Method Validation Plan Addendum 4

VSDVAC_65_VP_4

PPD Project Code: RPPF

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

Version 1.00

VSDVAC 65, v1.01, An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum

To be Conducted for Moderna

by PPD® Laboratories
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Issue Date: 13-Jan-2021

PPD Approval

Client:	Moderna
PPD Project:	RPPF
PPD Plan Title:	Addendum to the Validation of an ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum

This validation plan addendum has been reviewed and approved by the undersigned.

Jack Hester

Jack Hester
Associate Group Leader
I approve this document
14 Jan 2021 15:55:13 -05:00

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Signature/Date

Adrienne Howlett

Adrienne Howlett
Manager Labs
I approve this document
14 Jan 2021 16:44:39 -05:00

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Marie Bonhomme, Ph.D.

Marie Bonhomme
Associate Director
I approve this document
15 Jan 2021 08:02:36 -05:00

DocuSign

Signature/Date

Biostatistical Reviewer

Victoria A. Pisciella
Senior Biostatistician II
I reviewed this document
15 Jan 2021 07:03:20 -05:00

DocuSign

Signature/Date

Note: Analyst(s) performing these experiments are stating that she/he has read the document, has had an opportunity to ask questions on the design of the plan, and understands the expectations before performing the work by signing electronically in eSheet.

PPD QA Review

Client:	Moderna
PPD Project:	RPPF
PPD Plan Title:	Addendum to the Validation of an ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum

This validation plan addendum has been reviewed by the undersigned.

(b) (6)
(b) (6)
I reviewed this document
14 Jan 2021 19:14:01 -05:00
DocuSign

QA Reviewer

Signature/Date

Sponsor Approval

Client:	Moderna
PPD Project:	RPPF
PPD Plan Title:	Addendum to the Validation of an ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum

This validation plan addendum has been reviewed and approved by the undersigned.

Exception:
Approval of the original plan may be granted by the client in the form of written communication and will be stored in PPD's ECM system.

(b) (6)

(b) (6)
I confirm that this approval was given via email and housed in ECM under the project code(s) listed in the plan.
14 Jan 2021 15:51:16 -05:00

DocuSign

Bethany Girard
Clinical Biomarker Sr. Manager, Clinical Operations

Signature/Date

Method Validation Plan Addendum 4

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

Introduction

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® Laboratories, in Richmond, Virginia, USA. The qualification of this new method was conducted under PPD Project Code "ROQP2". The new method, VSDVAC 58[1] was finalized to Version 1.00 post qualification experiments[2].

At the request of Moderna, the PPD proprietary serological method, "An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum" was validated by PPD® Laboratories, in Richmond, Virginia, USA. The validation of this new method was conducted under PPD Project Code "RPPF". The new method, VSDVAC 65[3] was finalized to Version 1.01 post validation experiments[4].

This method describes the procedure for the analysis of the SARS-CoV-2 total IgG in human serum. This quantitative ELISA assay was designed to detect IgG antibody to the SARS-CoV-2 virus in human serum. Microtiter plates were coated with commercially available SARS-CoV-2 full-length spike (S) glycoprotein and serum containing the SARS-CoV-2 IgG antibody was added. Bound antigen-antibody complex was detected using purified goat anti-human IgG horseradish peroxidase (HRP) conjugate. Color development occurred during the addition of 3,3',5,5'tetramethylbenzidine (TMB) substrate and color intensity was measured spectrophotometrically (b) (4). The intensity of the color was directly proportional to the IgG antibody concentration. Quantitation of the human IgG antibody to SARS-CoV-2, or antibody concentration (AU/mL), was determined by interpolation from a standard curve analyzed on each assay plate.

