Validation Plan

VSDVAC 66 Version 0.00

PPD Project Code: TBD

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

Version 1.00

To be Conducted for Moderna

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

Issue Date: 04-Aug-2020

PPD Approval

PPD Project: TBD

PPD Report Title: 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.

Jack Hester _____
Signature/Date

Adrienne Howlett _____
Signature/Date

Marie Bonhomme, Ph.D. _____
Signature/Date

Tina Green _____
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

PPD Project:	TBD
PPD Report Title:	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 by the undersigned.

(b) (6)

_____
Signature/Date

Sponsor Approval

Client:	Moderna
PPD Project:	TBD
PPD Report Title:	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.

Rolando Pajon
Associate Director, Clinical Biomarkers

_____
Signature/Date

Validation Plan

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

Introduction

A PPD 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" will be validated by PPD® Laboratories, in Richmond, Virginia, USA. The new method, VSDVAC 66[3], will be approved for use as Version 0.00 prior to the execution of validation experiments by senior PPD laboratory management; the method will be finalized to Version 1.00 after validation. The client specific validation of this method will be conducted under PPD Project Code "TBD".

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)

The objectives of this validation plan are to:

(b) (4)

If additional experiments 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.*

Method version within the validation plan will remain consistent to the original method version approved for validation; version 0.00. Method versioning will not require validation plan amendments.

Sample (b) (4) and assay process (b) (4) will be performed as an addendum to the VSDVAC 64 assay qualification[2]. Stability will be assessed for the following but not limited to (b) (4)

(b) (4)

(b) (4)

Following the validation, the method, VSDVAC 66 will be finalized to Version 1.00 and used for Phase III or 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.

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.

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	Coating Antigen/ Homologous Antigen	(b) (4)				

(b) (4)

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

ADHS	Antibody-depleted Human Serum
ANOVA	Analysis of Variance
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

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.

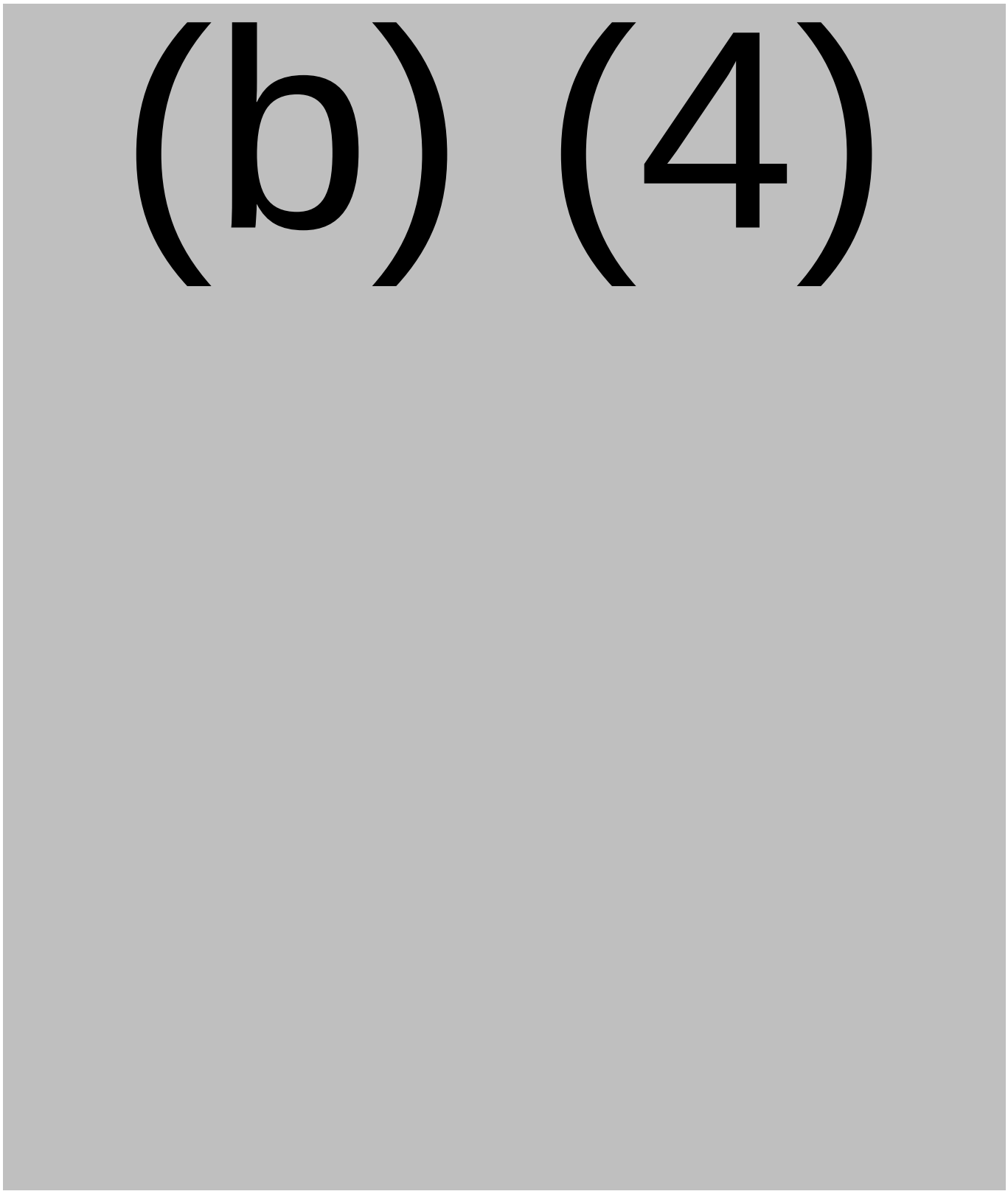
Validation Plan Specifications

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; neat concentration assigned 500 AU/mL
Run Acceptance	All system suitability criteria as defined in VSDVAC 66, V0.00 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. 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) *experiments will be prepared and confirmed during pre-work experimentation for use in validation.*

