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Performance Comparison of MK Buffered Solution for Diagnostic Based Detection of Sars-CoV-2 using quantitative PCR vs droplet digital PCR.

D. Brazeau [1,2], M. Casto[2], M. Karamchi[3],

[1] Joan C Edwards School of Medicine, [2]School of Pharmacy, Marshall University, [3]Puritan Medical Products, Guilford, ME

Brazeaud@Marshall.edu
716-425-0991

Abstract

Background: Recent genomic technologies have made possible the accurate and rapid assessment of specific pathogens based upon DNA/RNA. Droplet digital PCR (ddPCR) by partitioning a single reaction into many thousands of nano-droplets allows for the absolute quantitation of a given DNA/RNA target. The goal of this study was to assess the compatibility of a MK Buffered solution with ddPCR and to compare its performance against standard QPCR techniques.

Methods: Heat-inactivated Sars-CoV-2 (NR-52286) was obtained from bei Resources. Serial dilutions of Sars-CoV-2 were added to sample tubes containing 500 mL of MK buffered solution yielding final concentrations ranging from 8400 to 0.0084 genomes/µl. Serial dilutions were stored at 4º and 30º C for 0, 1, 7 and 28 days. For each sample 300 µl was processed for RNA isolation using standard RNA isolation kits (Zymo Viral RNA Kit), 9.9 µl of viral RNA was used for each assay. For QPCR, samples were quantified using Quantitative real-time PCR using Promega GoTaq® Probe 1- Step RT-qPCR System and primer set 2019-nCov CDC EUA Kit. For ddPCR samples were quantified using a BioRad® Droplet Digital PCR System (QXD200) with TAQMan® probes to detect the two specific regions of Sars-CoV-2 genome.

Results: The lower limit of detection by QPCR was 8.4 genomes/µl. The ddPCR assay was an order of magnitude more sensitive with detection to a concentration of 0.84 genomes/µl. QPCR Ct values ranged from 23.1 (8,400 genomes/µl) to 37.9 (0.84 genomes/µl). Ct values exhibited significant linear relationship ($p=0.00$, $r^2 = 0.726$). Droplet Digital PCR values ranged from 4928 (8400 genomes/µl) to 0.02 genomes/µl (0.84 genomes/µl). Values showed a significant linear relationship ($p=0.000$, $r^2 = 0.937$). Storage time at either 4º and 30º C had no effect on the ability to detect and quantify the viral pathogen.

Conclusions: While both PCR based assays were able to detect Sars-Cov-2 genomes over 4-5 orders of magnitude the ddPCR assay was an order of magnitude more sensitive than QPCR. There was a significant linear relationship among QPCR and ddPCR values ($p=0.000$, $r^2=0.702$).

Introduction

Recent genomic technologies have made possible the accurate and rapid assessment of specific pathogens based upon DNA/RNA targets. The timely and accurate determination of Sars-Cov-2 is particularly essential in these pandemic conditions. PCR-based detection of viral RNA provides a rapid and highly sensitive tool for clinicians as well as public health workers to track outbreaks.

Droplet digital PCR (ddPCR) by partitioning a single reaction into many thousands of nano-droplets allows for the absolute quantitation of a given DNA/RNA target.

The goal of this study was to assess the ability to detect and quantify known concentrations of heat-inactivated Sars-CoV-2 stored in MK inactivation buffer using both Droplet digital PCR (ddPCR) and standard Quantitative PCR (QPCR).

Methods

Heat-inactivated Sars-CoV-2 (ATCC# VR-1986HK) was obtained from bei Resources. Serial dilutions of Sars-CoV-2 were added to sample tubes containing 500 µL of MK inactivation buffer solution yielding final concentrations ranging from 8400 to 0.0084 genomes/µl. For each concentration two replicates were assayed.

For each sample 300 µl of the spiked MK inactivation buffer was processed for viral RNA isolation using standard RNA isolation kits (Zymo Viral RNA Kit) and eluted in 35 µl of RNase/DNase free water. For each PCR 8 µl of viral RNA was used for each assay.

