Quality Assurance
Duke Vaccine Immunogenicity Programs

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Standard Operating Procedure
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Title: Measuring Neutralizing Antibodies Against SARS-CoV-2 Using Pseudotyped Virus and 293T/ACE2 Cells

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 SOP document.

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Table of Contents

1.0 Purpose and Introduction

This assay measures neutralization of SARS-CoV-2 Spike-pseudotyped virus in ACE2-expressing 293T target cells as a function of a reduction in Luc reporter gene

expression. The pseudotyped virus consists of lentiviral particles that are deficient for lentiviral Env, but have surface SARS-CoV-2 Spike protein and package the gene for firefly luciferase. Infected cells express luciferase, and luciferase activity is quantified by relative light units (RLU) of luminescence. The magnitude of luminescence is directly proportional to the infectivity of the virus inoculum over a wide range of RLU values. 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 to quantify potency, which is recorded as 50% inhibitory dose (ID50), 80% inhibitory dose (ID80).

The assay is performed in a 96-well format for high throughput capacity and takes 3 days. Luciferase signal is measured in 96-well flat bottom black/white plates for enhanced luminescence with minimal bleed-over. Use of a clonal cell line provides enhanced precision and uniformity.

The assay was adopted using reagents 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 was formally optimized, qualified, and validated in Dr. Montefiori's laboratory at Duke University Medical Center, Durham, North Carolina, USA.

The assay requires 3 days to complete:

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

2.0 Scope and Application

This Standard Operating Procedure (SOP) applies to the assessment of SARS-CoV-2 neutralizing activity of serum and plasma samples from vaccine recipients and COVID-19 patients. It also applies to the assessment of the SARS-CoV-2 neutralizing activity of monoclonal antibodies.

3.0 Definitions

Term or Abbreviation	Definition
%CV	Percent coefficient of variation
ATCC	American Tissue Culture Collection
BSC	BioSafety cabinet
CFAR-GAP	Center for AIDS Research – GCLP Compliant AIDS Program
COVID-19	Coronavirus disease 2019
DHTS	Duke Health Technology Solutions
DMEM	Dulbecco's Modified Essential Medium

(b) (4)	(b) (4)
(b) (4)	(b) (4)
GCLP	Good Clinical Laboratory Practice
GM	Complete Growth Medium
ID	Identification
ID50	50 percent inhibitory dose
ID80	80 percent inhibitory dose
Luc	Luciferase
NIAID	National Institutes of Allergy and Infectious Diseases
NIH	National Institutes of Health
Operator	Personnel performing the procedure for the assay
PPE	Personal protective equipment.
PI	Principal Investigator
PLL	Poly-L-Lysine
QADVIP	Quality Assurance for Duke Vaccine Immunogenicity Programs
QC	Quality control
RLU	Relative Luminescence Units
SARS	Severe acute respiratory syndrome
SARS-CoV-2	SARS Coronavirus 2, the causative agent of COVID-19
SARS private drive	DHTS network storage in a compartment permissioned for COVID-19 NAb Lab members
SOP	Standard Operating Procedure
TCID	Tissue Culture Infectious Dose
Vol	Volume

4.0 Safety

4.1 Use universal safety precautions when handling samples. Treat all samples as potentially infectious.

4.2 Wear appropriate personal protective equipment (PPE) including gloves, protective eye wear, laboratory coat, shoe covers and face shields.

4.3 Handle 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.”

4.4 SOPs are to be followed at all times.

4.5 Puromycin is toxic; avoid exposure to eyes and skin, harmful if swallowed

5.0 Specimens

5.1 Serum and plasma

5.1.1 Serum and plasma samples should be heat-inactivated at 56°C as described in SOP CAVIMC-A0002.

5.1.2 Serum is preferred to use over plasma because the (b) (4) plasma are problematic in the assay. (b) (4) plasma (b) (4)

5.2 Purified antibodies

5.2.1 Heat inactivation of antibodies is not recommended.

6.0 Reagents and Materials

6.1 Critical Reagents:

6.1.1 Plasmids:

6.1.1.1 VRC5601: pHR' CMV Luc (luciferase reporter gene) (luc) (Naldini et al.:1996 PNAS(93) 11382-11388)
Supplier: Original plasmid produced by the Vaccine Research Center, National Institutes of Health (USA), additional preparations produced by GenScript and Dr. Montefiori's laboratory, Duke University Medical Center.

