TABLE OF CONTENTS

1. HISTORICAL REVISION SUMMARY 2
Table 1. Chronological Summary of Previous Versions 2
2. PURPOSE 2
3. SCOPE 2
4. GLOSSARY 2
Table 2. Terms and Definitions 2
5. GENERAL 3
6. RESPONSABILITIES 4
Table 3. Roles and Responsibilities 4
7. SAFETY 5
8. MATERIALS AND EQUIPMENT 5
9. PROCEDURE 6
9.1. Day 0: Preparation of ROPs 6
9.1.1. Seeding Cells 6
9.2. Day 1: Preparation of SAPs 8
9.2.1. Sera Dilution/Infection 9
9.2.2. Processing of SAPs 10
9.2.3. Preparation of Viral Stock Solution 11
9.2.4. Addition of Viral Stock Solution to SAPs and Neutralization of Virus 11
9.2.5. Transfer Neutralization Reaction onto ROPs and Decontamination of SAPs 11
9.3. Day 2: Acquire images 12
9.3.1. Preparation and Addition of Hoechst 33342 Solution to ROPs 13
9.3.2. Image Acquisition and Counting on the Cytation 7 13
9.4. Analysis of Data and Calculation of SARS-CoV-2_NT Titers 13
9.5. SARS-CoV-2_NT Data and Document Review 13
10. REFERENCES 14
Table 6. Form References 14
11. DOCUMENT VERSION MODIFICATIONS 14
Table 7. Detailed Changes 14

FDA-CBER-2021-5683-1072910

Document Approval Record 15

1. HISTORICAL REVISION SUMMARY

Table 1. Chronological Summary of Previous Versions

Version #	Effective Date	Originator	Summary
2.0	Current	Pei-Yong Shi	Approved.
1.1	20200614	Xuping Xie	Revised…
1.0	20200529	Camila Fontes	New Document

2. PURPOSE

This document describes the procedure for a manual 96-well SARS-CoV-2-mNG reporter virus neutralization assay (SARS-CoV-2_NT) designed to measure functional, neutralizing antibodies specific for SARS-CoV-2 in test serum using Cytation 5 Image Reader.

3. SCOPE

The scope applies to all personnel in the Shi Lab at UTMB responsible to support Pfizer clinical study.

NOTE: Procedures for other UTMB and Pfizer groups may vary in format, content, numbering, and approval procedures.

4. GLOSSARY

Table 2. Terms and Definitions

TERM	DEFINITION
Ab	Antibody
Batch	A sample list for an assay. One batch contains all samples tested in a single assay run.
BSC	Biological Safety Cabinet
Cytation 7	Cell imaging multi-mode reader manufactured by Biotek
FBS	Fetal Bovine Serum
FFU	Focus Forming Units
Gen5	Software associated with the Cytation 7

FDA-CBER-2021-5683-1072911

GMT	Geometric Mean Titer
HBSS	Hanks' Balanced Salt Solution
Hoechst 33342	Cell-permeant nuclear counterstain that emits blue fluorescence when bound to dsDNA
Monolayer	Cell culture in which cells are grown in a single homogenous layer
PPE	Personal Protective Equipment
PBS	Phosphate Buffered Saline
ROP	Read Out Plate
SARS-CoV-2_NT	Manual 96-well neutralization assay for the detection of functional antibodies to SARS-CoV-2
SARS-CoV-2 Critical Reagent Reference Form	SARS-CoV-2_NT reference document that contains clinical or developmental study-specific information, eg, viral bank number, viral bank date, viral stock solution dilution, and staining antibody information. One critical reagent form is created prior to the start of each clinical study or assay development activity.
QCS	Quality Control Sample
QNS	Quantity Not Sufficient
SAP	Sample Assay Plate
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SARS-CoV-2-mNG	SARS-CoV-2-mNeonGreen reporter virus
SOP	Standard Operating Procedure
Titer	The reciprocal of the dilution of a test serum required to neutralize virus at a specified percentage of the viral input in a neutralization assay
VSS	Viral Stock Solution
Vero	African green monkey kidney epithelial cells

5. GENERAL

The SARS-CoV-2_NT is a biofunctional assay designed to measure total serum antibody levels capable of neutralizing SARS-CoV-2 infection of cells in culture.

