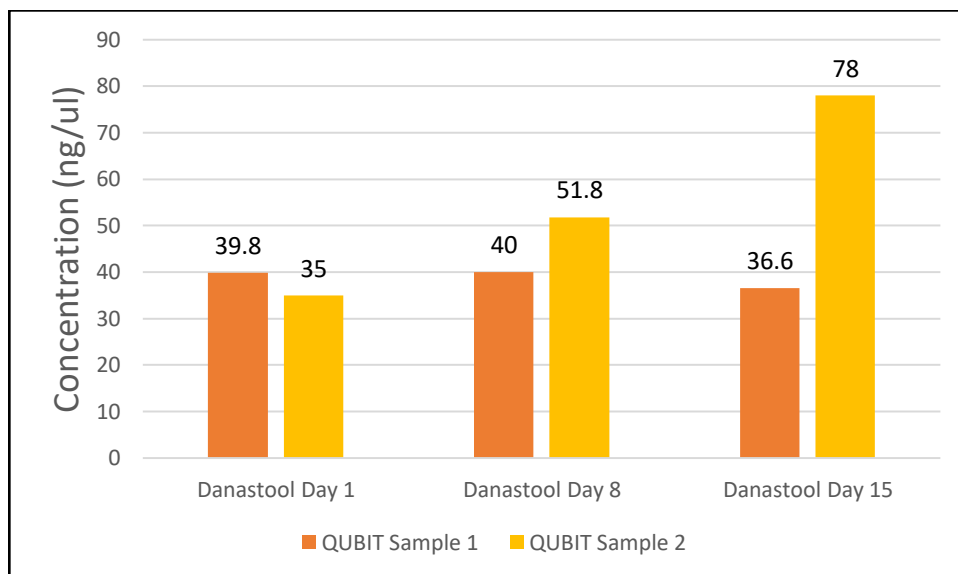


Figure 2. DNA concentrations of total DNA obtained by spectrophotometric analysis (Qubit)

DNA quality was assessed by spectrophotometry (Nanodrop) (**Figure 3**). All total nucleic acid isolated from all samples displayed A260/280 ratios > 1.75, demonstrating the high purity of the preparations. The A260/230 values are also good results if we take into account that they are stool samples.

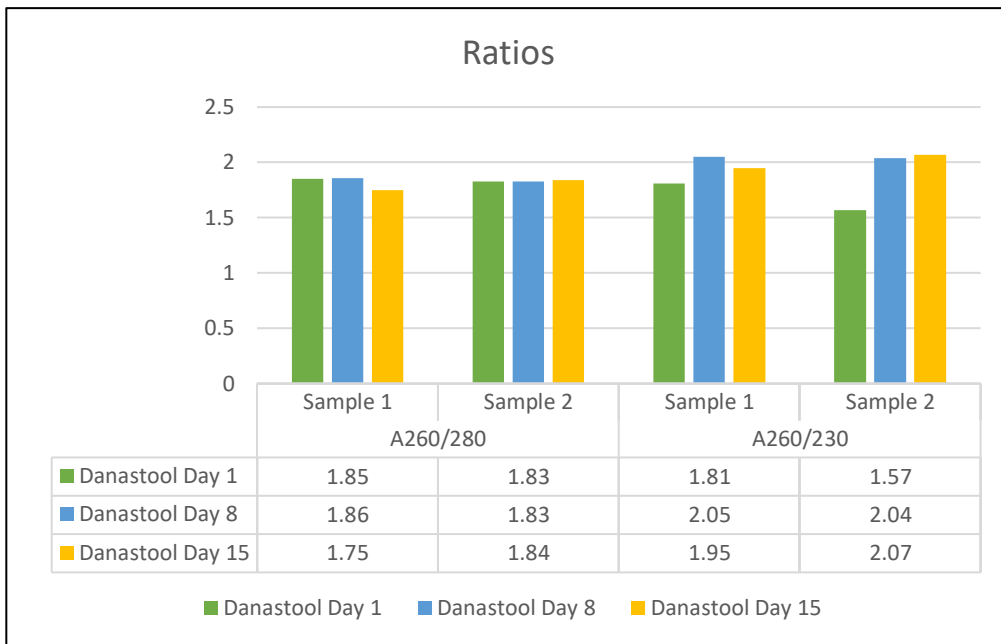


Figure 3. 260/280 and 260/230 ratios by spectrophotometric analysis (Nanodrop)

