The SARS-CoV-2 Panel (RUO): A high-throughput and robust assay with a low limit of detection for use on the MassARRAY® System

ABSTRACT

Agena Bioscience® has developed a Research Use Only (RUO) panel for the detection of SARS-CoV-2 virus on the MassARRAY® System. This assay provides a robust alternative to test for the presence of SARS-CoV-2 virus in human samples. This single well panel utilizes a one-step RT-PCR reaction to reverse transcribe viral RNA into cDNA and amplify the nucleic acid material in the same reaction. This new biochemistry is shown to work robustly with an input containing as few as 10 genome copy number equivalents (GCE) of the SARS-CoV-2 virus in the reaction. The high-throughput MassARRAY System enables laboratories to process up to 3,072 samples per day with a single instrument.

SARS-COV-2 PANEL

The Agena Bioscience SARS-CoV-2 Panel (RUO) consists of five SARS-CoV-2 specific assays - three in the *N* (nucleocapsid) gene, one in the *ORF1* gene, and one in the *ORF1ab* gene - as well as a control assay (RNase P [HsRP]), all multiplexed into a single reaction. Assay details are provided in Table 1.

Assay Design

A consensus sequence of the SARS-CoV-2 genome was generated using 165 complete genomes available as of 29th March 2020. Conserved regions were identified from the consensus sequence and used to verify primer homology (two PCR primers and one extension primer) for each assay in the panel.

NCBI nucleotide BLAST was used to test for cross reactivity of the primers *in silico* using a list of FDA-recommended micro-organisms (see Appendix A). The combination of primers for each assay (two PCR primers and one extension primer) exhibit 100% sequence homology to the conserved SARS-CoV-2 regions. However, individual primers (one of two PCR primers and/or the extension primer) for three of the assays exhibit higher than the 80% homology to a cross-reactive specie. As the individual primers will not have a template for extension, the likelihood of false positive results is extremely unlikely in each of these cross-reactive cases.

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Table 1. Agena Bioscience SARS-CoV-2 Panel (RUO) components

Assay name	Gene	Genome area covered
SC2-N1	Nucleocapsid	28,653 - 28,760
SC2-N2	Nucleocapsid	28,880 - 28,978
SC2-N3	Nucleocapsid	28,076 - 28,190
SC2-ORF1	ORF1	3,223 - 3,335
SC2-ORF1ab	ORF1ab	13,342 - 13,432
HsRP*	RPP30/RNAse P	chr10:92635825 - 92638839

*One PCR primer spans a 409 bp intron; the PCR product spans an additional 2.9kb intron controlling for RNA extraction & reverse transcription.

Special consideration was given to the design of the HsRP control assay to ensure amplification occurs only from cDNA template. The forward PCR primer is designed over a 409 bp intron, spanning exon 1 and 2, while the reverse primer is designed 2.9 kb away over exon 3. This design prevents the assay from amplifying genomic DNA and will only generate a product if RNA is converted to cDNA.

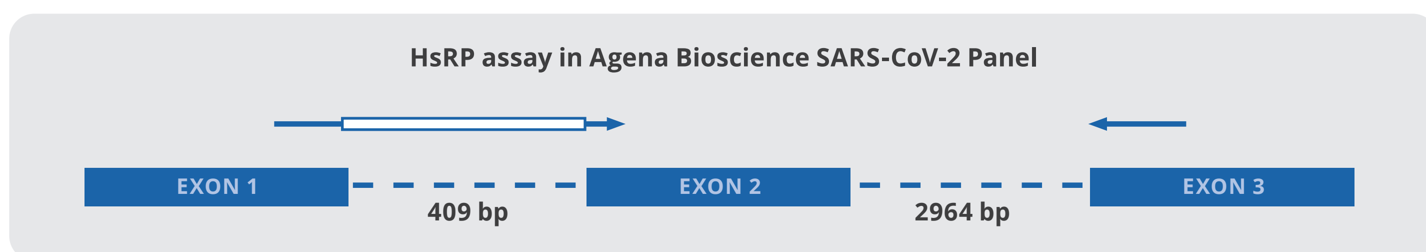

Figure 1. The HsRP assay showing forward and reverse PCR primers spanning 3 exons and 2 introns.

Analysis Software

Data analysis is simplified using supporting software which qualifies the RNA sample and determines the detection status of the virus. A sample passes QC based on the HsRP QC assay and the presence of the SARS-CoV-2 virus. The virus is reported as *SC2-Detected* if two or more SARS-CoV-2 assays generate an extension product in the sample. The thresholds for detection can be configured by the laboratory.

SARS-CoV-2 Interpretive Report (RUO)

Report version 1.0.0.36
Date Thu Apr 16 07:30:13 2020
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Sample	Location	QCStatus	QCMessage	SampleStatus
Sample01	chip_1*G02	FAIL	One or more QC markers failed.	SC2-Invalid
Sample02	chip_1*A02	PASS		SC2-Detected
Sample03	chip_1*D04	PASS		SC2-NotDetected
Sample04	chip_1*E04	PASS		SC2-NotDetected
NTC	chip_1*A01	PASS		
Sample05	chip_1*G06	Warning	One or more QC markers failed but SC2 target amplification was detected.	SC2-Detected

Figure 2. Example of the software output showing the variety of possible results.