The SARS-CoV-2-NT is a three-day assay, performed manually. On Day 0, African green monkey kidney epithelial cells (Vero) are seeded into 96-well tissue-culture treated plates. On Day 1, serial dilutions of heat-inactivated immune sera in sample assay plates (SAPs) are incubated with SARS-CoV-2-mNG reporter virus for 60 minutes to allow antigen specific antibodies to bind to the virus. The reaction is then transferred onto the Vero cell monolayer read-out plates (ROPs) and allowed to incubate overnight; only non-neutralized virus is able to infect the cells. On Day 2, productive viral infection is detected in live cells using the mNeonGreen expressed by

FDA-CBER-2021-5683-1072912

the reporter virus. Live cells are stained with Hoechst 33342. Both viral foci and live cells are enumerated from the ROPs on a Cytation 7 reader.

A sample titer is calculated as the reciprocal serum dilution at which a specific percentage of the virus is neutralized (eg, 50% or 90% of the virus). This value is referred to as the titer determining value (TDV). To calculate this percentage, an infection rate must first be determined by dividing the number of viral foci by live cells in any given well. The percent neutralization is then determined by comparing the infection rates of the serum containing wells to that of the control wells without serum samples. A titer is calculated for each of the two replicates of a sample and the geometric mean titer (GMT) of the two is reported as the final sample titer.

The initial serum dilution for the SARS-CoV-2_NT is 1:20, which, along with a 9-point 2-fold serum dilution series, enables a potential plate titer range of 20 to 5,120. Samples that receive titers greater than 5,120 are retested after pre-dilution in assay buffer. Samples that fail to neutralize at the TDV when tested at the lowest serum dilution of 1:20 are reported to have a neutralizing titer of 10, one half of the LOD of 20.

A quality control sample (QCS) is run on each assay plate to assess plate and run suitability. Plating of Vero onto the ROPs occurs at least 20 hours prior to the infection of the monolayer. This incubation time allows the cells to adhere to the plate surface and grow to a confluent monolayer to allow for consistent measurement of infection.

6. RESPONSABILITIES

- All personnel must be trained and knowledgeable with the process. Personnel can have multiple roles within the process but may only sign once with the most appropriate approval code.

Table 3. Roles and Responsibilities

Roles	Responsibilities
SARS-CoV-2_NT -Analyst -Coordinator -Data Analyst CF	• Read, understand, and complete training and qualification on this test method. • Read, understand, and complete training on applicable equipment operating manuals, procedures and test methods that are required to execute the procedure described in this document. • Execute this test method and complete SHI-TM-10011-FM01 and SHI-TM-10011-FM02. • Consult with the PI if there are any questions related to the procedure itself or serum samples, if an atypical event is

FDA-CBER-2021-5683-1072913

	observed or if a protocol deviation occurs. • Provides detailed scheduling of all SARS-CoV-2_NT runs. • Creates assay run batches and corrects run information with QNS information from the annotated SHI-TM-10011-FM01. • Prepare SARS-CoV-2_NT assay plates • Maintain and grow Vero cells. • Plate the appropriate concentration of Vero cells
Reviewer XP	• Review SARS-CoV-2_NT documentation to ensure completeness of data entry and compliance with Shi Lab policies. Direct the SARS-CoV-2_NT Analyst to correct documentation errors. • Verify that documentation is complete and compliant.
Supervisor PY	• Read and understand this test method. • Review and approve this test method. • Ensure that SARS-CoV-2_NT analysts executing this test method are trained in the appropriate procedures and test methods. • Provide guidance to SARS-CoV-2_NT analysts and reviewers and answer any questions that arise during the execution of this procedure.

