Title: **Moderna 1273 Biomarker Concordance Analysis Report for Protocol mRNA-1273-P301**

Sponsor Name: ModernaTX, Inc.
Legal Registered Address: 200 Technology Square
Cambridge, MA 02139

Date of Analysis Plan: 04 March 2021

Date (Version) of Report: 29 April 2021 (1.0)

DocuSigned by:
(b) (6)
30 April 2021
Author: (b) (6) (Signature) Date

DocuSigned by:
035ADF27488C419...
30 April 2021
Author: Katherine Kacena, PhD (Signature) Date

Reviewed and Approved by:

DocuSigned by:
Rolando Pajon
046725C1EE1146E...
03 May 2021
03 May 2021
Biomarker Lead: Rolando Pajon, PhD (Signature) Date

DocuSigned by:
Rolando Pajon
046725C1EE1146E...
DocuSigned by:
robert paris
2C38280C7FED4B8...
30 April 2021
Translational Medicine Lead: Robert Paris, MD (Signature) Date

DocuSigned by:
Brett Leav
7F997CA51B87496...
01 May 2021
Clinical Lead: Brett Leav, MD (Signature) Date

DocuSigned by:
Honghong Zhou
8D2CBD70CE87482...
30 April 2021
Biostatistics Lead: Honghong Zhou, PhD (Signature) Date

Background

Biomarkers of immune responses are key components for the clinical development and licensure of preventive vaccines. Immune bridging studies using these biomarkers are critical for expanding population coverage, establishing vaccine effectiveness when a validated correlate of protection is available, and assessment of the impact of vaccine co-administration on immunogenicity. Specifically, an immune bridging strategy is planned to establish vaccine effectiveness in:

1) the pediatric investigation plan, including mRNA-1273-P203 and mRNA-1273-P204,
2) immunocompromised and special populations,
3) in the evaluation of modified mRNA-1273 vaccines which encode antigens derived from emerging SARS-CoV-2 variants such as B.1.351, and
4) other development program objectives.

Although regulatory agencies have recently stated that a neutralizing antibody assay is preferred as a representation of antibody function (for example, neutralization or pseudotyped virus neutralization as cited in the recent FDA Guideline on EUA for COVID vaccines (February 2021)), its variability is higher than that of appropriately developed binding assays. Concordance between a neutralizing and binding assay can be established to allow bridging based on the more precise binding assay.

An analysis was proposed (Concordance Analysis Plan, ModernaTx) to determine the concordance of Anti-spike binding assays with the pseudotyped virus neutralization assay (PsVNA). This report includes both the Anti-spike by MSD (NIH, 2020) and the Anti-spike ELISA (PPD, 2020) assays for the detection of IgG specific to SARS-CoV-2 spike protein (Anti-S) to assess their concordance with PsVNA (Montefieri, 2020). Additional information on these assays is shown in Table 1.

Table 1: Assays Assessed in the Analysis

Report Test Name	Assay Lab	Category	Lab Test Name	LLOQ (Log10)	ULOQ (Log10)
PsVNT50	Duke/ Montefiori lab	nAb	*PsVNT50*	(b) (4)	
PsVNT80			*PsVNT80*		
Anti-spike MSD	VRC (NIH, McDermont lab)	bAb	SARSCOV2S2P		
Anti-spike ELISA	PPD vaccine	bAb	VAC65 Spike IgG Antibody		

Note: The PsVNA assays data points above the ULOQ were included in the analysis.

The principle of each assay has been accepted, with the binding assays seeking to determine the titers against virus epitopes related to entry into the cell and the PsVNA seeking to determine the inhibition of cellular entry and single-round of infection by lentivirus particles expressing SARS-CoV-2 Spike protein on their surface and containing a firefly luciferase (Luc) reporter gene. Concordance between the MSD and PsVNA, FRNT, PRNT and nLuc assays has been demonstrated in Study 20-0003 (Jackson, 2020).

Objectives:

The purpose of these analyses is to assess the concordance of both binding assays (the Anti-spike by MSD and Anti-spike by PPD) to the Pseudotyped Virus Neutralization Assay (PsVNA). Concordance between each of the binding assays and the neutralization assay is meant to be descriptive and not inferential.

Population:

As of 09-Mar-2021, there were 885 subjects (1554 observations) that received mRNA-1273 in the 301 clinical trial and had data from all four assays for a given timepoint (Day 29 or Day 57) and met the following constraints: 1) Above the LLOQ for all four assays, 2) below the ULOQ for the two binding assays, and 3) HIV negative. Table 2 provides a summary of the available data and the number of patients and observations for each subgroup and the associated model results.

