



21120.9109 SARS CoV 2 RT qPCR NW, NP SWAB, BAL and SERUM (using easyMAG 0.5 mL input) Validation Report  
3.0

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Validation Report**

Copy of version 3.0 (approved and current)

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Periodic review not required

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**Organization** Viracor

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Updates to stability through Day 120.

**Approval and Periodic Review Signatures**

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Approval

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Approver -

14-Jul-2020 8:12

2.0

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Approval

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10-May-2020  
21:58

1.0

(b) (6)

Approval

(b) (6)

Approver -

07-May-2020  
14:14

1.0

(b) (6)

#### Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
3.0	Approved and Current	Major revision	28-Sep-2020	15-Oct-2020	Indefinite
2.0	Retired	Major revision	12-Jul-2020	14-Jul-2020	15-Oct-2020
1.0	Retired	Initial version	29-Apr-2020	10-May-2020	14-Jul-2020

#### Linked Documents

- 21120.9043 SARS CoV2 Quantitative RT-PCR for BioPharma

# BioPharma Specific Validation Report to Establish the Performance Characteristics of the SARS Coronavirus 2 (SARS-CoV-2) Reverse Transcription Quantitative Real Time PCR assay in Human Upper/Lower Respiratory (Nasal Wash, NP Swab and BAL) and Serum Specimens (using easyMAG 0.5 mL input)

## 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 ability of SARS-CoV-2 virus (SARS-CoV-2) specific reverse transcription real-time PCR (RT-qPCR) to detect SARS-CoV-2 RNA in upper respiratory (nasal/nasopharyngeal wash and swab) and bronchoalveolar lavage (BAL). This assay is intended for quantitative 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 report was intended to provide documented evidence of (b) (4) of an in-house developed SARS-CoV-2 assay in human serum, human upper respiratory (nasal/nasopharyngeal wash and swab) and bronchoalveolar lavage (BAL).

## B. Scope

This validation report includes (b) (4) and acceptance criteria for each of these approaches, for the SARS-CoV-2 real-time RT-qPCR assay. This validation report 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 report including acceptance criteria, validation results and pass/fail status.

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**Table 1. Summary of validation criteria for SARS-COV-2 RT-qPCR assay in human serum, human upper respiratory (nasal/nasopharyngeal wash and swab) and bronchoalveolar lavage**

Performance Characteristic	Action and Acceptance Criteria	Validation Results	Pass/Fail
(b) (4)			Pass
(b) (4)			Pass
(b) (4)			As Found

**Table 1. Summary of validation criteria for SARS-COV-2 RT-qPCR assay in human serum, human upper respiratory (nasal/nasopharyngeal wash and swab) and bronchoalveolar lavage**

Performance Characteristic	Action and Acceptance Criteria	Validation Results	Pass/Fail
(b) (4)			Pass
(b) (4)			Pass

Table 1. Summary of validation criteria for SARS-COV-2 RT-qPCR assay in human serum, human upper respiratory (nasal/nasopharyngeal wash and swab) and bronchoalveolar lavage

Performance Characteristic	Action and Acceptance Criteria	Validation Results	Pass/Fail
(b) (4)			Pass
(b) (4)			Pass
(b) (4)			Pass

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**Table 1. Summary of validation criteria for SARS-COV-2 RT-qPCR assay in human serum, human upper respiratory (nasal/nasopharyngeal wash and swab) and bronchoalveolar lavage**

Performance Characteristic	Action and Acceptance Criteria	Validation Results	Pass/Fail
(b) (4)			As Found
(b) (4)			As Found
(b) (4)			As Found

**C. Materials**

The following materials (or suitable equivalents) were used:

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2. (b) (4)



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- 4.
- 5.
- 6.
- 7.
- 8.
- 9.
- 10.
- 11. RNase-, DNase-free water,
- 12.
- 13. Pipette tips with aerosol barrier: 10µL, 200µL, and 1000µL sizes
- 14. Pipettes to accommodate tip sizes listed above
- 15. (b) (4) statistical software (v. 2.7.8 (3/15/2010)
- 16. (b) (4) statistical software (b) (4)

(b) (4)

## D. Methods

The (b) (4) raw output files (.csv files) generated under validation protocol 21120.8890 SARS CoV 2 RT qPCR Validation Protocol URT LRT Serum was re-analyzed using the stored standard curve parameters generated after the March 2020 validation. All key detailed methods can be found in binder BP-2020-37.

### Sample preparation

All samples were prepared (b) (4)

### Nucleic acid extraction

Nucleic acid extraction for upper/lower respiratory samples was performed following instructions in SOP 21120.705 *NucliSens easyMAG Total Nucleic Acid Extraction*. (b) (4)

(b) (4) were established during validation experiments.

### 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.

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## Performance Characteristics Evaluation

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- Acceptance criteria

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Table 2.

