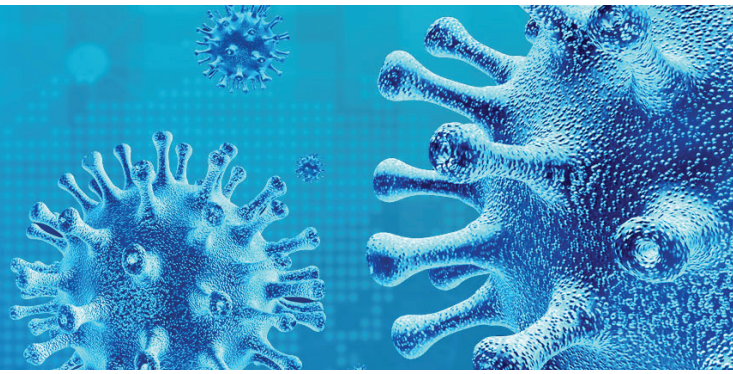
Empirical™ bioscience

Performance of QuantiTASE Plus One-Step RT-qPCR

mix for the detection of SARS-nCoV-2 Virus



STUDY OBJECTIVES

Using CDC and FDA Protocols and Guidance:

1) Assess the feasibility of detecting nCoV2 genomic material with Empirical Bioscience QuantiTASE PLUS One-Step RT-qPCR reaction mix with EUA approved Primer/Probe materials

2) Determine Limit of Detection (LoD) for QuantiTASE PLUS for detection of nCoV2 genomic material

3) Compare performance of QuantiTASE PLUS with that of competitor products for the detection of nCoV2 genomic material in clinical samples.

MATERIALS AND METHODS

Primer, Probe & Control Sets

- 2019 nCoV CDC EUA kit: qPCR assay primers & probes Integrated DNA Technologies

SARS-CoV-2 Synthetic RNA Template

- Quantitative Synthetic Severe acute respiratory syndrome-related coronavirus (SARSCoV-2) RNA: ORF, E, N

SARS-CoV-2 Genomic RNA Template

Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-WA1/2020

RNA Extraction

- Omega Biotek Total RNA Extraction Kit
- Thermofisher MagMax RNA Extraction Kit

One Step RT-qPCR Kit

- Empirical Bioscience QuantiTASE One Step RT-PCR Kit

EQUIPMENT USED

- Thermofisher Kingfisher Flex Nucleic Acid Extraction System
- Omega Biotek Total RNA Extraction Kit
- BioRad Touch CFX 96 Realtime PCR system
- ABI QuantStudio 3, QuantStudio 5 and QuantStudio 7 Real-time PCR systems

LIMIT OF DETECTION (LoD)

The LoD study established the lowest concentration of SARS-CoV-2 (genome equivalent copies(cp)/ reaction) that can be detected by the N1 and N2 assays at least 95% of the time using the Empirical Bioscience QuantiTASE One-Step reagents.

For each assay (N1 and N2), a preliminary LoD was established by testing serial 10-fold dilutions of SARS-CoV-2 RNA. Following the 10-fold dilution series, 20 replicates containing various genome equivalent copy number of the SARS-CoV-2 synthetic RNA were performed to establish an LoD. The lowest concentration in total cp/reaction at which 19/20 replicates resulted in a Ct of less than 40 was established as the LoD for each assay.

REACTION SET UP

Reagent	Final Concentration
Nuclease Free Water	N/A (Fill to 15µL)
InhibiTAQ Plus MasterMix	1X
RTScript Reverse Transcriptase	5U/µL
Primer/Probe Mix	500nM Primers, 125nM Probe
RNA Template	Added to specified genome equivalent copies/reaction

Data from the serial dilution LoD studies was used as a template for starting concentrations of genomic RNA to be added to RT-qPCR reactions for obtaining an LoD on SARS-CoV2 genomic RNA.

Reagent MasterMix was assembled as before and per CDC guidelines and distributed into each well.

