1 PURPOSE

1.1 Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a newly emerged coronavirus which manifested at the end of 2019 and caused a global pandemic since the beginning of 2020. In the effort to support vaccine development and clinical endpoint testing of vaccine samples a Meso Scale Discovery (MSD) 4-plex Custom Serology Assay was developed at the Vaccine Immunology Program (VIP) for the detection of immunoglobulin G (IgG) specifically recognizing SARS-CoV-2 viral proteins and subunits in serum samples derived from vaccination, natural infection or passive monoclonal antibody transfer. The assay will aid as a measure of immunogenicity endpoints, in different SARS-CoV-2 clinical vaccine trials and research projects.

1.2 The MSD® 384-well Custom Serology Assay/4-plex SARS-CoV-2 assay is a an Electrochemiluminescence Immunoassay (ECLIA) intended for the multi-plex simultaneous quantitative detection of IgG antibodies to SARS-CoV-2 distinct antigens in human serum. The 4-plex SARS-CoV-2 assay (detecting SARS-CoV-2 antigens Spike Protein (S-2P), Receptor Binding Domain (RBD), and Nucleocapsid (N), with a BSA control) is intended for use to aid in identifying volunteers with an adaptive immune response to SARS-CoV-2 viral proteins and subunits after vaccination with experimental SARS-CoV-2 vaccines

2 SCOPE

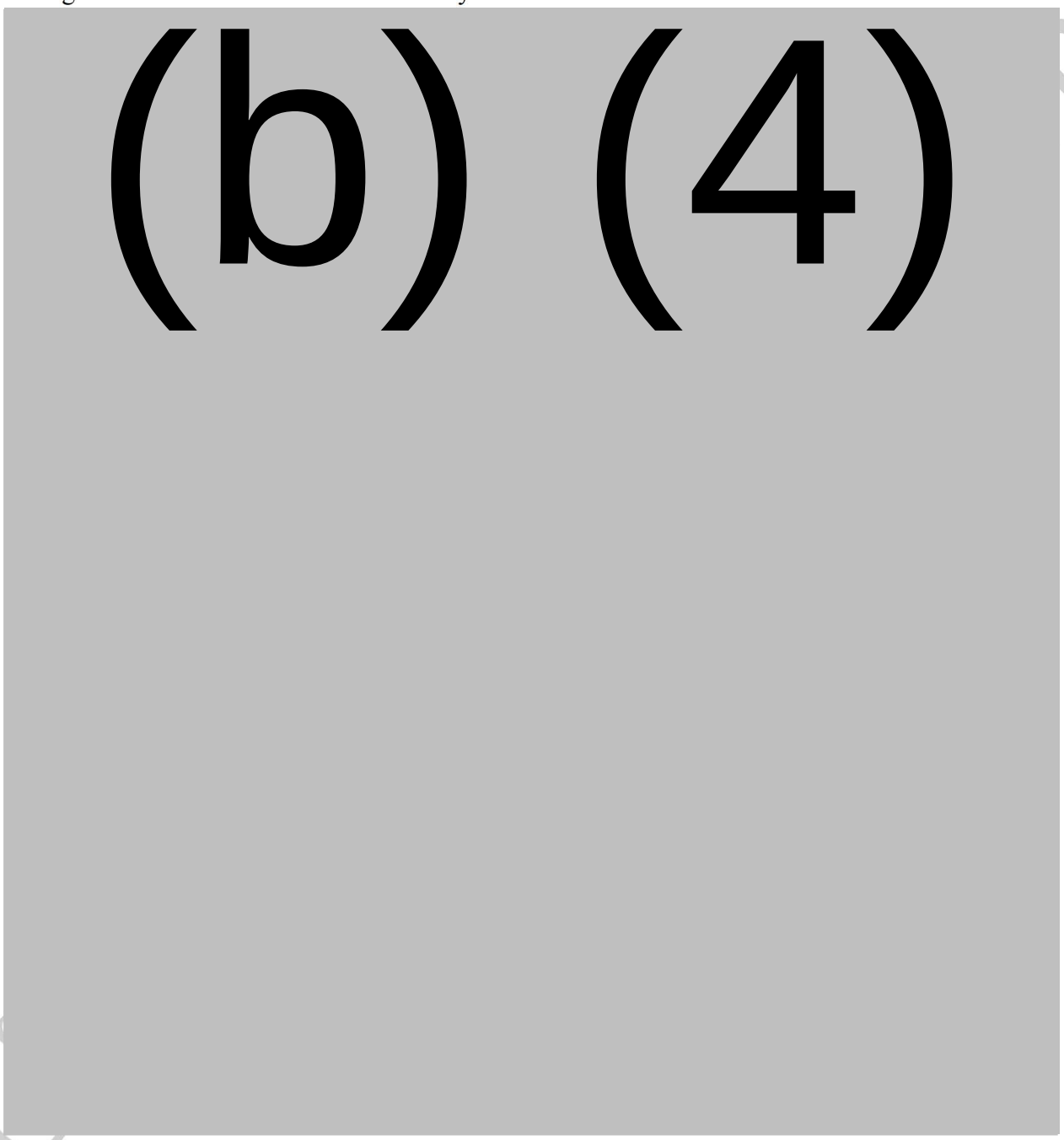
2.1 This SOP describes the necessary steps undertaken to conduct the semi-automated multiplex testing/4-plex SARS-CoV-2 at VIP.

