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Proprietary Information

Title: Report on the Validation of Multiplex Assay (4-plex) for the detection of IgG antibodies against SARS-CoV-2 proteins in human sera.			
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DOCUMENT DEVELOPMENT

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EXECUTIVE SUMMARY

The validation of the Multiplex Assay (4-plex) for the detection of IgG against SARS-CoV-2 S-2P protein was executed according to Document B1001.01: Validation Protocol of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins.

This validation report assesses the performance of the MSD® 384-well Custom Serology Assay/4-plex SARS-CoV-2 assay in the detection of IgG binding to SARS-CoV2 S-2P spike protein. The statistical analysis of the qualification data was provided by NIAID Biostatistics Research Branch (BRB). This assay demonstrated satisfactory performance as illustrated in the statistical report provided by NIAID BRB. The results and statistical analyses are summarized in the result section of this report and the overall determination found in the conclusion section. The statistical report is provided as an Attachment A to this document.

Taken together, the MSD® 384-well Custom Serology Assay/4-plex SARS-CoV-2 assay has been validated for use in the detection of human serum IgG reactive to SARS-CoV-2 S-2P sike protein.

BACKGROUND

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).

The MSD® 384-well Custom Serology Assay/4-plex SARS-CoV-2 assay is 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 MSD 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 spot) is intended for use to aid in identifying volunteers with an adaptive immune response to SARS-CoV-2 S-2P after vaccination with experimental SARS-CoV-2 vaccines in Phase I to Phase III clinical trials.

GENERAL METHODS AND VALIDATION PROTOCOL INFORMATION

Validation was set up and performed according to the latest versions of SOP 5525: Multiplex (4-Plex) Assay for the detection of IgG antibodies against SARS-CoV-2 proteins in human sera and B1001: Validation Protocol of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins.
The assay was performed with a (b) (4) based automation platform including the (b) (4) Plate Washer. In brief, serially dilution standards, control sera and human serum test samples are

added to the precoated wells, and specific antibodies complex with the coated antigens. Antibodies bound to the SARS-CoV-2 viral proteins are detected using a SULFO-TAG™ anti-human IgG detection antibody. A read solution (MSD GOLD™ read buffer) containing electrochemiluminescence (ECL) substrate is applied to the wells, and the plate is entered into the MSD (b) (4) detection system. An electrical current is applied to the plates and areas of well surface which form antigen-anti human IgG antibody SULFO-TAG™ complex will emit light in the presence of the ECL substrate. The (b) (4) detection system quantitates the amount of light emitted and reports the raw ECL signal and the as a result for each test sample and control and/or standard of each plate.

A. REFERENCE STANDARD AND CRITICAL CONTROL REAGENTS

Reference standard, MSD and (b) (4) assay controls as described in SOP 5525 (Multiplex 4-Plex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins in human sera), B1001 (Validation Protocol of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins) and in **Table 1** below were included in each run. The general assay plate layout is shown in Figure 1.

Performance of critical reagents was assessed using assay validity criteria established during development and qualification of the assay and are listed in the assay specific SOP 5525. The reference standard 1 also called calibrator (catalogue number C00ADK-2) and serology control pack 1 (catalogue number C4381-1) were provided by Meso Scale Discovery (MSD). Standard and controls were received at VIP in frozen aliquots on dry ice and immediately stored at -80ºC repository. (b) (4) reference standard and controls were thawed and used promptly and according to assay specific procedures.

(b) (4)

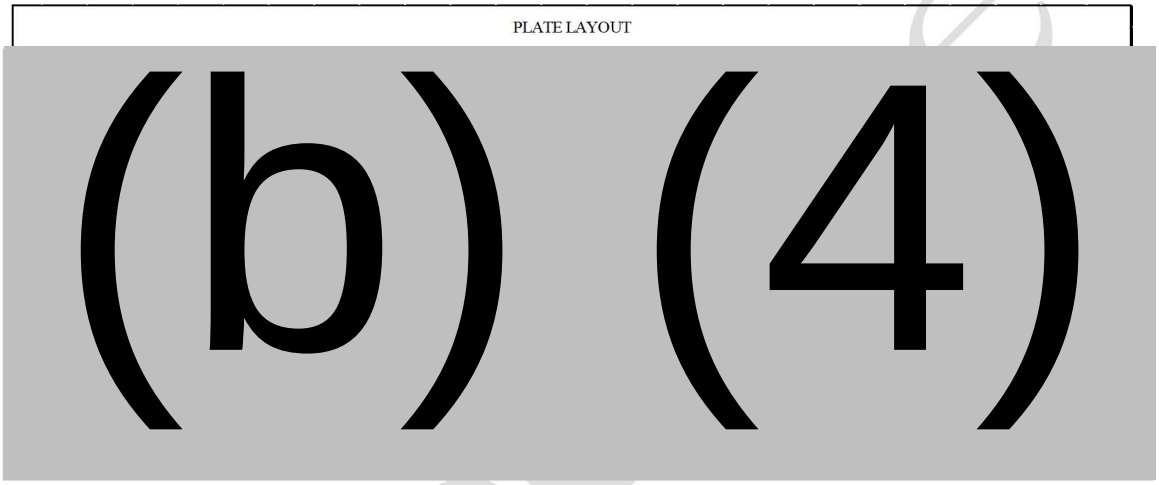
(b) (4) is described in B1001 (Validation Protocol of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins, Attachment A). Small volume aliquots for single use were prepared and stored at appropriate temperature at the (b) (4) (b) (4) controls were thawed and used immediately according to SOP and run specific instructions. An overview of all critical control material and their respective source is provided in Table 1 below. Preparations, dilutions and plating out of reference standard and controls were documented on the runs specific instructions and assay worksheets and were reviewed by Laboratory Management and Quality Assurance Unit (QAU).

