Validation Addendum 5 Statistical Report

Method: VSDVAC 65 Version 2.00, 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 at the (b) (4) Sample Dilution

Version: 1.0

Prepared for Moderna

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Experiment Background and Purpose

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[1], was finalized to version 2.00 post validation and validation addendum experiments.

The method was previously validated at sample dilutions 1:500, (b) (4) [2, 3]. Per client request, (b) (4) (b) (4)
For this reason, a validation plan addendum[4] was developed and approved to validate the SARS-CoV-2 Spike IgG ELISA at the (b) (4) sample dilution. The purpose of the validation addendum experiments was to (b) (4) confirm the lower limit of quantitation (LLOQ) using a (b) (4) dilution, evaluate assay precision at the (b) (4) dilution, and to evaluate the dilutional linearity of the assay between the (b) (4) and (b) (4) dilutions. The purpose of this report is to document the operating characteristics of the assay when using the (b) (4) sample dilution. The SARS-CoV-2 Spike IgG ELISA operating characteristics are summarized below and in Table 1.

Validation Addendum Results Summary

Assay Characteristic	Validation Addendum Results
(b) (4)	
Precision	• Precision estimates are provided in the Parameter Summary Table (Table 1). • The assay met the pre-specified acceptance criteria that (b) (4) of the samples within the (b) (4) and that the overall assay precision for samples within the (b) (4) for the (b) (4) dilution. • The assay is precise.
Dilutional Linearity	• The overall dilution fold-bias estimates per (b) (4) fold dilution was (b) (4)-fold. • The assay met the pre-specified acceptance criterion that the dilution bias per (b) (4) fold dilution must be less than (b) (4) fold. • The assay is dilutable between the (b) (4) dilutions.

Scientific Contribution

The SARS-CoV-2 Spike ELISA met the pre-specified acceptance criteria for both precision and dilutional linearity and is considered validated for analysis at the (b) (4) sample dilution.

Conclusion

The SARS-CoV-2 Spike ELISA is considered validated at the (b) (4) sample dilution with regard to precision and dilutional linearity.

The SARS-CoV2 Spike ELISA is considered acceptable for use in the assessment of Phase III (or higher) clinical samples and may be used for earlier phases as needed.

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

Assay Characteristic	SARS-CoV-2 Spike IgG ELISA	Acceptance Criteria
LLOQ (AU/mL, (b) (4))	(b) (4)	(b) (4)
ULOQ (AU/mL, (b) (4)(b) (4))		
Precision (b) (4) (b) (4)		
Precision (b) (4) (b) (4)		
Dilutional Linearity (Dilution Bias Per (b) (4) Fold)		

Green bold font indicates acceptance criteria were met.

TABLE OF CONTENTS

ACRONYMS AND DEFINITIONS

Acronyms	Definitions
Ab[C]	Antibody Concentration
Conc.	Antibody Concentration Measured in AU/mL
CoV	Coronavirus
ELISA	Enzyme-Linked Immunosorbent Assay
(b) (4)	
GM	Geometric Mean
GMC	Geometric Mean Antibody Concentration
GMedC	Geometric Median Concentration
IgG	Immunoglobulin-G
LLOQ	Lower Limit of Quantitation
(b) (4)	
mL	Milliliter(s)
NA	Not Applicable
NIH	National Institute of Health
OD	Optical Density
P	Precision Sample
QA	Quality Assurance
QC	Quality Control
QCS	Quality Control Serum or Samples
Rep	Replicate
(b) (4)	
Run	A group of analytical samples consisting of standard curve, QCS, blank and test samples processed across a minimum of one plate.
SARS	Sudden Acute Respiratory Syndrome
SAS	Statistical Analysis Software
(b) (4)	
VSD	Vaccine Sciences Department

SCOPE

The scope of this validation addendum 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 at the (b) (4) sample dilution. 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 addendum experiments were to:

(b) (4)

