



**Title:** Qualification Report for a Single-plex Direct Luminex Assay (dLIA) for Quantitation of IgG Antibodies to SARS-CoV-2 RBD Protein in Human Sera

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Pearl River, NY**

**Title:** Qualification Report for a Single-plex Direct Luminex Assay (dLIA) for Quantitation of IgG Antibodies to SARS-CoV-2 RBD Protein in Human Sera

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**Title:** Qualification Report for a Single-plex Direct Luminex Assay (dLIA) for Quantitation of IgG Antibodies to SARS-CoV-2 RBD Protein in Human Sera

**SYNOPSIS**

This report documents the results of the statistical analyses from assay qualification experiments of a single-plex direct Luminex immunoassay for quantitation of human IgG antibodies to the receptor binding domain (RBD) of the spike (S) protein of severe acute respiratory disease coronavirus 2 (SARS-CoV-2). The performance parameters examined in the assay qualification were (b) (4) linearity, precision and standard curve bias. From these data, the assay range, lower limit of quantitation, and descriptive statistics of assay parameters were determined. Key qualification outcomes are highlighted in the table below.

**Key Qualification Outcomes**

Antigen	Assay Range Lower Bound Plate Well IgG Concentration (U/mL) <sup>a</sup>	Assay Range Upper Bound Plate Well IgG Concentration (U/mL) <sup>a</sup>	LLOQ <sup>b</sup> Sample Dilution Adjusted IgG Concentration (U/mL) <sup>a</sup>	Assay Precision (b) (4) <sup>c</sup>
COVRBD <sup>d</sup>	0.002301	0.128000	1.1505	(b) (4)

- a. Units/mL.
- b. Lower Limit of Quantitation.
- c. (b) (4)
- d. Receptor binding domain of the spike protein of severe acute respiratory disease coronavirus 2.

The data provided in this report support the qualification of the single-plex dLIA for quantitation of human IgG antibodies that bind to the RBD protein of SARS-CoV-2 and confirm that the assay is suitable for its intended use when performed in accordance with standard operating procedures by qualified personnel.

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**Title:** Qualification Report for a Single-plex Direct Luminex Assay (dLIA) for Quantitation of IgG Antibodies to SARS-CoV-2 RBD Protein in Human Sera

**Study Number:** NA

**Functional Area:** Vaccine Research and Development

**Test Facility:** Pfizer Vaccine Research, 401 North Middletown Road,  
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## 1. OBJECTIVES

This report summarizes the methodology, results, and statistical analysis of the assay qualification for a single-plex direct dLIA for quantitation of IgG antibodies to the RBD protein of SARS-CoV-2 in human serum, described in VR-TM-10294 (see [Section 9](#)) Assay qualification provides documented evidence that the assay, when performed in accordance with standard operating procedures (SOPs) by qualified personnel, is suitable for the intended use to quantify RBD-specific IgG antibody concentrations in human sera.

## 2. INTRODUCTION

The single-plex SARS-CoV-2 RBD IgG dLIA measures specific IgG antibodies binding to the receptor binding domain (RBD) of the SARS-CoV-2 spike protein (S). This assay is described in the test method, VR-TM-10294 (see [Section 9](#)), and is based on the Luminex MagPlex xMAP technology.

Magnetic, fluorescent Luminex microspheres are coated with (b)(4) according to VR-SOP-LC-11295<sup>1</sup> and then (b)(4) RBD protein. RBD coated microspheres are added to serum samples, diluted in assay buffer and incubated, with shaking, in a 96-well microtiter assay plates for (b)(4) hours at (b)(4) °C. Unbound assay components are removed by washing and a purified (b)(4) anti-human IgG secondary antibody is added to the reaction wells. The secondary antibody is incubated for (b)(4) minutes at (b)(4) °C. Unbound assay components are removed by washing and the reaction is read on a Bio-Plex reader. The fluorescent protein coupled to the secondary antibody allows measurement of the specific antibody bound to the antigen coated microspheres. Fluorescence is expressed as median fluorescent intensities (MFI), and the assay results are calculated against a reference standard with arbitrary assigned concentration of 100.00 Units/mL. All sera are tested in duplicate from independently generated samples. Test samples are initially tested at (b)(4) dilutions, (b)(4) dilutions are used to increase the likelihood that at least one result for any sample will fall within the useable range of the standard curve. Using acceptance criteria established in this report, failed plates and samples may be repeated.

The final assay results are expressed as the geometric mean concentration (GMC) of all sample dilutions that produced a valid assay result within the assay range. GMC results that are below the Lower Limit of Quantitation (LLOQ) are reported as Below Limit of Quantitation (BLQ).

