Method

VSDVAC 65 Version 1.01

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

PPD Project Code: RPPF

Conducted for PPD® Laboratories

Confidentiality Statement

Signatures

Client: PPD

PPD Project: RPPF

PPD Method Title: **An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike 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.

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Method VSDVAC 65

Introduction

At the request of PPD® Laboratories, a bioanalytical method for the analysis of the SARS-CoV-2 total IgG in human serum was developed and qualified by PPD® Laboratories in Richmond, Virginia. The qualification of this method was conducted under PPD Project Code "ROQP2"[1]. The new method, VSDVAC 58, was finalized following qualification to Version 1.00[2]. At the request of Moderna, the bioanalytical method for the analysis of the SARS-CoV-2 total IgG in human serum, VSDVAC 58, was validated under PPD Project Code "RPPF"[3]. The new method, VSDVAC 65, was finalized following validation to Version 1.00.

This quantitative ELISA assay was designed to detect IgG antibody to the SARS-CoV-2 virus in human serum. Microtiter plates were coated with commercially available SARS-CoV-2 full-length spike (S) glycoprotein and serum containing the SARS-CoV-2 IgG antibody was added. Bound antigen-antibody complex was detected using purified goat anti-human IgG horseradish peroxidase (HRP) conjugate. Color development occurred during the addition of 3,3',5,5'tetramethylbenzidine (TMB) substrate and color intensity was measured spectrophotometrically (b) (4) . The intensity of the color was directly proportional to the IgG antibody concentration. Quantitation of the human IgG antibody to SARS-CoV-2, or antibody concentration (AU/mL), was determined by interpolation from a standard curve analyzed on each assay plate.

This assay requires a minimum of (b) (4) µL human serum. Undiluted samples are kept frozen at (b) (4) or below. As VSDVAC 13 [4], VSDVAC 58 and VSDVAC 65 methods are quantifying total IgG antibodies in human serum, sample stability performed in VSDVAC 13 will be followed until sample stability is assessed as an addendum to the qualification of method VSDVAC 58. Serum sample stability is supported up to (b) (4) and can be stored up to (b) (4) days at 2-8°C [5, 6].

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. 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

1. Deionized water (DI water) obtained from (b) (4), or equivalent. Store at ambient temperature. Assign expiry per SOP LP-PAL-5008.

2. Sterile distilled water, (b) (4) (b) (4). Store at ambient temperature. Follow manufacturer's expiry. If not provided, assign expiry per SOP LP-PAL-5008.

3. (b) (4) or equivalent. Store at ambient temperature. Follow manufacturer's expiry. If not provided, assign expiry per SOP LP-PAL-5008.

4. (b) (4) or equivalent. Store at ambient temperature. Follow manufacturer's expiry. If not provided, assign expiry per SOP LP-PAL-5008.

5. (b) (4) Blocking Buffer (b) (4) or equivalent. Store at 2-8°C. Follow manufacturer's expiry. If not provided, assign expiry per SOP LP-PAL-5008.

6. TMB [3,3',5,5'-tetramethylbenzidine] ELISA Substrate, (b) (4), or equivalent. Store at 2-8°C. Follow manufacturer's expiry. If not provided, assign expiry per SOP LP-PAL-5008.

7. (b) (4) Stop Solution (b) (4), or equivalent. Store at ambient temperature. Follow manufacturer's expiry. If not provided, assign expiry per SOP LP-PAL-5008.

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 LP-PAL-7012, 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 65 V1.00 and associated dilution schemes.

1. SARS-CoV-2 S-2P Spike Protein, (b) (4) Per manufacturer, store product at (b) (4) Expiration date SPAR (See Periodic Analysis Results) per SOP LP PAL-5008.

2. Goat Anti-Human IgG Antibody, HRP conjugate, (b) (4) Per the manufacturer, store lyophilized product at 2-8°C for up to (b) (4) after date of receipt. Reconstituted product is stable for up to (b) (4) at 2-8°C and (b) (4) at -90°C to -60°C.

3. Reference Standard, (b) (4) human sera, (b) (4) (b) (4) Store reference standard (b) (4). Expiration date SPAR (See Periodic Analysis Results) per SOP LP PAL-5008.

