Method Validation Plan Addendum 2

VSDVAC_65_VP_2

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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Richmond, Virginia 23230
(804) 359-1900

Issue Date: 18-Dec-2020

PPD Approval

Client:	Moderna
PPD Project:	RPPF
PPD Report 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
Associate Group Leader
I approve this document
18 Dec 2020 10:37:22 -05:00

DocuSign

Jack Hester

Signature/Date

Adrienne Howlett
Manager Labs
I approve this document
18 Dec 2020 10:24:58 -05:00

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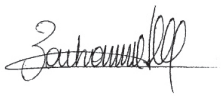
Adrienne Howlett

Signature/Date



Marie Bonhomme
Associate Director
I approve this document
18 Dec 2020 12:09:05 -05:00

DocuSign

Marie Bonhomme, Ph.D.

Signature/Date

Victoria A. Pisciella
Senior Biostatistician II
I reviewed this document
18 Dec 2020 10:31:47 -05:00

DocuSign

Biostatistical Reviewer

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 Report 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)
(b) (6)
I reviewed this document
18 Dec 2020 12:12:03 -05:00

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QA Reviewer

Signature/Date

Sponsor Approval

Client:	Moderna
PPD Project:	RPPF
PPD Report 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 hosted in ECM under the project code(s) listed in the plan.
18 Dec 2020 10:15:28 -05:00

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Bethany Girard
Clinical Biomarker Sr. Manager, Clinical Operations

Signature/Date

Method Validation Plan Addendum 2

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) (b) (4) Based on clinical testing observations, a (b) (4) sample dilution will be evaluated to (b) (4) (b) (4) As such, the objectives of this validation plan are to:

(b) (4)

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, 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. PPD Quality Assurance (QA) personnel will conduct an in-process inspection of the project. The data and report will be audited by the PPD Quality Assurance Unit in accordance with relevant PPD SOPs and this validation plan. Inspections and 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:

Compound	Purpose	Source	Lot	Conc.	Exp. Date	Storage Conditions
SARS-CoV-2 Spike S2P Protein	Coating 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)	
(b) (4)	
BB	Blocking Buffer (assay diluent)
CoV	Coronavirus
(b) (4)	
ELISA	Enzyme-Linked Immunosorbent Assay
(b) (4)	
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 Plan Specifications

Analyte Name(s)	IgG specific to SARS-CoV-2 Spike protein
Matrix, Species, and Additive	Human Serum
Sample dilution	(b) (4)
Quality Controls	(b) (4)
Blank	Blocking Buffer
Reference Standard	(b) (4) Human Serum, (b) (4)
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 sample panels for (b) (4) *Precision and Relative Accuracy experiments were prepared and confirmed during pre-work experimentation for use in validation.*

Validation Experiments

The ELISA assay validation addendum will be (b) (4) (b) (4) (b) (4) will evaluate Precision and Relative Accuracy at the (b) (4) dilution. Precision and Relative Accuracy from (b) (4) will be used to re-evaluate the (b) (4) (b) (4). (b) (4) will assess Dilutional Linearity. The (b) (4) will assess the (b) (4) of the assay at the (b) (4) dilution. Dilutional Linearity and (b) (4) experiments will use the (b) (4) data of the assay established in (b) (4) within the assay validation.

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) (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) (b) (4)
- (b) (4) (b) (4)