Validation Experiments

(b) (4)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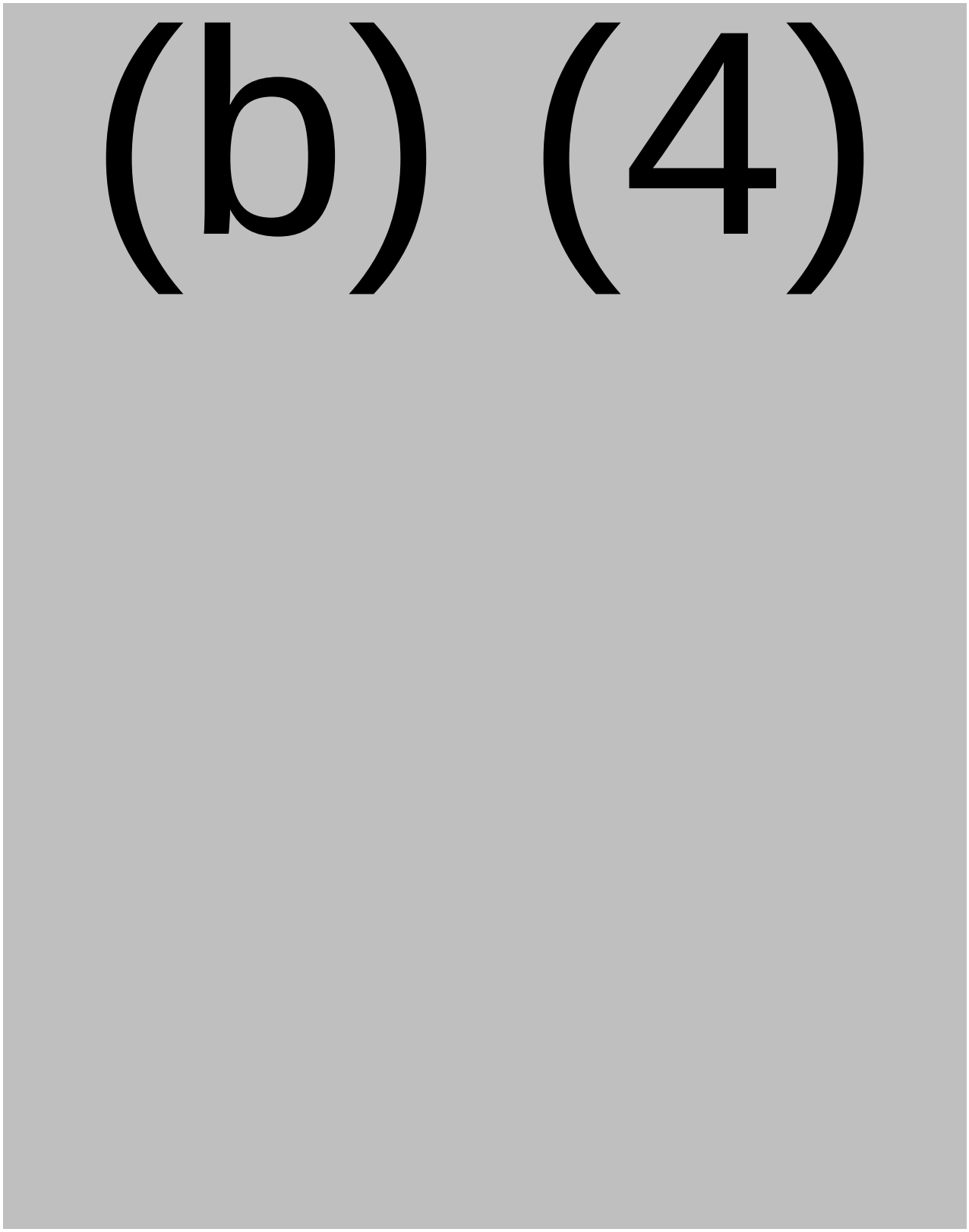
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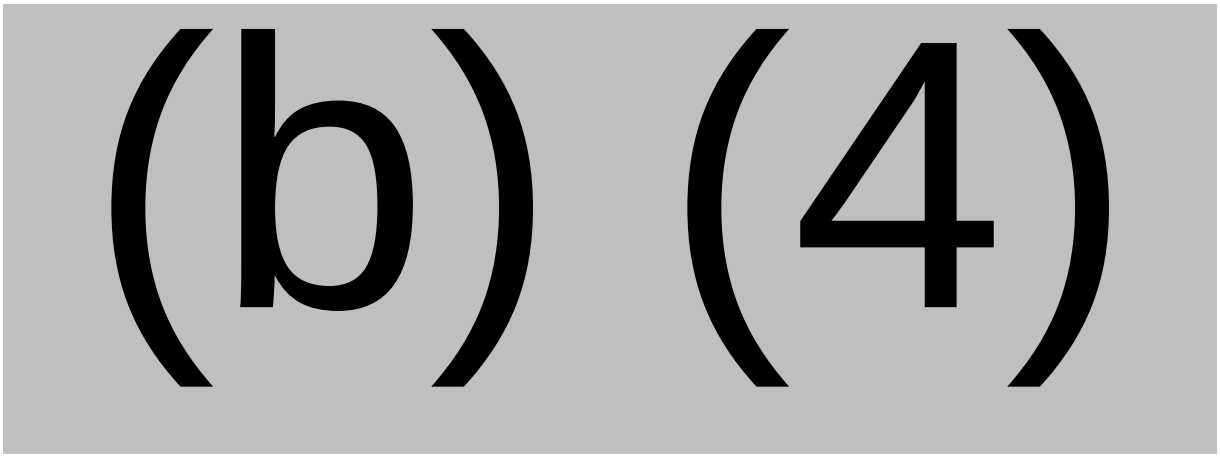


Table 1: Proposed Experimental Design for (b) (4)