7. SAFETY

- Biosafety level 3 (BSL-3) precautions are required for any work with SARS-CoV-2 virus and has to be handled in accordance with BSL-3 procedures.
    - Prior to starting work at BSL3:
    - Register as BSL3 worker and complete the training and access requirements for BSL3 facilities:
        - UTMB BSL3 training course
        - BSL3 facility specific training and access checklist form
        - BSL3 check off
        - BSL3 mentorship
- All laboratory work is to be carried out in compliance with UTMB safety policies and generally accepted laboratory safety guidelines.
- Refer to Laboratory Safety Reference Manual found on UTMB Website at https://liveutmb.sharepoint.com/:b:/r/sites/collaboration/webfiles/Shared%20Documents/EHS/Website_Files/UTMB%20Safety%20Manual%202019_3-5-20.pdf?csf=1&web=1&e=lKehru

8. MATERIALS AND EQUIPMENT

FDA-CBER-2021-5683-1072914

- Cell bank of Vero African green monkey kidney epithelial cells (ATCC CCL81). Each vial is used from p1 to p35.
- Viral stock of SARS-CoV-2 mNeonGreen, prepared in individual use aliquots (virus construction described in Xie et al 2020). Viral stock is at ~2x10^7 PFU/mL.
- Black µCLEAR flat-bottom 96-well plates (Greiner Bio-one™ 655090)
- Corning™ Clear Polystyrene 96-Well Microplates, round bottom (Corning Cat. No: 3799)
- DMEM (Gibco Cat. No: 11965-092)
- Fetal Bovine Serum (Hyclone Cat. No.: SH30071.03)
- Penicillium-Streptomycin (P/S) (10,000 U/ml) (Gibco Cat. No: 15140-122)
- Gibco™ Trysin-EDTA (0.25%), phenol red (Gibco Cat. No.: 25200072)
- Phosphate Buffered Saline (PBS) solution, pH 7.4 (Gibco Cat. No.: 10010-023)
- Gilson™ Accessory for PIPETMAN Complete Pipetting System
- Reagent Reservoirs 25 ml (Gilson Cat. No.: F267660) & 50 ml (Gilson Cat. No.: F267670)
- Cell Counting slides for TC10™/TC20™ Cell Counter, dual-chamber (Bio-Rad Cat. No.: 1450011)
- Corning™ Cell Culture Treated flasks .2µM Vent Cap T175cm2 (Corning™ Cat. No.: 431080)
- VACUBOY Hand Operator (INTEGRA Biosciences)
- Eppendorf Xplorer pipette, electronic 12 channel pipette 15-300 µL (Eppendorf Cat. No.: 4861000031)
- TC20™ Automated Cell Counter (Bio-Rad)
- Dualfilter 200ul (filtered tips) (Eppendorf Cat. No.:0030077.555)
- Hank's Balanced Salt Solution (HBSS) (1X) (Gibco Cat. No:14025-092)
- Breath-Easy sealing membrane. (Diversified Biotech Cat No.: BEM-1)
- Tissue culture CO_2 incubator
- Cell Imager or inverted microscope
- Microplate Reader (BioTek) (Cytation 7 Cell Imaging Multi-Mode Reader)
- Hoechst 33342 (20mM) (Thermo Scientific Prod # 62249)
- DMEM (1X) (Phenol Red Free) (Gibco Cat. No: 31053-028)
- GlutaMAXTM-1 (100X) (Gibco Cat. No: 35050-061)

9. PROCEDURE

9.1. Day 0: Preparation of ROPs

- Note: Refer to Tables 3-9 of SHI-TM-10011-FM01.
- Ensure the incubators are reading at 37°C/5% CO_2. Confirm that water reservoir is full.

9.1.1. Seeding Cells

- Seed cells into 96-well plate Black Microplates.