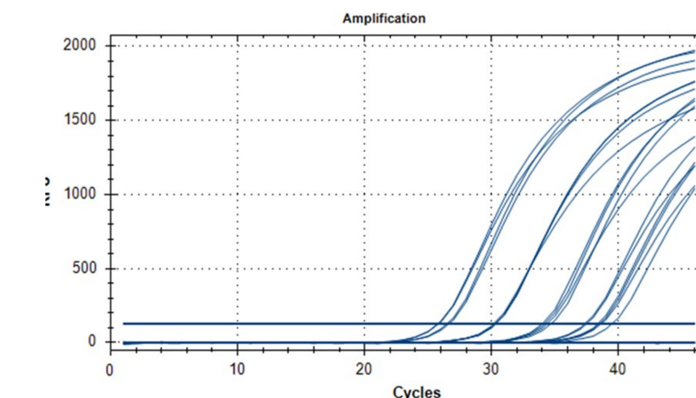
For ddPCR samples were quantified using a BioRad® Droplet Digital PCR System (QXD200) with TAQMan® probes to detect two regions of Sars-CoV-2 spike protein (N1 and N2 per 2019-nCoV CDC EUA Probe assays.

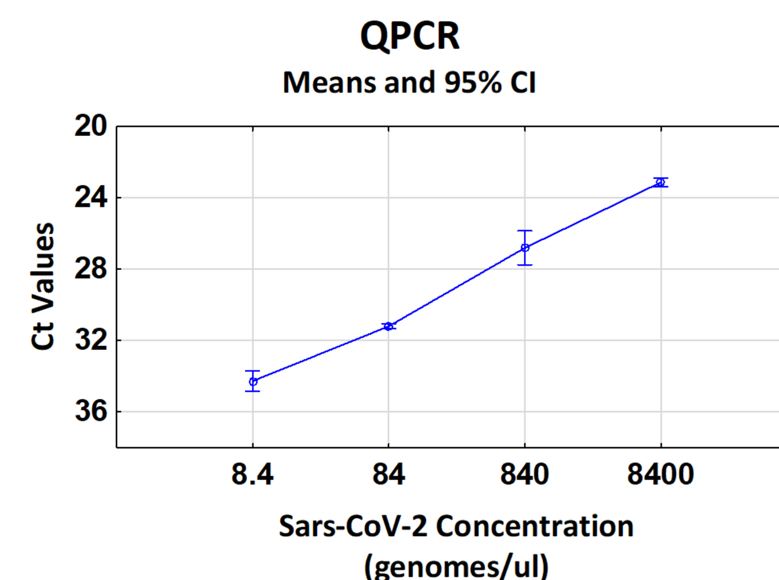
For QPCR, samples were quantified using Quantitative real-time PCR using Promega GoTaq® Probe 1- Step RT-qPCR System and primer set 2019-nCov CDC EUA Kit.

