



## **Validation Statistical Report**

**Method: VSDVAC 65 Version 0.00, An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum**

**PPD Project Code: RPPF**

**Validation of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum**

**Version: 1.0**

**Prepared for Moderna**

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## EXPERIMENT BACKGROUND AND PURPOSE

A proprietary serological method, *An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum* was developed and qualified by PPD<sup>®</sup> Laboratories, in Richmond, Virginia, USA. The qualification of this new method was conducted under PPD Project Code “ROQP2”. The new method, VSDVAC 58<sup>[1]</sup>, was finalized to version 1.00 post qualification experiments<sup>[2]</sup>.

At the request of Moderna, the PPD proprietary method, *An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum* was validated by PPD<sup>®</sup> Laboratories, in Richmond, Virginia, USA. The new method, VSDVAC 65<sup>[3]</sup>, will be finalized to version 1.00 after validation. The client specific validation of this method was conducted under PPD Project Code “RPPF”.

A validation plan<sup>[4, 5]</sup> was developed and approved to validate the SARS-CoV-2 Spike IgG ELISA. The purpose of the validation experiments were to re-evaluate the (b) (4)

(b) (4)

(b) (4) of the SARS CoV-2 spike proteins, and to re-evaluate assay quality control specifications for determining the validity of an assay run and the validity of individual test samples within that assay run. The purpose of this report is to document the operating characteristics of the assay. The SARS-CoV-2 Spike IgG ELISA operating characteristics are summarized below and in Table 1.

## Validation Results Summary

Assay Characteristic	Validation Results
	(b) (4) (b) (4)
Assay Validity Criteria	<p><i>The assay plate is considered invalid if</i></p> <p>(b) (4) (b) (4)</p> <p><i>The assay run is considered invalid if</i></p> <p>(6) (b) (4) of the plates fail (using the validity criteria described above).</p>

**Assay**

**Characteristic**

**Standard Curve  
Modeling**

**Validation Results**

(b) (4)

(b) (6)

## Scientific Contribution

The SARS-CoV-2 Spike ELISA met the pre-specified acceptance criteria and is considered validated with regard to ruggedness, relative accuracy, dilutional linearity and selectivity.

The SARS-CoV-2 Spike ELISA met the pre-specified acceptance criteria and is considered validated with regard to precision. (b) (4)

(b) (4)

Antibody concentrations associated with the QCS were established and provided in [Attachment III](#). As the data set is limited, (b) (4)

(b) (4)

The RMSE limit was (b) (4)

(b) (4)

The assay met the pre-specified acceptance criterion (b) (4)

(b) (4)

The SARS CoV-2 Spike ELISA assay is considered validated and is acceptable for use in the assessment of phase III, or higher, clinical samples. (b) (4)

(b) (4)

## Conclusion

The SARS-CoV-2 Spike ELISA is considered validated with regard to (b) (4)

(b) (4)

The SARS-CoV2 Spike ELISA is considered acceptable for use in the assessment of phase III (and higher) clinical samples.

Table 1  
Parameter Summary Table  
All limits are inclusive unless otherwise noted.

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

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## ACRONYMS AND DEFINITIONS

Acronyms	Definitions
Abs	Absolute
Ab[C]	Antibody Concentration
AD	ADHS Serum
(b) (4)	
Conc.	Antibody Concentration Measured in AU/mL
CoV	Coronavirus
Diff	Difference
EC50	Concentration Corresponding to the Median Response
ELISA	Enzyme-Linked Immunosorbent Assay
Exp.	Expected
(b) (4)	
GM	Geometric Mean
GMC	Geometric Mean Antibody Concentration
GMedC	Geometric Median Concentration
IgG	Immunoglobulin-G
(b) (4)	
mL	Milliliter(s)
NA	Not Applicable
NE	Not Estimable or Not Evaluable
NIH	National Institute of Health
Obs.	Observed
OD	Optical Density
P	Specificity Sample
PF	Plate Failure
(b) (4)	
QA	Quality Assurance
QC	Quality Control
QCS	Quality Control Serum or Samples
(b) (4)	
Ratio	Maximum(OD)/Minimum(OD)
Rep	Replicate
RMSE	Root Mean Square Error
Run	A group of analytical samples consisting of standard curve, QCS, blank and test samples processed across a minimum of one plate.
(b) (4)	
SARS	Sudden Acute Respiratory Syndrome
SAS	Statistical Analysis Software
(b) (4)	
VSD	Vaccine Sciences Department
Work Order	Unique run identifier assigned by LIMS
(b) (4)	