### 3. GLOSSARY

**Table 1. Terms and Definitions**

Term	Definition
Assay Range	Range of (b) (4) antibody concentrations that can be measured in the assay plate well that have (b) (4) linearity, precision, and standard curve bias within the limits described below. The assay range is generated from the most conservative lower and upper well concentration limits based on (b) (4) linearity, precision, and standard curve bias.
Beads	Luminex Microspheres
BLQ	Below Limit of Quantitation
(b) (4)	
CDAD	Clinical and Diagnostic Assay Development
COVID-19	Coronavirus Disease 2019
(b) (4)	
dLIA	Direct Luminex ImmunoAssay
(b) (4)	
GMC	Geometric Mean Concentration
HCID	High-throughput Clinical Immunoassays & Diagnostics
IgG	Immunoglobulin G
LIMS	Laboratory Information Management System
LLOQ	Lower Limit of Quantitation - the lowest dilution-adjusted sample concentration that can be determined with precision (b) (4) and falls within the linear portion of the assay range.
MFI	Median Fluorescent Intensity
mL	Milliliter
NHP	Non-human primate
QA	Quality Assurance
QCS	Quality Control Sample
(b)(4)	
RBD	Receptor binding domain
(b) (4)	
RT	Room Temperature 18-25°C
S	Spike glycoprotein of severe acute respiratory disease coronavirus 2
SARS-CoV-2	Severe acute respiratory disease coronavirus 2
Sample Concentration	(b) (4) antibody concentration calculated as the (b) (4) from the sample (b) (4) that fall within the assay range and the lower and upper parameter limits of the standard curve that is run on the same assay plate.

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**Table 1. Terms and Definitions**

Term	Definition
Sample Range	Range of (b) (4) sample concentrations that can be quantified without (b) (4) (b) (4) The sample range is bounded by the LLOQ and ULOQ. Results below LLOQ are reported as BLQ. (b) (4)
SOP	Standard Operating Procedure
TM	Test Method
ULOQ	Upper Limit of Quantitation - the highest dilution-adjusted sample concentration that can be determined with precision (b) (4) and falls within the linear portion of the assay range (b) (4)
VRD	Vaccine Research and Development
Well concentration	(b) (4) antibody concentrations in the assay plate wells

## 4. EXPERIMENTAL OUTLINE

### 4.1. Materials and Methods

Unique reagents prepared for this assessment are listed below, and all other materials are described in the SOPs referenced in Table 2. Specific details regarding reagent catalog and lot numbers and expiration, as well as the instrument identification numbers and maintenance details were documented in the assay worksheets within data packages.

#### 4.1.1. Methods

**Table 2. SOPs and Robotic Methods within Scope of this Protocol**

SOP Number	Work Described in SOP	Robotic Method Name
VR-TM-10294 (see Section 9)	Test method	N/A
VR-SOP-LC-11295 <sup>1</sup>	Bead coating method	N/A
VR-SOP-LC-11186 <sup>2</sup>	Sample preparation using (b)(4) robot	(b)(4)
VR-SOP-LC-10627 <sup>4</sup>	Preparation of buffers	N/A

#### 4.1.2. Critical Reagents

The critical reagents, including antigen-coated microspheres (beads), reference standard, QCS and PE-labeled secondary antibodies used in this assay qualification are listed in Table 3.

The reference standard serum is a (b) (4) human (b) (4) sera (COVID-19 (b) (4) sera drawn at least (b)(4) days after PCR-confirmed diagnosis from subjects (b) (4) years of age) and assigned an arbitrary concentration of 100.00 U/mL of IgG antibodies to the RBD antigen.

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**Table 3. List of Critical Reagents**

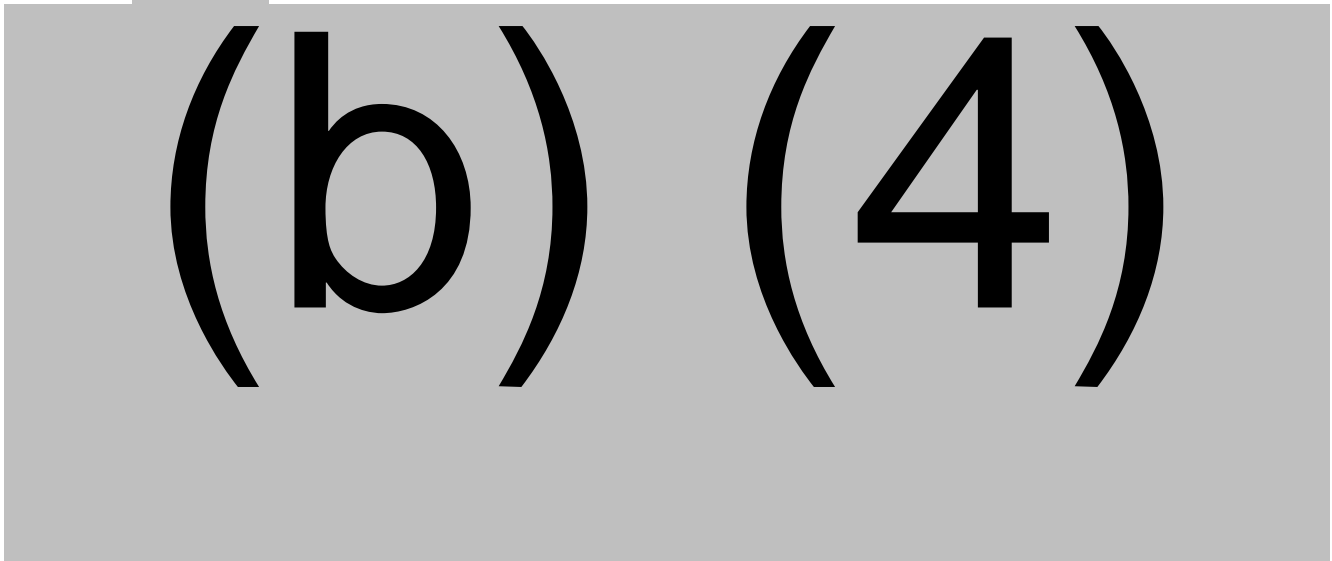
	(b) (4)		
RBD coated microspheres		(b) (4)	
Secondary antibody		(b) (4)	
	(b) (4)		
Reference Standard Serum COVID19 IgG STD <sup>a</sup>	(b) (4) PCR confirmed Covid-19 sera (b) (4)	(b) (4)	
QCS1 COVID19 IgG QC1 <sup>b</sup>	(b) (4)	(b) (4)	
QCS2 COVID19 IgG QC2 <sup>c</sup>	(b) (4)	(b) (4)	
QCS3 COVID19 IgG QC3 <sup>d</sup>	(b) (4)	(b) (4)	

