



21120.9142 SARS CoV 2 RT qPCR Validation Report Alt Ext & Amp methods 2.1

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Copy of version 2.1 (approved and current)

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Author
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Comments for version 2.0 (last major revision)

Addendum multiplexed RNase P with SARS CoV-2 and MS2 assays

Comments for version 2.1 (this revision)

Added conclusion statement of Director acceptance

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2.1	Approved and Current	Minor revision	17-Jul-2020	27-Jul-2020	Indefinite
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Validation report to establish equivalent performance of the SARS Coronavirus 2 (SARS-CoV-2) Reverse Transcription Quantitative Real Time PCR assay when performed with alternate extraction and amplification methods

A. Introduction / Objective

An outbreak of coronavirus disease 2019 (COVID-19) caused by the 2019 novel coronavirus (SARS-CoV-2) began in Wuhan, Hubei Province, China in December 2019, and has spread throughout China as well as numerous other countries, including the United States. The outbreak was declared a Public Health Emergency of International Concern on 30 January 2020 by the World Health Organization. Signs and symptoms of COVID-19 include fever, cough, and shortness of breath. Person-to-person spread of SARS-CoV-2 appears to occur mainly by respiratory transmission. How easily the virus is transmitted between persons is currently unclear. Based on the incubation period of illness for Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) coronaviruses, as well as observational data from reports of travel-related COVID-19, CDC estimates that symptoms of COVID-19 occur within 2–14 days after exposure. Preliminary data suggest that older adults and persons with underlying health conditions or compromised immune systems might be at greater risk for severe illness from this virus.

The primary objective of this study was to evaluate the performance of SARS-CoV2 virus (SARS-CoV2) specific reverse transcription real-time PCR (RT-qPCR) to detect SARS-CoV2 RNA in bronchoalveolar lavage (BAL) specimens using alternate extraction and amplification methods. This assay is intended for qualitative detection of RNA from SARS-CoV2 virus. The assay is intended for use with specimens collected from individuals meeting SARS-CoV2 virus clinical criteria (e.g., clinical signs and symptoms).

This validation report is intended to provide documented evidence of equivalent (b) (4) (b) (4) between the three methods.

B. Scope

This validation report includes the (b) (4) and acceptance criteria for each of these approaches, for the SARS-CoV2 real-time RT-qPCR assay. This validation plan was primarily composed using guidelines recommended by the US FDA (see: Policy for Diagnostics Testing in Laboratories Certified to Perform High Complexity Testing under CLIA prior to Emergency Use Authorization for Coronavirus Disease-2019 during the Public Health Emergency - Immediately in Effect Guidance for Clinical Laboratories and Food and Drug Administration Staff, document issued on February 29, 2020). Table 1 is a summary of the results including acceptance criteria.

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Table 1. Summary of validation results for SARS-COV-2 RT-qPCR assay using alternate extraction / amplification methods

Performance Characteristic	Action	Results	Pass/Fail
(b) (4)			PASS
			PASS

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C. Materials

The following materials (or suitable equivalents) will be used:

1. Biorad easyMAG instrument with disposables
2. (b) (4)
3. ThermoFisher KingFisher FLEX instrument with disposables
4. ThermoFisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit, cat # A42352, A48310, or A48383 – reagents for use with the KingFisher instrument
5. In-house developed SARS-CoV-2 real time RT-qPCR primers and probes part # 25 700143
6. SARS-CoV-2-negative human BAL
7. SARS-CoV-2 High positive (b) (4) control, part # 25 000415
8. SARS-CoV-2 Low positive (b) (4) control, part # 25 000414
9. (b) (4) Internal RNA Control (MS2). Stock (part # (b) (4)), (b) (4)
10. ABI 7500 Standard Instrument with disposables
11. ABI 7500 FAST Instrument with disposables
12. TaqPath™ 1-step RT-qPCR master mix, CG; (Life Technologies, catalog # A15299 or A15300)
13. RNase-, DNase-free water, Fisher Scientific, cat # BP561-1
14. (b) (4)
15. Pipette tips with aerosol barrier: 10µL, 200µL, and 1000µL sizes
16. Pipettes to accommodate tip sizes listed above
17. (b) (4) statistical software (b) (4)
18. (b) (4) statistical software (b) (4)

D. Methods

Sample preparation

All samples were spiked with SARS-CoV2 virus obtained and characterized from residual clinical sample(s) at pre-determined concentrations (b) (4). Volumes were prepared in sufficient quantities so that each sample prepared was able to be processed with each full-process method described.

Viral deactivation

(b) (4)

Nucleic acid extraction

Nucleic acid extraction for respiratory specimens will be performed for each sample following instructions in SOP 21120.705 *NucliSens easyMAG Total Nucleic Acid Extraction* with the following modifications:

Method 1 (b) (4)

Method 2 (b) (4)

Method 3 (b) (4)

Two positive extraction controls (high and low) will be used during the validation.

