Method
VSDVAC 66 Version 1.01

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

PPD Project Code: RPPF2

Conducted for Moderna

Signatures

Client: Moderna

PPD Project: RPPF2

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

This method has been reviewed by the undersigned and to the best of our knowledge is complete, accurate, and in compliance with applicable plans and SOPs. The method is effective as of the last signature date on this page.

Carl Breidenbach
Associate Group Leader
I approve this document
20 Jan 2021 13:00:14 -05:00

DocuSign

Carl Breidenbach

Signature/Date

Adrienne Howlett
Manager Labs
I approve this document
20 Jan 2021 12:52:42 -05:00

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Associate Director
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Marie Bonhomme, Ph.D.

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21 Jan 2021 12:55:26 -05:00

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Victoria A. Pisciella
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I reviewed this document
20 Jan 2021 12:55:03 -05:00

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Method VSDVAC 66

Introduction

At the request of PPD® Laboratories, a bioanalytical method for the analysis of total IgG specific to SARS-CoV-2 Nucleocapsid (NP) protein in human serum was developed and qualified by PPD® Laboratories in Richmond, Virginia. The qualification of this method was conducted under PPD Project Code "ROZD2"[1]. The new method, VSDVAC 64, was finalized following qualification to Version 1.00[2,3]. At the request of Moderna, the bioanalytical method for the analysis of the SARS-CoV-2 Nucleocapsid protein total IgG in human serum, VSDVAC 64, was validated under PPD Project Code "RPPF2"[4]. The new method, VSDVAC 66, was finalized following validation to Version 1.00.

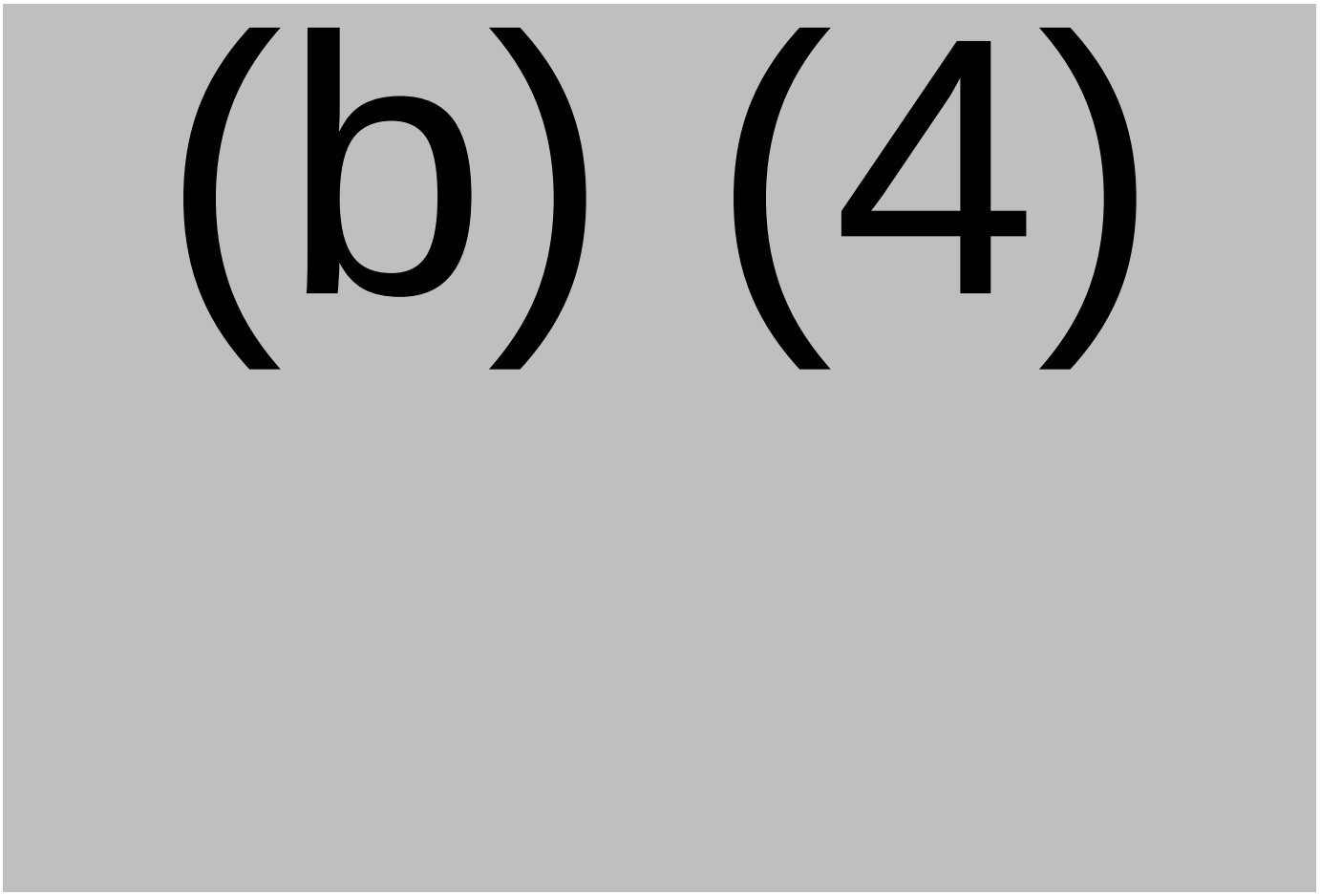
This quantitative ELISA assay was designed to detect IgG antibody to the SARS-CoV-2 NP protein in human serum. (b) (4)