FDA-CBER-2021-5683-1072915

- Remove the cotton filter from 2ml serological pipette and assembly on the hand operator of the VACUBOY
- Remove the medium from the cells using the VACUBOY.
- Briefly rinse the cell layer with 25ml PBS (same volume as the growth medium) to remove all traces of serum that contains trypsin inhibitor. Remove PBS using the VACUBOY. Repeat, wash twice.
- Note: make sure rinse every corner of the flask to get rid of any trypsin inhibitor.
- Add 3ml Trypsin-EDTA solution. Observe cells under an inverted microscope until cell layer is dispersed (usually within 2 minutes at RT).
- Note: The activity of trypsin decreases when stored at 4°C. Do not store trypsin at 4°C for > 1 week.
- Hit/tap the flask to detach cells, add 10 mL of complete growth medium and shake the flask to let the medium cover all the trypsinazed cells in order to inhibit the trypsin. Aspirate the cell suspension by gently pipetting and transfer the cell suspension into 50ml falcon tube.
- Centrifuge, RT, 3min, 1200rpm (300g)
- Remove media carefully don't get too close to the cells.
- Resuspend cells in phenol red free media (DMEM phenol red free, 2% FBS, 1% P/S, 2% GlutaMAX). Disperse cells by tapping the tube with finger gently, this is critical steps make sure cells are evenly dispersed. Add 5ml of media, keep tapping and swirling to resuspend, add more media (10-20 ml mix by pipetting up/down). Note: Using phenol red free media reduces the background when reading the plate.
- Count using the cell counter (C-Chip DHC-N01-5). Count live cells. Get a microcentrifuge tube and mix 50ul of trypan blue with 50ul of cell suspension. Add mixture to both sides of counting slide, count both sides to get the average of live cells.
- Dilute to 2.4×105 cells/ml. Add 50 µl of the diluted cell suspension to each well in the 96-well plate (ROP) to reach 1.2×104 cells/well. Note: to make sure the cells are evenly distributed in the 96-well plate. Leave the plate on the bench after seeding about 5-10min, then hit/tap the plate gently to make cells evenly distribute in the wells.
    - Example: 1-96W plate 50ul/well, live cell count= 1.0×106

FDA-CBER-2021-5683-1072916

Example: 1-96W plate 50ul/well, live cell count= 1.0×10^6

$50ul \times 96 = 4{,}800ul = 4.8ml$

Make 5ml cell suspension/plate

To get 1.2×10^4 cells/well we will dilute to 2.4×10^5 cells/ml, take 50ul, and add to each well

total vol $\times$ # cells/mL= total # of cells needed

$$(5ml)(2.4 \times 10^5) = 1{,}200{,}000 = 1.2 \times 10^6$$

$$\frac{\text{total \# of cells needed}}{\text{\# of live cells counted}} = \text{vol of cell suspension mL}$$

$$\frac{1.2 \times 10^6}{1.0 \times 10^6} = 1.2 \text{ mL}$$

total vol $-$ vol of cell suspension mL= volume of media mL

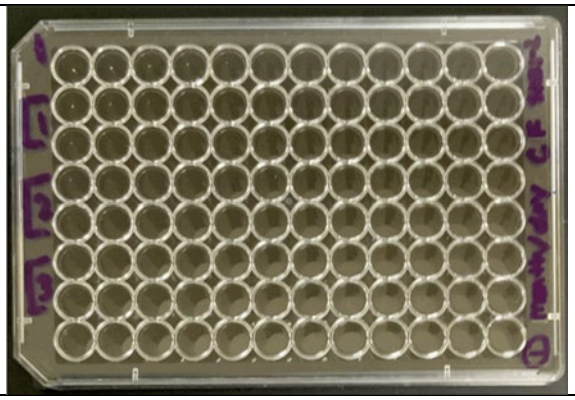
5ml $-$ 1.2ml=3.8ml

To dilute to 2.4×10^5 cells/ml, take 1.2ml of cell suspension and 3.8ml of media (DMEM phenol red free, 2% FBS, 1% P/S, 2% GlutaMAX)

- Add 1-5 ml of the remaining cell suspension to new culture flask to continue to grow cells depending on your needs.
- Incubate cell plates at 37°C. 6. Ensure ROPs have incubated at least 20 hours prior to execution of Transfer of Neutralization Reaction onto ROPs

9.2. Day 1: Preparation of SAPs

- Note: Refer to Tables 3-9 of SHI-TM-10011-FM01.
- Ensure the incubators are reading at 37°C/5% CO_2. Confirm that water reservoir is full.
- Inspect 6 wells at random of 2 ROPs per run under the light microscope to ensure the monolayer is healthy with no microbial contamination and has ≥80% confluency. A healthy Vero monolayer is evenly dispersed squamous cells adhered to the bottom of the ROP well. Record abnormalities in Table 13 of SHI-TM-10011-FM01.
- ROP. Label and number each plate appropriately (typically 1-12) in the Biosafety hood with marker and store in 37 °C/5% CO_2 until transfer.