4. Serum samples for experimental use including but not limited to (b) (4), reagent qualification and training. Any vendor. Store at -90°C to -60°C. Expiration date SPAR (See Periodic Analysis Results) per SOP LP PAL-5008. Serum sample stability is supported up to 10 freeze/thaw cycles and can be stored up to 60 days at 2-8°C [4, 5].

5. Quality Control Samples (QCs), Store all undiluted controls listed at -90°C to -60°C with an expiry of SPAR, per SOP LP-PAL-5008. Frozen undiluted controls can be thawed at ambient temperature or at 2-8°C. Serum sample stability is supported up to (b) (4) and can be stored up to (b) (4) days at 2-8°C [4, 5].

(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. (b) (4) 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, (b) (4)
   - 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 8, (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 (21 ± 2°C), (b) (4), or equivalent.
12. Repeater, 10µL-50mL, any vendor.
13. Single-channel pipette, 10-1000µL, any vendor.
14. 12-channel manual pipette, 30-300µL, any vendor.
15. 12- channel electronic pipette, 30-1200µL, any vendor.
16. 96-well benchtop pipettor, 0-250µL, (b) (4) or equivalent.
17. Biosafety cabinet

Reagent Preparation

Note: All reagents used for this method are from VSDVAC 58/VSDVACR 6.

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 [7].

SARS-CoV-2 ELISA Wash Buffer (Prepared from concentrate: (b) (4))

Prepared per the current version of VSDVACR 6 [7].

Secondary Antibody (Goat anti-human IgG, HRP conjugate) Rehydration

Prepared per the current version of VSDVACR 6 [7].

(b) (4) (b) (4) Stop Solution

Prepared per the current version of VSDVACR 6 [7].

Working SARS-CoV-2 Spike Protein Coating Solution

Dilute SARS-CoV-2 S2P Spike Protein to a concentration of (b) (4) Thoroughly mix by swirling or inversion. Refer to the *Reagent Qualification History and Calculation Reference* (RQHCR) document located in ECM (VSD/Methods/VSDVAC 65/RQH) for dilution schemes. Discard excess after use, same day.

Working Secondary Antibody Solution

Combine goat anti-human IgG, HRP conjugate with blocking buffer at the appropriate final concentration for the particular lot of goat anti-human IgG, HRP conjugate in use. Thoroughly mix by swirling or inversion. Refer to the *Reagent Qualification History and Calculation Reference* (RQHCR) document located in ECM (VSD/Methods/VSDVAC 65/RQH) for working dilution schemes. Discard excess after use, same day.

