Marcella Sarzotti-Kelsoe
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Method Validation Report for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay with Moderna Phase 1 Samples

Version No.:	1.0
Author(s):	David Montefiori Charlene McDanal
Effective Date:	**16/NOV/2020**

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

Signed:	Date:
See Electronic Signature in (b) (4)	
David C. Montefiori, Ph.D. *Professor*, Principal Investigator (PI) of the Duke Center for AIDS Research GCLP-compliant AIDS Program (CFAR-GAP) Neutralizing Antibody Assay Laboratory	
Signed:	Date:
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Marcella Sarzotti-Kelsoe, Ph.D *Director*, Quality Assurance for Duke Vaccine Immunogenicity Programs (QADVIP)	

Document Revision History:

Version	Replaces	Effective Date	Description of Change
Version 1.0	N/A	See Page 1	INITIAL VERSION

Table of Contents

1.0 Objective

This document describes the partial method validation of the SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay in 293T/ACE2 Cells for use with serum samples from phase 3 trial recipients of the Moderna mRNA-1273-P301 vaccine for SARS-CoV-2. Validation was performed with serum samples collected 2-4 weeks post 2nd inoculation from participants in a phase 1 trial of the identical vaccine, dose and schedule used in the phase 3 trial: "A

Phase 3, Randomized, Stratified, Observer-Blind, Placebo-Controlled Study to Evaluate the Efficacy, Safety, and Immunogenicity of mRNA-1273 SARS-CoV-2 Vaccine in Adults Aged 18 Years and Older" (IND: 19745).

2.0 Scope

This Method Validation report applies to the conduct of the experiments for partial validation with incurred vaccine sera 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 document applies to validation of the assay for precision, dilutional linearity and accuracy (including verification of the LLOQ and ULOQ).

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.

ModernaTX, Inc. (200 Technology Square Cambridge, MA 02139) developed a candidate vaccine for COVID-19 that is currently in a phase 3 clinical trial. The mRNA-1273 vaccine is a lipid nanoparticle (LNP) dispersion of an mRNA encoding the prefusion stabilized S protein of SARS-CoV-2 formulated in LNPs composed of 4 lipids (1 proprietary and 3 commercially available): the proprietary ionizable lipid SM-102; cholesterol; 1,2-distearoyl-sn-glycero-3 phosphocholine (DSPC); and 1 monomethoxypolyethyleneglycol-2,3-dimyristylglycerol with polyethylene glycol of average molecular weight 2000 (PEG2000-DMG). The mRNA-1273 is provided as a sterile liquid for injection and is a white to off- white dispersion in appearance, at a concentration of 0.2 mg/mL in 20 mM Tris buffer containing 87 mg/mL sucrose and 10.7 mM sodium acetate at pH 7.5. The placebo is 0.9% sodium chloride (normal saline) injection, which meets the criteria of the United States Pharmacopeia (USP). The vaccine is administered as an IM injection into the deltoid muscle on a 2-dose injection schedule on Day 1 and Day 29, with at least a 28-day

interval between doses. Each injection has a volume of 0.5 mL and contain mRNA-1273 100 µg, or saline placebo. Neutralizing antibodies are a secondary immunogenicity endpoint in the phase 3 trial.

4.0 Description of Method

Our strategy for quantifying SARS-CoV-2 neutralizing antibodies utilizes lentivirus particles expressing SARS-CoV-2 Spike protein on their surface and containing a firefly luciferase (Luc) reporter gene for quantitative measurements of infection by relative luminescence units (RLU). Infection takes place in transduced 293T cells expressing high levels of ACE2 (293T/ACE2 cells). Virus is applied to cells with or without pre-incubation with antibodies; neutralizing antibodies reduce infection, resulting in lower RLUs. Serial dilution of antibodies can be used to produce a dose-response curve. Neutralization is measured as the serum dilution at which RLU is reduced by 50% (ID50) and 80% (ID80) relative to mean RLU in virus control wells (cells + virus but no sample) after subtraction of mean RLU in cell control wells (cells only). Assay validation was performed with human serum samples and monoclonal antibodies using the D614G form of the Wuhan-1 Spike, which is the dominant form of Spike protein in the global epidemic. The assay was performed in 96-well flat-bottom clear Poly-L-Lysine treated culture plates for high throughput capacity. Luc signal (RLU) was measured in 96-well flat bottom black/white plates for enhanced luminescence with minimal bleed-over. Use of a clonal cell line provided enhanced precision and uniformity.

The assay was adopted and formally optimized and qualified using plasmids and an SOP obtained from Drs. Barney Graham, Kizzmekia Corbett, Nicole Doria-Rose, Adrian McDermott and John Mascola of the Vaccine Research Center, NIAID, NIH.

The assay requires 3 days:

- Day 0 dilute samples, incubate with pseudovirus, add cells
- Day 3 lyse cells, add luciferase reagents, read RLU in luminometer

All neutralization assays for this validation report were performed in accordance with SOP CFAR02-A0026 “Measuring Neutralizing Antibodies Against SARS-CoV-2 Using Pseudotyped Virus and 293T/ACE2 Cells”.

4.1 The following safety precautions were enforced in the conduct of these validation studies:

4.1.1 Use of universal safety precautions when handling samples. Treatment of all samples as potentially infectious.

4.1.2 Wearing appropriate personal protective equipment (PPE) including gloves, protective eye wear, laboratory coat, shoe covers and N95 mask.

4.1.3 Handling all materials according to "Standard Operating Procedure for Safe Handling of SARS-CoV-2-pseudotyped Virus, Whole Blood, Serum and Plasma from COVID-19 Patients, and Human Cell Lines at BSL2* Containment Laboratory."

4.1.4 Puromycin is toxic; avoiding exposure to eyes and skin.

5.0 Definitions

5.1 See Duke-02-MVPR-COVID0002 "Method Validation Protocol for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay wit Moderna Phase 1 Samples."

6.0 Facility Name and Address

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

7.0 Personnel

David C. Montefio , Ph.D. (Principal Investigator)
Charlene McDanal (Senior Lab R search Analyst)
Elizabeth D in (Lab Research Analyst I)
Jin Tong (Lab R search An lyst II)
Kendall Bradley, Re earch Technician II
Yund Huang (Statistician)

8.0 Method Validation Dates

Start Date: 04/Nov/20
End Date 14/Nov/20

9.0 Reagents and Materials

9.1 See Duke-02-MVPR-COVID0002 "Method Validation Protocol for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay with Moderna Phase 1 Samples." 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-MVPR-COVID0002 "Method Validation Protocol for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay with Moderna Phase 1 Samples."