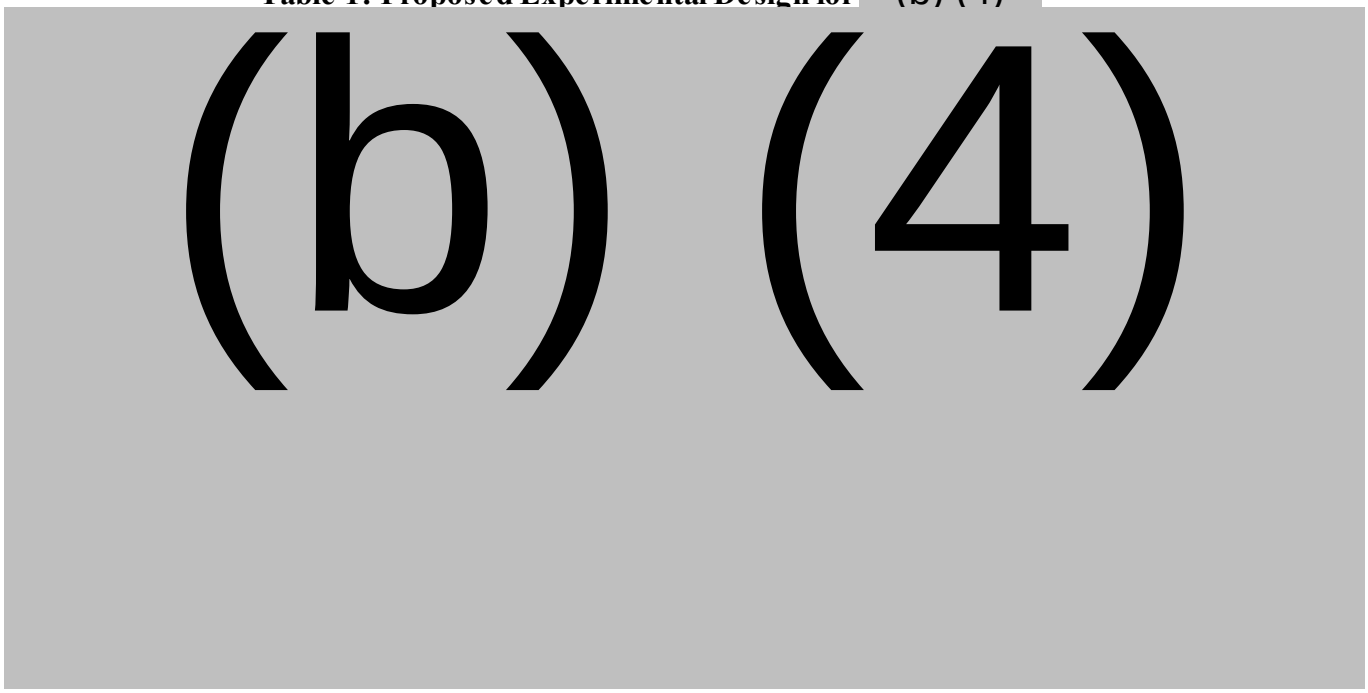


Table 2: Proposed Samples for (b) (4)

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

Table 3: (b) (4)

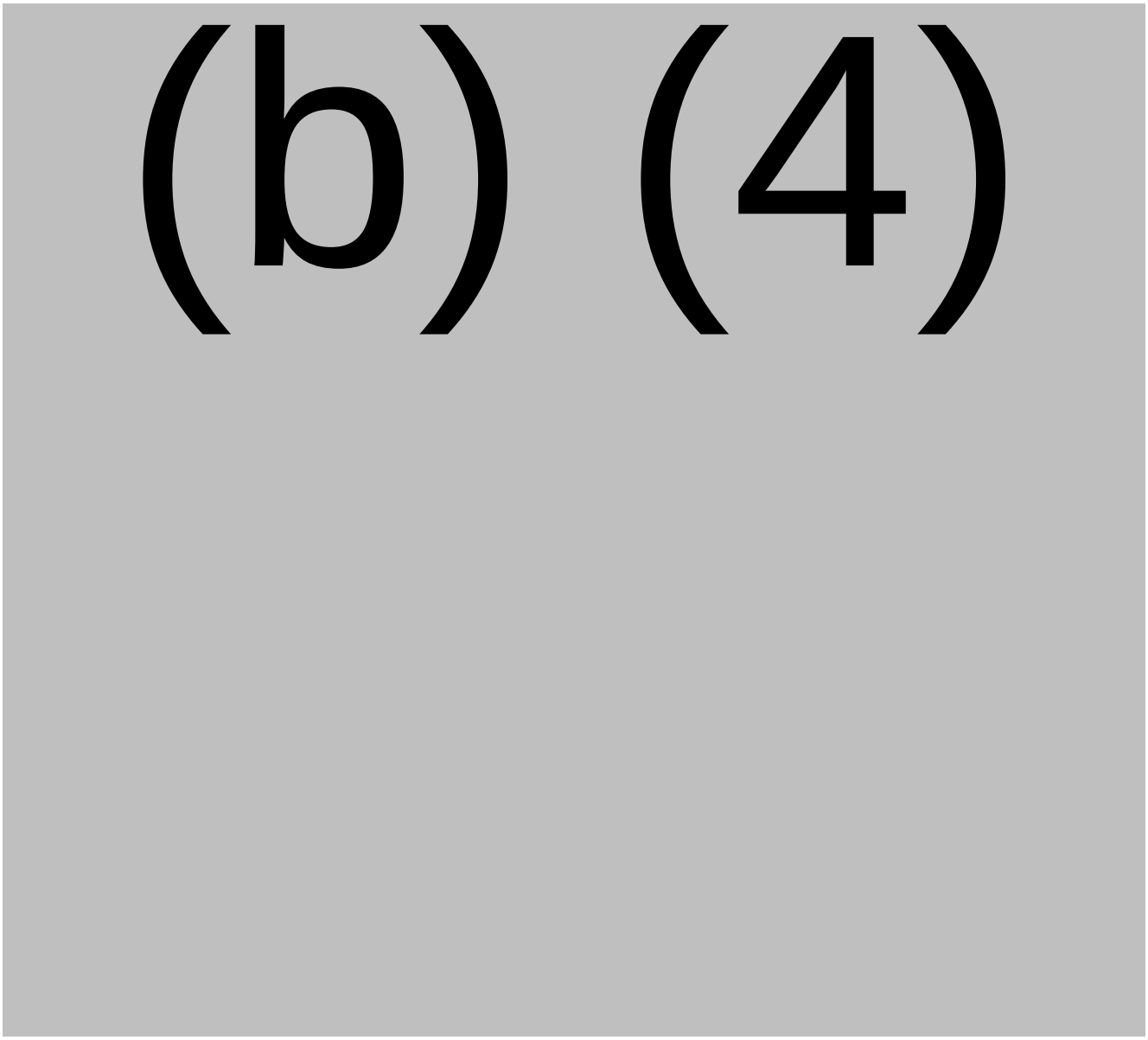
(b) (4)

Table 4: (b) (4)

(b) (4)

Figure 1: Proposed Plate Map for (b) (4)

(b) (4)


(b) (4)

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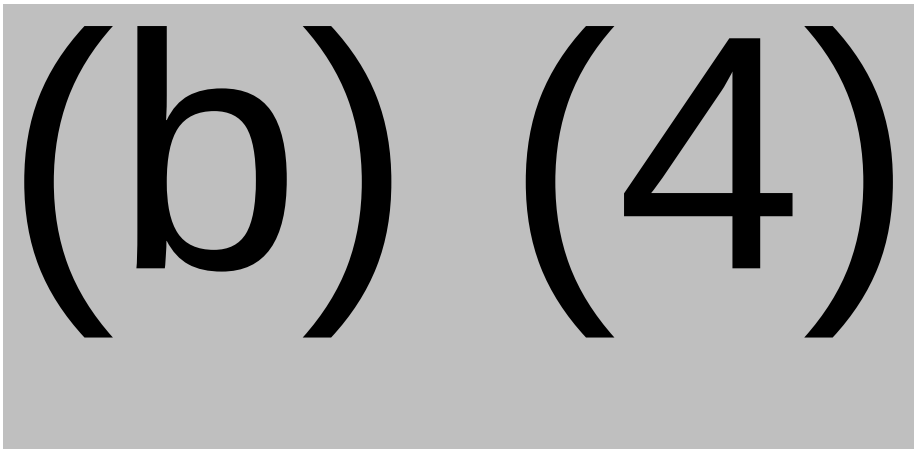


