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1. HISTORICAL REVISION SUMMARY

Table 1. Chronological Summary of Previous Versions

Version #	Effective Date	Originator	Summary
1.0	Current	Guy Singh	New Document

2. PURPOSE

This procedure describes the method to quantitate IgG antibody levels to subunit 1 (S1) of the spike (S) protein of severe acute respiratory disease coronavirus 2 (SARS-CoV-2). The assay method measures IgG antibody levels for the S1 protein in human serum.

3. SCOPE

Personnel in Vaccine Research & Development (VRD) that perform the single-plex SARS-CoV-2 S1 IgG dLIA in regulated testing are required to follow this test method.

Table 2. Vaccine Research Functional Units

Functional Units	Location
Vaccine Research & Development	Pearl River

4. GLOSSARY

Table 3. Terms and Definitions

Term	Definition
Ag	Antigen
Assay Plate	The 96-well flat-bottom opaque plate used to perform the assay.
Batch	(b) (4)
Beads	A term interchangeable with microspheres.
(b) (4)	
COV19	Coronavirus Disease 2019
Dilution Plate, (b) (4)	(b) (4)
dLIA	Direct Luminex Immunoassay
Expiration Date	Expiration dates of reagents will be assigned based on the available stability data. Specific expiration dates may be recorded on reagent labels, or within laboratory notebooks or documentation workbooks referenced by the reagent label.
IgG	Immunoglobulin G
Labware LIMS	The Labware brand of a Laboratory Information Management System which is a database used to track specimens and their associated tests.
LNB	Laboratory Notebook

Table 3. Terms and Definitions

Term	Definition
Metadata	Assay-related information that is captured in LabWare LIMS. This includes information pertaining to reagents, equipment and instruments used to complete the assay.
MFI	Median Fluorescent Intensity
(b)(4)	
QCS	Quality Control Sample
QNS	Quantity Not Sufficient. The designation given when a specimen has insufficient volume to complete testing.
RT	Room temperature defined as the range from 18 to 25°C
S	Spike glycoprotein of severe acute respiratory disease coronavirus 2
S1	Subunit 1 of the Spike protein
SARS-CoV-2	Severe acute respiratory disease coronavirus 2
VR-DWB	Vaccine Research Document Workbook
WS	Working Stock

5. GENERAL

The single-plex SARS-CoV-2 S1 IgG dLIA measures SARS-Cov-2 S1 specific IgG antibodies present in human serum samples. This assay is based on the Luminex MagPlex® xMAP® technology platform. MagPlex® microspheres are superparamagnetic carboxylated xMAP microspheres and allow assay multiplexing and automation. The S1 protein is (b) (4) and attached to (b) (4) coated microspheres (VR-SOP-LC-11295).

S1 coated microspheres (b)(4) are added to the assay plates with appropriately diluted reference standard serum, QCS and unknown serum samples. The assay plates are incubated for (b)(4) hours at (b)(4) °C with shaking. After washing of non-bound components, (b)(4) anti-human IgG secondary antibody is added to the microsphere mixture and incubated for (b)(4) minutes at RT. The fluorescent protein coupled to the secondary antibody allows for measurement of the antibody bound to the antigen coated microspheres by the Bio-Plex reader. Signals are expressed as median fluorescent intensities (MFIs) and read against a reference standard. The magnitude of the fluorescent (b)(4) signal is proportional to the amount of Ag-specific antibodies present in the sample.

6. RESPONSIBILITIES

- All personnel and positions referred to in this procedure are considered to have an alternate.
- An alternate must ensure that they are trained and knowledgeable with the process.

Table 4. Roles and Responsibilities

Role	Responsibilities
Analyst	• Performs the test method following the procedures described in this SOP. • Completes documentation of the work performed and any deviations that occurred. • Follows applicable departmental SOPs regarding safety, equipment use and documentation practices. • Initiates LDRs. • Notifies supervisor of any atypical events.
Supervisor or designee	• Reviews and signs documentation. • Ensures that analysts have been trained on appropriate SOPs. • Ensures that equipment and instruments have been properly calibrated and maintained. • Ensures LDRs are initiated and properly investigated.

7. SAFETY

- Biosafety level 2 precautions should be taken when handling human sera. For current guidelines refer to the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories Manual (BMBL), 5th Ed., 2009: http://www.cdc.gov/biosafety/publications/bmbl5/index.htm.