ROP lid labeling	

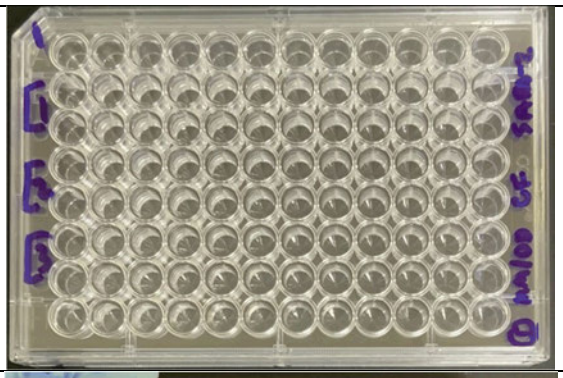
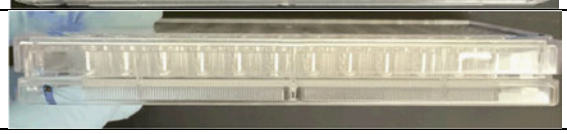
FDA-CBER-2021-5683-1072917

ROP side labeling	

- Heat inactive all the sera at 56°C, 30mins (If needed)

9.2.1. Sera Dilution/Infection

- SAP. Take a new 96-well plate with lid (clear plate, for dilutions). Label and number each plate appropriately (typically 1-12) in the Biosafety hood with marker.

SAP lid labeling	
SAP side labeling	

-
- Prepare serial dilutions of serum. Refer to SHI-SOP-10002 to process samples.
- Prepare 10 serials of 2-fold dilutions. The highest dilution is 10 folds. Prepare duplicates. See step 5

D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
10	20	40	80	160	320	640	1280	2560	0

D1: 6 µl + 56 µl 2% FBS medium.
D2-D9: 30 µl diluted samples + 30 µl medium.

- Transfer proper medium (according to cell type, phenol red free DMEM+2%FBS+1%P/S) to the 50ml reservoir to facilitate the use of a multichannel pipette (each plate needs 3.3ml, aliquot 4ml for each plate)
- Skip the first column. The outer wells are not used.
- Columns #3-11 add 30 µl DMEM+2%FBS+1%P/S.
- Notes: Columns 3-10, add 30 µl DMEM+2%FBS+1%P/S for 2-fold serial dilution from column 3. Column #11 on the 96W plate is control without any antibody.
- Columns #3-10, add 30 µl DMEM+2%FBS+1%P/S for 2-fold serial dilution from column 3
- Column #1 and 12, add 60 µl DMEM+2%FBS+1%P/S.

FDA-CBER-2021-5683-1072918

- Column #2 add 54 µl DMEM+2%FBS+1%P/S, then mix with 6 µl serum. Mix without creating bubbles. Note: the starting dilution is 1:10, then followed with 10, 20, 40, 80, 160, 320, 640, 1280, 2560 dilutions (refer to step 4). Overall dilution will be 1:20 once virus is added.
- Column #2, mix using an electronic 12-channel pipette (settings, P/M, 30/50, speed 6/4, 5X), discard the tips
- Load new tips, transfer 30 µl from column #2 to column #3 (settings, P/M, 30/50, speed 6/4, 5X), discard the tips
- Repeat step 12 for column #4 to #10
- Attention: remember to take 30 µl from column #10 after dilution and discard. The total volume of the dilute serum should be 30µl/well
- Sera dilution plate set-up (SAP). Samples are run in duplicates. The outer wells are not used for dilutions, just add media. Column 11 is reserved for viral input (wells B11-G11). Column 12 is reserved for cell control to monitor cell contamination (wells B12-G12). Row H is reserved for a QCS (wells H2 H10). The QCS is used to assess plate and run suitability.