Table 6: Proposed Samples for (b) (4)

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

Table 7: (b) (4)
(b) (4)

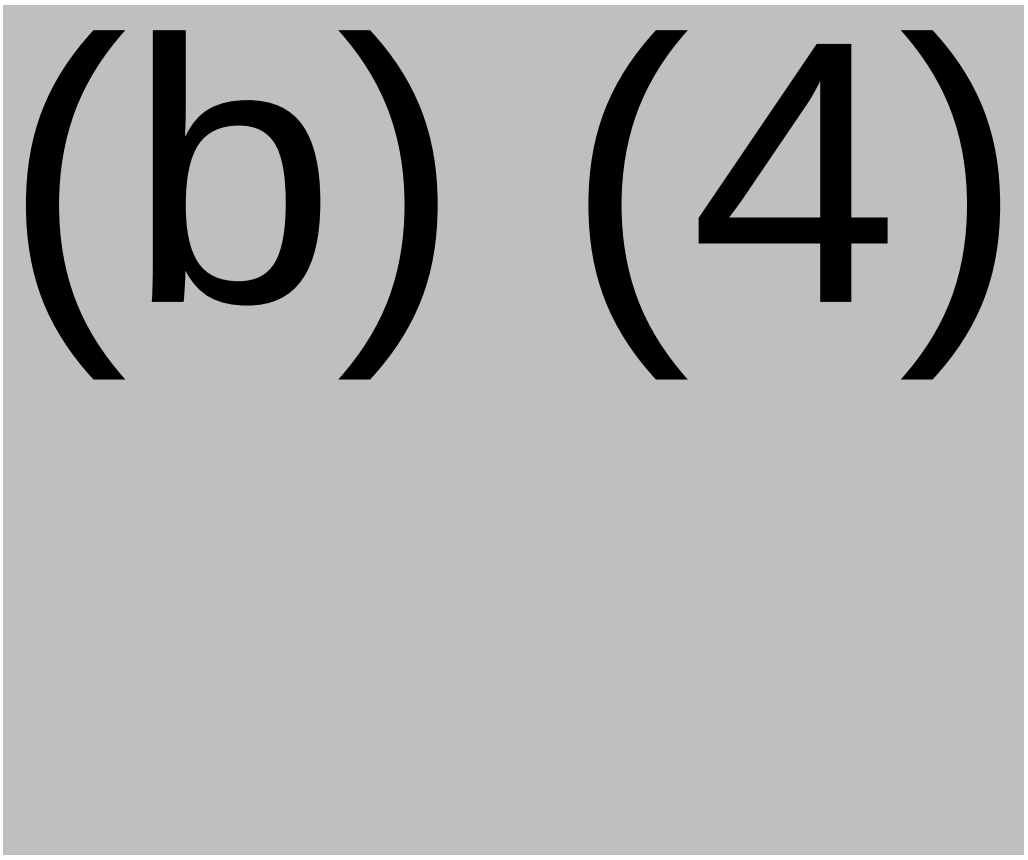
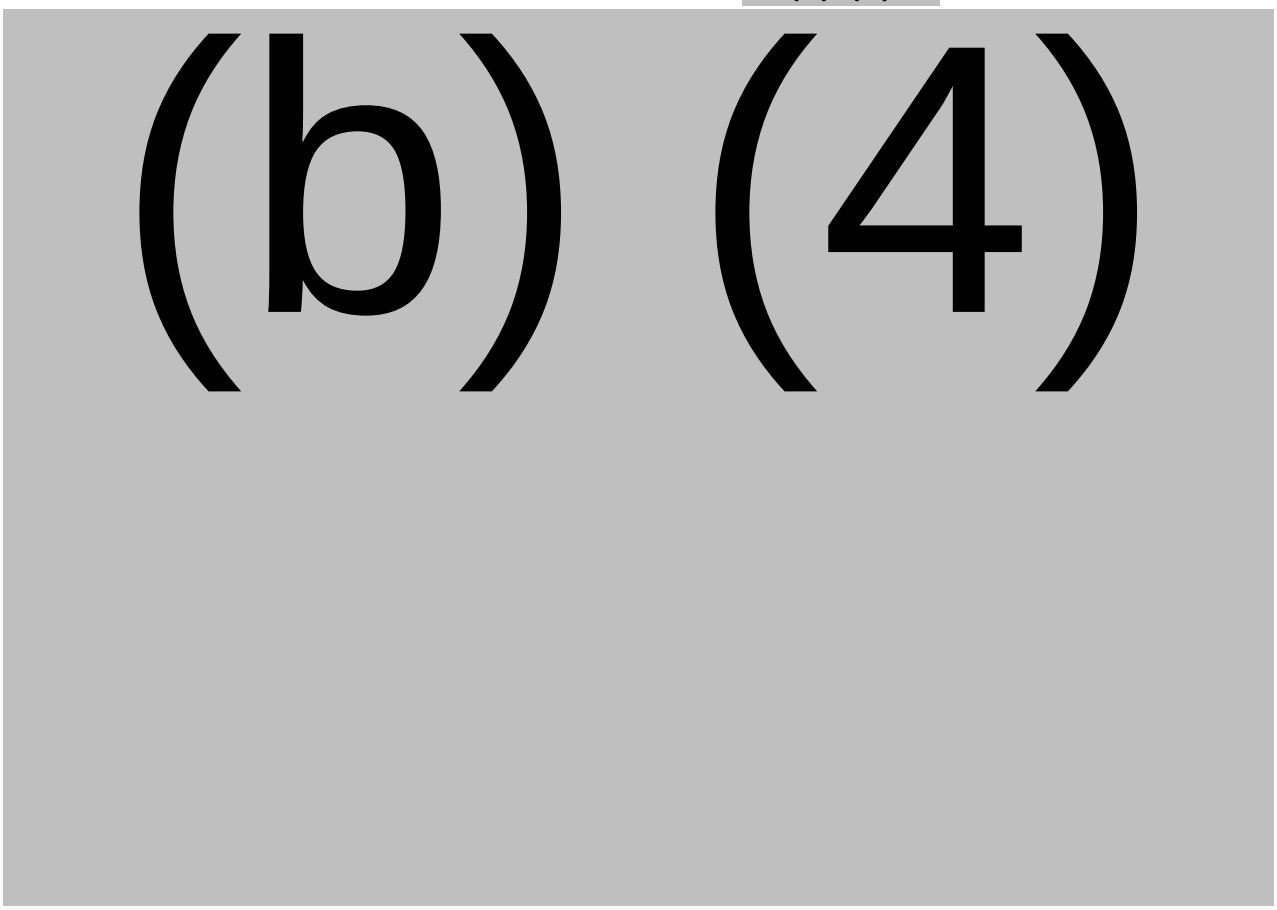
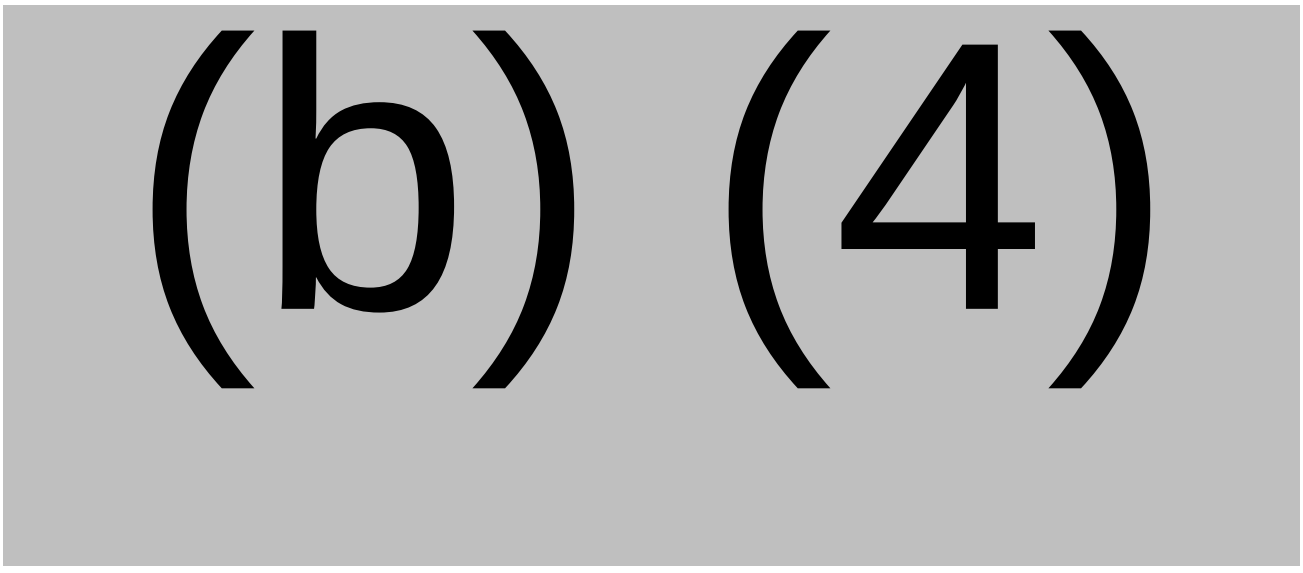


