Evaluating preservation and quantification of SARS-Cov-2 RNA From a Collection and Transport System for Rapid Point-of-Care Diagnostic Tests

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Introduction

Recent genomic technologies have made possible the accurate and rapid assessment of specific pathogens directly at the site of patient care without the time delays associated with diagnostic laboratories. One of the major advantages of recent Point-of-Care (POC) instrumentation is their ease of use, making diagnostic tests accessible to personnel without specialized laboratory training. Timely and accurate determination of the presence of Sars-CoV-2 is particularly essential in these pandemic conditions. The goal of this study was to assess the ability to detect and quantify Sars-CoV-2 RNA following long term storage in temperatures up to 30ºC following collection and preservation in a MK buffered solution.

The goal of this project is

- To evaluate Puritan MK buffer solution's ability to preserve known concentrations of Sars-CoV-2 and allow recovery and quantitation of RNA for genetic testing using standard CDC quantitative Polymerase Chain Reaction (QPCR) detection protocols.
- To assess the effect of storage Sars-CoV-2 from 1 to 30 days at both 4º and 30º C on the RNA concentrations as measured by QPCR.

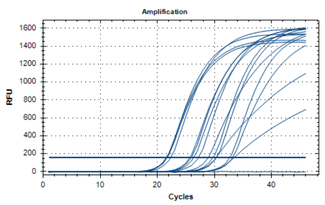
Methods

Heat-inactivated Sars-CoV-2 (NR-52286) was obtained from BEI® Resources. Known concentrations of Sars-CoV-2 were added to sample tubes containing 1 mL of MK buffered solution for final concentrations ranging from 0.22 to 2200 genomes/µl. Two storage temperatures were evaluated, 4º and 30º C for 0, 1, 7 and 30 days. Following storage 375 µl was removed at each time point and temperature and processed for RNA isolation using standard RNA isolation kits (Zymo Viral RNA Kit). For each concentration and temperature three replicates were quantified using Quantitative real-time PCR using Promega GoTaq® Probe 1- Step RT-qPCR System and primer set 2019-nCov CDC EUA Kit.

ProMega GoTAQ Probe 1-step RT-qPCR

	one rxn		
GoTAq qPCR Master Mix	10.0 ul		
GoScript RT Mix	0.4 ul		
Combined Primer/Probe Mix	1.5 ul		
Nuclease-free water	3.1 ul		
	15.0 ul		
	15.0 ul/sample + 5 ul RNA		
Thermocycle Program			
Reverse Transcriptase	45 ºC	15 min	
RT Inactivation	95 ºC	2 min	
Denaturation	95 ºC	15 sec	45 X
Annealing/Extension *	60 ºC	1 min	* Detect FAM

QPCR fluorescence readings were measured by a BioRad CFX 96 Real-Time PCR System. The threshold for determining Ct value for each sample is based on the baseline threshold – above background and within the exponential phase of amplification curve.



QPCR curves for Sar-CoV-2 at four concentrations.

Results

QPCR

Yields of RNA were more than sufficient for multiple QPCR assays at all concentrations and time points. The MK buffered solution allowed the recovery and detection of Sars-CoV-2 down to a concentration of 0.22 genomes/µl even following 30 days storage at 30ºC. QPCR detected a linear positive relationship between the initial sample concentration and detected concentration regardless of storage temperature or storage time. For samples stored at 4ºC there was no significant difference in Ct values versus time (ANOVA, F=0.47, P=0.49). There was a small, though significant difference in Ct values for samples stored at 30ºC due to some degradation of samples stored for 30 days (ANOVA, F=11.81, P<0.001).