The method, VSDVAC 65, was previously validated at sample dilutions (b) (4)[4]. Based on clinical testing observations, a (b) (4) sample dilution will be evaluated to expand the (b) (4) quantitative range of the assay. While the assay was considered sufficiently specific after the original validation, one sample tested showed reproducible low level of cross-reactivity and two samples tested showed non-reproducible low level of cross-reactivity with the (b) (4) (b) (4) spike protein[4]. As such, the objectives of this validation plan addendum is to confirm specificity of the assay.

Assay runs that are rejected or aborted due to a documented technical error will be repeated within the confines of this plan. Clear documentation of the need for a repeat run will be made on the assay bench sheet.

If additional evaluations not covered by the original plan are required, an addendum to the plan can be written. A plan amendment documents an intended change to the plan after the evaluation was initiated. Amendments and addenda to plans must be approved at the same levels as the original plan or higher. Any exceptions to this plan will be evaluated to determine if an Event is required per SOP-GQC-42, *Quality Event Management.*

Following the validation addendum, the method, VSDVAC 65 will be finalized to Version 2.00 and may be used in support of Phase I through Phase III (and higher) sample analysis.

Responsibilities

1. PPD Richmond Vaccine Sciences Department (VSD) scientists will oversee the design of the validation plan addendum, including performance of the analysis, collection of the data, transmission of the data to Biostatistics and Quality Assurance, and scientific contributions to the statistical report. The instrument raw data files for each run will be provided to the Biostatistical team for statistical analysis.

2. PPD Biostatistics personnel will assist in the design of the validation plan with regard to the statistical requirements and analysis of the assay data. PPD Biostatistics will provide a statistical report documenting the operating characteristics of the assay to PPD VSD scientists. The validation report will be issued in standard PPD format. The report will contain a project summary and data tables (where applicable).

3. The data and report will be audited by the PPD Quality Assurance Unit in accordance with relevant PPD SOPs and this validation plan addendum. Audits will be conducted by Quality Assurance personnel independent of staff involved in the project.

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.

Reference Standard and Critical Reagents

The following reference standard and critical reagents will be used during this validation addendum:

Compound	Purpose	Source	Lot	Conc.	Exp. Date	Storage Conditions
SARS-CoV-2 Spike S2P Protein	Coating Antigen Homologous Antigen	(b) (4)				
Goat Anti-Human IgG Antibody, HRP Conjugate	Conjugate					
(b) (4) Human Serum	Reference Standard					

Note: Any changes to the lot of reagent will be included in the validation statistical report. If the content of the Certificate of Analysis differs from this plan, updated information will be included in the final report.

Definitions and Formulas

(b) (4)	
BB	Blocking Buffer (assay diluent)
CoV	Coronavirus
(b) (4)	
ELISA	Enzyme-Linked Immunosorbent Assay
(b) (4)	
HCoV	Human Coronavirus
IgG	Immunoglobulin-G
LLOQ	Lower Limit of Quantitation
(b) (4)	
NIH	National Institute of Health
OD	Optical Density
QA	Quality Assurance
QC(s)	Quality Control Samples
(b) (4)	
Run	A group of analytical samples consisting of standard curve, QCs, and test samples processed across a minimum of one plate
SARS	Sudden Acute Respiratory Syndrome
SOP	Standard Operating Procedure
SPAR	See Periodic Analysis Results (trending)
ULOQ	Upper Limit of Quantitation
VSD	Vaccine Sciences Department

Validation Addendum Specifications

Analyte Name(s)	IgG specific to SARS-CoV-2 Spike protein
Matrix, Species, and Additive	Human Serum
Sample dilution	(b) (4)
(b) (4)	(b) (4)
Quality Controls	
Blank	Blocking Buffer
Reference Standard	(b) (4) Human Serum, 500 AU/mL
Run Acceptance	All system suitability criteria as defined in VSDVAC 65, V1.01 (current version or higher) will be followed. Exceptions to the method are provided in the experimental design section for each of the experiments. When reanalysis runs are needed due to system suitability failure, the same analyst will be used to perform the repeat runs if available. Experimental plate layouts and schedules detailed below are tentative and may be subject to change to accommodate repeats or additional testing as needed.