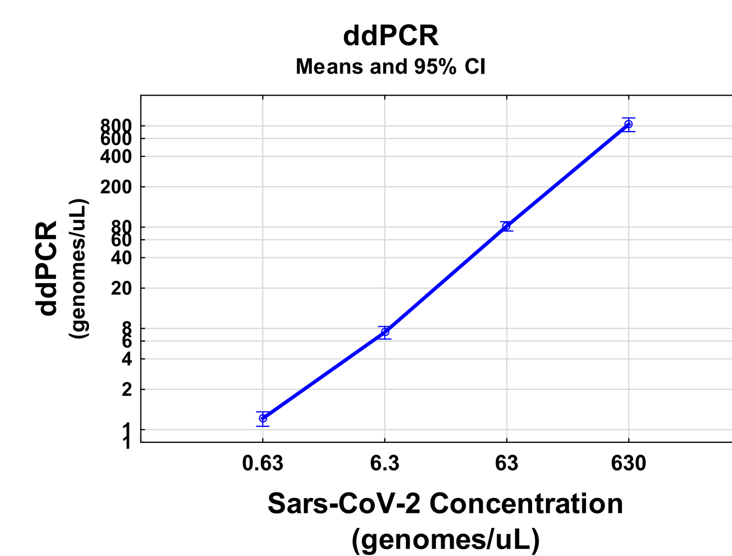
Results

Quantitative PCR

QPCR Ct values ranged from 23.1 (8400 genomes/ µl) to 37.9 (0.84 genomes/µl). The lower limit of detection by QPCR was 0.84 genomes/µl though Ct values were quite high (>37) indicating positive detection but low accuracy. Within the range from 8400 to 8.4 genomes/ µl Ct values exhibited significant linear relationship ($p=0.011$, $r^2 = 0.997$).

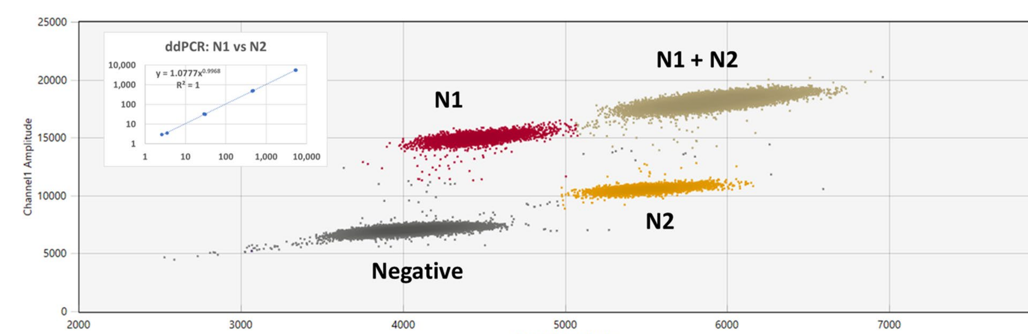






Droplet Digital PCR

The ddPCR assay was an order of magnitude more sensitive with detection to a concentration of 0.84 genomes/µl (Table 1). Droplet Digital PCR values ranged from 4928 (8400 genomes/ µl) to 0.194 genomes/µl (0.84 genomes/ µl). Values showed a significant linear relationship ($p=0.000$, $r^2 = 0.999$).



Results (continued)

Table 1. Results of ddPCR and QPCR assays on identical samples. Estimated concentrations ranged from 8400 to 0.0084 genomes/µl. ddPCR results in genomes/µl. QPCR results in Ct values. Values in red were not significantly above background.

Est Conc		ddPCR (copies/uL)			QPCR (Ct values)	
Copies/uL	N	Mean	Std Dev	N	Mean	Std Dev
8,400	4	4,928.2	230.7	4	23.1	0.2
840	4	402.0	97.7	4	26.1	0.4
84	4	27.2	1.4	4	30.2	0.1
8.40	4	1.91	0.3	4	34.3	0.2
0.84	4	0.2	0.1	4	37.9	0.5
0.084	4	0.1	0.2	4	N/A	--
0.008	4	N/A	--	4	N/A	--

Stability of Sars-CoV-2 RNA stored in MK Buffered solution for 0, 7, and 28 days .