Statistical Methodology

(b) (4) was used to describe the relationship between the binding and neutralization assays (in Log10 scales). The (b) (4) (b) (4) (b) (4) (b) (4)

There are, however, multiple reasons that the (b) (4) might deviate from the ideal. These include the impacts of different test conditions on the neutralization assay and the range of titers in the two assays. For these and the lack of a scientific basis for an acceptance criterion on the (b) (4), the analysis was proposed to be descriptive with the objective to estimate the (b) (4) support a conclusion that the binding assay is a suitable alternative to the neutralization assay.

This approach is furthermore supported by the principles of the assays (both related to the ability of serum antibodies to inhibit infections of cells), and the superior precision of the binding assays. For each model, the (b) (4) (b) (4) are provided in Table 2. Sixteen figures are shown, which include the (b) (4) models specified in the Biomarker Concordance Analysis Plan. Each figure includes a slope reference line of 1 (thick dotted gray line), the raw data points, and the (b) (4) model estimate.

Table 1: Summary of Available Data for each Model (885 subjects, 1554 observations)

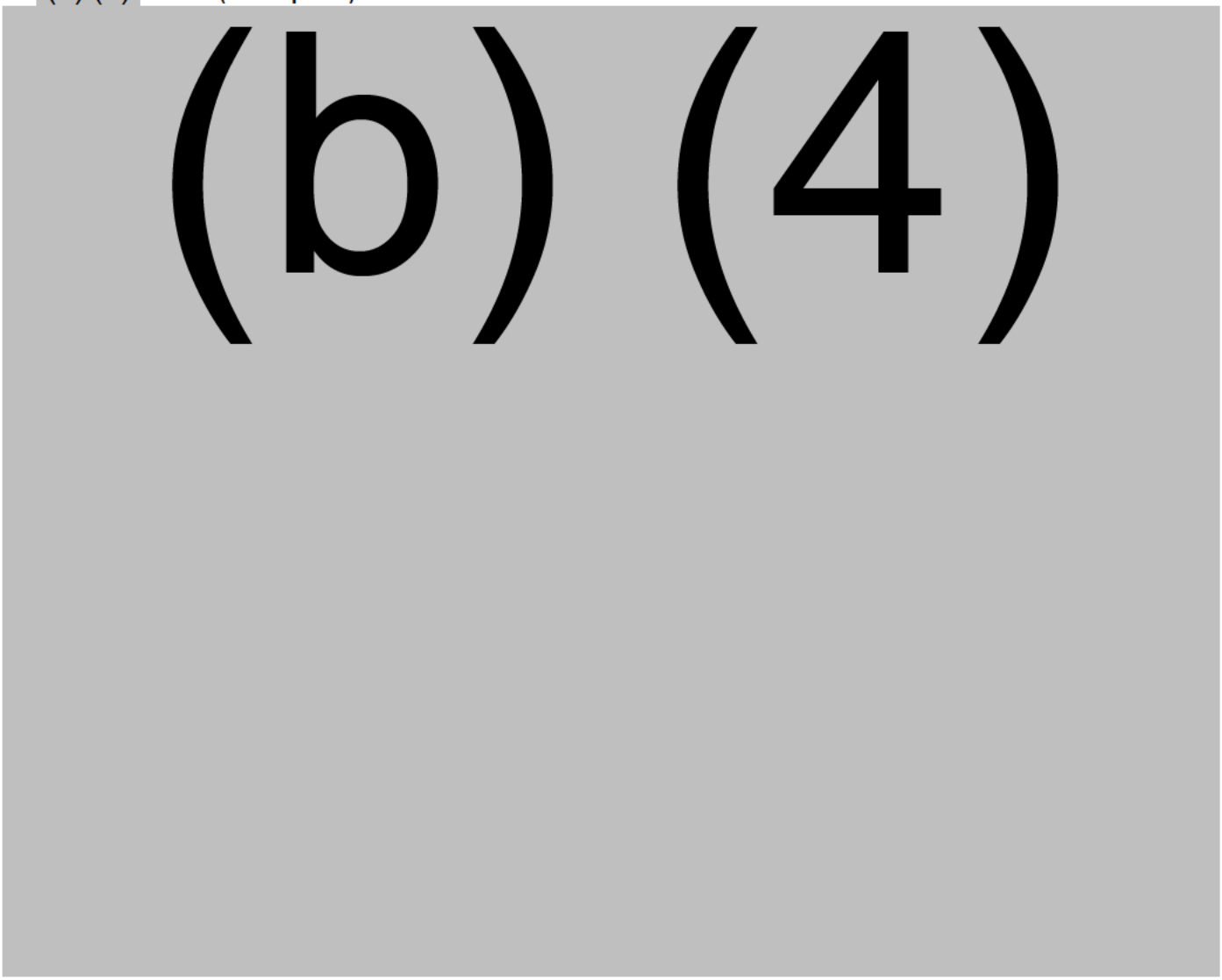
	Anti-spike MSD vs PsVNA50	Anti-spike MSD vs PsVNA80	Anti-spike ELISA vs PsVNA50	Anti-spike ELISA vs PsVNA80
Overall Day 29 Day 57	(b) (4)			
Baseline SARS-CoV-2 status Negative Positive				
Age (years) 18 to <65 ≥ 65				