11.0 Test and Control Articles

11.1 Incurred Serum Samples from Vaccine Recipients

Serum samples ((b) (4) aliquots) from (b) (4) participants in the Moderna phase 1 trial of mRNA-1273 vaccine for SARS-CoV-2 (Protocol 20-0003) were obtained from (b) (4) and received by Dr. Montefiori's laboratory on September 16, 2020. The serum samples were collected 2 weeks post 2nd inoculation (Day 57) from participants in the phase 1 trial of the identical vaccine, dose and schedule used in the phase 3 trial, and represent high, medium and low neutralization titers. The samples were stored at -80°C, thawed and heat-inactivated for 30 minutes at 56°C on September 18, 2020. The heat-inactivated samples were stored at 4°C until assayed. Testing also did not begin within (b) (4) of thawing the samples (see Deviation 19.1).

11.2 (b) (4) Positive Control

(b) (4) was obtained from Drs. Leonidas Stamatatos and Kristen Cohen, Fred Hutchinson Cancer Research Center Vaccines and Infectious Diseases Division, Seattle, WA. This is a human monoclonal antibody that binds an epitope in the receptor binding domain (RBD) of the Spike protein. The antibody was (b) (4) (b) (4) Acceptable ranges for ID50 and ID80 were established as described in "Reagent Qualification Testing Report: (b) (4) as a Positive Control for SARS-CoV-2 Neutralization Assays in 293T/ACE2 Cells" (see Attachment 20.1).

(b) (4) was used as a positive control in place of (b) (4) for Experiment #1 because (b) (4) was not qualified in time (see Deviation 19.2). Experiment #1 was originally scheduled to begin after the qualification of (b) (4) was completed. Operation Wrap Speed instructed the lab to begin validation experiments sooner than originally scheduled. (b) (4) was the only positive control qualified at the time Experiment #1 was performed. (b) (4) qualification was completed in time for Experiment #2.

11.3 (b) (4) Positive Control

(b) (4) is comprised of human monoclonal antibody DH1043 (Ab026116-LS) (b) (4) (b) (4)(b) (4). This reagents was used as a positive control on (b) (4) for

Experiment #2. DH1043 is a potent SARS-CoV-2 neutralizing antibody that was isolated from PBMC of a COVID-19 convalescent individual by Dr. Barton Haynes at 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 It was produced and QC'd by the Duke Protein Production Facility (see Attachment 20.2). (b) (4) (b) (4) (b) (4)) (see Attachment 20.3). (b) (4) (b) (4) (b) (4) Acceptable ranges for ID50 and ID80 were established as described in "Reagent Qualification Report: (b) (4) as a Positive Control for SARS-CoV-2 Neutralization Assays in 293T/ACE2 Cells" (see Attachment 20.4).

12.0 Validation Parameters and Pre-set Acceptance Criteria

Table 1. Validation parameters and pre-set acceptance criteria.

Parameter	Acceptance Criteria
Accuracy	(b) (4) (b) (4)
Precision	(b) (4) (b) (4)
Limits of Quantitation	The upper and lower limits or quantitation will be defined as the highest and lowest ID50 and ID80 titers measured in the provided sample set that meet all acceptance criteria for (b) (4)
Dilutional Linearity	The ID50 and ID80 neutralizing activity of serum samples is (b) (4) (b) (4)

13.0 Validation Experiments

13.1 Experiment 1 – Range and Repeatability

The (b) (4) serum samples (heat-inactivated at 56ºC for 30 minutes) were assayed (b) (4) The assays used a 1:20 start dilution and a 5-fold dilution series for 8 dilutions total. (b) (4) was assayed on (b) (4) as a positive control. ID50 and ID80 titers were recorded and used to determine the range of values.

13.2 Experiment 2 – Dilutional Linearity, Precision and Limits of Quantit ..ion

(b) (4)

14.0 Validation Results

14.1 Range and Repeatability

Results of the (b) (4) serum samples assayed (b) (4) are shown in **Figure 1**.

(b) (4)

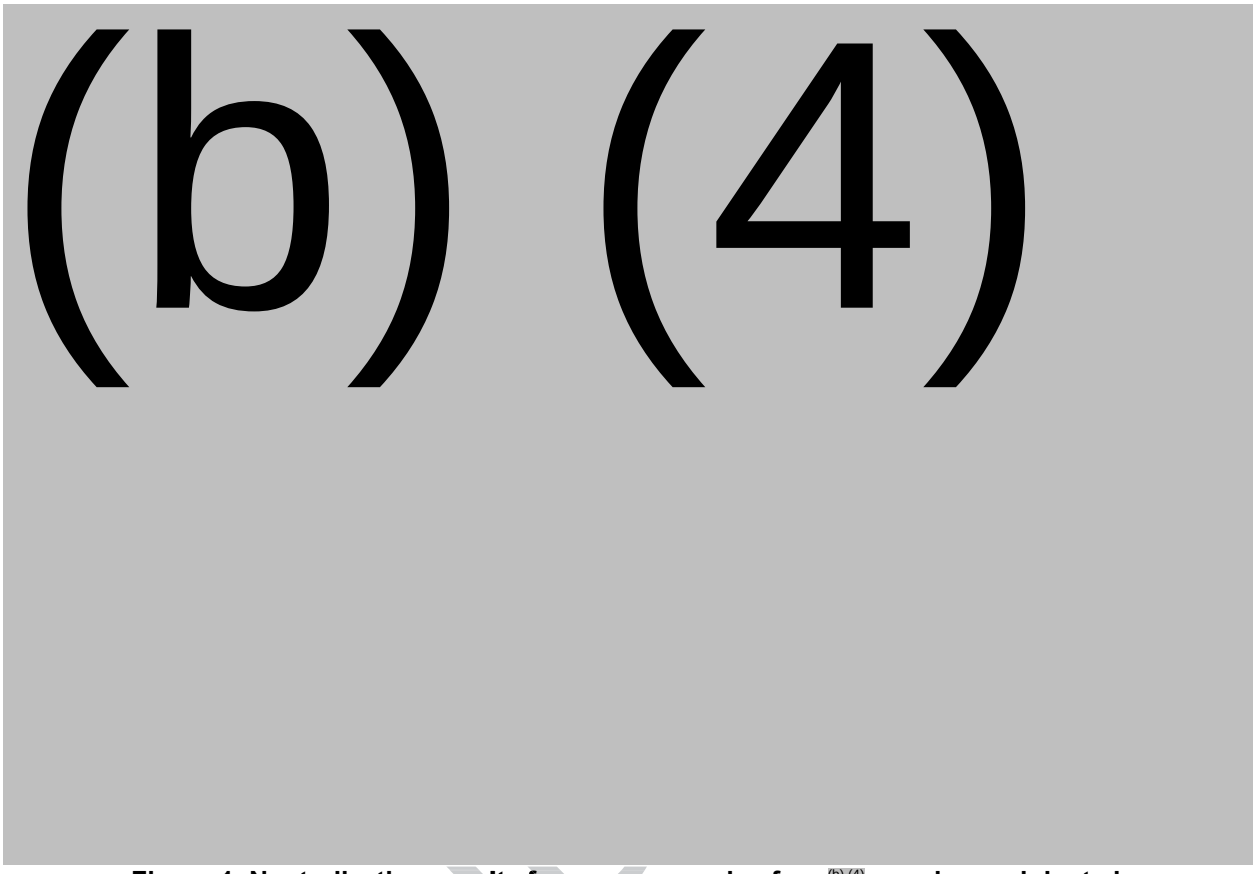
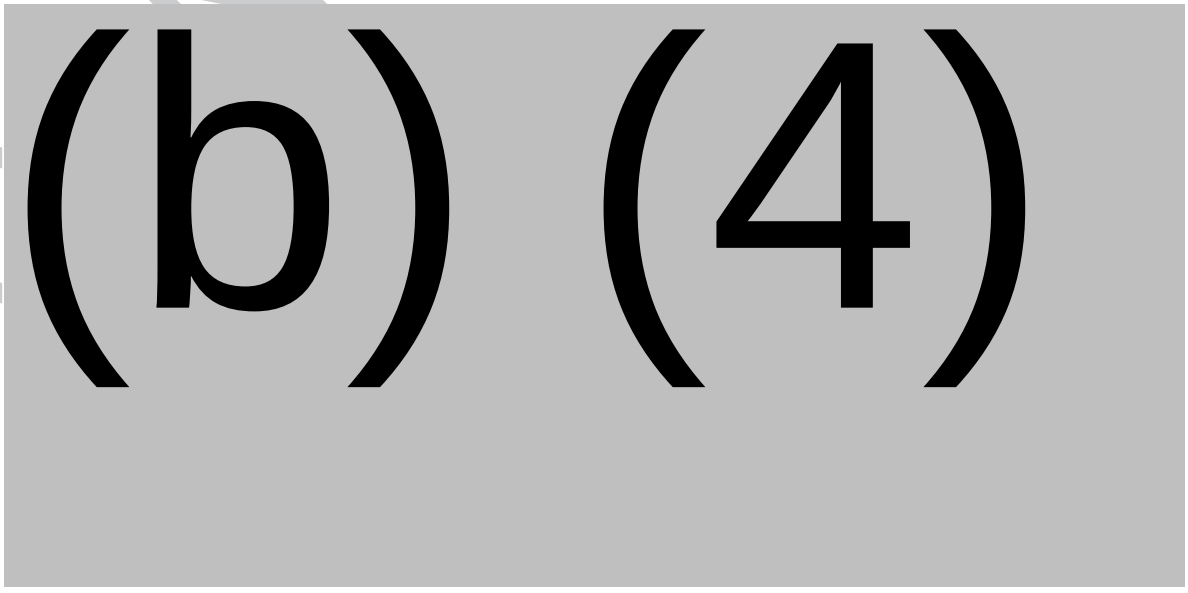