- a. Assay Standard.
- b. Assay Quality control serum 1.
- c. Assay Quality control serum 2.
- d. Assay Quality control serum 3.

**4.2. Experimental Design**

The following sections describe the experimental designs for evaluating the (b) (4) linearity, precision and standard curves.

**4.2.1. (b) (4) Linearity**



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#### 4.2.2. Precision

Precision describes the closeness of measurements for a sample tested multiple times. Precision is a measure of assay variability that contains both repeatability and intermediate precision. Repeatability measures the assay variability, usually within one assay run, whereas intermediate precision measures the variability within the same laboratory while taking into account relevant sources of variation due to operating conditions (eg, different analysts, time, reagent lots).

- The precision of the assay was examined using a panel of <sup>(b) (4)</sup> COVID-19 <sup>(b) (4)</sup> sera with IgG antibody concentrations to the RBD protein intended to span the expected assay range and <sup>(b) (4)</sup> pre-pandemic human serum samples that have low or no specific IgG to the RBD protein for a total of <sup>(b) (4)</sup> samples. Refer to Supportive Table 12.2 for the precision samples used.
- All samples were tested in the assay at <sup>(b) (4)</sup> dilutions performed by a single <sup>(b) (4)</sup> workstation, using the methods developed for clinical testing as described in VR-SOP-LC-11186.<sup>2</sup> Refer to Supportive Figure 11.2 for the assay plate layout used for the precision experiments.
- Precision measurements of the <sup>(b) (4)</sup> samples were performed following VR-TM-10294 (see Section 9) over <sup>(b) (4)</sup>  
<sup>(b) (4)</sup> Results were analyzed as described in Section 5.2.2 and Section 5.2.5.

#### 4.2.3. Standard Curve Bias

<sup>(b) (4)</sup> <sup>(4)</sup>

### 5. STATISTICAL METHODS

#### 5.1. Sample Results

<sup>(b) (4)</sup> <sup>(4)</sup>

#### 5.2. Statistical Analyses

<sup>(b) (4)</sup> <sup>(4)</sup>

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**5.2.1. (b) (4) Linearity**

(b) (4)

**5.2.2. Precision**

(b) (4)

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(b) (4)

### 5.2.3. Standard Curve Bias

(b) (4)

### 5.2.4. Assay Range

(b) (4)

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### 5.2.5. Assay Precision (Intermediate Precision)

(b) (4)

### 5.2.6. Sample Quantitation Range

The quantitation limits are defined as the sample concentrations that are between the LLOQ and the ULOQ.

#### 5.2.6.1. Lower Limit of Quantitation

The lower limit of quantitation (LLOQ) is the lowest sample concentration in the assay that can be determined with precision (b) (4) and falls within the linear portion of the assay range. (b) (4)

(b) (4)

### 5.2.6.2. Upper Limit of Quantitation

The upper limit of quantitation (ULOQ) is the highest sample concentration that can be determined with precision (b) (4) and falls into the linear assay range. (b) (4)

### 5.2.7. Assay Run Performance

(b) (4)

#### 5.2.7.1. (b) (4)

(b) (4)

#### 5.2.7.2. Standard Curve Parameters

(b) (4)

#### 5.2.7.3. Quality Control Samples

(b) (4)

## 6. RESULTS AND DISCUSSION

Qualification data used for the results of the analyses described herein are listed in VR-MQR-10212-ATT01 (see [Section 9](#)).

