WHITE PAPER December 2020

High Sensitivity and Specificity Detection of SARS-CoV-2 on Food Packaging and Environmental Surfaces

Brandt, Alex[1], Stephenson, Patrick[2], David Crabtree[2], Daniele Sohier[2], Amanda Manolis[2]

[1]Food Safety Net Services, San Antonio, TX, USA; [2]Thermo Fisher Scientific, Basingstoke, UK

Introduction

The outbreak of SARS-CoV-2 in 2019 and the resulting pandemic has led to significant loss of life, as well as massive economic decline and widespread disruption to daily life. Controlling the spread of the virus has largely focused on human testing comprising nasopharyngeal swabbing, RNA extraction, and RT-qPCR. A positive test result triggers isolation of the individual, and any possible human-to-human transmission contacts, until symptoms have subsided.

While this strategy has largely proven successful, outbreaks within businesses have resulted in large numbers of positive cases and so-called 'virus hot spots'. Active monitoring of businesses is undertaken to prevent the economic impact of a site shutdown following a localised outbreak. Numerous studies have shown that the SARS-CoV-2 virus remains viable on environmental surfaces for extended periods. For example, viral particles were detected on stainless steel and common surfaces for up to 28 days[1] post-inoculation, while it took more than seven days to be lost from the surface of a face mask[2]. It is therefore likely that infection is also occurring as a result of surface-to-human transmission. Food production and hospitality segments such as slaughterhouses, hotels and restaurants have faced contamination issues in the working environment and among co-workers. Environmental testing of contact surfaces is one of the relevant control measures that support decision-making in allowing employees to safely use shared areas, while also providing data on the effectiveness of cleaning regimes.

With the pandemic expansion, scientists are tracking new coronavirus variants to learn from the mutation rate of the virus. Tens of thousands of SARS-CoV-2 sequences are available in international and open-access databases. The observed homology among viral strains is higher than 99.9%, at the nucleotide level and at the amino acid level. But 13 variations sites are showing mutation rates close to 30%[3]. It is most likely that selective mutations are occurring in SARS-CoV-2 genome, and certain regions should be of course avoided when designing primers and probes. Additionally, targeting more than one genomic sequence is certainly a strategy to adopt when dealing with pandemic coronavirus. Indeed, a variant of SARS-CoV-2 has emerged

during summertime in Spain and has since spread to multiple European countries. The sequences of this new phylogenetic cluster differ from ancestral sequences at six or more positions[4].

Thermo Fisher Scientific has developed a Real-Time PCR Solution to detect SARS-CoV-2 in the production environment and to support the industry in evaluating the efficiency of the control measures in the work place. The Thermo Scientific™ SARS-CoV-2 Real-Time PCR Workflow for detection of the virus on food packaging and environmental surfaces (candidate method) is designed to target three different viral genomic regions, ORF1ab, N-gene and S-gene, by the application of three Real-Time PCR assays in order to ensure a high specificity.

With the pandemic expansion, scientists are closely tracking new coronavirus variants to learn from the mutation rate of the virus. Tens of thousands of SARS-CoV-2 sequences are available in international and open-access databases. An in silico analysis was run in September 2020 to assess the inclusivity of the primers and probes of the Thermo Scientific SARS-CoV-2 Real-Time PCR Workflow.

In parallel, an experimental study was performed to evaluate the limit of detection (LOD) of the candidate method, and compare performance between the PCR master mixes used in the candidate and the reference method based on the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel[5].

AOAC INTERNATIONAL activated an accelerated program to evaluate test kits for detection of the SARS-CoV-2 virus on surfaces, bringing confidence to end-users for detection workflows. The candidate method has been submitted for validation in accordance with the AOAC Research Institute Performance Tested Methods℠ (PTM) Program for the detection of SARS-CoV-2 on environmental surfaces[6].

Method

In silico analysis

The in silico analysis of the primer and probe sequences was conducted according to the Validation Outline provided by AOAC Research Institute.

This analysis was performed to determine inclusivity (reactivity) and exclusivity of the primer and probe sequences according to the Wuhan *in silico* Inclusivity Study Protocol. BLASTN 2.6.0+ version was used to assess 15,756 SARS-CoV-2 genomes from both the GISAID and GenBank Viral NCBI databases.

Individual components (forward and reverse primers and probe) of each target were realigned against the sequence database. Alignments were reported for all of the three components (forward and reverse primers and probe) resulting in an alignment signal with the blast parameters listed above.