Nucleic acid amplification and detection

Nucleic acid amplification was performed as described in SOP 21120.461 *Real-Time PCR and RT-PCR Using (b) (4) Instruments* with the following modifications:

Method 1 (b) (4)

(b) (4)

Method 2 (b) (4)

(b) (4)

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(b) (4)

Table 2.

(b) (4)

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Table 3.

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Table 4.

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Table 5.

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G. Exceptions

(b) (4)

H. Conclusions

The performance characteristics of the SARS CoV-2 RT-qPCR assay with human upper and lower respiratory specimens met acceptance criteria specified in *Validation protocol to establish equivalent performance of the SARS Coronavirus 2 (SARS-CoV-2) Reverse Transcription Quantitative Real Time PCR assay when performed with alternate extraction and amplification methods* 21120.9037. I have reviewed the validation and the performance of the method is considered acceptable for patient testing.

I. References

Policy for Diagnostics Testing in Laboratories Certified to Perform High Complexity Testing under CLIA prior to Emergency Use Authorization for Coronavirus Disease-2019 during the Public Health Emergency Immediately in Effect Guidance for Clinical Laboratories and Food and Drug Administration Staff. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Devices and Radiological Health. February 29, 2020.

Basic Method Validation, 3rd Edition. JO. Westgard, Ph.D. Westgard QC, Inc. Madison, WI. 2008.

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CLIA Interpretive Guidelines 493.1252. CDC, DHHS. CLIA Current Regulations. 01/24/2004. www.cdc.gov/clia/regs/toc.aspx

(b) (4)

(b) (4)

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(b) (4)

(b) (4)

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Guidance for Industry; Bioanalytical Method Validation. U.S. Department of Health and Human Services, FDA Food and Drug Administration, Center for Drug Evaluation and Research Center for Veterinary Medicine, May 2001 BP.

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MM06-A2, Vol. 30 No.22. Quantitative Molecular Diagnostics Methods for Infectious Diseases; Approved Guideline, Second Edition, Clinical and Laboratory Standards Institute. Wayne, PA. 2010

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Molecular Microbiology: Diagnostic Principles and Practice, Second Edition. David H. Persing. ASM Press. 2011. Washington, D.C.

(b) (4)

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J. Amendment Performance Characteristics Evaluation of SARS CoV-2 RNase P Multiplex assay

K. Introduction / Objective

In the process of developing a self-collection nasal swab test, Viracor multiplexed an RNase P assay with the SARS CoV-2 RT qPCR assay. One aspect of self-collection that differs from the existing testing method is that self-collection requires a check for sample validity. This has been achieved by other groups by incorporating a separate PCR assay targeting a human housekeeping gene. RNase P is a constitutively expressed ribonuclease that cleaves RNA and is required for normal and efficient transcription of various small noncoding RNAs. Therefore, a 'valid' nasal swab sample (one containing human epithelial cells and/or cells infected with virus) will contain this target sequence and should be detectable by real-time PCR. The CDC's published RNase P assay was using a probe labeled with a (b) (4)

In order to streamline the clinical lab workflow and minimize the number of different reagents used for the SARS CoV2-2 assay, using the recently validated multiplexed SARS-CoV2 + MS2 + RNase P assay for all amplifications submitted for testing regardless if the sample type requires RNase P assessment (i.e. self-collected nasal swabs) was considered.

The primary objective of this study was to evaluate the performance of SARS-CoV2 + MS2 + RNase P multiplexed assay to detect SARS-CoV2 RNA in BAL specimens. This assay is intended for qualitative detection of RNA from SARS-CoV2 clinical samples collected from individuals infected with the 2019 novel strain of coronavirus. The assay is intended for use with specimens collected from individuals meeting SARS-CoV2 virus clinical criteria (e.g., clinical signs and symptoms).

L. Scope

This validation experiment was designed to (b) (4)

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Table 6. Summary of validation results for SARS-COV-2 RNase P RT-qPCR assay			
Performance Characteristic	Action	Results	Pass/Fail
(b) (4)			PASS
			PASS

M. Materials (In addition to materials in section C)

(b) (4)

N. Methods

Nucleic acid amplification and detection

Nucleic acid amplification was performed as described above for Method 2 and Method 3 (b) (4)

Performance Characteristics Evaluation

(b) (4)

- **Acceptance criteria**

(b) (4)

Clinical evaluation

(b) (4)

- **Acceptance criteria**

(b) (4)

(b) (4)

O. Analysis

(b) (4)

Table 7.

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Table 8. (b) (4)

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Table 8 (cont'd).

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