Figure 1. Neutralization results for serum samples from (b) (4) vaccine recipients in Moderna phase 1 t ial of the COVID-19 vaccine mRNA-1273. Top: ID50 (left) and ID80 (right) titers of (b) (4) for each sample. Bottom: Corresponding %CVs with a horizontal dotted line designating the acceptable limit.

14.2 Precision



(b) (4)

(b) (4)

(b) (4) (**Table 3**). (b) (4)
(b) (4)
(b) (4)

Table 2: Summary of (b) (4)
(b) (4)
(b) (4)
(b) (4)

(b) (4)	(b) (4)	(b) (4)	
		(b) (4)	
		(b) (4)	
		(b) (4)	
		(b) (4)	
		(b) (4)	

Table 3: Summary of ID80 titers and percent coefficient of variation (%CV) for the esti ation of i termediate precision (inter-operator) and repeatability (intra-assay) of the assay. (b) (4)
(b) (4)

(b) (4)	(b) (4)	
(b) (4)		
(b) (4)	(b) (4)	

14.3 Dilutional Linearity and Limits of Quantitation

As shown in **Table 4**, (b) (4)
(b) (4) in Experiment #2 ranged from (b) (4)

(b) (4)

(b) (4)

Table 4. Summary of ID50 and ID80 titers and percent coefficient of variation (%CV) for t e estimation of dilutional linearity of the assay.

(b) (4)

(b) (4)

(b) (4)

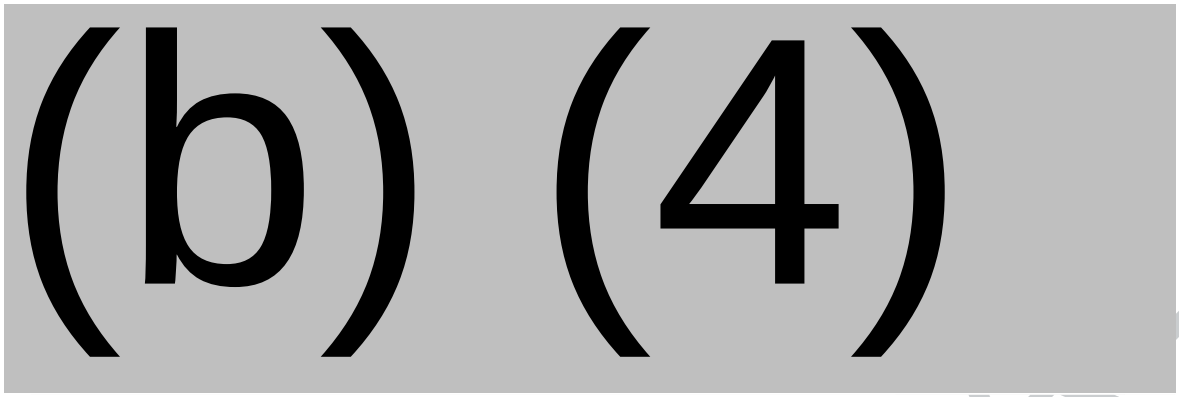
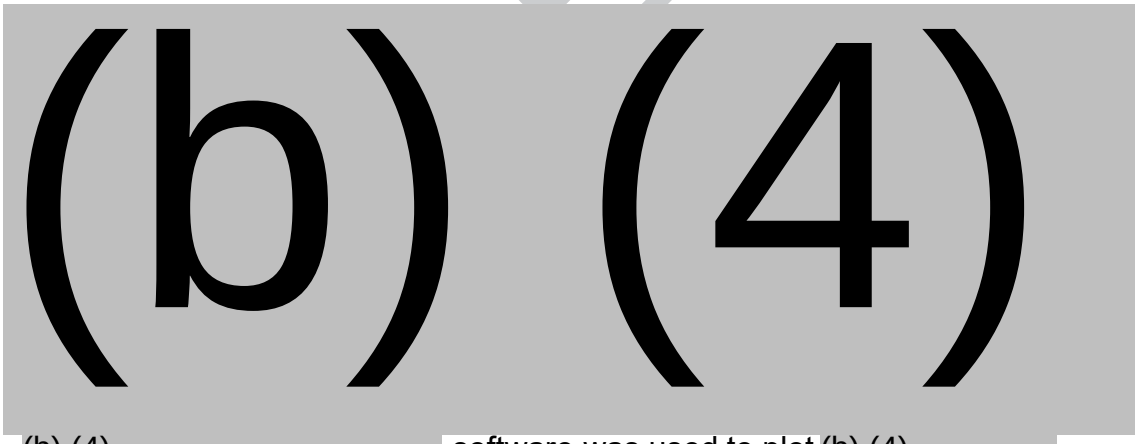


Figure 2. Dilutional linearity of neutralizing activity. Assays were performed with di'utions of (b) (4) Results are shown only for titers that were in the detectable range (b) (4). Each datapoint is the mean ± SD.

14.4 Accuracy

All experimental results for precision and linearity pass d the pre-set criteria.

15.0 Statistical Analysis of Re ults



(b) (4) software was used to plot (b) (4) (b) (4)

16.0 Conclusions

All pre-set acceptance criteria for accuracy, precision, and limits of quantitation in Table 1 were met in these experiments except for the (b) (4) experiment for (b) (4) (b) (4)

17.0 Plan for Storage and Archival of Validation Data

All electronic validation data will be stored as electronic files on a secure server at Duke University Medical Center. File identifiers and contents will be 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.

18.0 References

18.1 Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, G , et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 395(10223):497-506. doi: 1 .1016/S0140-6736(20)30183-5.

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

18.3 Zhu N, Zhang D, Wang W, Li X, Yang B, Song , Zhao X, Huang B, Shi W, Lu R., et al. (2020). A Novel Coronavirus from P tients with Pneumonia in China, 2019. N Engl J Med. 38 , 727–733.

18.4 Li Q, Guan X, Wu , Wang X, Zhou , Tong Y, et al. (2020) Early transmission dyn mics in Wu an, hina, of novel coronavirus-infected pneumonia. N Engl J Med. (202) 382(13):1199-1207. doi: 10.1056/NE Moa2001316.

18.5 de Wi , E., van Dorema n, N., Falzarano, D., and Munster, V.J. (2016). SA S and MERS: recent insights into emerging coronaviruses. Nat. Rev. Microbiol. 14, 523- 34.

18.6 Fehr AR, Channappanavar R., and Perlman S. (2017). Middle East Respiratory Syndrome: Emergence of a Pathogenic Human Coronavirus. Annu. Rev. Med. 68, 387–399.

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 China. Cell Host Microbe, S1931-

3128(1920)30072-X, doi:10.1016/j.chom.2020.02.001.

18.10 Duke-02-MVPR-COVID0002 “Method Validation Protocol for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay with Moderna Phase 1 Samples.”, version 1.0

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

18.12 SOP QADVIP-M008 “Archives”

19.0 Deviations

19.1 Deviation 02-3VP: The (b) (4) portion of experiment #1 of the Method Validation Protocol for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay with Moderna Phase 1 Samples,(Du e-02-MVPR-COVID0002) was not able to be performed due to the sam les being previo sly (b) (4) (b) (4) Testing also did not begin (b) (4) the samples (electronic record in (b) (4)).

19.2 Deviation 02-2VP: In the Meth d Validation Prot col for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay with oderna Phase 1 Samples, it was planned that (b) (4) control be used. However, for experiment #1, (b) (4) of (b) (4) was not complete (electronic record in (b) (4)).

20.0 Attachments

20.1 Reagent esting Qualificat on Report: (b) (4) as a Positive Control for SARS-CoV-2 Neutralization Assays in 293T/ACE2 Cells

20.2 Certificate of Analysis of human monoclonal antibody DH1043 (Ab026116-LS)

20.3 Certificate of Analysis of (b) (4)

20.4 Reag nt Qualification Report: (b) (4) as a Positive Control for SARS-CoV-2 Neutralization Assays in 293T/ACE2 Cells

Reagent Qualification Testing Report:
(b) (4) as a Positive Control for SARS-CoV-2 Neutralization Assays in 293T/ACE2 Cells

I. Objective:

This document describes the qualification of (b) (4) as a positive control for SARS-CoV-2 Spike-pseudotyped virus neutralization assays in 293T/ACE2 cells. The reagent is intended to be used in part to determine the acceptability of assay results.

II. Scope:

This Report applies to experiments used to define the acceptable ranges of IC50 and IC80 neutralizing values for the positive control reagent (b) (4). The work was performed by the "Neutralizing Antibody Core" Laboratory, under the GCLP oversight of the Quality Assurance for Duke Vaccine Immunogenicity Programs (QADVIP).

III. Reagent Description:

(b) (4) is a monoclonal antibody isolated from a patient infected with SARS-CoV-2 that binds an epitope in the receptor binding domain (RBD) of the SARS-CoV-2 Spike protein (1). The antibody was obtained from Drs. Leonidas Stamatatos and Kristen Cohen, Fred Hutchinson Cancer Research Center, Vaccines and Infectious Diseases Division, Seattle, WA, USA.

IV. Reagent Preparation and Storage:

The antibody arrived at the Duke University Medical Center on June 25, 2020 on dry ice (temperature confirmed upon receipt: -79.6°C). It was received by Kristy Long and stored immediately at -80°C in room 133 freezer (GCLP 06-00-12).

On July 10, 2020 the antibody was placed at 4°C in room 215 GCLP refrigerator 04-02-19. On July 13, 2020 it was (b) (4) by Jin Tong and stored at (b) (4) in room 215 GCLP refrigerator 04-02-24. On July 28, 2020 the (b) (4) (b) (4) by Jin Tong and stored immediately at (b) (4) in room 215 GCLP freezer 06-02-23.

The reagent (b) (4) (b) (4)

Table 1. Materials.

(b) (4)
(b) (4)
(b) (4)
(b) (4)

V. Establishing Acceptable Ranges

Neutralization assays were conducted in accordance with SOP CFAR02-A0026 "Measuring Neutralizing Antibodies Against SARS-CoV2 Using Pseudotyped Virus and 293T/ACE2 Cells" using the Spike pseudotyped virus CoV-2 VRC7480.D614G.1[CMVΔR8.2]/293T/17. The assays were performed between July 16, 2020 and August 31, 2020 by (b) (4) (b) (4) (b) (4) The acceptable ranges reflect a (b) (4)

Table 2. (b) (4) **IC50 and IC80 values.**

(b) (4)			
(b) (4)	(b) (4)	(b) (4)	
		(b) (4)	
		(b) (4)	
	(b) (4)	(b) (4)	
	(b) (4)	(b) (4)	

Table 3. (b) (4) **Acceptance Criteria.**

Virus	(b) (4) IC50 (ug/ml)	Acceptance Criteria (ug/ml)
CoV-2 VRC7480.D614G.1[CMVΔR8.2]/293T/17	(b) (4)	(b) (4)
Virus	**(b) (4) IC80 (ug/ml)**	**Acceptance Criteria (ug/ml)**
CoV-2 VRC7480.D614G.1[CMVΔR8.2]/293T/17	(b) (4)	(b) (4)

VI. Trendline Analysis

The positive control data for (b) (4) were analyzed for consistency in accordance with SOP CFAR02-D0004 "An lysis and Tracking of Positive Control Trendlines in Neutralizing Antibody Assays." Figure 1 shows the (b) (4) plot for IC50 values and Figure 2 shows the (b) (4) plot for IC80 values. The (b) (4) (b) (4) plots ere visually checked for violations of standards based on the (b) (4). No errors were observed. The Quality Control Checklists for Analysis of Positive Control Trendlines can be viewed in Attachment #1 and Attachment #2.