2.2 This SOP applies to all staff testing human sera samples to investigation of SARS-CoV-2 specific antibody responses captured in a multiplex assay format in support of clinical trials or research projects.

2.3 The semi-automated workflow of the 4-plex assay is illustrated below in Figure 1.

Note: The protocol steps described here can also be performed as a manual process only if needed for research projects or exploratory objectives. If used for clinical routine testing the manual version of the assay will need to be qualified as per SOP SOP 2036: Method Development, Qualification and Validation.

Figure 1: Semi-automated 4-Plex Assay Workflow



3 RESPONSIBILITES

3.1 VIP Quality Assurance unit (QAU) is responsible for the control of this SOP, the implementation of this procedure and for ensuring that all appropriate personnel are trained.

3.2 VIP staff assigned as initiators in EDMS have the authority to establish and revise this procedure

3.3 All VIP staff working on this procedure are responsible for reading and understanding this SOP prior to performing the procedures detailed herein

4 REFERENCES

4.1 MSD Custom Serology Assay Protocol 384-well, 26May2020

4.2 SOP 2036: Method Development, Qualification and Validation

4.3 SOP 2037: Sample Management

4.4 SOP 3013: Operation and Maintenance of Automation Robotic Arms

4.5 SOP 3202: Operation and Maintenance of the (b) (4)

4.6 SOP 3536: Use and Maintenance of the (b) (4)

4.7 SOP 3538: Use and Maintenance of MSD Multi-Array System

4.8 SOP 3541: Operation and Maintenance of the (b) (4)

4.9 SOP 3544: Operation and Maintenance of the (b) (4) Automated Plate washer and (b) (4)

4.10 SOP 3545 Operation and Maintenance of the (b) (4) Water Purification System

4.11 SOP 4106 Preparation of MSD Blocking Buffer, Wash Buffer and Assay Diluent

4.12 SOP 4109: (b) (4) of Clinical Samples

4.13 SOP 5510: Automated Sample Addition to (b) (4) Plate

4.14 SOP 6018: Analysis, QC and Data Output of SARS-CoV-2 Multiplex ECLIA Data for clinical trials

4.15 SOP 8000: Sample Receipt and Check in of Biological Samples

4.16 SOP 8004 Accessioning of Specimens into (b) (4)

4.17 FDA Bioanalytical Method Validation, Guidance for Industry, May 2018 (Biopharmaceutics)

5 RELATED FORMS/ATTACHMENTS

5.1 F-5525A. 4-plex SARS-CoV-2 Assay Data Review Form

5.2 F-5525B. 4-plex SARS-CoV-2 Assay Sample Retesting Form

5.3 F-5525C .4-plex SARS-CoV-2 Assay Specific Run Instructions

5.4 4-plex SARS-CoV-2 Automation Report from (b) (4)

6 ABBREVIATIONS USED IN THIS DOCUMENT

6.1 BSA: Bovine Serum Albumin

6.2 ECLIA: Electrochemiluminescence Immunoassay

6.3 EDMS: Electronic Document Management System

6.4 IgG: Immunoglobulin G

6.5 MSD: Meso Scale Discovery

6.6 N: nucleocapsid protein

6.7 PPE: Personal Protective Equipment

6.8 QA: Quality Assurance

6.9 RBD: Receptor Binding Domain

6.10 RPM: Rounds per Minute

6.11 S-2P: SARS-CoV-2 spike protein

6.12 SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

6.13 VIP: Vaccine Immunology Program

7 SAFETY

7.1 Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Additional product-specific safety information is available in the safety data sheet (SDS), which can be obtained from MSD Customer Service

7.2 Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

7.3 Follow SOP for Sample Receipt and Check in of Biological Samples (SOP 8000) and Treat all specimen/aliquots as potential infectious and wear appropriate PPE.