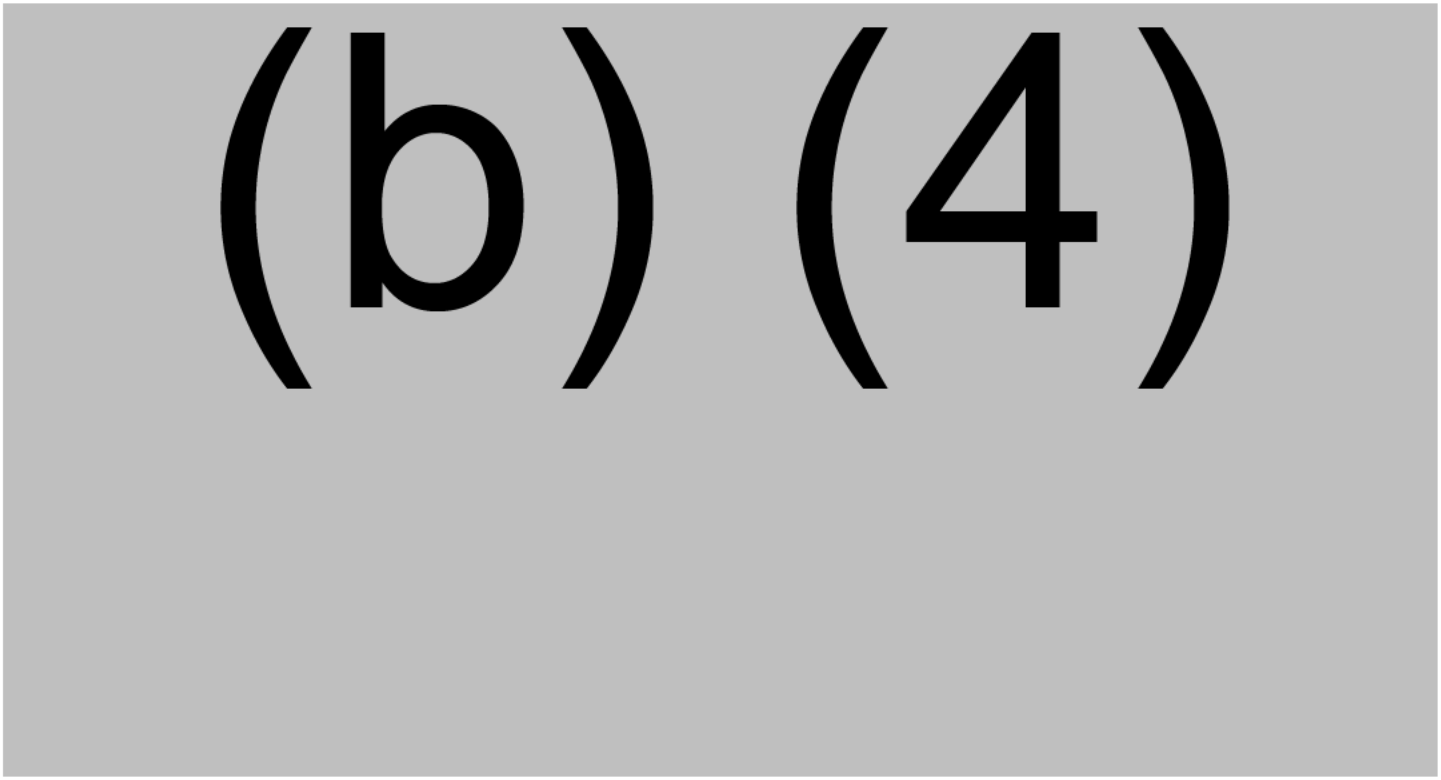
Plate Layout

The following sample types were analyzed in each validation addendum run:

(b) (4)

An example of the typical plate layout is provided in Figure 1.

Figure 1
Generic Plate Layout



EXPERIMENTAL DESIGN

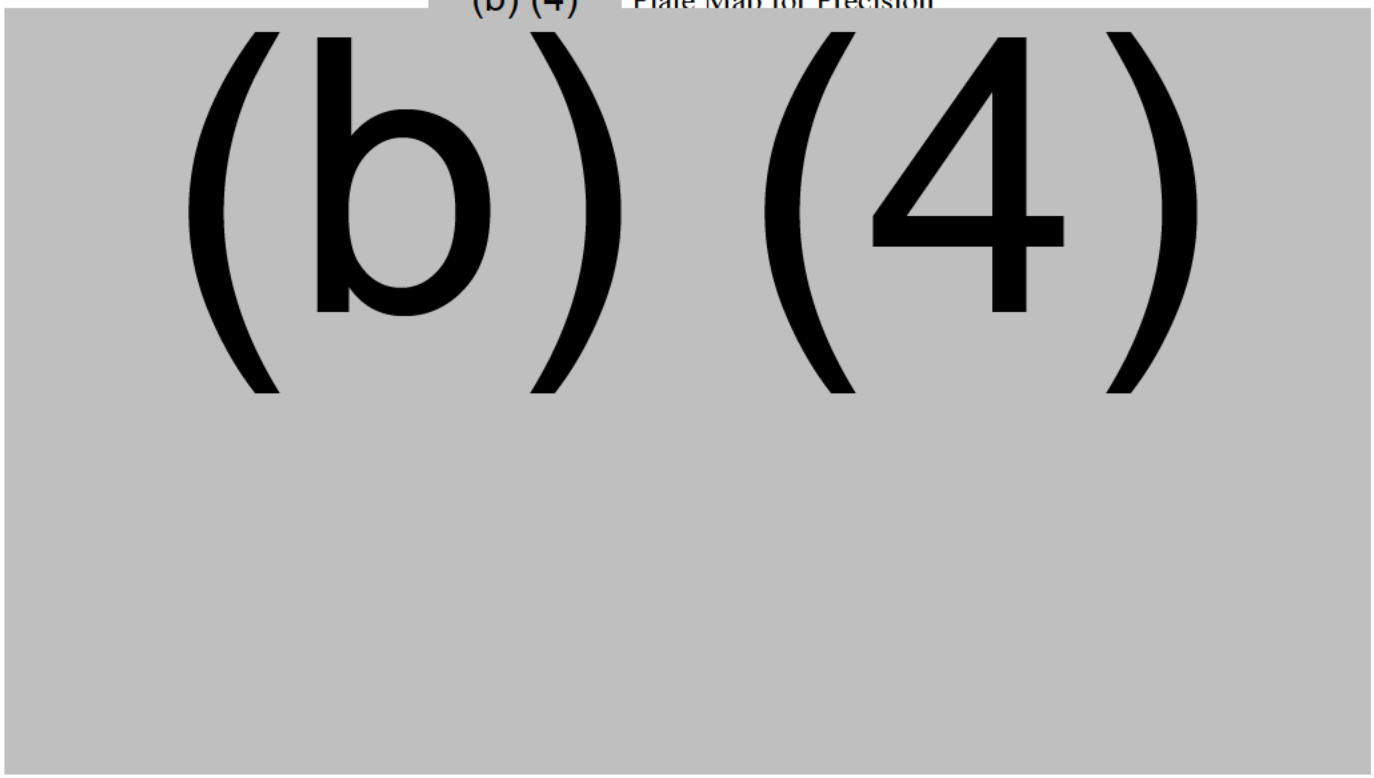
The ELISA assay validation addendum was (b) (4) evaluated Precision at the (b) (4) dilution. Precision from (b) (4) was then used to confirm the (b) (4) (b) (4) assessed the Dilutional Linearity of the assay.