Figure 2: Proposed Plate Map for (b) (4)





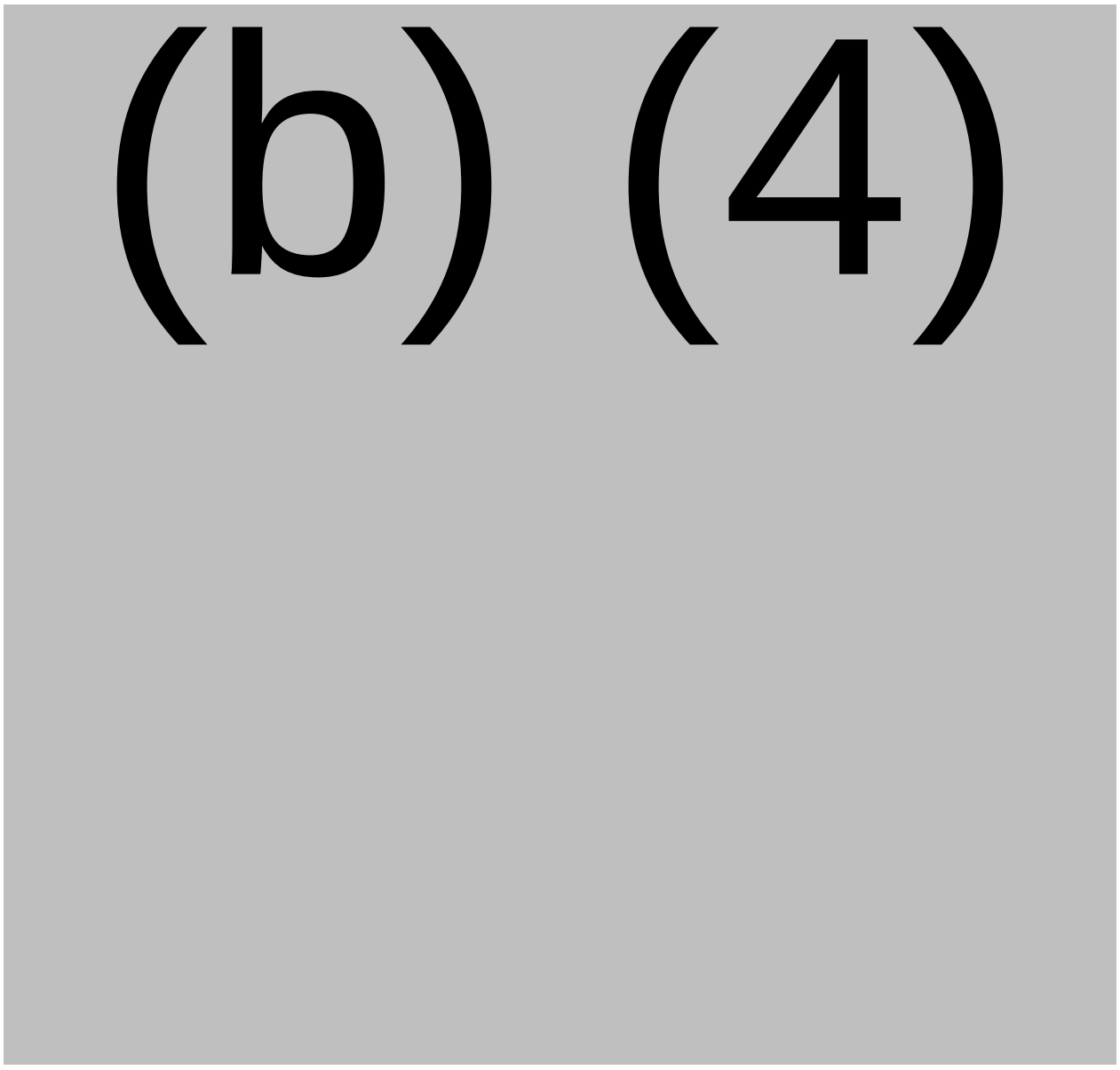


Table 8: Proposed Experimental Design for (b) (4)