Limit of Detection

Heat-treated SARS-CoV-2 (ATCC® VR-1986HK™) was diluted to concentrations of 9.6 $x10^4$ through 1.5 $x10^3$ genome units (GU; a measure of virus number) in a doubling dilution series. One hundred microliter volumes of each dilution were used to evenly inoculate 2” x 2” stainless steel surfaces in triplicate. Surfaces were dried at 22 ºC and 50% relative humidity for 16-24 hours.

Surface swabbing and extraction of viral RNA was carried out according to the Thermo Scientific SARS-CoV-2 Real-Time PCR Workflow (Figure 1). The Applied Biosystems™ MagMAX™ Express-96 Magnetic Particle Processor was used for RNA extraction; PCR was carried out on the Applied Biosystems™ 7500 Fast Food Safety System with Applied Biosystems™ 7500 Software SDS v.1.4.2.

PCR was conducted four times for each extraction with duplicate reactions carried out for two different two-channel designs (Table 1).

Table 1: Two-channel designs used in the LOD study

PCR Detector Channel	FAM	VIC
Design 1 Targets	2019-nCoV N protein, S protein and ORF1ab targets	Human RNase P RPPH1 (IPC)
Design 2 Targets	2019-nCoV N protein	Human RNase P RPPH1 (IPC)

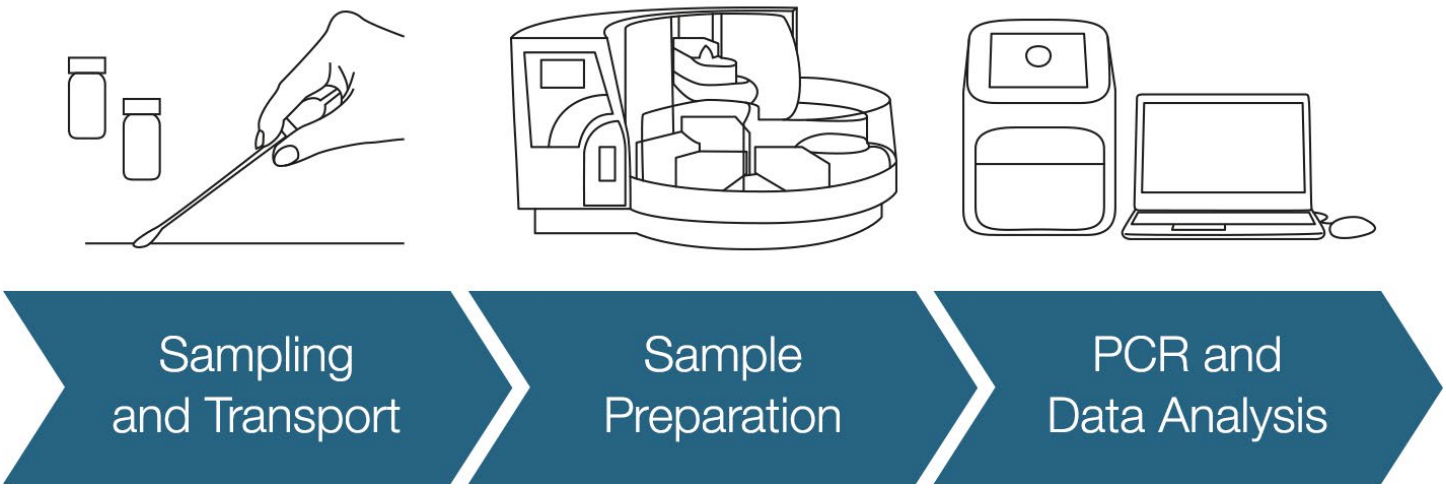


Figure 1. The Thermo Scientific SARS-CoV-2 Real-Time PCR Workflow for detection of the virus on food packaging and environmental surfaces

Master Mix Comparison

The Thermo Scientific™ Applied Biosystems™ TaqMan™ 2019-nCoV Control Kit v1 was diluted to concentrations of 1.0 x10^4 through 1.0 x10^1 per microliter in a log-dilution series. One microliter of each dilution was added to each PCR reaction.