<table>
<tr><td>Well</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td><td>8</td><td>9</td><td>10</td><td>11</td><td>12</td></tr>
<tr><td>Reciprocal Serum Dilution</td><td>N/A</td><td>20</td><td>40</td><td>80</td><td>160</td><td>320</td><td>640</td><td>1,280</td><td>2,560</td><td>5,120</td><td>N/A</td><td>N/A</td></tr>
<tr><td>A</td><td rowspan="8">Media + Virus</td><td colspan="9">Media + Virus</td><td colspan="2"></td></tr>
<tr><td>B</td><td colspan="9">Test Sample 1 (Virus+Serum)</td><td rowspan="7">Virus input control</td><td rowspan="7">Cell control</td></tr>
<tr><td>C</td><td colspan="9">Test Sample 1 replicate (Virus+Serum)</td></tr>
<tr><td>D</td><td colspan="9">Test Sample 2 (Virus+Serum)</td></tr>
<tr><td>E</td><td colspan="9">Test Sample 2 replicate (Virus+Serum)</td></tr>
<tr><td>F</td><td colspan="9">Test Sample 3 (Virus+Serum)</td></tr>
<tr><td>G</td><td colspan="9">Test Sample 3 replicate (Virus+Serum)</td></tr>
<tr><td>H</td><td colspan="9">Quality Control Sample (Virus+Serum)</td><td colspan="2">Media + Virus</td></tr>
</table>

9.2.2. Processing of SAPs

- Transfer SAPs and ROPs to BSL3.
- Note: Refer to Tables 3-9 of SHI-TM-10011-FM01.
- Ensure the incubators are reading at 37°C/5% CO_2. Confirm that water reservoir is full.
- Confirm a correct set of assay plates.
- Inspect plates to confirm that all wells of each SAP contain approximately 30 µL liquid and that volumes are consistent from well to well. Use the Atypical Event(s) box (Table 13 of SHI-TM-10011-FM01) to record the location of any wells that have no liquid or appear to have a volume other than 30 µL. Do not use a pipette to measure well volumes; this step is a visual inspection only.

FDA-CBER-2021-5683-1072919

- Number each SAP with a marker. The hand-written number dictates the order in which the SAPs will receive a reagent and corresponds to its respective ROP.
- Place ROPs in incubator.

9.2.3. Preparation of Viral Stock Solution

- Note: Refer to Table 7 of SHI-TM-10011-FM01.
- Perform all manipulations of reagents and active virus in a Level II biosafety cabinet within the BSL-3 laboratory using sterile techniques.
- In BSC add 30 µl diluted SARS-CoV2-mNG virus. 1µl reporter virus + 29µl media/well (phenol red free). Any new batch of virus should be tested before use. Use the volume of virus that can cause about 30% infection rate at 24 h post-infection.
- Complete Viral Stock Solution (VSS) Table; verify with another analyst.
- Remove the appropriate number of frozen viral stock vials from -80 °C freezer.
- Thaw virus in distilled water at room temperature.
- If multiple vials are needed, pool all vials into a larger tube and pipette up and down 3 times to ensure proper mixing.
- Label a sterile bottle “VSS”. Add the appropriate volume of Assay Media.
- Add the appropriate volume of SARS-CoV-2-mNG into VSS bottle and gently swirl to mix.

9.2.4. Addition of Viral Stock Solution to SAPs and Neutralization of Virus

- Transfer diluted virus to the 25ml reservoir to facilitate the use of a multichannel pipette (each plate needs ~2ml, 60 wellsx30µl=1800µ, always make 1ml extra to the total volume to avoid running out)
- Add 30µl of diluted virus to each well of the plate which containing diluted sera (SAP). Mix using an electronic 12-channel pipette (settings, P/M, 30/50, speed 6/4, 5X), after each column discard the tips
- Incubate at 37°C/5% CO_2 for 1 hour.
- Record the start and end times for this incubation in Table 8 in SHI-TM-10011-FM01.