Isolation and detection of Human Genomic DNA in stool samples is a challenge, to demonstrate that our solution allows its preservation and our kits can isolate it from preserved stool samples, we performed 2 Real-Time PCR assays to detect the human GAPDH gene and the Total Human DNA (**Figure 4**).

We can observe a little variation in Ct, indicating that our stabilization solution is capable of stabilizing Human Genomic DNA intact at room temperature for at least 15 days. The results are mean values of 2 samples for each time.

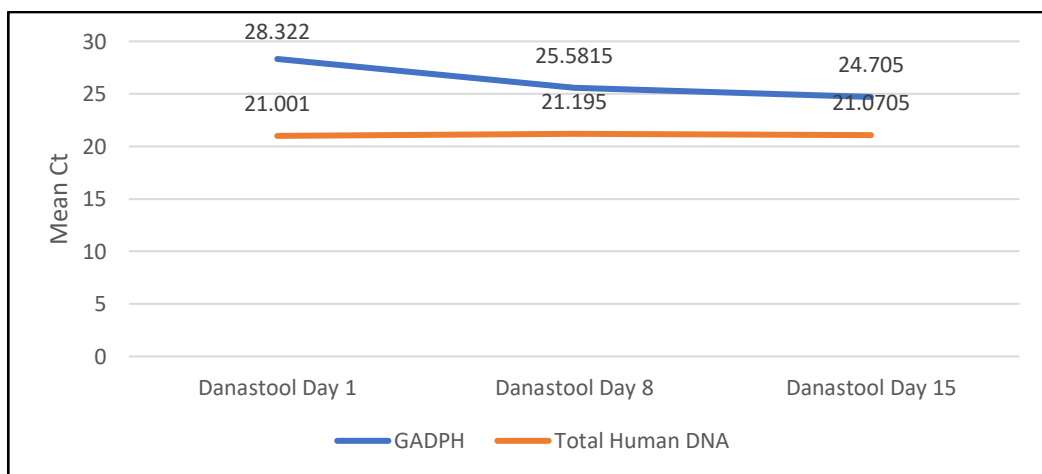


Figure 4. Amplification of the GAPDH gene and Total Human DNA by qPCR analysis

Detection of SARS-Cov-2

Through a Real-Time PCR of SARS-CoV-2, we obtained results indicating the Ct value over a period of 15 days for the inactivated SARS-CoV cells (**Figure 5**).

We can observe a little variation in Ct, indicating that our stabilization solution is capable of stabilizing SARS-CoV-2 cells or RNA from coronavirus intact at room temperature for at least 15 days. The results are the mean value of 2 samples for each time.

