21120.8918 SARS-CoV-2 (COVID-19) RT-qPCR Validation Report

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Periodic review not required

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Organization ViraCor

Comments for version 3.0
Added Suitability for Clinical Use statement.

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	(b) (6)	21-Jul-2020 20:11	3.0	(b) (6) (b) (6)	
Approval	(b) (6) Approver -	21-Jul-2020 18:43	3.0	(b) (6) (b) (6)	
Approval	(b) (6)	27-Apr-2020 16:45	2.0	(b) (6) (b) (6)	
Approval	(b) (6) Approver -	24-Apr-2020 12:32	2.0	(b) (6)	

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Approval	(b) (6)	12-Mar-2020 20:52	1.0	(b) (6)	
					(b) (6)
Approval	(b) (6)	Approver - 12-Mar-2020 20:47	1.0	(b) (6)	
					(b) (6)

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
3.0	Approved and Current	Major revision	21-Jul-2020	21-Jul-2020	Indefinite
2.2	Retired	Minor revision	17-Jul-2020	21-Jul-2020	21-Jul-2020
2.1	Retired	Minor revision	24-Jun-2020	24-Jun-2020	21-Jul-2020
2.0	Retired	Major revision	24-Apr-2020	27-Apr-2020	24-Jun-2020
1.0	Retired	Initial version	12-Mar-2020	12-Mar-2020	27-Apr-2020

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 and Serum 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 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 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 report was intended to provide documented evidence of (b) (4) (b) (4) (b) (4) of an in-house developed SARS-CoV-2 assay in human upper respiratory (nasal/nasopharyngeal wash and swab) and bronchoalveolar lavage (BAL).

B. Scope

This validation report includes the (b) (4) (b) (4) (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, 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	Acceptance Criteria	Validation Results	Pass/Fail
(b) (4)			Pass
			Pass
			Pass
			Pass
			Pass
			Pass

(b) (4)	Pass (with additional work required)

C. Materials

The following materials (or suitable equivalents) were used:

1. (b) (4)
2. (b) (4)
3. (b) (4)
4. (b) (4)
5. (b) (4)
6. (b) (4)
7. (b) (4)
8. (b) (4)
9. (b) (4)
10. (b) (4)
11. RNase-, DNase-free water, (b) (4)
12. (b) (4)
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)

D. Methods

Sample preparation

All samples were prepared by (b) (4)

(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)

(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.

(b) (4)

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

(b) (4)

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Performance Characteristics Evaluation

(b) (4)

- **Acceptance criteria**

(b) (4)

Clinical evaluation

(b) (4)

- **Acceptance criteria**

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(b) (4)

- **Acceptance criteria**

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- **Acceptance criteria**

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- **Acceptance criteria**

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- **Acceptance criteria**

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- **Acceptance criteria**

(b) (4)

Table 2. (b) (4)

(b) (4)

E. Analysis

(b) (4)

(b) (4)

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(b) (4)

F. Results and Conclusions

Results are summarized graphically and/or in tables.

(b) (4) 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.

Controls and negative samples

(b) (4)

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Table 3. (b) (4)

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Table 4. (b) (4)

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Table 5. (b) (4)

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Table 6. (b) (4)

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Table 7. (b) (4)

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Table 8. (b) (4)

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Table 9. (b) (4)

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Table 10. (b) (4)

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Table 11. (b) (4)

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Table 12. (b) (4)

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Table 13. (b) (4)

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Table 14. (b) (4)

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Table 15. (b) (4)

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Table 16. (b) (4)

