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Method Validation Report Amendment #1 for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay in 293T/ACE2 Cells

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By signing the "Approved By" section below, the person attests that he/she has personally conducted a review of the document for completeness and accuracy and approves the contents of the Method Validation Report.

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Document Revision History:

Version	Replaces	Effective Date	Description of Change
Version 1.1	Version 1.0	See Page 1	11.2 New (b) (4) reagent 11.3 New (b) (4) reagent 12.0 Revised pre-set acceptance criteria 13.0 Repeat validation experiments 14.0 Repeat validation results 15.0 Revised statistical analysis 16.0 Revised conclusions 17.0 Revised list of raw data files

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1.0 Objective

This document provides an amendment to the Method Validation Report for SARS-CoV-2 Spike-Pseudotyped Virus Neutralizaton Assays in 293T/ACE2 Cells (Duke-02-MVR-COVID0001). The purpose of this amendment is to describe results of validation experiments that were repeated because of a failure to meet pre-defined acceptance criteria for precision, and because some of the earlier experiments were potentially(b) (4)

(b) (4)

2.0 Scope

This amendment applies to the conduct of the experiments for precision in support of the "SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay in 293T/ACE2 Cells" by the "Neutralizing Antibody Core" Laboratory, under the GCLP oversight of the Quality Assurance for Duke Vaccine Immunogenicity Programs (QADVIP). This amendment also applies to the upper and lower limits of quantitation of ID50 and ID80 neutralization titers, specificity, linearity, and inference of accuracy.

3.0 Introduction

SARS-CoV-2, the etiologic agent of COVID-19 (Ref 18.1-18.4), is one of three related beta-coronaviruses that have caused highly pathogenic epidemics in humans; the others are Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV), all of which are believed to have originated in bats and transmitted to humans through intermediate hosts (Ref 18.5-18.9). SARS-CoV-2 infects cells through binding of its surface Spike protein to the angiotensin-converting enzyme 2 (ACE2) receptor on susceptible cells. Antibodies to Spike protein can neutralize the virus by blocking entry. Candidate COVID-19 vaccines incorporate the viral Spike protein with the goal of eliciting protective neutralizing antibodies. It is therefore critical to monitor the SARS-CoV-2 neutralizing antibody response in clinical trials. Assays are needed that are high throughput and rigorously optimized and validated to facilitate vaccine licensure and implementation after completion of phase 3 clinical trials.

(b) (4) in the intra-assay, inter-operator and inter-day precision was observed in the initial validation experiment (Experiment #3, Duke-02-MVR-COVID0001) ((b) (4) CV acceptance criteria for (b) (4). QA investigation indicated that (b) (4) was in part the cause of the (b) (4)

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(b) (4) however, it was determined this was not the sole source of the (b) (4) observed. No other root cause could be identified at a technical level. Statistical modeling of the precision results reported in the Duke-02-MVR-COVID0001 indicate that the acceptance criteria initially established for precision (b) (4) for this assay. New acceptance criteria were established as described in the amended Validation Plan/Protocol Duke-02-MVPPR-COVID0001.1. The acceptance criteria were applied to a repeat of Experiments #3 and #4 that were conducted under Duke-02-MVPPR-COVID0001 and reported in the Validation Report (Duke-02-MVR-COVID0001) here. In addition, during the conduct of validation experiments it was discovered that COVID-19 convalescent serum samples from (b) (4)
(b) (4)

(b) (4)

4.0 Description of Method

This validation report amendment follows the test plans outlined in Duke-02-MVPPR-COVID0001 "Method Validation Plan (Protocol) for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay in 293T/ACE2 Cells: Precision, LOQ and Specificity Verification." All neutralization assays were conducted in accordance with SOP CFAR02-A0026 "Measuring Neutralizing Antibodies Against SARS-CoV-2 Using Pseudotyped Virus and 293T-ACE2 Cells."

5.0 Definitions

5.1 See Duke-02-MVR-COVID001 "Method Validation Report for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay in 293T/ACE2 Cells" for definitions.

6.0 Facility Name and Address

6.1 Neutralizing Antibody Core Laboratory
SORF Building
915 S. LaSalle St
Duke University Medical Center
Durham, NC 27710lls.