9.2.5. Transfer Neutralization Reaction onto ROPs and Decontamination of SAPs

- Note: Refer to Table 9 of SHI-TM-10011-FM01.
- Remove ROPs from incubator.
- Transfer 50µl virus-Ab diluted plate (SAP) to 96-well plate seeded on day 0 (ROP) (plates containing cells 1.2x104 in 50 µl/well, after adding the diluted reporter virus the total volume of each well will be 100µl). Mix using an electronic 12-channel pipette (settings, P, 50/80, speed 3/3, 3X), after each column discard the tips. Note: do not touch the bottom of the plate, add the mixture on the side of the well, make sure the pipette tip is touching the media to avoid making bubbles when mixing. It is very important to avoid bubbles, mix well, and do not disturb the cells to get an even infection.

FDA-CBER-2021-5683-1072920

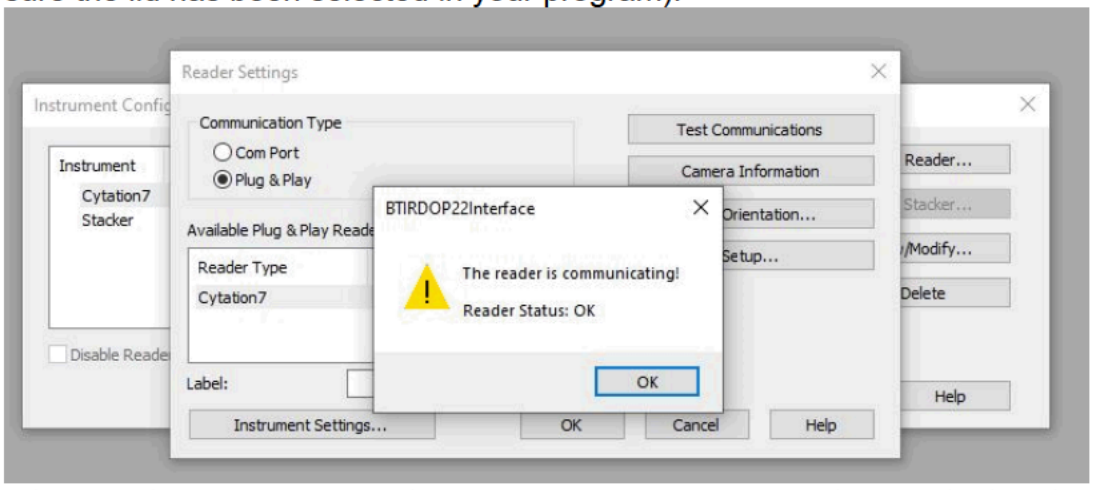
- ROP. Cells+Ab+reporter plate set up (black plate)

		1	2	3	4	5	6	7	8	9	10	11	12
	A												
Sample 1	B		+V	+V	+V	+V	+V	+V	+V	+V	+V	+V	No virus
Sample 1	C		+V	+V	+V	+V	+V	+V	+V	+V	+V	+V	No virus
Sample 2	D		+V	+V	+V	+V	+V	+V	+V	+V	+V	+V	No virus
Sample 2	E		+V	+V	+V	+V	+V	+V	+V	+V	+V	+V	No virus
Sample 3	F		+V	+V	+V	+V	+V	+V	+V	+V	+V	+V	No virus
Sample 3	G		+V	+V	+V	+V	+V	+V	+V	+V	+V	+V	No virus
	H												

- Incubate ROPs at 37°C5% CO_2 for 16-24HR.
- Record the time the ROPs were placed in incubator in Table 9 of SHI-TM-10011-FM01.

9.3. Day 2: Acquire images

- Note: Refer to Tables 10-12 of SHI-TM-10011-FM01.
- At 16-24 h post-infection, read the plate using Cytation 7 Cell Imaging Multi-Mode Reader
- Prior to imaging, carefully examine the instrument CytationTM 7, bioStackTM 4 robotor and the workplace. Please ensure no used plates or any boxes blocking the plate carrier.
- Power on the instrument, wait about 5 minutes to warm up the instrument. Green light will show on the machine if instrument if it is ready. Open the Gen5 software from the instrument attached computer and make sure the software communicates with the instrument. Choose the right program for reading (Since all the reading will be performed with plate lid-on, please make sure the lid has been selected in your program).