(b) (4)

This assay requires a minimum (b) (4) human serum aliquot. Undiluted samples are kept frozen at (b) (4) or below. As VSDVAC 13[5], VSDVAC 64, and VSDVAC 66 methods are quantifying total IgG antibodies in human serum, sample freeze/thaw stability performed in VSDVAC 13 will be followed until sample and process stability is assessed as an addendum to the qualification of method VSDVAC 64. Serum sample stability is supported up to (b) (4) (b) (4) (b) (4)[6,7] and can be stored up to (b) (4) at 2-8°C [10].

This method applies to the testing of clinical serology samples at PPD. Any analyst who is expected to conduct the assay should be trained in this method or on an approved equivalent procedure. Any (b) (4) operator who is expected to execute the programs for sample preparation should be trained in Appendix 1 - Preparation of Samples, QCS and Reference Standard using the (b) (4) Automated Platform. Once the training in each section is completed, the training can be documented in the (b) (4) or in a training Memo saved in ECM. All samples, controls and reagents should be considered potentially infectious. It is recommended they be handled using established universal precautions. Wear personal protective gear (lab coats, gloves and safety glasses) when working with open sera. Work in an approved biosafety cabinet when possible, especially if creating aerosols is probable. Refer to Appendix 1 for additional safety notes related to the automated method.

Reagents and Chemicals



Critical Reagents

Note: Lot(s) listed were used during validation and were current at the time of use. A change to a critical reagent requires a performance qualification run at a minimum using the new and current critical reagent in accordance with PPD SOP-18196, Qualification of Critical Reagents Lots, and applicable PPD SOP(s) and may require calculations for working solutions using the new critical reagent to be adjusted. See Reagent Qualification History and Calculation Reference (RQHCR) in ECM for lots qualified after the effective date of VSDVAC 66 V1.00 and associated dilution schemes.

1. (b) (4)

2. (b) (4)

3. Reference Standard, (b) (4) Store reference standard (b) (4) (b) (4) Expiration date SPAR (See Periodic Analysis Results) per SOP LP PAL-5008. The Reference Standard neat solution has been assigned a concentration of (b) (4) AU/mL.

4. Serum samples for experimental use include but not limited to QC panels, reagent qualification and training panels. Any vendor. Store at (b) (4) Expiration date SPAR (See Periodic Analysis Results) per SOP-18199. Serum sample stability is supported up to (b) (4) (b) (4)[6,7] and can be stored up to (b) (4) at 2-8°C [10].

5. Quality Control Samples (QCs), (b) (4)

(b) (4)

Materials and Equipment

Note: Substitutions for materials and equipment are to be made in accordance with applicable PPD SOP(s) and should be deemed equivalent prior to substitution unless marked "no substitutions".

1. (b) (4) microplate reader equipped with (b) (4) software, or equivalent technology.
2. 96-well transparent flat bottom (b) (4) microtiter plates, (b) (4), or equivalent.
3. PVC adhesive plate sealers, (b) (4), or equivalent.
4. Reagent reservoirs, (b) (4), or equivalent.
5. Microfuge tubes, (b) (4), or equivalent.
6. Plate washer: (b) (4) Microplate Washer with (b) (4).
7. Polypropylene centrifuge tubes, Falcon
   - 5 mL, (b) (4), or equivalent.
   - 15 mL, (b) (4), or equivalent.
   - 50 mL, (b) (4), or equivalent.
8. Dilution Tubes
   - 1.2 mL polypropylene cluster dilution tubes, strips of (b) (4) (b) (4), or equivalent.
9. Analog vortex mixer, (b) (4), or equivalent.
10. (b) (4) Water Treatment/Purification System.
11. Controlled Temperature Incubator, Reach-In Incubator set at (b) (4) or equivalent.
12. Repeater, 10µL-50mL, any vendor.
13. 12-channel manual pipette, 30-300µL, any vendor.
14. 12- channel electronic pipette, 30-1200µL, any vendor.
15. Single-channel electronic pipette, 10-1000µL, any vendor.
16. Single-channel manual pipette, 10-1000µL, any vendor.
17. 96-well benchtop pipettor, 0-250µL, (b) (4) (b) (4) or equivalent.
18. Biosafety cabinet

Reagent Preparation

Note: All reagents used for this method are from VSDVAC 64/VSDVACR6.

Note: The prepared volume of a reagent may be adjusted according to the needs of the analyst unless otherwise specified in the method. All reagents/components will be allowed to remain at ambient temperature immediately prior to use. Preparation of all reagents may occur within a biosafety cabinet or on a bench top.

Note: All reagents are prepared as instructed below or with an equivalent approved procedure.

(b) (4)

Prepared per the current version of VSDVACR 6[8]

(b) (4)

Prepared per the current version of VSDVACR 6[8]

Secondary Antibody (b) (4)

Prepared per the current version of VSDVACR 6[8]

(b) (4) Stop Solution

Prepared per the current version of VSDVACR 6[8]

(b) (4)

Assay Controls

Reference Standard

(b) (4)
(Refer to Appendix 1) will be analyzed as a reference standard curve using human serum, (b) (4) . Refer to Table 4 for standard dilutions and associated concentrations.