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7.0 Personnel

David C. Montefiori, Ph.D. (Principal Investigator)
Charlene McDanal (Senior Lab Research Analyst)
Elizabeth Domin (Lab Research Analyst I)
Jin Tong (Lab Research Analyst II)
Yunda Huang (Statistician)

8.0 Method Validation Dates

Start Date: 16/Oct/2020
End Date: 27/Oct/2020

9.0 Reagents and Materials

9.1 See Duke-02-MVR-COVID001 “Method Validation Report for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay in 293T/ACE2 Cells.” All neutralization assays were conducted in accordance with SOP CFAR02-A0026 “Measuring Neutralizing Antibodies Against SARS-CoV-2 Using Pseudotyped Virus and 293T-ACE2 Cells.”

10.0 Equipment

10.1 See Duke-02-MVR-COVID001 “Method Validation Report for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay in 293T/ACE2 Cells.”All neutralization assays were conducted in accordance with SOP CFAR02-A0026 “Measuring Neutralizing Antibodies Against SARS-CoV-2 Using Pseudotyped Virus and 293T-ACE2 Cells.”

11.0 Test and Control Articles

11.1 COVID-19 convalescent serum samples. Obtained from HIV-1 uninfected subjects enrolled HVTN 405/HPTN 1901 “Characterizing SARS-CoV-2-specific immunity in convalescent individuals”. This is an ongoing clinical trial conducted jointly by the HIV Vaccine Trials Network and the HIV Prevention Trial Network, both funded by the US National Institutes of Health. The trial is designed in part to provide critical serum samples needed to formally qualify and validate a suite of immunologic assays and reference reagents in preparation for phase 3 trials of COVID-19 vaccines. Serum is being collected from males and females who are 18-55 years of age and >55 years of age and who experienced a spectrum of COVID-19 disease severities, from asymptomatic to requiring advanced medical care in the intensive care unit (ICU). The protocol is expected to enroll >800 participants total. The specific samples selected from this protocol for the experiments described in this

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report had not been used previously for method qualifcation or validation. The laboratory was blinded to the clinical status of the donors but not to HIV-1 infection status. Only samples from HIV-1 uninfected individuals were used in this amended protocol. Upon receipt of samples in this laboratory, the samples were heat-inactivated at 56°C for 30 minutes according to CFAR02-A0026 (SOP for method) and stored at 4°C for up to (b) (4) prior to assay.

11.2 (b) (4) . A SARS-CoV-2 positive control consisting of human monoclonal antibody DH1043 (Ab026116-LS) (b) (4) (b) (4) DH1043 is a potent SARS-CoV-2 neutralizing antibody that was isolated from PBMC of a COVID-19 convalescent individual by Dr. Barton Haynes in the Duke Human Vaccine Institute, Duke University Medical Center. Antibody IgH and IgK/L genes were recovered from single-cell sorted cells and cloned into human IgG1 constant region backbone. The antibody is IgG isotype, RBD-specific and ACE2-blocking (see supplemental materials). It was produced and QC'd by the Duke Protein Production Facility. (b) (4)

(b) (4)

(b) (4) stock concentration of DH1043. (b) (4) was prepared immediately before use and stored at 4°C during the duration of validation experiments.

11.3 (b) (4) (b) (4) COVID-19 convalescent serum samples from HIV-1 uninfected subjects enrolled in HVTN 405/HPTN 1901. The (b) (4) samples were selected as having (b) (4) neutralization titers against SARS-CoV-2 Spike-pseudotyped virus in the assay.

12.0 Revised Validation Parameters and Pre-set Acceptance Criteria (from Duke-02-MVPPR-COVID001.1-INV001)

Table 1. Revised validation parameters and pre-set acceptance criteria

Parameter	Acceptance Criteria
Accuracy	(b) (4)
Precision	

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Specificity	(b) (4)
Limit of Detection	
Limit of Quantitation	
Linearity	
Range	

(b) (4)

13.0 Repeat Validation Experiments

13.1 Experiment 3R - Intra-assay, inter-operator, inter-day variability (repeatability and intermediate precision), LLOQ and ULOQ

(b) (4)

13.2 Experiment 4R1- Dilutional Linearity (b) (4)

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(b) (4)

13.3 Experiment 4R2 - Dilutional Linearity (b) (4)

(b) (4)

13.4 Experiment 5R – (b) (4)

Duke-02-MVPPR-COVID0001 proposed to repeat the validaton experiment for (b) (4)

(b) (4)

(b) (4) It was deemed that repeating this expriment was no longer warranted (see sections 11.2 and 15.8.1 in Duke-2-MVR-COVID0001).