Concentration (copies/µl)	Rep 1 Ct	Rep2 Ct	Rep 3 Ct	Rep 4 Ct	**Mean Ct**	**Std. Dev.**
2.2 x 10^3	21.7	21.9	22.5	21.6	**21.9**	**0.41**
2.2 x 10^2	26.4	26.1	27.6	26.4	**26.6**	**0.67**
2.2 x 10^1	30.6	29.7	31.5	30.4	**30.5**	**0.73**
0.22 x 10^1	31.9	31.9	31.0	33.0	**31.9**	**0.82**

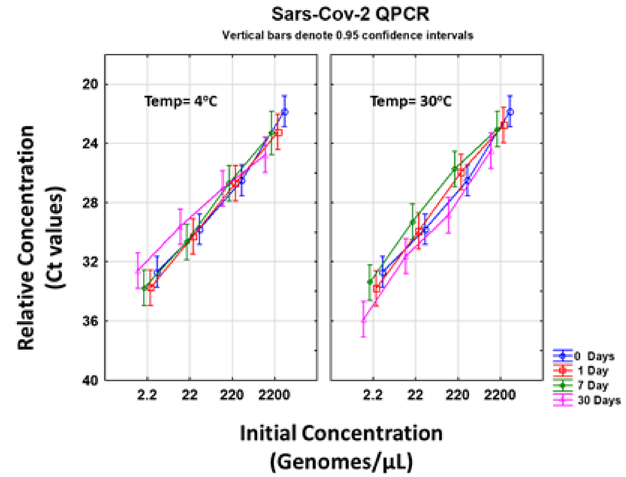
Limit of Detection for replicate QPCR runs.

Plot of QPCR curves for 25 replicates for Sars-Cov-2 at 2.2 x 10^3 copies/µl.

Final Concentration 2.2 genomes/µL							
		Stability at 4ºC			**Stability at 30ºC**		
	Day 0	24 hrs	7 Days	30 Days	24 hrs	7 Days	30 Days
Mean Ct	32.7	33.7	33.8	32.6	33.8	33.4	35.9
Std Dev Ct	1.70	0.45	1.05	0.76	0.45	0.48	1.44
Final Concentration 2.2 x 10^1 genomes/µL							
		Stability at 4ºC			**Stability at 30ºC**		
	Day 0	24 hrs	7 Days	30 Days	24 hrs	7 Days	30 Days
Mean Ct	29.8	30.3	30.6	29.6	29.9	29.3	31.6
Std Dev Ct	0.93	0.33	1.79	1.04	0.28	0.10	1.14
Final Concentration 2.2 x 10^2 genomes/µL							
		Stability at 4ºC			**Stability at 30ºC**		
	Day 0	24 hrs	7 Days	30 Days	24 hrs	7 Days	30 Days
Mean Ct	26.5	26.7	26.7	27.0	25.9	25.7	28.8
Std Dev Ct	0.79	0.25	0.83	0.59	0.20	0.73	2.55
Final Concentration 2.2 x 10^3 genomes/µL							
		Stability at 4ºC			**Stability at 30ºC**		
	Day 0	24 hrs	7 Days	30 Days	24 hrs	7 Days	30 Days
Mean Ct	21.8	23.2	23.3	24.8	22.8	23.0	24.5
Std Dev Ct	0.52	0.25	0.19	2.41	0.27	0.32	0.36

Results (continued)

QPCR curves for Sars-Cov-2 at 4º and 30ºC following storage for 1, 7, and 30 days



Univariate Tests of Significance for Ct values.

There was a significant difference among concentrations as expected. There was a small significant effect due to storage temperature indicating some degradation after storage for 30 days at 30ºC.

Effect	SS	Degr. of	MS	F	p
Conc	1488.29	3	496.10	459.72	0.000000
Time	38.23	3	12.74	11.81	0.000002
Temp	0.51	1	0.51	0.47	0.495522
Conc*Time	12.20	9	1.36	1.26	0.276101
Conc*Temp	3.35	3	1.12	1.04	0.381985
Time*Temp	20.85	3	6.95	6.44	0.000641
Conc*Time*Temp	8.32	9	0.92	0.86	0.567279
Error	76.62	71	1.08		

Conclusions

These results indicate that MK buffered solution allows for the detection and accurate quantification of Sars-Cov-2 RNA following long term storage up to 30 days at sub-optimal temperatures.

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