Note: All Critical Reagents and the sample panel for this Specificity experiment were prepared and confirmed during pre-work experimentation for use in validation.

Validation Experiments

The ELISA assay validation addendum will confirm Specificity.

As shown in the generic plate map in Figure 1, at a minimum the following sample types will be analyzed in each validation run:

- Blocking buffer only (Sample Blank) in (b) (4)
- Reference standard analyzed as an 11-point, 2.5-fold dilution series starting at 1:500 and ending at 1:4,768,371.58203125; all curve points will be evaluated in replicate per plate. The reference standard has been arbitrarily assigned a concentration of 500 AU/mL. The concentration of each standard curve point is provided in Table 1.

(b) (4)

- Up to (b) (4) experimental samples per plate, in (b) (4), analyzed as detailed below in the experimental plan.

Figure 1. Suggested Plate Map for VSDVAC 65

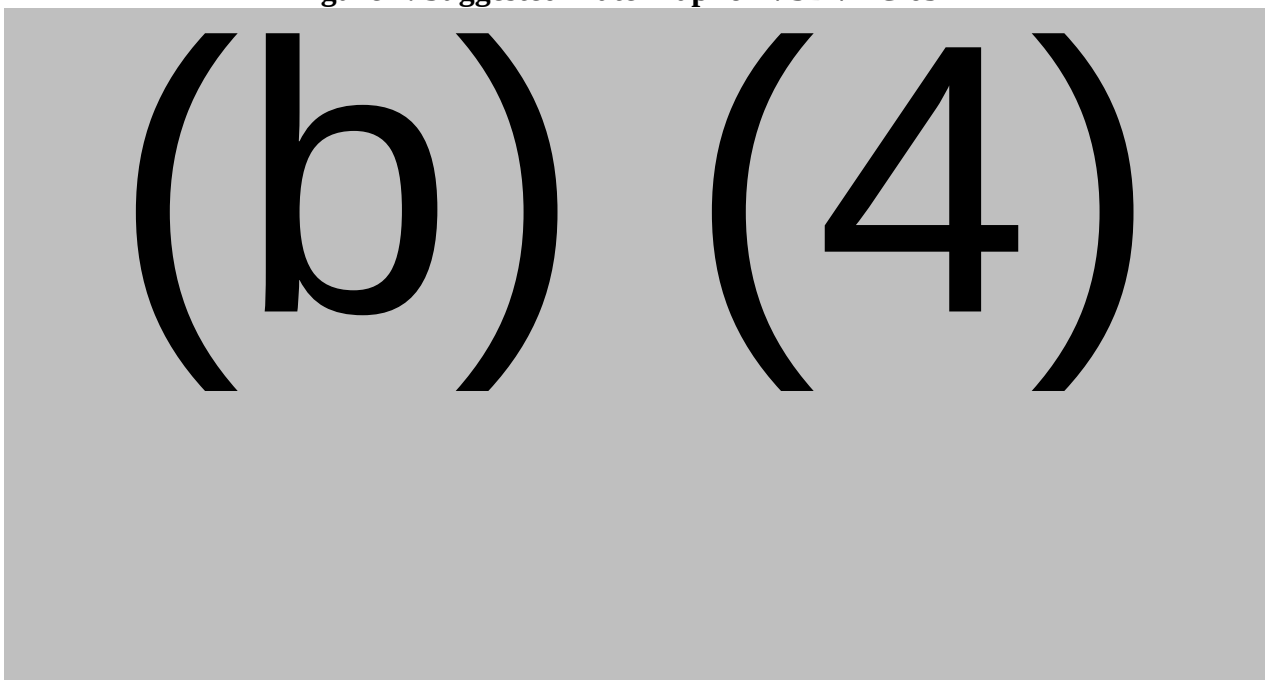


Table 1: Expected Concentrations of Standard Curve Levels

Solution	Dilution	Concentration (AU/mL)
STD 1	1:500	1
STD 2	1:1250	0.4
STD 3	1:3125	0.16
STD 4	1:7812.5	0.064
STD 5	1:19531.25	0.0256
STD 6	1:48828.125	0.01024
STD 7	1:122070.3125	0.004096
STD 8	1:305175.78125	0.0016384
STD 9	1:762939.453125	0.00065536
STD 10	1:1907348.6328125	0.000262144
STD 11	1:4768371.58203125	0.0001048576