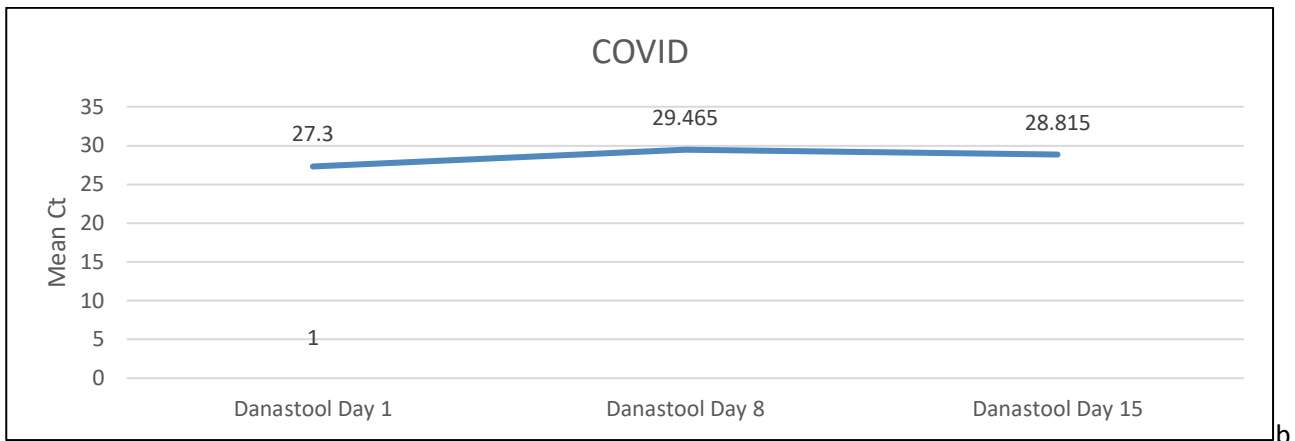


Figure 5. Detection of SARS-Cov-2 in preserved stool samples by qPCR analysis

Microbiome Analysis

The effect of storage conditions on fecal microbiome was assessed based on the relative abundance of bacterial genera (**Figure 6**). Microbial composition of preserved stool samples at room temperature is unchanged after 15 days. Samples are more stabilized and consistent at genus level when preserved using our DANASTOOL Sample Collection MICROBIOME Kit.

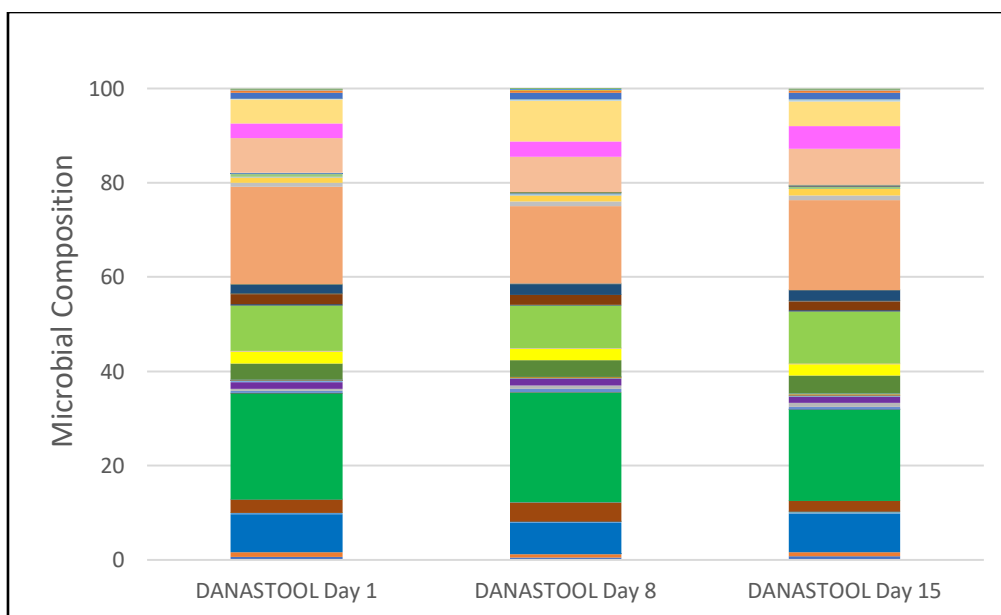


Figure 6. Microbial composition (Genus) of preserved stool samples at room temperature

Conclusion

- A) **Microbial and host human DNA from preserved stool samples are effectively stabilized in DANASTOOL Sample Collection Microbiome Kit at room temperature for at least 15 days.**
- B) **SARS-CoV-2 spiked in preserved stool samples is effectively stabilized in DANASTOOL Sample Collection Microbiome Kit at room temperature for at least 15 days.**
- C) **DANAGEN´ s isolation kits can be used to successfully isolate microbial and human host DNA and viral nucleic acids from stool preserved samples in DANASTOOL Sample Collection Microbiome Kit.**
- D) **Microbial composition of preserved stool samples is unchanged after 15 days at room temperature with DANASTOOL Sample Collection Microbiome Kit.**



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