Microbiomics

APPLICATION NOTE 5 **DANASWAB Sample Collection MICROBIOME Kit**

Detection of SARS-CoV-2, Human Genomic DNA and Microbiome Analysis from preserved Stool Samples in DANASWAB 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 DANASWAB Sample Collection MICROBIOME Kit containing a liquid solution which allows for the long-term preservation of stool samples at ambient room temperatures.

DANASWAB Sample Collection MICROBIOME Kit is designed for the collection, ambient storage and transport of samples collected using a swab.

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 used 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 200 mg was collected from healthy donor using our DANASWAB Sample Collection MICROBIOME Kit and stored at room temperature.

DNA isolation

Human genomic DNA was isolated from 400 µl of preserved stool sample. Isolation was carried out on day 1, day 8 and day 15. Briefly, 800 µl of Lysis Buffer LS + 25 µl of proteinase K were added. The mixture was incubated at 55ºC for 30 minutes. Following the incubation, the microtubes were centrifuged and the supernatant was transferred into a Mini Spin column. The microtubes were centrifuged for 1 minute and the flow-through was discarded. Next, 500 µl of Desinhibition buffer were added to the column and centrifuged for 1 minute, the flow-through was discarded. Next, 700 µl of Wash buffer was added to the column and centrifuged for 1 minute, the flow-through was discarded. The column was spin for 3 minutes in order to thoroughly dry the resin. The spin column was transferred to a fresh 1.5 ml microtube and 50 µl of Elution Buffer was applied to the column and centrifuged for 1 minute. All samples were done in duplicate.

Microbial DNA was isolated from 800 μl of preserved stool sample. Isolation was carried out on day 1, day 8 and day 15 and processed following the recommended protocol of our DANAGEN MICROBIOME Swab DNA Kit. All samples were done in duplicate.

Viral RNA isolation

Preserved stool samples were spiked with a positive sample for SARS-CoV-2. Isolation was carried out on day 1, day 8 and day 15 and processed following the recommended protocol modification of our DANAGEN MICROBIOME RNA Kit. All samples were performed 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 Genomic DNA and Microbial DNA size and integrity, 20 μl 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. GADPH 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 was 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 30 days. 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 1.6 to 5.0 μg from 400 μl of preserved stool sample (total input 2.0 ml) and increases over time, probably due to the lytic power of the stabilization solution.

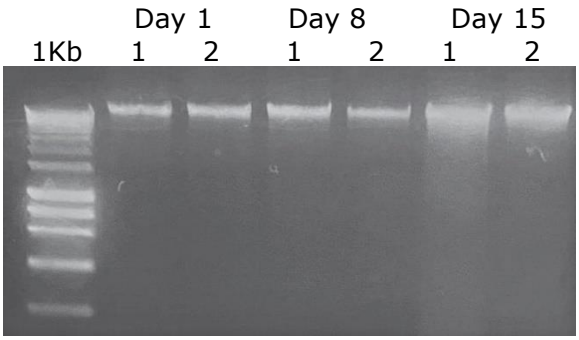

Figure 1. Gel electrophoresis of microbial and host DNA purified form preserved stool samples

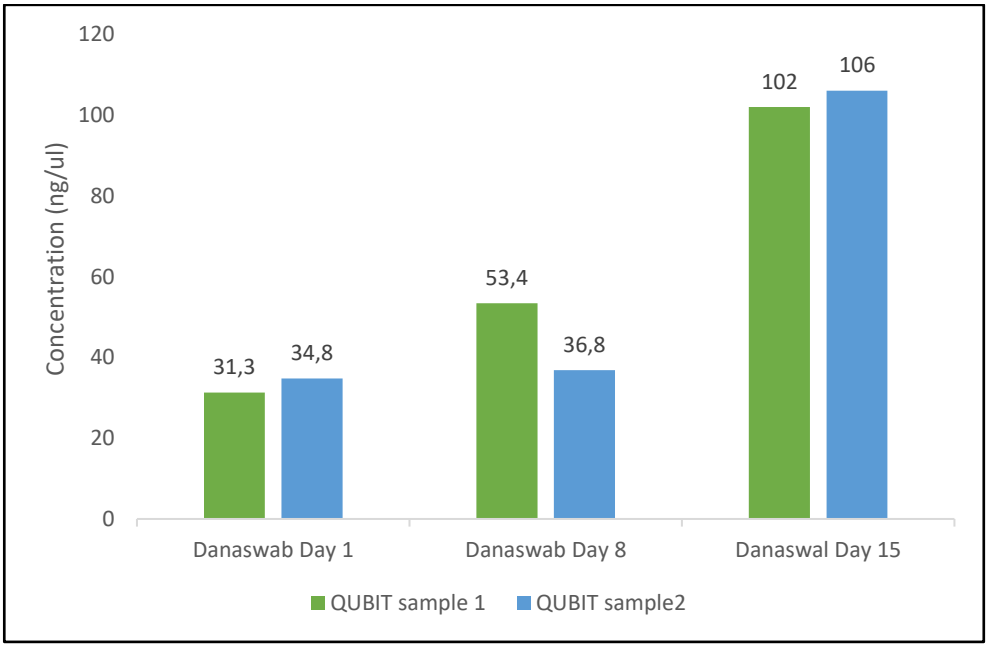

Figure 2. DNA concentrations of total DNA obtained by spectrophotometric analysis (Qubit)

DNA quality was assessed by spectrophotometry (Nanodrop) **(Figure 3)**. All total nucleic acid isolated from all samples displayed A260/280 ratios > 1.7, demonstrating the high purity of the preparations.