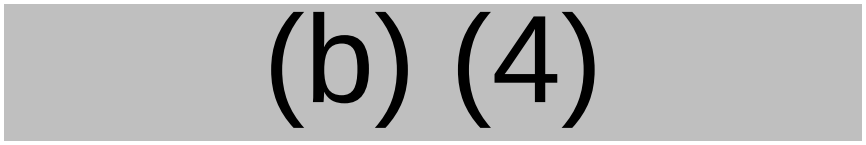
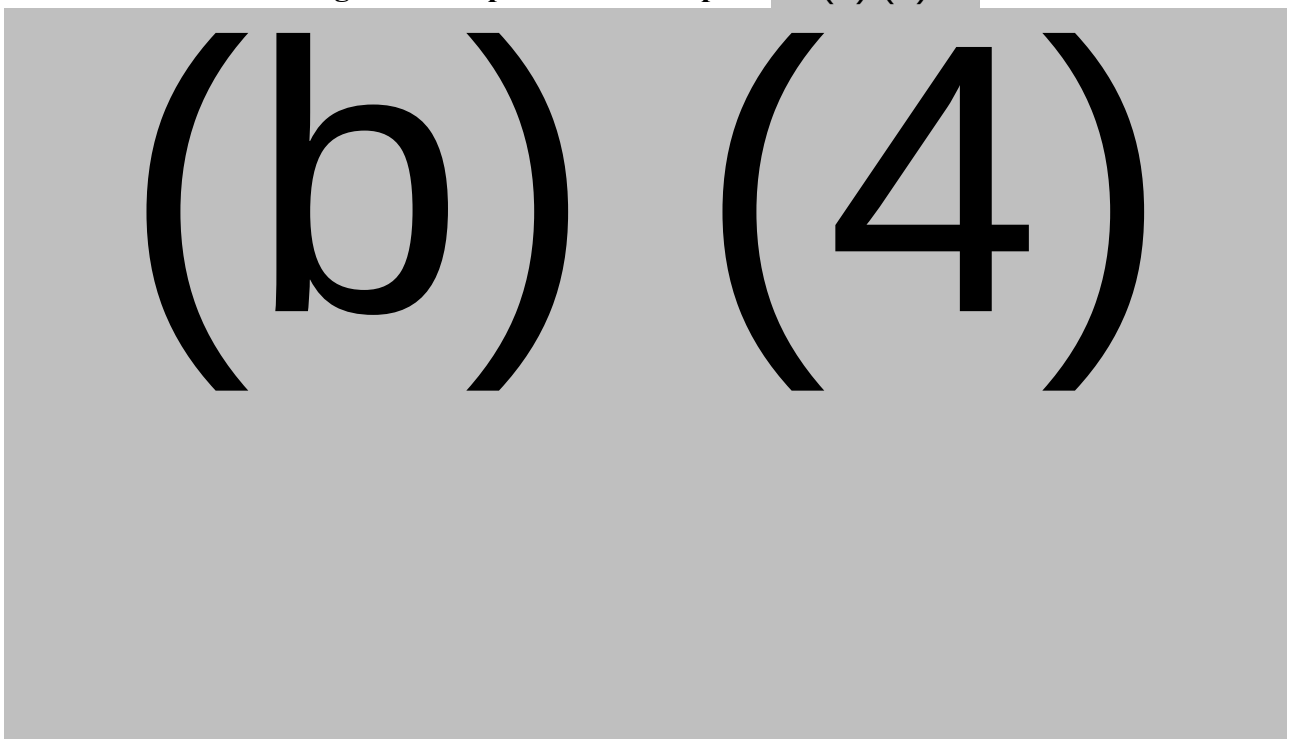


Table 9. Proposed Samples for (b) (4)

Sample ID	Barcode
(b) (4)	

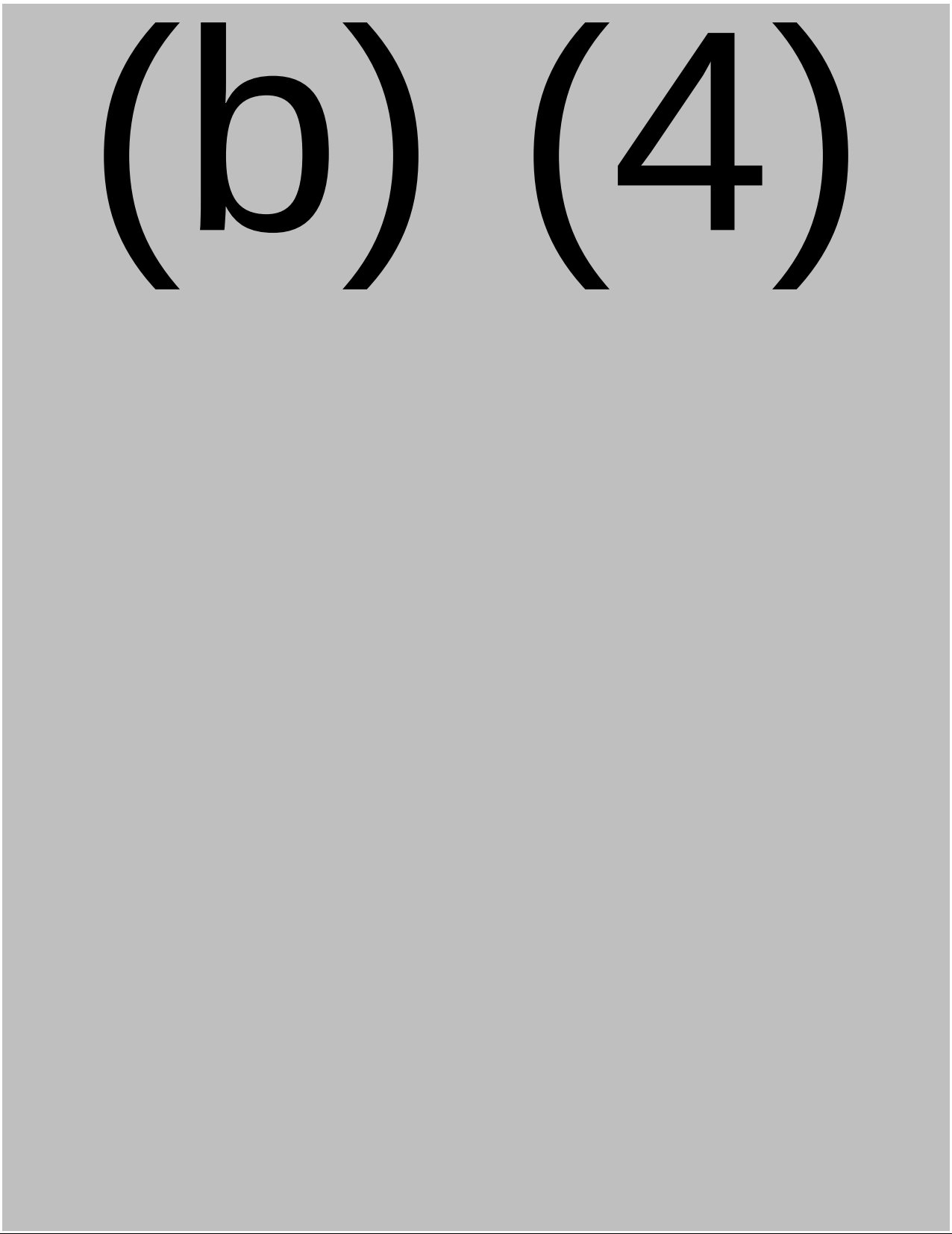
Figure 3: Proposed Plate Map for (b) (4)