Table 1: Standard and control material used during validation and routine testing

Item	Source/Catalogue#	Expiration Date	Storage
Reference Standard	MSD/C00ADK-2	30 JUN 2025	Initially freeze at -80ºC, after thawing keep at 2-8ºC
Positive control 1.1 (high)	MSD/C4381-1	30 JUN 2025	
Positive control 1.2 (medium)	MSD/C4381-1	30 JUN 2025	
Positive control 1.3 (low)	MSD/C4381-1	30 JUN 2025	
(b) (4)			
Blank (MSD Diluent 100)	MSD/R50AA-3	30 NOV 2022	2-8ºC

Figure 1: 4-plex Assay Plate Layout used during validation and routine testing.

Shown is the layout for a 384-well plate, testing (b) (4) serum samples in duplicates in an (b) (4) dilution series (dark blue). The reference standard (CAL, light blue), three MSD controls (orange) and (b) (4) (yellow) are being tested in (b) (4) A (b) (4) the serum test samples (yellow, sample control). Blank negative control (assay diluent) is shown in green.



B. ASSAY REAGENTS

The MSD 4-plex SARS-CoV-2 assay was performed on precoated MSD 4-spot 384-well plates, using reagents and buffers provided by MSD as part of their specific test kit (Table 2). Buffers and solutions were prepared according to SOP 5525 (Multiplex (4-Plex) Assay for the detection of IgG antibodies against SARS-CoV-2 proteins in human sera) and assay specific run instructions. Preparations were documented on worksheets and forms and reviewed by Laboratory Management and QAU.

Table 2: MSD Reagents used in the 4-plex SARS-CoV-2

Item	Source	Catalogue #	Expiration Date	Storage
SULFO-TAG™ anti human IgG Detection Antibody	MSD	D21ADF-3	31 DEC 2023	2-8ºC
Assay Diluent 100	MSD	R50AA-3	30 NOV 2022	2-8ºC
MSD Wash buffer (20X)	MSD	R61AA	31 MAR 2022	RT*
MSD Phosphate Buffer (5X)	MSD	R93SA-Series	31 OCT 2022	RT*
MSD Blocker A Kit	MSD	R93BA-Series	31 DEC 2022	RT*
MSD GOLD™ Read Buffer	MSD	R60AM-4	31 DEC 2022	RT*

*RT = Room Temperature, 20-25 ºC

C. SERUM SAMPLES

(b) (4) normal human serum samples were randomly chosen for testing of Selectivity. All serum samples were collected under protocol VRC 500: Screening of Volunteers for Clinical Trials of Investigational Products and Licensed Products Evaluated for Research Purposes(NIH 11-I-0164, ClinicalTrials.gov Identifier: NCT01375530). Samples were chosen from a total list of (b) (4) available serum samples, using the *RAND()* function in Microsoft Excel. The first (b) (4) samples on the randomized list were chosen for testing (refer to Table 3 below).

Table 3: NHS Sample Selection for Selectivity and Specificity testing

Study Number	Sample ID	Collection Date	Thaw Date	Run Number
(b) (4)				

Several validation parameters were tested with COVID-19 (b) (4) samples from PCR positive participants enrolled under VRC protocol 200 (20A): Apheresis and Specimen Collection Procedures to Obtain Plasma, Peripheral Blood Mononuclear Cells (PBMCs) and Other Specimens for Research Studies (ClinicalTrials.gov Identifier: NCT00067054).

COVID-19 (b) (4) serum samples from VRC 200 for Selectivity and Specificity testing were selected according to their binding response levels seen in VIP's qualified standard IgG SARS-CoV-2 S-2P ELISA and qualified MSD® 384-well custom serology ECLIA assay. All available (b) (4) samples were screened and samples with (b) (4) binding to SARS-

CoV-2 S-2P identified. COVID-19 (b) (4) samples were then sorted according to their Endpoint titer, Area under the curve (AUC) or AU/ml results for binding to S-2P. The (b) (4) (b) (4) responses were in the (b) (4) percentile range of all samples tested, (b) (4) samples were based in the (b) (4) percentile. (b) (4) serum from (b) (4) responder and (b) (4) (b) (4) responder were chosen for (b) (4) the normal human serum (NHS) samples at (b) (4) dilution for Selectivity testing (Table 4). A total of (b) (4) sample aliquots with different response levels (Table 5) were used for Specificity and (b) (4) testing. An additional (b) (4) samples previously showing (b) (4) levels in the ELISA and 4-plex ECLIA, with (b) (4) each were used to asses (b) (4) (Table 6).