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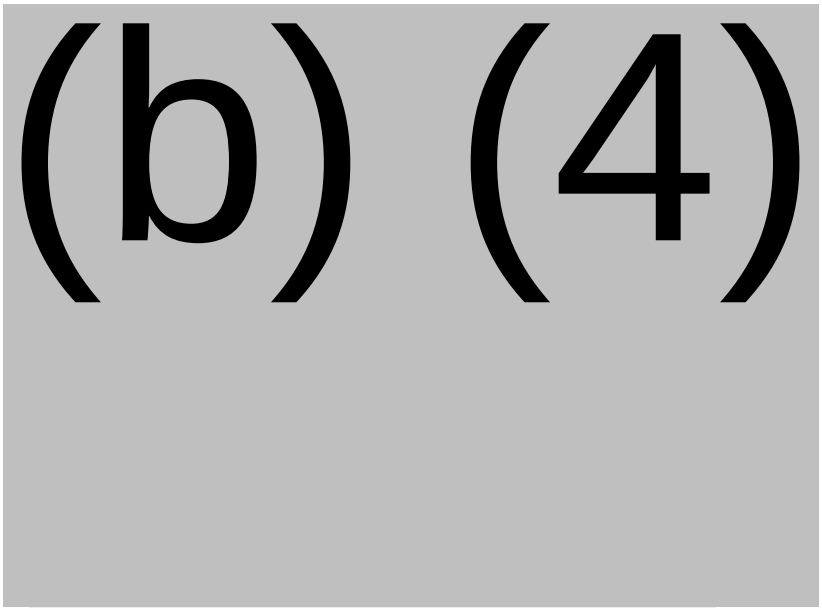
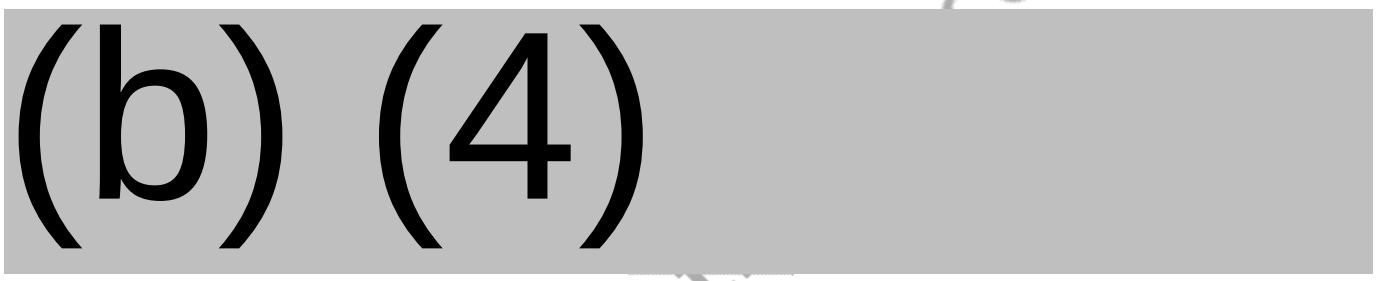
Figure 1. (b) (4)





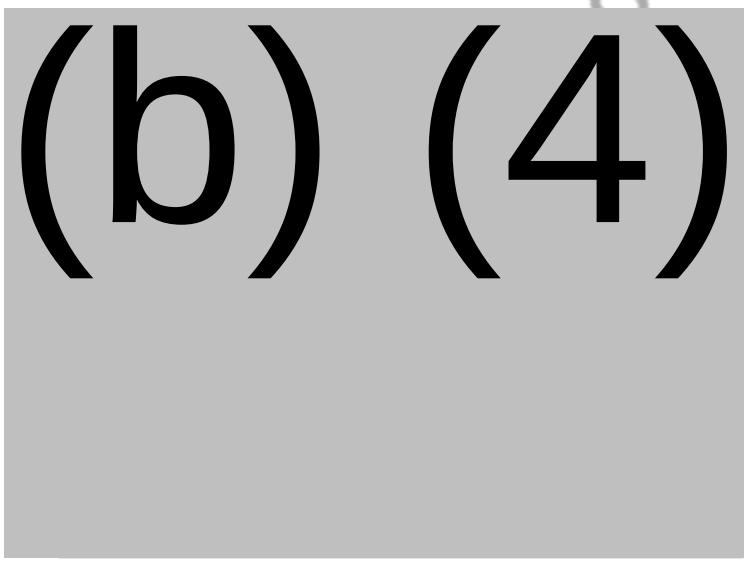


Figure 2. (b) (4)