(b) (4) Precision

(b) (4) Precision samples (b) (4) pre-screened to have antibody concentrations that target the quantifiable range of the (b) (4) dilution were used to assess assay precision (b) (4) total assay precision) at the (b) (4) dilution. Refer to Table 2 and Table 3 for the experimental design and list of samples used in each experiment.

(b) (4) assay runs were performed by (b) (4) analysts. Each assay consisted of (b) (4) analyzed at (b) (4) dilution. Across the runs, except where noted in the experimental design, all critical reagents remained the same. The plate layout is provided in Figure 2.

Figure 2
(b) (4) Plate Map for Precision



(b) (4) Dilutional Linearity

(b) (4) assay runs, each consisting of (b) (4) per run (b) (4) were performed by (b) (4) on (b) (4) separate days. The experimental design, samples used in this experiment, and plate layout are provided in Table 2, Table 3, and Figure 3, respectively.

The purpose of (b) (4) was to evaluate the dilutional linearity of the assay, which is often referred to as dilutability. A dilutional linearity panel of (b) (4) was analyzed across (b) (4) Each sample was pre-diluted in sample assay diluent (blocking buffer) at dilutions of (b) (4) (b) (4) dilutions. The dilutional linearity of the assay was evaluated across a (b) (4) dilution series between (b) (4) (b) (4)

Figure 3
(b) (4) Plate Map for Dilutional Linearity

(b) (4)

Table 2
Experimental Design

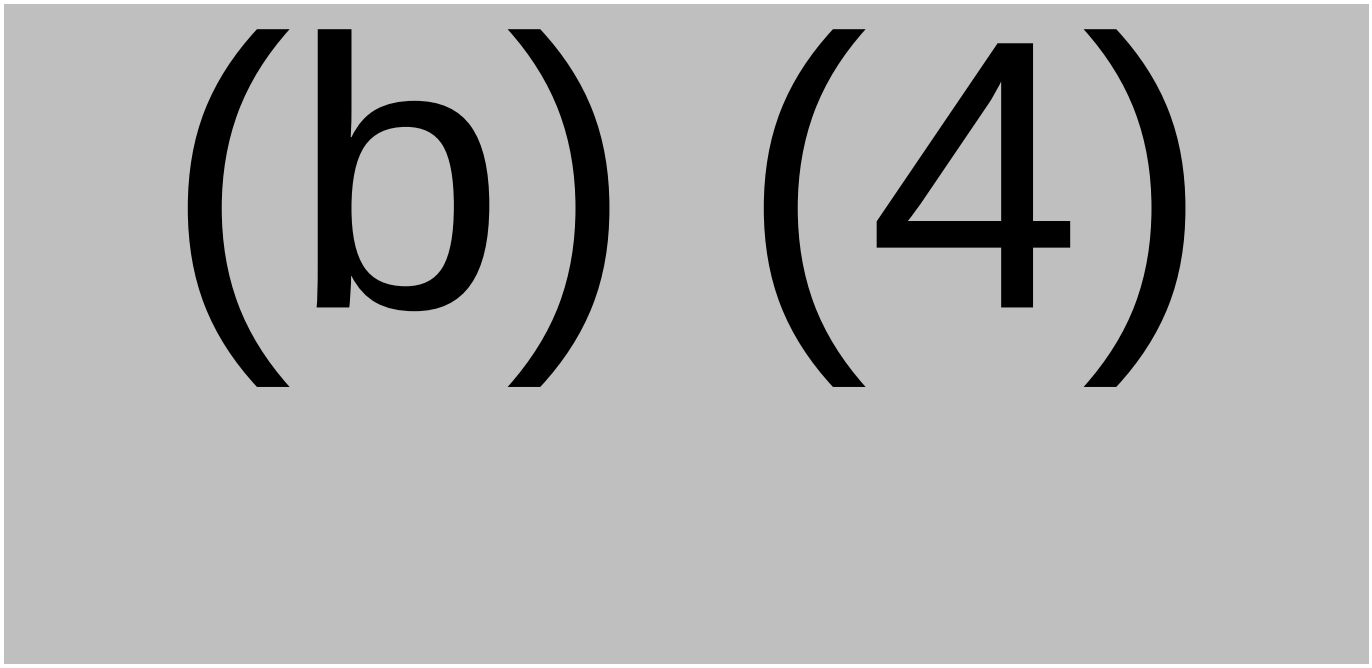


Table 3
Sample Description for Samples Used within Each of the Experiments

Experiment	Sample	Serum ID	Experiment	Sample	Serum ID
(b) (4)					

STATISTICAL METHODS AND RESULTS

(b) (4)

The standard curves associated with each of the runs are displayed graphically in Attachment I, the QCS graphs are provided in Attachment II, and the (b) (4) parameters are provided in Attachment III. All plates met the system suitability criteria.