8 SUPPLIES, REAGENTS, and EQUIPMENT

8.1 MSD 4-plex SARS-CoV-2 Kit components:

8.1.1 MSD 384-well, 4-Spot Custom Serology SECTOR® plates pre-coated with SARS-COV2 S-2P, N, RBD and BSA protein (MSD Part No. N35356A-1)

8.1.2 MSD SULFO-TAG™ Anti-human IgG Detection antibody (MSD Part No. D21ADF-3)

8.1.3 MSD Blocker A Kit (MSD Part No. R93BA-1)

8.1.4 MSD Diluent 100 (MSD Part No. R50AA-1)

8.1.5 MSD Wash Buffer (20x) (MSD Part No. R61AA-1)

8.1.6 MSD GOLD™ Read Buffer B (MSD Part No. R60AM-4)

8.1.7 MSD Kit Reference Standard 1 Adhesive Plate seals

Note: Refer to Procedure Section, 10 below for reconstitution, standard, control and reagent preparation

8.2 Reference Standard and control samples

8.2.1 MSD Kit Reference Standard 1 (MSD Part No. C00ADK-1)

8.2.2 MSD Kit Serology Control Pack 1 (MSD Part No. 3-3081-420276-A, 3-3081-420277-A, 3-3081-420278-A)

8.2.3 Blank control - MSD Diluent 100 (MSD Part No. R50AA-1)

(b) (4)

(b) (4)

8.3 Consumables and reagents not provided by the MSD 4-plex SARS-CoV-2 kit are listed in Table 1 below.

Note: Vendors and catalogue numbers listed are representative; equivalent items may be used by the laboratory.

Table 1. 4-plex Assay Reagent and Material

Reagent/Material	Manufacturer	Catalogue number
96 and 384 polypropylene (b) (4) plates for stock sample preparation	(b) (4)	
96 and 384 polypropylene plates for sample dilution		
Deionized water for buffer reconstitution		
Media Reagent Bottle (125mL, 250mL and 500mL		
(b) (4) pipet tips (96 and 384 well tips, P 250, P50, P30XL)		
15 and 50mL tubes		
Single and Multichannel Micropipette		
Pipet tips (20µL to 1000µL)		
Serological pipets (5-50mL)		
Reagent Reservoirs (5-50mL)		
Flat bottom reagent reservoir		
Plate sealing film		

8.4 Equipment

8.4.1 (b) (4) dual pod liquid handler w/ 96-well (Enhance Selective Tip) and 384-well pipetting heads. (b) (4) (b) (4)

8.4.2 (b) (4) robotic arm robotic arm (b) (4) (b) (4)

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

8.4.4 Automated Plate Washer (b) (4)

8.4.5 (b) (4) Plate Shaker (b) (4)

8.4.6 MSD (b) (4) Instrument and Workbench Discovery Software

8.4.7 Ultra-Low Temperature Freezer (b) (4)

8.4.8 Refrigerator (b) (4)

8.4.9 Water Bath (b) (4)

8.4.10 Models: (b) (4)

8.4.11 Thermometer (b) (4)

8.4.12 Biological Safety Cabinet (b) (4) (b) (4)

8.4.13 Three Display Timer (b) (4)

8.4.14 Single and Multichannel Micropipette (b) (4) (b) (4)

9 PROCEDURES

9.1 Best Practices

9.1.1 Practices apply for manual as well as semi-automated procedures. Refer to Figure 1 for an overview of the stepwise workflow

9.1.2 Assay incubation steps should be performed between (b) (4) to maximize consistency in signals between runs.

9.1.3 Avoid prolonged exposure of detection antibody (stock or diluted) to light. Thaw and add detection antibody shortly before the detection step. During the antibody incubation step, plates do not need to be shielded from light except for direct sunlight.

9.1.4 For manual pipetting: avoid bubbles in wells at all pipetting steps. Bubbles may lead to variable results; bubbles introduced when adding read buffer may interfere with signal detection.

9.1.5 For manual pipetting: Use reverse pipetting when necessary to avoid introduction of bubbles, and for empty wells, pipette to the bottom corner.

9.1.6 For manual pipetting: Gently tap the plate to remove residual fluid after washing

9.2 All workflow steps will be recorded in LIMS (b) (4) in the Automation Entry Form for SARS-CoV-2 4-plex. For the Automation Entry Form, a report with key quality indicators will be printed from LIMS and reviewed by the laboratory management and QAU. In the event the LIMS system is down or cannot be used, the data is also recorded on the run specific instructions, provided by laboratory management.