Figure 1. (b) (4) **Plot – IC50 Values**

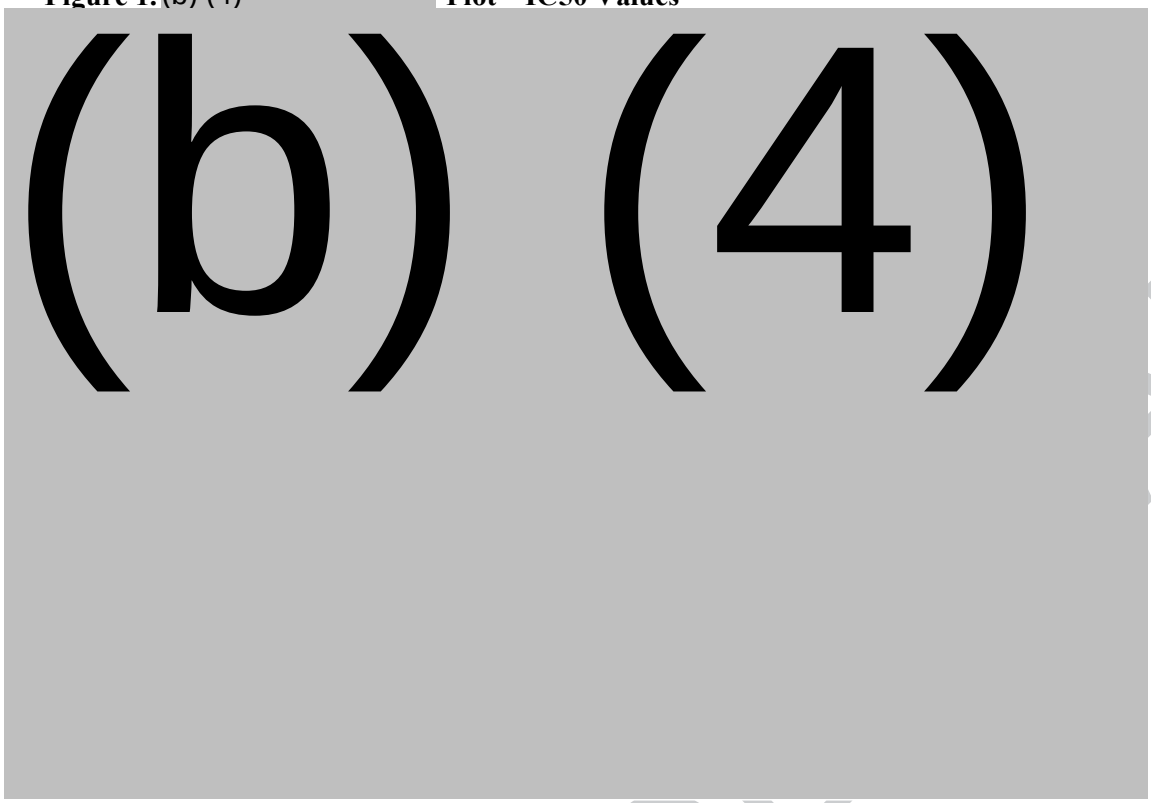
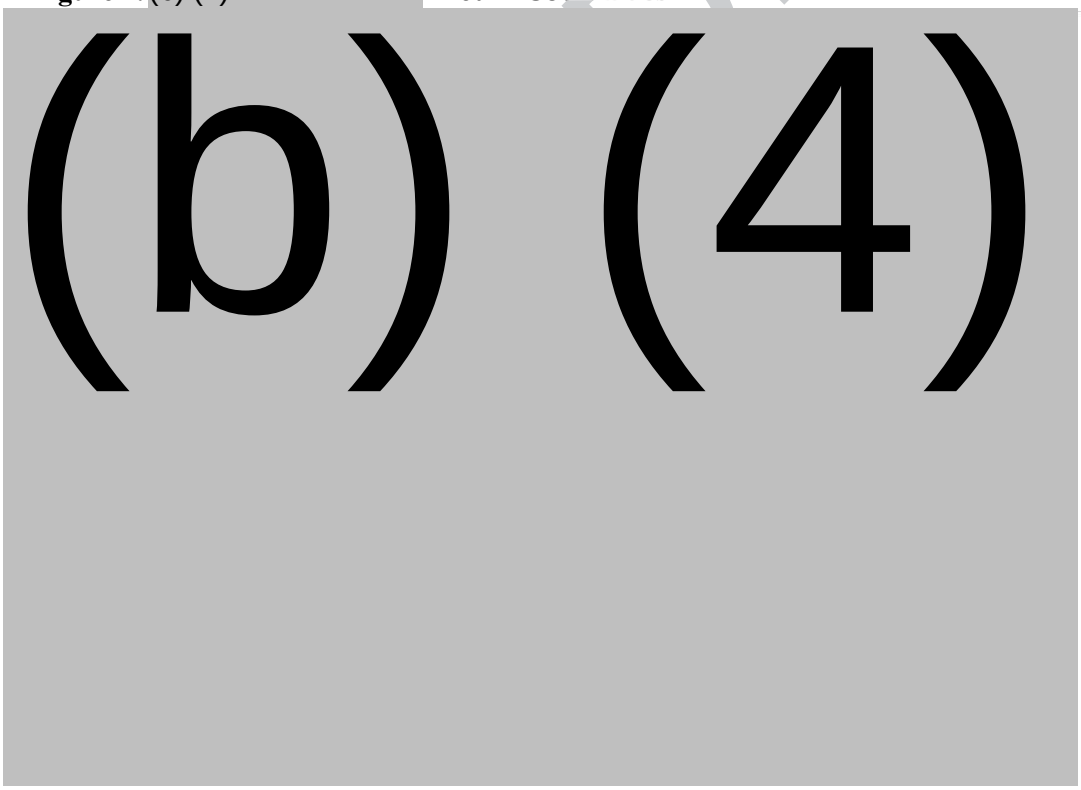


Figure 1. (b) (4) **Plot – IC80 Values**



VII. References

1. Hurlburt, N. K., Wan, Y. H., Stuart, A. B., Feng, J., McGuire, A. T., Stamatatos, L., & Pancera, M. (2020). Structural basis for potent neutralization of SARS-CoV-2 and role of antibody affinity maturation. bioRxiv : the preprint server for biology, 2020.06.12.148692. https://doi.org/10.1101/2020.06.12.148692
2. SOP CFAR02-A0026 "Measuring Neutralizing Antibodies Against SARS-CoV2 Using Pseudotyped Virus and 293T/ACE2 Cells"
3. SOP CFAR02-D0004 "Analysis and Tracking of Positive Control Trendlines in Neutralizing Antibody Assays"