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- Acceptance criteria

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## E. Analysis

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(b) (4)

## F. Results and Conclusions

The (b) (4) raw output files (.csv files) generated under validation protocol 21120.8890 SARS CoV 2 RT qPCR Validation Protocol URT LRT Serum was re-analyzed using the stored standard curve parameters generated after the March 2020 validation. All key detailed results can be found in BP-2020-37.

### Controls and negative samples

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**Table 3.**

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Table 6.

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Table 8.

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Table 9.

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Table 10.

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Table 11.

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(b) (4)

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Table 14.

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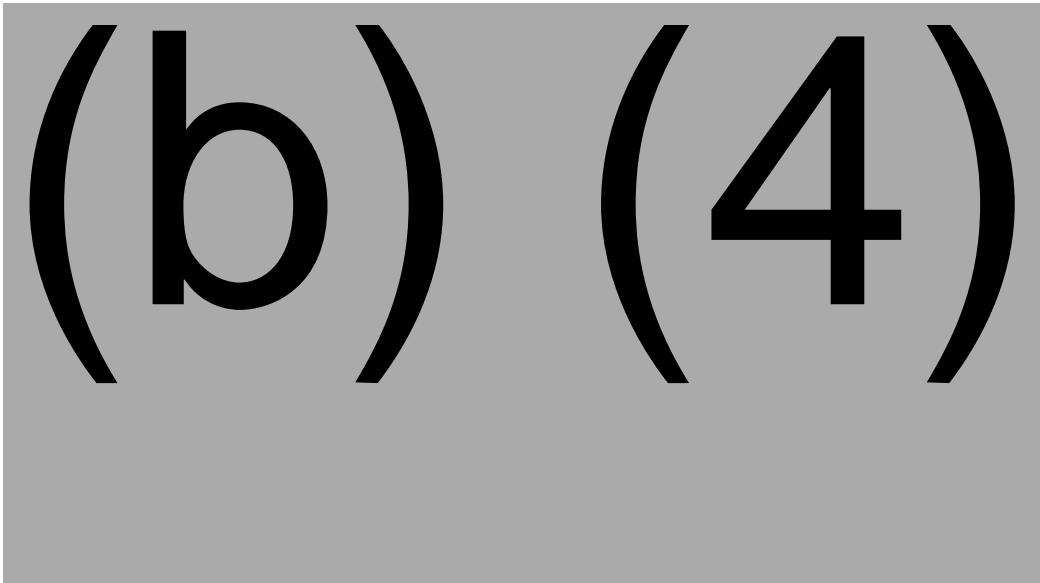
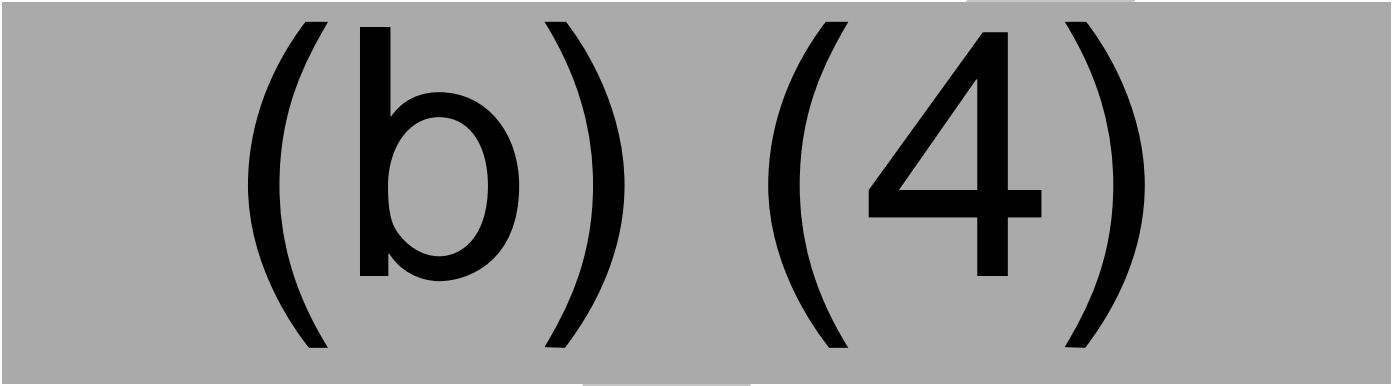


Figure 4. (b) (4)



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(b) (4)



**Table 20.**

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**Table 20.**

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(continued)

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**Table 24.** (b) (4)

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## G. Deviations

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3. Due to safety concerns when receiving high titer SARS CoV-2 specimens (b) (4)

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, a change in processing was

implemented to

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4.

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Table 27. (b) (4)

(b) (4)

- **Acceptance criteria**

(b) (4)

## H. References

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(b) (4)

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