Manual Reference Standard Preparation

Note: The manual reference standard preparation below is not used for clinical testing and is not a qualified method procedure but is included in the method for experimental use where appropriate and for future evaluation of the manual method. The reference standard preparation should be conducted in a biosafety cabinet.

(b) (4)

Table 1: Suggested Reference Standard Dilution Scheme

Solution	Dilution	Serum volume (µL)	Blocking Buffer Volume (µL)	Final volume (µL)	Final dilution (µL)	Assigned concentration (AU/mL)
(b) (4)						

Note: The volumes above are the volumes used unless otherwise specified. Analysts may use other volumes provided the dilutions remain the and dilution scheme used is documented in the applicable batch sheet.

Sample Blank

(b) (4) blocking buffer will be added by automated means (Refer to Appendix 1) and analyzed as the sample blank. Refer to recommended plate map in Figure 1.

Quality Controls (QCs)

(b) (4)
(b) (4)
Refer to Table 2.

All QCs are prepared prior to use by automated means (Refer to Appendix 1) and analyzed as run acceptance controls on each plate (b) (4).

Manual Quality Control Preparation

Note: The manual quality control sample preparation below is not used for clinical testing and is not a qualified method procedure but is included in the method for experimental use where appropriate and for future evaluation of the manual method. The quality control preparation should be conducted in a biosafety cabinet.

Table 2: Manual Quality Control Sample Preparation

	Transfer Volume (µL)	**Blocking Buffer Volume** (µL)	**Total Volume** (µL)	**Dilution**	**Final Dilution**	**Applicable QCs**
(b) (4)						

Note: The volumes in this table are the volumes used unless otherwise specified. Analysts may use other volumes provided the dilutions remain the same and the dilution scheme used is documented in the applicable batch sheet.

Batch Sheets & Run Names

(b) (4)

Sample Preparation

Serum samples are prepared by automated means (see Appendix 1).

Manual Sample Preparation

Note: The manual sample preparation below is not used for clinical testing and is not a qualified method procedure but is included in the method for experimental use where appropriate and for future evaluation of the manual method. Sample preparation should be conducted in a biosafety cabinet.

Dilute all serum samples in dilution cluster tubes. A pre-dilution of (b) (4) is prepared to achieve (b) (4) (b) (4) (b) (4) Remove samples from storage. Thaw as needed at ambient temperature or at 2-8°C. See Table 3 for Suggested Sample Dilution Scheme.

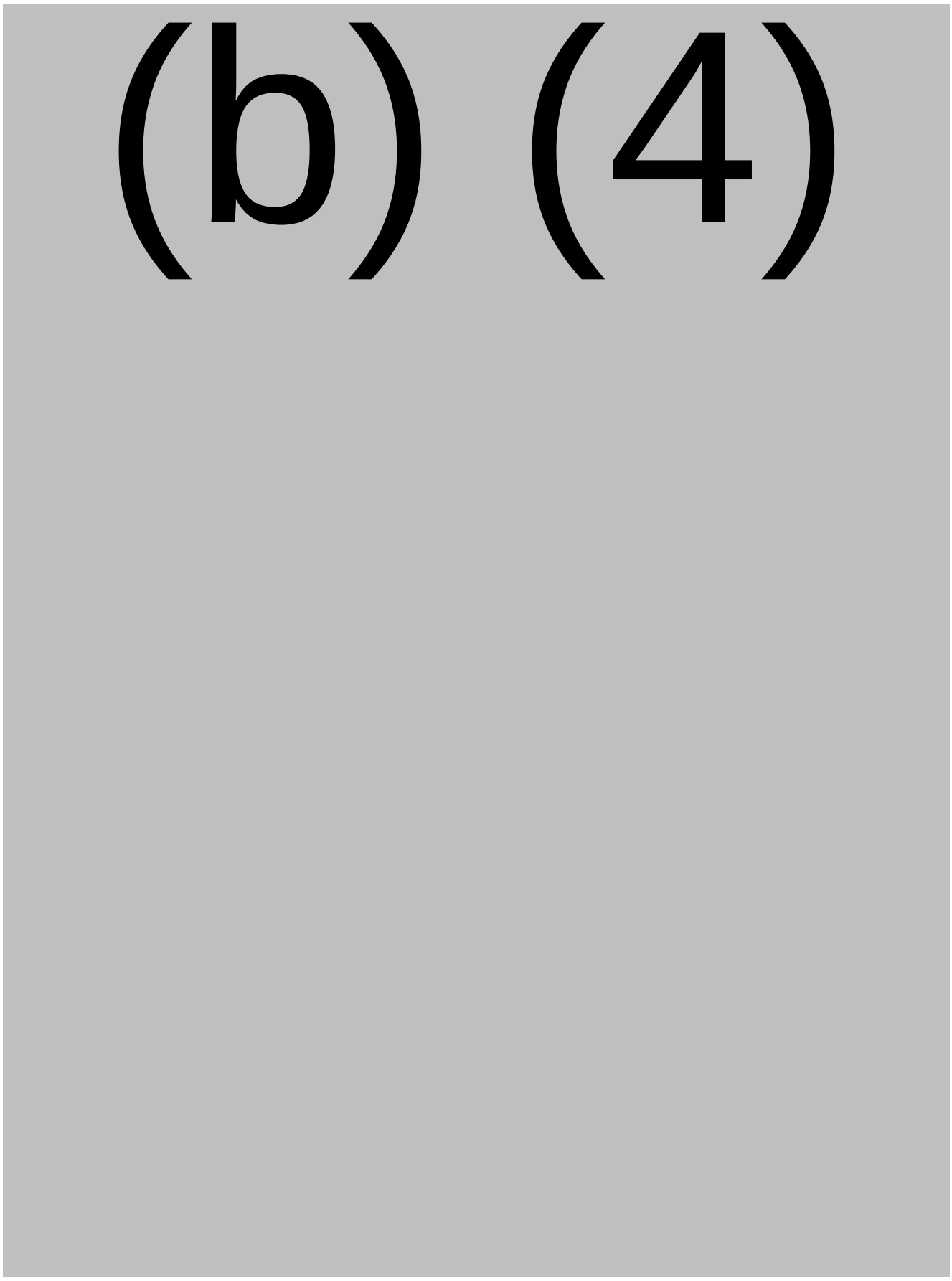
Table 3: Suggested Sample Dilution Scheme

