Evaluation of Saliva in Glycerol

Introduction

There were question about adding Glycerol to saliva. If there is any interference or inhibition in the SARS-CoV-2 PCR tests.

Material & Methods

Saliva is collected by using a saliva collector Oracol S010 (Malmed, Worchester, United Kingdom). For the spiking experiment we used inactivated SARS-CoV-2 (hCoV19/Netherlands/Noord_Braband_0117R/2020) in MEM with Hanks' salts; heat inactivated at 60°C for 2 hours; 5.62*10^7 TCID50/ml or 8.26*10^8 digital copies RdRP positive strand RNA/ml. The SARS-Cov-2 virus is spiked into saliva, collected from a SARS-CoV-2 negative donor. From each saliva sample 200 µl was mixed with 200 µl 20% glycerol and vortex shortly. After incubating one hour at room temperature, +4 °C or -20 °C, 200 µl was drawn and mixed with 275 µl MagNA Pure lysis buffer with EAV included and 450 µl was extracted on a MagNA Pure 96 Instrument (Roche) using the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche) and eluted in a volume of 50 µl. The E-gene/EAV multiplex PCR was used to test inhibition of EAV amplification. E-gene and RdRp-gene primers and probes were as described by Corman et al (1). EAV primers and probe were as described by Scheltinga et al (2).Reaction condition are described in Table 2 and 3. The Equine Arteritis Virus (EAV) is used as standard internal control for the qRT-PCR to check for inhibition.

***Table 1**. For the SARS-CoV-2 the primers and probes obtained from Biolegio were premixed at a final concentration of 10 µM primers and 5 µM probes.*

E-gene/EAV qRT PCR	**µl**	**RdRp**-gene qRT-PCR	**µl**
4x Taqman Fast Virus MM	5	**4x Taqman Fast Virus MM**	5
E+EAV Mix	3	**nCoV Mix**	3
PCR grade water	7	**PCR grade water**	7
Specimen nucleic acid	5	**Specimen nucleic acid**	5
Total volume	20	**Total volume**	20

***Table 2** . Amplification temperature protocol LC480 II.*

PCR Program	Segment number	Temp Target (°C)	Hold Time (sec.)	Slope (°C/sec.)	Acquisition mode	
Reverse Transcription	**1**	**50**	**900**	**EXTERNAL**		
Denaturation/Inactivation	**1**	**95**	**120**	**EXTERNAL**		
Denaturation	1	95	60	4.4	None	LC 480
Amplification	1	95	10	4.4	None	
(cycles:50)	2	**60**	30	2.2	Single	
Cooling	1	40	30	4.4	None	

Results

Presence of inhibition factors

The saliva panel with six simulated clinical samples in which heat-inactivated SARS-CoV-2 virus or no virus in SARS-CoV-2 negative donor saliva were tested in the EAV qRT-PCR. The RNA from these samples were tested in a duplex E-gene and EAV qRT-PCR. Each of the saliva panel samples with glycerol incubated at room temperature, +4 °C or -20 °C and the original saliva panel samples were tested in triplicate. The original saliva panel incubated at +4 °C for one hour.

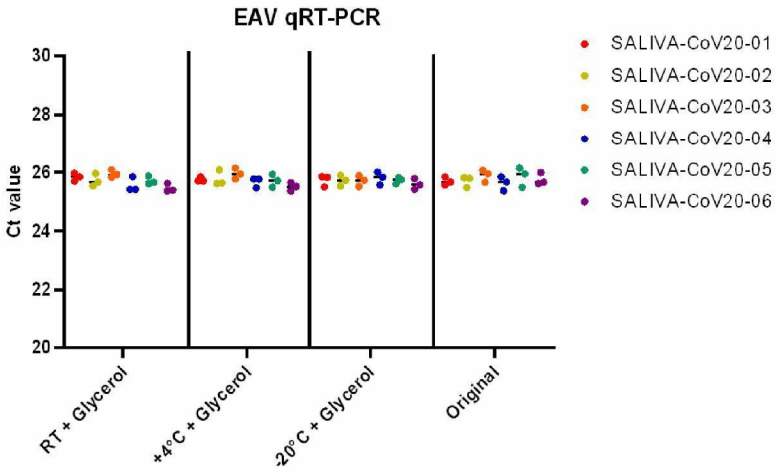

Figure 1. *EAV qRT-PCR results to determine if there are no interfering substances in glycerol compared with the original saliva panel*

In general there is no inhibition in all 3 saliva panels with glycerol compared with the original saliva panel samples. Storage temperature did not affect amplification of EAV in the added lysis buffer.

