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

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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 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

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Assay Range Lower Bound Plate Well IgG Concentration (U/mL)</th>
<th>Assay Range Upper Bound Plate Well IgG Concentration (U/mL)</th>
<th>LLOQ(^b) Sample Dilution Adjusted IgG Concentration (U/mL)</th>
<th>Assay Precision ((b) (4))(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COVRBD(^d)</td>
<td>0.002301</td>
<td>0.128000</td>
<td>1.1505</td>
<td>(b) (4)</td>
</tr>
</tbody>
</table>

\(^a\) Units/mL.
\(^b\) Lower Limit of Quantitation.
\(^c\) 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, Pearl River, NY 10965

Study Initiation Date: 15 Apr 2020

Study Completion Date: 01 Jul 2020

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 RBD protein according to VR-SOP-LC-11295\(^1\) and then 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 hours at °C. Unbound assay components are removed by washing and a purified anti-human IgG secondary antibody is added to the reaction wells. The secondary antibody is incubated for minutes at °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 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

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Range</td>
<td>Range of antibody concentrations that can be measured in the assay plate well that have 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 linearity, precision, and standard curve bias.</td>
</tr>
<tr>
<td>Beads</td>
<td>Luminex Microspheres</td>
</tr>
<tr>
<td>BLQ</td>
<td>Below Limit of Quantitation</td>
</tr>
<tr>
<td>CDAD</td>
<td>Clinical and Diagnostic Assay Development</td>
</tr>
<tr>
<td>COVID-19</td>
<td>Coronavirus Disease 2019</td>
</tr>
<tr>
<td>dLIA</td>
<td>Direct Luminex ImmunoAssay</td>
</tr>
<tr>
<td>GMC</td>
<td>Geometric Mean Concentration</td>
</tr>
<tr>
<td>HCID</td>
<td>High-throughput Clinical Immunoassays &amp; Diagnostics</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>LIMS</td>
<td>Laboratory Information Management System</td>
</tr>
<tr>
<td>LLOQ</td>
<td>Lower Limit of Quantitation - the lowest dilution-adjusted sample concentration that can be determined with precision and falls within the linear portion of the assay range.</td>
</tr>
<tr>
<td>MFI</td>
<td>Median Fluorescent Intensity</td>
</tr>
<tr>
<td>mL</td>
<td>Milliliter</td>
</tr>
<tr>
<td>NHP</td>
<td>Non-human primate</td>
</tr>
<tr>
<td>QA</td>
<td>Quality Assurance</td>
</tr>
<tr>
<td>QCS</td>
<td>Quality Control Sample</td>
</tr>
<tr>
<td>RT</td>
<td>Room Temperature 18-25°C</td>
</tr>
<tr>
<td>S</td>
<td>Spike glycoprotein of severe acute respiratory disease coronavirus 2</td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>Severe acute respiratory disease coronavirus 2</td>
</tr>
<tr>
<td>Sample Concentration</td>
<td>antibody concentration calculated as the from the sample 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.</td>
</tr>
</tbody>
</table>
Table 1. Terms and Definitions

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Range</td>
<td>The sample range is bounded by the LLOQ and ULOQ. Results below LLOQ are</td>
</tr>
<tr>
<td></td>
<td>reported as BLQ.</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>ULOQ</td>
<td>Upper Limit of Quantitation - the highest dilution-adjusted sample</td>
</tr>
<tr>
<td></td>
<td>concentration that can be determined with precision and falls within the</td>
</tr>
<tr>
<td></td>
<td>linear portion of the assay range</td>
</tr>
<tr>
<td>VRD</td>
<td>Vaccine Research and Development</td>
</tr>
<tr>
<td>Well concentration</td>
<td>antibody concentrations in the assay plate wells</td>
</tr>
</tbody>
</table>

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

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

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 human sera drawn at least days after PCR-confirmed diagnosis from subjects years of age and assigned an arbitrary concentration of 100.00 U/mL of IgG antibodies to the RBD antigen.
Table 3. List of Critical Reagents

<table>
<thead>
<tr>
<th>RBD coated microspheres</th>
<th>[b] (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary antibody</td>
<td>[b] (4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reference Standard Serum</th>
<th>[b] (4)</th>
<th>PCR confirmed Covid-19 sera</th>
<th>[b] (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COVID19 IgG STD(a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QCS1</td>
<td>[b] (4)</td>
<td></td>
<td>[b] (4)</td>
</tr>
<tr>
<td>COVID19 IgG QC1(b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QCS2</td>
<td>[b] (4)</td>
<td></td>
<td>[b] (4)</td>
</tr>
<tr>
<td>COVID19 IgG QC2(c)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QCS3</td>
<td>[b] (4)</td>
<td></td>
<td>[b] (4)</td>
</tr>
<tr>
<td>COVID19 IgG QC3(d)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 linearity, precision and standard curves.