Assay Controls

Reference Standard

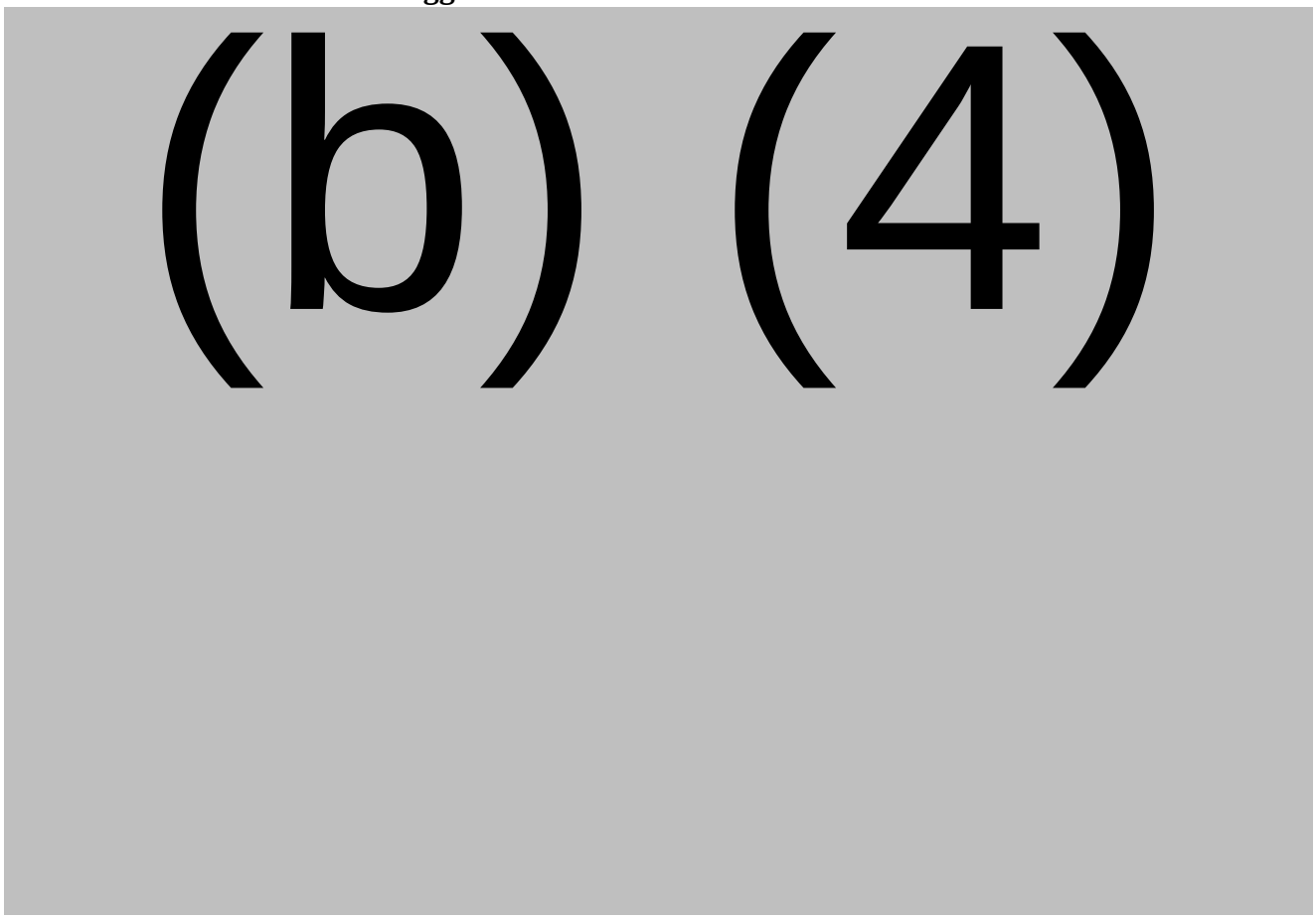
(b) (4) an 11-pt. standard curve diluted at 2.5-fold by (b) (4) (Refer to Appendix 1) will be analyzed as a reference standard curve using (b) (4) human sera, (b) (4) Refer to Table 4 for standard dilutions and associated concentrations.

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.

Remove reference standard, (b) (4) human sera from storage and thaw at ambient temperature. Using blocking buffer as diluent, an initial (b) (4) dilution is performed to create the Dilution 1 solution at (b) (4) The Dilution 1 solution is then used to create a 1:500 dilution followed by a (b) (4)-pt, 2.5 dilution series. Refer to Table 1 for Suggested Reference Standard Dilution Scheme.

Table 1: Suggested Reference Standard Dilution Scheme



Sample Blank

(b) (4)
(b) (4) Refer to recommended plate map in Figure 1.

Quality Controls (QCs)

(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

(b) (4)

Batch Sheets & Run Names

Create work order in (b) (4) as per SOP LP-PAL-7007. Complete either a hard copy batch sheet or utilize an electronic batch record (e-sheet) to capture all relevant assay information. TestName ID_XXX (VSDVAC 65_817). Work orders created and processed in (b) (4) will utilize the following Run Name convention: Date (MMMDDYYYY)_username*_assayname (VAC65_IgG_ S)_MTM(40400)_N_assay# (i.e. 1, 2, 3…).

*Username of analyst who created WO.