Limits of Quantitation

The lower and upper limits of quantitation (LLOQ and ULOQ, respectively) define the range of concentrations over which the assay is acceptably accurate, and the assay precisely quantitates samples.

The LLOQ at the (b) (4) dilution was confirmed by evaluating the precision profile of the (b) (4) (b) (4) (See Precision Section). Details outlining the method used to set the (b) (4) are provided below:

(b) (4)

Following the guidelines listed above, the assay ranges are provided in Table 4. As shown in Table 5, all (100%) of the samples with GMC both (b) (4) and just below the determined LLOQ had precision estimates (b) (4) (b) (4) for the (b) (4) dilution.

Table 4
Assay Limits of Quantitation (LLOQ and ULOQ)
(All Units in AU/mL)
Note: (b) (4)

(b) (4)

Precision

(b) (4)

Overall Assay Precision: (b) (4)

(b) (4)

The assay met the pre-specified acceptance criteria that (b) (4) % of the samples (b) (4) must have (b) (4) % (b) (4) and that the overall assay precision for samples (b) (4) must be (b) (4) % (b) (4); therefore, the assay is considered precise at the (b) (4) dilution.

Figure 4
Precision Profiles

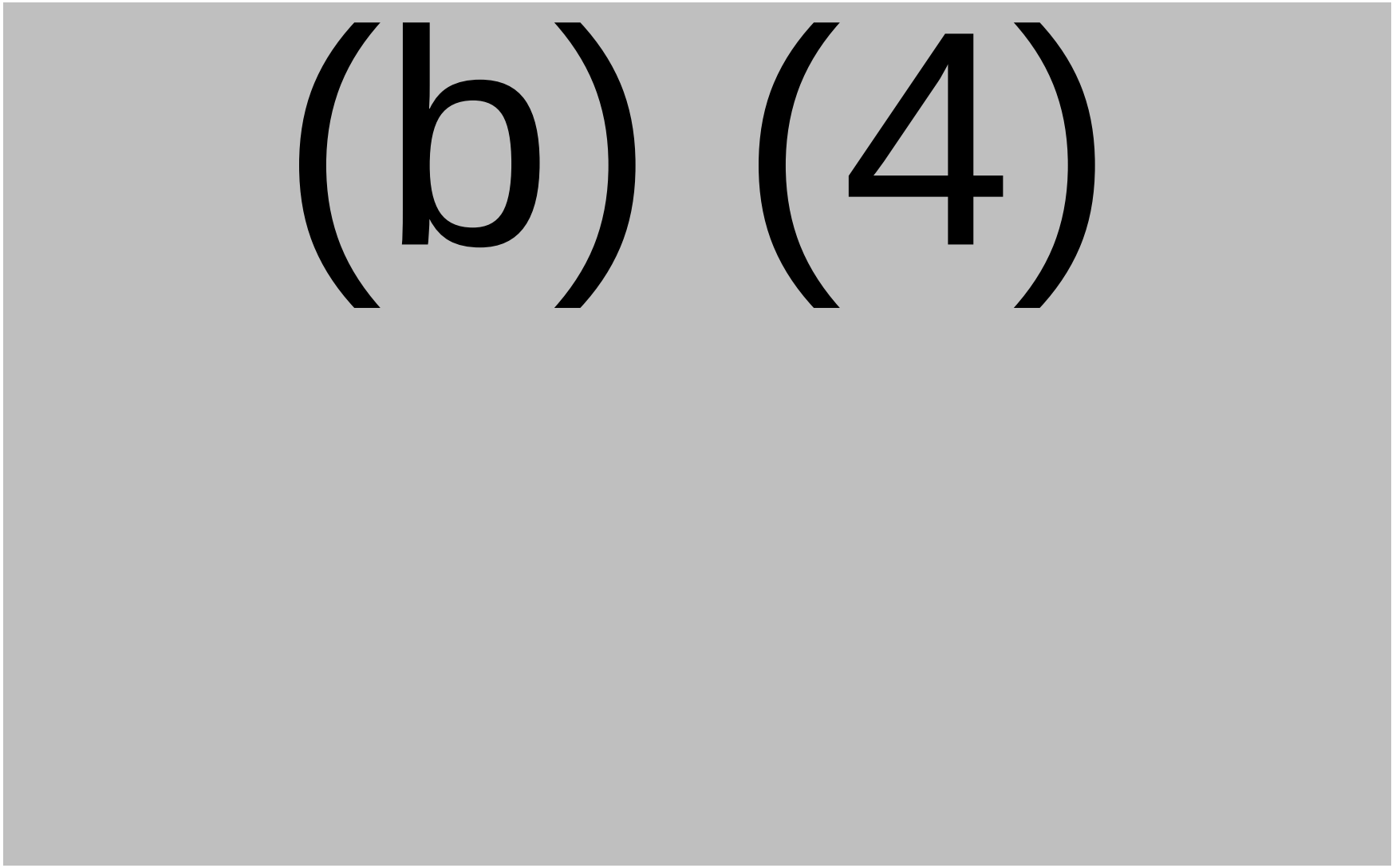


Table 5
Assay Precision (Measured in %(b) (4))

