



21120.9184 SARS CoV 2 RT qPCR Swab and Saliva Validation Protocol 4.0

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### Copy of version 4.0 (approved and current)

**Last Approval or  
Periodic Review Completed** 07-Oct-2020

Periodic review not required

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**Organization** Viracor

### Comments for version 4.0

This version changes the number of concentrations of samples in stability and the number of replicates at each time point per client request.

### Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	(b) (6)	07-Oct-2020 20:58	4.0	(b) (6)	
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#### Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
4.0	Approved and Current	Major revision	07-Oct-2020	07-Oct-2020	Indefinite
3.0	Retired	Major revision	06-Oct-2020	06-Oct-2020	07-Oct-2020
2.0	Retired	Major revision	26-May-2020	26-May-2020	06-Oct-2020
1.0	Retired	Initial version	18-May-2020	19-May-2020	26-May-2020

#### Linked Documents

- 21120.9249 SARS CoV 2 RT qPCR Swab and Isohelix-Saliva Validation Report

# BioPharma Specific Validation Protocol to Establish the Performance Characteristics of the SARS Coronavirus 2 (SARS-CoV-2) Reverse Transcription Quantitative Real Time PCR assay in Human Swab and Saliva Specimens

## 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 ability of SARS-CoV-2 virus (SARS-CoV-2) specific reverse transcription real-time PCR (RT-qPCR) to detect SARS-CoV-2 RNA in swab and saliva specimens. This assay is intended for quantitative detection of RNA from SARS-CoV-2 virus.

This validation protocol is intended to provide a record of (b) (4)

an in-house developed SARS-CoV-2 assay in human swab and saliva specimens.

The amendment to this protocol will show (b) (4)

## B. Scope

This validation protocol includes (b) (4)

and acceptance criteria for each of these approaches, for the SARS-CoV-2 real-time RT-qPCR assay. [Table 1a](#) is a summary of the original protocol including acceptance criteria; [Table 1c](#) is a summary of the original stability testing including stability criteria.

This amendment to the protocol adds an equivalency study for the swab type used due to issues with availability for the swab originally specified by this protocol. The (b) (4) swab type originally validated (b) (4) is no longer available, so a different (b) (4) swab type (b) (4) will be used ([Table 1b](#)).

The amendment also adds a repeat of the (b) (4) study due to questions surrounding the original (b) (4) study results. This study will be performed only if the equivalency testing of the new swab meets acceptance criteria. The previous (b) (4) study showed apparently instability (b) (4) (b) (4), see Document ID 21120.9249 *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 Swab and Saliva Specimens*. It is possible that this was due to an anomalously high (b) (4) value. The study will be repeated in this amendment using the swab that is in use in the clinical study ([Table 1d](#)).

### C. Abbreviations and Definitions

-80°C	-64°C to -90°C, standard storage condition unless otherwise stated
-20°C	-15°C to -35°C
Refrigerated (4°C)	2°C to 8°C
Ambient	15°C to 25°C
37°C	36°C to 38°C
CPS/mL	Copies per milliliter
LOD	Limit of Detection
LLOQ	Lower Limit of Quantification
LDR	Linearity and Dynamic Range
mL	milliliter
NEC	Negative Extraction Control
NTC	No Template Control
qPCR	Quantitative Real-Time PCR
SD	Standard Deviation
SOP	Standard Operating Procedure
TAE	Total Analytical Error
UIC	Universal Internal Control
μL	microliter
ULOQ	Upper Limit of Quantification

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Performance Characteristic	Purpose	Action	Acceptance Criteria
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Table 1a. Summary of validation criteria for SARS-COV-2 RT-qPCR assay in swab and saliva specimens			
Performance Characteristic	Purpose	Action	Acceptance Criteria
(b) (4)			

Table 1b. Summary of equivalency criteria for SARS-COV-2 RT-qPCR assay in swab specimens			
Performance Characteristic	Purpose	Action	Acceptance Criteria
(b) (4)			







## D. Materials

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

1. KingFisher™ Flex Instrument, Applied BioSystems (b) (4)
2. MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit, Applied BioSystems #A42352/A48310
3. MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit, Applied BioSystems (b) (4)
4. (b) (4)
5. (b) (4)
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12. (b) (4)
13. (b) (4)
14. (b) (4)
15. RNase-, DNase-free water, (b) (4)
16. (b) (4)
17. Pipette tips with aerosol barrier: 10µL, 200µL, and 1000µL sizes
18. Pipettes to accommodate tip sizes listed above
19. (b) (4) statistical software (v. 2.7.8 (3/15/2010))
20. (b) (4) statistical software (b) (4)

## E. Methods

### General Methods

#### Nucleic acid extraction

For the evaluation of (b) (4)

MagMax kit will be used (cat #A42352/A48310). Details are recorded further below in this methods section (see *Evaluation of* (b) (4))

For the assessments of (b) (4)  
(b) (4) MagMax kit will be used (b) (4) Details are recorded further below in this methods section (see *Assessments of* (b) (4))

(b) (4)

Nucleic acid extraction for swab and saliva specimens will be performed following instructions in SOP 21120.9152 *KingFisher MagMax Viral Pathogen Nucleic Acid Isolation*.