Table 4: COVID Sample Selection for Selectivity and Specificity testing. (b) (4) NHS

Study Number	Sample ID	Collection Date	Days since Onset of Symptoms	AU/ml S-2P	Thaw Date	Run Number
(b) (4)						

Table 5: COVID Sample Selection for Selectivity and Specificity testing. (b) (4) (b) (4) **Testing**

Study Number	Sample ID	Collection Date	Days since Onset of Symptoms	Thaw Date	Run Number
(b) (4)					

Table 6: COVID Sample Selection for (b) (4) testing

Study Number	Sample ID	Collection Date	Days since Onset of Symptoms	AU/ml S-2P	Number of vials used	Thaw Date	Run Number
(b) (4)							

D. (b) (4)

To test for (b) (4) the following (b) (4) (b) (4) COVID-19 (b) (4) samples at (b) (4) during specificity and selectivity testing (Table 7). (b) (4) were selected from the VIP biorepository whereas the (b) (4) and (b) (4) were generously provided by the (b) (4)

Table 7: (b) (4) **Selection for Selectivity and Specificity testing**

(b) (4)	Source	Concentration	Storage	Thaw Date	Run Number
(b) (4)					

MODIFICATIONS EVALUATED IN THIS REPORT

The following modifications were made from the B1001.00 Qualification Protocol prior to execution of the validation experiments. Where appropriate, justification for the change is also noted. All changes were added to version 01 of the validation protocol. The validation protocol revision was started to include suggestion received from the FDA in regard to DDMF 23422, 23 September 2020.

Test Plan Outline (Table 10, B1001.01)

1. Testing for Specificity was expanded to include testing (b) (4) (b) (4) as per FDA recommendation. (b) (4) COVID-19 human (b) (4) sera to address (b) (4)

2. (b) (4) of (b) (4) was changed to baseline (b) (4) for testing method and acceptance criteria. Evaluation of the 4-plex workflow during testing and possible repeat testing ruled out an occurrence where the serum samples were stored at 4ºC in a (b) (4) interval.

3. Testing for (b) (4) Precision was added as validation parameter as per FDA suggestion. (b) (4) samples, reference standard and controls were evaluated on a total of (b) (4) plates, (b) (4)

Statistical Analysis and Design

1. Acceptance criteria for Selectivity and Specificity was separated as per FDA suggestion.
2. Description of calculation of AU/mL was expanded in the Statistical and Analysis Design. Explanation for exploratory Endpoint titer analysis was separated from AU/mL as per FDA recommendation.
3. Data Analysis and Acceptance Criteria for Selectivity, Specificity and Precision were changed to address FDA comments.

DEVIATIONS FROM PROCEDURE AND REPEATS

Runs for short-term (b) (4) at (b) (4) had to be repeated. Run (b) (4) failed due to technical error (refer to Deviation D2020-046), run (b) (4) failed the assay validity criteria as set in the assay specific SOP. As seen in Table 8 below, runs (b) (4) were part of the (b) (4) (b) (4) and had therefore to be repeated, despite the fact that run (b) (4) (b) (4) passed the assay validity criteria. The (b) (4) at (b) (4) was repeated during run (b) (4) (b) (4), samples from the different (b) (4) (b) (4) were repeated in run (b) (4) (both shown in green below).

Table 8: Repeat Run Summary for (b) (4) Testing

Run number	(b) (4)
	(b) (4)

During Selectivity testing, one (b) (4) sample (500-2347-01) (b) (4) (b) (4) sample showed a recovery of (b) (4) The sample was repeated in Run (b) (4) and with a recovery of (b) (4) met the preset acceptance criteria of having a %recovery between (b) (4)

RESULTS

A. RUN SUMMARY

A total of (b) (4) assay runs with (b) (4) plates were analyzed for the validation of the 4-plex SARS-CoV-2 assay. (b) (4) QC samples, including COVID-19 (b) (4) serum samples were run in (b) (4) on each plate to evaluate the Reference Standard, Precision, Accuracy, (b) (4) and the (b) (4) (b) (4) serum samples were run to evaluate Selectivity; Selectivity was also evaluated using (b) (4) samples spiked with varying levels of COVID-19 (b) (4) serum. In addition to the (b) (4) assay runs listed above, (b) (4) was performed for Selectivity testing.
Specificity was evaluated by (b) (4) serum samples with (b) (4) (b) (4) was evaluated using (b) (4) individual (b) (4) serum samples, (b) (4) (b) (4) anti-S-2P IgG binding responses. The antibody concentration (AU/mL) of (b) (4) test samples ((b) (4) serum under (b) (4) (b) (4) was compared at different (b) (4) .

A total of (b) (4) runs were set up for validation. Run (b) (4) failed due to a technical error (refer to Deviation D2020-046), run (b) (4) failed the assay validity criteria and run (b) (4) showed a low ECL signal (b) (4) compared to an average of (b) (4)). All three were excluded from analysis.

