Marcella Sarzotti-Kelsoe
Digitally signed by Marcella Sarzotti-Kelsoe
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Method Validation Plan (Protocol) for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay in 293T/ACE2 Cells

Version No.:	Version 1.0
Author(s):	David Montefiori, PhD., (b) (6), (b) (6) (b) (6)
Effective Date:	**14/OCT/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.

Approved By:

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	
See Electronic Signature in (b) (4)	
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

1.0 Objective

This document is merging the Method Validation Plan for the SAPS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay in 293T/ACE2 Cells (Duke-02-MVP-COVID001 version 1.0) with the Method Validation Protocol for the SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay in 293T/ACE2 Cells (Duke-02-MVPR-COVID001 version 1.0) and provides an addendum to these two existing documents. The purpose of this addendum is to describe a protocol for repeating the validation experiment that did not meet the pre-defined acceptance criteria of (b) (4) CV for (b) (4) and/or were potentially (b) (4) in COVID-19 convalescent serum samples from people who are being (b) (4) This addendum also describes a protocol for ID80 precision and LOQ.

2.0 Scope

This addendum 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 addendum also applies to the upper and lower limits of quantitation of ID50 and ID80 neutralization titers in the assay, linearity, inference of accuracy and (b) (4) (b) (4)

3.0 Introduction

SARS-CoV-2, the etiologic agent of COVID-19 (Ref 16.1-16.4), 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 16.5-16.9). 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)
was observed in the initial validation experiment ((b) (4) C√ acceptance criteria for (b) (4). QA investigation indicated that(b) (4) partially related to the (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 MVR indicate that the resolution resides mostly in re-establishing the acceptance criteria based on the larger set of existing data. The new acceptance criteria will be applied to a repeat of Experiments #3 and #4. In addition, during the conduct of validation experiments it was discovered that COVID-19 convalescent ser m samples from (b) (4)
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4.0 Desc ption of Meth d

This addendum fol'ows the test plans outlined in Duke-02-MVPR-COVID001 "Method Validation Protocol for SARS-CoV-2 Spike-Pseudotyped Virus Neutralizati n Assay in 293T/ACE2 Cells". All neutralization assays will be 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-MVP-COVID001 "Method Validation Plan for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay in 293T/ACE2 Cells." All neutralization assays will be conducted in accordance with SOP CFAR02-A0026 "Measuring Neutralizing Antibodies Against SARS-CoV-2 Using Pseudotyped virus and 293T-ACE2 Cells."

6.0 Facility Name and Address

6.1 See Duke-02-MVP-COVID001 "Method Validation Plan for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay in 293T/ACE2 Cells." All neutralization assays will be conducted in accordance with SOP CFAR02-A0026 "Measuring Neutralizing Antibodies Against SARS-CoV-2 Using Pseudotyped virus and 293T-ACE2 Cells."

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: 13-14/Oct/20

End Date: 1/Nov/20

9.0 Reagents and Materials

9.1 See Duke-02-MVP-COVID001 "Method Validation Plan for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay in 293T/ACE2 Cells." All neutralization assays will be 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-MVP-COVID001 "Method Validation Plan for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay in 293T/ACE2 Cells." All neutralization assays will be 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

COVID-19 convalescent serum samples (set 1 - HVTN). 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 (HVTN) and the HIV Prevention Trial Network (HPTN), 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 disease severities, from asymptomatic to ICU. The protocol is expected to enroll >800 participants total. A separate set of serum samples not used previously in method qualification and validation experiments will be used here. We are blinded to the clinical status of the donors but not to HIV-1 infection status. Samples were heat-inactivated at 56ºC for 30 minutes according to CFAR02-A0026 (SOP for method) and stored at 4ºC for up to (b) (4) (b) (4) prior to assay.

11.1 (b) (4) 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 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) (b) (4) (b) (4) (b) (4) (b) (4) stock concentration of DH1043. (b) (4) was made immediately before use and stored at 4ºC during the duration of validation experiments.

12.0 Revised Validation Parameters and Pre-set Acceptance Criteria

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

Parameter	Acceptance Criteria
Accuracy	(b) (4)
Precision	

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

13.0 Original Validation Experiments to be Repeated

13.1 Experiment 3 – (b) (4)

(b) (4)

13.2 Experiment 4 – (b) (4)

(b) (4)

13.3 Experiment 5 – (b) (4)

(b) (4)

14.0 Replacement Validation Experiments

14.1 Experiment 3R/4R - (b) (4)

(b) (4) LLOQ, ULOQ (b) (4)

(b) (4)

14.2 Experiment 5R – (b) (4)

(b) (4)

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NOTE 1: (b) (4)

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15.0 Statistical Analysis of Results

(b) (4)

(b) (4)

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16.0 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.

17.0 References

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

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

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

17.4 SOP QADVIP-M011”Method Qualification and Validation”

17.5 SOP QADVIP-M008 “Archives”

18.0 Attachments

18.1 Not Applicable