## SCOPE

The scope of this validation is limited to documenting the operating characteristics of the method for the detection of IgG specific to SARS-CoV-2 Spike protein in human serum. All sample test results will be used for assay validation purposes only and will not be included in the analysis of any clinical trial or epidemiology study. The assay and data are not designed for medical or diagnostic purposes.

## STUDY OBJECTIVES & DESIGN

### Study Objectives

For the assay under evaluation, the objectives of the validation experiments were to:

(b) (4)

### Plate Layout

The following sample types were analyzed in each qualification run:

(b) (4)

An example of the typical plate layout is provided in [Figure 1](#).

Figure 1  
Generic Plate Layout

(b) (4)

#### EXPERIMENTAL DESIGN

(b) (4)

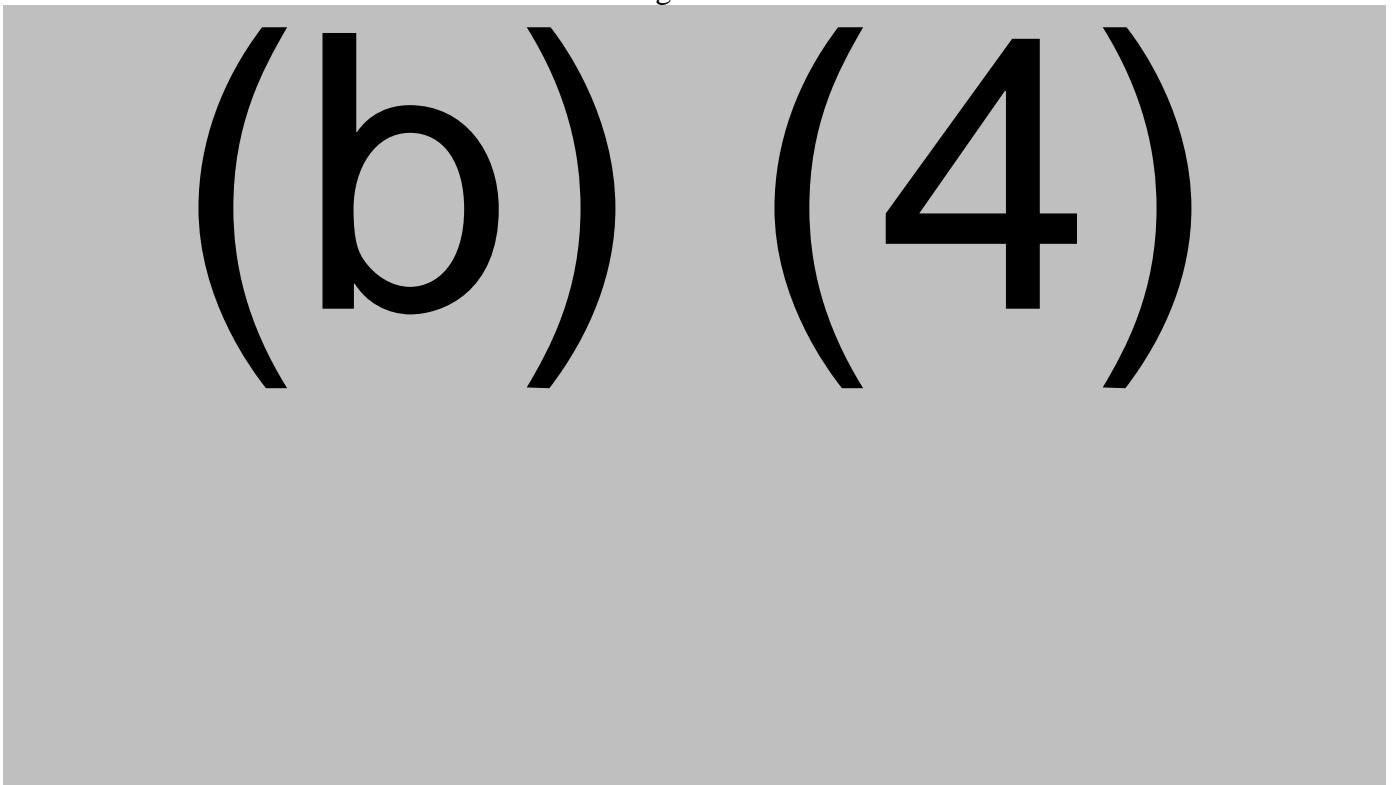
(b) (4)