Run (b) (4) was part of the (b) (4) testing (b) (4), but despite passing the assay validity criteria, data from run (b) (4) was only used for the analysis of the reference standard and QC samples and controls but not for the (b) (4) test samples. (b) (4) testing was repeated in runs (b) (4). One sample outside of the acceptable recovery rate for Selectivity was repeated in run (b) (4)

Table 9: RUN SUMMARY

RUN NUMBER	RUN DATE	ANALYSIS DATE	ASSAY RESULT
(b) (4)			

B. ASSAY VALIDITY

As shown in Attachment B to this report, the assay validity criteria as stated in SOP 5525, section 12 were met for runs (b) (4) and (b) (4). Run (b) (4) failed due to a technical error (refer to Deviation D2020-046) and run (b) (4) failed several assay validity criteria. As seen in Attachment B, for run (b) (4) the calibrator curve fit was (b) (4) the calibrator replicate ECL (b) (4) for CAL1-CAL6 (b) (4) and (b) (4) for CAL7 (b) (4) The calibrator over recovered with a range of CV between (b) (4) The same pattern was seen for the MSD controls, where the (b) (4) All (b) (4) MSD controls also over recovered with (b) (4) CV. In addition, all (b) (4) fell outside of the established acceptance range, for (b) (4). In summary for Run (b) (4) failed (100%) and the assay was repeated.

All controls were trended over time and a summary is shown in Figure 2 below. Shown is the % recovery over time for the MSD controls (b) (4) different plates. Red lines indicated that the controls recovered in the expected range of (b) (4) and in the required (b) (4) (black dotted lines). All MSD controls fell within the acceptance range. (b) (4) ranged in the established acceptable (b) (4) nominal AU/mL concentration (red dotted line) and in the range of (b) (4), black dotted line). (b) (4) runs showed a concentration of (b) (4) AU/mL, on the (b) (4) ranked within the (b) (4) (b) (4) AU/mL, as did (b) (4) (both within the red dotted line showing the (b) (4) concentration (b) (4) For (b) (4) all values ranked within the (b) (4) nominal AU/mL, with two values close to the (b) (4), respectively. In addition to the assay controls, a QC test sample was (b) (4)
The average AU/mL was calculated and plotted in Figure 2 below. As seen in the respective graph below, the AU/mL concentration measured over (b) (4) runs fell into the established and expected range of (b) (4) nominal AU/mL (red dotted line) and well within the (b) (4)

Figure 2: Control Trending over (b) (4) Plates run during Validation

(b) (4)

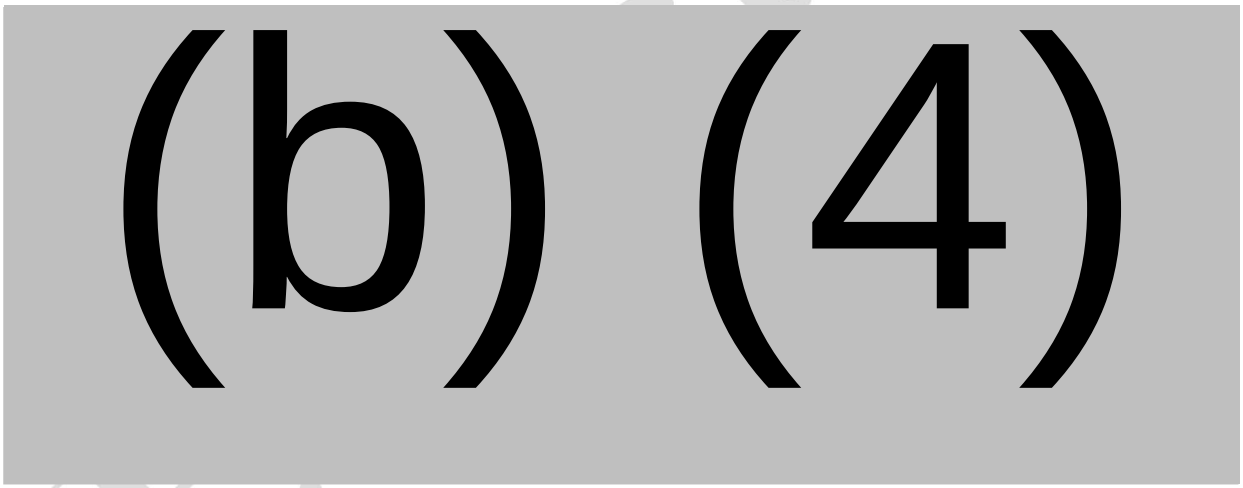
(b) (4)

C. REFERENCE STANDARD

The MSD reference Standard is run in (b) (4). The reference standard includes one blank (CAL8) and 7 non-zero calibrators (CAL1-7) in a 4-fold dilution series covering the (b) (4) and range of the assay, including the (b) (4) Data from validation runs (b) (4) were analyzed for the preset acceptance criteria. Run (b) (4) failed due to a technical error and hence did not meet the assay validity criteria and weren't included in the analysis.

Figure 3: Recovery of the Reference Standard over all (b) (4) validation runs.

Shown is the %recovery over time for each calibrator (b) (4) Data for CAL1-CAL6 is shown on the left, CAL7 recovery for (b) (4) on the right. Red lines indicate the preset acceptance criteria for %recovery, (b) (4)% for CAL1-6 and (b) (4)% for CAL8. Data was analyzed by the NIAID biostatistical branch (refer to Table 1, pages 3-6 in statistical report, Attachment A) and %recovery values were plotted in GraphPad Prism V8.0.