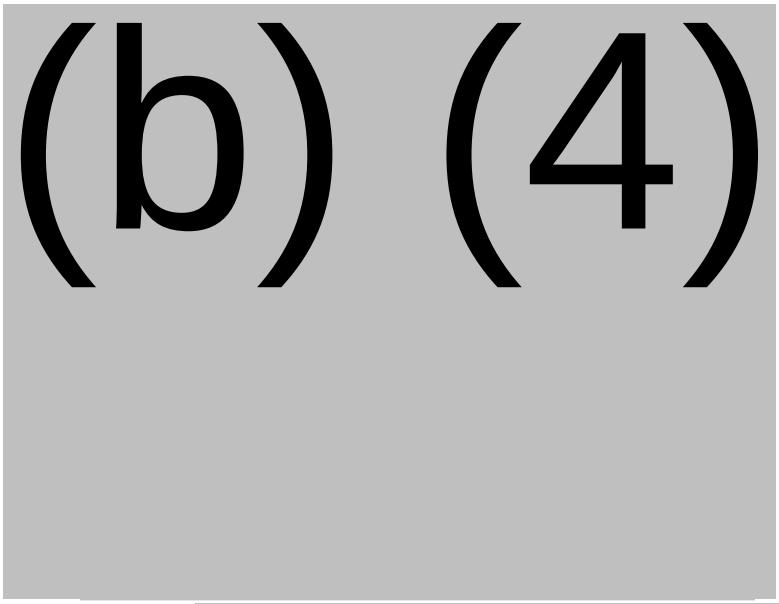


Figure 3. (b) (4)

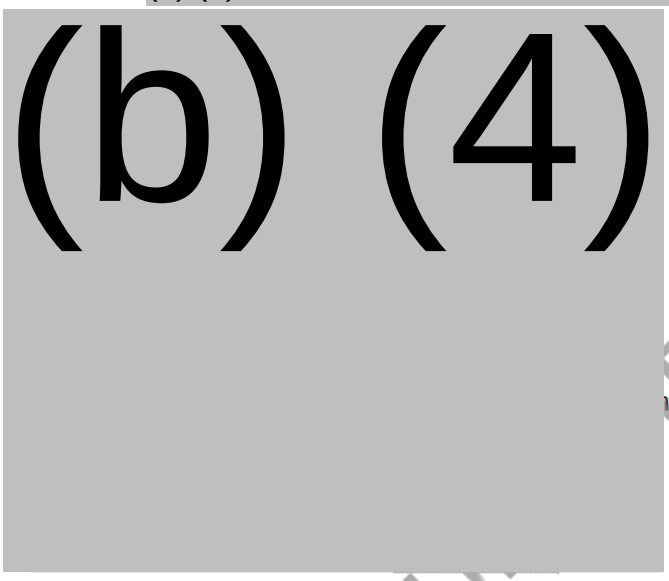


Figure 4. (b) (4)

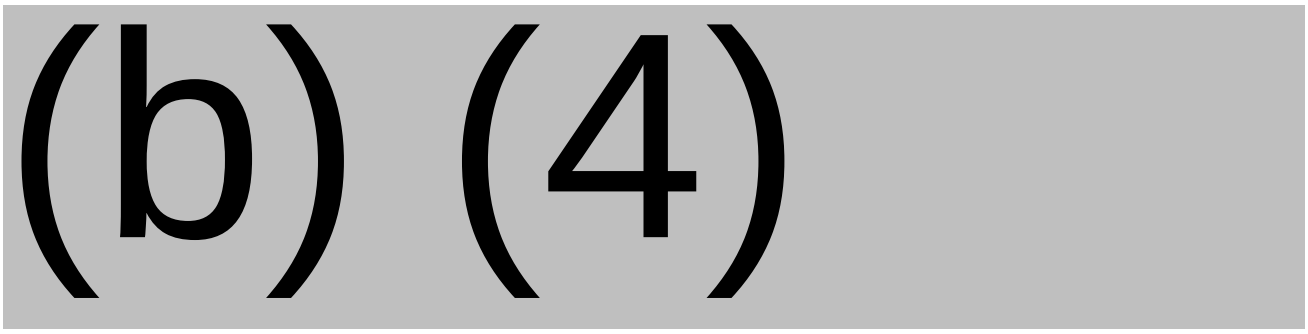


Table 17. (b) (4)

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Table 18. (b) (4)

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Table 18. (b) (4) (*continued*)

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Table 19. (b) (4)

(b) (4)

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. 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 technical team.

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 21120.8890 *Validation Protocol 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 and Serum Specimens.* I have reviewed this validation data and conclude that it meets the acceptance criteria for this protocol and the performance of this method is acceptable for patient testing of human upper and lower respiratory specimens for the SARS CoV-2 RT-qPCR.

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.

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(b) (4)

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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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

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

(b) (4)

Submission guidelines for nucleic acid amplification tests for infectious agents, State of New York Department of Health. February, 2011.

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J. Amendment - Performance Characteristics Evaluation

(b) (4)

Table 20. (b) (4)

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- **Acceptance criteria**

(b) (4)

K. Results and Conclusions

(b) (4)

Figure 5.