(b) (4)

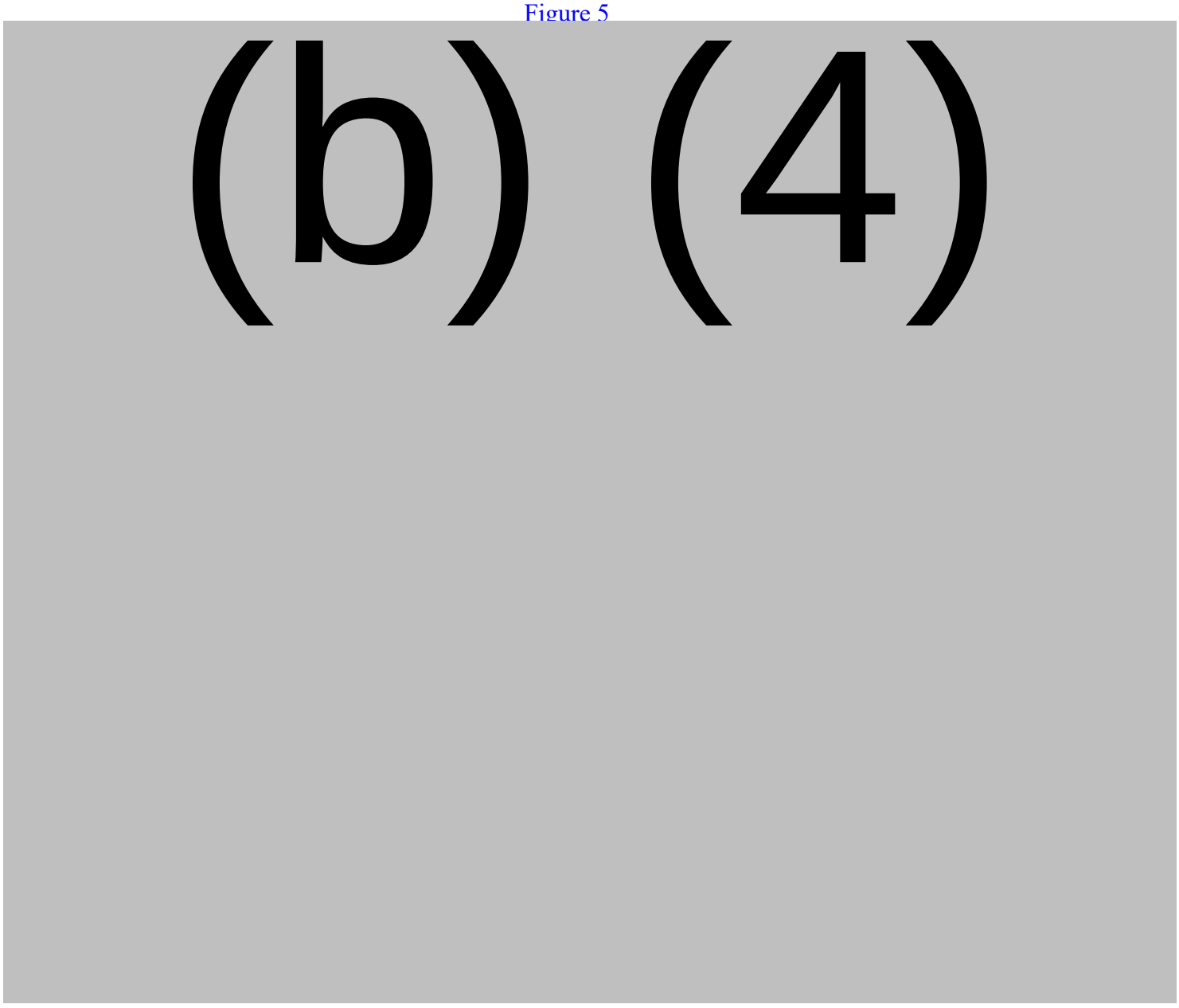
Dilutional Linearity

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. To evaluate the dilutional linearity of the assay, (b) (4)

(b) (4)

The assay met the pre-specified acceptance criterion that the dilution bias per(b) (4)fold dilution (b) (4) must be less than(b) (4)fold; therefore, the assay is considered dilutable.

Figure 5



References

1. VSDVAC65: *An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum,* v2.00.

2. PPD Validation Statistical Report: *Validation of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum,* RPPF, v1.0, 16-October-2020.

3. PPD Validation Addendum 2 Statistical Report: *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,* RPPF, v1.0, 01-March-2021.