Figure 1. Suggested Plate Map for VSDVAC 65

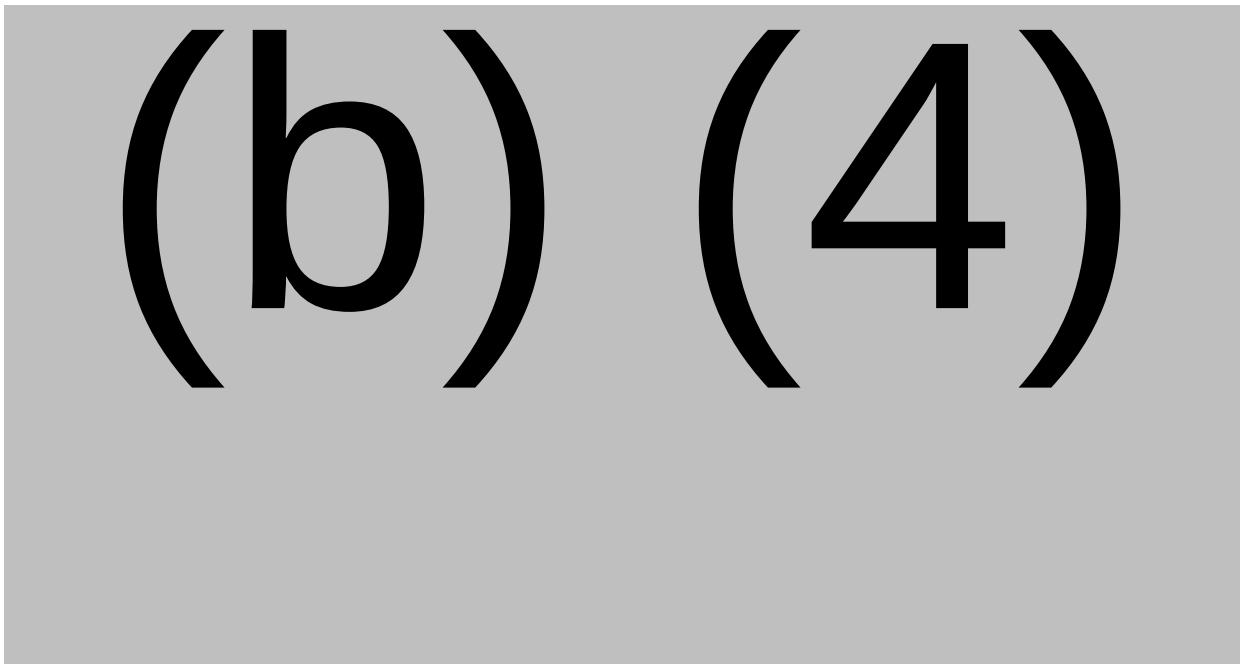


Table 1: Expected Concentrations of Standard Curve Levels

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. Experiment 1: Precision and Relative Accuracy (RA)

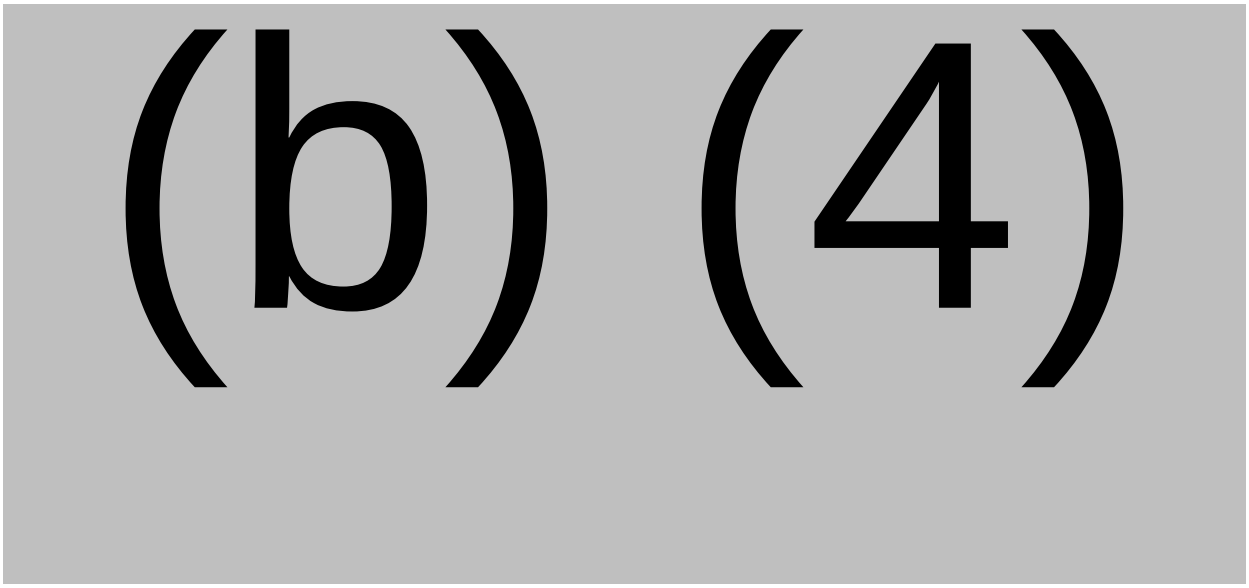
Precision and Relative Accuracy will be assessed (b) (4) (b) (4) A minimum of (b) (4) Precision samples (b) (4) pre-screened to have antibody concentrations that span the quantifiable range of the assay, will be used to confirm assay precision (b) (4) assay precision) at the (b) (4) dilution. Relative Accuracy samples (b) (4) will be used to confirm the ability of the assay to reproducibly measure a known concentration of antibody within sample assay diluent (blocking buffer). Refer to Table 2 and Table 3 for the proposed experimental design and list of samples used in each experiment.