Validation experiments may require the use of custom (b) (4) program: *VSDVAC65_*(b) (4), or higher, to facilitate the mapping of diluted samples, reference standard and/or quality controls. The documentation associated with the instrument processes, will include the program(s) used during each experiment, where applicable, and references for sample traceability and mapping.

1. (b) (4) Specificity

The assay was considered sufficiently specific after the original validation; however, two of the samples tested during validation showed a non-reproducible but low level of cross-reactivity with the (b) (4) spike protein[4]. Additional competing antigen conditions will be conducted in this addendum to confirm the specificity of the assay using, in addition to (b) (4) spike protein, (b) (4) other (b) (4) proteins: (b) (4)

The specificity experiment will consist of (b) (4) (b) (4) containing samples will be analyzed at (b) (4) dilution. The experimental design and plate layout are depicted in Table 2 and Figure 2 respectively.

(b) (4)

All analysis will be performed following method VSDVAC 65, V1.01 (current version or higher), with the following exceptions:

(b) (4)

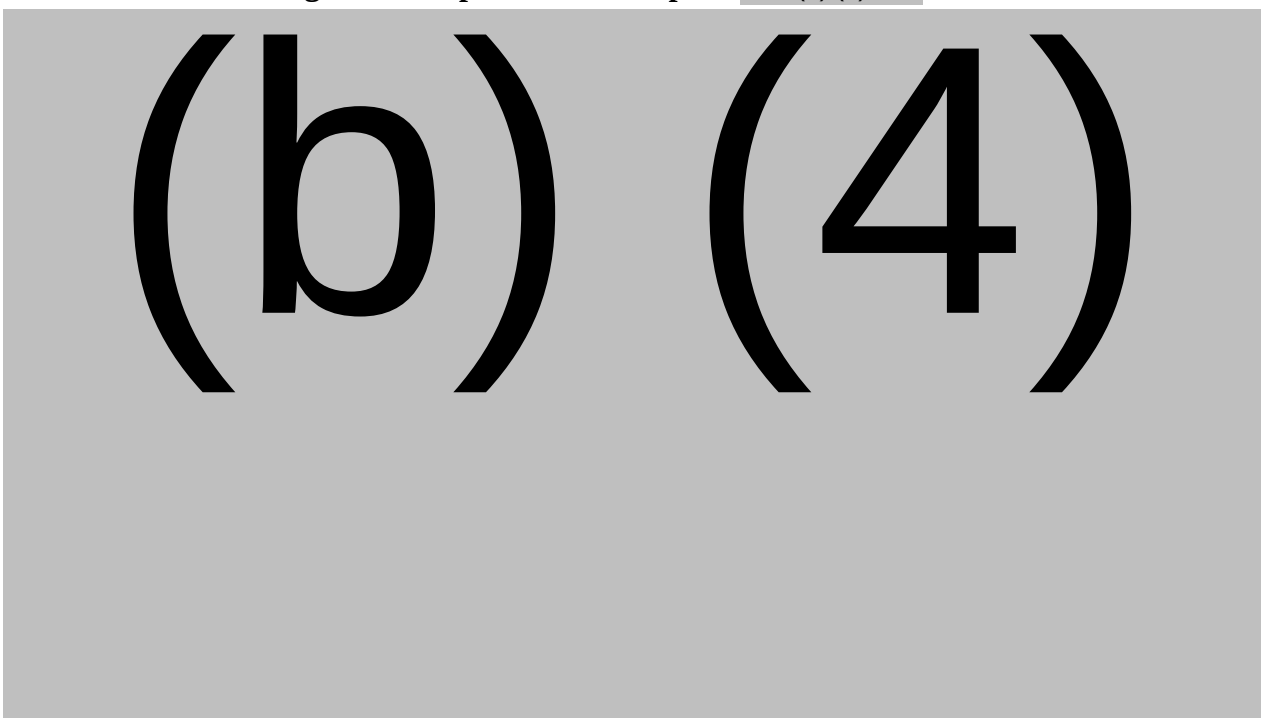
Table 2: Proposed Experimental Design for (b) (4)