(b) (4) values for the (b) (4) wells for each dilution on each plate were averaged and compared to the expected (known) concentration to determine %recovery. Three samples upon analysis showed air bubbles in the well contributing to increased variability in that particular well and were excluded from the analysis (Validation run (b) (4) (b) (4) Table 1, pages 3-5, Table 2 and Figure 1 on page 6 of the Statistical Report for the Validation of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins (Attachment A) shows a summary of the %recovery of each calibrator. In summary, for CAL1-CAL6, all mean of (b) (4) values had a %recovery between (b) (4) % (range (b) (4) %),

and CAL7 had a %recovery between (b) (4) % (range (b) (4)), see figure 5 below. All 7/7 (100%) of the non-zero calibrators met the percent recovery criteria, which meets the validation criteria for the reference standard.

D. SELECTIVITY/SPECIFICTY

(b) (4) normal human serum samples from various donors randomly selected from VRC screening protocol 500 were tested for selectivity. As shown in Table 10 below, the concentration (AU/mL) was calculated and reported for each sample. As defined during assay qualification, the (b) (4) range of (b) (4) AU/mL for S-2P was used as the (b) (4) to assess the (b) (4) naive samples (refer to document R1013: Report on the qualification of the Multiplex Assay (4-plex) for the detection of IgG against SARS-CoV-2 proteins).

(b) (4) (95%) of the naive serum samples had an AU/mL of less than (b) (4), meeting the preset acceptance criteria of (b) (4) being below (b) (4) AU/mL. Sample 500-2347-01 was repeated as part of the selectivity testing, the repeat also showed an AU/mL (b) (4) One sample, 500-2307-01, showed a concentration of (b) (4) AU/mL (Table 10).

(b) (4) normal human serum samples from various donors randomly selected from VRC screening protocol 500 were (b) (4) COVID-19 (b) (4) serum. The AU/mL of the (b) (4) samples was calculated and reported. Responses from the naive samples were multiplied by the ratio of naive sample volume/total sample ((b) (4) + naive sample volume) to normalize the response of the naive sample in the (b) (4) sample. Then, that normalized value was subtracted from the response of the (b) (4) sample to determine the response from the (b) (4) Normalized responses from the (b) (4) material were divided by responses from the diluent (neat) (b) (4) material to determine the percent recovery.

Table 11 shows the results for the (b) (4) samples sets and the neat diluent. Also refer to table 3 and 4, page 7, Statistical Report, Attachment A. (b) (4) (10%) naive sample (b) (4) COVID-19 (b) (4) sample had a % recovery (b) (4) Naïve sample 500-2347-01 showed an increased biding response to S-2P (Table 10) and was therefore repeated. The repeat %recovery was (b) (4) and therefore the preset acceptance criteria were met for %recovery (b) (4) serum samples, respectively.

Table 10: Selectivity testing of (b) (4) normal human serum samples in the 4-plex SARS-CoV-2 assay

Shown is the interpolated concentration in AU/ml for each of the (b) (4) normal human serum samples from pre-pandemic samples drawn from volunteers of VRC screening protocol 500. (b) (4) = concentration lies (b) (4) (b) (4) of the assay. Concentrations were calculated using MSD Discovery Workbench V4.0 and Microsoft Excel. Test Sample 500-2347-01 was repeated for Selectivity testing, concentrations from both runs are shown in bold.

Sample Type	Sample	Evaluation	Time Point/Condition	SARS-COV2 S-2P AU/mL
(b) (4)				

Table 11: Selectivity testing of (b) (4) normal human serum samples in the 4-plex SARS-CoV-2 assay

Shown is the interpolated concentration in AU/ml for each of the (b) (4) (b) (4) normal human serum samples from pre-pandemic samples drawn from volunteers of VRC screening protocol 500. In addition, the (b) (4) assay diluent is shown (Neat). Concentrations were calculated using MSD Discovery Workbench V4.0 and Microsoft Excel. The repeat sample and neat Diluent for the (b) (4)) is shown in bold.

Plate No.	Sample Type	Sample	Evaluation	Time Point/Condition	SARS-COV2 S-2P AU/mL	Recovery
(b) (4)						

(b) (4) COVID-19 (b) (4) serum samples of (b) (4) (b) (4) were tested for (b) (4) (b) (4) as well as (b) (4) protein were (b) (4) into normal human sera at different concentration (see Table 7). The AU/mL of (b) (4) and (b) (4) samples was calculated and reported.
The response from the (b) (4) material was adjusted for the (b) (4) (b) (4) These normalized responses from (b) (4) material were divided by values from the (b) (4) material to determine the percent recovery (refer to Tables 5 and 6, page 8 in the Statistical report, Attachment A).

All (b) (4) samples (b) (4) had a % recovery between (b) (4), and all (b) (4) recovery range was observed to be between (b) (4) samples (b) (4) showed a % recovery between (b) (4) In summary, all samples met the preset validation criteria.

(b) (4) serum samples of (b) (4) SARS-CoV-2 S-2P protein and as expected the %recovery decreased when competitive specific S-2P was added. For (b) (4) serum samples, the %recovery ranged between (b) (4) and for (b) (4) (refer to Table 7, page 9, Statistical Report, Attachment A).

