Vaccine Immunology Program (VIP)
Proprietary Information

Title: Report for Amendment to Partial Validation of Incurred Samples for OWS - Moderna			
Document No: R1024	**Revision:** 00	**Effective Date:** 14 DEC 2020	Page **1** of **14**

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 for incurred samples was executed according to Document P1013.01: Protocol for Validation of Incurred Samples for Operation Warp Speed (OWS). Incurred samples from ModernaTX Inc. and NIH/NIAID/DMID Phase I clinical trial: Safety and Immunogenicity Study of 2019-nCoV Vaccine (mRNA-1273) for Prophylaxis of SARS-CoV-2 Infection (COVID-19) - ClinicalTrials.gov Identifier: NCT04283461, were evaluated for this report.

1.2 This amendment to the partial validation report for incurred samples (document R1022) 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 partial 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 P1013.01, to include a panel of incurred test 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 incurred samples from the Moderna/DMID Phase I trial.

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

1.6 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, clinical endpoint testing of vaccine samples and correlate of protection analysis a MSD® 384-well Custom Serology Assay/4-plex SARS-CoV-2 assay was developed at the VIP for the detection of immunoglobulin G (IgG) specifically recognizing SARS-CoV-2 S-2P spike protein in human serum samples. The validated assay will aid as a secondary and/or exploratory measure of binding antibody levels, in different SARS-CoV-2 clinical Phase III vaccine trials under the Operation Warp Speed (OWS) initiative.

1.7 A full bioanalytical method validation was performed for the newly developed and qualified MSD® 384-well Custom Serology Assay/4-plex SARS-CoV-2 assay using COVID-19 (b) (4) samples. Incurred samples were not available at time of method validation and the decision to perform additional partial validation studies on incurred clinical samples once they became available was made.

1.8 Both reports, the validation report for (b) (4) samples (Document B1004.00) and the partial validation report for incurred samples (R1022.00) were sent to the FDA in December 2020 and during the review of the results the FDA asked for an expansion of testing and revision of the experimental design, to re-validate the (b) (4), accuracy via dilutional linearity and precision parameters for incurred samples.

1.9 The results of the re-testing of the partial validation of the incurred samples are described in this amendment to document R1022.00 Report for Partial Validation of Incurred Samples for OWS – Moderna.

3. GENERAL METHODS AND PROTOCOL INFORMATION

1.10 The re-validation assay was set up according to SOP 5525: Multiplex (4-Plex) Assay for the detection of IgG antibodies against SARS-CoV-2 proteins in human sera and run specific instruction provided by VIP Management

1.11 In brief, the assay was performed with a (b) (4) based automation platform including (b) (4) Plate Washer. Plate were blocked for (b) (4) (b) (4) with MSD blocker A solution (b) (4) Plates were washed and serial diluted standards, control sera and human serum test samples were added to the precoated wells and incubated at (b) (4) (b) (4) SARS-CoV-2 specific antibodies present in the sera or controls bind to the coated antigens. Plates were washed to remove unbound antibodies. Antibodies bound to the SARS-CoV-2 viral proteins were detected using a SULFO-TAG™ anti-human IgG detection antibody incubated for (b) (4) (b) (4) Plates were washed and a read solution (MSD GOLD™ read buffer) containing electrochemiluminescence (ECL) substrate is applied to the wells, and

the plate was entered into the MSD (b) (4) detection system. A current was 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 ECL unit response for each test sample and control and/or standard of each plate.

1.12 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.

1.13 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.

1.14 (b) (4)
(b) (4)
(b) (4) and negative human serum samples. Small volume aliquots for single use were prepared and stored at appropriate temperature at the (b) (4) biorepository. (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).

1.15 Incurred serum samples from DMID 20-0003 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 incurred samples from different timepoints during the vaccine trial were screened and samples with (b) (4) binding to SARS-CoV-2 S-2P identified. The incurred sample aliquots with different response levels, taken from different visits and timepoints during the protocol used for re-validation testing are listed in ***Table 1***below.

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

Study Number	Sample ID	Visit (Timepoint)	Thaw Date	Run Number
(b) (4)				

1.16 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.

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

1.18 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 is (b) (4) no concentration was be interpolated.

1.19 Arbitrary units (AU/mL) have been assigned for the MSD reference standard and MSD serology control pack. Ranges are established for all (b) (4) and QC samples tested on each plate. Performance of control 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

1.20 All available incurred samples prior to partial 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.

1.21 The volume of samples received was too low to just (b) (4) AU/mL concentration for (b) (4) testing. Instead, (b) (4) participants with (b) (4) (b) (4) binding responses were (b) (4) and used for (b) (4) testing (***Table 1***, runs 31-33).

1.22 For precision testing of incurred samples, a total of (b) (4) samples of different QC levels were tested instead of the (b) (4) listed in the protocol P1013.01.

5. RESULTS

1.23 Run summary

1.23.1 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 incurred samples (***Table 2***).