6.1.1.2 VRC5602: pCMV ΔR8.2 (lentivirus backbone) (backbone) (Naldini et al.:1996 PNAS(93) 11382-11388)
Supplier: Original plasmid produced by the Vaccine Research Center, National Institutes of Health (USA), additional preparations produced by GenScript and Dr. Montefiori's laboratory, Duke University Medical Center.

6.1.1.3 VRC9260: (b) (4)
Supplier: Original plasmid produced by the Vaccine Research Center, National Institutes of Health (USA) , additional preparations produced by GenScript and Dr. Montefiori's laboratory, Duke University Medical Center.

6.1.1.4 VRC7480: (b) (4)
Supplier: Original plasmid produced by the Vaccine Research Center, National Institutes of Health (USA), additional preparations produced by

GenScript and Dr. Montefiori's laboratory, Duke University Medical Center.

6.1.1.5 VRC7480.D614G: (b) (4)
Supplier: Original plasmid produced by the Vaccine Research Center, National Institutes of Health (USA) with mutation introduced by Dr. Montefiori's laboratory, Duke University Medical Center. Preparations produced by GenScript and Dr. Montefiori's laboratory, Duke University Medical Center.

NOTE 1: Other Spike plasmids may be used for research purposes.

6.1.2 Cell lines:

6.1.2.1 293T/17 [HEK 293T/17] cells (cat. # CRL-11268). Cells are maintained in DMEM, 10% FBS, 50 ug/ml Gentamicin and 25 mM HEPES (GM)
Supplier: ATCC

6.1.2.2 293T cell line stably overexpressing the human ACE2 cell surface receptor protein. These cells, here called 293T/ACE2 (also called 293T-hACE2.MF), are maintained in DMEM, 10% FBS, 50 ug/ml Gentamicin, 25 mM HEPES and 3 µg/ml puromycin
Supplier: Drs. Mike Farzan and Huihui Mu at Scripps.

6.1.3 Spike-pseudotyped virus:

6.1.3.1 (b) (4)
Supplier: Spike-pseudotyped virus is prepared by Dr. Montefiori's laboratory, Duke University Medical Center according to the procedure outlined in section 8.3 of this procedure and bridged according to SOP CFAR02-A0011.

6.1.4 Positive controls:

6.1.4.1 COVA1-18
Supplier: Obtained from (b) (6)
(b) (6) *This mAb binds an epitope in the receptor binding domain (RBD) of the Spike protein. The mAb was* (b) (4)
(b) (4)

6.1.4.2 (b) (4)

Supplier: Obtained from (b) (6) *and Kristen Cohen, Fred Hutchinson Cancer Research Center, Vaccines and Infectious Diseases Division, Seattle, WA. This mAb binds an epitope in the receptor binding domain (RBD) of the Spike protein. The mAb was* (b) (4) (b) (4)

6.1.4.3 (b) (4)

Supplier: DH1043 was produced by the Duke Protein Production Facility (PPF) with permission from Dr. Barton Haynes, Duke Human Vaccine Institute, Duke University Medical Center. This mAb binds an epitope in the receptor binding domain (RBD) of the Spike protein. (b) (4) (b) (4)

6.1.5 Assay run positive controls:

6.1.5.1 High (ID50 (b) (4)), medium (ID50 (b) (4)), and low (ID50 (b) (4) (b) (4) titer COVID-19 convalescent serum samples (b) (4) serum samples.

Supplier: Obtained from HIV-negative subjects enrolled in 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 COVID-19 disease severities, from asymptomatic to requiring advanced medical care in the intensive care unit (ICU). Serum samples are heat inactivated and stored in aliquots at -80°C. Thawed aliquots are stored at 4°C for up to (b) (4)

Supplier: (b) (4)
Serum samples are heat inactivated and stored in aliquots at -80°C. Thawed aliquots are stored at 4°C for up to (b) (4)

6.1.6 Assay run negative controls:

6.1.6.1 Normal Human Serum (NHS)
Supplier: Obtained from (b) (4) *(product number* (b) (4) (b) (4) *This is human serum from* (b) (4)

(b) (4)

6.1.7 Other critical reagents:

6.1.7.1 FBS: Fetal Bovine Serum, also called Fetal Calf Serum, heat inactivated and triple 0.1-µm filtered
Manufacturer: (b) (4) *(product number* (b) (4) *lot number* (b) (4))

6.1.7.2 Bright-Glo Luciferase Assay System
Manufacturer: Promega (product number (b) (4)

6.1.7.3 Luciferase Cell Culture Lysis 5X Reagent
Manufacturer: Promega (product number PAE1531)

6.2 Non-critical Reagents and Materials

NOTE 2: Recommended vendors are listed. Products of equal or better quality than the recommended ones can be used whenever necessary.