9.3 Each run will be assigned a unique run number which will be recorded in the study folder and on the 4-plex SARS-CoV-2 Master Run List, located on the VIP T-drive.

9.4 Reagent preparation

9.4.1 4-plex Blocker A Solution

9.4.1.1 Prepare 4-plex Blocker A solution according to SOP 4106 Preparation of MSD Blocking Buffer, Wash Buffer and Assay Diluent.

9.4.2 4-plex Wash Buffer

9.4.2.1 Prepare 4-plex Wash Buffer according to SOP 4106 Preparation of MSD Blocking Buffer, Wash Buffer and Assay Diluent.

9.4.3 4-plex MSD Assay Diluent 100

9.4.3.1 MSD Assay Diluent 100 is provided by the vendor as ready to use solution without further dilution.

9.5 Preparation of calibrator and control (b) (4) plate

9.5.1 Prepare an assay calibrator and control (b) (4) (b) (4) in a 96-well (b) (4) -plate.

Note: The reference standard/calibrator and controls must be prepared fresh each day. Discard any remains at the end of the assay day.

9.5.2 The assay calibrator and control (b) (4) plate contains serially diluted calibrator, MSD control, and (b) (4) as indicated in Figure 2 below:

Figure 2. Layout of Assay Calibrator and Control (b) (4) Plate

(b) (4)

9.5.3 The following reference and control material will be used during routine testing of samples in the MSD® 384-well Custom Serology Assay/4-plex SARS-CoV-2 assay.

9.5.4 MSD Calibrator

(b) (4)

9.5.5 MSD positive controls

9.5.5.1 Serology positive control pack 1 is provided by MSD as listed below:

9.5.5.1.1 MSD Control 1.1 (High)

9.5.5.1.2 MSD Control 1.2 (Medium)

9.5.5.1.3 MSD Control 1.3 (Low)

9.5.5.2 (b) (4)

(b) (4)

9.5.6

(b) (4)

(b) (4)

9.5.6.3 Blank control wells will receive MSD Assay Diluent 100 only.

9.5.7 Add MSD and VIP controls to well location as indicated in Figure 2: Layout of Assay Calibrator and Control (b) (4) Plate in Section 9.5.2.

9.5.8 The volumes of controls to be added are listed below in Table 2 as required for (b) (4)

9.6 Assay Preparation

9.6.1 (b) (4)

9.6.2

Table 2. Required Control Volumes

(b) (4)

9.7 Sample Preparation

9.7.1 Test Serum samples should be (b) (4) according to SOP 4109: (b) (4) of Clinical Samples prior to assay day, except for VIP (b) (4) Test sample (b) (4) (b) (4) or otherwise indicated) which was (b) (4) already.

9.7.2 Sample addition to (b) (4) plate

9.7.2.1 (b) (4)

9.7.2.2

9.7.2.3

9.7.2.4

9.7.2.5

9.7.2.6

Table 3. Layout of Sample (b) (4) Plate

(b) (4)

(b) (4)

(b) (4)

9.8 Automation instrument preparation and automated method instruction

9.8.1 Plate Washer daily set up

9.8.1.1 Follow SOP 3544: Operation and Maintenance of the (b) (4) Automated Plate Washer and (b) (4) Automated for daily set up (b) (4) plate washer.

9.8.1.2 (b) (4)

9.8.2 Liquid handler initial daily set up

9.8.2.1 Follow SOP 3202: Operation and Maintenance of the (b) (4) for daily set up of (b) (4) (b) (4) liquid handlers.

9.8.3 Automated method setup:

9.8.3.1 Automated methods are used in the assay workflow as illustrated in Figure 1. Refer to Table 4 below for specific method for each step:

9.8.4 Open the automated method as indicated in the table above for each specific step and set up the required labware as described in SOP 3202: Operation and Maintenance of the (b) (4)

Table 4. Automated Methods

Workflow Step	Method	Description
(b) (4)		

9.8.5 Place required tip boxes, reagent reservoirs w/ indicated volume of buffers, sample (b) (4) plates, (b) (4) plates, and assay plates on the deck positions as indicated by the Labware Report generated by the automated method. Label all plates and reservoirs before placing them on the deck positions.

9.8.6 Run the automated method after confirming correct position of the labware and the volumes of reagent in the labware.

9.9 4-plex SARS-CoV-2 assay set up

9.9.1 Follow the same workflow steps as described below if the assay is conducted manually. Exchange the robotic pipetting steps on the automated handler for manual pipetting.