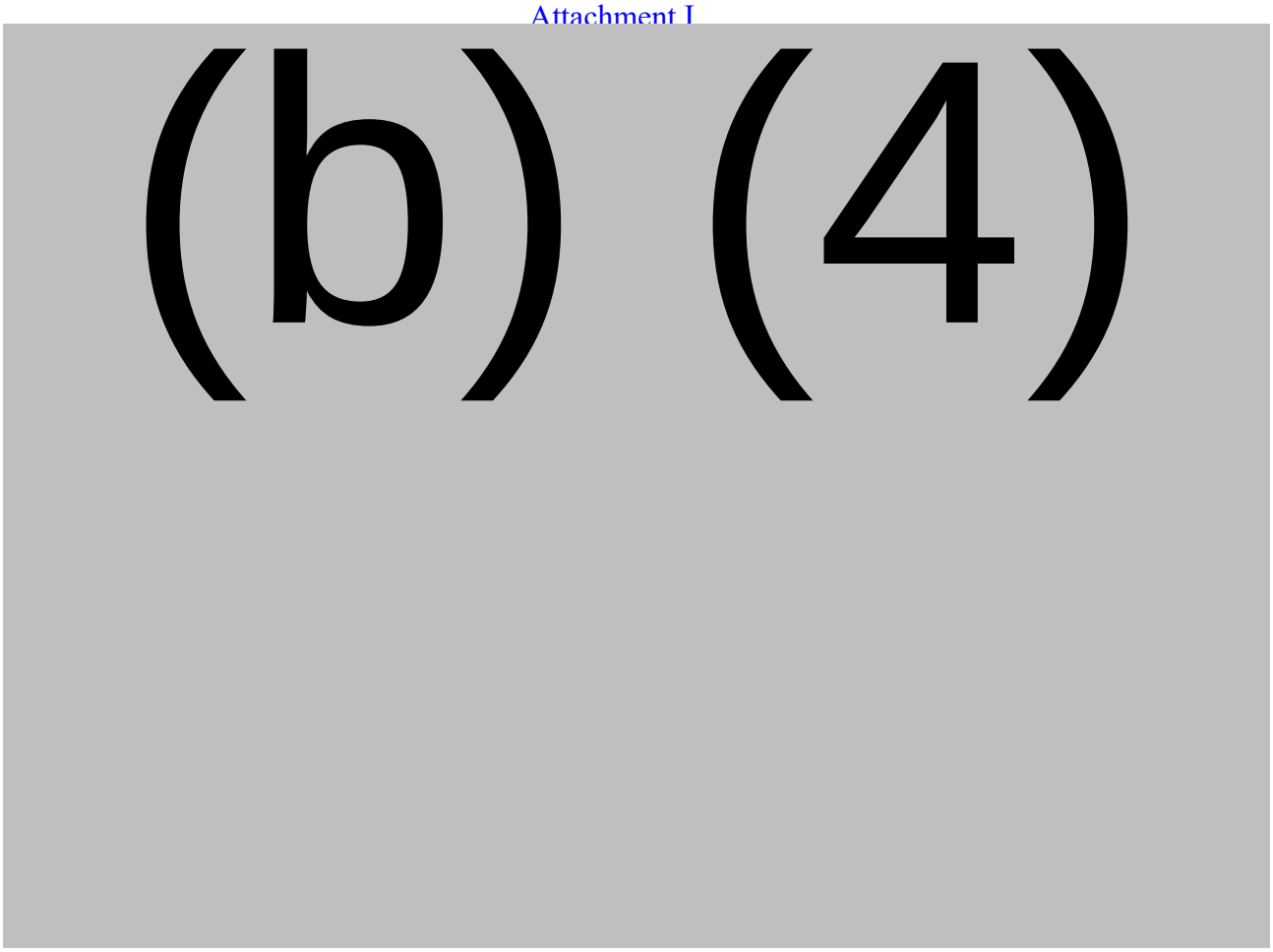
4. PPD Method Validation Plan Addendum 5: *Validation of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum at the* (b) (4) *Dilution*, RPPF, 23-April-2021.