6.2.1 FuGENE 6
Manufacturer: Promega

6.2.2 (b) (4)
Manufacturer: (b) (4)

6.2.3 Puromycin
Manufacturer: (b) (4)

6.2.4 Gentamicin solution, 10mg/ml
Manufacturer: (b) (4)

6.2.5 HEPES buffer
Manufacturer: (b) (4)

6.2.6 TrypLE™ Select Enzyme (1X), no phenol red
Manufacturer: Thermo Fisher Scientific

6.2.7 (b) (4)
Manufacturer: (b) (4)

6.2.8 DMEM: Dulbecco's Modified Eagle's Medium with L-glutamine, sodium pyruvate, glucose and pyridoxine
Manufacturer: Thermo Fisher Scientific

6.2.9 Opti-MEM reduced-serum medium (Life Technologies Catalog # (b) (4))
Manufacturer: Thermo Fisher Scientific

6.2.10 96-well black/white plates
Manufacturer: PerkinElmer

6.2.11 96-well Poly-L-Lysine (PLL) plates
Manufacturer: Corning

6.2.12 Microliter pipette tips, sterile
Manufacturer: (b) (4)

6.2.13 Combitips, sterile
Manufacturer (b) (4)

6.2.14 Disposable serologic pipettes, sterile, individually wrapped
1 ml pipettes
2 ml pipettes
5 ml pipettes
10 ml pipettes
25 ml pipettes
50 ml pipettes
100 ml pipettes
Manufacturer: (b) (4)

6.2.15 Cryogenic vials, 2.0 ml sterile screw cap, frosted label
Manufacturer: (b) (4)

6.2.16 Conical tubes (15 ml and 50 ml), sterile
Manufacturer: (b) (4)

6.2.17 T-25, T-75, or T-225 Culture flasks with vented caps, sterile
Manufacturer: (b) (4)

6.2.18 150ml Filter unit, 0.45uM filter size
Manufacturer: (b) (4)

7.0 Equipment

Recommended vendors are listed. Unless otherwise specified, products of equal or better quality than the recommended ones can be used whenever necessary.

7.1 Critical Equipment

7.1.1 Luminometer: GloMax® Navigator Microplate Luminometer
Manufacturer: *Promega*

NOTE 3: Recommended vendors are listed. Products of equal or better quality than the recommended ones can be used whenever necessary.

7.1.2 Non-critical Equipment

7.1.2.1 Biological Safety Cabinet
Manufacturer: (b) (4)

7.1.2.2 Incubator (37°C, 5% CO2 standard requirements)
Manufacturer: (b) (4)

7.1.2.3 Centrifuge and Microcentrifuge (low speed capable of up to 500 x g)
50 ml tube holder
15 ml tube holder
Microtitration plate holder
Manufacturer: (b) (4)

7.1.2.4 18 place standard rotor (b) (4) for 1.5ml microcentrifuge tubes.
Manufacturer: (b) (4)

7.1.2.5 Computer
Manufacturer: (b) (4)

7.1.2.6 Water bath
Manufacturer: (b) (4)

7.1.2.7 Scientific Counting Chamber Levy Double
Manufacturer: (b) (4)

NOTE 4: An automated cell counting device (e.g. (b) (4) Manufacturer: (b) (4) (b) (4) Manufacturer: (b) (4)) may be used in lieu of a light microscope/hemocytometer for cell counting and viability calculation.

7.1.2.8 Pipettors:
Single channel electronic pipettor, 10-300 µl
12-channel electronic pipettor, 50-1200 µl
12-channel electronic pipettor, 10-300 µl
Single channel manual, 0.5-10 µl
Single channel manual, 2-20 µl
Single channel manual, 20-200 µl
Single channel manual, 100-1000 µl
Manufacturer: (b) (4)

7.1.2.9 PipetteAid XP
Manufacturer: (b) (4)

7.1.2.10 Light Microscope
Manufacturer: (b) (4)

7.1.2.11 Ultra Low Temperature Freezer (−70°C or lower)
Manufacturer: (b) (4)

7.1.2.12 4°C Refrigerator
Manufacturer: (b) (4)