Table 2: Summary of (b) (4)

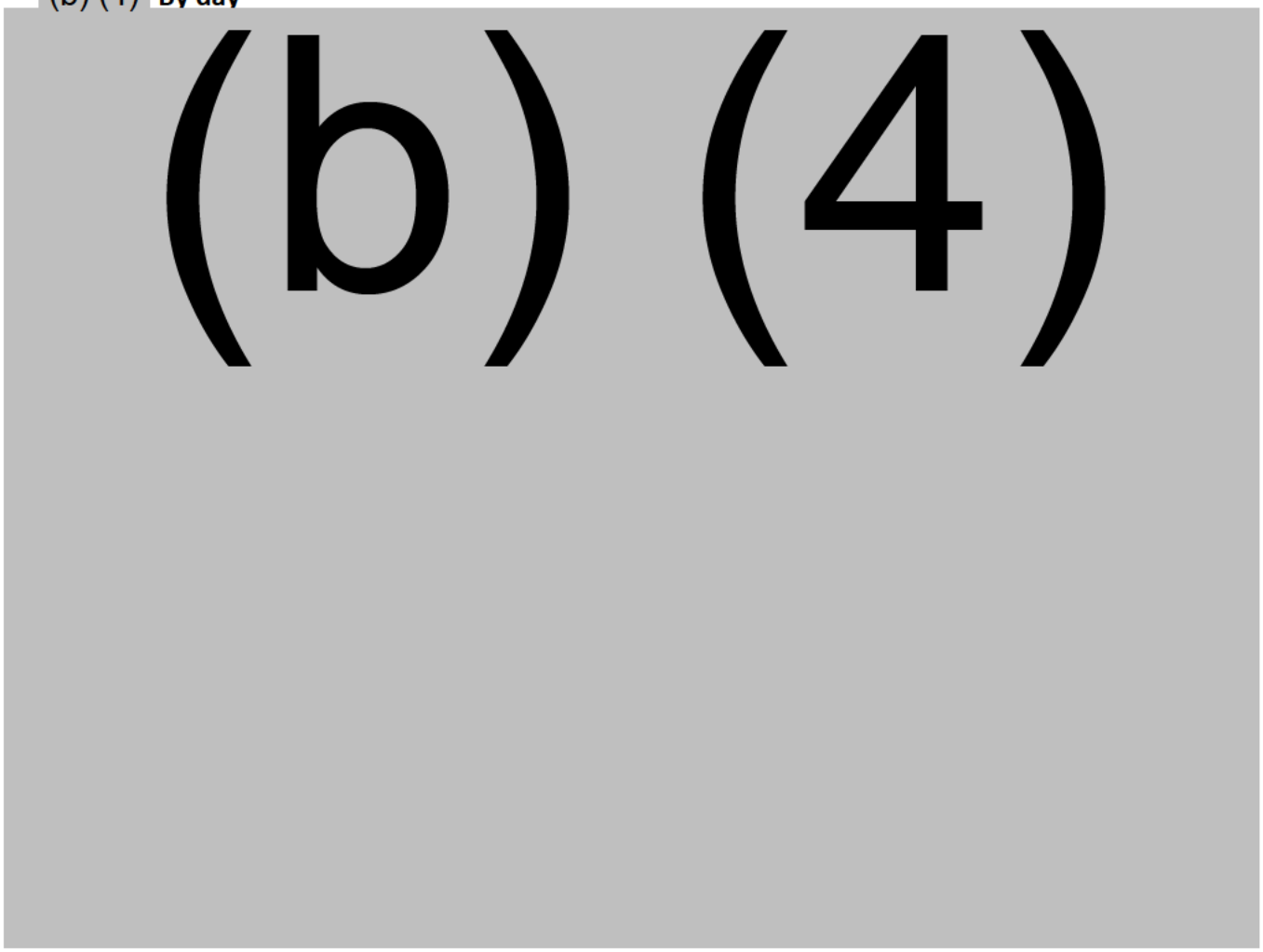
Model	Anti-spike MSD vs PsVNA50	Anti-spike MSD vs PsVNA80	Anti-spike ELISA vs PsVNA50	Anti-spike ELISA vs PsVNA80
Overall (all timepoints) Day 29 Day 57	(b) (4)			
Age Ages 18 to <65 years Ages 65 years and older				
Baseline SARS-CoV-2 status Negative Positive				

