Validation Addendum 1 Statistical Report

Method: VSDVAC 66 Version 0.00, An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum

PPD Project: RPPF2

Reagent ID: VSDVAC_66_VS_Adden1_(b) (4)_01

Qualification of a New (b) (4) (Lot (b) (4)) for Use in the Nucleocapsid IgG ELISA

Version: 1.0

Prepared for Moderna

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Confidentiality Statement

PPD Signatures

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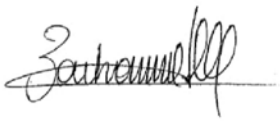

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Client approval of this report may be granted in the form of written communication and will be stored in PPD's ECM system.

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Experiment Summary

Background: The currently qualified lot of (b) (4) (Lot (b) (4)), which is used in the Nucleocapsid IgG ELISA, is nearly depleted. For this reason, an experiment was performed to qualify a new lot of (b) (4) (Lot (b) (4)) as a replacement for the current lot. The objective of this statistical report is to assess whether the new lot produces assay results that are acceptably similar to those produced when using the qualified lot.

Experimental Design: Per the Validation Plan Addendum 1[1], the new lot and the qualified lot were tested

(b) (4)

(b) (4) All testing was performed following VSDVAC 66 Version 0.00[3].

Statistical Results: Sample antibody concentrations are provided in Attachment I. QCS antibody concentrations are displayed graphically in Attachment II. The experimental results and their respective pre-specified acceptance criteria are summarized in the table below.

(b) (4)

Conclusion

All pre-specified acceptance criteria were met. Therefore, the new (b) (4) lot (b) (4) at a working concentration of (b) (4) (b) (4) is qualified for use in the Nucleocapsid IgG ELISA.

Assay Information

The quantitative ELISA (referred to as Nucleocapsid IgG ELISA) was designed to detect IgG antibody to the SARS-CoV-2 virus in human serum. (b) (4)

(b) (4)

(b) (4)