Effect on SARS-CoV-2 detection

The same panels were tested in the SARS-CoV-2 qRT-PCR tests. This will show whether the glycerol and different storage temperatures have an effect on the qRT-PCR tests. These samples are tested for E-gene and RdRp-gene.

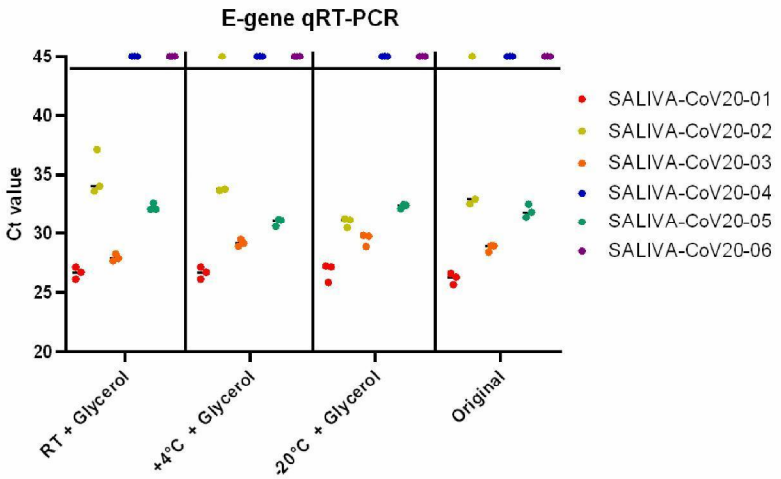

Figure 2. *E-gene qRT-PCR results of the saliva panel with glycerol incubated at room temperature, at +4°C or -20 °C and the original saliva panel*

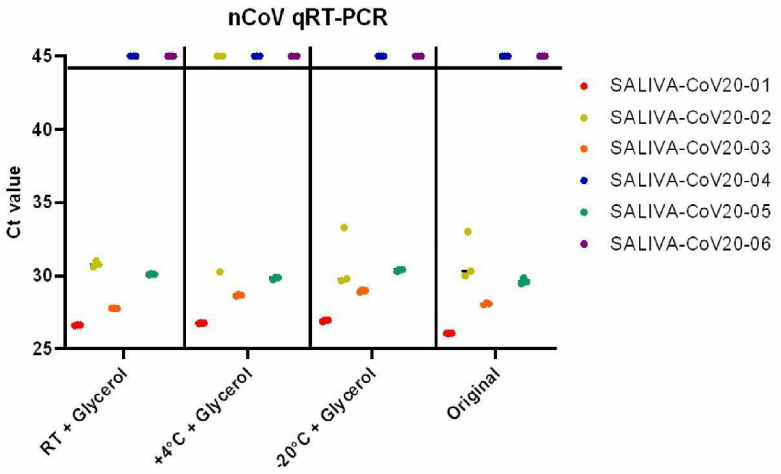

Figure 3. *RdRp-gene qRT-PCR results of the saliva panel with glycerol incubated at room temperature, at +4°C or -20 °C and the original saliva panel.*

The results of the E-gene qRT-PCR and the results of the RdRp-gene qRT-PCR show that all 3 saliva panels with glycerol have similar results compared the original saliva panel samples.

Conclusion

Each of the saliva panel samples with glycerol incubated at room temperature, +4 °C or -20 °C show no inhibition in the EAV qRT-PCR. The samples show similar SARS-CoV-2 detection results compared with the original saliva panel samples. Glycerol can be added to the saliva samples.