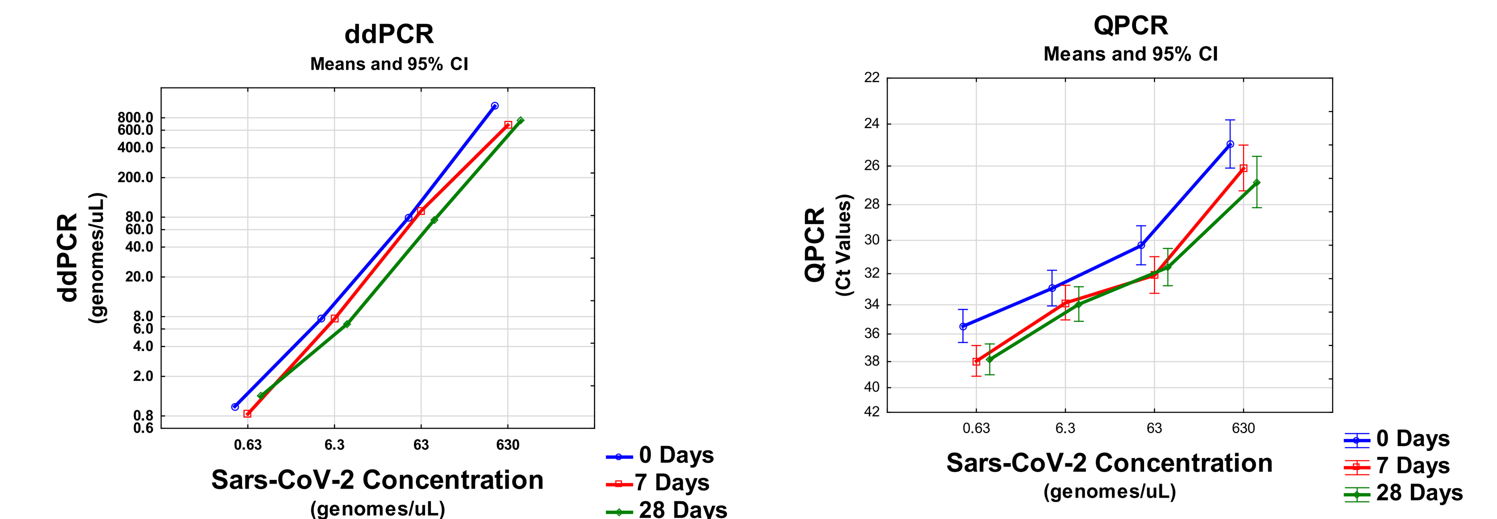
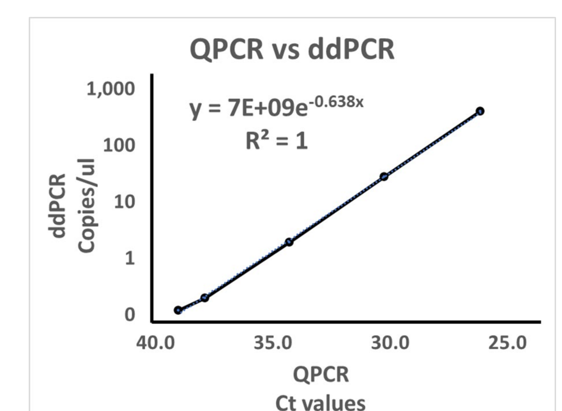


Table 2. Results of univariate tests of significance for ddPCR assays for samples stored for 0, 7, and 28 days for 4º and 30º C . There were no significant differences among samples as a function of storage time or temperature.

Nasal: Univariate Tests of Significance for Sars-Cov-2 N1					
Effect	SS	Degr. of	MS	F	p
Lot	3629.47	3	1209.82	1.32	0.30
Temp	462.20	1	462.20	0.51	0.49
Time	4954.58	2	2477.29	2.71	0.10
Lot*Temp	416.15	3	138.72	0.15	0.93
Lot*Time	4838.67	6	806.45	0.88	0.53
Temp*Time	232.87	2	116.44	0.13	0.88
Lot*Temp*Time	3931.48	6	655.25	0.72	0.64
Error	14613.55	16	913.35		

There was a significant linear relationship among QPCR and ddPCR values ($p=0.000$, $r^2=0.702$).



Conclusions

While both PCR based assays were able to detect Sars-Cov-2 genomes over 5 orders of magnitude. The ddPCR assay was an order of magnitude more sensitive than QPCR. There was a significant linear relationship among QPCR and ddPCR values ($p=0.000$, $r^2=1$).

Acknowledgements

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