	Transfer Volume (µL)	**Blocking Buffer Volume** (µL)	**Total Volume** (µL)	**Dilution**	**Final Dilution**
(b) (4)					

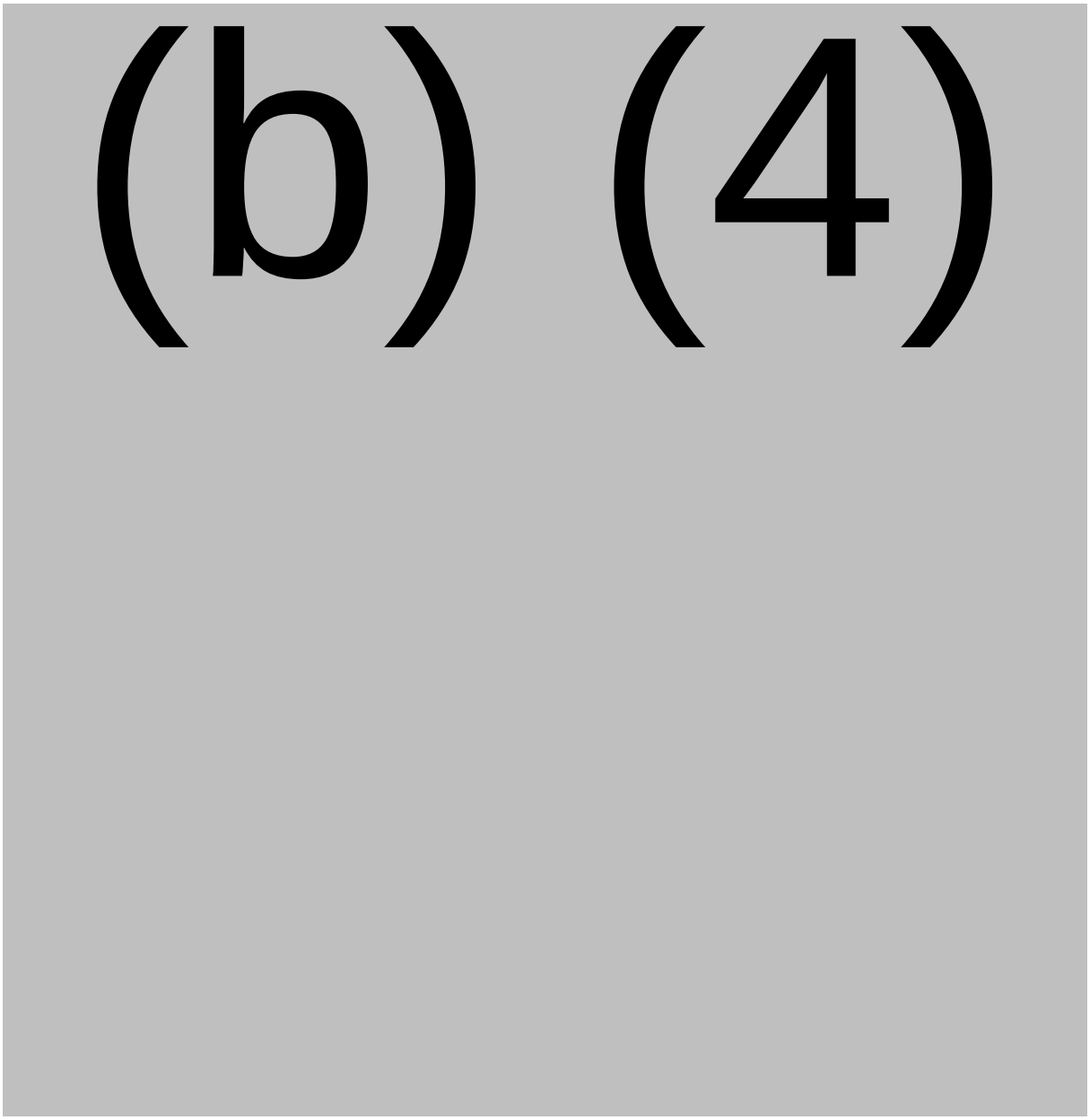
Note: The volumes above are the volumes used unless otherwise specified. Analysts may use other volumes provided the dilutions remain the and dilution scheme used is documented in the applicable batch sheet.