- Personnel must be trained on current laboratory safety procedures and made aware of the Safety Data Sheets (SDS) for chemicals being used, before performing this procedure. For SDS information, refer to link at http:/ (b) (4)

- All laboratory work is to be carried out in compliance with Pfizer safety policies. For general laboratory safety guidelines consult the Pearl River Environmental Health and Safety Laboratory Manual located at:
  http:// (b) (4)

  Lab coats, safety glasses and disposable gloves must be worn at all times when handling human specimens.

8. MATERIALS/EQUIPMENT

All equipment and materials used in regulated testing must conform to current departmental guidelines for calibration, maintenance and qualification.

8.1. Equipment

- Bio-Plex (b) (4) Reader, Bio-Rad Cat. # (b) (4) or equivalent
- Platform Shaker, (b) (4) or equivalent
- Scientific freezers, -80°C ±15°C and -20°C -15/+5°C
- Scientific refrigerator, 2 to 8°C

- (b) (4) (magnetic bead) plate washer or equivalent
- Alarm timer
- Single channel pipettes, various sizes
- Multi-channel pipettes, manual and electronic, various sizes
- (b) (4) Pipetting Device, (b) (4) or equivalent
- Vortex mixer
- Nutating mixer

8.2. Materials

- (b) (4)
- (b) (4)
- 96-well flat bottom opaque white plates, (b) (4) or equivalent
- Polypropylene pipette tips, various sizes
- Sterile disposable serological plastic pipettes, various sizes
- Disposable polypropylene tubes or containers, various sizes
- Disposable light protective amber polypropylene centrifuge tubes or containers, various sizes
- Aluminum sealing tape for 96 well plates
- Reagent Reservoirs, various sizes
- Polypropylene micro snap cap tubes
- Tube racks
- Magnetic separators
- Biohazardous waste receptacle
- Biohazard sharps waste containers

8.3. Reagents

8.3.1. Standard and QCS, Prepared as Described in VR-RGR-RS-10747

- Standard reference serum: COVID19 IgG STD
- QCS
  - QC1: COVID19 IgG QC1
  - QC2: COVID19 IgG QC2
  - QC3: COVID19 IgG QC3

8.3.2. Microspheres

- Preparation of antigen coated microspheres is described in VR-SOP-LC-11295. Coated microspheres are (b) (4) (b) (4)

8.3.3. Secondary Antibody

- (b)(4) anti-human IgG, (b) (4) (store at 2 to 8°C).

8.3.4. (b) (4)

- (b) (4)

8.3.5. Buffers

(b) (4)

9. PROCEDURE

- (b) (4) secondary antibodies are added to the plates, incubated as described below and plates are read on Bio-Plex (b) (4) instruments.

- Any step of this procedure may be performed manually, or by a qualified automated method. Follow VR-SOP-LC-11186 for the automated serum sample preparation by a (b)(4) robotic unit.

(b) (4)

- Incubator temperatures stated in this method are temperature ranges as follows: 5°C = 2-8°C; room temperature = 18-25°C.

- Bring all reagents and supplies to room temperature before use in all of the following steps. All assay steps are to be performed at room temperature unless otherwise specified.

- Movement of specimens from and back to cold storage is logged using LIMS following VR-SOP-LC-10774. For batches in LIMS the reagent, equipment and incubation information will be captured as metadata in LIMS. Raw data will be uploaded to LIMS.

- VR-TM-10293-FM01 is the assay completion worksheet used when sample preparation is performed either manually or by automated methods. An assay completion worksheet is not required for fully electronic/paperless batches where metadata is recorded in LIMS by a (b)(4) robotic unit or manually.

- VR-TM-10293-FM02 is the worksheet used to prepare sample dilution plates manually. An assay completion worksheet is not required for fully electronic/paperless batches where metadata is recorded in LIMS.

- Refer to VR-SOP-LC-10601 for instruction on maintaining the documentation of the data package for paper-based documentation, if required. A Chain of Custody form is not required for fully electronic/paperless batches where all metadata and raw data is recorded in/uploaded to LIMS.

- Raw data CSV files will be transferred to the appropriate secure network drive upon assay completion. Any other file types do not need to be transferred, and raw data is not required to be printed.