Note: (b) (4)

(b) (4) **Overall (all timepoints)**

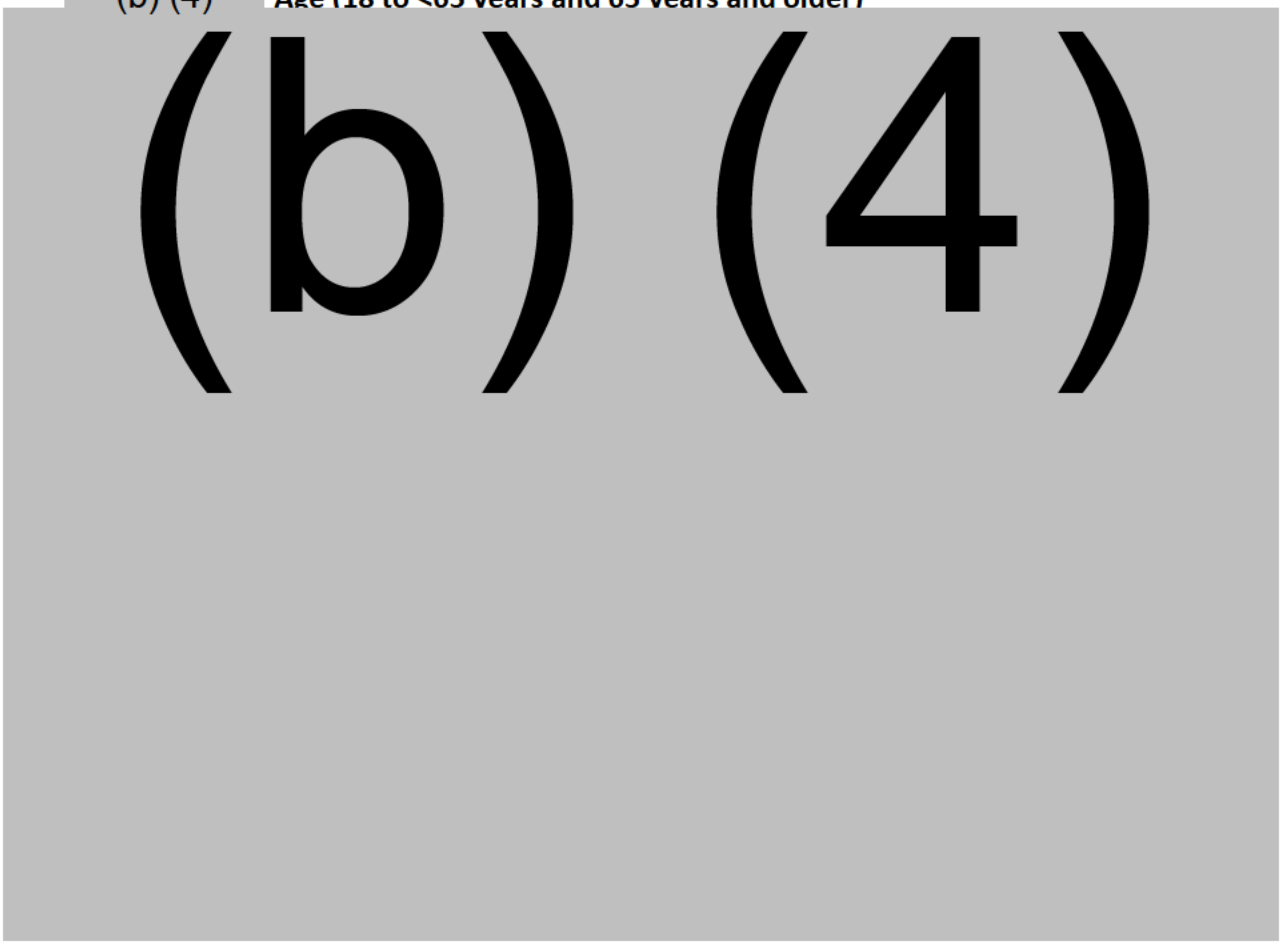