Sample Preparation

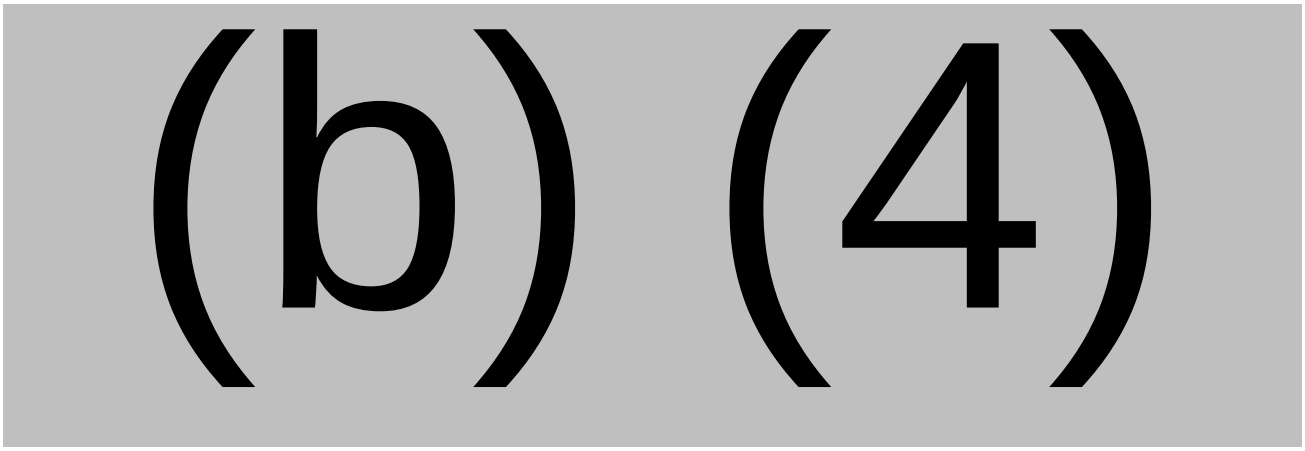
Serum samples are prepared by automated means (see Appendix 1).

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. (b) (4) the starting dilution factor (default dilution) used in this assay of (b) (4) Samples may require (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



Assay Procedure

Note: The secondary antibody should be brought to ambient temperature before use. Assay procedure should be conducted within a biosafety cabinet.

(b) (4)

Coating Incubation

(b) (4)

Blocking

(b) (4)

Sample Incubation

(b) (4)

Conjugate Incubation

(b) (4)

(b) (4)

Substrate (TMB) Incubation

(b) (4)