9.9.2 Blocking of assay plate

9.9.2.1 Add 4-plex Blocker A solution using (b) (4) Method: CoV MSD Blocking as described in Section 9.8.

9.9.2.2 Add sufficient 4-plex Blocker A solution to the blocking buffer reservoir according to Table 5 below.

9.9.2.3 (b) (4)

(b) (4)

Table 5. 4-plex Blocker A Solution Volume

Buffer	Volume (mL) to Add to Reservoir
Block A Solution	(b) (4)

9.9.3 (b) (4)

9.9.4

9.9.5

9.9.5.2 Run (b) (4) Method: CoV (b) (4) Transfer as described in Section 9.8.

Figure 4. Assay Plate Sample Layout

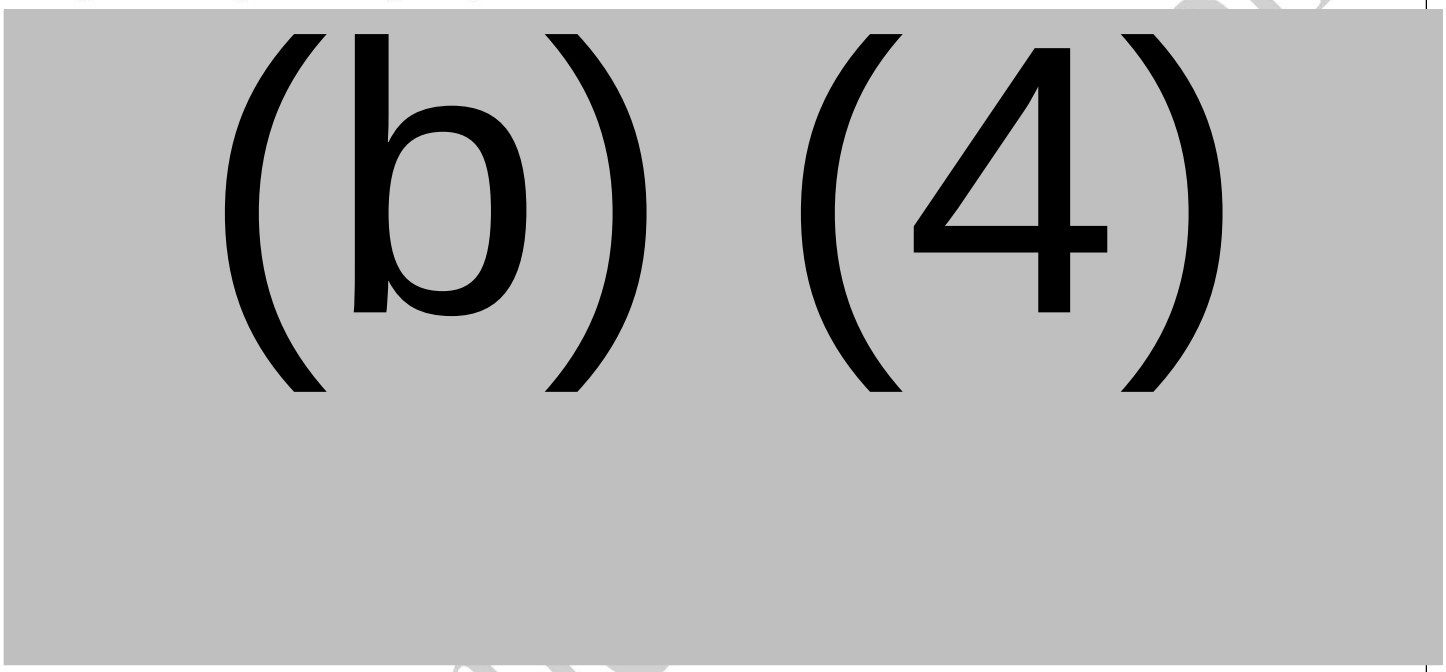


Figure 5. Assay Plate (b) (4) Layout

(b) (4)

9.9.5.3

9.9.5.4

(b) (4)

Figure 6.

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

9.9.6 Addition of detection antibody solution to assay plate(s).

9.9.6.1 Shortly before end of test sample and control incubation, prepare 1X working detection antibody solution within 30 minutes of usage.

9.9.6.2 Remove stock 200X MSD sulfo-tag anti-human IgG antibody from refrigerator.

9.9.6.3 Prepare 1X working detection antibody solution be diluting stock 200X MSD sulfo-tag anti-human IgG antibody in MSD Assay Diluent 100 according to volumes indicated in Table 6 below.

Table 6. 1X Working Detection Preparation

(b) (4)

9.9.6.4 Remove plate sealer manually from plates after (b) (4) (b) (4) incubation.

9.9.6.5 Run (b) (4) Method: CoV Detection as described in Section 9.8. Add the 1X Detection Solution into the detection solution reservoir as indicated in Table 6 above.