VIII. Attachments

1. "Quality Control Checklist for Analysis of Positive Control Trendline" IC50 Values - **from** SOP CFAR02-D0004
2. "Quality Control Checklist for Analysis of Positive Control Trendline" C80 **Values - from** SOP CFAR02-D0004

IX. Revision History

Report Date	Report Info
	Original Report
n/a	Revised Report Summary of Changes:

October 23, 2020

David C. Montefiori, Ph.D.
Professor, Division of Surgical Sciences
Director, Laboratory for AIDS Vaccine Research & Development
Phone: 919-684-5278
E-mail: monte@duke.edu

Date:

Duke Human Vaccine Institute

Protein Production Facility

Certificate of Analysis

Antibody name: (b) (4)

Lot #:

Date of Manufacture:

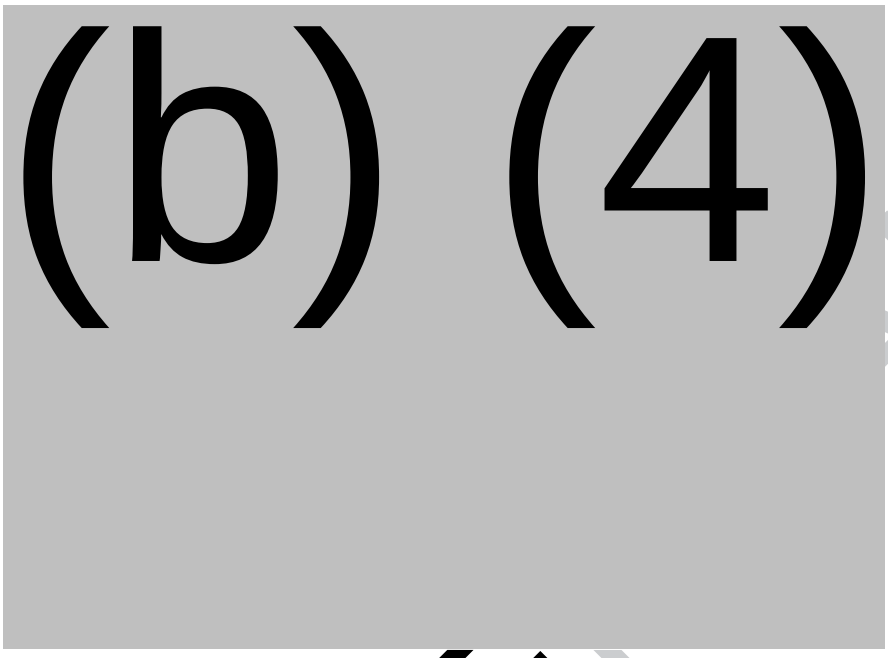
Concentration:

Description:

(b) (4)

Quality of Antibody:

Sterility Test	Result
(b) (4)	
(b) (4)	
(b) (4)	
(b) (4)	



Additional Notes: N/A

Viral Inactivation: This Material has not undergone any process to inactivate potential viral contaminant and should therefore be handled at the discretion of the user.

Disclaimer: This Material meets the requirements of our Facility in effect at the time of release.

This Material is for in vitro research use only and it is not intended for use in humans. This material is experimental in nature and is likely to have hazardous properties. The Material and associated information are provided without any warranties, express or implied, including any warranty of merchantability or fitness for a particular purpose, or that the use of the material and associated information will not infringe upon any patent, copyright, trademark, or other rights, or that the material will not pose a health or safety risk.

In no event shall Duke University (including the Protein Production Facility) be liable for any use by the recipient of the Material or for any loss, claim, damage, or liability of any kind or nature that may arise from or in connection with the use, handling, storage, or disposal of the Material".

PPF Signature:

James Peacock James Peacock 3 Oct 2020

(Printed name) **(Signature)** **(Date)**

QA Signature: Signature on file with QADVIP (12/Oct/2020)

(Printed name) **(Signature)** **(Date)**

(b) (4)

(b) (4), (b) (6)

Reagent Qualification Report: (b) (4) as a Positive Control for SARS-CoV-2 Neutralization Assays in 293T/ACE2 Cells

I. OBJECTIVE

This document describes the qualification of (b) (4) as a positive control for SARS-CoV-2 Spike-pseudotyped virus neutralization assays in 293T/ACE2 cells. The reagent is intended to be used in part to determine the acceptability of assay results.

II. SCOPE

This report applies to experiments used to define the acceptable ranges of ID50 and ID80 neutralizing values for the positive control reagent (b) (4). The work was performed by the "Neutralizing Antibody Core" Laboratory in the Surgical Oncology Research Facility, under the GCLP oversight of the Quality Assurance for Duke Vaccine Immunogenicity Programs (QADVIP).

III. REAGENT DESCRIPTION

(b) (4) is comprised of human monoclonal antibody DH1043 (also called: Ab026116_LS/293i) (b) (4) Ab02611 _LS/293i is a potent SARS-CoV-2 neutralizing antibody that was isolated from PBMC's of a COVID-19 convalescent individual by Dr. Barton Haynes in 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, receptor binding domain (RBD)-specific and ACE2-blocking. It was produced and QC'd by the Duke Protein Production Facility (see Attachment #1). The protein was produced in 293i cells by transient transfection, purified using Protein A chromatography. The protein was further purified by FPLC Size Exclusion Chromatography, filtered using (b) (4) filtration, and stored in (b) (4) (b) (4) (b) (4) (b) (4) (see Attachment #).

IV. REAGENT PREPARATION AND STORAGE

The (b) (4) shipment from (b) (4) was received by Charlene McDanal on 16/SEP/20; the (b) (4) was received (b) (4) and immediately placed in (b) (4) in Cold Room 133, Surgical Oncology Research Facility.

To determine fit-for-purpose, the (b) (4) was tested for (b) (4)

(b) (4)

Ab026116_LS/293i (also called: DH1043) was received frozen from the Duke Human Vaccine Institute Protein Production Facility on 18/SEP/20 by (b) (6). Shipment was processed and placed immediately in -80°C (Rm 133 freezer GCLP 06-00-12) until (b) (4) reagent preparation date on 22/OCT/20 (See Attachment #4).

On 09/OCT/20 (b) (4)
(b) (4)
(b) (4)
Francesca Suman. (b) (4)
(b) (4)

(b) (4)

TABLE 2. MATERIALS

Antibody Name	Production Date	Concentration (mg/ml)	Lot No.
Ab026116_LS/293i (DH1043)	(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4) (b) (4)	(b) (4) (b) (4)	(b) (4) (b) (4)

V. ESTABLISHING ACCEPTABLE RANGES

Neutralization assays were conducted in accordance with SOP FAR02-A0026 "Measuring Neutralizing Antibodies Against SARS-CoV2 Using Pseudotyped Virus and 293T/ACE2 Cells" using the Spike pseudotyped virus CoV-2 VRC7480.D614G.1[CMVΔR8.2]/293T/17. The assays were performed between October 27, 2020 and November 5, 2020 by (b) (4)

(b) (4), (b) (6)

TABLE 3. (b) (4) **ID50 AND ID80 VALUES**

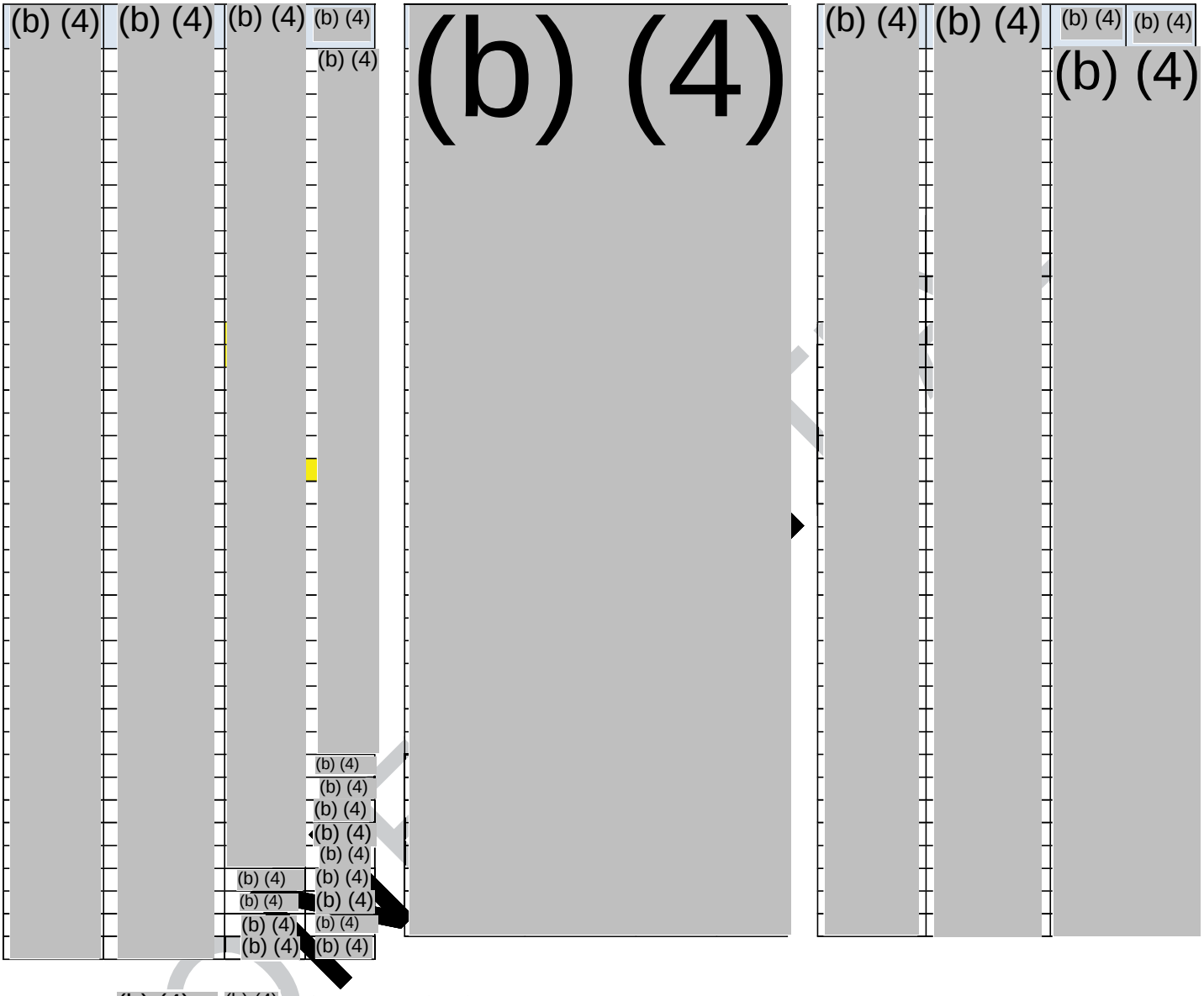


TABLE 4. (b) (4) (b) (4) **ACCEPTANCE CRITERIA.**

Virus: CoV-2 VRC7480.D614G.1[CMVΔR8.2]/293T/17			
(b) (4) ID 0:	(b) (4)	Acceptance Criteria:	(b) (4)
(b) (4) ID80:	(b) (4)	Acceptance Criteria:	(b) (4)

VI. TRENDLINE ANALYSIS

The positive control data for (b) (4) were analyzed for consistency in accordance with SOP CFAR02-D0004 "Analysis and Tracking of Positive Control Trendlines in Neutralizing Antibody Assays." Figure 1 shows the (b) (4) plot for ID50 values and Figure 2 shows the (b) (4) plot for ID80 values. The (b) (4) plots were visually checked for violations of standards based on the (b) (4) No errors were observed. The Quality Control Checklists for Analysis of Positive

Control Trendlines can be viewed in Attachment #5 and Attachment #6.