(b) (4)

Table 3. Proposed Specificity Panel Samples for (b) (4)

Sample ID	Barcode
(b) (4)	

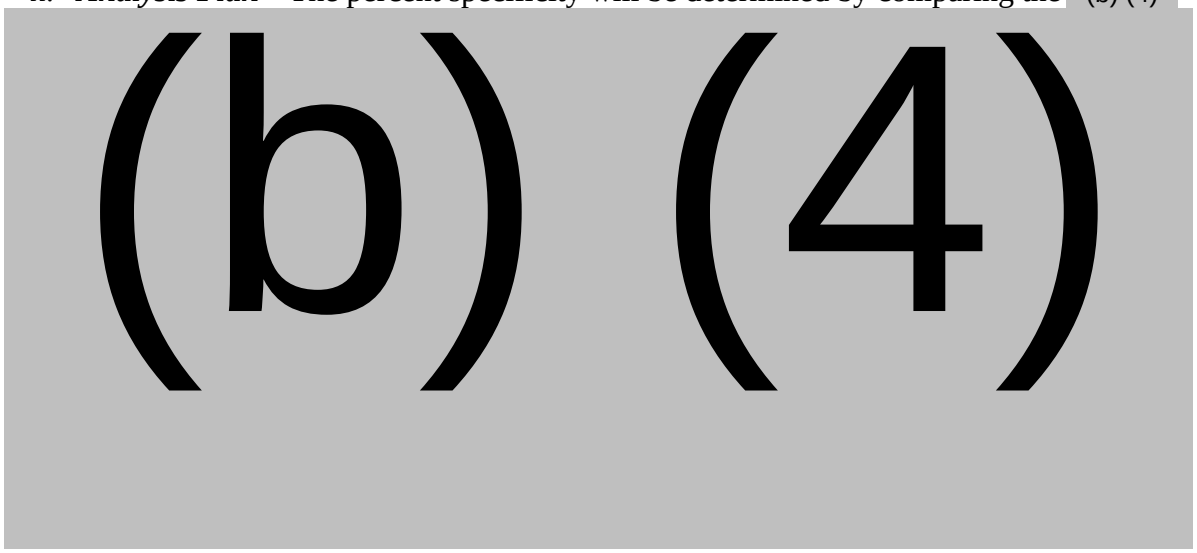
Figure 2: Proposed Plate Map for (b) (4)



Method Validation Data Analysis Plan

a. Analytical Specificity
   i. ***Rationale*** – The ability of an analytical method to determine only the component it purports to measure or the extent to which the assay responds only to all subsets of a specified analyte and not to other substances present in the sample.

   ii. ***Analysis Plan*** – The percent specificity will be determined by comparing the (b) (4)



   iii. ***Acceptance Criteria*** - (b) (4) % of the evaluable samples should have: (b) (4)
(b) (4)
(b) (4)

References

1. VSDVAC 58: *An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum,* v1.00.

2. PPD Statistical Report: *Qualification of an ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum*, ROQP2, v1.00, 19-Jun-2020.

3. VSDVAC 65: *An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum,* v1.01.

4. PPD Statistical Report: *Validation of an ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum*, RPPF, v1.00, 16-Oct-2020.

5. (b) (4)
(b) (4)