PCR was conducted four times for each extraction, using a duplex reaction (Table 1, Design 2) in each case duplicate reactions were prepared using the following master mix reagents:

- Thermo Scientific™ RNA UltraSense™ One-Step RT-PCR System master mix (candidate method)
- Applied Biosystems™ TaqPath™ 1-Step RT-qPCR Master Mix, CG (reference method)

PCR reagent volumes and cycling parameters for the RNA UltraSense master mix were as described in the Thermo Scientific SARS-CoV-2 Real-Time PCR Workflow instructions for use. PCR volumes and cycling parameters for the TaqPath master mix were as described in the TaqMan 2019nCoV Assay Kit v1 instructions for use.

Results

In silico analysis

Of the 15,756 SARS-CoV-2 sequences analyzed, more than 90% had 100% homology to all three assays (ORF1ab, N-gene, S-gene) in the Thermo Scientific SARS-CoV-2 Real-Time PCR Workflow and 99% have 100% homology to at least two of the three assays. All the 15,756 tested genomes are predicted to be detected by at least one of the three assays (ORF1ab, N-gene, S-gene).

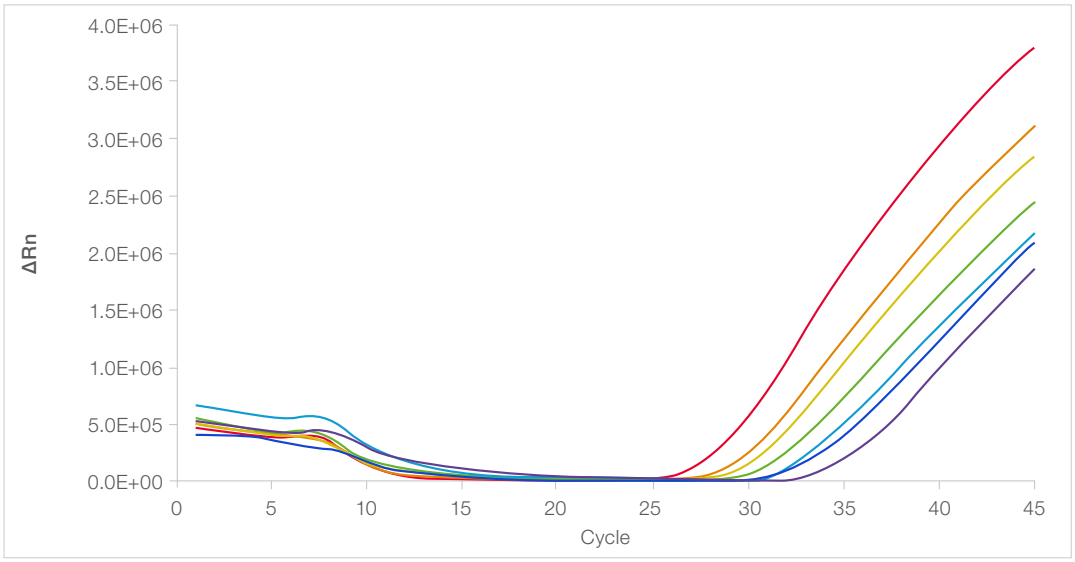
These data will be used in the validation study coordinated within the AOAC Research Institute Performance Tested Methods℠ (PTM) Program for the detection of SARS-CoV-2 on environmental surfaces[6].

Limit of Detection Results

Table 2. LOD data for the Thermo Scientific SARS-CoV-2 Real-Time PCR Assay

Surface inoculation GU/sample	Combined assay		N protein-only	
	Number positive calls	Average C_T value	Number positive calls	Average C_T value
9.6 x10^3	6/6	27.27	6/6	28.62
4.8 x10^3	6/6	28.76	6/6	30.06
2.4 x10^3	6/6	29.36	6/6	30.52
1.2 x10^3	6/6	30.92	6/6	31.80
6.0 x10^2	6/6	32.01	6/6	32.98
3.0 x10^2	6/6	32.19	6/6	33.53
1.5 x10^2	6/6	33.35	6/6	35.26