Assay Procedure

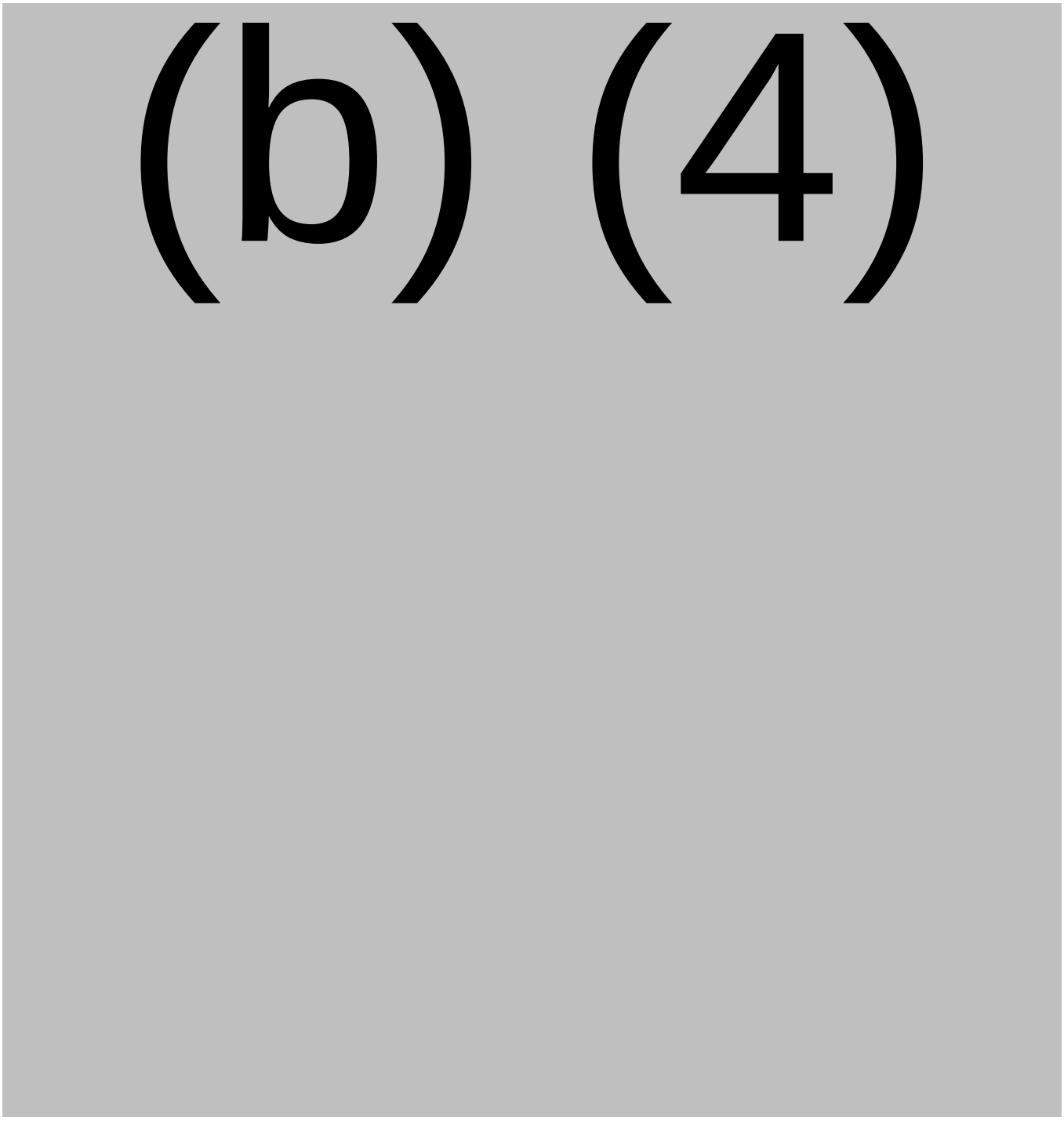
(b) (4)



Instrument Parameters



Data Regression



Assay Acceptance Criteria

Plate Validity Criteria:

(b) (4)

Assay Run Validity Criteria:

(b) (4)

(b) (4)

Data Analysis

Determining the Antibody Concentration

(b) (4)

Test Flags

(b) (4)

Final Antibody Concentration = (dilution)(titer)

Samples are analyzed at one dilution through (b) (4) therefore the final result will be determined as follows:

(b) (4)

Completion of the Assay

1. After the assay run is completed, an assay report (Lab Report or Summary Report) must be generated by the assay analysis system.
2. The raw data file will be retained ECM (Enterprise Content Manager). If a hard copy of the raw data-printout is needed, the file name for the assay raw data, the wavelength at which the plate was read, the date, and the signature of the performing analyst must be recorded.
3. The batch sheet and assay report must be reviewed by another qualified analyst and/or the PI or the Lab Manager (or designee).
4. Completion of Assay Run
   a. No further action is required by the analyst that executed the assay.
   b. Acceptance of the assay results will be performed by the PI or Lab Manager (or designee).
5. Following finalization or acceptance in the assay analysis system, final results will be transferred based on client requirements.