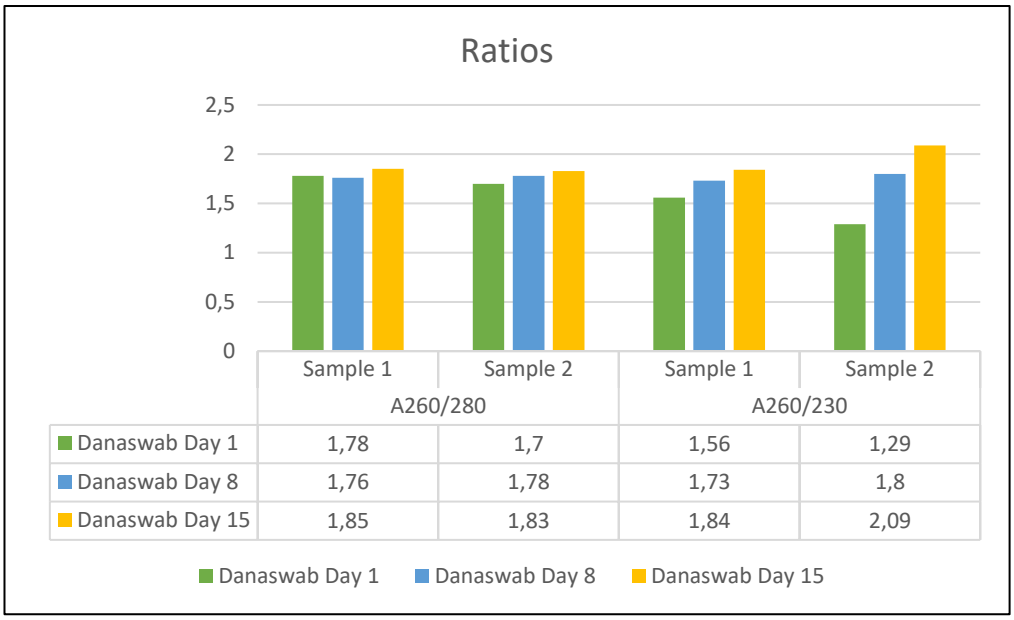

Figure 3. 260/280 and 260/230 ratios by spectrophotometric analysis (Nanodrop)

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.

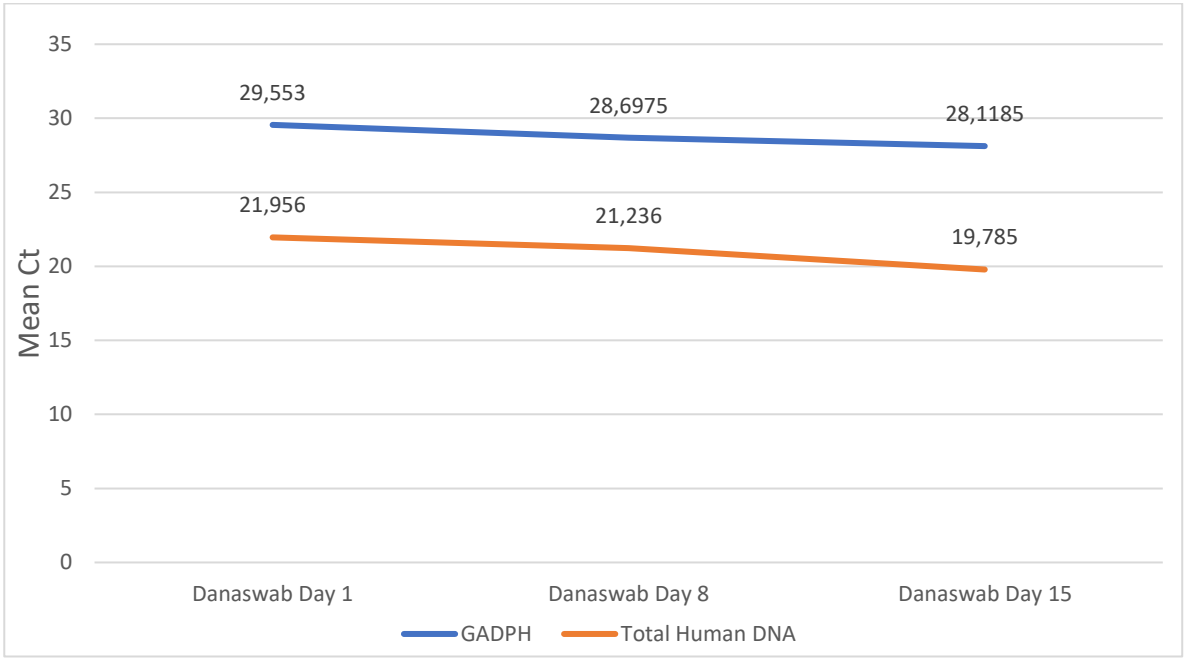

Figure 4. Amplification of the GADPH gene and Total Human DNA by qPCR analysis

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.

