Method Validation Plan Addendum 3

VSDVAC_66_VP_Adden3

PPD Project Code: RPPF2

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

Version 1.00

VSDVAC 66, v1.00, An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum

To be Conducted for Moderna

by PPD® Laboratories
2244 Dabney Road
Richmond, Virginia 23230
(804) 359-1900

Issue Date: 15-Dec-2020

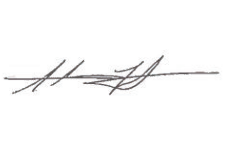
PPD Approval

Client:	Moderna
PPD Project:	RPPF2
PPD Plan Title:	Addendum to the Validation of an ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum

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

Carl Breidenbach

Signature/Date



Adrienne Howlett

Signature/Date

Marie Bonhomme, Ph.D.

Signature/Date

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:	RPPF2
PPD Plan Title:	Addendum to the Validation of an ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum

This validation plan addendum has been reviewed by the undersigned.

(b) (6)
(b) (6)
I reviewed this document
16 Dec 2020 08:14:50 -05:00
DocuSign

(b) (6)

QA Reviewer

Signature/Date

Sponsor Approval

Client:	Moderna
PPD Project:	RPPF2
PPD Plan Title:	Addendum to the Validation of an ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum

This validation plan 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 located in ECM under the project code(s) listed in the plan.
15 Dec 2020 19:27:58 -05:00

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Bethany Girard, Ph.D.
Senior Manager, Clinical Assays and Biomarkers

Signature/Date

Method Validation Plan Addendum 3

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

Introduction

A proprietary serological method, "An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid 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 "ROZD2". The new method, VSDVAC 64[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 Nucleocapsid 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 "RPPF2". The new method, VSDVAC 66[3] was finalized to Version 1.00 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. (b) (4)

(b) (4)

(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 66 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).

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 Nucleocapsid 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 Nucleocapsid Protein	(b) (4)					

Definitions and Formulas

ADHS	Antibody-depleted Human Serum
(b) (4)	
BB	Blocking Buffer (assay diluent)
CoV	Coronavirus
(b) (4)	
ELISA	Enzyme-Linked Immunosorbent Assay
(b) (4)	
IgG	Immunoglobulin-G
(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)
(b) (4)	
VSD	Vaccine Sciences Department

Validation Plan Specifications

Analyte Name(s)	IgG specific to SARS-CoV-2 Nucleocapsid protein
Matrix, Species, and Additive	Human Serum
Sample dilution	(b) (4)
(b) (4)	(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 66, V1.00 (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 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) (b) (4) *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)

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

(b) (4)

Figure 1. Suggested Plate Map for VSDVAC 66

(b) (4)

Table 1: Expected Concentrations of Standard Curve Levels

Solution	Dilution	Concentration (AU/mL)
(b) (4)		

(b) (4)

(b) (4)