References

[1] VSDVAC 64, *An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum,* v1.02.

[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, 06Jul2020.

[3] PPD Statistical Report Addendum: *Qualification of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum – LOD & Selectivity Addendum,* ROZD2, 24Aug2020.

[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, 01Dec2020.

[5] VSDVAC 13, (b) (4) *Total IgG Assay* (b) (4) *, V3.01, 30Jun2020.*

[6] PPD Statistical Report: *Assessment of Reference Standard and Serum Sample Stability at 2-8°C for the* (b) (4) *Total IgG Assay* (b) (4) *IgG),* RDC09, 01Jun2018

[7] PPD Statistical Report: *Assessment of Reference Standard and Serum Sample Stability by Freeze/Thaw Cycle, for the* (b) (4) *Total IgG Assay* (b) (4) RDC09, 06Feb2018

[8] VSDVACR 6: *SARS-CoV-2 ELISA Generic Reagent Preparation Procedure*.

[9] PPD Statistical Report, *Assessment of Antigen Stability for Storage at 2-8°C for* (b) (4) (b) (4) *for Use in an ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum*, ROZD2, 18Dec2020.

[10] PPD Statistical Report, *Assessment of Sample Stability for Storage at 2-8°C for* (b) (4) *for Use in an ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum*, ROZD2, 11Jan2021.

Method History

Version 1.00
Draft Method VSDVAC 66 Version 0.00 was modified by Christopher Hammond on 19Nov2020 to include the following updates:

1. Version updated from 0.00 to 1.00 throughout
2. Recommended Plate Map numbering updated to (b) (4) samples
3. Capitalization and grammar throughout assay steps. Added (b) (4) naming convention to blocking buffer references for clarity.
4. Updated wording in Reference Standard and Manual Reference Standard Preparation
5. Updated Manual Sample Preparation intermediate to align with Table 3.
6. Added note to reagent preparation noting reagents shared between methods.
7. Procedure: Updated number of wash cycles per wash step.
8. Assay Acceptance Criteria: Updated (b) (4) (b) (4) per the statistical validation report.
9. Test Flags: Updated LLOQ and ULOQ.
10. References: Added reference to statistical validation report. Added reference to VSDVAC 64 method.
11. Batch Sheets & Run Names and Assay Procedure Step 15: Updated format of Run Naming convention and plate reader file naming convention.
12. Instrument Parameters: Updated number of wash cycles
13. Appendix 1, Program Overview for Standard, Quality Control and Unknown Sample Preparation, Item 5: Required RS and QCS barcode formats updated.

Version 1.01
VSDVAC 66 Version 1.00 was modified by Carl Breidenbach on 14Jan2020 to include the following updates, as documented in VSDVAC_66_v1-00_MCR and CCF-VSD-0266:

1. Version updated from 1.00 to 1.01 on title page and footers.
2. Updated SOP numbers throughout document to align with updated document control numbers assigned in (b) (4) .
3. In the Introduction section, a statement referencing the VSDVAC 13 and VSDVAC 64 methods for purpose of sample stability was reworded to include process stability.
4. Updated nucleocapsid antigen critical reagent section to include established 2-8°C storage information.

5. Updated Materials and Equipment section to include (b) (4) benchtop pipettor.
6. In the Data Regression section, a formula was inserted within the last sentence to describe (b) (4)
7. Added reference to stability reports for 2-8°C antigen[1] stability and 2-8°C sample stability[2].
8. In Appendix 1, Materials and Equipment section 3, numbers 3.2 and 3.3 were added to include additional consumables.

Change History

1. N/A

Appendix 1: Preparation of Samples, QCS and Reference Standard using the (b) (4) Automated Platform

Introduction

The (b) (4) platform and custom (b) (4) programs are used as part of the sample, and quality control and reference standard preparation processing for PPD Method VSDVAC 66.

This method attachment outlines the procedure for the execution of custom (b) (4) programs. The scope of this attachment is limited to the trained and authorized (b) (4) Lab Operator role, and those whom have qualified on the SOPs defining the role; SOP LE-BA-010. Additionally, (b) (4) operators trained in this procedure should have the training captured as appropriate (such as an OJT for example).

During (b) (4) operation, DO NOT place any part of the body in the way of moving parts. Before and after processing samples, the accessible working surfaces of the (b) (4) should be disinfected following SOP LE-BA-010.

Reagents and Chemicals:

1. Assay reagents and chemicals per method VSDVAC 66, An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum

Critical Reagents:

2. Assay reagents and chemicals per method VSDVAC 66, An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Nucleocapsid Protein in Human Serum

Materials and Equipment:

Note: All materials and equipment cannot be substituted without review and evaluation by (b) (4) and/or Automation Specialist (or higher). This review and evaluation will be documented, for example through a memo, signed by Automation, lab management, and QA.

1. (b) (4) Carriers, Racks and Modules:

   1.1. (b) (4) platforms equipped with the same hardware are considered equivalent platforms, carriers, racks and modules may have different part numbers, as applicable.

2. (b) (4) Tips:

   2.1. Irradiated Conductive, sterile tips, manufactured by the instrument manufacturer. No substitutes.

3. Consumables:

   (Note: Part number may differ from part number listed if sourced from alternate distributor but the same manufacturer.)