### 6.1. (b) (4) Linearity Evaluation

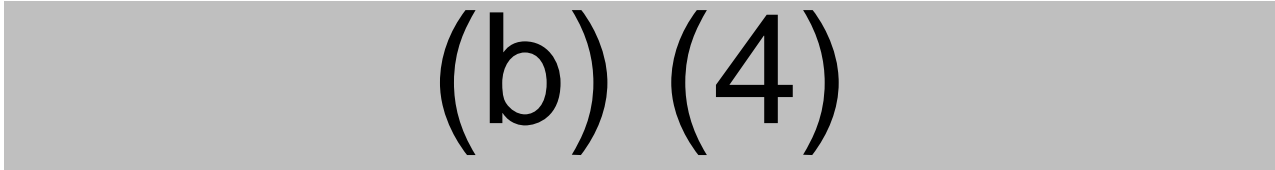


Figure 1. (b) (4) Linearity Plot for RBD IgG dLIA

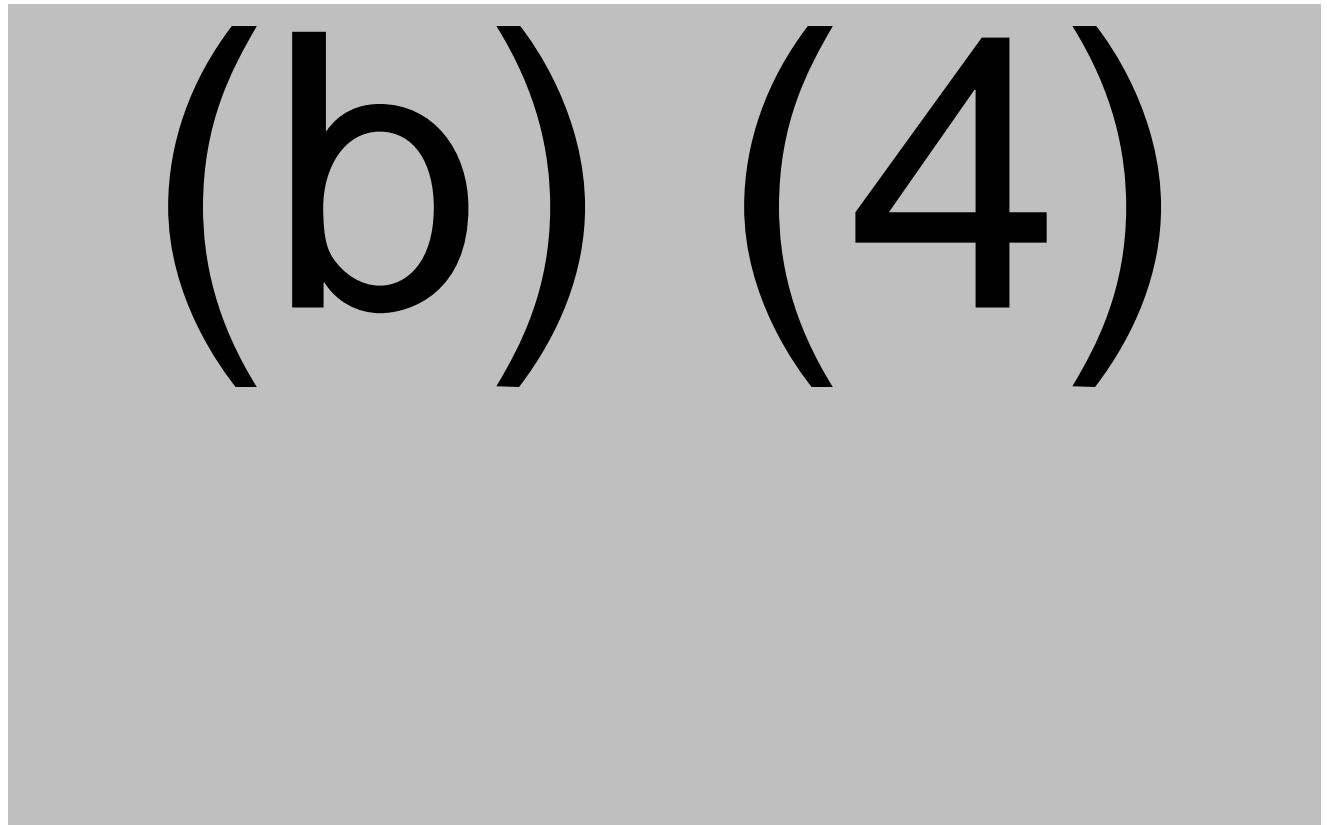
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Table 4. Well Concentration Range Based on (b) (4) Linearity Data

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## 6.2. Precision Evaluation

