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Sample collection and stabilization from saliva samples for SARS-CoV-2 detection by qPCR

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INTRODUCTION

The severe acute respiratory syndrome-associated coronavirus (SARS-CoV-2) is a novel coronavirus that has caused the COVID-19 pandemic. Currently, naso and oropharyngeal swabs are two ways to collect specimens of COVID-19 from the respiratory tract to carry out diagnostics. Saliva is a promising candidate for SARS-CoV-2 diagnostics because (1) collection of samples is minimally invasive and can be reliably done by untrained subjects, and (2) there are publications from different research groups that used saliva for SARS-CoV-2 detection with good results. However, it is necessary to develop some tools to use saliva as the elected sample: 1) A device that prevents the creation and expansion of drops during sampling, 2) a saliva stabilization solution, as it is known that degradation of saliva components can occur under certain storage conditions. Taking advantage of our experience in collecting saliva samples, DANAGEN has developed a system to overcome these challenging tasks.

MATERIALS AND METHODS

Sample Processing

2 × 1ml of saliva samples was collected from three donors using our funnel and a 5ml collection tube. We added 1ml of Saliva Stabilization Solution to every sample, mixed and spiked with the following external quality controls for nucleic acids detection:
1) Purified SARS-CoV-2 genome. AMPLIRUN® CORONAVIRUS RNA CONTROL (Vircell, SPAIN).
2) Inactivated SARS-CoV-2 cells. AMPLIRUN® TOTAL SARS-COV-2 CONTROL (Vircell, SPAIN).

Isolation of Viral RNA

Viral RNA was isolated from aliquots of 200µl of preserved samples. These samples were preserved at room temperature for 30 days. Isolation was carried out on day 0, day 7, day 14, day 21 and day 30 using our **DANAGENE SPIN VIRAL RNA Kit**.

Quantitative Real-Time PCR

One-step RT-PCR for SARS-CoV-2 was performed using the **GPS™ CoVID-19 dtec-RT-qPCR Test**. The validation of this kit has been carried out by the Instituto de Salud Carlos III (ISCIII) SPAIN and Public Health England (PHE; Colindale, London, UK). A diagnostic sensitivity of 100% and a diagnostic specificity of 100% was assigned.

Results

Fig. 1 Real-time PCR of SARS-CoV-2 results indicating the Ct value over a period of 30 days for the (A) Purified coronavirus RNA and (B) Inactivated SARS-CoV-2 cells.
The results are a mean value of three donors for each time. We can observe little variation in Ct, indicating that our stabilization solution is capable of stabilizing both external quality controls (SARS-CoV-2 cells and RNA from coronavirus).

Fig. 2 shows the stability of the Beta Actin housekeeping gene over 30 days.
The Beta Actin gene was monitored by qPCR analysis of RNA purified from saliva samples stored at room temperature in our Saliva Stabilization Solution, demonstrating that the human RNA remained intact at room temperature for at least 30 days. The results are mean values of three donors at each time.

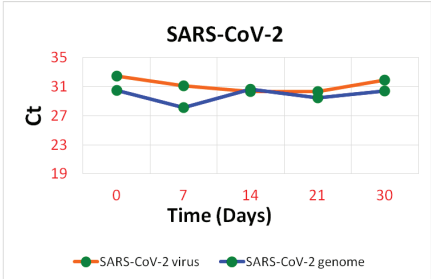

Fig. 1

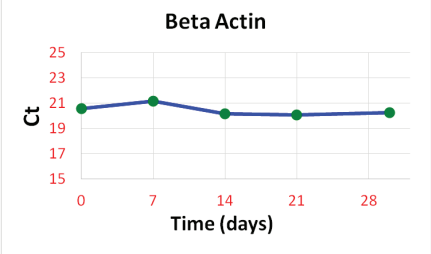

Fig. 2

Conclusion

In conclusion, our data demonstrate that the **DANASALIVA VIRAL Sample Collection Kit** is a non-invasive RNA self-collection kit that can be used by untrained subjects to collect saliva samples and able to stabilize total RNA at room temperature for up to 30 days.
It has also been verified that the sample becomes non-infective by pathogen inactivation and it is possible to isolate the human genomic DNA present in the preserved saliva sample (data not shown).
Our system could be very useful if saliva samples are considered for SARS-CoV-2 detection.

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