A



B

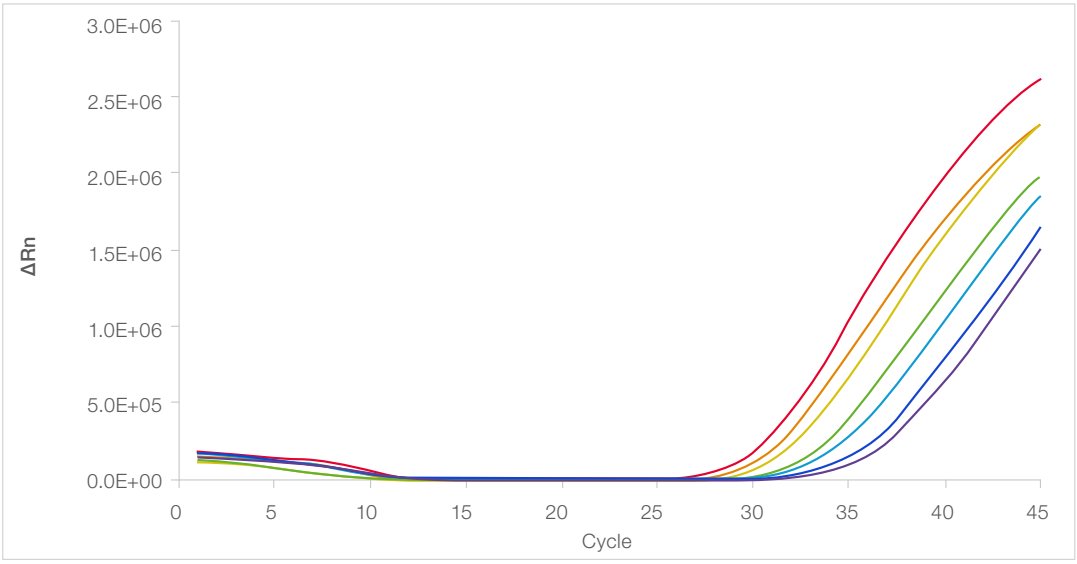


Figure 2. Typical amplification plots in the LOD study.
A) 2019-nCoV target in the combined assay. B) 2019-nCoV N protein-only target. Amplification of signal at increasing cycle number correlates with decreasing level of virus inoculation from 9.6 x10^3 through 1.5 x10^2 GU/sample.

The Thermo Scientific SARS-CoV-2 Real-Time PCR Workflow identified 3/3 positive reactions at all inoculum levels, down to 1.5 x10^2 GU/sample, for both the combined and N Protein-only assay (Table 2). Amplification plots give a typical shape and the expected distribution (Figure 2). The human RNase P RPPH1 IPC was also detected in all reactions (data not shown). These data suggest the candidate method provides exceptional sensitivity, with the ability to detect < 37.5 GU/square inch of surface or 0.075 GU/µL transport media. The average C_T difference between the combined assay and N Protein-only assay is consistent with the former simultaneously amplifying three targets rather than only one in the latter; this provides additional sensitivity over alternative singleplex or duplex assays.

The RNA UltraSense One-Step RT-PCR System master mix gives earlier C_T values and stronger fluorescence signal compared to TaqPath 1-Step RT-qPCR Master Mix, CG. This indicates that the master mix selected for the candidate method performs comparably to others selected for use with the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel.

Master Mix Comparison Results

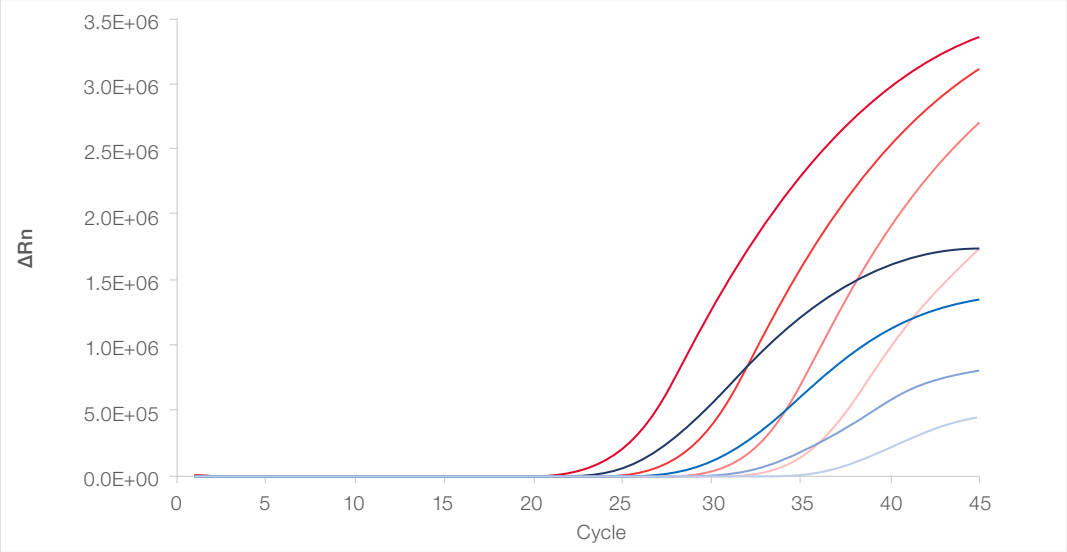


Figure 3. Comparison of RNA UltraSense (red) and TaqPath (blue) master mix performance.
Decreasing colour intensity indicates decreasing level of DNA input into the PCR reaction from 1.0 x10^4 through 1.0 x10^1 GU/reaction.