14.0 Repeat Validation Results

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14.1 Precision

Both the intermediate precision and the intra-assay variability of the ID50 titer readout of the assay passed the preset precision acceptance criteria of (b) (4)

(b) (4)

The intermediate precision and the intra-assay variability of the ID80 titer readout of the assay also passed the preset acceptance criteria of (b) (4)

(b) (4)

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Table 1a: Summary of ID50 titers and percent coefficient of variation (%CV) for the estimation of intermediate precision (inter-operator and inter-day) and repeatability (intra-assay) of the assay.

(b) (4)

(b) (4)

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Table 1b: Summary of ID80 titers and percent coefficient of variation (%CV) for the estimation of intermediate precision (inter-operator and inter-day) and repeatability (intra-assay) of the assay.

(b) (4)

(b) (4)

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Table 2a: Summary of ID50 titers and percent coefficient of variation (%CV) for the estimation of intermediate precision (inter-operator) and repeatability (intra-assay) of the assay.

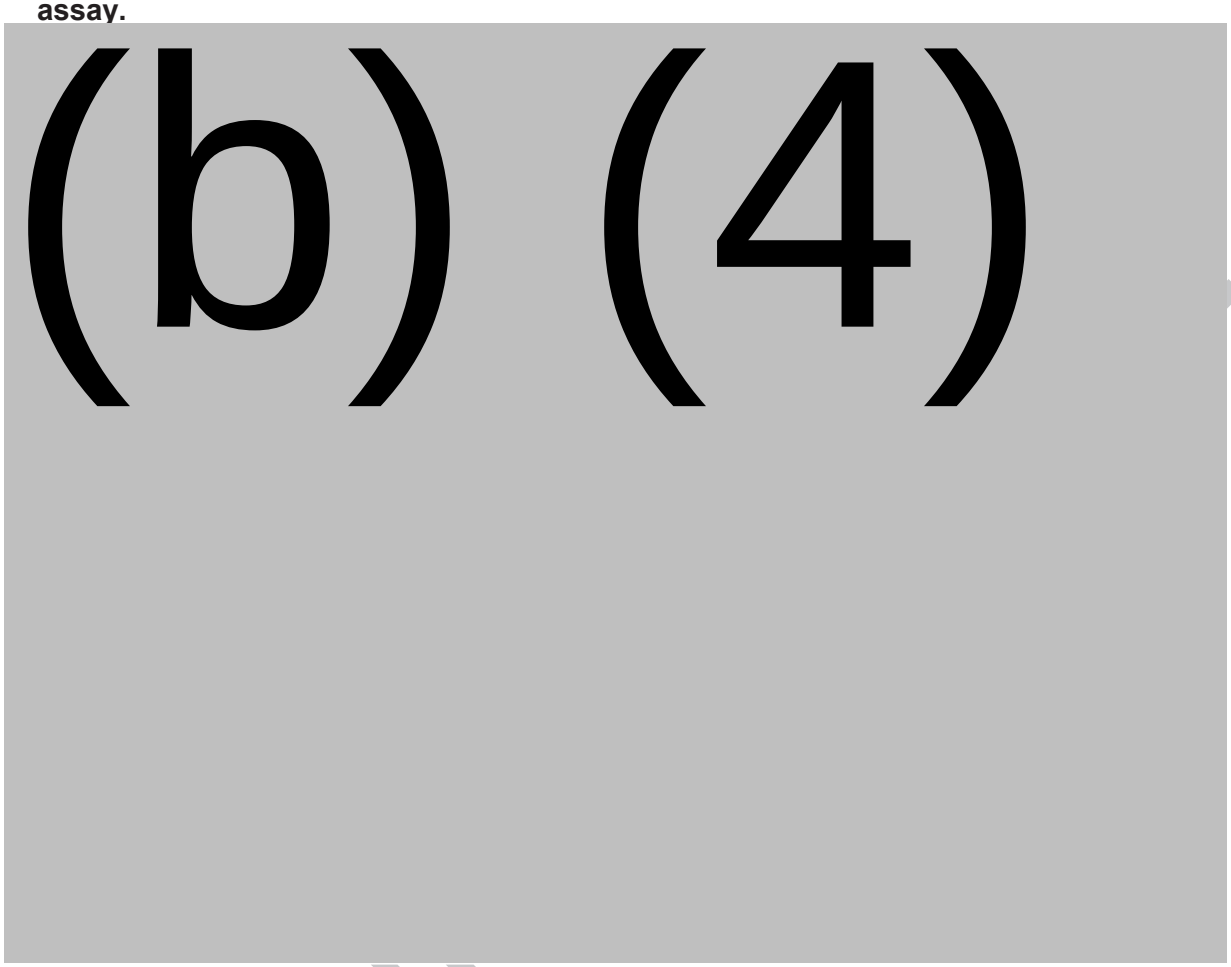

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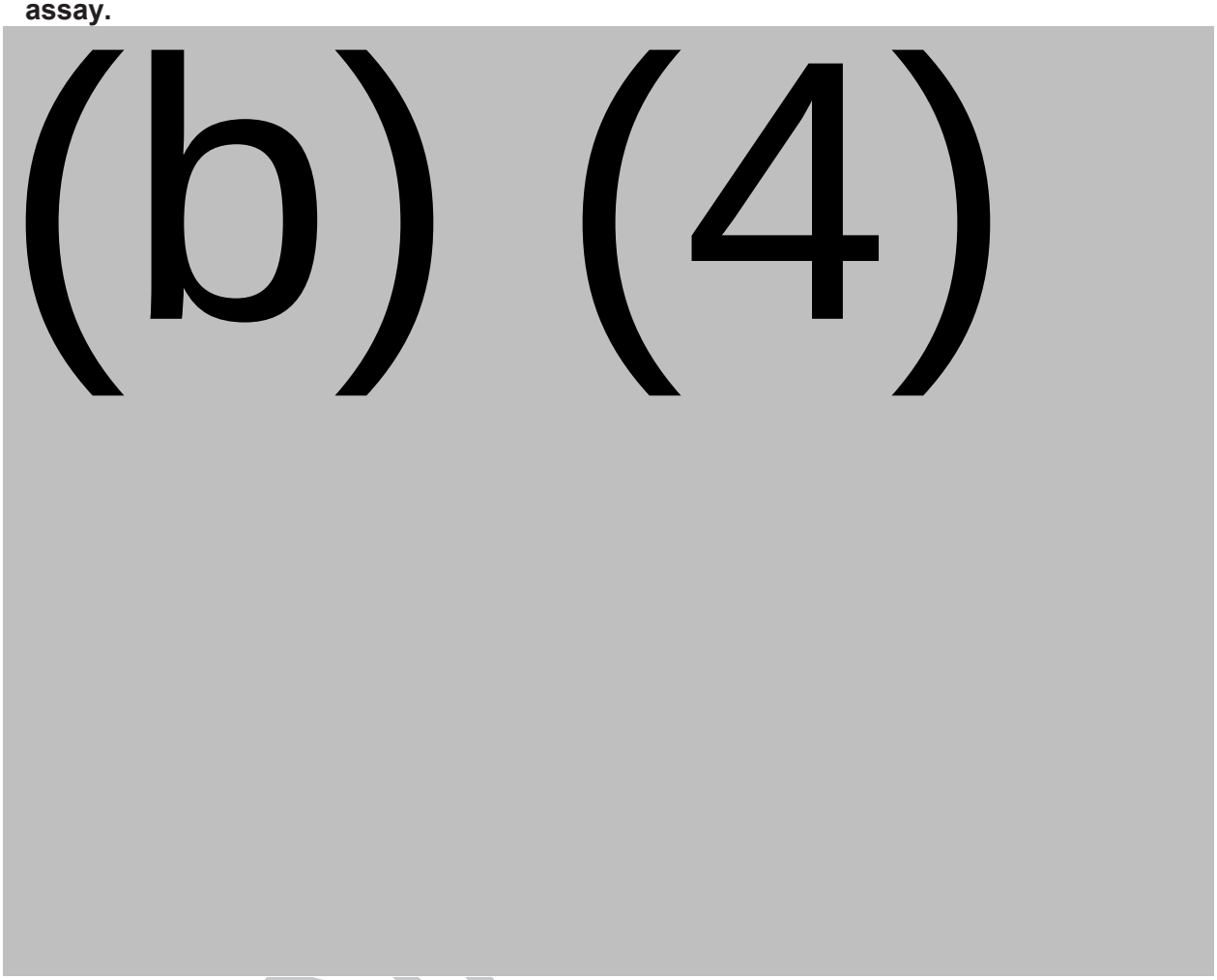
Table 2b: Summary of ID80 titers and percent coefficient of variation (%CV) for the estimation of intermediate precision (inter-operator) and repeatability (intra-assay) of the assay.