9.9.6.6 The assay plate will be washed and (b) (4) per well of the 1X detection antibody solution will be added to the assay plate.

9.9.6.7 Seal assay plate manually with plate sealer and incubate for (b) (4) (b) (4)

9.9.7 Addition of read buffer to assay plate.

9.9.7.1 Remove plate sealer manually.

9.9.7.2 Run (b) (4) Method: CoV Read Buffer as described in Section 9.8.

9.9.7.3 Add sufficient volume of MSD GOLD Read Buffer B to the read buffer reservoir as indicated in Table 7 below.

Table 7. MSD GOLD Read Buffer B Volume

Buffer	Volume (mL) to Add to Reservoir
MSD GOLD Read Buffer B	(b) (4)

9.9.7.4 The assay plate will be washed and (b) (4) per well of the read buffer will be added to the assay plate.

9.9.8 Reading of assay plate.

9.9.8.1 No additional incubation time is required before reading the plate.

9.9.8.2 Manually load the assay plate on MSD reader.

9.9.8.3 Read assay plate on the MSD instrument within (b) (4) of adding read buffer.

9.9.8.4 The raw data file is automatically saved in MSD Discovery Workbench software's database. Save an additional copy

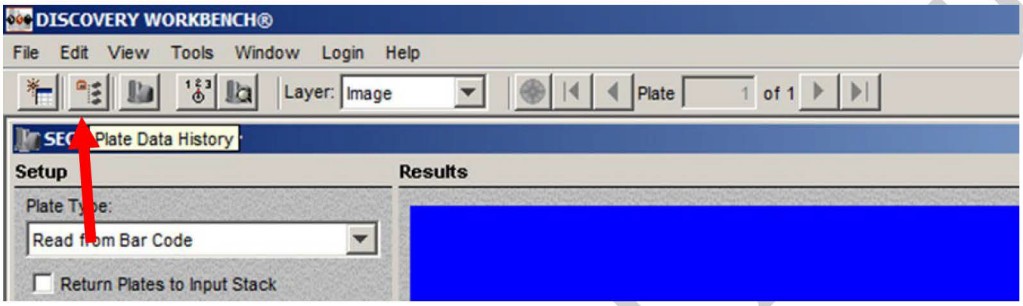
of the raw data file in the shared drive assay specific folder located here: (b) (4)

10 CALCULATIONS and INTERPRETATIONS of RESULTS

10.1 Data analysis on MSD Discovery Workbench software

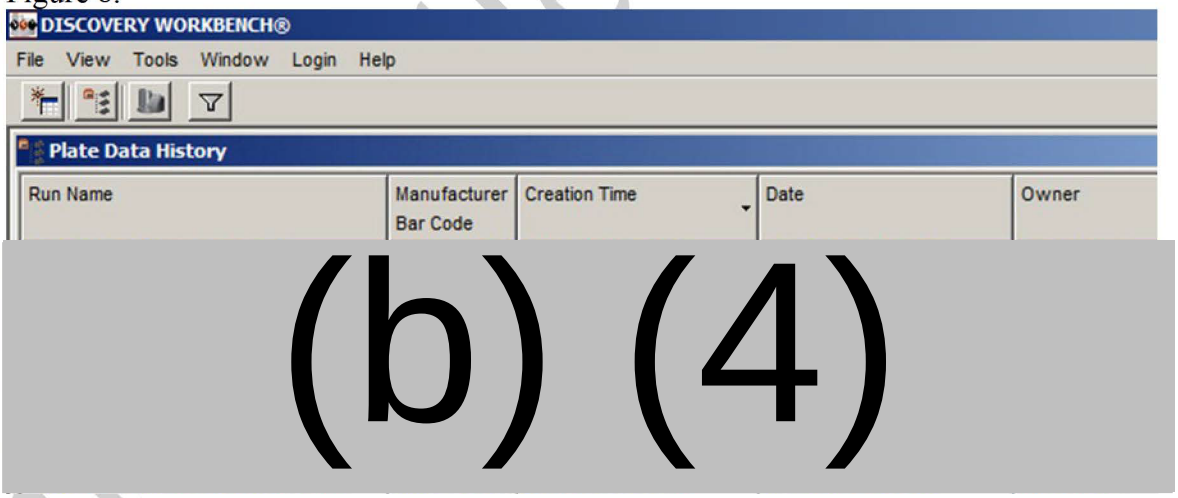
10.1.1 Open MSD Discovery Workbench software and select Plate Data History button (Figure 7).

Figure 7.