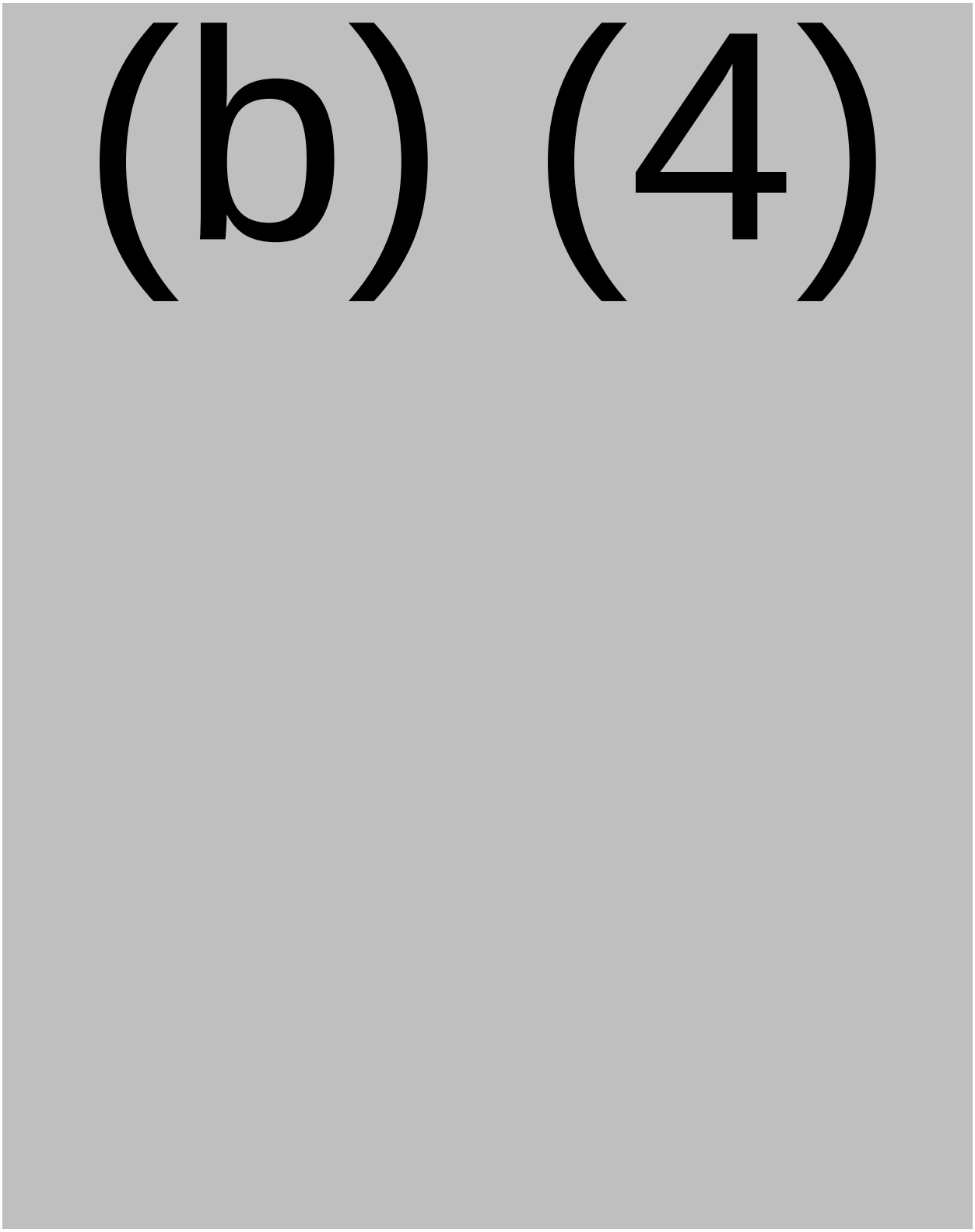
(b) (4)

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

Run #	Day	Analyst	Samples	Dilution
(b) (4)				

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

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

Table 4: (b) (4)

(b) (4)

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

(b) (4)