14.2 Limits of Quantitation

The ID50 and ID80 values of samples (b) (4) **(Fig 1)** and (b) (4) **(Fig 2)** were the best approximators of the lower and upper limits of quantitation, having the lowest and highest titers, respectively, among the samples tested. The LLOQ defined by sample (b) (4) was (b) (4) for ID50, and (b) (4) for ID80. The ULOQ defined by sample (b) (4) was (b) (4) for ID50, and (b) (4) for ID80. These LLOQ and ULOQ are within the pre-set acceptance criteria for precision and specificity **(see Table 2)** and afford a (b) (4) range of quantifiable titers for assessments of vaccine immunogenicity and immunologic correlates analyses. Results of the dilutional linearity experiment shown in **Table 3 and Figure 2** indicate LLOQ titers of (b) (4) and (b) (4) for ID50 and ID80, respectively, and ULOQ titers of (b) (4) and (b) (4) for ID50 and ID80, respectively. These values fall within the acceptance criteria for precision (section 14.1) and dilutional linearity (section 4.3) and are specific (Duke-02-MVR-COVID001, section 15.3).

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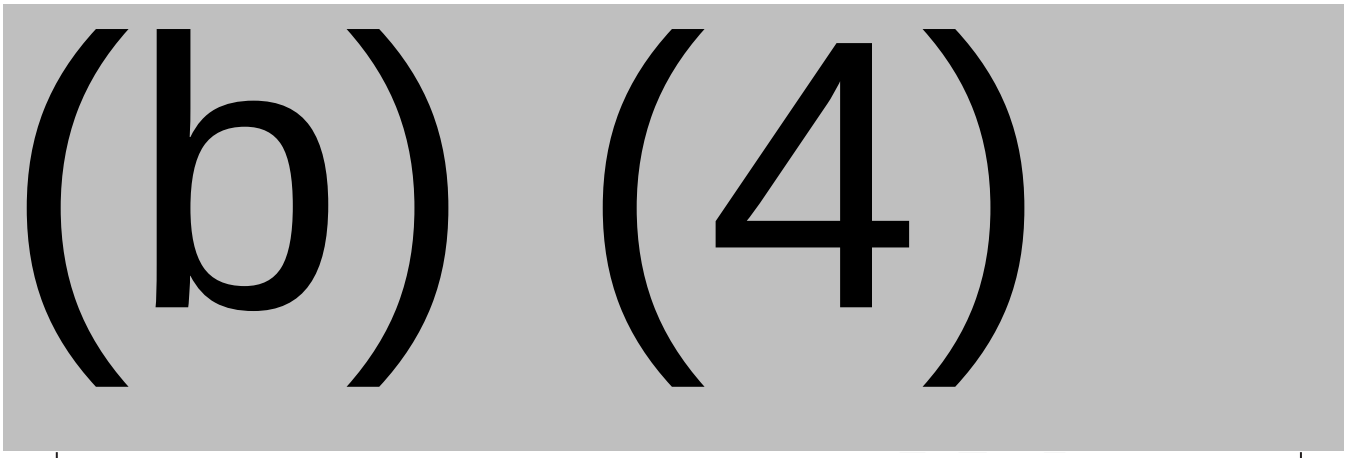
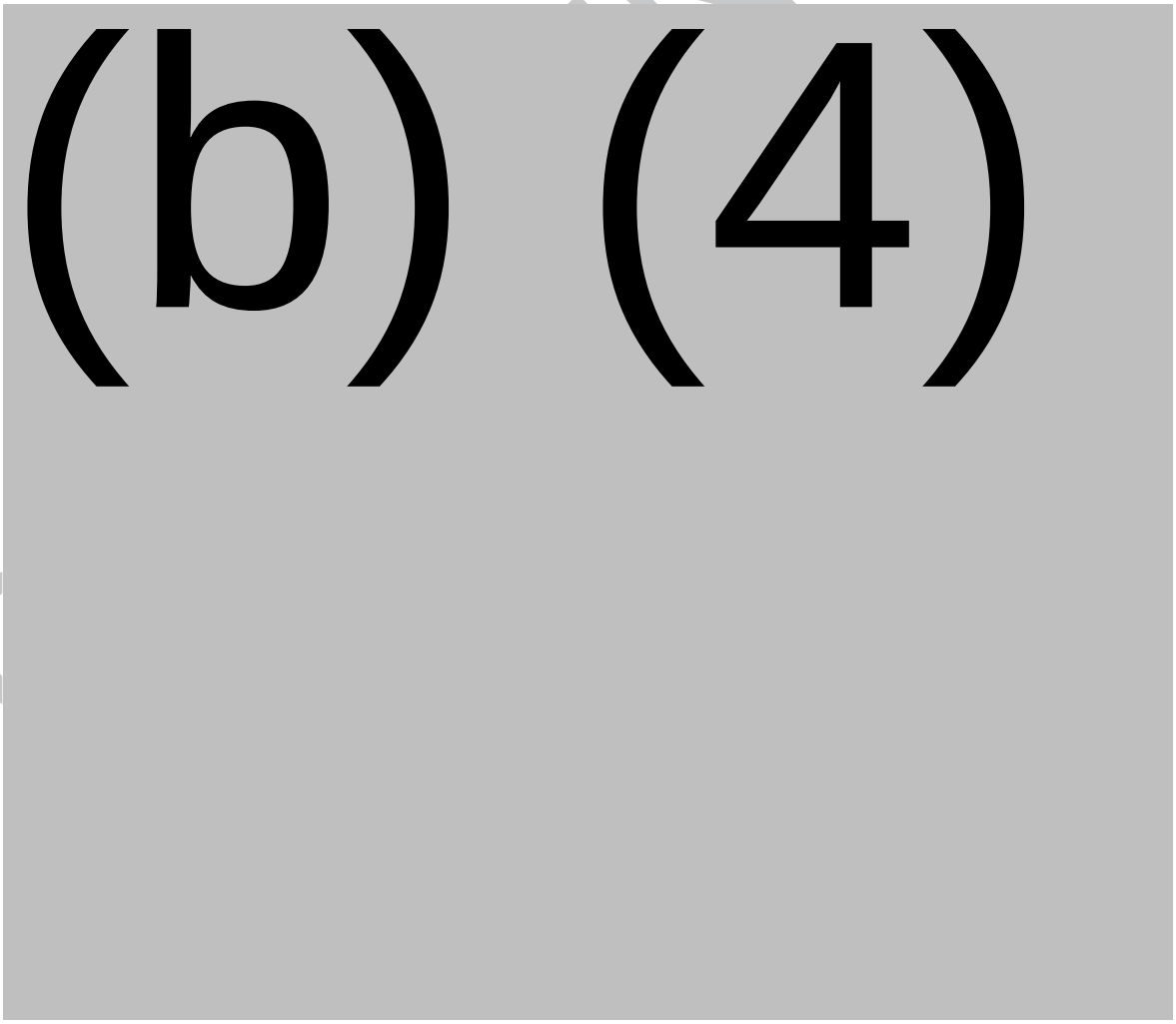