THERMAL CYCLING PARAMETERS

Stage	Time	Number of Steps
1. 25°C	2 min	1 rep
2. 50°C	15 min	1 rep
3. 95°C	2 min	1 rep
4. Step 1, 95°C	3 sec	
4. Step 2, 55°C	30 sec	45 reps for stage 4, steps 1/2

Thermal cycling parameters were run according to CDC guidelines. Reactions were amplified and analyzed with BioRad CFX96 Touch Real-Time PCR detection system (96 well) Thermofisher QuantStudio 3 (96 well), QuantStudio 5 (384 well) and QuantStudio 7 (384 well) Real-Time PCR detection systems.

LoD FOR ALL SYSTEMS EVALUATED

Assay	LoD (genome equivalent copies/reaction)	Mean Ct (± std. dev.) of positive replicates
N1	5 copies/reaction – 100% positive (20/20)	37.05 ± 0.68
N2	10 copies/reaction – 100% positive (20/20)	37.01 ± 0.50

PERFORMANCE DATA

QuantiTASE PLUS & TaqPATH

			=X/20	Average	Std. Dev
N1	Empirical QuantiTASE	20 copies	20/20	34.58	0.308
		10 copies	20/20	36.28	0.554
		5 copies	20/20	37.05	0.682
	ThermoFisher TaqPATH	20 copies	20/20	34.04	0.338
		10 copies	20/20	35.21	0.443
		5 copies	19/20	37.59	1.005

LoD samples were prepared by spiking known copies of Genomic RNA into the RT-PCR reaction mix at the time of analysis. Thermal cycling parameters were run according to CDC guidelines. Reactions were amplified and analyzed with BioRad CFX96 Touch Real-Time PCR detection system (96 well) using the CDC recommended cycling conditions.

			=X/20	Average	Std. Dev
N2	Empirical QuantiTASE	20 copies	20/20	35.91	0.328
		10 copies	20/20	37.01	0.502
	ThermoFisher TaqPATH	20 copies	20/20	37.08	0.854
		10 copies	20/20	37.94	0.491

			=X/20	Average	Std. Dev
RPP	Empirical QuantiTASE	50,000 copies	20/20	27.50	0.095
	ThermoFisher TaqPATH	50,000 copies	20/20	26.54	0.121

TaqPATH is a trademark and is the property of Thermo Fisher Scientific and its subsidiaries.