7.1.2.13 −20°C Freezer
Manufacturer: (b) (4)

8.0 Procedure

8.1 Reagent Preparation

8.1.1 Complete Growth Medium (GM)

8.1.1.1 Complete GM consists of DMEM containing 10% heat-inactivated FBS, 50 µg gentamicin/ml, and 25mM HEPES.

8.1.1.2 Example: To make 500ml of GM, combine and mix in a sterile bottle:

- 435 ml DMEM
- 50 ml FBS
- 2.5 ml gentamicin
- 12.5 ml HEPES

8.1.1.3 Store the GM at 4°C for up to (b) (4) or to the earliest expiration date of any one of the constituent reagents, whichever comes first.

8.1.1.4 Reagents used to prepare GM must be labeled with the date that the sterile bottle is opened and thawed (if applicable).

8.1.1.5 Label GM bottle according to SOP CFAR02-R0003.

8.2 Cell Cultures

NOTE 5: The 293T/17 cells are maintained in GM in T-75 vented culture flasks. The 293T/ACE2 cells are maintained in GM+puromycin in T-75 culture flasks to maintain ACE2 expression.

8.2.1 Maintenance and Expansion of 293T/17 and 293T/ACE2 cells:

8.2.1.1 (b) (4)

8.2.1.2

8.2.1.3

8.2.1.4

NOTE 6: (b) (4) can be substituted for (b) (4) when maintaining 293T/17 cells but should not be used when culturing 293T/ACE2 cells.

8.2.1.5 (b) (4)

8.2.1.6

8.2.1.7

8.2.1.8 293T/17 cells may be used up to passage (b) (4) in culture or (b) (4) in culture, whichever comes first.

8.2.1.9 293T/ACE2 cells may be used up to passage 46 in culture or 4 months in culture, whichever comes first.

8.3 Production of SARS-CoV-2 S-Pseudotyped Lentivirus

NOTE 7: Before beginning pseudovirus preparation, the operator must obtain a copy of Attachment #1: "SARS CoV-2 Pseudovirus Preparation Checklist." As each step of the preparation is performed, the checklist should be filled out appropriately. See SOP CFAR02-D0006 for record keeping specifics.

8.3.1 Day 0

8.3.1.1 (b) (4)

8.3.1.2 (b) (4)

Table 2.

(b) (4)

8.3.2 Day 1

NOTE 8: The following steps pertain to (b) (4)

(b) (4)

8.3.2.1 (b) (4)

NOTE 9: (b) (4)

(b) (4)

8.3.2.2 (b) (4)

8.3.2.3 Add plasmids (b) (4) to a polystyrene tube:

(b) (4)

- VRC7480 (b) (4)

(b) (4)

8.3.2.4 (b) (4)

NOTE 10: (b) (4)

8.3.2.5 (b) (4)

8.3.2.6

8.3.3 (b) (4)

8.3.3.1

8.3.3.2

8.3.4 (b) (4)

8.3.4.1

8.3.4.2

8.3.4.3

8.3.4.4

8.4 Titration of Pseudovirus

NOTE 11: Before beginning a titration (TCID) assay, the operator must obtain a copy of Attachment #4: "SARS CoV-2 Pseudovirus Titration Checklist." As each step of the assay is performed, the checklist should be filled out. See SOP CFAR02-D0006 for record keeping specifics.

8.4.1 Day 0

8.4.1.1 (b) (4)

8.4.1.2

(b) (4)

(b) (4) See Figure A below for plate layout.

Figure A: Assay template for measuring TCID50, (b) (4)

(b) (4)

8.4.1.3 (b) (4)

8.4.1.4

8.4.1.5

8.4.1.6

8.4.2 Day 3

8.4.2.1 (b) (4)

8.4.2.2

8.4.2.3

8.4.2.4 (b) (4)

8.4.2.5

8.4.2.6

8.4.2.7

8.4.2.8

8.4.2.9 (b) (4)

8.4.2.10

8.4.2.11

8.4.3 Analyzing TCID results

8.4.3.1 Analyze and save the TCID data on DHTS SARS private drive using the Excel based TCID Macro and the .xlxs file from the GloMax. Select the pseudovirus dilution that yields an average of (b) (4) RLU. Refer to SOP CFAR02-D0006 for record keeping specifics.