(b) (4) **By day**



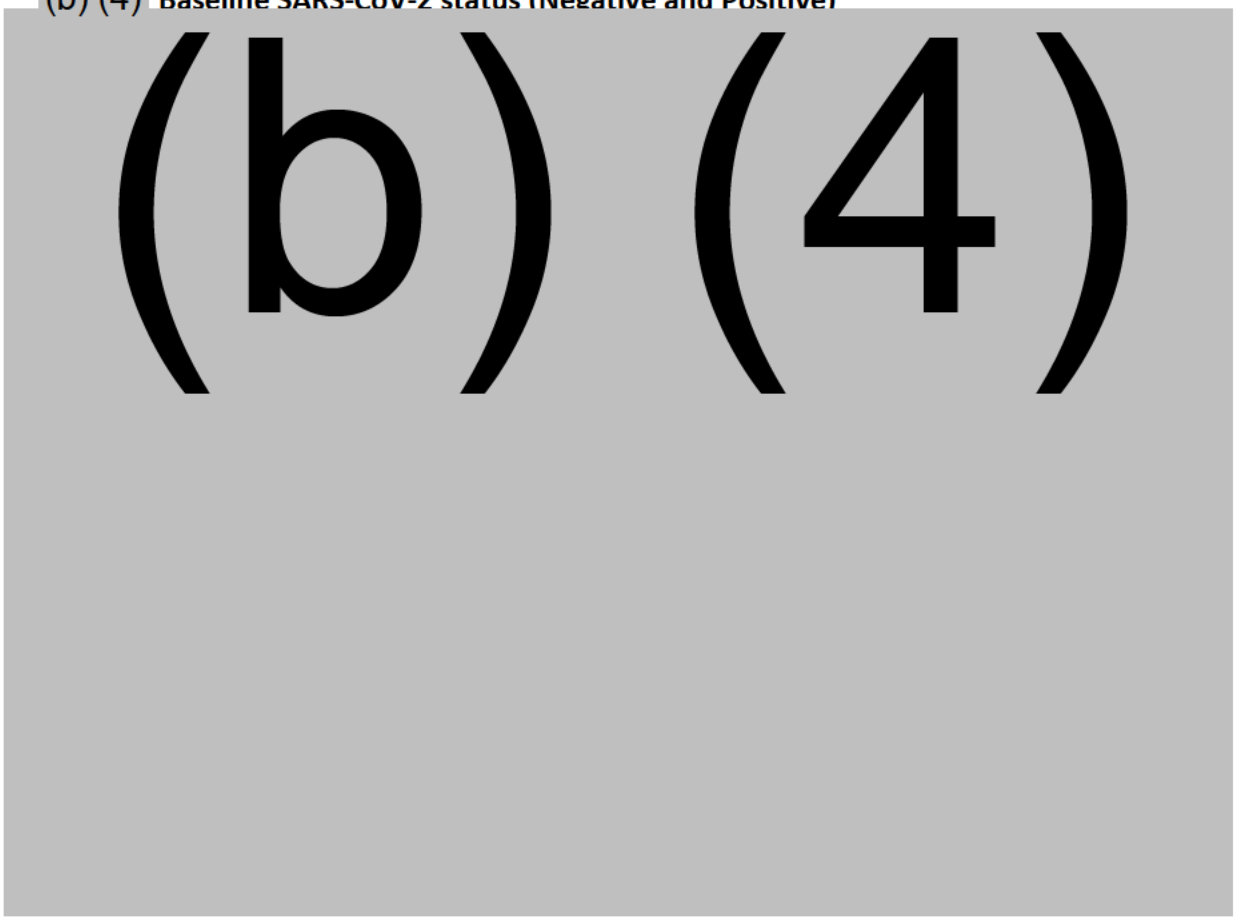
Key: Day 29 and Day 57, (b) (4) for Each Subgroup