Table 2

(b) (4)

(b) (4)

Figure 2



(b) (4)

Table 3

(b) (4)



Figure 3

(b) (4)

(b) (4)

Figure 4

(b) (4)

Table 4  
Experimental Design

(b) (4)

Table 5  
Sample Description for Samples Used within Each of the Experiments

Experiment	Sample	Serum ID	Experiment	Sample	Serum ID
------------	--------	----------	------------	--------	----------

(b)

(4)

**STATISTICAL METHODS AND RESULTS**

(b) (4)

Figure 5

(b) (4)

Table 7

(b) (4)

(b) (4)

Table 8

(b) (4)

(b) (4)



Quality Control Samples (QCS)

(b) (4)

Run Suitability

Using the criteria stated above, the assay run is considered invalid if (b) (4) of the plates fail.

Validation Data Validity

(b) (4)

Table 9

(b) (4)

Table 10

(b) (4)

(b) (4)

Table 11

(b) (4)

Figure 6

(b) (4)

(b) (4)

Table 12

(b) (4)

(b) (4)

Figure 7

(b) (4)

Table 13

(b) (4)



Table 14a

(b) (4)

Figure 8

(b) (4)

(b) (4)

Table 15

(b) (4)

(b) (4)

Figure 9

(b) (4)

Table 16

(b) (4)

(b) (4)

Figure 10

(b) (4)



Table 17a

(b) (4)

Table 17b

(b) (4)

(b) (4)

Table 18

(b) (4)

## References

1. VSDVAC58: *An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum*, v1.00.
2. PPD Qualification Statistical Report: *Qualification of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum*, ROQP2, v1.0, 19-June-2020.
3. Draft VSDVAC65: *An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum*, v0.00.
4. PPD Validation Plan: *Validation of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum*, RPPF, 21-August-2020.
5. PPD Method Validation Plan Amendment 1: *Validation of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum*, RPPF, 21-September-2020.
6. (b) (4)
7. Event QEI #5614: VSDVAC 65: RPPF: (b) (4) Sample Storage not Documented after Preparation, RPPF, Date opened 14-September-2020.
8. Event QEI #5858: VSDVAC 65: VSDVAC 65: RPPF: (b) (4) Unable to be Processed at Completion of Assay, RPPF, Date opened 17-September-2020.

## Revision History

Version	Date	Author	Reason for Revision
1.0	16-October-2020	Victoria Pisciella	Original Version

## Attachment I

(b) (4)

## Attachment II

(b) (4)

## Attachment III

(b) (4)

## Attachment IV

(b) (4)

## Attachment V

(b) (4)



## Attachment V

(b) (4)

## Attachment VI

(b) (4)

## Attachment VI

(b) (4)

## Attachment VII

(b) (4)

## Attachment VII

(b) (4)

## Attachment VIII

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## Attachment VIII

(b) (4)

## Attachment VIII

(b) (4)



## Attachment IX

(b) (4)