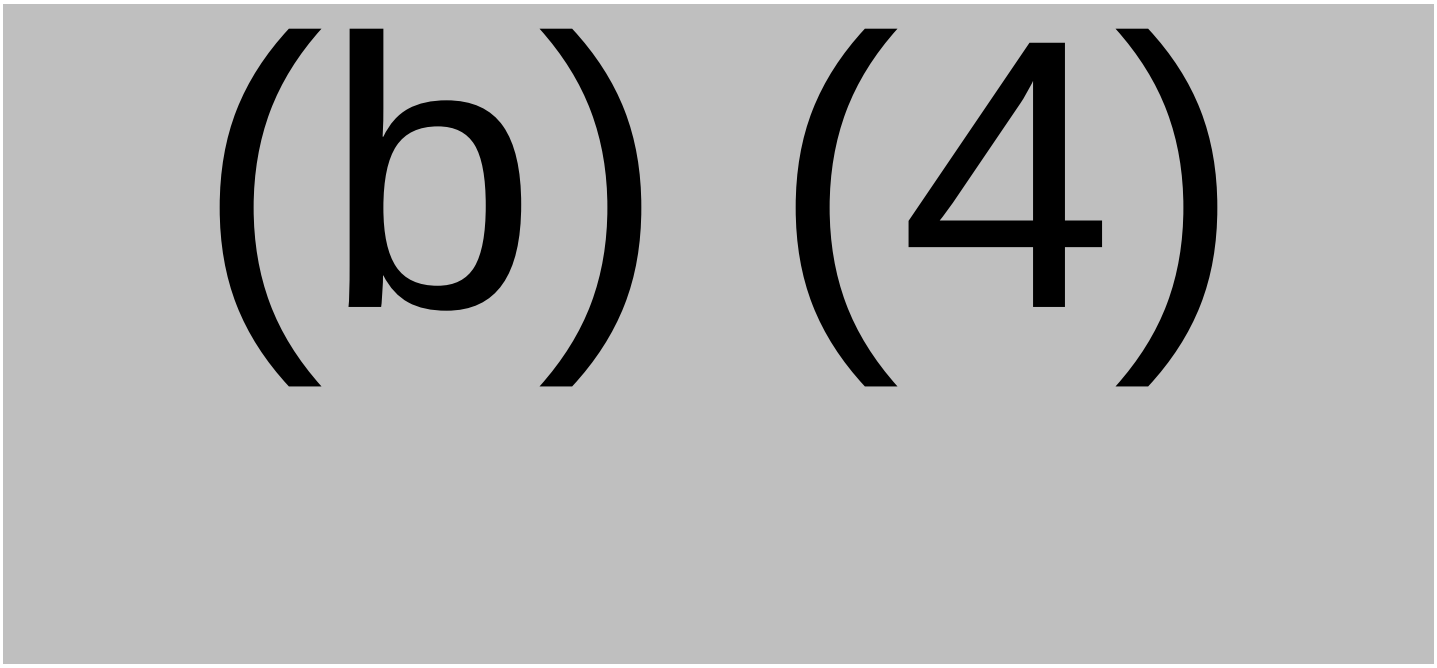
Statistical Results

Statistical methods utilized to analyze data within this experiment are provided in the Statistical Methods Reference Guide[4].

Plate Status:
One plate failed to meet the plate acceptance criteria detailed in the Validation Statistical Report[5]. Run (b) (4) Plate (b) (4) tested with the new lot, failed due to too few valid standard curve points. The plates were repeated pairwise, qualified versus new, and the repeat results replaced the original results in all analyses. The inclusion status for each plate is provided in Table 2.

Standard Curves:
The standard curve fits for each of the runs are graphically depicted in Attachment III. As shown in Attachment III, the (b) (4) are in line with those determined during assay validation[5].

(b) (4)



Scientific Contribution

During review of experimental Run (b) (4) and Run (b) (4) Repeat (b) (4) it was found that the preparation of the (b) (4) for use with the candidate (b) (4) followed an incorrect dilution factor. It was noted upon review of experimental Run (b) (4) Repeat (b) (4) the plate was significantly overdeveloped, resulting in elevated background and elevated responses across the plate. Due to these noted technical errors, these three runs were rejected and repeat experiments performed. All pre-specified acceptance criteria were met and the (b) (4) lot (b) (4) is considered acceptable for use in the assay at a working concentration of (b) (4) using a (b) (4)

The validation plan addendum listed the antigen lot number as (b) (4) as per the Certificate of Analysis (CoA) from (b) (4) The vendor, (b) (4) clarified this was an error. The lot number should be listed as (b) (4)

It was noted that there was variation in the standard curves, especially within the qualified lot (b) (4); however, all runs used in statistical analysis passed validity criteria. Standard curves analyzed on plates using the new lot of (b) (4) produced more consistent results across plates when compared to plates coated with the qualified (b) (4) All runs used in statistical analysis passed method acceptance criteria established during the validation. It is recommended that standard curve performance be monitored during routine testing to assess variability.

References

[1] Validation Plan Addendum 1: *Addendum to the Validation of an ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum*, RPPF2, VSDVAC_66_VP_Addend1, 21-October-2020.

[2] Validation Plan Addendum 1 Amendment 1: *Amendment to the Addendum to the Validation of an ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum*, RPPF2, VSDVAC_66_VP_Addend1_Amend1, 02-November-2020.

[3] VSDVAC 66: *An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum*, v0.00.

[4] PPD Nonclinical Statistics: *Statistical Methods Reference Guide v1.0*, 21-December-2009.

[5] Validation Statistical Report: *Validation of an ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum*, RPPF2, v1.0, 30-November-2020.