E. PRECISION

QC samples were run in (b) (4) and data from all validation runs were analyzed for acceptance criteria. Three samples (b) (4) were excluded from the analysis as the (b) (4) in the plate showed air bubbles, causing increased variability in those wells. Precision was analyzed for the reference standard, the MSD controls and (b) (4), all either negative serum or COVID-19 (b) (4) samples (b) (4)

(b) (4)

(b) (4)

F. ACCURACY

Data for analysis of Accuracy was generated using (b) (4) independent preparations of the reference standard, CAL1-CAL7 from a total of (b) (4) validation runs with a total of (b) (4) plates. In summary, there were (b) (4)

accuracy for the reference standard is listed in Table 1, page 3-5, Statistical Report, Attachment A and all recoveries were observed to be within (b) (4) of the nominal value (b) (4) The accuracy for all seven calibrators across all runs is within (b) (4) as seen in Table 13, page 19, which meets the preset validation criteria.

G. (b) (4) AND DYNAMIC RANGE

Data generated during the assessment of accuracy was also used for (b) (4) analysis. (b) (4) was assessed by graphing the (b) (4)

(b) (4)

Pages 20-25 of the Statistical Report in Attachment A to this report summarize the (b) (4) analysis.(b) (4)

(b) (4)

(b) (4) All %CVs were within (b) (4) and the percent recovery was within(b) (4) for all samples. This meets the (b) (4) criteria. However, there were only(b) (4) points in the (b) (4) range, which does not meet the preset acceptance criteria of having(b) (4) points in the (b) (4) range of the assay.

H. (b) (4)

(b) (4)

Figure 8 above). The %CV (b) (4) was (b) (4) for all (b) (4) plates (b) (4) Table 14, page 26 of the Statistical Report). These CV values met the preset validation criteria for LLOQ.

(b) (4)

DISCUSSION

The MSD® 384-well Custom Serology Assay/4-plex SARS-CoV-2 assay for the detection of Immunoglobulin (IgG) antibodies against SARS-CoV2 S-2P spike protein has met the validation acceptance criteria stated in Document B1001: Validation Protocol of Multiplex (4-plex) Assay for the detection of IgG antibodies against SARS-CoV2 proteins in human serum.

The validation samples (human (b) (4) and COVID-19 negative human serum) were run on a total of (b) (4) plates over (b) (4) runs where (b) (4) (b) (4) for the assay setup.

(b) (4) QC serum samples from (b) (4) and COVID-19 negative sera were run in (b) (4) on each plate to evaluate the reference standard, precision, accuracy, (b) (4), and the (b) (4) Naive pre-pandemic serum samples were run to evaluate selectivity; selectivity was also evaluated using naive samples (b) (4) COVID-19 (b) (4) sample. Specificity was evaluated by (b) (4) naive samples (b) (4). (b) (4) was evaluated using (b) (4) individual serum samples, (b) (4) The AU/mL of (b) (4) test samples (b) (4) (b) (4)

The reference standard means of the (b) (4) values for each dilution (b) (4) from all (b) (4) plates tested showed a recovery between (b) (4) and all seven non-zero calibrators met the validation acceptance criteria. In general, the calibrator curve fit for all standard curves showed an (b) (4) All assay controls (MSD as well as the (b) (4)) recovered in the concentration of (b) (4) expected. The blank negative control showed no recovered assigned unit and all ECL signals in the blank wells fell below the calibrator range. In summary, as already shown during assay development, qualification and testing of research samples, the reference standard and controls are (b) (4) and recover well. Controls can be trended well over time and in real time to assess the assay status. Three wells of (b) (4) during run (b) (4) had to be excluded form analysis as they showed high variability, due to air bubbles in the plate during reading on the MSD instrument. VIP as part of Atypical Investigation ARR2020-002 has put measures in place to prevent air bubbles during the assay workflow on the automated handler, and technicians have been trained to look for signs of air bubbles in the plate.

The % recovery for CAL7 (LLOQ) on (b) (4) was within (b) (4) of the nominal concentration. The %CV between triplicates on the same plate was (b) (4) for (b) (4). This met the validation criteria for the (b) (4)

(b) (4) of the naive serum samples had AU/mL less than (b) (4) for Selectivity testing, which met the validation criteria of having at least (b) (4) of naive serum samples with an AU/mL less than (b) (4) We have observed during assay development and assay qualification, that background and potential (b) (4) (b) (4) For the DMID 20-0003 Phase I trial we have observed high background in some instances for baseline, Day 0 samples, tested with VIP's qualified SARS-CoV-2 S-2P ELISA. We anticipate certain samples where (b) (4) can be observed at baseline, but those responses measured should not influence the fold-rise increase of responses after primary and booster vaccination. The same naïve (b) (4) (b) (4) samples all recovered in the expected range of (b) (4) (b) (4) samples. In summary all samples recovered between (b) (4) (b) (4) The assay can therefore selectively pick up SARS-CoV-2 specific IgG responses, despite background in naïve serum.

More specifically, (b) (4) COVID-19 (b) (4) samples were tested for (b) (4)

(b) (4)

recovery between (b) (4) which met the pre-set validation criteria. The assay is capable of specifically detecting SARS-CoV-2 S-2P specific IgG antibodies, even when (b) (4) are present. As expected, when COVID-19 (b) (4) samples were (b) (4) (b) (4) the recovery was decreased due to the (b) (4) in the sera.

Assay precision was tested for the following parameters: (b) (4) precision. In addition, total (b) (4) (b) (4) This met the validation criteria for all %CVs (excluding at the (b) (4)

(b) (4)

(b) (4)

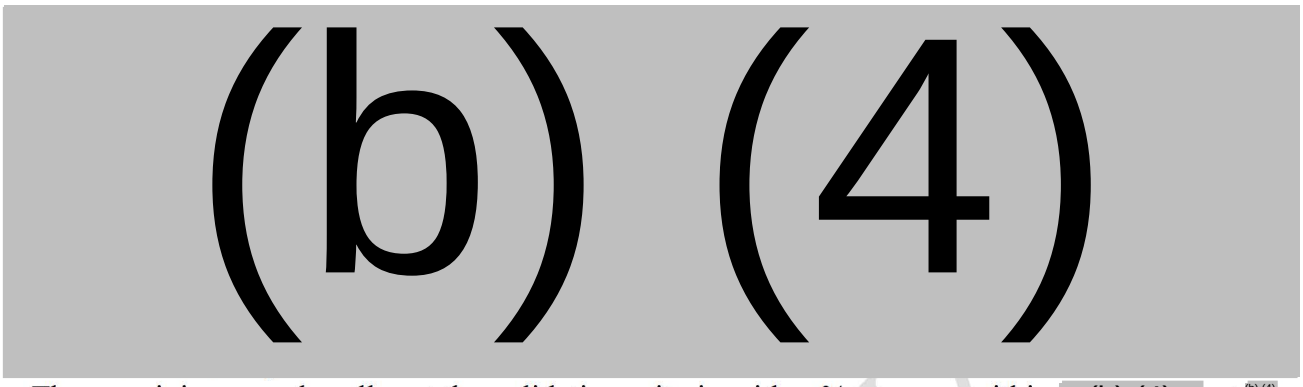
Data for Accuracy was generated from 7 independent preparations of the reference standard (CAL1 to CAL7). For CAL1-CAL6, the mean of the (b) (4) values from (b) (4) had a % recovery between (b) (4) meeting the validation criteria. For CAL7, the mean of the (b) (4) values from (b) (4) had a % recovery between (b) (4) which met the validation criteria. The accuracy for all 7 calibrators averaged across all runs is within (b) (4), which met the pre-set acceptance criteria for validation.

All data found to be (b) (4)

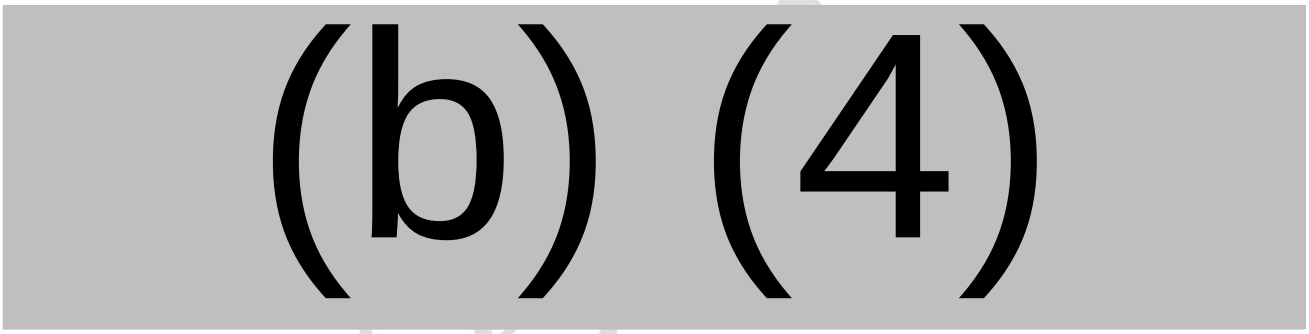
(b) (4)

(b) (4) All %CVs were within (b) (4) and the percent recovery was within (b) (4) for all samples. This meets the (b) (4) criteria. However, there were only (b) (4) points in the (b) (4) range, which does not meet the pre-set acceptance criteria of having (b) (4) points in the (b) (4) range of the assay. MSD, the assay developer recommends to only use the data from the (b) (4) (b) (4) the reference standard for SARS-CoV-2 S-2P. After the initial functionality testing MSD set their specification standards for production verification and validation of the 384-well assay platform and recommended calibrators which should be included in signal ratio average calculations, e.g. (b) (4) for RBD (refer to Figure 4 below). VIP, for testing of clinical trial samples will follow those recommendations and only interpolate the concentration if the signal of the test and QC samples are within the range of the particular (b) (4) However, the whole curve for the reference standard will be fit using CAL1 to CAL7. A note has been added to the latest version of the SOP under section 10 for data reporting.

(b) (4)



The remaining samples all met the validation criteria with a %recovery within (b) (4) at (b) (4) relative to baseline, (b) (4)



CONCLUSIONS

With the evaluations reported in this document, the MSD® 384-well Custom Serology Assay/4-plex SARS-CoV-2 assay has been validated at VIP for use in the detection of human serum IgG antibodies reactive to SARS-COV2 S-2P spike protein.

Notably, the assay passed all acceptance criteria as set in the Validation Protocol. (b) (4)

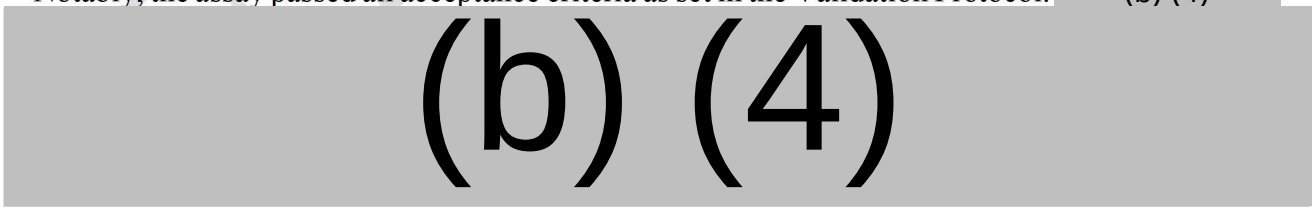
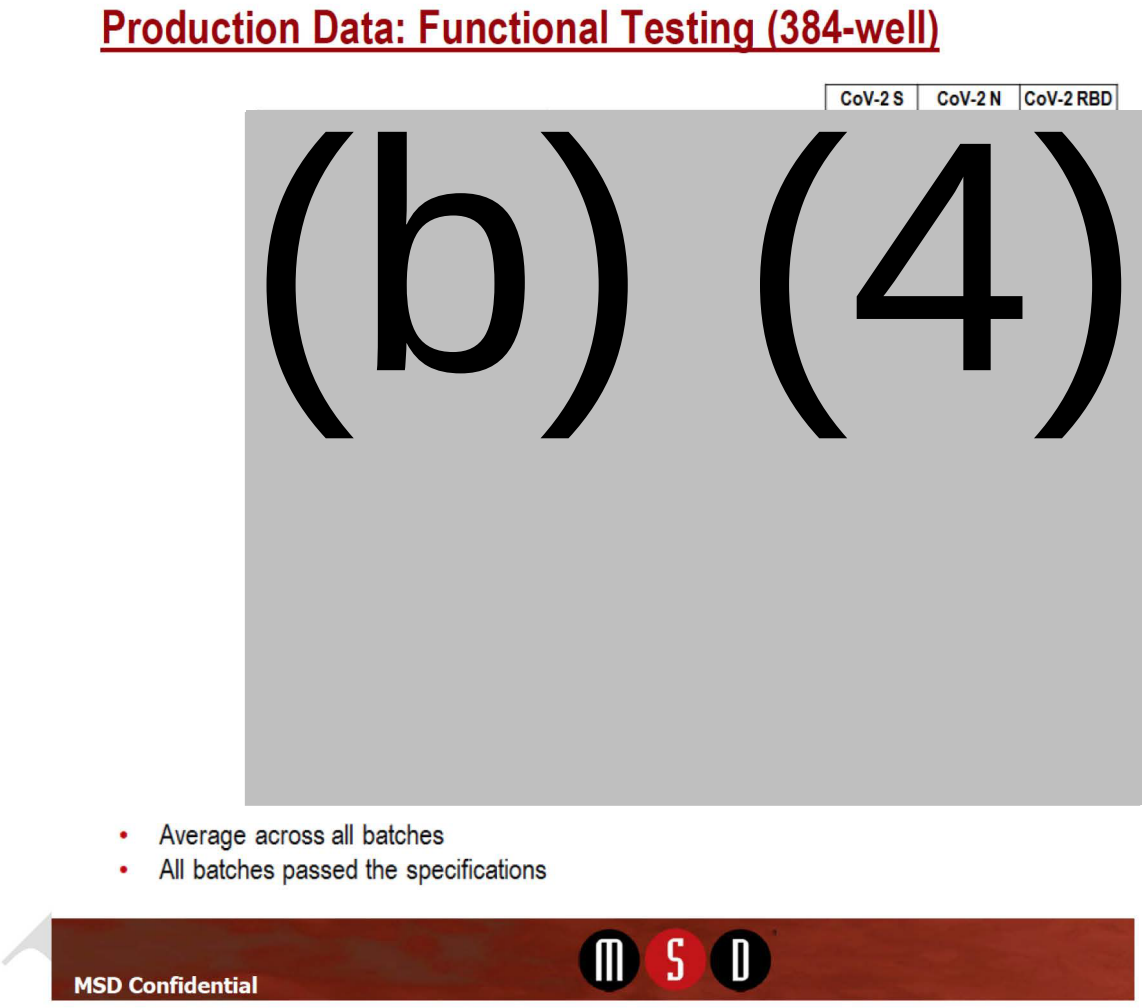


Figure 4: MSD Functionality Testing of the 384-well custom serology assay production lot (b) (4)



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ATTACHMENT

a) STATISTICAL REPORT
b) ASSAY VALIDITY SUMMARY

REVISION HISTORY

Date of Revision	Description/Revisions Made	Initials and Date
19 Oct 2020	• New Version	BF, BCL. NMD 19 Oct 2020

Attachment B: Assay Validity Summary

Shown is the summary of all assay validity criteria, as specified in SOP 5525, section 12, for each of the validation runs performed. The first 6 columns on page 28 and 29 show the assay information and columns 7 – 13 on page 28 and columns 7 – 17 on page 29, the assay data. In orange, parameters that did not meet the plate validity ranges, in yellow the values that fell outside the expected but inside the required nominal values and ranges.

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

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