Fig 1. ID50 and ID80 titers of COVID-19 convalescent serum samples with (b) (4) (b) (4) Samples are order left to right by descending mean titer. Bars are mean ± SD.

14.3 Linearity (dilutional)



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(b) (4)

Table 3. Summary of ID50 and ID80 titers and percent coefficient of variation (%CV) for the estimation of dilutional linearity of the assay (second experiment, (b) (4)).

(b) (4)

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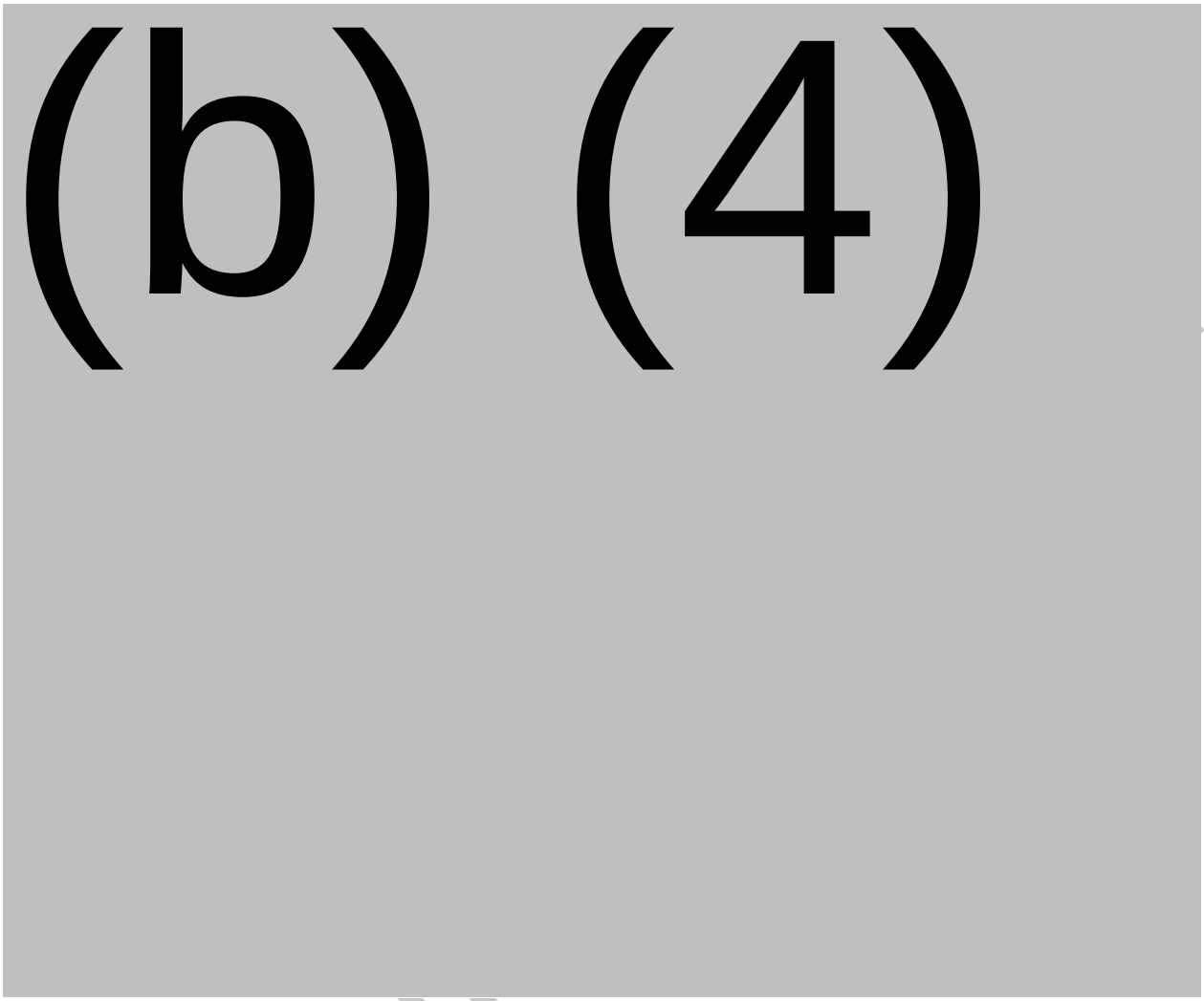
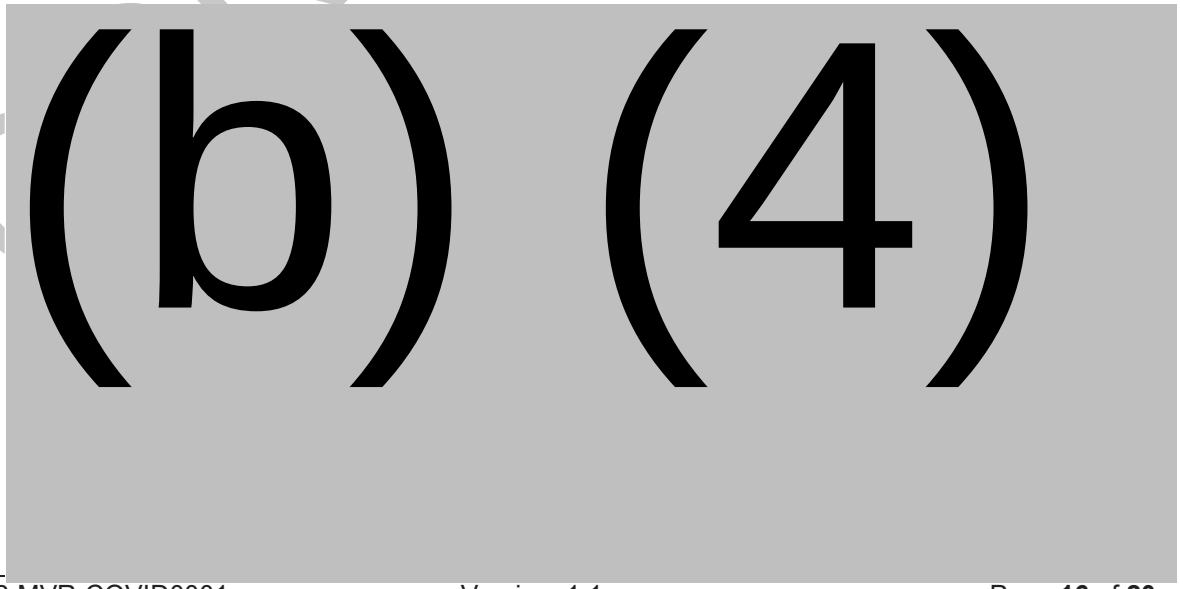