FIGURE 1. (b) (4) (b) (4) **PLOT – ID50 VALUES**

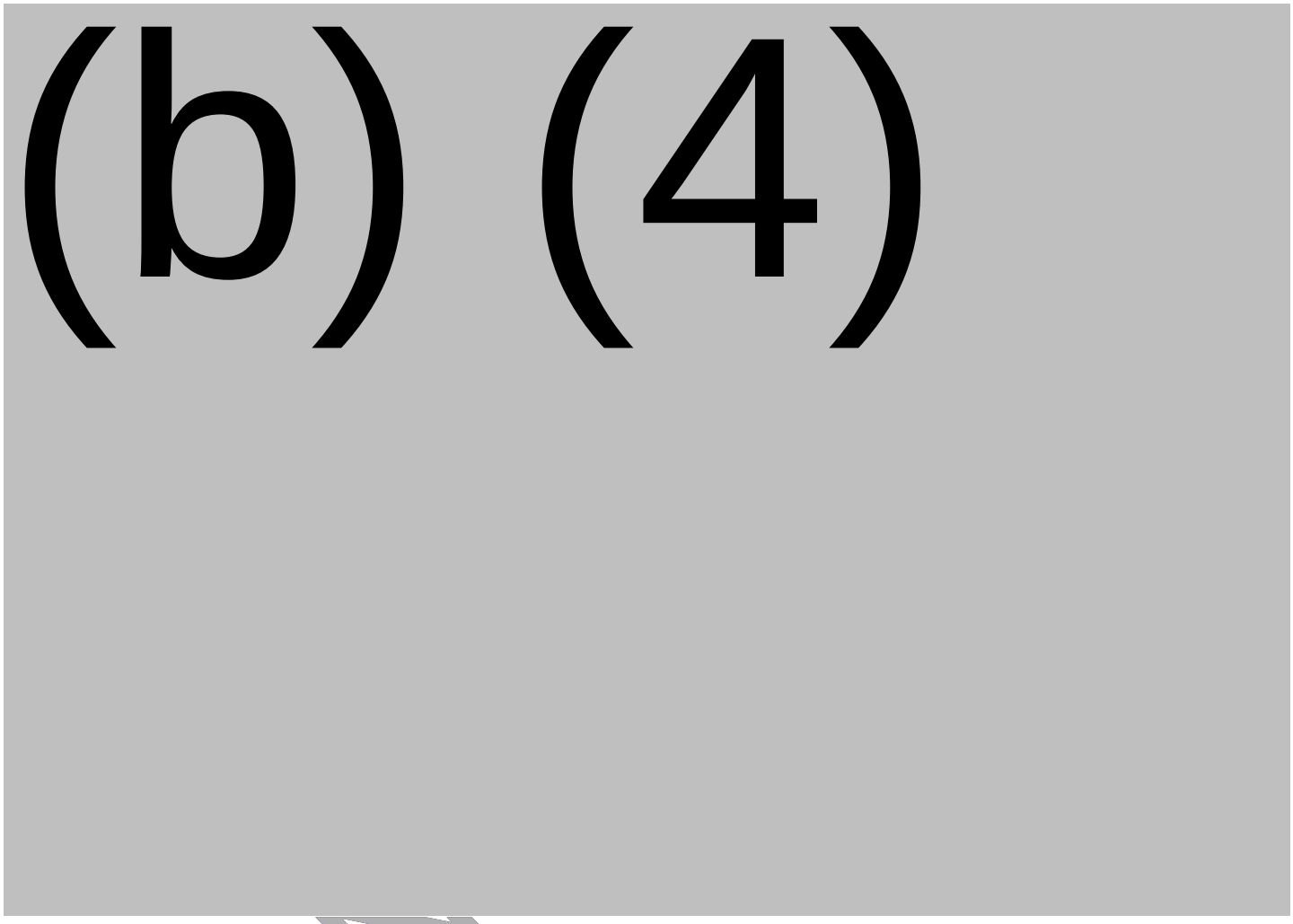
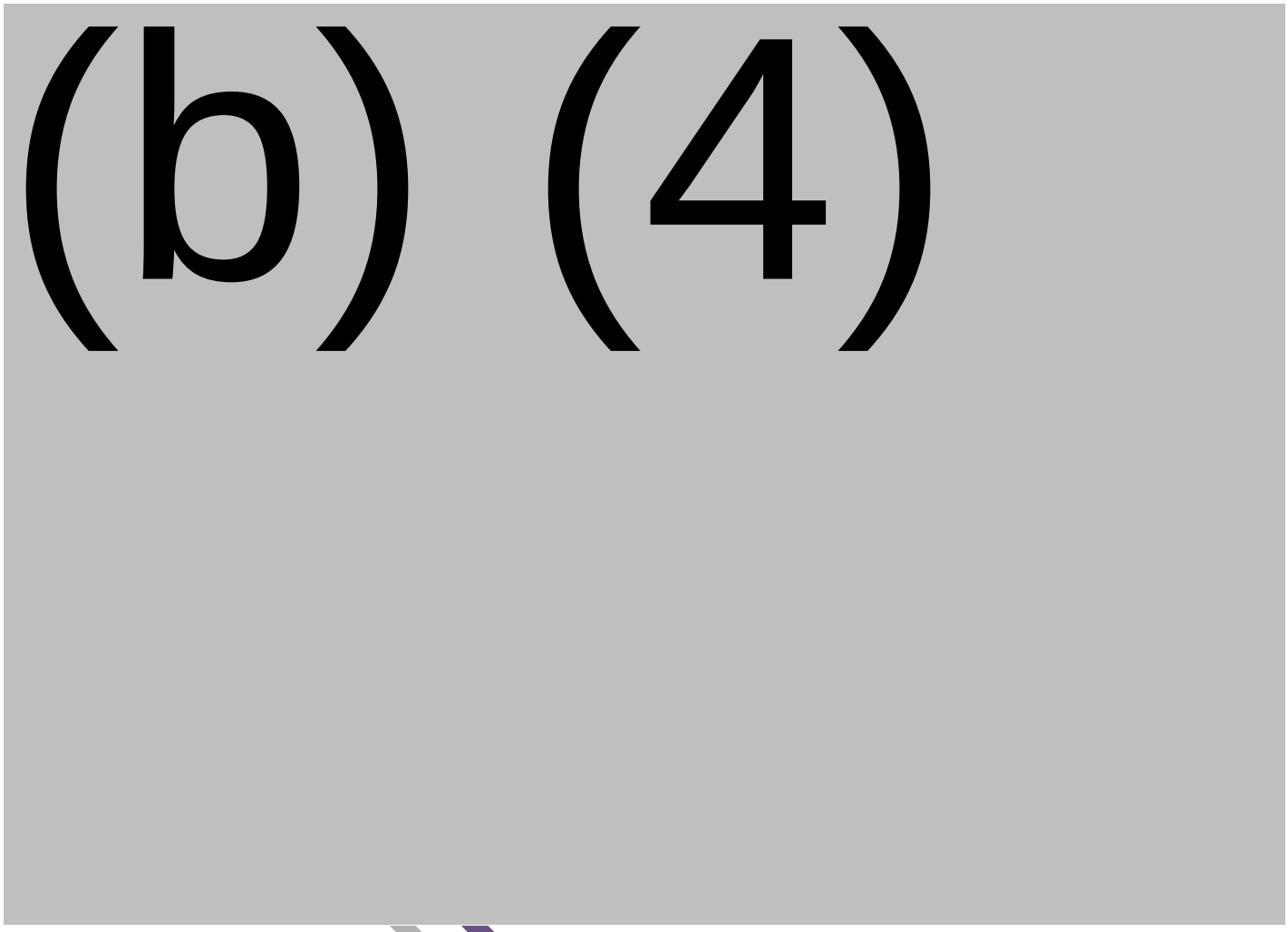


FIGURE 2. (b) (4) (b) (4) **PLOT – ID80 VALUES**



VII. REFERENCES

1. SOP CFAR02-A002 "Measuring Neutralizing Antibodies Against SARS-CoV2 Using Pseudotyped Virus and 293T/ACE2 Cells"
2. SOP CFAR02-D0004 "Analysis and Tracking of Positive Control Trendlines in Neutralizing Antibody Assays"
3. SOP CAVIMC-A0002 "Heat-Inactivation of Serum and Plasma Samples"

VIII. ATTACHMENTS

1. Duke Human Vaccine Institute Protein Production Facility Certificate of Analysis: Ab026116_LS/293i
2. (b) (4) Certificate of Analysis: (b) (4)
3. Report for (b) (4) of (b) (4) experiment: (b) (4) Testing
4. Ab026116_LS293i Shipping Manifest
5. Quality Control Checklist for Analysis of Positive Control Trendline ID50
6. Quality Control Checklist for Analysis of Positive Control Trendline ID80

IX. REVISION HISTORY

Report Date	Report Info
	Original Report
n/a	Revised Report Summary of Changes:

David C Montefiori

Digitally signed by David C Montefiori
DN: cn=David C Montefiori, o, ou, email=david.montefiori@duke.edu, c=US
Date: 2020.11.12 11:08:35 -05'00'

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