Samples processed on the ThermoFisher KingFisher FLEX

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(b) (4)

## Nucleic acid amplification and detection

Nucleic acid amplification will be 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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Acceptance criteria for controls and negative samples:

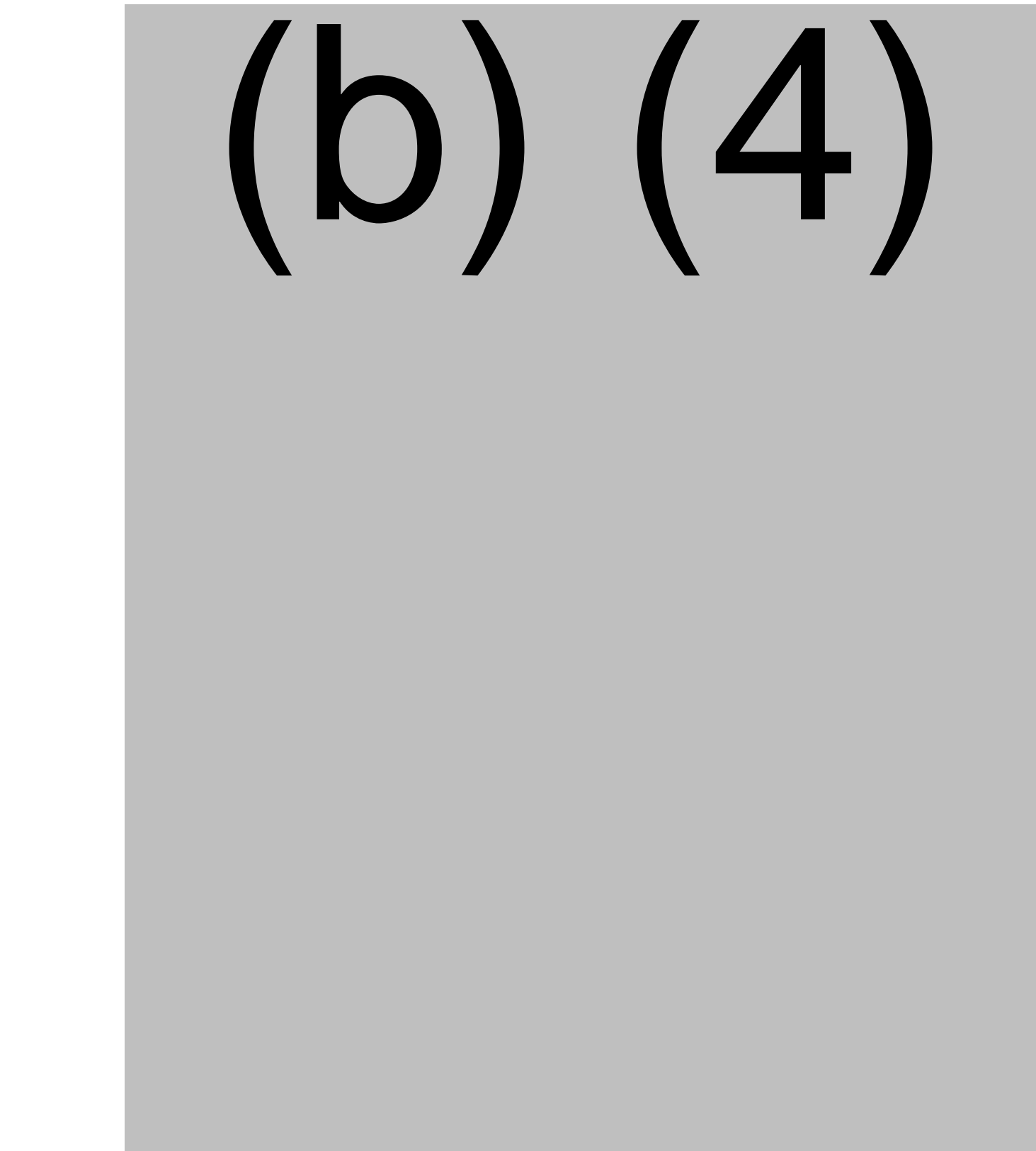
(b) (4)

## Performance Characteristics Evaluation

### Methods

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Acceptance criteria:

(b) (4)

(b) (4)

Acceptance criteria:

(b) (4)





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(b) (4)



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(b) (4)

## F. Analysis

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(b) (4)

## G. 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.

Footnotes will be included in the validation report with the run packet numbers from which the data originated, and a table will be included showing the fate of runs that includes the run number, experimental objective, outcome of the run, and any repeat analysis runs conducted in support of this protocol.

## H. Exceptions

Any deviations or exceptions to this protocol will be documented both in our NCE program and 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's technical team.

## I. References

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

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