15.0 Statistical Analysis of Results



(b) (4)

(b) (4) software was used to plot (b) (4) (b) (4) and to calculate (b) (4) This software also was used to calculate (b) (4) values for the second dilututional linearity experiment (b) (4).

16.0 Conclusions

Table 4 lists the findings of the validation experiments described in the Validation Report (Duke-02-MVR-COVID0001) that failed to pass the pre-set acceptance criteria. Previous findings showed that specificity, limits of detection, linearity, range and (b) (4) all passed the pre-set acceptance criteria. This Amendment was a re-evaluation of accuracy, precision and limits of quantitation, where precision was the major failing that affected accuracy, ULOQ and LLOQ. The repeat of experiments to assess precision was justified based on the root cause analysis and statistical assessment of the results of the first Experiment #3. This Amendments is also an evaluation of ID80. All pre-set acceptance criteria for accuracy, precision, and limits of quantitation were met in these experiments. Notably, as was seen during qualification and earlier validation experiments, ID80 continued to exhibit (b) (4) than ID50 and may be a (b) (4) for assessments of vaccine immunogenicity and immunologic correlates analyses.

Table 4. Validation parameters and pre-set acceptance criteria (Modified)

Parameter	Acceptance Criteria	Previous Findings	New findings	Revised Outcome
Accuracy	(b) (4)			PASS
Precision				PASS

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	(b) (4)			
Limit of Quantitation	(b) (4)			PASS

17.0 Plan for Storage and Archival of Validation Data

All electronic validation data are stored as electronic files on a secure server at Duke University Medical Center. File identifiers and contents are described in a Table that makes reference to the specific assay parameter addressed by the data. Paper records will be archived by QADVIP following SOP QADVIP-M008.

Table 5. Raw data files.

File Identifier	Experiment #	Figure or Table	Assay Parameter(s)
(b) (4)			

18.0 References

18.1 Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 395(10223):497-506. doi: 10.1016/S0140-6736(20)30183-5.

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18.2 Zhou P, Yang XL, Wang XG, Hu B., Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, et al. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 579(7798):270-273. doi: 10.1038/s41586-020-2012-7.

18.3 Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R., et al. (2020). A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 382, 727–733.

18.4 Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. (2020) Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med. (2020) 382(13):1199-1207. doi: 10.1056/NEJMoa2001316.

18.5 de Wit, E., van Doremalen, N., Falzarano, D., and Munster, V.J. (2016). SARS and MERS: recent insights into emerging coronaviruses. Nat. Rev. Microbiol. 14, 523–534.

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18.7 Cui J, Li F, Shi Z-L. (2019). Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol, 17:181-192.

18.8 Lu R. et al. (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 395, 565-574, doi:10.1016/S0140-6736(20)30251-8.

18.9 Wu A. et al. (2020) Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in shina. Cell Host Microbe, S1931-3128(1920)30072-X, doi:10.1016/j.chom.2020.02.001

18.10 Method Validation Plan for the SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay in 293T/ACE2 Cells (Duke-02-MVP-COVID001 version 1.0)

18.11 Method Validation Protocol for the SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay in 293T/ACE2 Cells (Duke-02-MVPR-COVID001 version 1.0)

18.12 SOP CFAR02-A0026 “Measuring Neutralizing Antibodies Against SARS-CoV-2 Using Pseudotyped Virus and 293T/ACE2 Cells”

19.0 Attachments

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19.1 Not Applicable

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