9.1. Preparation of Single-plex S1 Microspheres

The preparation of S1 antigen coated microspheres is described in VR-SOP-LC-11295. (b) (4)

(b) (4)

9.2. Preparation of Sample Dilution Plates

In the single-plex SARS-CoV-2 S1 IgG dLIA, each assay plate is derived from a corresponding dilution plate. Each dilution plate contains three QCS, serially-diluted reference standard serum, blank and up to (b)(4) test samples. For initial testing, the (b)(4) dilutions of test sera are (b)(4). Additional dilutions can be evaluated if needed. A dilution diagram of the plate is in Figure 1.

9.2.1. Robotic Preparation of Dilution Plates

Refer to VR-SOP-LC-11186 for sample and dilution plate preparation using the (b)(4) (b)(4) robot. (b) (4)

9.2.2. Manual Preparation of Dilution Plates

(b) (4)

4. See Figure 1 for the Dilution Plate Diagram.

Figure 1. Dilution Plate Diagram

(b) (4)

9.2.2.1. Reference Standard

(b) (4)

9.2.2.2. Unknown Specimen and QCS

(b) (4)

9.2.2.3. Samples Requiring Pre-dilutions

(b) (4)

9.2.2.4. Dilution Plate Completion

(b) (4)

9.3. Preparation of Assay Plates

9.3.1. Robotic Preparation of Assay Plates

Refer to VR-SOP-LC-11186 for assay plate preparation using the (b)(4) Robot. Continue to Section 9.4 to begin assay completion steps.

9.3.2. Manual Preparation of Assay Plates

(b) (4)

9.4. Primary Incubation of S1 Coated Microspheres with Sera

(b) (4)

9.5. Preparation of (b) (4) Plate Washer

1. Prepare (b) (4) plate washer for use as described in VR-SOP-FE-10887. Record the activities, as required, in the appropriate equipment logbook.

2. Ensure the Magnetic Bead Plate Adaptor is inserted for magnetic bead plate wash.

9.6. PE-conjugated Secondary Antibody Preparation and Incubation

(b) (4)

(b) (4)

9.7. Assay Completion

(b) (4)

9.8. Reading Assay Plates on the Bio-Plex (b) (4) Instrument

1. Prepare the Bio-Plex reader for use as described in VR-SOP-FE-10111. Record the activities, as required in the appropriate equipment logbook.

(b) (4)

(b) (4)

9.9. Rereading of Assay Plates Due to Errors

(b) (4)

6. Prepare assay plates for disposal by emptying the liquid off the plates. Discard empty assay plates in a Biohazard waste receptacle.

9.10. Electronic Documentation

- VR-TM-10293-FM01 is the assay completion worksheet used when sample preparation is performed either manually or by automated methods. An assay completion worksheet is not required for fully electronic/paperless batches where metadata is recorded in LIMS.

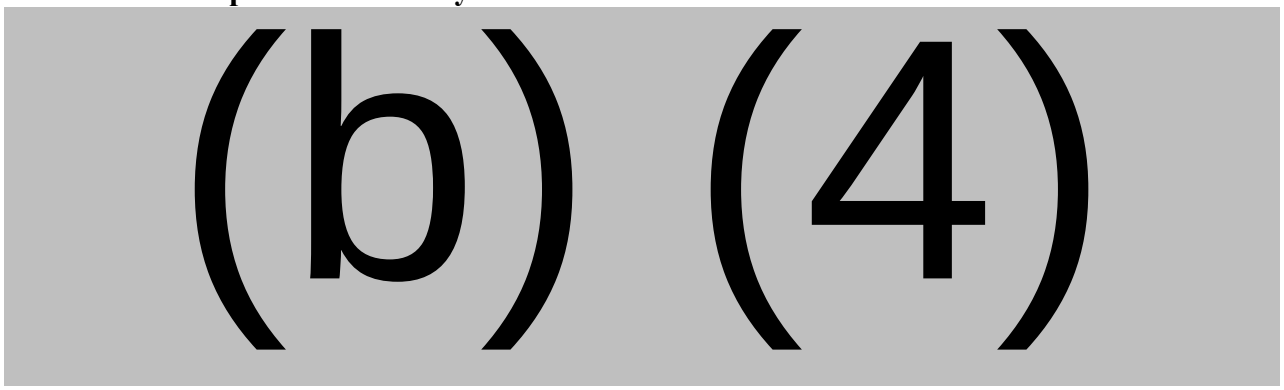
- VR-TM-10293-FM02 is the manual sample preparation worksheet used when automated sample preparation is not performed. An assay completion worksheet is not required for fully electronic/paperless batches where metadata is recorded in LIMS.