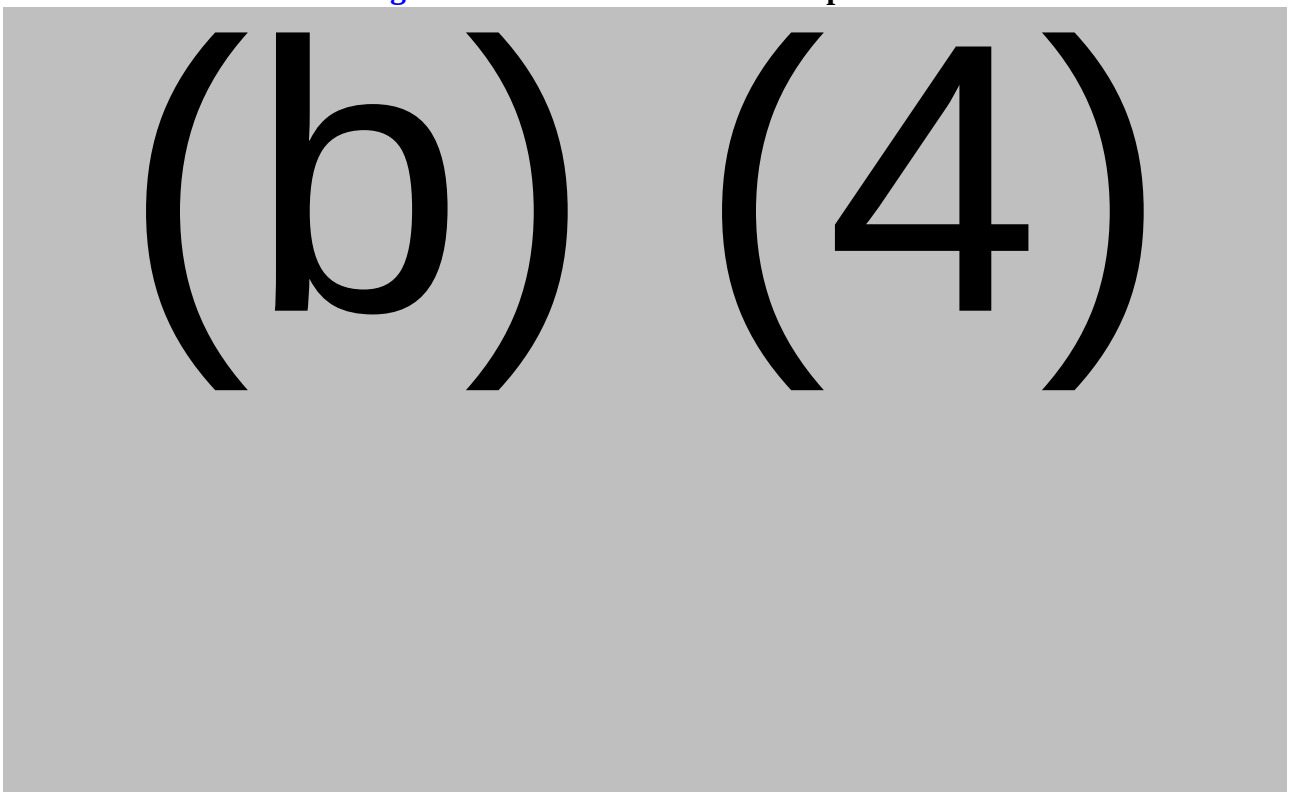
Stopping and Plate Development

(b) (4)

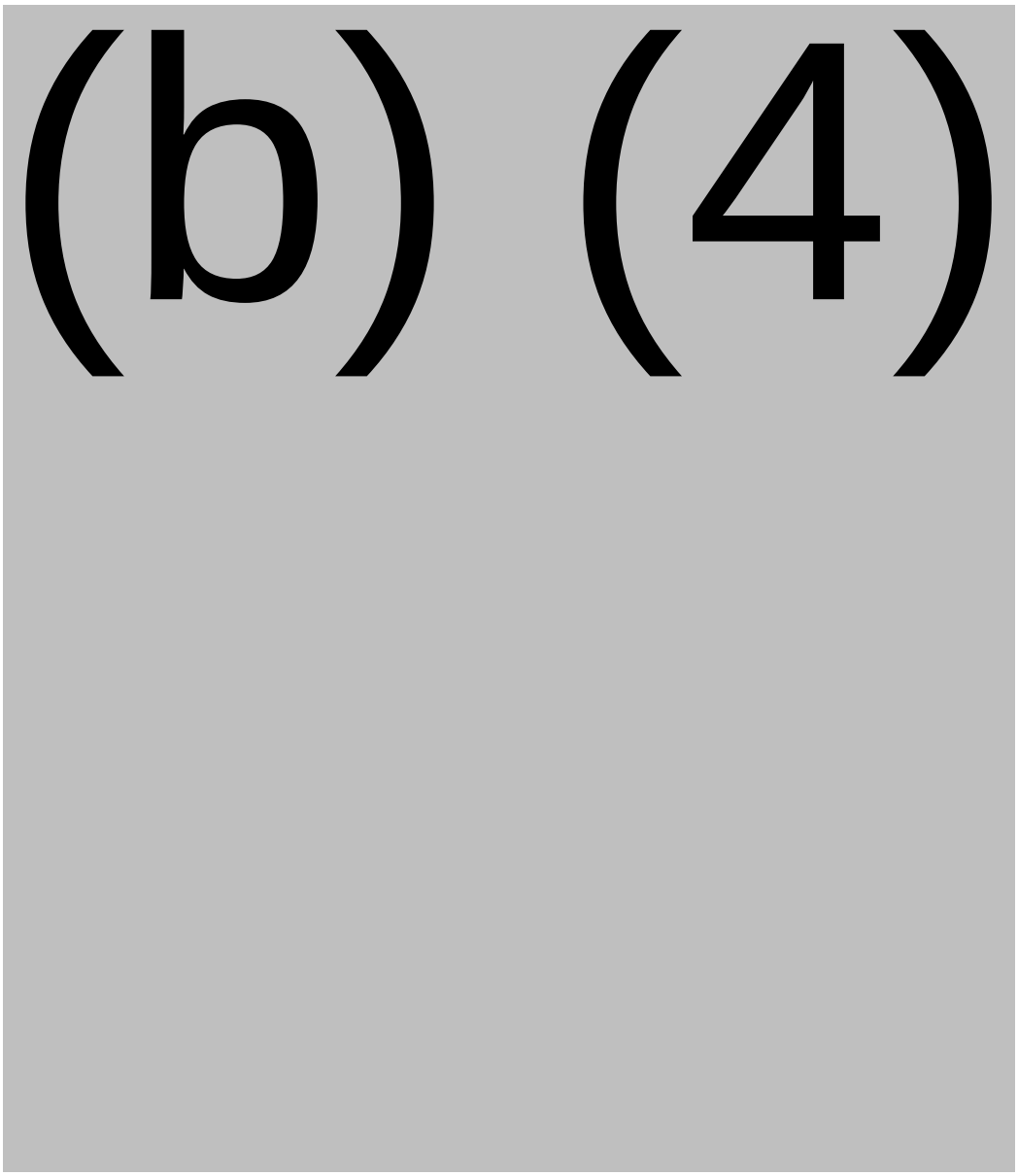
15. Plate reader experimental file name should use the following nomenclature: Date (MMMDDYYYY)_username*_assayname (VAC65_IgG_S)_MTM(40400) _N_assay# (i.e. 1, 2, 3…). During export of each plates raw data file, a plate number should be appended to the end of the experimental file name using the following nomenclature: _Plate # (i.e. 1, 2, 3…).