[6] (b) (4)
(b) (4)

Figures, Tables, and Attachments

Table 1a
Reagent Details

Reagent Type	Source	Lot #	Conc.	Expiration	Storage Conditions
(b) (4)					

Table 1b
Sample Information

Sample	Lot
(b) (4)	

Table 2
Experimental Design

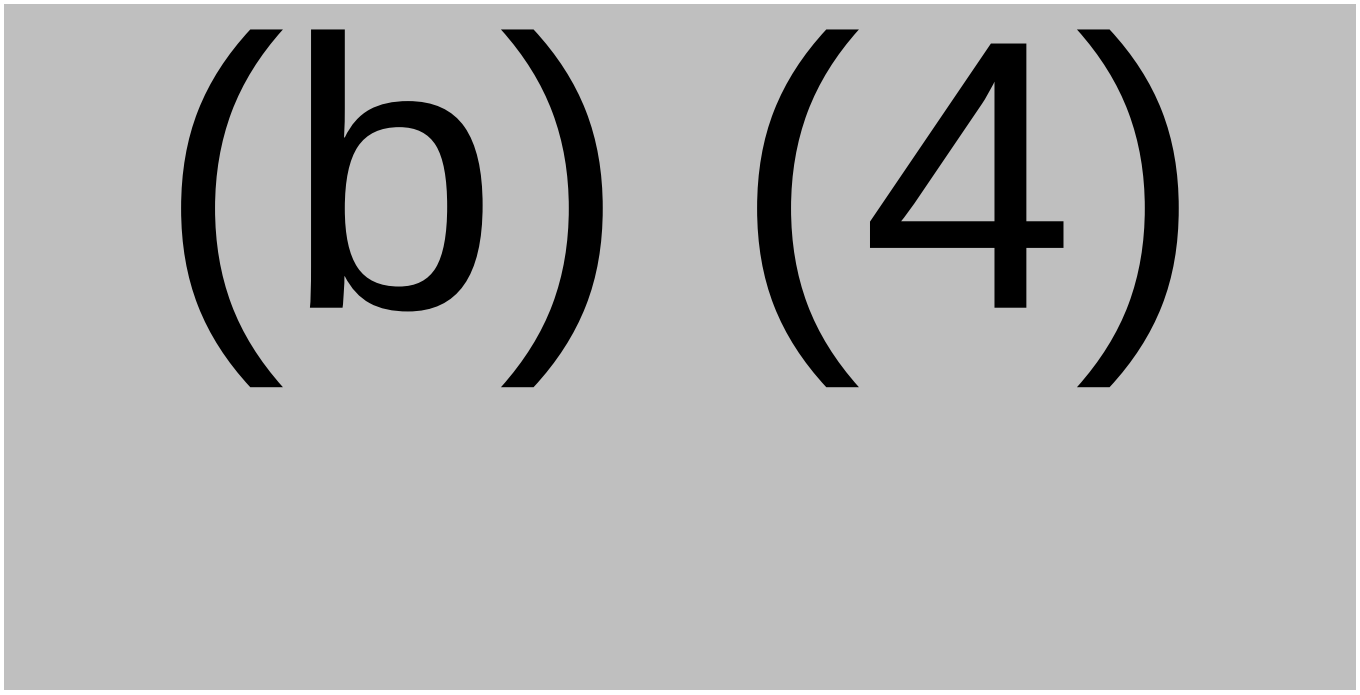


Figure 1