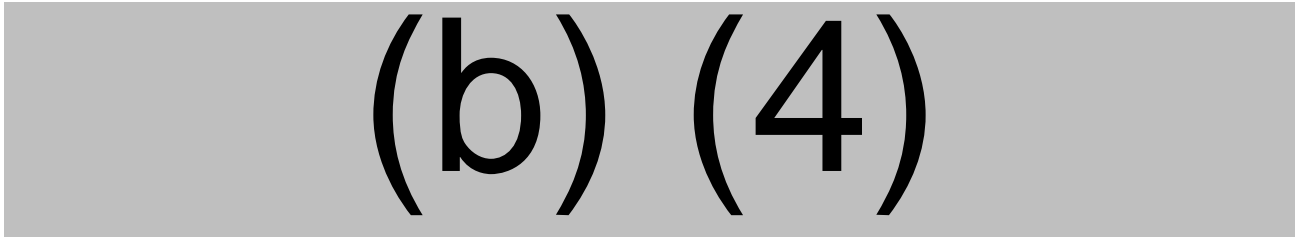


Figure 2. Precision Plot for RBD IgG dLIA

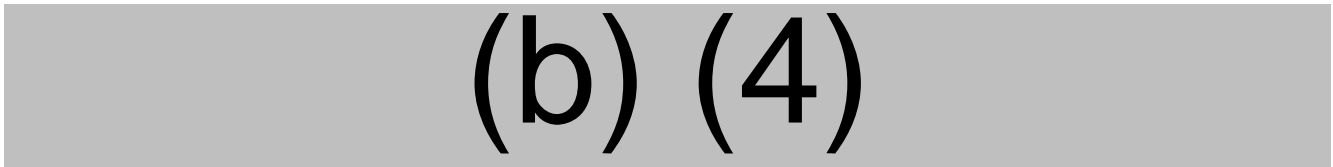
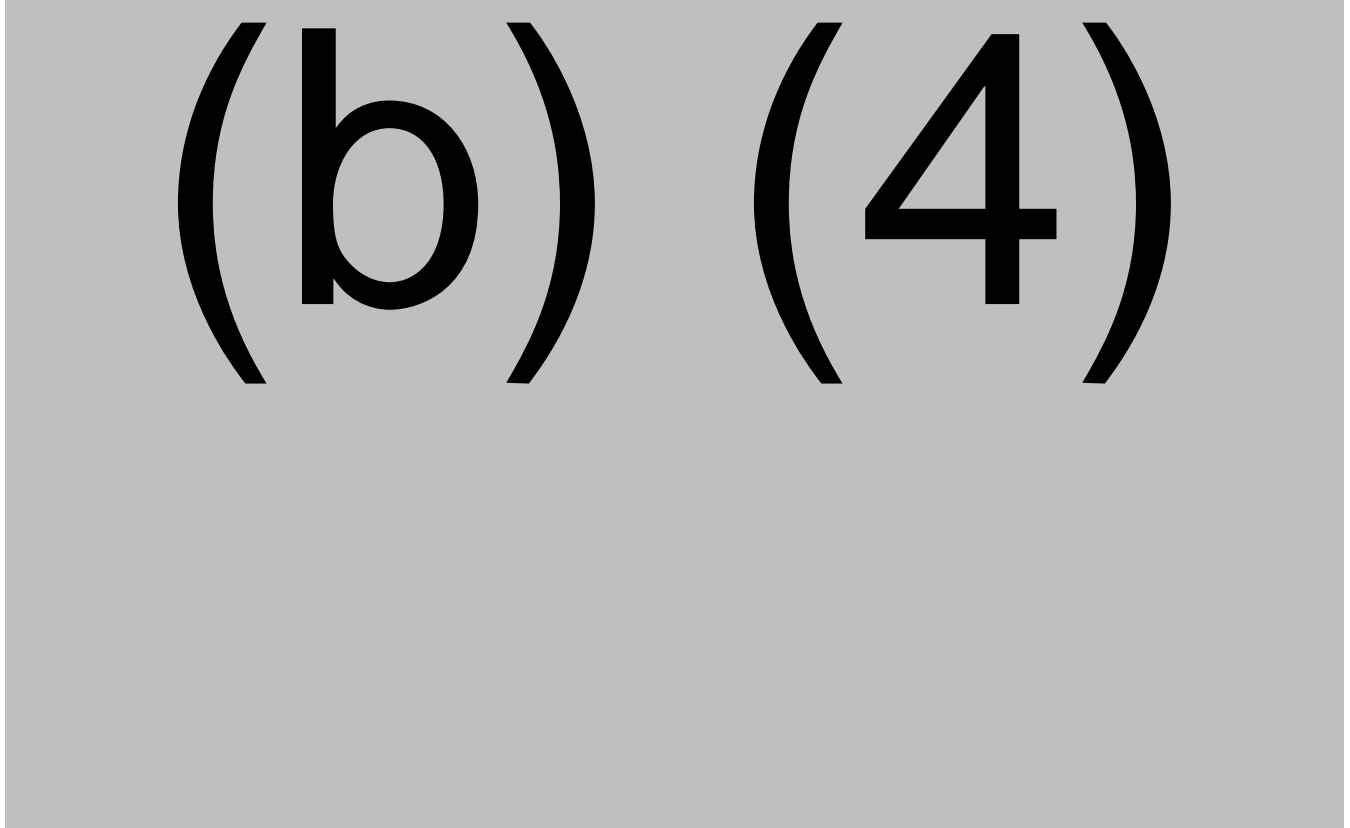
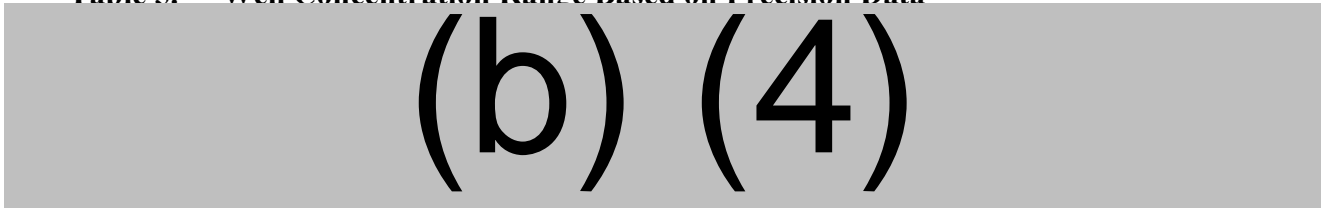


Table 5. Well Concentration Range Based on Precision Data



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### 6.3. Standard Curve Bias Evaluation