(b) (4) **Age (18 to <65 years and 65 years and older)**



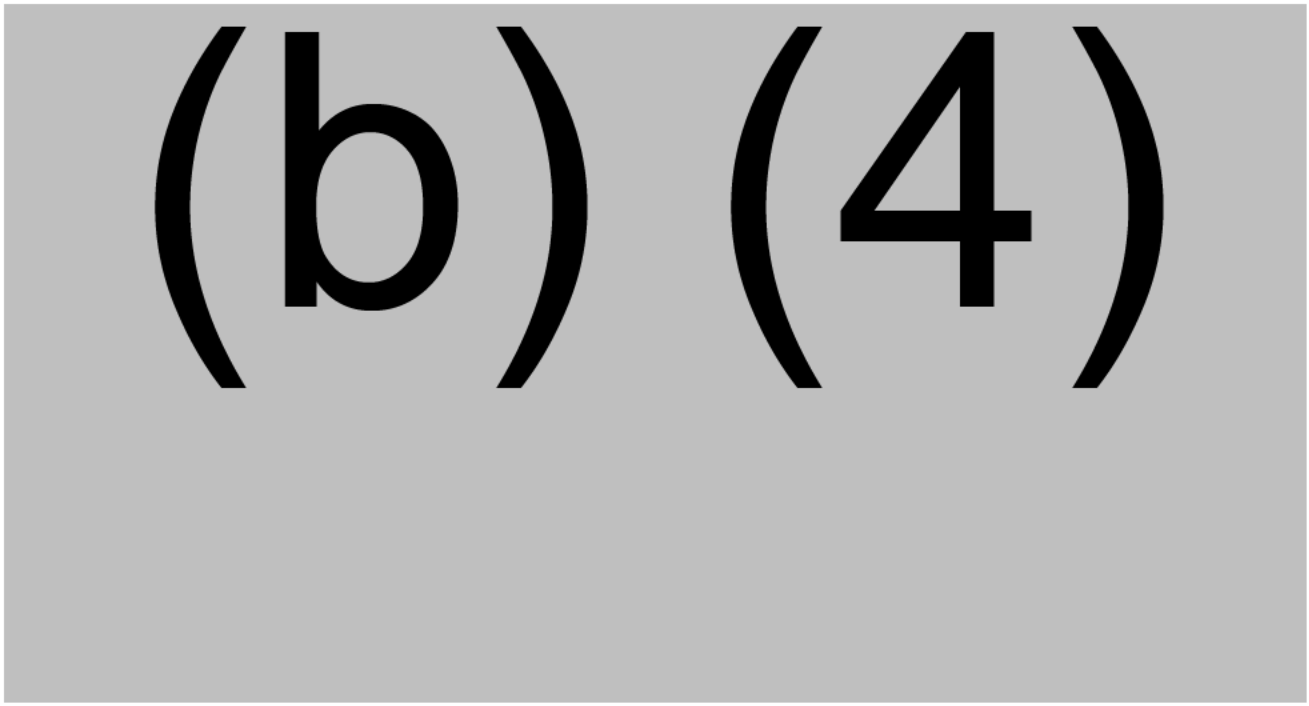
Key: Age 18 to 65y, Age 65 years or more, (b) (4) for Each Subgroup

(b) (4) **Baseline SARS-CoV-2 status (Negative and Positive)**



Key: SARS-CoV-2 status Negative, Positive, (b) (4) for Each Subgroup

Summary



Thus, the concordance (b) (4) can be used to adjust fold difference criteria (e.g., (b) (4)) for purposes of clinical sample evaluations.

References

Jackson, L et al. An mRNA Vaccine against SARS-CoV-2— Preliminary Report, N Engl J Med 2020; 383:1920-31, 2020.

ModernaTx. Moderna mRNA-1273 Biomarker Concordance Analysis Plan. March 4, 2021.

Montefiori D and McDanal C. Method Validation Report for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay with Moderna Phase 1 Samples. November 16, 2020.

NIH. Amendment to Validation of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins (December 14, 2020).

PPD. Validation of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein (Anti-S). Version 1 (16 Oct 2020).

Schofield, T., "Assay Validation,", Chow, Shein-Chung (Ed). Biopharmaceutical statistics. 2020. Marcel-Dekker, Inc. New York.

U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research. Emergency Use Authorization for Vaccines to Prevent COVID-19 Guidance for Industry Document issued on February 22, 2021.

Title: **Moderna 1273 Biomarker Seroresponse Analysis Report: SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay**

Sponsor Name: ModernaTX, Inc.
Legal Registered Address: 200 Technology Square
Cambridge, MA 02139

Date (Version) of Report: 29 April 2021 (Version 1.1)

DocuSigned by: (b) (6) 03 May 2021

Author: (b) (6) (Signature) Date

DocuSigned by: 035ADF27488C419... 30 April 2021

Author: Katherine Kacena, PhD (Signature) Date

Reviewed and Approved by:

DocuSigned by: Rolando Pajon 046725C1EE1146E... 03 May 2021

Biomarker Lead: Rolando Pajon, PhD (Signature) Date

DocuSigned by: robert paris 2C38280C7FED4B8... 30 April 2021

Translational Medicine Lead: Robert Paris, MD (Signature) Date

DocuSigned by: Brett Leav 7F997CA51B87496... 02 May 2021

Clinical Lead: Brett Leav, MD (Signature) Date

DocuSigned by: Honghong Zhou 8D2CBD70CE87482... 02 May 2021

Biostatistics Lead: Honghong Zhou, PhD (Signature) Date

Background

Fold rise in antibody titer has been used historically both in lieu of a correlate of protection and as a measure of response in initially seropositive individuals. The threshold for fold-rise was originally postulated on the basis "distinguishing titers" obtained from a doubling dilution method. As ratios of reciprocal titers, fold rises can take on values of 1 (i.e. no fold rise), 2, 4, 8 and so forth, and even ½, ¼, etc. A rule was established based on the assumption that the titer of a sample (b) (4)
(b) (4)
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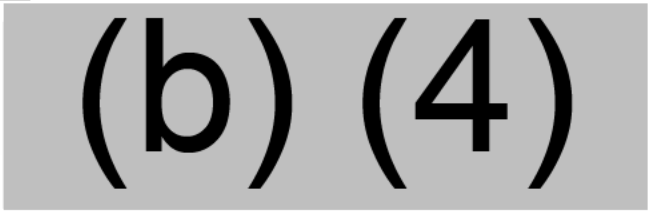
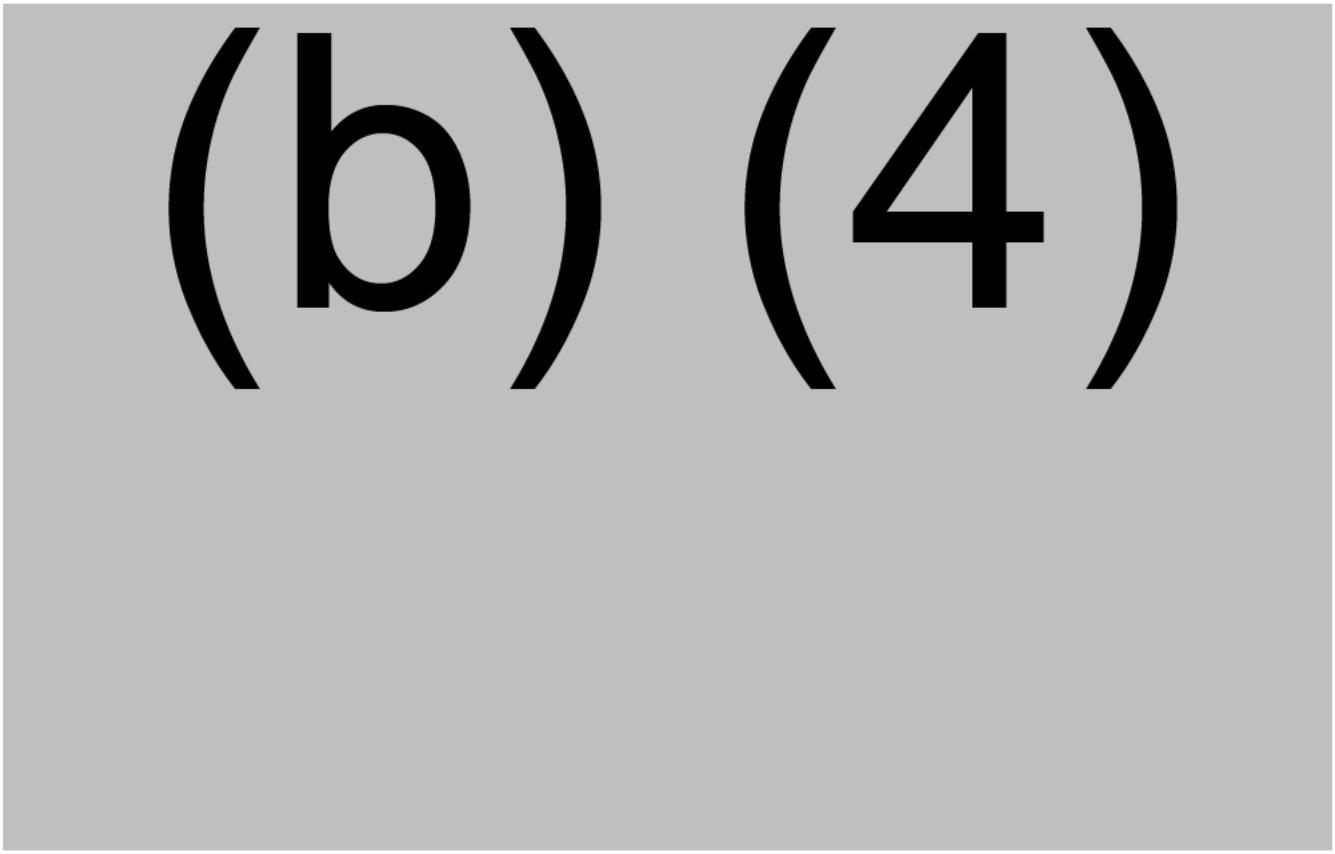


This led to the rule that (b) (4) to conclude that an individual seroconverted.



Summary measures obtained from the validation of the pseudovirus typed neutralization assay and their corresponding CFR based on the above methodology are given in Table 1.

