Validation of Sema4 SARS-CoV-2 RT-PCR (COVID-19) Assay

OVERVIEW

Sema4 has validated the Perkin Elmer Coronavirus Nucleic Acid Detection Assay which has been authorized for emergency use by the FDA. The Coronavirus Nucleic Acid Detection Assay can detect and measure Coronavirus SARS-CoV-2 RNA that is associated with the worldwide pandemic. The clinical validation of the Coronavirus Nucleic Acid Detection Assay demonstrated that extraction, preparation and detection of Coronavirus SARS-CoV-2 RNA on the PerkinElmer platform generated reproducible and accurate results. The validation adhered to the established standard operating procedures as applicable.

TEST METHODS

Sample preparation was performed in the Baker Level 2 Biosafety cabinet while reagent preparation was performed on the PerkinElmer® Janus™. Viral RNA isolation was accomplished using the Perkin Elmer chemagic™ viral DNA/RNA 300 kit executed on the chemagic™ 360 instrument. The isolated SARS-CoV-2 RNA was prepared manually or on the PerkinElmer Janus G3 for subsequent analysis with real-time PCR via the PerkinElmer New Coronavirus Nucleic Acid Detection kit on the Roche LightCycler 480. This method used *in vitro* reverse transcription of the SARs-CoV-2 viral RNA resulting in the synthesis of cDNA. The resulting cDNA underwent PCR amplification followed by fluorescent detection using FAM and ROX tagged TaqMan probes targeting the nucleocapsid (N) gene and ORF1ab of the SARs-CoV-2 virus. In addition to the two virus specific probe sets, an internal VIC/HEX tagged control probe set was included with each reaction which amplifies the internal extraction and amplification control spiked-in to each specimen. Results were established by analyzing the Ct values of the three probe sets for each sample and were reviewed by a clinical laboratory director.

Three validations runs were performed by one of two technologists during the validation. All runs were processed with the PerkinElmer nCoV Positive Control and PerkinElmer nCoV Negative Control as intended in a clinical run. Validation runs one and two were prepared manually, while validation run three was prepared using the Janus and chemagic 360. All validation runs were proceeded on the LightCycler 480 II.

The control materials outlined below were run across all three of the validation runs and used to assess the accuracy, inter-assay repeatability, and intere-assay reproducibility:

- PerkinElmer nCoV Positive Control contains SARS-CoV-2 RNA fragments capsulated in bacteriophage
- PerkinElmer nCoV Negative Control contains buffer
- SeraCare AccuPlex™ SARS-CoV-2 positive and negative reference material
- PerkinElmer nCoV Internal Control contains TE Buffer, bacteriophage MS2

Results

The three validation runs generated data from three positive controls, three negative controls, which were processed in replicate within runs and across 3 separate runs to generate a total of sixteen positive samples and sixteen negative samples. The results of all controls and samples were 100% concordant with expected results.

The summary results across all replicates on the three validation runs can be found in **Table 1** below and additional details can be found in **Supplemental Table 1**.

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Table 1: Summary of Validation Results

Sample ID	Replicate	Run 1	Run 2	Run 3	Concordance
PE Kit Positive	1	Detected	Detected	Detected	100%
	2	Detected	Detected	Detected	100%
	3	Detected	Detected	Detected	100%
	4	N/A	N/A	Detected	100%
PE Kit Negative	1	Not Detected	Not Detected	Not Detected	100%
	2	Not Detected	Not Detected	Not Detected	100%
	3	Not Detected	Not Detected	Not Detected	100%
	4	N/A	N/A	Not Detected	100%
SeraCare Positive	1	Detected	Detected	Detected	100%
	2	Detected	Detected	Detected	100%
	3	Detected	Detected	Detected	100%
SeraCare Negative	1	Not Detected	Not Detected	Not Detected	100%
	2	Not Detected	Not Detected	Not Detected	100%
	3	Not Detected	Not Detected	Not Detected	100%

CONCLUSION

The Coronavirus Nucleic Acid Detection Assay extraction, preparation and detection of Coronavirus SARS-CoV-2 RNA satisfied all acceptance criteria set forth in the validation plan and has been deemed acceptable for clinical testing.

REFERENCES

1. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-diagnostic-tests-coronavirus-disease-2019-during-public-health-emergency
2. https://www.who.int/health-topics/coronavirus
3. https://perkinelmer-appliedgenomics.com/home/products/sars-cov-2-real-time-rt-pcr-assay-ce-ivd/
4. https://www.seracare.com/AccuPlex-SARSCoV2-Reference-Material-Kit-0505-0126/

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