Table 2: Run summary for Re-Validation Testing

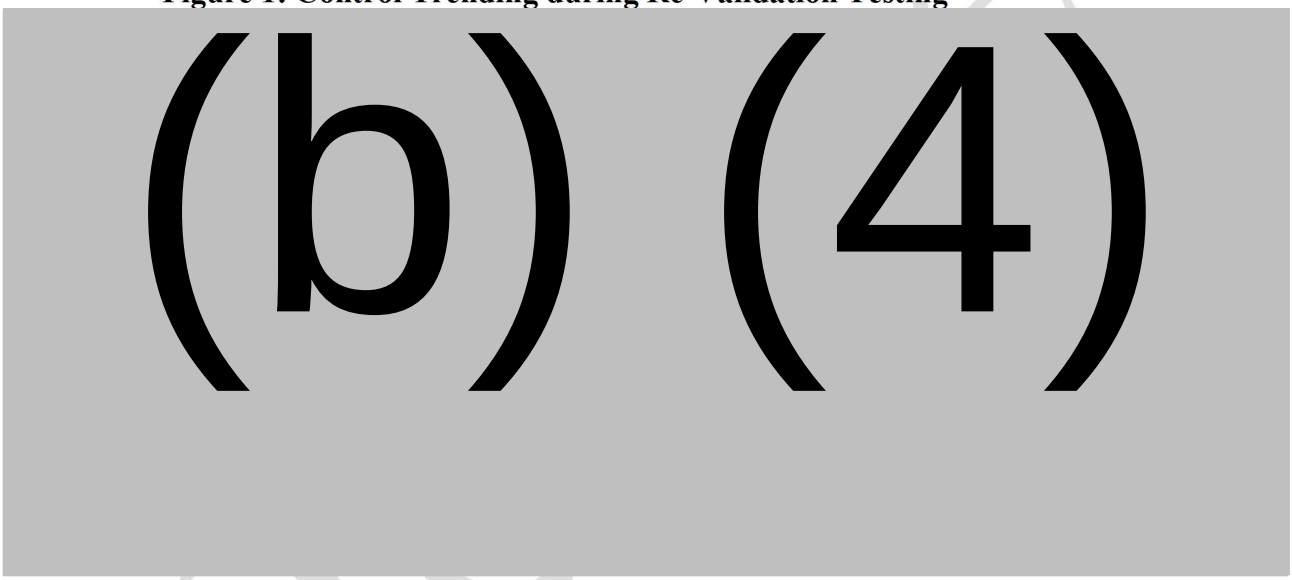
RUN NUMBER	RUN DATE	ANALYSIS DATE	ASSAY RESULT
(b) (4)			

1.24 Assay (b) (4)

1.24.1 The assay (b) (4) criteria as stated in SOP 5525, section 12 was met for all runs performed.

1.24.2 All controls were trended over time and a summary is shown in ***Figure 1***. 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 the controls recovered in the expected range of (b) (4) and in the required (b) (4) (b) (4) (black dotted lines). All MSD controls fell within the acceptance range.

Figure 1: Control Trending during Re-Validation Testing



1.24.3 (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).

1.24.4 In addition to the assay controls, a QC test sample was (b) (4) (b) (4) 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 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)

1.25 Reference Standard

1.25.1 The reference standard is run in (b) (4) The reference standard includes one blank (CAL8) and 7 non-zero calibrators (CAL1-7). Data from all re-validation runs (for (b) (4) and incurred test samples (document R1023) will be analyzed for acceptance criteria. There was a total of (b) (4) re-validation runs; (b) (4)

(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 ± 20% of nominal concentration. ULOQ and LLOQ should be ± 25% of nominal concentration. 75% and a minimum of 6 non-zero calibrators should meet the above criteria. As seen in Table 1, table 2 and %recovery graph on pages 1-5 in the Statistical Report attached to document R1023, for CAL1-CAL7, the mean of the triplicate values from all 24 plates have a % recovery between 80% and 120%, which meet the re-validation criteria. All 7 of the non-zero calibrators meet the 20% recovery criteria, which meet the re-validation criteria for the reference standard.

1.26 (b) (4)

1.26.1 (b) (4)

1.1.1 (b) (4)

1.1.2 (b) (4)

(b) (4)

1.1.3 (b) (4)

1.27 Precision

1.1.4 6 COVID-19 (b) (4) test samples (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.

1.1.5 (b) (4)

1.1.6

1.28 (b) (4)

1.1.7 (b) (4)

(b) (4)

6. DISCUSSION

1.29 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.

1.30 The re-validation test samples (incurred human serum samples) were run on a total of (b) (4) plates over (b) (4) runs. Additional plates were run for the re-validation of the (b) (4) samples (document R1023). The reference standard means of the (b) (4) values for each dilution (b) (4) from all plates 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.

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

(b) (4)

1.32 (b) (4)

(b) (4)

1.33 (b) (4)

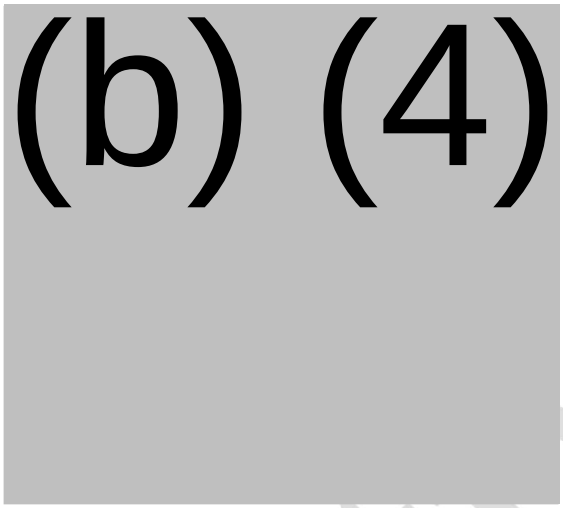
(b) (4)

1.34

(b) (4)

1.35

1.36



7. CONCLUSIONS

1.37 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.

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

8. REFERENCES

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

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

1.41 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

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

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

1.44 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

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

1.46 Document R1023: Report for Amendment to Validation Protocol of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins[1]

9. ATTACHMENTS

1.47 Statistical Report for Partial Validation of Incurred Samples for operation Warp Speed (OWS), Allyson Mateja, December 14th, 2020

10. REVISION HISTORY

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