4.2.1. Linearity

(b) (4)
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 (e.g., different analysts, time, reagent lots).

- The precision of the assay was examined using a panel of COVID-19 sera with IgG antibody concentrations to the RBD protein intended to span the expected assay range and pre-pandemic human serum samples that have low or no specific IgG to the RBD protein for a total of samples. Refer to Supportive Table 12.2 for the precision samples used.

- All samples were tested in the assay at dilutions performed by a single workstation, using the methods developed for clinical testing as described in VR-SOP-LC-11186. Refer to Supportive Figure 11.2 for the assay plate layout used for the precision experiments.

- Precision measurements of the samples were performed following VR-TM-10294 (see Section 9) over Results were analyzed as described in Section 5.2.2 and Section 5.2.5.

4.2.3. Standard Curve Bias

5. STATISTICAL METHODS

5.1. Sample Results

5.2. Statistical Analyses
5.2.1. Linearity

(b) (4)

5.2.2. Precision

(b) (4)
5.2.3. Standard Curve Bias

5.2.4. Assay Range
5.2.5. Assay Precision (Intermediate Precision)

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 and falls within the linear portion of the assay range.
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 and falls into the linear assay range.

5.2.7. Assay Run Performance

5.2.7.1. (b) (4)

5.2.7.2. Standard Curve Parameters

5.2.7.3. Quality Control Samples
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. Linearity Evaluation

Figure 1. Linearity Plot for RBD IgG dLIA

Table 4. Well Concentration Range Based on Linearity Data
6.2. Precision Evaluation

Figure 2. Precision Plot for RBD IgG dLIA

Table 5. Well Concentration Range Based on Precision Data
6.3. Standard Curve Bias Evaluation

Figure 3. Standard Curve Bias Plot for RBD IgG dLIA

(b) (4)
Table 6. Well Concentration Range Based on Reference Standard Curve Bias

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Linearity Range</th>
<th>Precision Range</th>
<th>Standard Curve Range</th>
<th>Final Assay Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>COVRBD</td>
<td>(b) (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6.4. Assay Range Based on Linearity, Precision, and Standard Curve Bias

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


6.5. Assay Precision (Intermediate Precision)

The intermediate precision of the assay was evaluated using the as described in Section 5.2.5 and the results are summarized in Table 8.
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 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)

<table>
<thead>
<tr>
<th>Antigen</th>
<th>LLOQ (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COVRBD\textsuperscript{a}</td>
<td>1.1505</td>
</tr>
</tbody>
</table>

\( \textsuperscript{a} \) Receptor binding domain of the spike protein of severe acute respiratory disease coronavirus 2.

6.6.2. Upper Limit of Quantitation

6.7. Assay Run Performance

6.7.1. (b) (4)
6.7.2. Standard Curves

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

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
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. 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
2. VR-MQR-10212-ATT01, Supportive Data for VR-MQR-10212.
10. SUPPLEMENTAL INFORMATION


5. VR-DTN-11380, Planned Deviation from VR-SOP-FE-10111 Bio-Plex Operation and Maintenance.
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The assay plate layout in Supportive Figure 11.2 is used for routine testing. (b) (4)
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12.4. .........................................................................................................................................................28

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

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Sample ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>(b)</td>
<td>(4)</td>
</tr>
</tbody>
</table>
12.2. Sample Panel for Precision Evaluation

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>ID</th>
<th>Sample Number</th>
<th>ID</th>
<th>Sample Number</th>
<th>ID</th>
<th>Sample Number</th>
<th>ID</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

12.3. Precision of COVRBD Well Titers

<table>
<thead>
<tr>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>(b) (4)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>(b) (4)</td>
</tr>
</tbody>
</table>
(b) (4)
**Document Approval Record**

**Document Name:** VR-MQR-10212

**Document 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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<th>Date(GMT)</th>
<th>Signing Capacity</th>
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<td>Pride, Michael</td>
<td>15-Aug-2020 04:08:57</td>
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<td>15-Aug-2020 13:38:53</td>
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<td>18-Aug-2020 18:13:12</td>
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Approved On: 18-Aug-2020 23:10 (GMT)