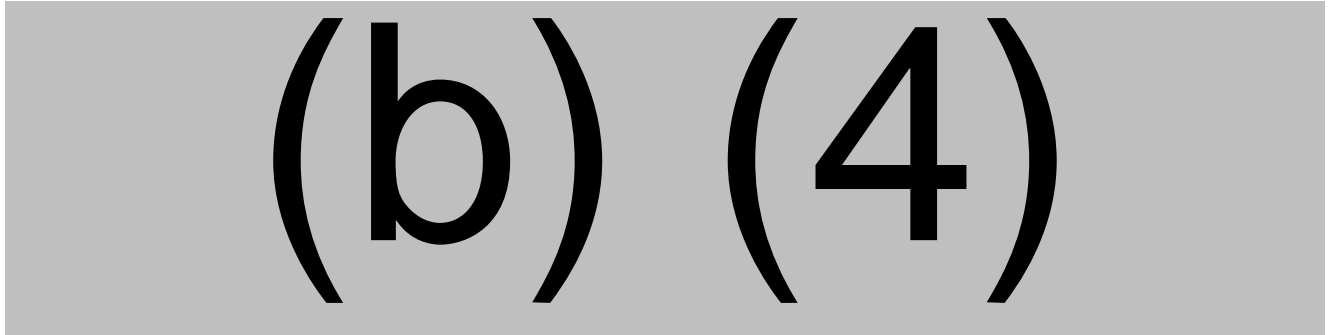
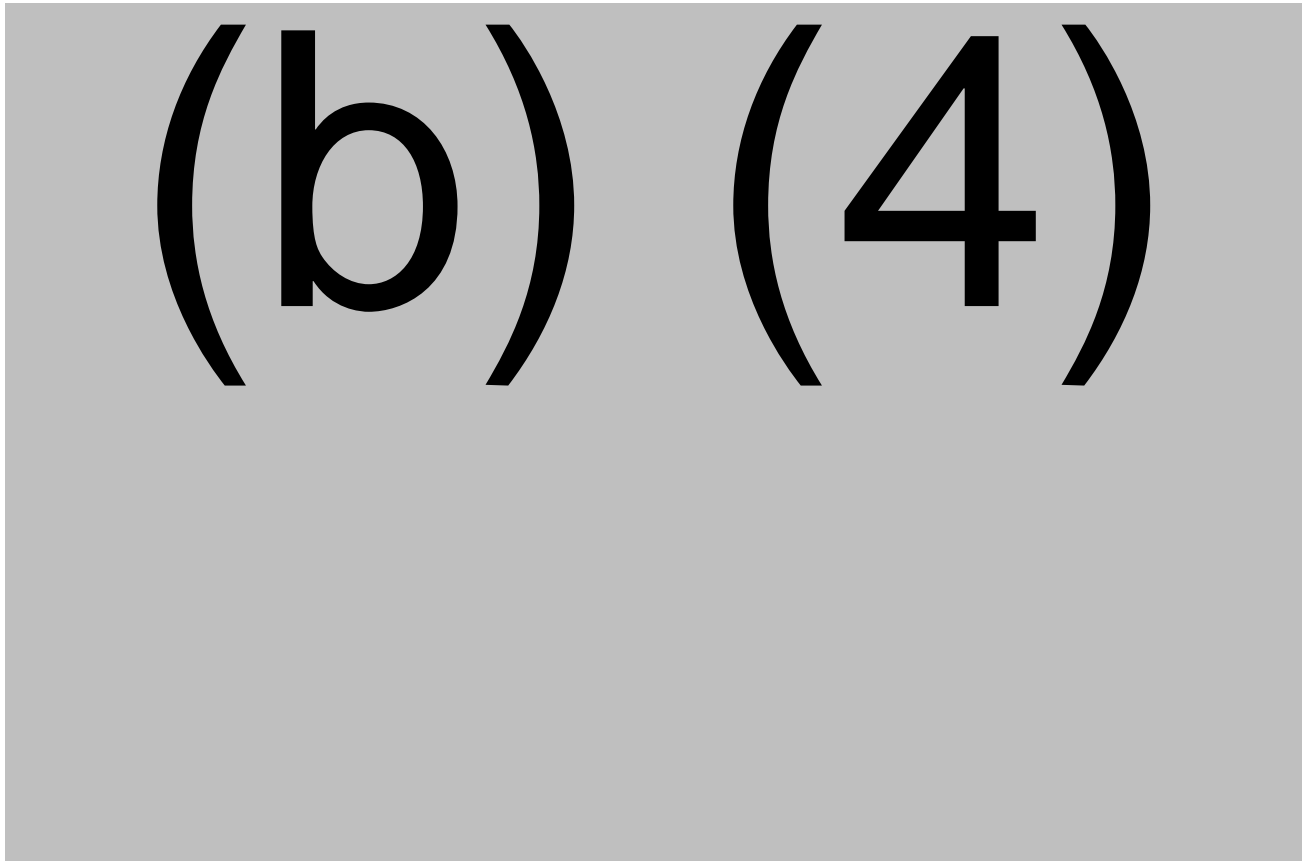


Figure 3. Standard Curve Bias Plot for RBD IgG dLIA



(b) (4)

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**Table 6. Well Concentration Range Based on Reference Standard Curve Bias**

(b) (4)

**6.4. Assay Range Based on (b) (4) Linearity, Precision, and Standard Curve Bias**

(b) (4)

**Table 7. Final IgG Assay Range – Well Concentration (Units/mL)**

Antigen	(b) (4) Linearity Range	Precision Range	Standard Curve Range	Final Assay Range	
				Lower	Upper
COVRBD <sup>a</sup>	(b) (4)			0.002301	0.128

a. Receptor binding domain of the spike protein of severe acute respiratory disease coronavirus 2.