8.4.3.2 Complete Attachment #1 with relevant information.

8.4.3.3 After pseudovirus preparation is complete, the virus must be bridged according to SOP #CFAR02-A0011 prior to use in GCLP assays.

8.5 Neutralization Assay

NOTE 12: Before beginning a neutralization assay, the operator must obtain a copy of Attachment #2: "SARS CoV-2 Neutralizing Antibody Assay in 293T/ACE2 Cells Checklist." As each step of the assay is performed, the checklist should be filled out. See SOP CFAR02-D0006 for record keeping specifics.

8.5.1 Day 0

8.5.1.1 (b) (4)

8.5.1.2 Using the format of a 96-well flat-bottom PLL culture plate as illustrated in Figure B below, place 150 µL of GM in all wells of column 1 (cell control). (b) (4) (column 2 will be the virus control). (b) (4)
(b) (4)

Figure B: Assay template for measuring neutralization titers, (b) (4)

(b) (4)

8.5.1.3

8.5.1.4 (b) (4)

NOTE 13: (b) (4)
(b) (4) (Figure B above). (b) (4)
(b) (4)

NOTE 14: Appropriate adjustments may be made to test a different range of dilutions (refer to "Sample Dilution Charts" in Figure C below). Dilution scheme will be noted on Attachment #2.

Figure C: Sample Dilution Charts

STANDARD DILUTION CHART FOR (b) (4) **SAMPLE DILUTIONS**

(b) (4)

(b) (4)

STANDARD DILUTION CHART FOR (b) (4) **SAMPLE DILUTIONS**

(b) (4)

(b) (4)

STANDARD DILUTION CHART FOR (b) (4) **SAMPLE DILUTIONS**

(b) (4)

(b) (4)

(b) (4)

NOTE 15: (b) (4)

(b) (4)

8.5.1.5 (b) (4)

NOTE 16: (b) (4)

(b) (4)

NOTE 17: (b) (4)

(b) (4)

8.5.2 Prepare virus/GM suspension at the recommended dilution as described below:

8.5.2.1 Virus Calculations:

(b) (4)

(b) (4)

8.5.2.2 (b) (4)

8.5.2.3 (b) (4)

8.5.2.4 During the incubation, prepare a suspension of 293T/ACE2 cells at a concentration of 100,000 cells/ml in GM as described below.

8.5.2.4.1 Perform Viable Cell Count

8.5.2.4.1.1 Follow procedures for viable cell count using SOP CFAR00-E0053 or CFAR00-E0084. Cells must be (b) (4) viable to be used in the assay.

8.5.2.4.1.2 Cell Calculations (if using a hemocytometer):

(b) (4)

For example:

(b) (4)

(b) (4)

8.5.2.5 (b) (4)

8.5.2.6

8.5.3 Day 3

8.5.3.1 (b) (4)

8.5.3.2

8.5.3.3

8.5.3.4

8.5.3.5

8.5.3.6

8.5.3.7

8.5.3.8

8.5.3.9

8.5.3.10 NAb assay plates should be saved as: luminometer#-Lumi YYYY.MM.DD HH_MM_SS_experiment ID_Plate#, where luminometer#, date, and time are auto populated by the luminometer at the time the plate is read. For example:

01-Lumi 20201212 13_40_20_XY01-02_PL01

8.5.3.11 The GloMax software program associated with the luminometer automatically or manually exports the raw data in .xlxs, .csv, and .raw formats in a specified location on Duke Health Technology Solutions (DHTS) network storage in a compartment permissioned for COVID-19 NAb Lab members (SARS private drive), after each plate is read.

8.5.4 Analyzing and saving neutralizing antibody assay results

8.5.4.1 Analyze the data using the (b) (4) (SOP CFAR02-D0003) and the .xlxs file from the GloMax.

NOTE 18: (b) (4)

(b) (4)

8.5.4.2 Prepare a data packet to provide to the data reviewer(s) and/or PI. The data packet should include, at a minimum, the following items:

8.5.4.2.1 Attachment #3 CFAR02-A0026_3_Att#3 - SARS CoV-2 Neutralizing Antibody Assay QC Checklist.

8.5.4.2.2 The data run summaries include at least the following information: i) experiment number, ii) protocol and/or study number, iii) cells used in the assay, iv) length of incubation in hours, v) name, date, and dilution of the virus stock used, vi) luminometer file ID, vii) experiment date and performer, and viii) all pertinent sample information.