*Username of analyst who created WO.

Figure 1. Recommended Plate Map



Instrument Parameters



Data Regression

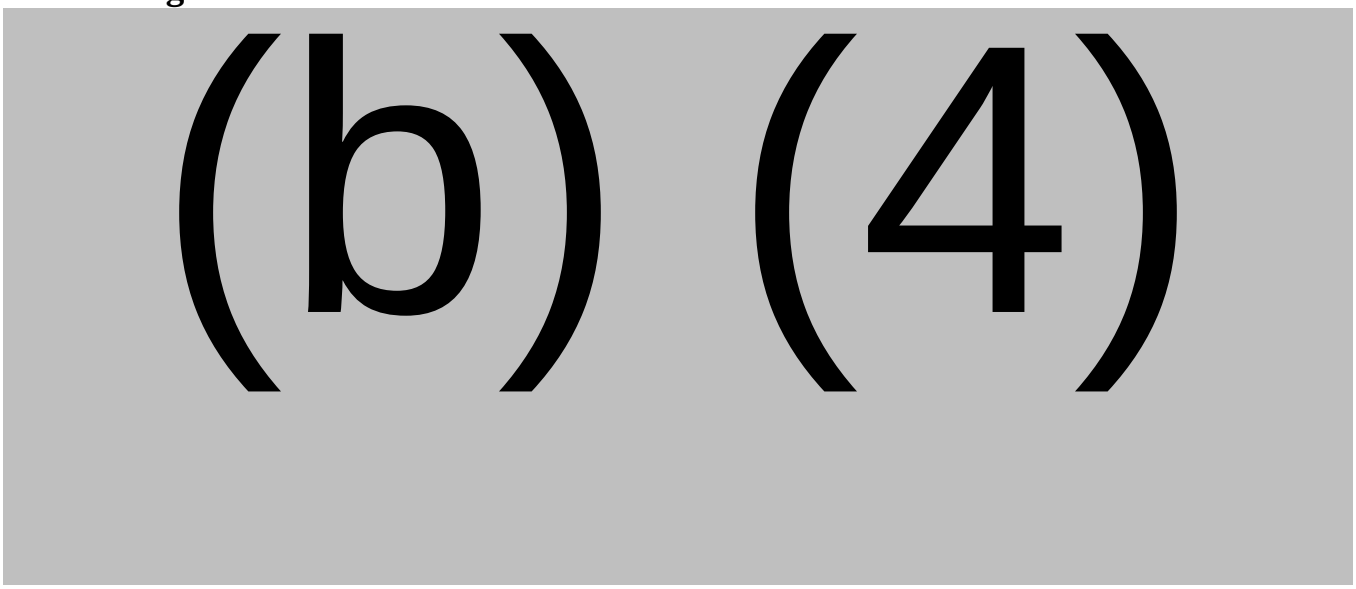


Table 4. Standard Curve Concentration

Level	Final Dilution	Concentration (AU/mL)
Neat	N/A	500
STD 1	1:500	1
STD 2	1:1250	0.4
STD 3	1:3125	0.16
STD 4	1:7812.5	0.064
STD 5	1:19531.25	0.0256
STD 6	1:48828.125	0.01024
STD 7	1:122070.3125	0.004096
STD 8	1:305175.78125	0.0016384
STD 9	1:762939.453125	0.00065536
STD 10	1:1907348.6328125	0.000262144
STD 11	1:4768371.58203125	0.0001048576