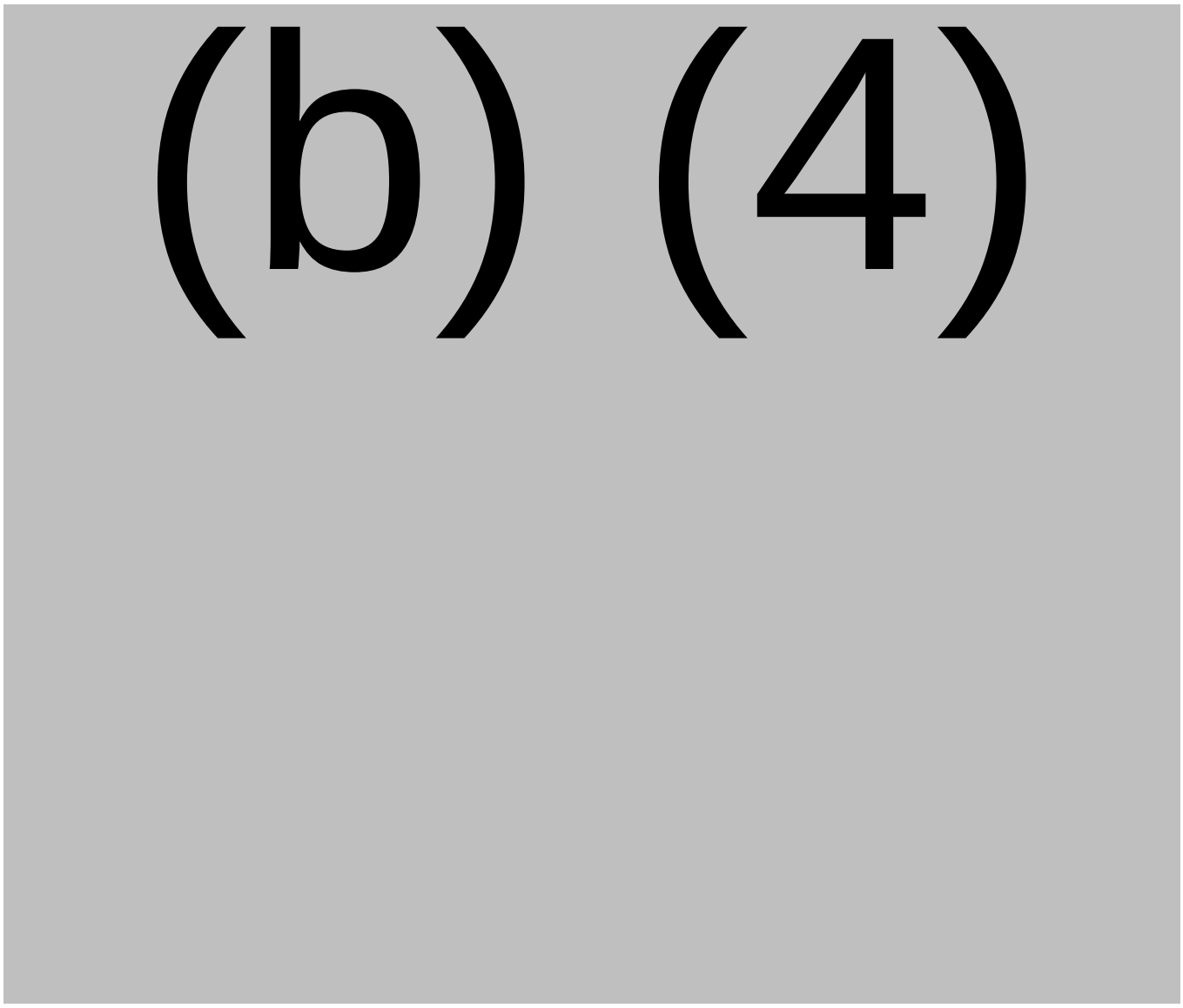

Table 5: Proposed Experimental Design for (b) (4)

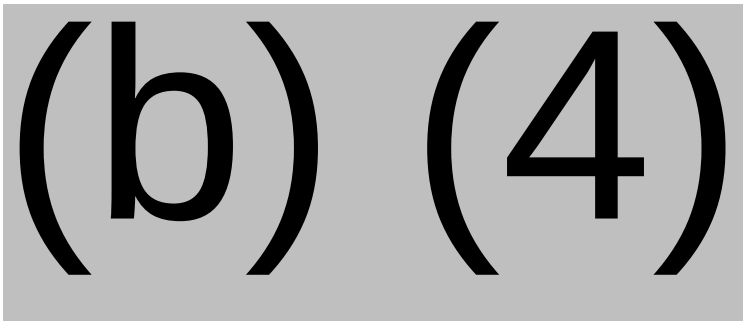

Table 6: Proposed (b) (4) **Panel Samples for** (b) (4)

Sample ID	Barcode (b) (4)	Barcode (b) (4)	Barcode (b) (4)
(b) (6)			

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

(b) (4)