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

Revision History

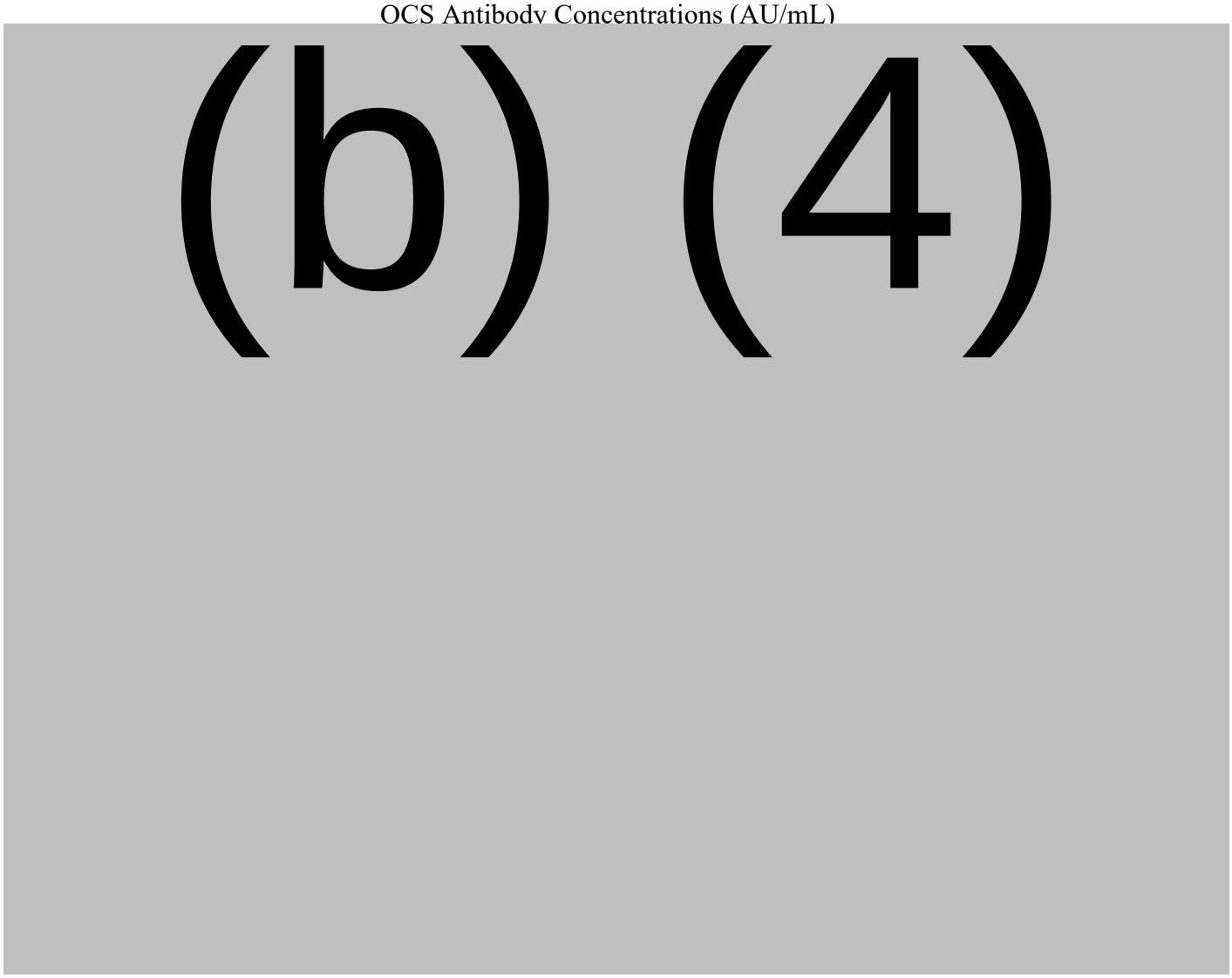
Version	Date	Author	Reason for Revision
1.0	24-May-2021	Victoria Pisciella	Original Version