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If the HsRP QC assay and the five SARS-CoV-2 assays all fail, the result outcome for the sample is *SC2-Invalid*. If the QC assay passes, the sample may either be *SC2-Detected* or *SC2-NotDetected*, depending on the number of SARS-CoV-2 assays detected. If a user specified minimum number of SARS-CoV-2 assays are detected but the QC assay fails, the report will show *Warning* instead of *FAIL* for the sample QC status. This allows the user to take appropriate action.

Table 2. Logic for sample qualification and virus detection

	Minimum # of SARS-CoV-2 assays detected	Less than minimum # of SARS-CoV-2 assays detected
QC assay result = ***PASS***	*SC2-Detected*	*SC2-NotDetected*
QC assay result = ***FAIL***	*SC2-Detected* Sample QCStatus = *Warning*	*SC2-Invalid*

METHODS AND RESULTS

Several experiments were designed to verify the panel performance as described below. Positive samples were generated using SARS-CoV-2 negative human RNA spiked with the Synthetic SARS-CoV-2 RNA Controls from Twist Bioscience (SKU 102019).

Limit of Detection (LOD) Study

Guidelines from the U.S. Food & Drug Administration (FDA) were followed to determine the LOD for the assay.[1] SARS-CoV-2 dilutions of 0, 5, 10, 20, 100, 1000, and 10,000 GCE in a background of 100 ng of human RNA were used as input for the RT-PCR reaction. Results showed that the LOD of the assay was 10 viral copies added to RT-PCR reaction.

An input of 3 µL of RNA was used for the RT-PCR reaction, therefore indicating that the sensitivity of the assay is as low as 3 copies of SARS-CoV-2 per µL of input RNA. The overall sensitivity of detecting SARS-CoV-2 may be higher depending on whether the RNA was concentrated during the extraction process, prior to being added into the RT-PCR reaction.

Table 3. Results from the LOD study

SARS-COV-2 COPIES	DETECTED	NOT DETECTED	DETECTION RATE
0	0	3	0%
5	2	1	67%
10	3	0	100%
20	3	0	100%
100	3	0	100%
1,000	3	0	100%
10,000	3	0	100%

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Analytical Sensitivity & Specificity Studies

Two 96-well plates containing a total of 100 samples were processed to determine analytical sensitivity and specificity.

PLATE 1: 40 human RNA samples

- 20 human RNA samples spiked with 10 GCE of SARS-CoV-2
- 20 human RNA samples negative for SARS-CoV-2

PLATE 2: 60 human RNA samples

- 30 replicates of human RNA negative for SARS-CoV-2
- 20 replicates of human RNA spiked with 15 copies of SARS-CoV-2 RNA at 1.5x the LOD threshold established in the previous experiment
- 10 samples with 20 – 100 copies of SARS-CoV-2

The results show that the SARS-CoV-2 Panel (RUO) achieves 100% sensitivity and specificity with a limit of detection of 10 viral copies.

Table 3. Results from the sensitivity and specificity study

SARS-CoV-2 Copies	# Samples Tested	# Samples with SARS-CoV-2 Detected	# Samples with SARS-CoV-2 Not Detected
0	50	0	50
10	20	20	0
15	20	20	0
20-100	10	10	0

Accuracy Study

An external clinical laboratory was used to determine accuracy by comparing results with an established TaqMan based assay. Samples that were positive or negative on the alternative platform were tested using the SARS-CoV-2 Panel (RUO) on the MassARRAY System with Chip Prep Module 96. Results for the 50 samples tested showed 100% concordance with expected results.

Table 4. Comparison of SARS-CoV-2 Panel (RUO) with a TaqMan-based assay.

	MassARRAY Positive	MassARRAY Negative
TaqMan positive	41	0
TaqMan negative	0	9

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CONCLUSIONS

Results of the limit of detection study and analytical sensitivity and specificity studies demonstrate that the Agena Bioscience SARS-CoV-2 Panel (RUO) can be used to detect SARS-CoV-2 viral RNA with an input as low as 10 GCE. The assay is equivalent to TaqMan-based detection as seen in the accuracy study conducted at an external clinical site. The MassARRAY workflow offers a sensitive, high-throughput and robust method for the detection of SARS-CoV-2.

References

1. U.S. FDA Guidance Document: Policy for Diagnostic Tests for Coronavirus Disease-2019 during the Public Health Emergency. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Devices and Radiological Health. March 16, 2020. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-diagnostic-tests-coronavirus-disease-2019-during-public-health-emergency

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Appendix A. Organism list used for BLAST similarity evaluation

High priority pathogens from the same genetic family	High priority organisms likely in circulating areas
Human coronavirus 229E	**Adenovirus (e.g., C1 Ad. 71)**
Human coronavirus OC43	**Human Metapneumovirus (hMPV)**
Human coronavirus HKU1	**Parainfluenza virus 1-4**
Human coronavirus NL63	**Influenza A & B**
SARS-coronavirus	**Enterovirus (e.g., EV68)**
MERS-coronavirus	**Respiratory syncytial virus**
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Pooled human nasal wash – to represent diverse microbial flora in the human respiratory tract
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermis
	Staphylococcus salivarius

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