Method Validation Data Analysis Plan

All system suitability criteria will be applied per the method VSDVAC 66, v0.00, (unless otherwise specified in the Experimental Design section of this plan).

(b) (4)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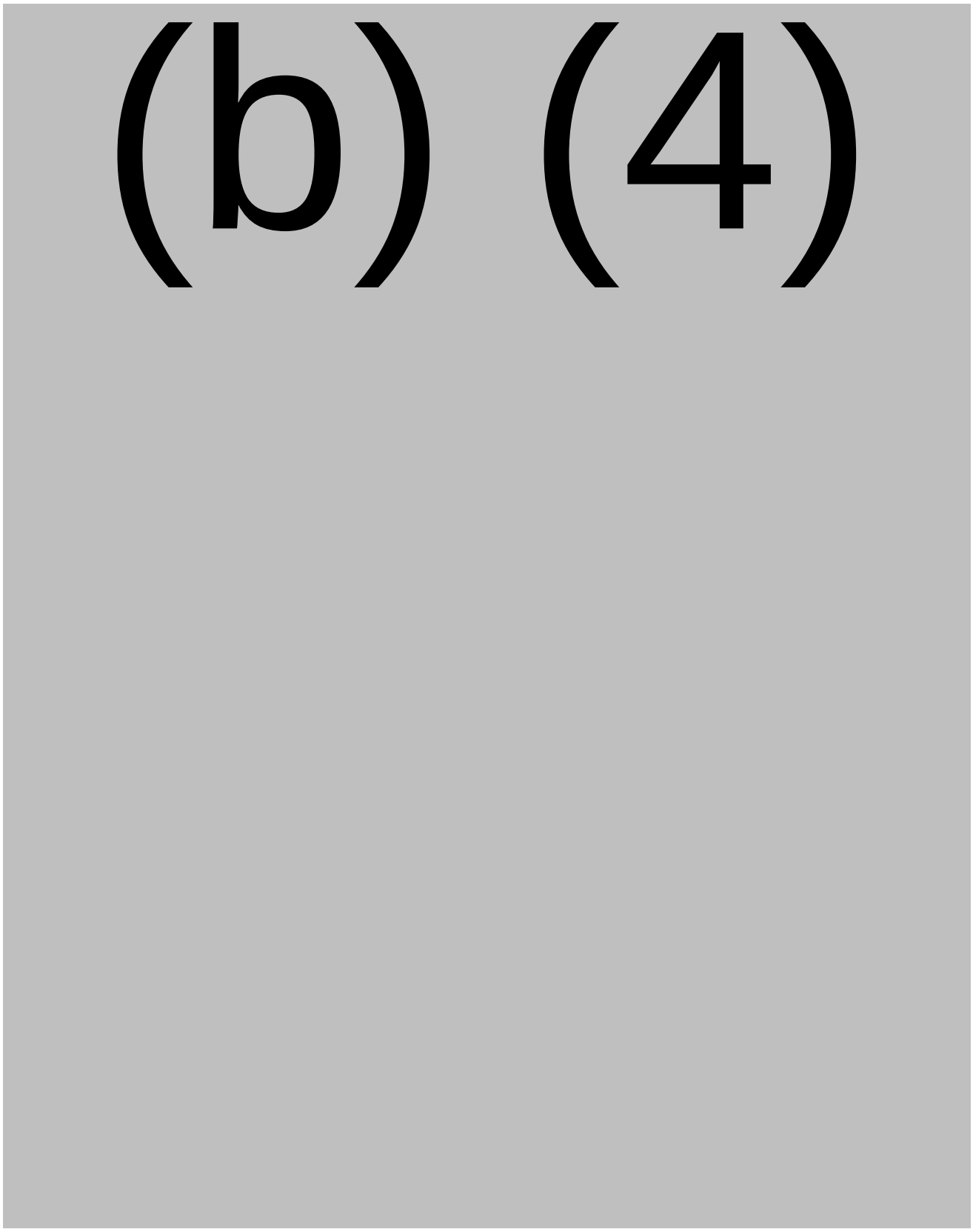
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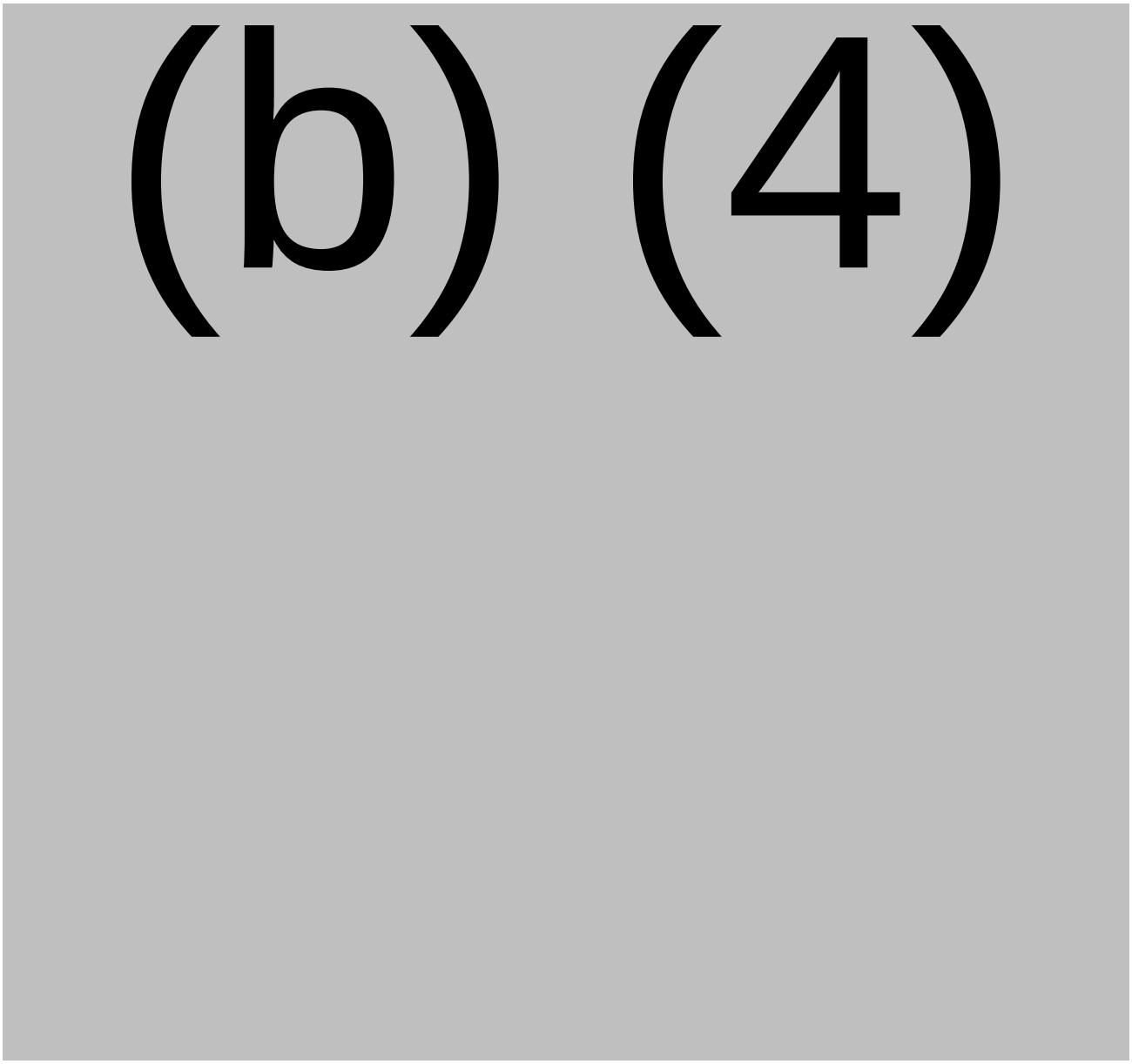