8.5.4.2.3 See SOP CFAR02-D0006 for record keeping specifics.

NOTE 19: A signature on every data sheet is not required if the web-based (b) (4) available on the (b) (4) was used to analyze data (see SOP CFAR02-D0003).

8.5.1 QC of neutralizing antibody assay results

8.5.1.1.1 Review of pass-fail criteria and QC of data packet for each experiment is recorded on Attachment #3. See SOP CFAR02-D0006 for record keeping specifics.

8.5.1.2 Pass-fail criteria

8.5.1.2.1 Assay plates, samples, and/or experiments that do not meet the validity criteria specified below should not be included in the data transfer to investigators/sponsors. Additional experiments should be performed to replace the invalid data. In general, (b) (4) assay (b) (4) are considered permissible. Additional re-runs may require considerations of the PI and consultation with the investigator/sponsor. When a valid result is not able to be acquired during the execution of the protocol, the rational for the result exclusion will be communicated to the investigator/sponsor.

8.5.1.2.2 Plate level QC – if a plate does not pass any of the below criteria, it must be repeated:

8.5.1.2.2.1 (b) (4)

8.5.1.2.2.2

8.5.1.2.2.3

8.5.1.2.3 Sample level QC – if a sample does not pass any of the below criteria, it must be repeated:

8.5.1.2.3.1 (b) (4)

8.5.1.2.3.2

8.5.1.2.4 Assay run level QC – if an experiment does not pass the below criteria, it must be repeated:

8.5.1.2.4.1 (b) (4)

8.5.1.3 Data Packet QC Checks

8.5.1.3.1 (b) (4)

8.5.1.3.2

8.5.1.3.3

8.6 Transferring Results

8.6.1 After data approval by the PI/Final Reviewer, acceptable study data can be transferred to investigators and sponsors (SOP #CFAR02-D0003).

8.6.2 If applicable, refer to the protocol/sponsor specific Data Transfer Plan or Agreement for specific data transfer instructions.

8.7 Calculations & Interpretations of Results

8.7.1 Calculating % neutralization

8.7.1.1 (b) (4)

8.7.1.2

8.7.2 Calculating ID50 and ID80.

8.7.2.1 Plasma or serum. Neutralizing plasma or serum antibody titers are expressed as the dilution required to achieve 50% neutralization (50% inhibitory dilution, also called ID50) or 80% neutralization (ID80).

9.0 References

9.1 Naldini *et al*. [1996] PNAS(93) 11382-11388

9.2 Vaccine Research Institute SOP# HIC 003 version 1.0, “Protocol for Measuring Neutralizing Antibodies Against SARS-CoV-2 Using Pseudotyped Virus and 293T/ACE2 Cells”

9.3 SOP CAVIMC-A0002 “Heat-Inactivation of Serum and Plasma Samples”

9.4 SOP CFAR02-A0011 “Neutralizing Antibody Assay Reagent Bridging Studies”

9.5 SOP CFAR00-E0083 “GloMax® Navigator Microplate Luminometer”

9.6 SOP CFAR00-E0053 “Countess Automated Cell Counter”

9.7 SOP CFAR00-E0084 (b) (4) Automated Cell Counter”

9.8 SOP CFAR02-D0003 “Neutralizing Antibody Assay Data Analysis and Transfer Using the (b) (4)

9.9 SOP CFAR02-D0006 “Electronic Data Management for the Neutralizing Antibody Assay”

9.10 SOP CFAR02-R0003 “Reagent Labeling in the Neutralizing Antibody Laboratory”

10.0 Attachments

NOTE 20: The following attachments are documents that are required to be used in conjunction with this SOP. All required documents are uploaded in (b) (4) under the SOP-specific record.

10.1 Attachment #1: CFAR02-A0026_4_Att#1 – SARS-CoV-2 Pseudovirus Preparation Checklist

10.2 Attachment #2: CFAR02-A0026_4_Att#2 - SARS CoV-2 Neutralizing Antibody Assay in 293T/ACE2 Cells Checklist

10.3 Attachment #3: CFAR02-A0026_4_Att#3 - SARS CoV-2 Neutralizing Antibody Assay QC Checklist

10.4 Attachment #4: CFAR02-A0026_4_Att#4 - SARS CoV-2 Pseudovirus Titration Checklist

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

SARS CoV-2 Neutralizing Antibody Assay QC Checklist

The following items must be inspected and approved by the operator, study manager and the PI or designee .

(b) (4)

(b) (4)