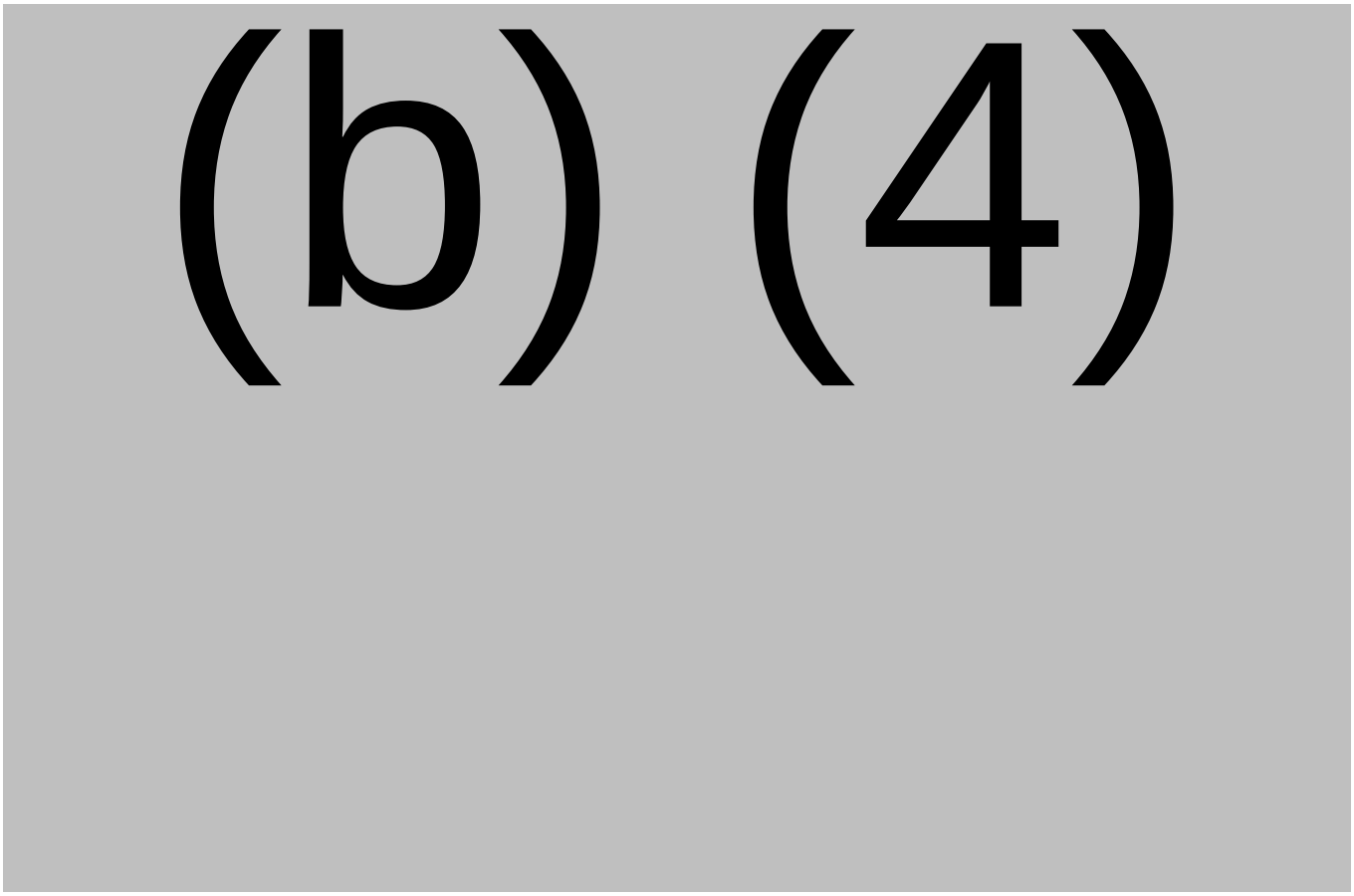


Table 3

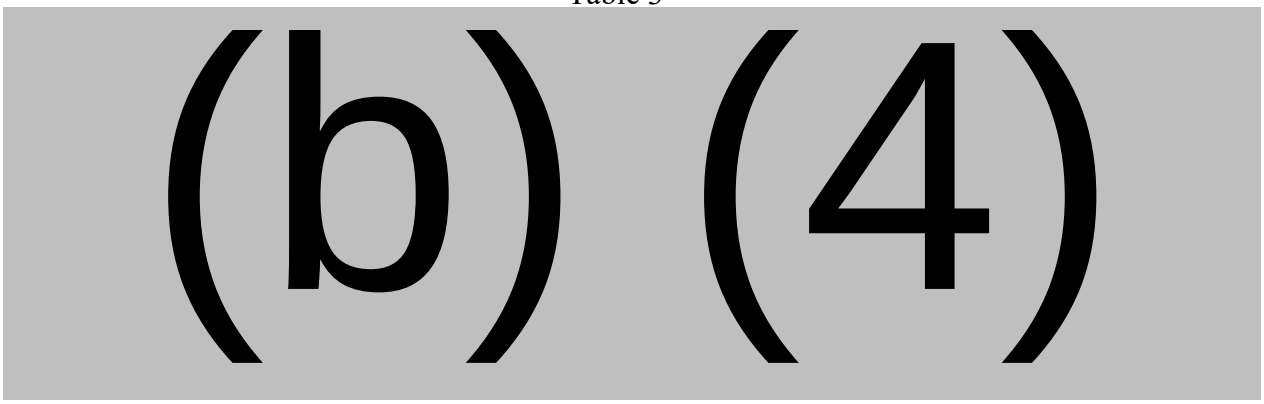


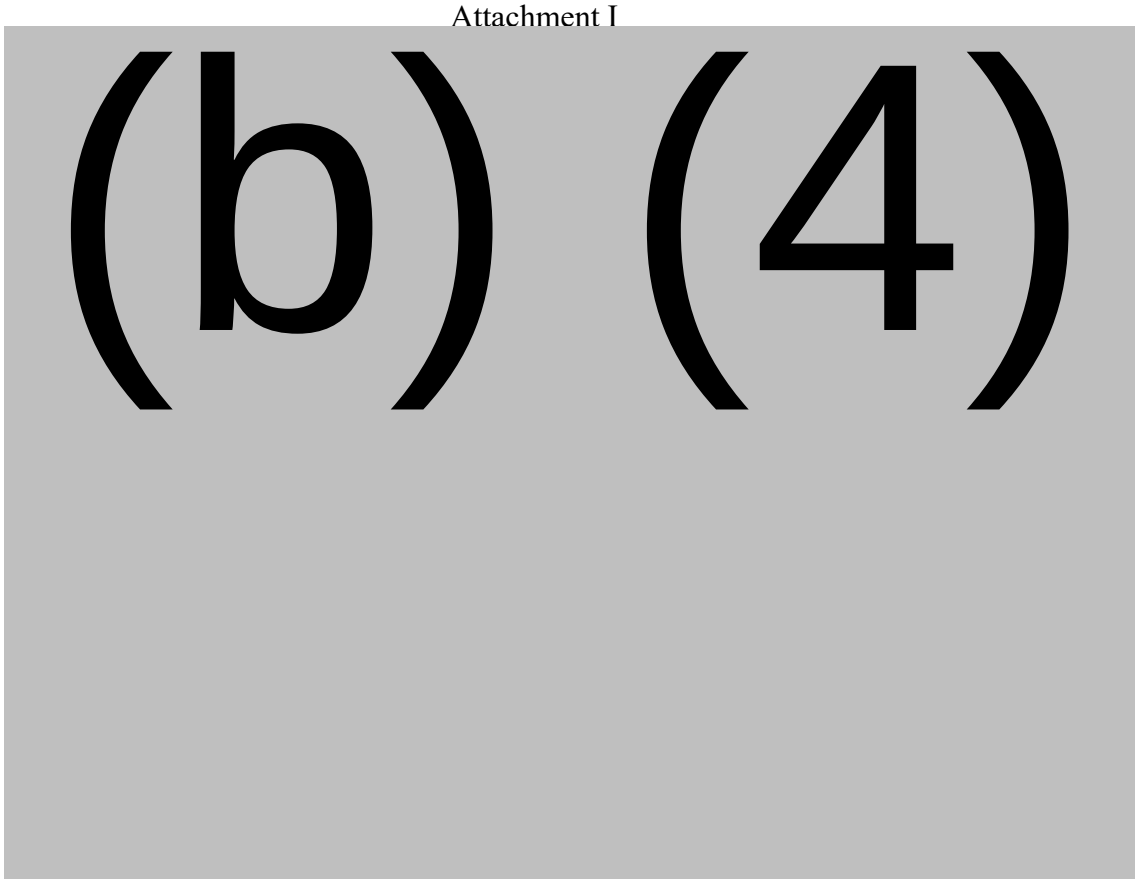
Table 4

(b) (4)