PERFORMANCE COMPARISON

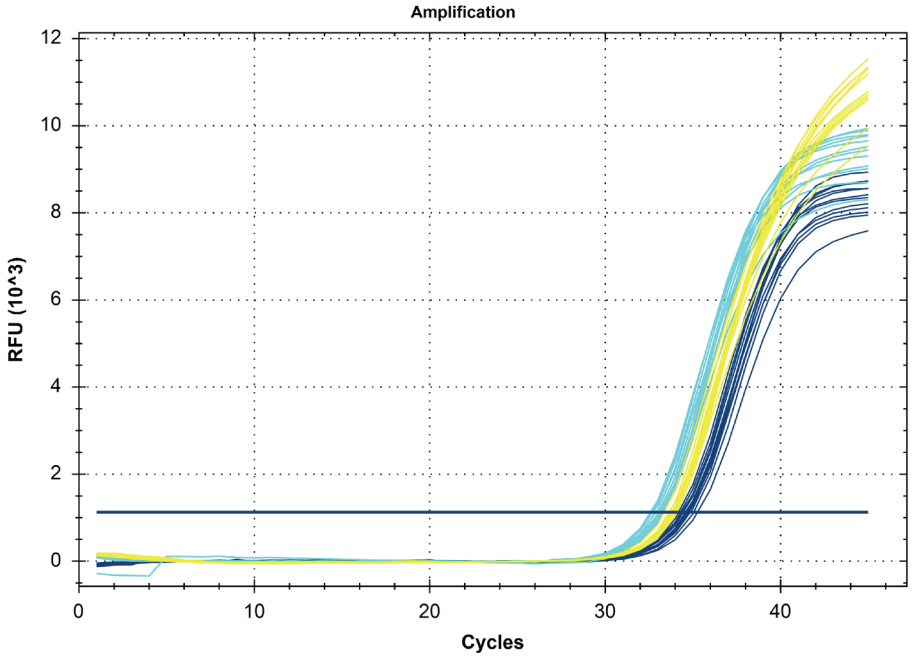
Contrived Clinical Samples

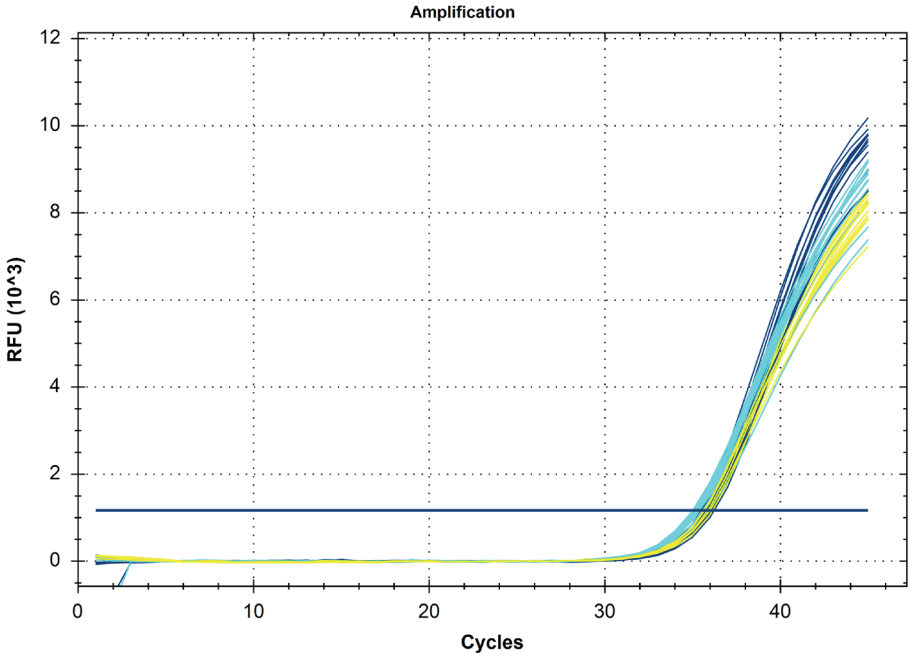
Targets	N1			N2		
Master Mix	Empirical QuantiTASE Plus 1-Step RT-qPCR System	Thermo Fisher TaqPath 1-Step RT-qPCR MasterMix, CG	Promega GoTaq Probe 1-Step RT-qPCR System	Empirical QuantiTASE Plus 1-Step RT-qPCR System	Thermo Fisher TaqPath 1-Step RT-qPCR MasterMix, CG	Promega GoTaq Probe 1-Step RT-qPCR System
Positives/ Total	12/12	12/12	12/12	12/12	12/12	12/12
Mean Ct.	34.78	33.12	33.97	35.89	35.38	35.87
Standard Deviation Ct	0.31	0.24	0.20	0.27	0.25	0.16

The QuantiTASE PLUS One-Step Universal RT-qPCR Kit SARS-CoV-2 was determined to be compatible with RNA extraction workflow at concentrations at or near LoD. Pooled negative OP swabs were spiked with genomic RNA. Input volume for extraction was 250µL and elution volume was 80µL. A 5-µL aliquot of the eluate was added to the PCR reaction.

PERFORMANCE COMPARISON

Competitor Products





Empirical Bioscience QuantiTASE PLUS

ThermoFisher TaqPath

Promega GoTaq

n= 12 samples/product

Detection on N1 and N2 Genes of nCoV2: 50 copies of Genomic RNA added in a 20uL reaction. Reaction set up and RT-qPCR protocol were in accordance with CDC guidance.

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GoTaq is a trademark and is the property of the Promega Corporation