Title

Validation of Viral Sample Preservation Solution transport medium (LOT 354)

Introduction

The Purpose of this validation is to validate if the Viral Sample Preservation Solution (VSPS) transport medium show no inhibition in the in-house qPCR. This will be compared with viral transport medium GLY. If there is no inhibition compared with GLY, the VSPS can be used for diagnostic testing.

Material & Methods

Viral Sample Preservation Solution (VSPS)
VSPS is manufactured by CoWin Biotech
LOT: 11140/50512
EXP: 20210308

Virale transport medium GLY
GLY-medium is the viral transport medium used by RIVM National institute for Public Health and the Environment of the Netherlands. This Viral transport medium is manufactured by Mediaproduct BV.

Clinical specimen
The clinical specimen that has been selected for the validation is positive for SARS-CoV-2. The Ct value for this clinical specimen is for the Sarbeco-RdRp 20.2; Sarbeco-E 17.0; nCoV-RdRp 19.2.

qRT-PCR
From each dilution of the clinical specimen 200 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. E-assay and RdRP-assay primers and probes as described in the provided protocol by Corman et al.
Reaction conditions were as follows

***Table1**. For primers and probes obtained from Biolegio primers and probes were premixed at a final conancentration of 10 µM each except for the SJVR probes for 5 µM each.*

E-gene qRT PCR	µl	RdRP-gene qRT-PCR	µl
4x Taqman Fast Virus MM	5	4x Taqman Fast Virus MM	5
SJVE Mix (10 µM)	3	SJVR Mix (10 µM)	3
PCR grade water	7	PCR grade water	7
Specimen nucleic acid	5	Specimen nucleic acid	5
Total volume	20 µl	Total volume	20 µl

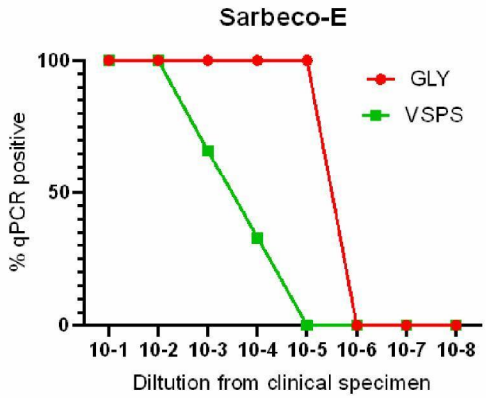
***Table 2.** Amplification temperature protocol.*

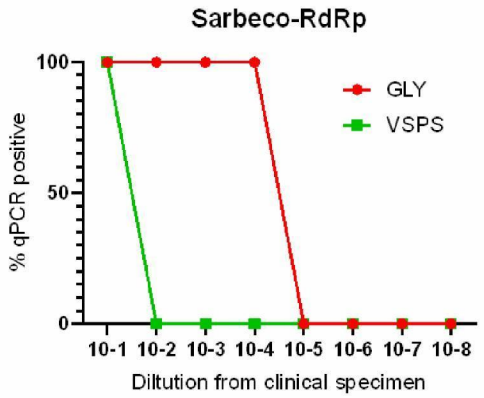
PCR Program	Segment number	Temp Target (°C)	Hold Time (sec.)	Slope (°C/sec.)	Acquisition mode	
Reverse Transcription	**1**	**50**	**900**	**EXTERNAL**		
DVSPSuration/Inactivation	**1**	**95**	**120**	**EXTERNAL**		
DVSPSuration	1	95	60	4.4	None	LC 480
Amplification	1	95	10	4.4	None	
(cycles:50)	2	**60**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

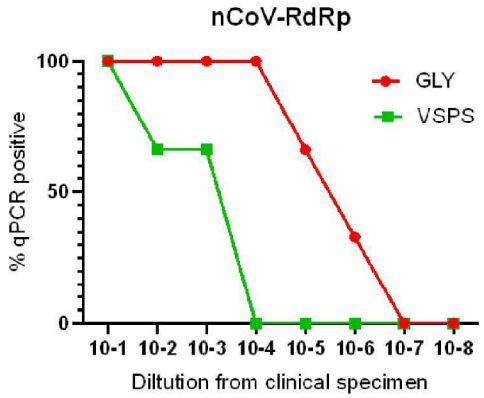
Standard EAV external control qRT-PCR was performed to control for inhibitors. (IDS/VIR/F351)

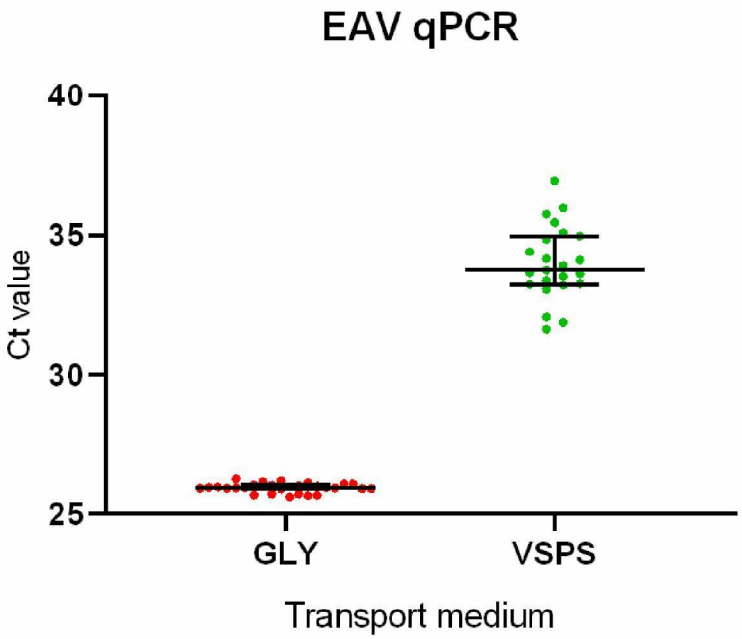
Results

The Clinical specimen is diluted 100-fold before it is diluted in a 10-fold series in VSPS and GLY. The VSPS and GLY are tested pure as negative control. The RNA from these samples where tested in triplicate in a duoplex Sarbeco-E and EAV qPCR and the duoplex Sarbeco-RdRP and nCoV-RdRp qPCR.









Conclusion

In the diluted clinical specimen there is a more than 10-fold difference in the Sarbeco-E. In Sarbeco-RdRp and nCoV-RdRp qPCR there is a 1000-fold difference between GLY and VSPS. In the In the EAV qPCR you see there is inhibition in the VSPS, there is a 9 CT-value difference between the samples from GLY and VSPS. In the EAV qPCR 4 of the intern controls did not have a signal in the qPCR. Because there is a more than 10-fold inhibition in VSPS, the VSPS can't be used for further diagnostic testing.

Archive raw data

Excel: Raw data CoWin Biotech_Viral Sample Preservation Solution validation_09042020