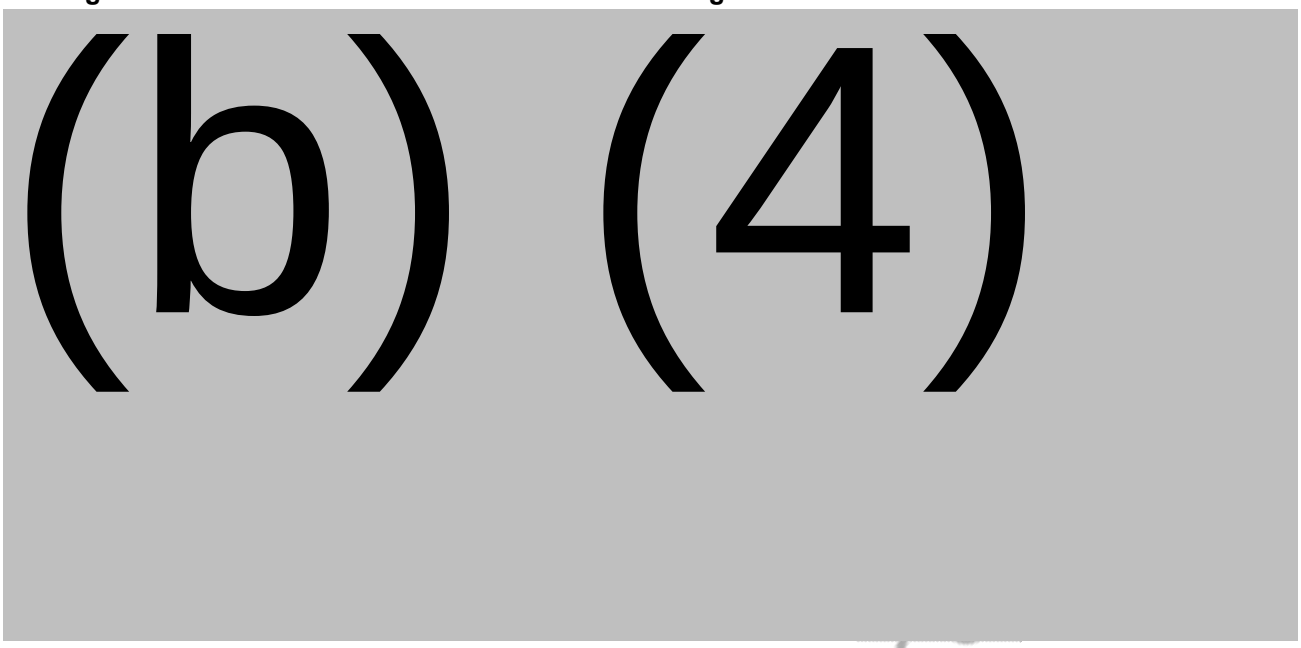

Figure 6.

Figure 7.

