THE UNDERSIGNED AGREE WITH THE CONTENT AND CONCLUSIONS OF THE REPORT DETAILED WITHIN

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1 EXECUTIVE SUMMARY

1.1 The partial validation of the Multiplex Assay (4-plex) for the detection of IgG against SARS-CoV-2 S-2P protein of 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 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 partial 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 in incurred samples from vaccine recipients. The statistical analysis of the qualification data was provided by NIAID Biostatistics Research Branch (BRB).

1.3 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 A to this document.

2 BACKGROUND

2.1 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 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.

2.2 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 convalescent samples. The validation results are summarized in 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.

2.3 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. Addendums to the validation protocol and report such as this present protocol and subsequent report(s) will serve as reporting tools for the partial validation

3 GENERAL METHODS AND PROTOCOL INFORMATION

3.1 The 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

3.2 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 wass 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.

3.3 The 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.

3.4 Statistical analysis of assay partial validation results was performed by a biostatistician and the report generated was attached to the partial validation report.

3.5 Standard arbitrary units (AU/mL) will be assigned using interpolation to the MSD standard curve included in the assay. The antibody concentrations are calculated using the MSD Discovery Workbench version 4.0 software. The concentration AU/mL will be assigned from interpolation of the reference standard curve for each well and antigen spot. For (b) (4) control wells the mean (average) is calculated and reported. For test samples the average of the (b) (4) for each dilution are calculated and subsequently the overall mean across all dilutions. If the test and QC

sample signal is below the range of (b) (4) and above (b) (4) no concentration will be interpolated.

3.6 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.

3.7 The following plate Layout was used, controls were assessed as described in section 6.2, page 5, document P1013.

PLATE LAYOUT

(b) (4)

4 MODIFICATIONS EVALUATED IN THIS REPORT

4.1 As described in the legend of table 3, all incurred samples prior to partial validation were tested in the validated MSD® 384-well Custom Serology Assay/4-plex SARS-CoV-2 assay to determine the binding responses and concentration in AU/mL.

4.2 During the specificity testing of all (b) (4) incurred samples the laboratory observed high AU/mL levels in some of the participants. Therefore, one samples of the highest concentration pre-diluted into (b) (4) dilution steps would not have been sufficient enough to reach the low linearity range needed for the analysis and the dilution range was changed to (b) (4) steps. In addition, the (b) (4) was increased to (b) (4) This way, (b) (4) of dilutional linearity were (b) (4) across a total of (b) (4)

4.3 The volume of samples received was too low to just use one samples of (b) (4) AU/mL concentration for dilutional linearity testing. Instead the (b) (4) participants with (b) (4) (Table 1) were (b) (4) and used for (b) (4) testing.

5 RESULTS

5.1 Screening of Incurred Samples was performed prior to partial validation. The samples were selected by ModernaTX Inc. and sent to VIP blinded. All samples were coded with a LIMS text ID (e.g. 0045-1613-01) and run in the validated MSD® 384-well Custom Serology Assay/4-plex SARS-CoV-2 assay to determine the binding responses and concentration in AU/mL. Table 1 shows the results of the binding assay. The IgG antibody concentration in AU/mL ranged from (b) (4) to (b) (4) AU/mL (Average of (b) (4) AU/mL). Samples from the screening assay were chosen for the partial validation parameter, precision, accuracy and (b) (4)

Table 1: Screening of (b) (4) incurred samples from peak Immunogenicity timepoint, ModernaTX/DMID 20-0003 trial.

Sample	SARS-COV2 S-2P Calc. Conc. Mean
(b) (4)	

5.2 (b) (4)

5.3

5.4

5.5 The values for precision were also used for analysis of accuracy. The accuracy across all (b) (4) samples form concentration of (b) (4) AU/mL were less than (b) (4) and passed the partial validation criteria.

5.6 (b) (4)

5.7 In addition, the dilutional linearity was assessed. The %CV of (b) (4) (AU/mL) between (b) (4) per dilution was below (b) (4) and therefore met the acceptance

criteria. As seen in Table 3, page 18,Attachment A, Statistical Report, the CV of the data in the linear range of (b) (4) was between (b) (4)

6 DISCUSSION

6.1 This partial validation was performed to demonstrate assay reliability and was conducted to evaluate a panel of incurred samples from recipients of vaccine product mRNA-1273 (ModernaTx, Inc.) covering the potential/expected analytical range of the assay (b) (4) samples). (b) (4) samples from the peak immunogenicity timepoint of the Phase I Moderna trial were run on the validated MSD® 384-well Custom Serology Assay/4-plex SARS-CoV-2 assay to determine the binding responses and concentration in AU/mL.

6.2 A total of (b) (4) samples, (b) (4) AU/mL were run in (b) (4) over (b) (4) runs to evaluate precision and accuracy. A (b) (4) samples after screening (table 1) of the (b) (4) AU/mL concentration from the peak immunogenicity timepoint was used for the (b) (4) assessment. The assay and partial validation of incurred samples passed the previously established acceptance criteria for precision, accuracy, (b) (4) and dilutional linearity.