Attachment I



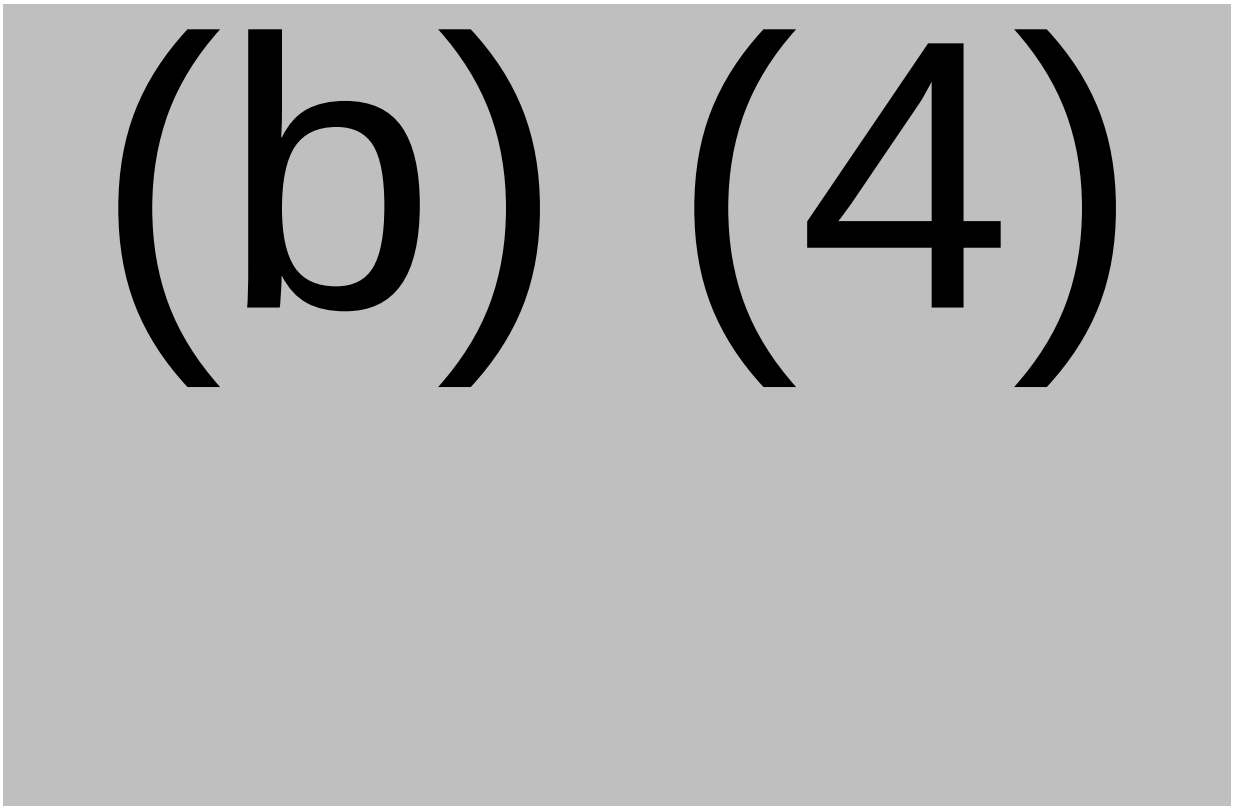
Attachment II

OCS Antibody Concentrations (AU/mL)



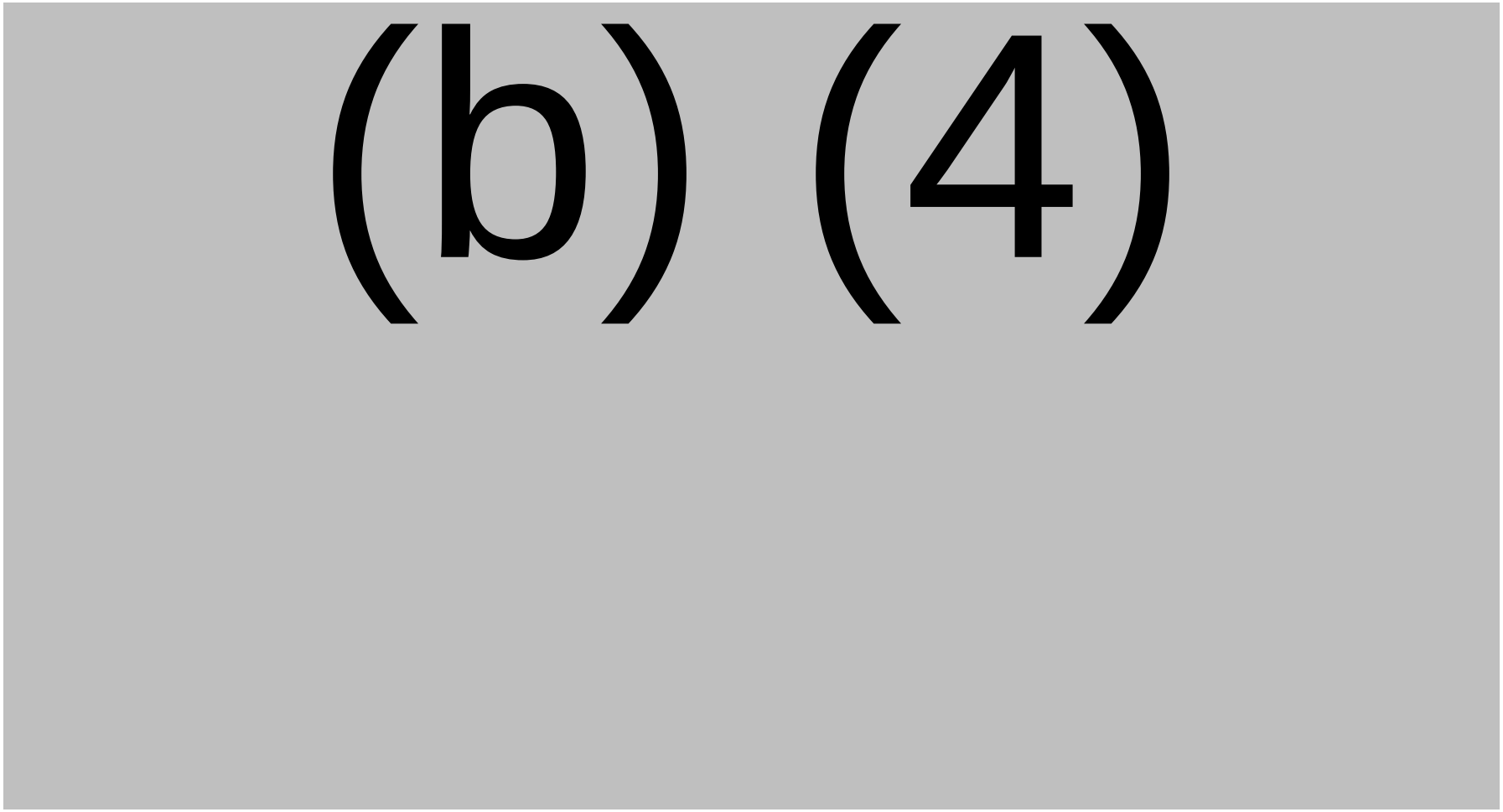
Attachment III

Standard Curve and QCS Summary



Attachment IV

Precision Antibody Concentrations (AU/mL)



Attachment V

Antibody Concentrations (AU/mL) and Dilution Bias per (b) (4)-Fold Dilution for Dilutional Linearity