



## 21120.9037 SARS CoV 2 RT qPCR Validation Protocol Alt Ext & Amp methods 1.0

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#### Copy of version 1.0 (approved and current)

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#### Comments for version 1.0

Updated (clean) version - added (b) (4) extraction platform

#### Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	(b) (6)	04-May-2020 7:23	1.0	(b) (6)	
				(b) (6)	
Approval	(b) (6) Approver -	03-May-2020 22:50	1.0	(b) (6)	
				(b) (6)	

#### Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
1.0	Approved and Current	Initial version	10-Apr-2020	04-May-2020	Indefinite

# 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

## 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 is to evaluate the performance of SARS-CoV-2 virus (SARS-CoV-2) specific reverse transcription real-time PCR (RT-qPCR) to detect SARS-CoV-2 RNA in bronchoalveolar lavage (BAL) specimens using alternate extraction and amplification methods. This assay is intended for qualitative detection of RNA from SARS-CoV-2 virus. The assay is intended for use with specimens collected from individuals meeting SARS-CoV-2 virus clinical criteria (e.g., clinical signs and symptoms).

This validation protocol is intended to provide documented evidence of (b) (4)

## B. Scope

This validation protocol includes the (b) (4) and acceptance criteria for each of these approaches, for the SARS-CoV-2 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 protocol including acceptance criteria.

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

Performance Characteristic	Purpose	Action	Acceptance Criteria
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## C. Materials

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

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(b) (4)

with disposables

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13. RNase-, DNase-free water,

(b) (4)

(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 will be

(b) (4)

Volumes will be prepared in sufficient quantities so that each sample prepared will be able to be processed with each full-process method described.

### Viral deactivation

Prepared samples will be subjected to

(b) (4)

(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:

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(b) (4)

## Nucleic acid amplification and detection

(b) (4)

*Instruments with the following modifications:*

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- **Acceptance criteria for controls and negative samples**

## Performance Characteristics Evaluation

### Limit of detection

(b) (4)

- **Acceptance criteria**

(b) (4)

## Clinical evaluation

(b) (4)

- **Acceptance criteria**

**(b) (4)**

E. Analysis

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(b) (4)

## F. Results and Conclusions

Results will be summarized graphically and/or in tables.

A validation report will be generated with all the results obtained from this validation protocol.

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will be included in the validation report.

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will be included in the validation report.

Footnotes will be included in the validation report with the run packet numbers from which the data originated.

## G. Exceptions

Any deviations or exceptions to this protocol will be documented on the appropriate laboratory records and data packets, and addressed in the validation report. Instances in which the pre-specified acceptance criteria are not met will be identified and evaluated in the validation report. Validation approval/rejection will not be exclusively determined based on pass/fail outcome. Rather, criteria failures will be investigated and evaluated based on the nature of the violation and its assessed impact in the context of clinical testing after further discussion between the design review committee members and Viracor-Eurofins technical team.

## H. 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.

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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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