   3.1. 2 mL Polypropylene, Sterile, (b) (4), Part No.: (b) (4), or equivalent.

   3.2. 2 mL Cryvial, Sterile, (b) (4), Part No.: (b) (4), or equivalent

   3.3. 1.8 mL Sterile, (b) (4), Part No.: (b) (4), or equivalent

   3.4. (b) (4) Reservoir, (b) (4), Part No.: (b) (4) or (b) (4), Part No.: (b) (4) (b) (4) (b) (4)

   3.5. (b) (4) 96Well (b) (4), (b) (4), Part No.: (b) (4)

   3.6. (b) (4) 96Well (b) (4), (b) (4), Part No.: (b) (4)

   3.7. Sterile Foil Sealers, (b) (4), Part No.: (b) (4), or equivalent

   3.8. (b) (4) 96Well (b) (4), (b) (4) Part No.: (b) (4) or (b) (4) Part No.: (b) (4)

   3.9. 1.5 mL Self Standing Cryo Tubes, (b) (4), Part No.: (b) (4), or equivalent

General Procedural Comments

1. When applicable, sample storage, scheduling and data processing should be managed within the (b) (4) LIMS system. If (b) (4) or other systems are not available or required for program execution, then input files may need to be generated manually and the procedural steps with (b) (4) are not required.

2. If (b) (4) is used to track the movement of samples and is not available, relevant sample movement information should be captured in the electronic TestSheet, or appropriate documentation, until (b) (4) is available.

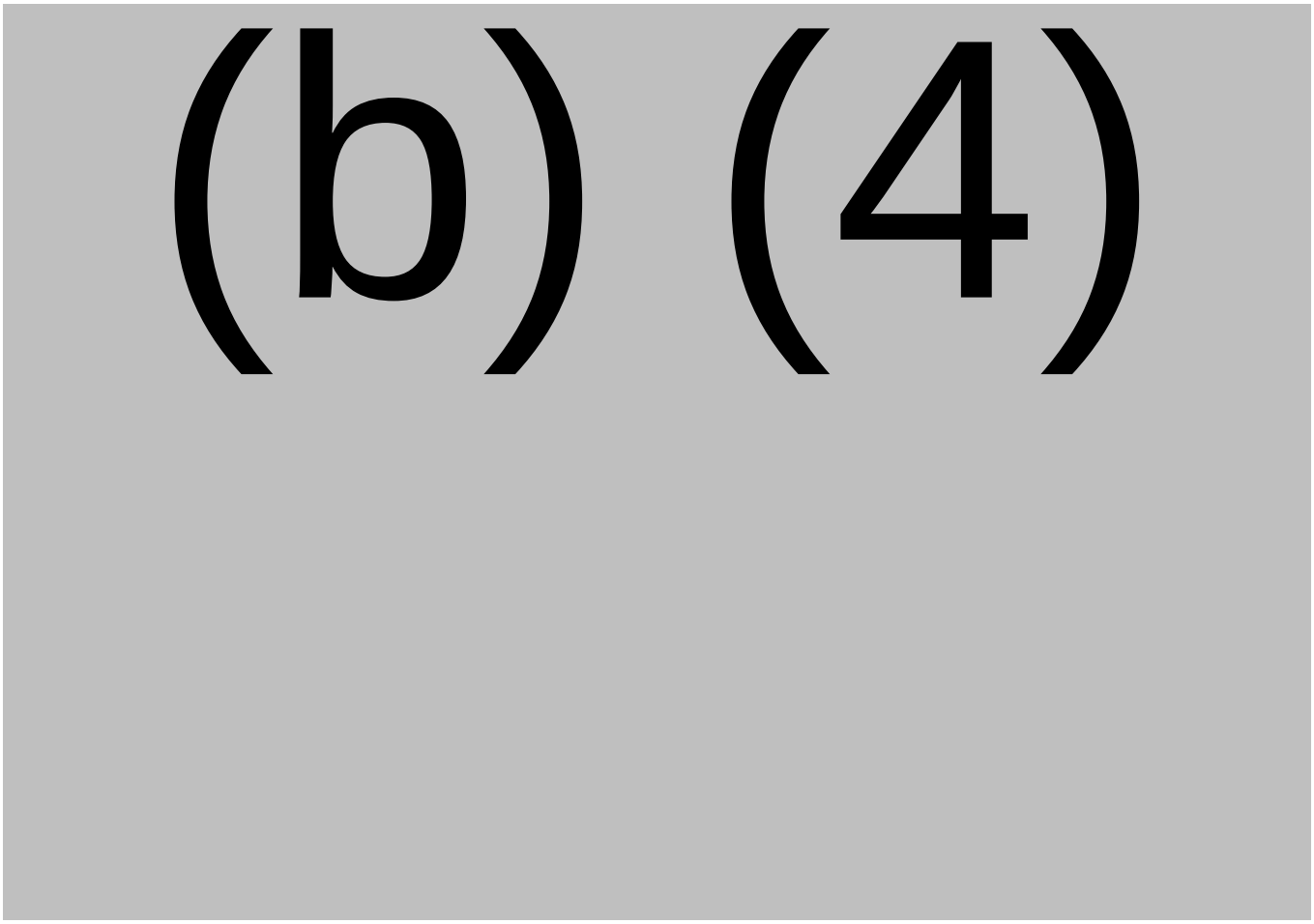
3. When applicable, documentation of each program execution or run should be performed within the eSheet application. If this system is not available for prompt data entry, then documentation may be completed and signed manually, creating hard copy records, which should then be transcribed into eSheet when the application is available. The original hardcopy records can be scanned and attached to eSheet, as applicable. The original hardcopy must be maintained and bound to appropriate laboratory notebooks, as applicable.