- Refer to VR-SOP-LC-10601 for instruction on maintaining the documentation of the data package, if required. A Chain of Custody form is not required for fully electronic/paperless batches where all metadata and raw data is recorded in/uploaded to LIMS.

- For batches created in LIMS the reagents, equipment, and incubation information will be captured as metadata in LIMS, therefore those sections can be N/A if the worksheets are used, and the raw data will be uploaded to LIMS.

- Raw data CSV files will be transferred to the appropriate secure network drive upon assay completion. Any other file types do not need to be transferred, and raw data is not required to be printed.

9.11. Data Export and Primary Data Review



3. For LIMS runs, upload the data files back to LabWare LIMS and verify that all equipment and reagent information for assay completion steps is accurately completed.

4. Complete the Data Package chain of custody form, VR-SOP-LC-10601-FM14, if required.

5. Review assay documentation and/or LIMS metadata entry for accuracy and completeness prior to submitting documentation for secondary review.

NOTE: If technical problems occurred with the assay plate (plates are spilled or dropped, etc.) (b) (4)

10. INTERPRETATION OF RESULTS

A qualified data reviewer analyzes the data according to pre-specified acceptance criteria, as described in VR-SOP-LC-11120.

11. REFERENCES

Table 5. General References

Document	Title
VR-SOP-QU-10004	Documentation Practices in Vaccine Research
VR-GWP-10041	Metadata Entry Procedure in LabWare LIMS for Luminex Immunoassays
VR-SOP-QU-10726	Laboratory Deviation Report
VR-SOP-LC-10788	Labeling and Traceability of Reagents and Solutions

Table 6. Form References

Form	Title
VR-TM-10293-FM01	Assay Completion Worksheet for SARS-CoV-2 S1 IgG dLIA
VR-TM-10293-FM02	Manual Sample Preparation Worksheet for SARS-CoV-2 S1 IgG dLIA
VR-SOP-LC-10601-FM14	Generic Luminex Assay Data Package Chain of Custody

Table 7. Specific References

Document	Title
VR-SOP- LC-10222	Procedure for Making Sample Predilutions with (b)(4) Robot
VR-SOP-LC-10627	Preparation of Assay Buffers and Solutions for (b) (4) Assays
VR-SOP-LC-11114	Data Review Procedures for Direct Luminex Immunoassays Using Input Files or LabWare v5 LIMS
VR-SOP-LC-11120	Data Review Procedures for Direct Luminex Immunoassays in LIMS v6.
VR-SOP-LC-11186	Running the (b)(4) Method Using (b)(4) Robot
VR-SOP-LC-11190	Standard Operating Procedure for Running Predilute Sample Method using (b)(4) Robot
VR-SOP-LC-11295	Preparation of and Evaluation of (b) (4) Microspheres for use in Direct Luminex Assays
VR-RGR-RS-10747	Preparation of Reference Standard Serum and Quality Control Samples for SARS-CoV-2 S1 and RBD Direct Luminex Immunoassays
VR-SOP-FE-10111	Bio-Plex System Operation and Maintenance
VR-SOP-LC-10586	Liquid Handling Techniques for Regulated Laboratory Procedures
VR-SOP-LC-10601	Data Package Chain of Custody
VR-SOP-LC-10774	Specimen Management Record Using LabWare LIMS
VR-SOP-FE-10887	Operation, Maintenance and Performance Verification of (b) (4) Microplate Washers

12. DOCUMENT VERSION MODIFICATIONS

12.1. CRIF Number: VR-CRIF-20-14611

Table 8. Detailed Changes

List detailed changes for document(s) Include section number(s) for each	List rationale for each change
New	NA

Document Approval Record

Document Name:	VR-TM-10293
Document Title:	Single-plex Luminex Assay for Quantitation of IgG Antibodies to SARS -CoV-2S1 Protein in Human Serum

Signed By:	Date(GMT)	Signing Capacity
(b) (6)	08-Jul-2020 12:22:38	Final Approval
Kalina, Warren	08-Jul-2020 12:27:54	Manager Approval
Pavliakova, Danka	08-Jul-2020 12:34:00	Manager Approval
(b) (6)	08-Jul-2020 14:24:01	Quality Assurance Approval
Singh, Guyanand	09-Jul-2020 11:49:56	Author Approval