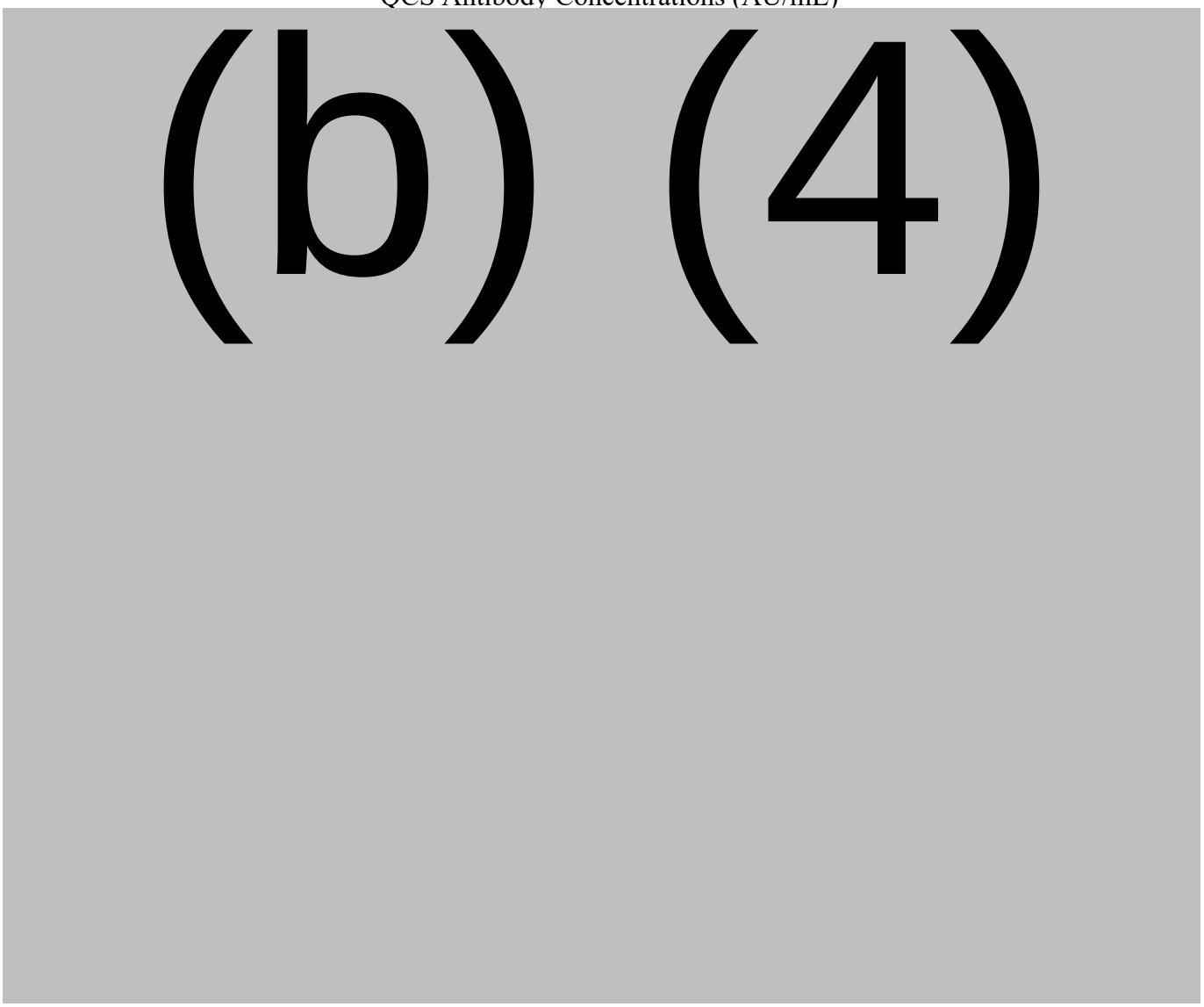
Table 5

(b) (4)

Attachment I



Attachment II
QCS Antibody Concentrations (AU/mL)



Attachment III
Standard Curves