The Precision samples will be stored at (b) (4) throughout the experiments. Samples may be stored at (b) (4) for prolonged storage if needed.

Samples for Relative Accuracy were prepared from an 11-point, 2-fold dilution series of the reference standard (referred to as "Accuracy Panel") starting at a (b) (4) (b) (4) in sample assay diluent (blocking buffer). Each point will be interpolated from the Reference Standard curve, which is tested once, in replicate, on each plate. The expected concentrations associated with each of the spike levels are provided in Table 4. Samples for RA will be stored at (b) (4) upon preparation. Post thaw samples will be stored at (b) (4) throughout the experiments. The samples may be stored at (b) (4) for prolonged storage if needed.

(b) (4)
(b) (4)
except where noted in the experimental design, all critical reagents will remain the same. Refer to example plate layout in Figure 2.

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



Sample, plate, and run acceptance criteria outlined in the method will be used to qualify a sample, plate, or run as valid. Data from samples or runs that do not meet the assay acceptance criteria will be excluded from the analyses and all rejected runs will be repeated following the design details outlined within this plan. If a given plate failed (b) (4) times for the same criteria, the lab will investigate the reason for failure and determine a path forward. The evaluation will be documented as an Event as per SOP-GQC-42, *Quality Event Management*. The data from this experiment will be forwarded to the Statistics group for analysis.

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

(b) (4)

Table 3: Proposed Precision Panel Samples and Barcodes for (b) (4)

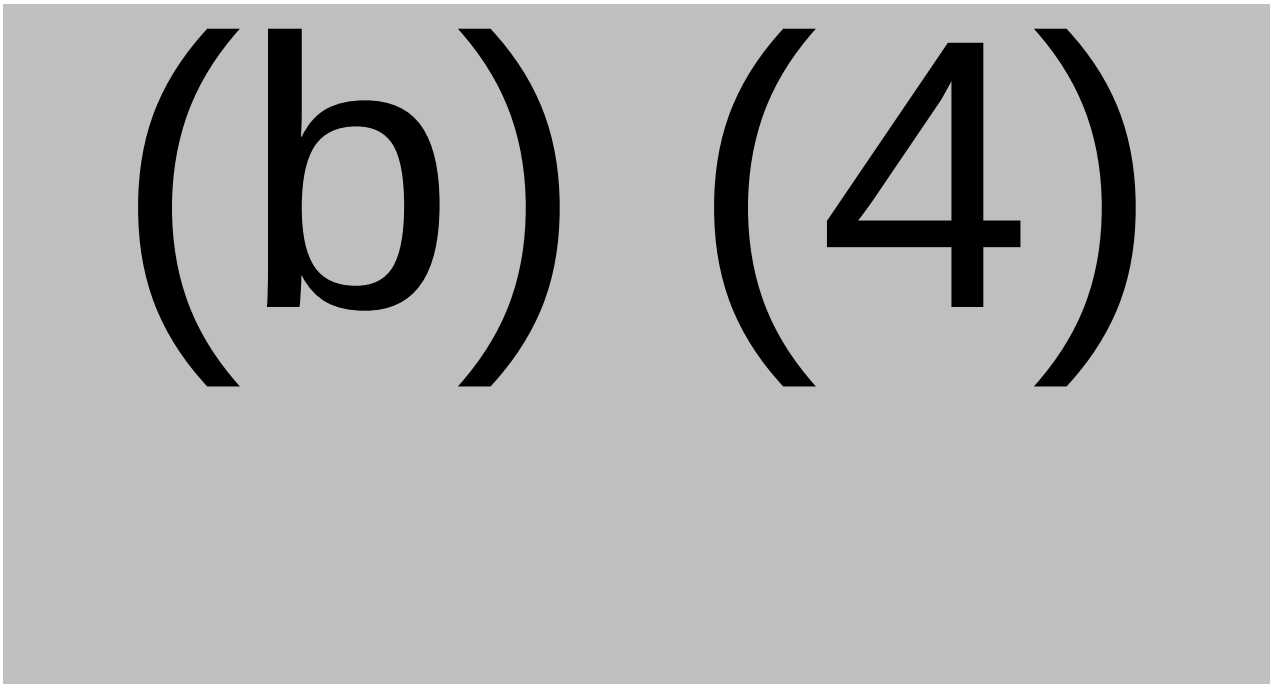
Sample ID	Barcode	Sample ID	Barcode
(b) (4)			