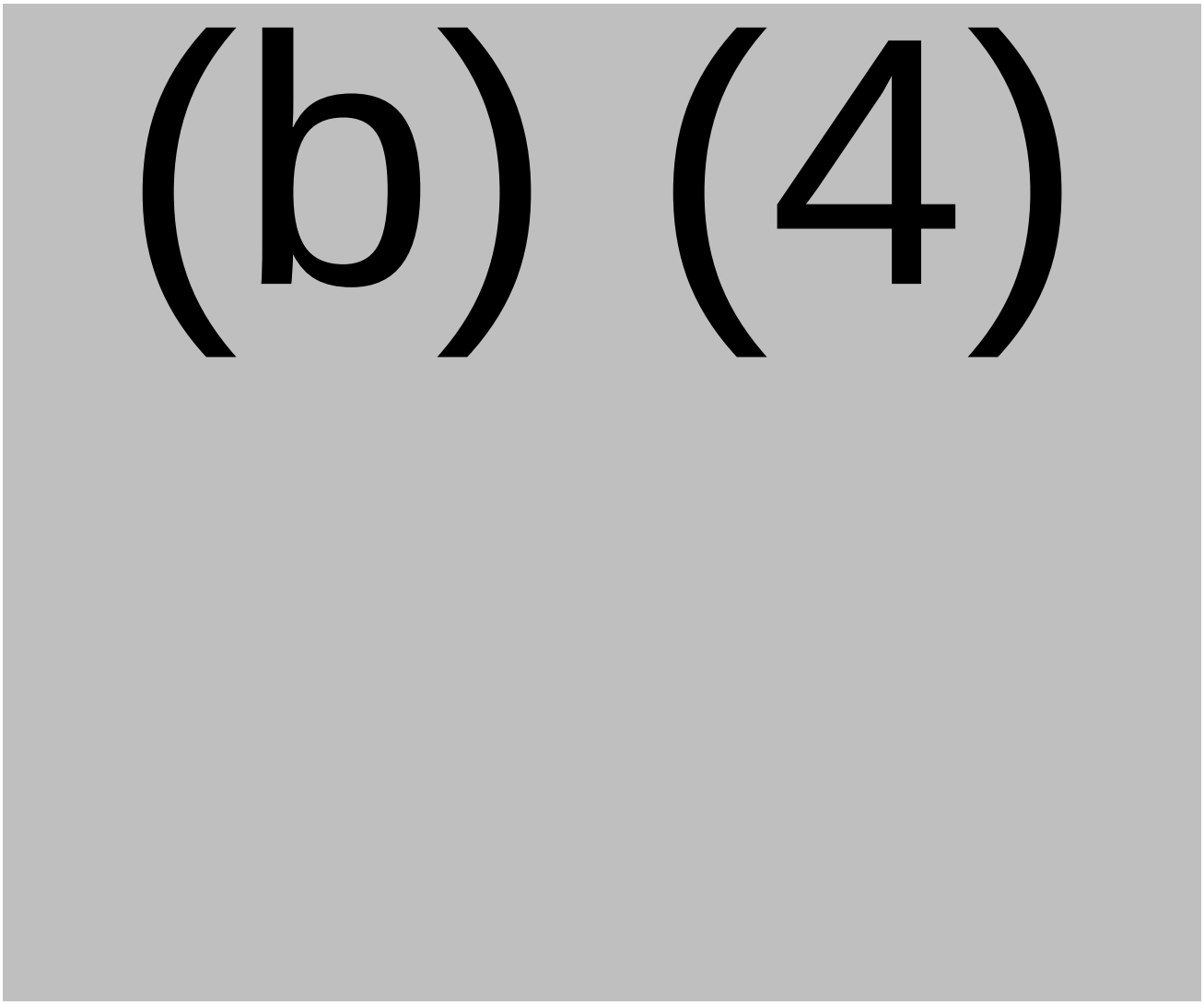

Table 7: Proposed Experimental Design for (b) (4)

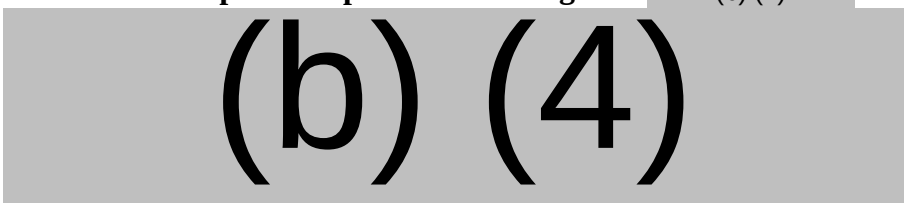

Table 8: (b) (4)

