MicroGen DX
Next-Gen DNA Sequencing
FOR ACCURATE MICROBIAL IDENTIFICATION
Superior Diagnostics. Targeted Treatments. Better Outcomes.

SARS-CoV-2 Molecular Diagnostic Assay Validation Summary

The MicroGen DX SARS-CoV-2 Molecular Diagnostic Assay is a modification of the CDC's EUA-approved assay. The modifications have been shown not to impact the performance of the assay. The validation testing meets or exceeds the requirements of the FDA for submission of EUA request.

Specificity (Cross-Reactivity)

Primer and probe specificities were confirmed by testing the primers and probes against plasmid controls as well as:

Human Coronavirus 229E	Parainfluenza virus 1-4	*Streptococcus pyogenes*
Human Coronavirus NL63	Influenza A & B	*Bordatella pertusis*
Human Coronavirus OC43	Enterovirus*	*Mycoplasma pneumoniae*
Human Coronavirus HKU1	Respiratory Syncytial Virus	*Pneumocystis jirovecii (PJP)**
SARS	Rhinovirus	*Candida albicans*
MERS	*Haemophilus influenza*	*Pseudomonas aeruginosa*
Adenovirus	*Mycobacterium tuberculosis*	*Chlamydia pneumoniae*
Human Metapneumovirus	*Streptococcus pneumoniae*	*Legionella pneumophila**

*These species were not readily available in-house but were analyzed *in silico* and assay primers and probes were determined to be specific to assay targets.

The assay was determined to be 100% specific to SARS-CoV-2.

Limit of Detection

The FDA generally does not have concerns with spiking RNA or inactivated virus into artificial or real clinical matrix for LoD determination. The FDA recommends that laboratories test a dilution series of 3 replicates per concentration and confirm the final concentration with 20 replicates.

The assay limit of detection (LoD) was determined by spiking SARS-CoV-2 control plasmids into real clinical matrix (swab and sputum) and testing a dilution series of 8 concentrations with 3 replicates.

Swabs: 283.76 copies/mL, C_t cutoff 35.00

Sputum: 283.76 copies/mL, C_t cutoff 35.00

The determined LoD was confirmed using 20 replicates. The FDA requirement that a minimum of 19/20 replicates are positive at the lowest concentration was met; actual value 20/20.

Precision

Precision was evaluated by running the dilution series in replicates of three. The precision plates were loaded by another technician on another machine and performed on another day. The curves and dilutions were compared to original curves and limit of detection results. The cycle thresholds were within 2 standard deviations and deemed precise.

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Extraction

60 swab and 60 sputum de-identified patient samples were extracted using manual and automated methods. 30 of the 60 samples for each sample type were spiked with positive controls to determine efficacy of the extraction, sensitivity, specificity and accuracy of the assay. Data shown in Clinical Evaluation section.

Clinical Evaluation

60 contrived samples created by spiking target RNA (30 positive) or molecular grade water (30 negative) into clinical specimens comprised of nasopharyngeal swabs and sputum to determine assay accuracy. 20 of the positive specimens will be spiked at 1x-2x LoD and the remainder will be spiked to span the assay analytical measurement range. 95% accuracy at 1x-2x LoD and 100% accuracy for negative samples are recommended by the FDA.

Manual Extraction:

SARS-CoV-2 Sputum

	SPIKE	NO-SPIKE	
POSITIVE	30	0	30
NEGATIVE	0	30	30
	30	30	60

Accuracy	100%
Specificity	100%
Sensitivity	100%

SARS-CoV-2 Swab

	SPIKE	NO-SPIKE	
POSITIVE	30	0	30
NEGATIVE	0	30	30
	30	30	60

Accuracy	100%
Specificity	100%
Sensitivity	100%

Automated Extraction:

SARS-CoV-2 Sputum

	SPIKE	NO-SPIKE	
POSITIVE	30	0	30
NEGATIVE	0	30	30
	30	30	60

Accuracy	100%
Specificity	100%
Sensitivity	100%

SARS-CoV-2 Swab

	SPIKE	NO-SPIKE	
POSITIVE	30	0	30
NEGATIVE	0	30	30
	30	30	60

Accuracy	100%
Specificity	100%
Sensitivity	100%

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Inclusivity

Inclusivity of the primers and probes was confirmed *in silico* demonstrating that all publicly available SARS-CoV-2 sequences can be detected by the assay.

100% detection of published sequences achieved.

Confirmatory Testing

The MicroGen DX SARS-CoV-2 Molecular Diagnostic Assay has been validated, and patient samples will be accepted starting Monday March 23rd, 2020. The first 5 positive and 5 negative patient samples will be tested by an EUA-approved method to confirm the results of the assay.