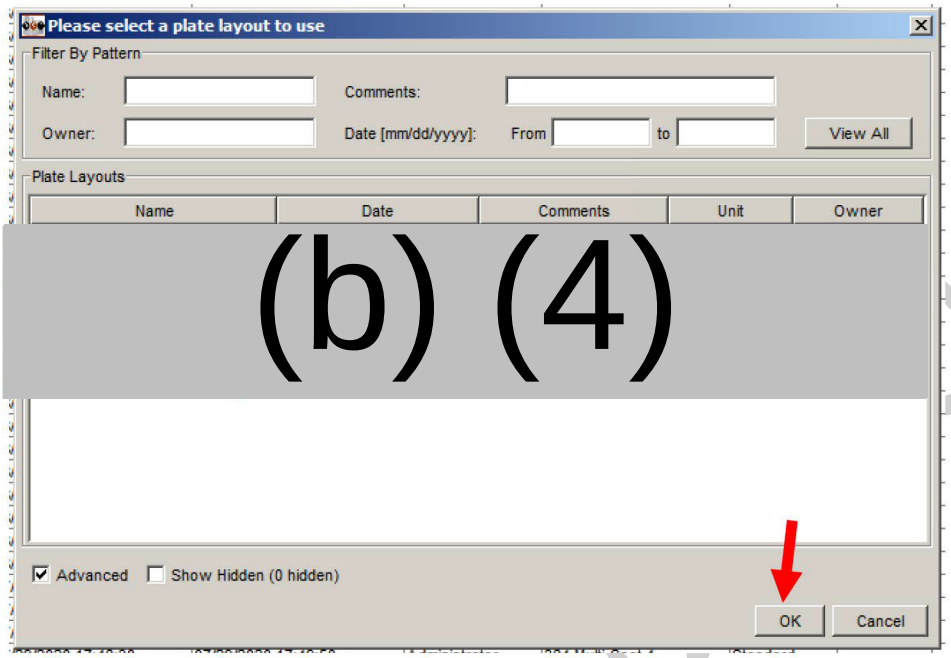
10.1.2 Select the run/plate, use right click on mouse and select Analyze Plates for analysis (Figure 8)

Figure 8.



10.1.3 Select SARS-COV 4Plex (b) (4) plate layout and click on OK. (Figure 9)

Figure 9.



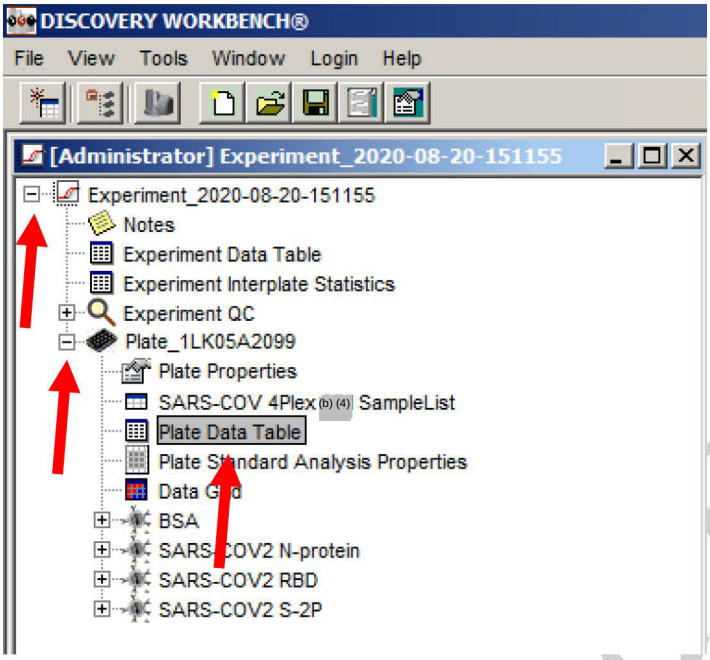
10.1.4 MSD Discovery Workbench software will analyze the ECL and assigned unit of MSD calibrator with non-linear 4-parameter logistic (pl) regression.

10.1.5 The ECL measurement from each well of the controls and samples will be interpolated on the calibration curve for assigned unit (AU/mL). The interpolated assigned unit will be calculated with the dilution factor to obtain a recovered assigned unit.

10.1.6 The analyzed data will be rendered in an automatically generated Experiment (Figure 10).

10.1.7 Expand the Plate and navigate to Plate Data Table, double click to open the table (Figure 10).

Figure 10.