Table 1: %GCV and associated ln standard deviation (S) for PsVNA50 and PsVNA80 at various dilutions

Test	Dilution Category	Mean Titer	(b) (4)	(b) (4)	(b) (4)	(b) (4)	Total (S)	Critical Fold-rise
(b) (4)								
PsVNA 50	Overall						(b) (4)	
(b) (4)								
PsVNA 80	Overall						(b) (4)	
(b) (4)								

References

David Montefiori and Charlene McDanal. Method Validation Report for SARS-CoV-2 Spike-Pseudotyped Virus Neutralization Assay with Moderna Phase 1 Samples. November 16, 2020.

(b) (4)

Title: **Moderna 1273 Biomarker Seroresponse Analysis Report: ELISA Detection of IgG Specific to SARS-CoV-2 Spike Protein (Anti-S)**

Sponsor Name: ModernaTX, Inc.
Legal Registered Address: 200 Technology Square
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DocuSigned by:
(b) (6)

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DocuSigned by:
035ADF27488C419...

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Author: Katherine Kacena, PhD (Signature) Date

Reviewed and Approved by:

03 May 2021

DocuSigned by:
Rolando Pajon
0467BC1E514E...

Biomarker Lead: Rolando Pajon, PhD (Signature) Date

DocuSigned by:
robert paris
2C38280C7FED4B8...

30 April 2021

Translational Medicine Lead: Robert Paris, MD (Signature) Date

DocuSigned by:
Brett Leav
7F997CA51B87496...

02 May 2021

Clinical Lead: Brett Leav, MD (Signature) Date

DocuSigned by:
Honghong Zhou
8D2CBD70CE87482...

02 May 2021

Biostatistics Lead: Honghong Zhou, PhD (Signature) Date

Background

Fold rise in antibody titer has been used historically both in lieu of a correlate of protection and as a measure of response in initially seropositive individuals. The threshold for fold-rise was originally postulated on the basis "distinguishing titers" obtained from a doubling dilution method. As ratios of reciprocal titers fold rises can take on values of 1 (i.e. no fold rise), 2, 4, 8 and so forth, and even ½, ¼, etc. A rule was established based on the assumption that the titer of a sample (b) (4) (b) (4) (b) (4) This is illustrated in the following figure.

(b) (4)

This led to the rule that (b) (4) to conclude that an individual seroconverted.

(b) (4)

The scope of this report is to use the (b) (4) "Overall Precision," (b) (4) (b) (4) as shown below in Tables 1a and 1bfrom the validation reports for the ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein (Anti-S) as the sole data for analysis.

Table 1a

Assay Precision (Measured in (b) (4)

(b) (4)

Reference. Table 13: PPD. Validation of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein (Anti-S). Version 1 (16 Oct 2020).

Table 1b

Assay Characteristic	SARS-CoV-2 Spike IgG ELISA	Acceptance Criteria
Precision (b) (4) (b) (4)	(b) (4)	(b) (4)

Reference. Table 1: VSDVAC 65: Validation Addendum 2 Statistical Report RPPF: Validation of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum at the (b) (4) Dilution

(b) (4)

Additional Statistical Methodology to Account for the "critical fold rise" between the (b) (4) to (b) (4) dilutions.

(b) (4)

Table 2: Critical Fold Rise Between Samples

Dilution	CFR
(b) (4)	

(b) (4)

References

PPD. Validation of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein (Anti-S). Version 1 (16 Oct 2020).

PPD. VSDVAC 65: Validation Addendum 2 Statistical Report RPPF: Validation of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum at the (b) (4) Dilution (DRAFT Version: 0.4).

(b) (4)

Title: **Moderna 1273 Biomarker Seroresponse Analysis Report: Multiplex Assay for Detection of IgG Specific to SARS-CoV-2 Spike Protein (Anti-S)**

Sponsor Name: ModernaTX, Inc.
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DocuSigned by: Honghong Zhou 02 May 2021

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Biostatistics Lead: Honghong Zhou, PhD (Signature) Date

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(b) (4)

(b) (4)

(b) (4)

This led to the rule that (b) (4) to conclude that an individual seroconverted.

(b) (4)

The scope of this report is to use the Total %CV as shown in Table 7 of the statistical report for Amendment to Validation of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins.

Table 7: Total Assay Variability

Sample	Inter-Run Variance	Intra-Run Variance	Total Variance	N	Mean	Inter-Run %CV	Intra-Run %CV	Total %CV
(b) (4)								

Reference. Table 7: PPD. Amendment to Validation of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins (December 14, 2020).

Statistical Methodology to Account for the (b) (4) [(b) (4)]" in the MSD assay

(b) (4)

References

NIH. Amendment to Validation of Multiplex Assay for the detection of IgG antibodies against SARS-CoV-2 proteins (December 14, 2020).

(b) (4)

USP General Chapter <1033> *Biological Assay Validation*

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