Note: Additional samples may be sequentially numbered and will be included in the validation statistical report.

Table 4: Expected Concentrations of Relative Accuracy

Sample ID	Barcode	Dilution	Expected Conc. (AU/mL)
(b) (4)			

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



2. (b) (4) Dilutional Linearity

(b) (4)
(b) (4) The experimental design and plate layout are depicted in Table 5 and Figure 3, respectively.

The purpose of (b) (4) is to evaluate the dilutional linearity of the assay which is often referred to as parallelism or dilutability. A dilutional linearity panel of (b) (4) samples ("Panel D", depicted in Table 6) will be analyzed across (b) (4) runs. Each sample will be pre-diluted in sample assay diluent (blocking buffer) at dilutions of (b) (4) (b) (4)
(b) (4)
(b) (4)
(b) (4) The Dilutional Linearity Panel will be prepared in advance and stored at (b) (4) until use. Post thaw samples will be stored at (b) (4) throughout the experiments. The samples may be stored at (b) (4) for prolonged storage if needed.

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

1) Since the purpose of this experiment is to evaluate dilutional linearity and all treatments for each sample are intentionally analyzed on the same plate within the same run, samples with (b) (4) as per the method. These samples will be excluded from analysis. It should be noted that the system suitability rule applying to the (b) (4) samples within a plate will be applied and any plate exceeding the maximum allowable (b) (4) sample percentage as defined in the method will be repeated. The purpose of this exception is to ensure that the results from samples that are repeated due to (b) (4) in a future run do not bias the overall analysis.