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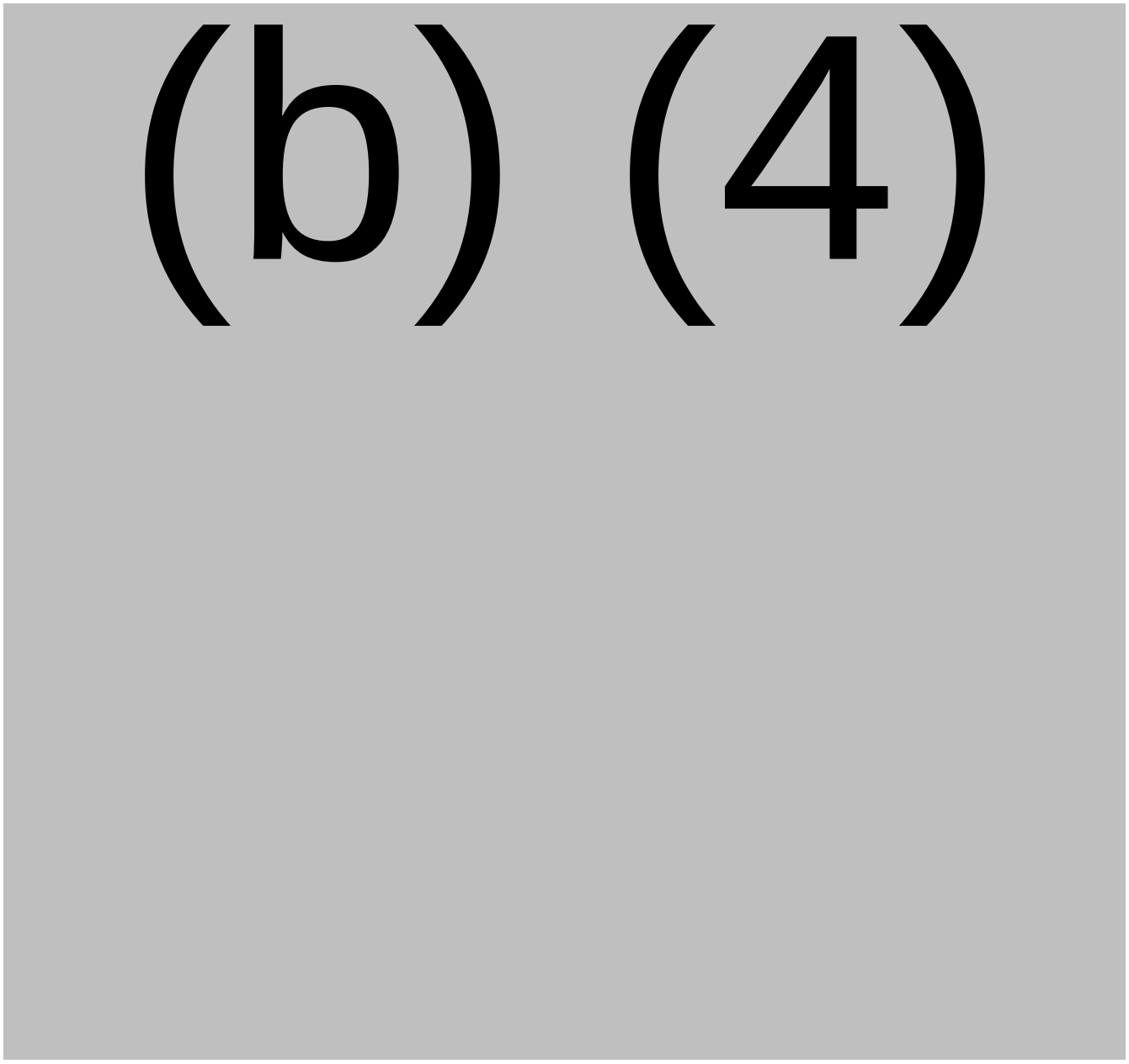
Figure 4: Proposed Plate Map for (b) (4)

(b) (4)

Method Validation Data Analysis Plan

(b) (4)


(b) (4)

References

1. VSDVAC 64: *An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum,* v1.00.

2. PPD Statistical Report Addendum: *Qualification of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum –* (b) (4) *Addendum,* ROZD2, 24Aug2020.

3. VSDVAC 66: *An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum,* v1.00.

4. PPD Statistical Report: *Validation of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum,* RPPF2, 02Dec2020.

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