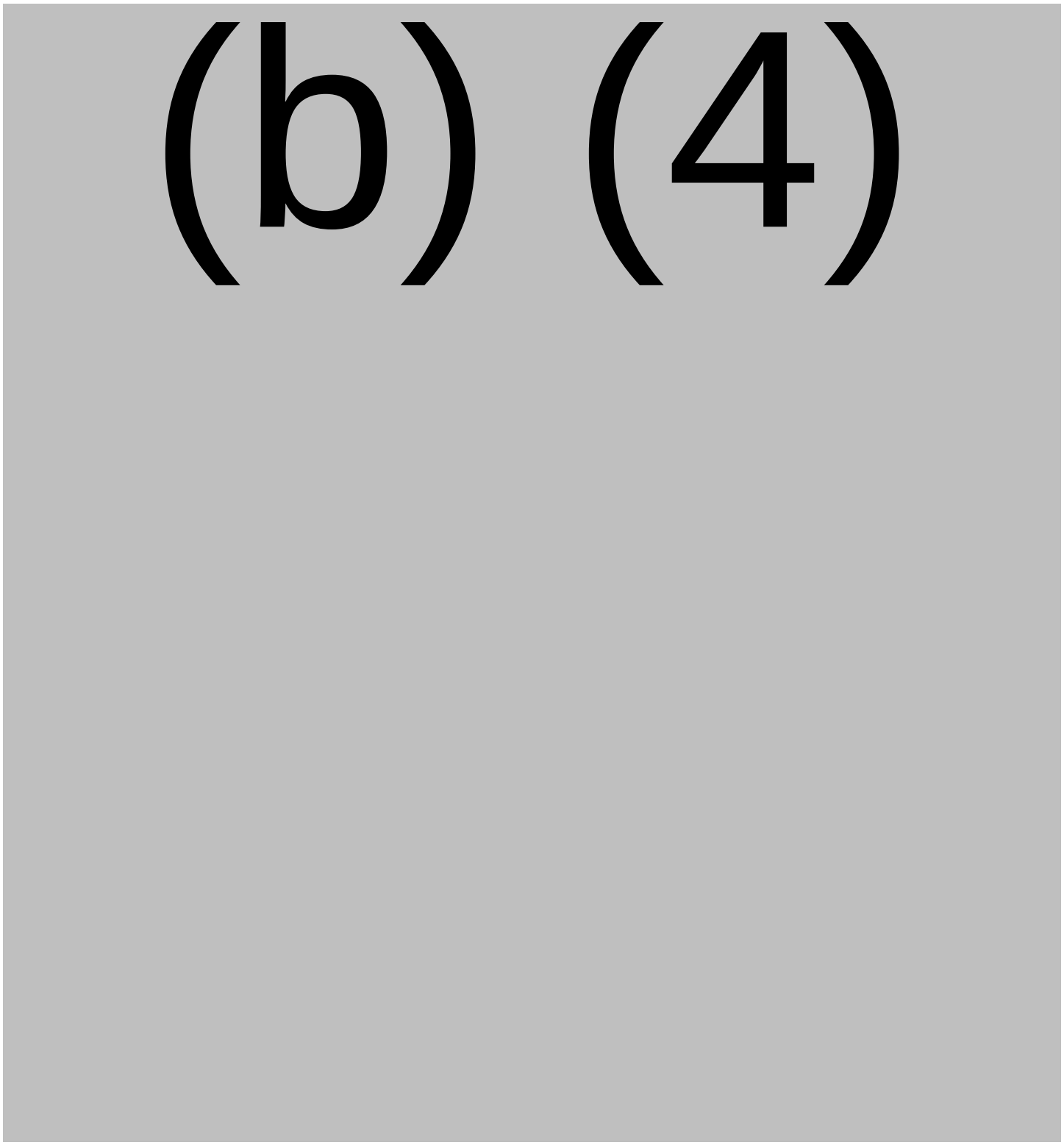
4. Procedural steps which are required to be executed in a specific order should be indicated appropriately, as applicable.

Program Overview for Standard, Quality Control and Unknown Sample Preparation

1. The custom program (b) (4) (current version or higher, where platform is instrument, i.e. (b) (4), prep, etc.) is used to create (b) (4) (b) (4), which contains the diluted reference standard, blank and quality controls. The custom program is also used to create the sample dilutions (b) (4) and map the diluted standard, quality controls and samples into (b) (4), which are then provided to the assay group for assay steps. The custom program can perform the preparation of the diluted standards, controls and samples independently or within the same program execution.

Note: The (b) (4) *program listed in VSDVAC 66 share common processes such as aliquoting, that can be used to prepare the aliquots in place of the method listed in VSDVAC 65. Other custom programs may be suitable for use if qualified as per SOP-18077.*




(b) (4)

Program Execution Steps for Standard, Quality Controls and Unknown Sample Preparation