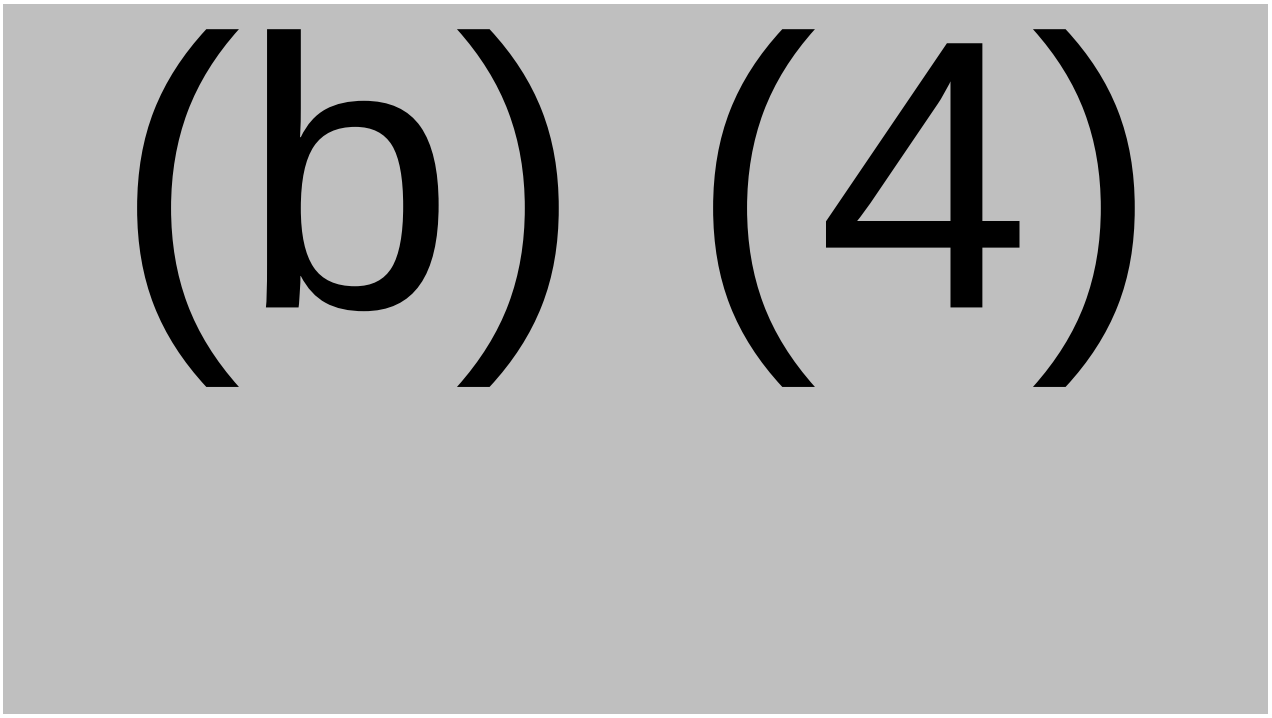
Table 5: Proposed Experimental Design for (b) (4)

Run #	Plate	Samples	Dilution
(b) (4)			

Table 6: Proposed Dilutional Linearity Panel Samples for (b) (4)

Sample ID	Barcode (b) (4)	Barcode (b) (4)	Barcode (b) (4)
(b) (4)			

Figure 3: Proposed Plate Map for (b) (4)



3. (b) (4)

(b) (4)

(b) (4) The experimental design and plate layout are depicted in Table 7 and Figure 4, 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 7: Proposed Experimental Design for (b) (4)

(b) (4)

Table 8: (b) (4)

(b) (4)

(b) (4)

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

(b) (4)

(b) (4)

c. Precision (b) (4)

i. ***Rationale*** – (b) (4)

(b) (4) The estimate of assay precision is used to calculate a statistically meaningful fold-increase in antibody concentration for an individual sample.

ii. ***Analysis Plan*** – (b) (4)

(b) (4)

iii. ***Acceptance Criteria*** – Precision: (b) (4)% of the test samples must have a (b) (4)

(b) (4)

d. Relative Accuracy

i. ***Rationale*** – Accuracy is the closeness of agreement between the value which is accepted either as the conventional true value or an accepted reference value and the value determined.

ii. ***Analysis Plan*** – An 11-point, 2-fold dilution series of reference standard will be analyzed (as test samples) in replicate on each plate in (b) (4) Antibody concentrations will be determined by interpolating each of the 11 relative accuracy sample points off the 11-point reference standard that is also analyzed on each plate.

(b) (4)

iii. ***Acceptance Criteria*** – The assay will be defined as accurate across the ranges in which the relative accuracy estimates are between (b) (4) and (b) (4)

e. Dilutional Linearity (dilutability)

i. ***Rationale*** – Dilutability is an attribute of a biological assay which demonstrates that a test sample can be diluted through a series, yielding equivalent dilution corrected antibody concentrations across that series.

ii. ***Analysis Plan*** – Samples analyzed in the dilutional linearity panel within (b) (4)

(b) (4)

iii. ***Acceptance Criteria-*** The assay will be considered acceptably dilutable if the dilution-bias is less than (b) (4) fold for each (b) (4) -fold range in dilution. If the dilution bias exceeds (b) (4) old per (b) (4) fold dilution, the assay will be considered non-dilutable and sample testing procedures will be modified to minimize the potential for bias that can result when making comparisons between dilutions.

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)