6.3 (b) (4)

6.4

(b) (4)

(b) (4)

6.5 (b) (4)

(b) (4)

6.6

6.7

(b) (4)

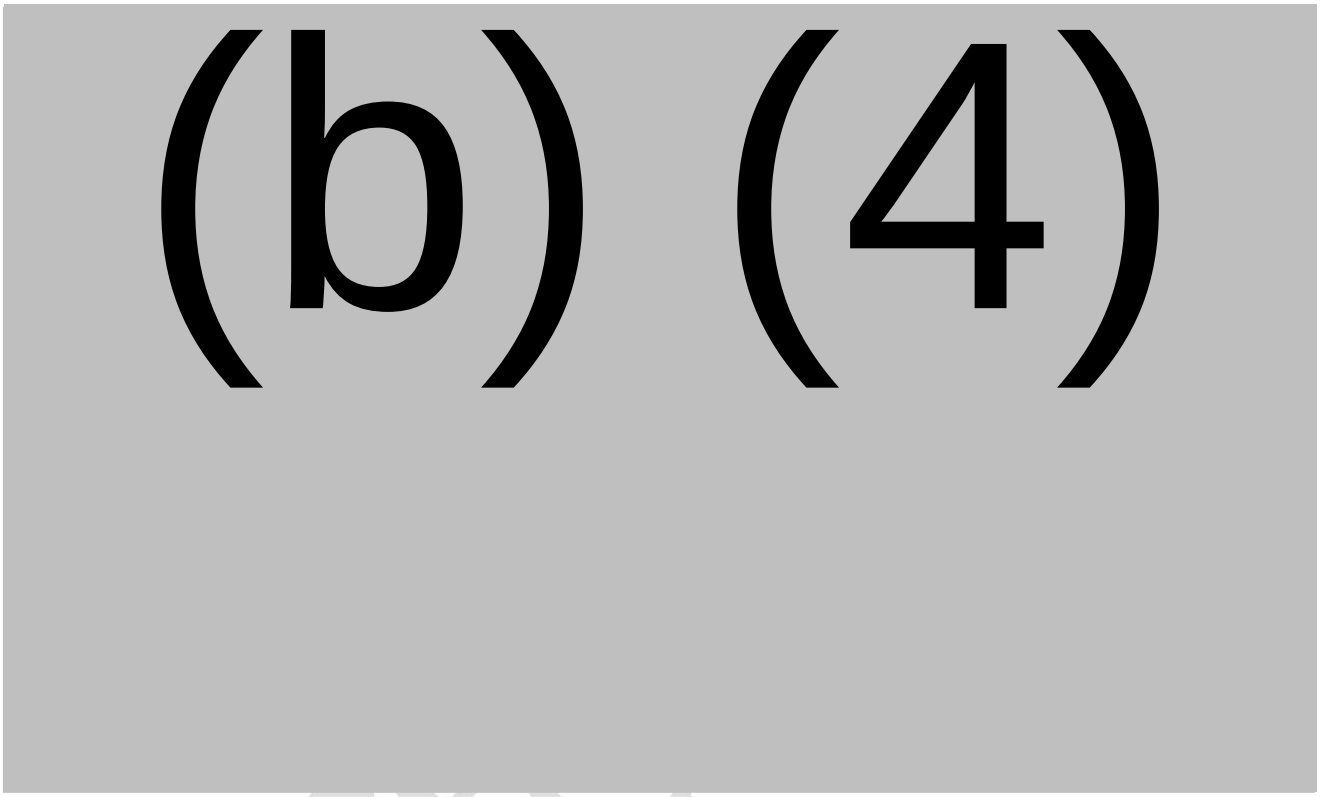
(b) (4)

6.8 (b) (4)

6.9

6.10 By evaluating the (b) (4) we conclude that the assay demonstrated acceptable precision and accuracy across the range of concentration (b) (4) AU/mL) evaluated in the partial validation using incurred samples.

(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 for the quantitation of Immunoglobulin IgG against SARS-CoV2 S-2P spike protein has been partially validated for the testing of incurred samples for the Moderna vaccine trial.

8 REFERENCES

8.1 P1013.00 Protocol for Partial Validation of Incurred Samples for OWS

8.2 R1013.00 Report on the qualification of the Multiplex Assay (4-plex) for the detection of IgG against SARS-CoV-2 proteins

9 ATTACHMENTS

9.1 Statistical Report for Partial Validation of Incurred Samples for Operation Warp Speed (OWS), Allyson Mateja, November 13, 2020

10 REVISION HISTORY

Date of Revision	Description/Revisions Made	Initials and Date
13 NOV 2020	• New report	BF, BCL, 13 NOV 2020