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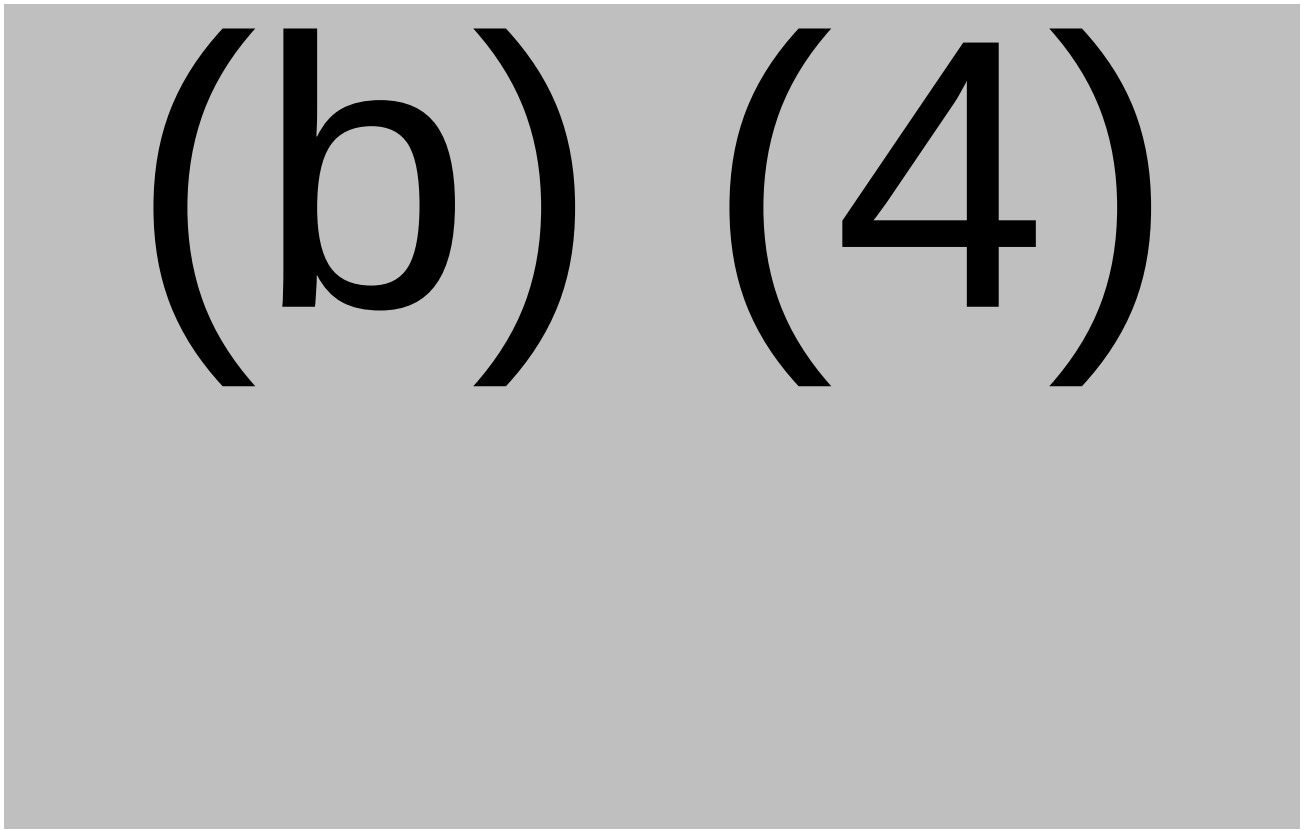

References

1. VSDVAC64: *An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid 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 Nucleocapsid Protein in Human Serum, ROZD2, 06-Jul-2020.

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

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