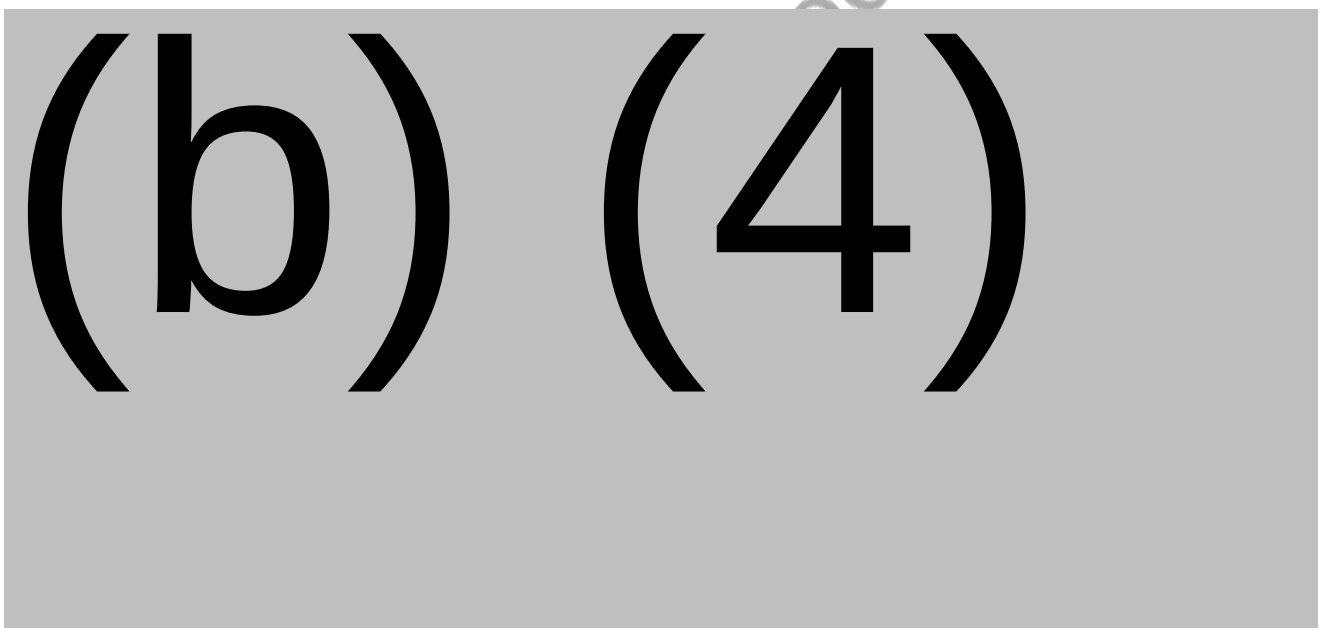

Figure 8.

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Figure 9.

Figure 10.

Figure 11.

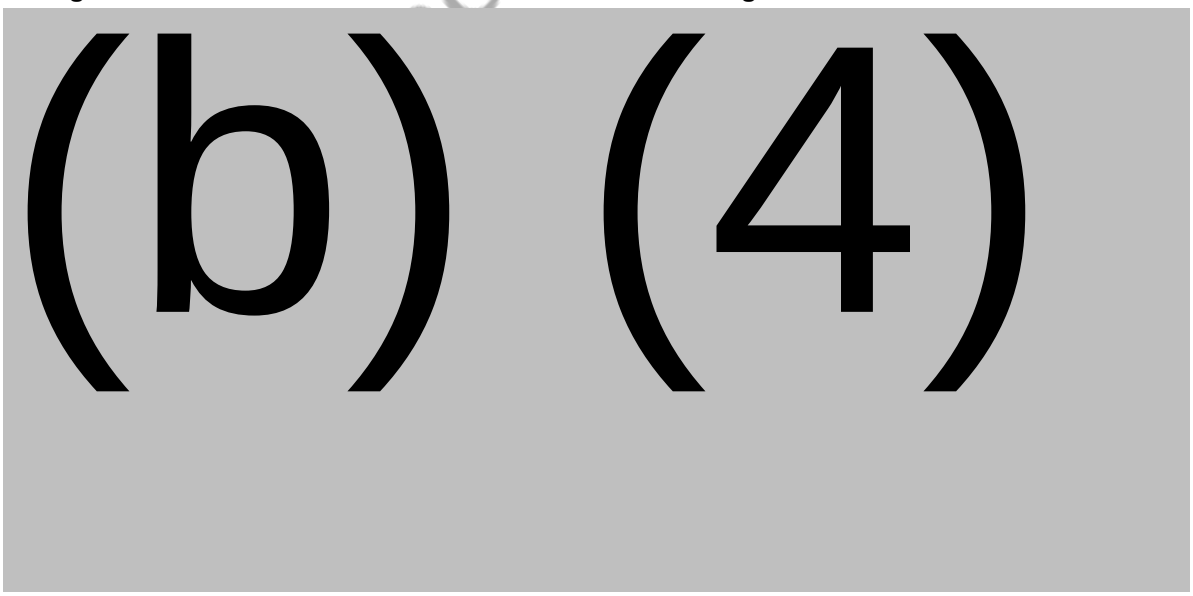

Figure 12.

(b) (4)

Figure 13.

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L. Exceptions

Any deviations or exceptions to this Amendment was documented on the appropriate laboratory records and data packets. Instances in which the pre-specified acceptance criteria are not met were identified and evaluated. Validation approval/rejection was not exclusively determined based on pass/fail outcome. Rather, criteria failures were 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 technical team.

Due to safety concerns when receiving high titer SARS CoV-2 specimens a change in processing was implemented to (b) (4)

(b) (4)

Table 21. (b) (4)

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- **Acceptance criteria**

(b) (4)

M. Results and Conclusions

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Figure 14.

(b) (4)

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