- If the bioStackTM 4 is going to be used for your experiment, place test-plates (set of empty plates dedicated for testing only) on the bioStackTM 4 robotor

FDA-CBER-2021-5683-1072921

and run a quick test to verify the system and protocol. Ensure the bioStackTM 4 robotor transfers the plates smoothly.

- If the instrument is under any abnormal conditions, do not use the instrument. Notify the lab manager or PI immediately.
- Weekly System Checks and Calibration plate instructions are described in SHI-SHOP-10006

9.3.1. Preparation and Addition of Hoechst 33342 Solution to ROPs

- Carefully transfer the plates in a secondary container from the incubator to the biosafety cabinet.
- Add 25 µl diluted Hoechst 33342 Solution (500-fold in HBSS) to each well of the 96-well plates.
- Seal the plates carefully with films.
- Incubate plate at 37°C for about 20 minutes.

9.3.2. Image Acquisition and Counting on the Cytation 7

- After surface decontamination with calvicide, carefully transfer the plate with a secondary container to CytationTM 7 or BioStackTM 4 to acquire images by following the operation manual attached to the instrument.
- The individual who performed scanning and counting completes the “Cytation 7 Analyst” section of Table 1 of SHI-TM-10011-FM01 and enters the date that these steps were completed.
- At the end of the experiment, remove the plate from the carrier and transfer it to the biosafety cabinet. Use 70% ethanol to wipe out the plate carrier. And power off the instrument. Disinfect and dispose all the plates and liquid properly according to the laboratory waste disposal procedures.

9.4. Analysis of Data and Calculation of SARS-CoV-2_NT Titers

- The individual who performs data analysis completes the “SAS Operator/Data Analyst” section on Table 1 of SHI-TM-10011-FM01 and enters the date the steps were completed.
- SARS-CoV-2_NT serum titers are calculated, and data is interpreted according to the instructions outlined in SHI-SOP-10011
- Complete Table 14 of SHI-TM-10011-FM01.

9.5. SARS-CoV-2_NT Data and Document Review

- The individual who performs data and document review completes the “Reviewer” section on Table 1 of SHI-TM-10011-FM01 and enters the date the steps were completed.
- Assembly of SARS-CoV-2_NT data packages, review of worksheets to confirm that proper documentation practices are followed, and review and processing of data are described in SHI-SOP-10012. Checklists and comments for the documentation reviews are provided in SHI-TM-10011-FM01 Tables 15 and 16.

FDA-CBER-2021-5683-1072922

10. REFERENCES

Xie X, Muruato M, Lokugamage KG et al, An Infectious cDNA Clone of SARS-CoV-2. *Cell Host & Microbe* 2020, 27 (5): 841-848.

Table 6. Form References

Document	Title
SHI-SOP-10011	NT50 and NT90 calculations for SARS-CoV-2 Neutralization Assay
SHI-SOP-10012	Data transferring between UTMB and Pfizer
SHI-SOP-10006	Equipment Management
SHI-TM-10011-FM01	Manual 96-well SARS-CoV-2 Neutralization Assay Worksheet
SHI-TM-10011-FM02	Manual Sample Preparation for the 96-well SARS-CoV-2 Neutralization Assay Worksheet

11. DOCUMENT VERSION MODIFICATIONS

Table 7. Detailed Changes

List detailed changes for documents	List rationale for each change
N/A	N/A

FDA-CBER-2021-5683-1072923

Document Approval Record

Document Name:	SHI-SOP-10011
Document Title:	Manual 96-well Neutralization Assay for the Detection of Functional Antibodies to SARS-CoV-2 in Test Serum using Cytation 7 Image Reader

Signed By:	Date	Signing Capacity
Fontes, Camila	20200614	Author Approval
Shi, Pei-Yong		Final Approval

FDA-CBER-2021-5683-1072924

PERSONS TRAINED IN THIS PROTOCOL:

The following persons state that they have read and understood the protocol and the risk assessment and have been properly trained in the procedures.

Name:	Date:	Signature:	Name and Signature of Trainer: