Title: Report for Amendment to Validation Protocol of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins			
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THE UNDERSIGNED AGREE WITH THE CONTENT AND CONCLUSIONS OF THE REPORT DETAILED WITHIN

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1. EXECUTIVE SUMMARY

1.1 The re-validation of the Multiplex Assay (4-plex) for the detection of IgG against SARS-CoV-2 S-2P protein was executed according to Document P1015: Amendment to Validation Protocol of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins.

1.2 This amendment to the initial validation report (document B1004) 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. It was recommended by the U.S. Food and Drug Administration (FDA) to expand on the experiments performed for the initial assay validation (see MF23422, Date of Communication 12/04/2020).

1.3 A revision of the (b) (4) Precision and Accuracy test plan was generated in document P1015.00, to include a panel of COVID-19 (b) (4) samples covering the (b) (4) (b) (4) The specimen panel will first be tested for (b) (4) and then undergo additional precision and accuracy testing, verifying the (b) (4) of the assay when tested on COVID-19 (b) (4) samples.

1.4 The statistical analysis of the re-validation data was provided by NIAID Biostatistics Research Branch (BRB). The 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 to this document.

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

2. BACKGROUND

2.1 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, clinical endpoint testing of vaccine samples and correlate of protection analysis a Meso Scale Discovery (MSD) 4-plex Custom Serology Assay was developed at the Vaccine Immunology Program (VIP).

2.2 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.

3. GENERAL METHODS AND PROTOCOL INFORMATION

3.1 Re-validation was set up and performed according to the latest version of SOP 5525: Multiplex (4-Plex) Assay for the detection of IgG antibodies against SARS-CoV-2 proteins in human sera and document P1015: Amendment to Validation Protocol of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins.

3.2 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 were added to the precoated wells, and specific antibodies complex with the coated antigens. Antibodies bound to the SARS-CoV-2 viral proteins were detected using a SULFO-TAG™ anti-human IgG detection antibody. A read solution (MSD GOLD™ read buffer) containing electrochemiluminescence (ECL) substrate was applied to the wells, and the plate was entered into the MSD MESO (b) (4) detection system. An electrical current was applied to the plates and areas of well surface which form antigen-anti human IgG antibody SULFO-TAG™ complex 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.

3.3 Reference standard, MSD controls and (b) (4) as described in SOP 5525 (Multiplex 4-Plex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins in human sera), in document B1001 (Validation Protocol of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins) and in document P1015: Amendment to Validation Protocol of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins were included in each run.

3.4 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.

3.5 (b) (4) (b) (4) (b) (4) 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. 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).

3.6 COVID-19 convalescent serum samples from VRC 200 study protocol for (b) (4) and Precision testing were selected according to their binding response levels seen in the qualified MSD® 384-well custom serology ECLIA assay. All available (b) (4) samples were screened and samples with (b) (4) (b) (4) binding to SARS-CoV-2 S-2P identified. The (b) (4) sample aliquots with different response levels used for re-validation testing are listed in Table 1 below.

Table 1: COVID Sample Selection for (b) (4) and Precision Testing

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

3.7 The data analysis was performed using appropriate statistical and graphical software (MSD Discovery Workbench, Version 4.0, Microsoft Excel Version 16.36). Raw data generated during assay validation was accumulated by a subject matter expert.

3.8 Statistical analysis of assay partial validation results was performed by a biostatistician and the report generated is attached to this re-validation report.

3.9 Standard arbitrary units (AU/mL) were assigned using interpolation to the MSD standard curve included in the assay. The antibody concentrations were calculated using the MSD Discovery Workbench version 4.0 software. The concentration AU/mL was assigned from interpolation of the reference standard curve for each well and antigen

spot. For (b) (4) control wells the mean (average) was calculated and reported. For test samples the average of the (b) (4) for each dilution were calculated and subsequently the overall mean across all dilutions. If the test and QC sample signal was (b) (4) no concentration was interpolated.

3.10 Arbitrary units (AU/mL) have been assigned for the MSD reference standard and MSD serology control pack. Ranges are established for all (b) (4) controls and QC samples tested on each plate. Performance of controls and test samples was assessed using assay validity criteria established during development and qualification of the assay and are listed in the assay specific SOP 5525.

4. MODIFICATIONS EVALUATED IN THIS REPORT

4.1 No modifications were made from the P1015 protocol during execution of the re-validation runs. No deviations to existing procedures were observed.

4.2 All available (b) (4) samples prior to re-validation were screened in the qualified MSD® 384-well Custom Serology Assay/4-plex SARS-CoV-2 assay to determine the binding responses and concentration in AU/mL.

5. RESULTS

5.1 Run summary

5.1.1 A total of (b) (4) assay runs with (b) (4) plates were analyzed for the re-validation of the 4-plex SARS-CoV-2 assay using (b) (4) t samples (Table 2).