**6.5. Assay Precision (Intermediate Precision)**

The intermediate precision of the assay was evaluated using the (b) (4) as described in [Section 5.2.5](#) and the results are summarized in Table 8.

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

(b) (4)

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(b) (4)

## 6.6. Sample Quantitation Range

### 6.6.1. Lower Limit of Quantitation

The LLOQ is the lowest sample IgG concentration in the assay that is precise (b) (4) and falls into the linear assay range. Unlike the lower limit of the assay range that is defined as a well concentration, the LLOQ is a dilution-adjusted sample concentration. As described in [Section 5.2.6.1](#), LLOQ is defined as the dilution-adjusted lower limit of the assay range. Table 9 lists the LLOQ value for the RBD IgG dLIA.

**Table 9. Lower Limit of Quantitation (LLOQ)**

Antigen	LLOQ (U/mL)
COVRBD <sup>a</sup>	1.1505

a. Receptor binding domain of the spike protein of severe acute respiratory disease coronavirus 2.

### 6.6.2. Upper Limit of Quantitation

(b) (4)

## 6.7. Assay Run Performance

(b) (4)

### 6.7.1. (b) (4)

(b) (4)

**Table 10.** (b) (4)

(b) (4)

### 6.7.2. Standard Curves

Descriptive statistics for the reference standard curves for RBD IgG dLIA are listed in Table 11. (b) (4)

(b) (4)

Table 11 presents suitability limits on standard curves which were calculated as described in [Section 5.2.7.2](#).

**Table 11. Descriptive Statistics for Standard Curve Parameters**

(b) (4)

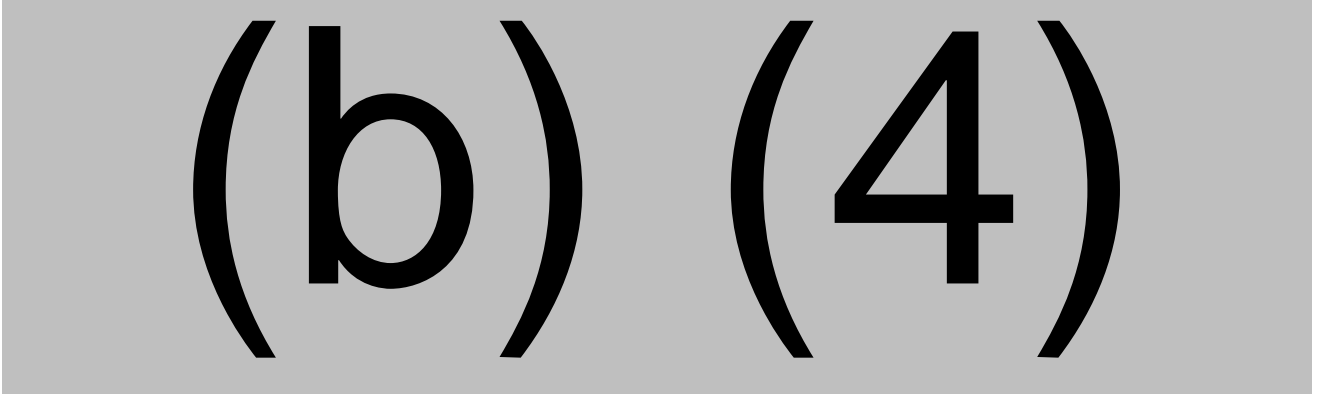
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### 6.7.3. Quality Control Samples

Descriptive statistics for the QCS samples from the qualification data for the RBD IgG dLIA are listed in Table 12. (b) (4)

(b) (4) Table 12 also shows QCS limits on the plates (refer to [Section 5.2.7.3](#)).

**Table 12. Descriptive Statistics for QCS**



## 7. CONCLUSION

The data provided in this report support the qualification of the single-plex SARS-CoV-2 RBD IgG dLIA for quantitating RBD specific IgG in human sera. The assay is suitable for the intended use when performed in accordance with standard operating procedures by qualified personnel. The assay limits established from the data generated in this qualification will be used to support assay suitability during clinical testing until such limits are refined in a future validation.