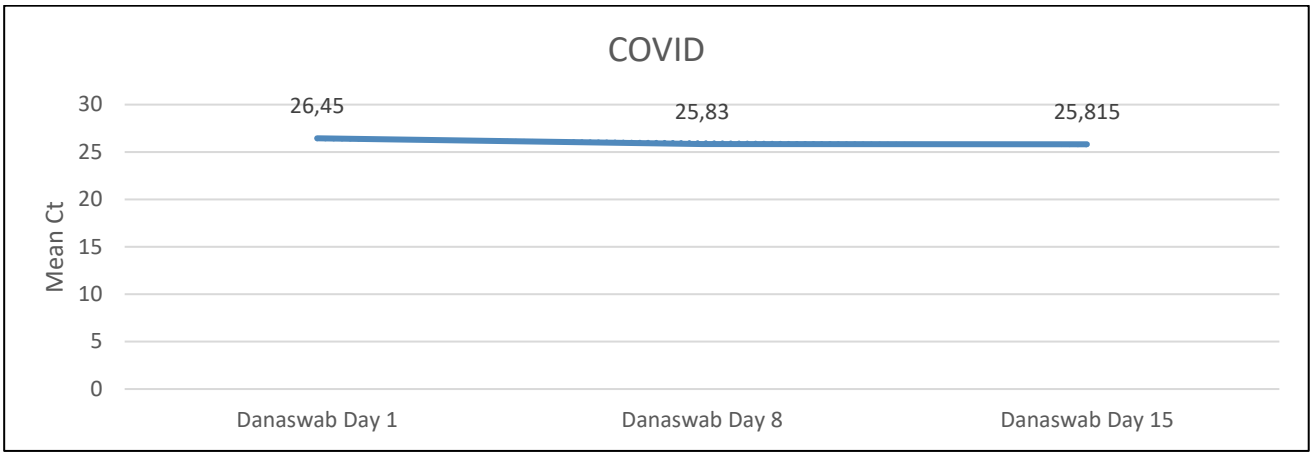

Figure 5. Detection of SARS-Cov-2 in preserved stool samples by qPCR analysis

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 DANASWAB Sample Collection MICROBIOME Kit.

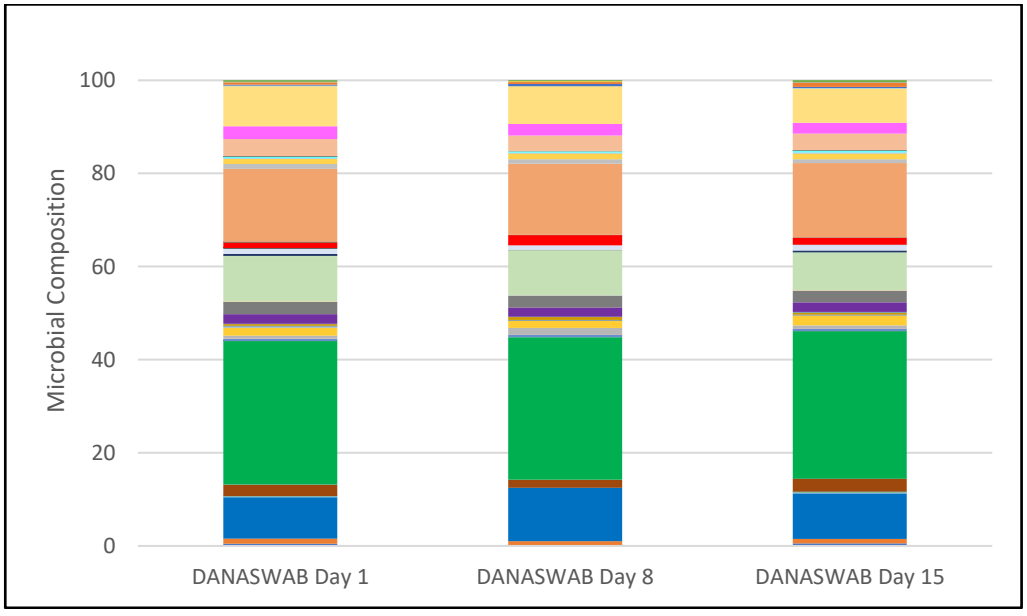

Figure 6. Microbial composition (Genus) of preserved stool samples at room temperature

Conclusion

A) Microbial and host human DNA from preserved stool samples are effectively stabilized in DANASWAB 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 DANASWAB 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 DANASWAB Sample Collection Microbiome Kit.

D) Microbial composition of preserved stool samples is unchanged after 15 days at room temperature with DANASWAB Sample Collection Microbiome Kit.

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