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:

Requirement #	Dilution (Dil_1)	Outcome
(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 in 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, and the date and signature of the performing analyst must be recorded.
3. The batch sheet and assay report must be reviewed by another qualified analyst or quality control reviewer 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 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] PPD Statistical Report: *Qualification of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum*, ROQP2, 19Jun2020.

[2] VSDVAC 58 *An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum, V1.01*, 22Jul2020.

[3] PPD Statistical Report: *Validation of An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum*, RPPF, TBD.

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

[5] 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

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

[7] VSDVACR 6 *SARS-CoV-2 ELISA Generic Reagent Preparation Procedure, V1.00, 17Jul2020.*

[8] Rockx B, Corti D, Donaldson E, Sheahan T, Stadler K, Lanzavecchia A, Baric R. Structural basis for potent cross-neutralizing human monoclonal antibody protection against lethal human and zoonotic Severe Acute Respiratory Syndrome Coronavirus challenge. J Virol. 2008 Apr;82(7):3220-35. doi: 10.1128/JVI.02377-07. Epub 2008 Jan 16. PMID:18199635

[9] ter Meulen et al. 2006. Human Monoclonal Antibody Combination against SARS Coronavirus: Synergy and Coverage of Escape Mutants PLoS Med. Jul; 3(7): e237 PMID:16796401

[10] Tian et al., 2020. Potent binding of 2019 novel coronavirus spike protein by SARS coronavirus-specific human monoclonal antibody. bioRxiv posted online 28Jan2020. doi: http://dx.doi.org/10.1101/2020.01.28.923011

Method History

Updates from Version 0.00 to 0.01 was modified by Jack Hester on 27Aug2020 to include the following updates:

1. Version updated from 0.00 to 0.01.
2. Critical Reagents, (b) (4) (b) (4)
3. Reagent Preparation: Added note to indicate use of reagents from VSDVAC 58 and VSDVACR 6.
4. Assay Acceptance Criteria: Updated with (b) (4)
5. References: Added references to VSDVAC 13 and VSDVACR 6.
6. Appendix 1, Table A: Updated QC dilutions to reflect actual QC dilutions used.
7. Appendix 1, Table B: Updated (b) (4) map to align with automated process.

Version 1.00
Draft Method VSDVAC 65 Version 0.01 was modified by Christopher Hammond on 13Oct2020 to include the following updates:

1. Version updated from 0.01 to 1.00
2. Assay Acceptance Criteria: Updated (b) (4) per the statistical validation report.
3. Test Flags: Updated LLOQ and ULOQ.
4. Other minor grammatical and formatting.
5. References: Replaced reference to validation plan to the statistical report.
6. Appendix 1, Program Overview for Standard, Quality Control and Unknown Sample Preparation, Item 5: Required RS and QCS barcode formats updated.
7. Batch Sheets & Run Names and Assay Procedure / Assay Procedure (b) (4) Updated format of Run Naming convention / plate reader file naming convention.

Version 1.01
Method VSDVAC 65 Version 1.00 was modified by Jack Hester on 29Oct2020 to include the following updates:

1. Version updated from 1.00 to 1.01
2. In Batch Sheet and Run Names section, updated naming convention of the work order run name to remove "_Plate # (1, 2, 3…)"
3. In step 15 of Procedure, updated naming convention of the plate reader data file to remove "_Plate # (i.e. 1, 2, 3…)"

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 VSDVAC65.

