Emergency AOAC PTM Certification of a Method to Detect for SARS-CoV-2 from Environmental Surfaces

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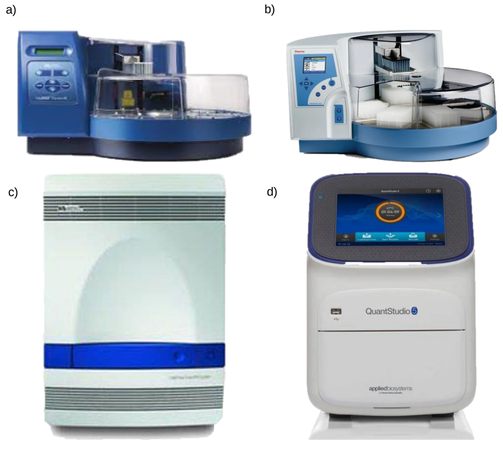
INTRODUCTION

A significant number of SARS-CoV-2 outbreaks have been traced back to food processing plants, and studies have shown long-term viability of the virus on environmental surfaces, leading to concerns that surface-to-human transmission may be occurring[1].

The Thermo Scientific™ Real-Time PCR Detection of SARS-CoV-2 on Food Packaging and Environmental Surfaces Assay (candidate method) was submitted for emergency validation in accordance with the AOAC Research Institute Performance Tested Methods℠ Program. Performance was evaluated versus the CDC 2019-Novel Coronavirus Real-Time Diagnostic Panel Instructions for Use[2] (reference method) for the detection of SARS-CoV-2 on a 2" x 2" stainless steel surface.

Figure 1. Candidate workflow instrumentation



a) MagMAX™ Express-96 Deep Well Magnetic Particle Processor (MM)
b) KingFisher™ Flex Purification System (KF)
c) Applied Biosystems™ 7500 Fast Food Safety Real-Time PCR System (7500F)
d) Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR System (QS5)

METHOD

Inclusivity/Exclusivity

In silico analysis for target specificity was performed through comparison to 15,756 SARS-CoV-2 sequences and 65 exclusivity organisms identified in the GISAID and GenBank Viral NCBI databases.

RESULTS

Table: Inclusivity results

	Total of sequences analyzed	Three-target^ 100% homology	Two-target^ 100% homology
Inclusivity	15,756	90%	99%

^Candidate workflow comprises a three-target multiplex design: ORF1ab, N-gene, S-gene

Figure 3:

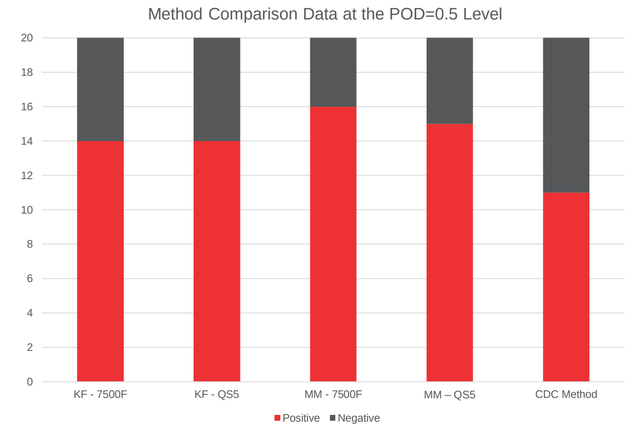
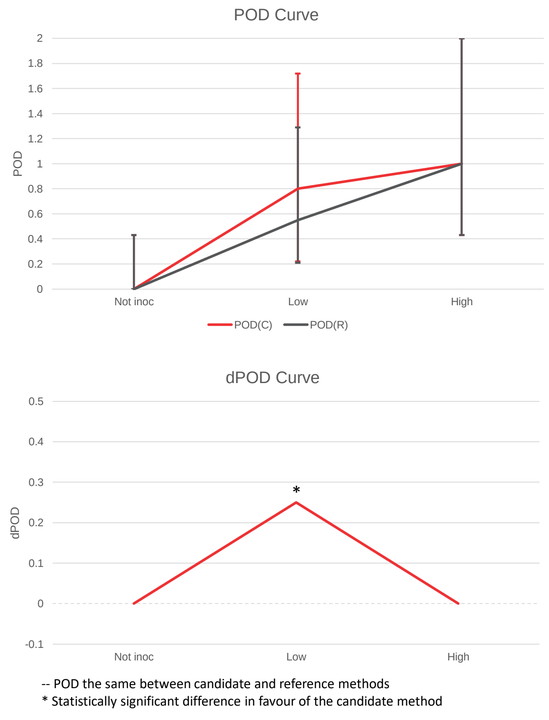


Figure 4: POD and dPOD Plots for the Candidate vs Reference Methods (MM – 7500F workflow):



-- POD the same between candidate and reference methods
* Statistically significant difference in favour of the candidate method

Table 1: POD and dPOD Data for the Candidate vs Reference Methods (Matrix Study)

Method	Candidate				Reference			dPOD$_C$	95% CI
	GC[a] per Test Area	X[d]	POD$_C$	95% CI	X[d]	POD$_R$	95% CI		
All	0	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	-0.43, 0.43
KF*	1.8×10^3	14	0.70	0.48, 0.86				0.15	-0.14, 0.41
MM – 7500F	1.8×10^3	16	0.80	0.58, 0.92	11	0.55	0.34, 0.74	0.25	**-0.04, -0.49**
MM – QS5	1.8×10^3	15	0.75	0.53, 0.89				0.20	-0.09, 0.45
All	1.8×10^4	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

*Both thermal cyclers gave equal performance. Bold: statistically significant difference.
X[d] = Number of positive test portions.

METHOD CONTINUED

Matrix Study

Probability of detection (POD) was evaluated in an unpaired study. Two-by-two-inch stainless-steel test areas were contaminated with heat-treated SARS-CoV-2: five with a high level (POD=1), twenty with a low level (POD=0.5) and five uncontaminated (POD=0). The candidate method utilizes RNA extraction and reverse transcription qPCR with an option of two different extraction devices and two different thermal cyclers (Figure 1). All combinations of equipment were evaluated in this study.

DISCUSSION

In silico analysis showed that primers and probes used in the candidate method matched ninety-nine percent of the SARS-CoV-2 sequences analyzed (Table 1); none of the exclusivity sequences showed sequence matches.

In the matrix study, for all combinations of extraction device and thermal cycler, the candidate method showed comparable or superior detection to the reference method. The candidate workflow consistently detected SARS-CoV-2 more frequently (Figure 2) with the MagMAX extraction followed by 7500 Fast thermal cycling achieving a significant improvement to the reference method (Figure 3 and Table 2).

CONCLUSIONS

The candidate method offers a specific and sensitive option to detecting SAR-CoV-2 on environmental surfaces, allowing directed hygiene intervention strategies to reduce latent health risks and plant shutdowns.

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).
2. Centers for Disease Control and Prevention (2020). CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. Revision 4, Effective 6/12/2020;2.

TRADEMARKS/LICENSING

IAFP, July 2021 LT2660A