10.1.8 A result table will be displayed, right click on the title of any column and select Save Table As to save the table as CSV file in in the shared drive assay specific folder located here: (b) (4) (b) (4)

10.1.9 For data reporting, if the control or sample ECL is less than the Calibrator's lowest ECL range, then report (b) (4) AU/mL.

10.1.10 If the control or sample ECL is greater than the Calibrator's highest ECL range, then repeat the sample at higher starting dilution.

10.1.11 (b) (4)

(b) (4)

10.2 Data Review will be performed using F-5525A: 4-plex SARS-CoV-2 Assay Data Review Form

10.2.1 Enter 4-plex SARS-CoV-2 Run number onto the Review Form

10.2.2 Laboratory Manager checks:

10.2.2.1 that the data package provided by the technician is complete

10.2.2.1.1 Test Sample Pull list

10.2.2.1.2 (b) (4) Worksheet

10.2.2.1.3 Automation (b) (4) Report

10.2.2.1.4 Reagent Preparation Worksheets

10.2.2.2 Controls passed and QC Test sample are in range

11 MAINTENANCE, CALIBRATIONS, and CLEANING

N/A

12 CRITERIA for VALID TEST

12.1 Assay Validity

12.1.1 Plate Validity

12.1.1.1 Calibrator curve fit with (b) (4)

12.1.1.2 Calibrator replicate ECL with %CV (b) (4) (b) (4) Calibrators) should be within the %CV range.

12.1.1.3 (b) (4)

12.1.1.4

12.1.1.5

12.1.1.6 (b) (4) controls are expected to fall within the established acceptance range as per document R1020, Addendum I.

12.1.1.6.1 (b) (4)

12.1.1.6.2

12.1.1.7 (b) (4)

	Low	Medium	High
Control	(b) (4)		

12.1.1.8 Blank control has no recovered assigned unit (ECL signal falls below Calibrator's range, <CAL7)

12.1.2 Sample Validity

12.1.2.1 Sample (b) (4) ECL with %CV (b) (4) for signal range covered by the Calibrator's signal range.

12.1.2.2 Sample (b) (4) interpolated concentration with %CV ≤ (b) (4)

12.1.3 If any assay plate fails to satisfy the assay validity criteria, an investigation may be performed to determine an assignable cause, and corrective action will be taken which will necessitate repeating the affected plate or assay run. Use Form F-5525B: 4-plex SARS-CoV-2 assay Sample Retesting Form

12.1.4 If any sample fails to satisfy the sample validity criteria, the sample should be repeated with stated justification. Use Form F-5525B: 4-plex SARS-CoV-2 assay Sample Retesting Form

12.1.5 Assay validity criteria will be exported on the qualified 4-plex Excel Worksheet as per SOP 6018 – Analysis, QC and Data Output of SARS-CoV-2 Multiplex ECLIA Data for clinical trials.

13 REVISION HISTORY

Date of Revision	Description/Revisions Made	Initials and Date
21 Aug 2020	• NEW SOP	(b) (6) 21 Aug 2020
18 SEP 2020	• FDA Validation Guidelines were added to the reference section. • A note was added to describe what will happen in the event that LIMS is unavailable. Form F-5525C was generated and added to the SOP. • Assay Validity criteria were adjusted after pre-validation run.	(b) (6) 18 SEP 2020
26 SEP 2020	• Revision started to include modifications to the SOP as suggested by the FDA review of DMF from 24 SEP2020.	(b) (6) 26 SEP 2020

	• Minor changes in grammar throughout text. Note was added to distinguish between manual and semi-automated procedures. SOP 2036 was added as a reference. • Section 8.2 was added to list information about the reference standard and the controls used. • Note was added under 9.5.1 that the standard and controls are used for routine testing. • Table 4 and 5 were revised to include the new automation dispending method to reduce bubbles in the plates and increase the volume in the reservoir by 5uL. • 10.1.10 and 11 were revised to describe the process of excluding outliers in the raw data due to high CV. • 12.1.1.3 was changed to include a (b) (4) recovery range for (b) (4)	
09 JAN 2021	• Assay Validity Checklist was removed from Form F-5525C. Sentence was added to refer to the data export worksheet, which highlights the assay validity criteria • SOP 6018 – Analysis, QC and Data Output of SARS-CoV-2 Multiplex ECLIA Data for clinical trials was added as reference • Updated Blocker A volumes in Table 5, 1X detection solution volumes in Table 6, and Read Buffer volumes in Table 7. • Added instruction for storing left over sample (b) (4) plate at (b) (4) at section 9.9.3.4. • Included additional criteria in section 12.1.1 plate validity. • Added SOP 8000 Sample Receipt and Check in of Biological Samples in Section 4.	(b) (6) 09 JAN 2021