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 VSDVAC65, An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum

Critical Reagents:

2. Assay reagents and chemicals per method VSDVAC65, An ELISA Method for the Detection of IgG Specific to SARS-CoV-2 Spike Protein in Human Serum

Materials and Equipment:

(b) (4)

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. Deep Well Reservoir, (b) (4), Part No.: (b) (4) or (b) (4), Part No.: (b) (4) (b) (4)

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

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

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

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

   3.7. 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

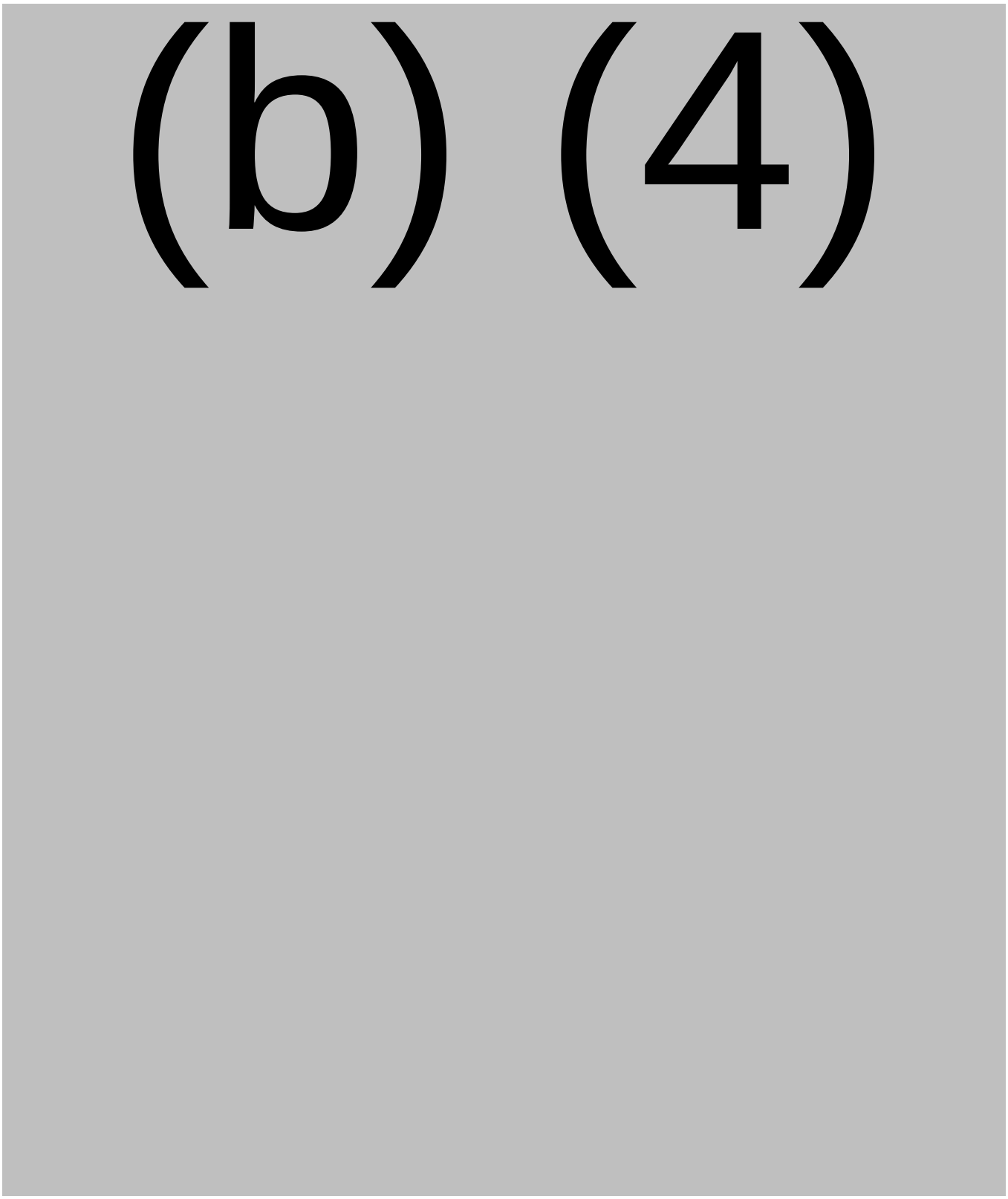


Table A. **Example Standard and Control** (b) (4)

(b) (4)

Table B. **Example Sample** (b) (4)

(b) (4)

Table C. **Example** (b) (4)

(b) (4)

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

(b) (4)