Conclusion

The Thermo Scientific SARS-CoV-2 Real-Time PCR Workflow is highly specific. Of the 15,756 target SARS-CoV-2 genomes analyzed, 100% are predicted to be detected by at least one of the three SARS-CoV-2 assays as 100% of the strains are matching with at least one of the three assays (ORF1ab, N protein, S protein), confirming the relevance of targeting more than one single genomic sequence to ensure high specificity.

The use of a combined assay that simultaneously amplifies three SARS-CoV-2 targets, providing one cumulative amplification plot, should reduce the likelihood of false negatives when viral copy numbers are low and provide a more economic PCR setup than single-target designs.

The Thermo Scientific SARS-CoV-2 Real-Time PCR Workflow provides highly sensitive detection of SARS-CoV-2 viral particles on environmental surfaces, with an LOD of at least 150 GU in a 2” x 2” area of stainless steel per the conditions in this study.

The candidate method provides better detection capabilities than the reference method. It is suggested that this is achieved partly through the use of the RNA UltraSense One-Step RT-PCR System master mix which gives earlier C_T values and stronger fluorescence signal than another master mix recommended in the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel.

The minimum infective dose of intact SARS-CoV-2 virus in humans, is higher than 100 particles in respiratory microdroplets that are directly transmitted from human to human by aerosol with droplets[7]. The estimated LOD for an environmental surface that is considerably bigger than respiratory microdroplets guaranties a high sensitivity regarding the contamination risk in working environments.

The combination of the RNA UltraSense One-Step RT-PCR System Master Mix ensures strong PCR parameter signals to the multiple SARS-CoV-2 targeted sequences and robustness ensure of the Thermo Scientific SARS-CoV-2 Real-Time PCR Workflow.

thermo scientific

Note: The Probability of Detection (POD) has been determined within the AOAC-PTM program through independent laboratory testing at GlobalMRI (MO, USA[6]). Replicate stainless steel test areas were inoculated with an intact, SARS-CoV-2 virus. Surface sampling was run prior to analysis with both, the GlobalMRI method, used as the reference method and the candidate method. Thermo Fisher Scientific has made the choice to run a fully independent study, with inoculated surfaces prepared by the Food Safety Net Services (TX, USA) for analysis with the Thermo Scientific SARS-CoV-2 Real-Time PCR Workflow. The difference between the POD values of the compared methods clearly demonstrates the acceptability of the candidate method. The report is expected to be available early 2021. The Robustness, Product Consistency and Stability studies will be run in a second timeframe.

References:

1. Riddell, S., Goldie, S., Hill, A. et al. The effect of temperature on persistence of SARS-CoV-2 on common surfaces. Virol J 17, 145 (2020). https://doi.org/10.1186/s12985-020-01418-7
2. Chin A W H et al. Stability of SARS-CoV-2 in different environmental conditions. The Lancet. 2020;1:e10.
3. Wang C et al, 2020. The establishment of reference sequence for SARS-CoV-2 and variation analysis. J Med Virol. 92(6):667-674. doi: 10.1002/jmv.25762. Epub 2020 Mar 20.
4. Hodcroft E et al, 2020. Emergence and spread of a SARS-CoV-2 variant through Europe in the summer of 2020. medRxiv preprint doi: https://doi.org/10.1101/2020.10.25.20219063
5. US Centers for Disease Control and Prevention. Real-time RT-PCR panel for detection 2019-novel coronavirus, instructions for use. 2020 July 13 [cited 2020 Sept 28] https://www.fda.gov/media/134922/download
6. https://www.aoac.org/news/aoac-international-now-accepting-applications-for-emergency-response-validation-of-novel-coronavirus-test-kits/
7. Karimzadeh S et al. Review of Infective Dose, Routes of Transmission, and Outcome of COVID-19 Caused by the SARS-CoV-2 Virus: Comparison with Other Respiratory Viruses. Version 2 Online: 22 October 2020.

ThermoFisher SCIENTIFIC