## 8. DEVIATIONS

NA

## 9. SUPPORTING DOCUMENTATION

1. [VR-TM-10294, Single-plex Luminex Assay for Quantitation of IgG Antibodies to SARS-CoV-2 RBD protein in Human Serum.](#)
2. [VR-MQR-10212-ATT01, Supportive Data for VR-MQR-10212.](#)
3. [VR-SOP-LC-11120, Data Review Procedures for Direct Luminex Immunoassays in LIMS v6.](#)

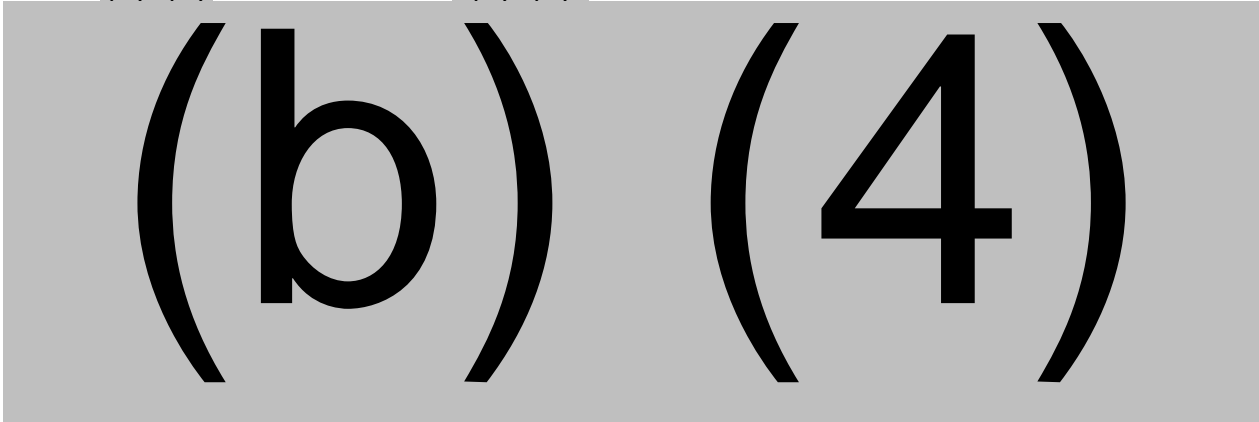
## 10. SUPPLEMENTAL INFORMATION

1. VR-SOP-LC-11295, Preparation and Evaluation of (b) (4) Coated Microspheres for use in Direct Luminex Assays.
2. VR-SOP-LC-11186, Standard Operating Procedure for Running (b)(4) Method Using (b)(4) Robot.
3. (b) (4)
4. VR-SOP-LC-10627, Preparation of Assay Buffers and Solution.
5. VR-SOP-FE-10111, BioPlex (b) (4) System Operation and Maintenance.
6. VR-DTN-11380, Planned Deviation from VR-SOP-FE-10111 Bio-Plex (b) (4) Operation and Maintenance.

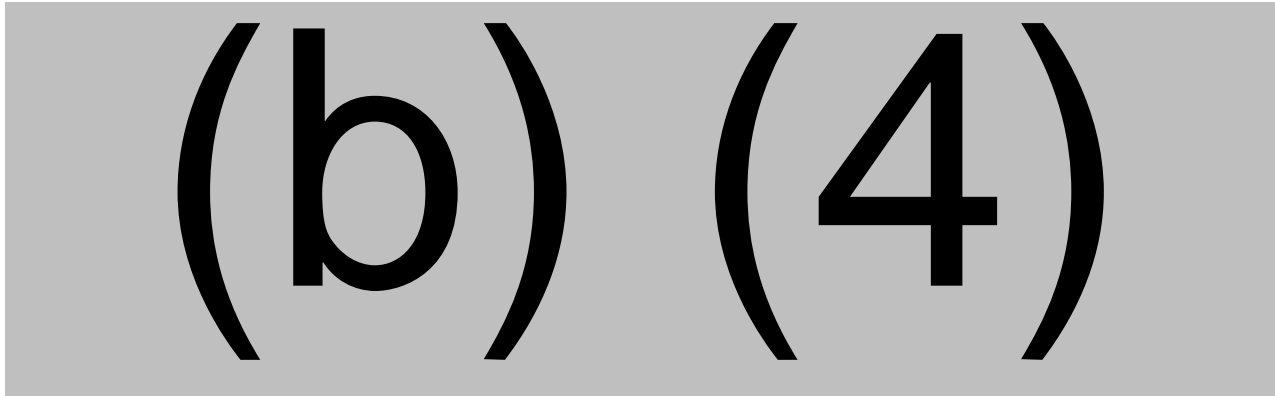
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### 11.1. (b) (4) Plate Layout for (b) (4) Linearity Experiments



### 11.2. SARS-CoV-2 RBD IgG dLIA Assay Plate Layout for Routine Testing



The assay plate layout in Supportive Figure 11.2 is used for routine testing. (b) (4)  
(b) (4)

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### 12.1. (b) (4) Linearity Samples

Sample Number	Sample ID
(b)	(4)

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### 12.2. Sample Panel for Precision Evaluation

Sample Number	ID	Sample Number	ID	Sample Number	ID	Sample Number	ID
(b) (4) (4)							

### 12.3. Precision of COVRBD Well Titers (b) (4) Sample

(b) (4)		Sample
(b) (4) (4)		

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**12.3. Precision of COVRBD Well Titers** (b) (4) **Sample**

(b) (4) (4)

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(b) (4)

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Kalina, Warren	16-Aug-2020 14:29:43	Manager Approval
Singh, Guyanand	17-Aug-2020 12:20:11	Author Approval
Pavliakova, Danka	17-Aug-2020 15:14:51	Manager Approval
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Tan, Charles	18-Aug-2020 17:52:25	Manager Approval
(b) (6)	18-Aug-2020 18:13:12	Business Line Approver
Cooper, David	18-Aug-2020 23:10:31	Final Approval

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