Table 2: Run summary for Re-Validation Testing

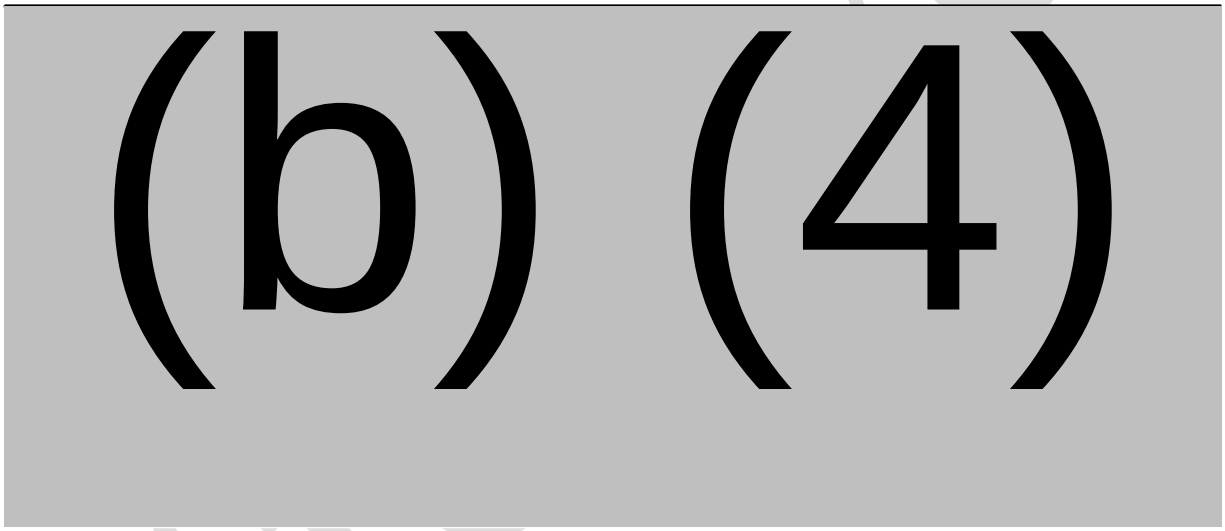
RUN NUMBER	RUN DATE	ANALYSIS DATE	ASSAY RESULT
(b) (4)			

5.2 Assay Validity

5.2.1 The assay validity criteria as stated in SOP 5525, section 12 was met for all runs performed.

5.2.2 All controls were trended over time and a summary is shown in Figure 1 below. Shown is the % recovery over time for the MSD controls 1.1, 1.2 and 1.3 on (b) (4) different plates. Red lines indicated that all 3 controls recovered in the expected range of (b) (4)

Figure 1: Control Trending during Re-Validation Testing



5.2.3 (b) (4)

5.2.4 In addition to the assay controls, a QC test sample was (b) (4) in the same pattern as the test samples. The average AU/mL was calculated and plotted in Figure 1 above. As seen in the respective graph, the AU/mL concentration measured for (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) (b) (4) runs were marginally out of the (b) (4) with an AU/mL concentration of (b) (4)

5.3 Reference Standard

5.3.1 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. Data from a total of (b) (4) runs was analyzed for the preset acceptance criteria. Data from all re-validation runs (for (b) (4) and incurred test samples (document R1023) were analyzed for acceptance criteria. There was a total of (b) (4) re-validation runs; (b) (4) (b) (4) values for the control wells on each plate were averaged and compared to the expected (known) concentration to determine % recovery. Non-zero calibrators should be (b) (4) of nominal concentration. (b) (4) should be (b) (4) of nominal concentration. (b) (4) non-zero calibrators should meet the above criteria. As seen in Table 1, table 2 and %recovery graph on pages 1-5 in the attached Statistical Report for CAL1-CAL7, the mean of the (b) (4) values from (b) (4) have a % recovery between (b) (4) (b) (4), which meet the re-validation criteria. All 7 of the non-zero calibrators meet the (b) (4) recovery criteria, which meet the re-validation criteria for the reference standard.

5.4 (b) (4)

5.4.1 (b) (4)

5.4.2

5.4.3

(b) (4) All pre-specified re-validation criteria for linearity were met.

5.4.4 (b) (4)

5.5 Precision

5.5.1 COVID-19 (b) (4) test samples covering different response levels (b) (4) (b) (4) were run in (b) (4) in (b) (4) independent runs performed by (b) (4) operators over (b) (4) days. All runs were analyzed for acceptance criteria.

5.5.2 (b) (4)

5.5.3

5.6 (b) (4)

5.6.1 (b) (4)

6 DISCUSSION

6.1 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 all re-validation acceptance criteria stated in document P1015: Amendment to Validation Protocol of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins.

6.2 The re-validation test samples (COVID-19 (b) (4) human serum) were run on a total of (b) (4) plates over (b) (4) runs. Additional plates were run for the partial re-validation of the incurred samples (document R1023). The reference standard means of the (b) (4) values for each dilution (b) (4) tested for re-validation 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) (data not shown). All assay controls (MSD as well as the (b) (4)) recovered in the concentration of (b) (4) expected. In summary, as already shown during assay development, qualification and testing of research samples, the reference standard and controls are stable and recover well. Controls can be trended well over time and in real time to assess the assay status.

6.3 Assay precision was tested for the following parameters: (b) (4)

(b) (4)

6.4

(b) (4)

(b) (4)

6.5 (b) (4)

6.6 (b) (4)

6.7 (b) (4)

(b) (4)

7 CONCLUSIONS

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

7.2 Notably, the assay passed all acceptance criteria as set in the Amendment to the Validation protocol (P1015).

8 REFERENCES

8.1 B1001: Validation Protocol of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins

8.2 P1015: Amendment to Validation Protocol of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins

8.3 Document B1004. Report on the Validation of Multiplex Assay (4-plex) for the detection of IgG antibodies against SARS-CoV-2 proteins in human sera

8.4 SOP 5525: Multiple(4-plex) Assay for the detection of IgG antibodies against SARS-CoV-2 proteins in human serum

8.5 SOP 5301: Establishment, Use and Monitoring of Immunological Assay Controls

8.6 Document R1020: Addendum I to Report R1013 - Report on the qualification of the Multiplex Assay (4-plex) for the detection of IgG against SARS-CoV-2 proteins

8.7 FDA Guidance for Industry: Bioanalytical Method Validation, May 2018

9 ATTACHMENTS

9.1 Statistical Report for Amendment to Validation of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins, Allyson Mateja, December 14th, 2020

10 REVISION HISTORY

Date of Revision	Description/Revisions Made	Initials and Date
	• new version	