Quantitative Bio-Plex Pro Human IgG, IgA, and IgM SARS-CoV-2 Serology Assays Using a Standard Curve

Candice Cox, Vanitha Margan, Woei Tan, and Qian Gao
Bio-Rad Laboratories, Inc., 2000 Alfred Nobel Drive, Hercules, CA 94547

Abstract

Bio-Plex Pro SARS-CoV-2 Serology Assays can be used to determine severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) exposure rates, assess vaccine immunogenicity, and investigate the magnitude and duration of the humoral response to the virus. The magnetic bead–based multiplex assays are for research use only and enable simultaneous measurement of human immunoglobulin antibodies against SARS-CoV-2 nucleocapsid (N), receptor binding domain (RBD), spike 1 (S1), and spike 2 (S2) antigens. Here, we demonstrate that Bio-Plex Pro Human IgG, IgA, and IgM SARS-CoV-2 Serology Assays can be used to quantitate levels of SARS-CoV-2–specific immunoglobulins when the VIROTROL SARS-CoV-2 Single Level Control and IgA and IgM Positive Controls are validated for use as reference materials. The standard curves generated with use of VIROTROL SARS-CoV-2 Single Level Control and IgA and IgM Positive Controls as reference materials showed excellent intra- and inter-assay precision (<4%) and accuracy (71–119% analyte recovery). The IgG standard curve enabled IgG quantitation of most (>79% detectability) 500- and 1,000-fold diluted anti–SARS-CoV-2 IgG-positive samples. The IgA and IgM standard curves enabled the IgA and IgM quantitation of most (>86% detectability for both assays) 100-fold diluted anti–SARS-CoV-2 positive samples. These data support the use of Bio-Plex Pro Human IgG, IgA, and IgM SARS-CoV-2 Serology 4-Plex Panels as quantitative assays for serological studies in patients with coronavirus disease 2019 (COVID-19) or vaccinated individuals.

Introduction

The SARS-CoV-2 outbreak in December 2019 quickly transformed into a global pandemic, causing the deaths of more than 4 million people worldwide as of August 2021, according to the WHO Coronavirus (COVID-19) Dashboard (World Health Organization 2021). Defining the humoral response can help inform ongoing therapeutic efforts aimed at combatting COVID-19. By enabling simultaneous measurement of antibodies against the SARS-CoV-2 N, RBD, S1, and S2 antigens, Bio-Plex Pro Human IgG, IgA, and IgM SARS-CoV-2 Serology Assays can be utilized (for research use only) to study the duration and magnitude of the humoral response in patients with COVID-19 and vaccinated individuals.

Quantitating the abundance of SARS-CoV-2 antibodies against S protein subunits (RBD, S1, S2) and N protein in human sera can provide important insights about an individual's exposure to SARS-CoV-2, vaccination, or both (Dai and Gao 2021). For example, because SARS-CoV-2 vaccines largely use S protein subunits to elicit immunity to SARS-CoV-2, antibodies against the N protein identify individuals with an immune response against naturally acquired infection (Burbelo et al. 2020). Also, patients with mild COVID-19 cases have been shown to have higher levels of IgG antibodies against S protein subunits than against the N protein (Röltgen et al. 2020). Together, these findings indicate that quantitation of antibodies against S and N proteins can be used to draw conclusions about the vaccination status of an individual or the disease severity of a patient with COVID-19.

Serological quantitation is also useful in seroprevalence studies, where it aids in the modeling of SARS-CoV-2 transmission by determining exposure rates. Anti–SARS-CoV-2 antibodies persist in the sera of patients with COVID-19 longer than viral nucleic acids, making serological quantitation useful for longitudinal studies. Seroprevalence studies can also capture asymptomatic or mild COVID-19 cases, which are less likely to be tested in the clinic by PCR-based tests.

The Bio-Plex Pro Human IgG, IgA, and IgM SARS-CoV-2 N/RBD/S1/S2 4-Plex Panels are indirect serology assays (Figure 1) that provide qualitative results in less than 3 hours (Figure 1). The kits contain ready-to-use positive and negative controls and provide median fluorescence intensity (MFI) cutoff values to help users interpret their sample results and determine whether there are detectable IgG, IgA, and IgM levels against the four viral antigens. The IgG assay can be made quantitative by using the VIROTROL SARS-CoV-2 Single Level Control as a reference material to generate a standard curve providing U/ml values. The IgA and IgM assays can be made quantitative with the respective kit positive control to generate the standard curve that provides ng/ml values. This application note highlights the performance of standard curves made with the VIROTROL SARS-CoV-2 Single Level Control and IgA and IgM Positive Controls, demonstrating the assays' ability to accurately and precisely quantitate anti–SARS-CoV-2 antibodies in SARS-CoV-2–positive samples.

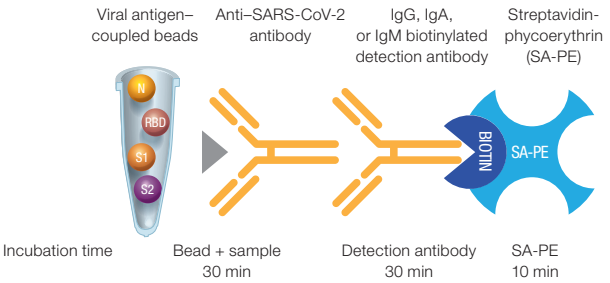

Fig. 1. Qualitative Bio-Plex Pro SARS-CoV-2 Assay format and incubation times. Four SARS-CoV-2 viral antigens, N, RBD, S1, and S2, were coated individually on magnetic beads to capture antibodies specific to them. Simultaneous antibody response profiling against each viral antigen through a biotinylated detection antibody against IgG, IgA, and IgM, followed by binding of SA-PE, results in an MFI readout that can be interpreted as positive or negative through assigned cutoff values.

Materials and Methods

A seven-point IgG standard curve was generated using the VIROTROL SARS-CoV-2 Single Level Control (Bio-Rad Laboratories, Inc., catalog #200300A) following the instructions in the Bio-Plex Pro Human IgG SARS-CoV-2 Serology Assays Protocol (bulletin 7459). The VIROTROL SARS-CoV-2 Single Level Control was diluted fivefold from the stock concentration; this was called standard 1. Standards 2–7 were prepared as threefold serial dilutions from standard 1.

Seven-point standard curves for the IgA and IgM assays were generated using the respective ready-to-use kit positive control (Bio-Rad, #12014775, 12014776) following the instructions in the Bio-Plex Pro Human IgA and IgM SARS-CoV-2 Serology Assays Protocol (bulletin 3224). The ready-to-use positive controls were used as stock concentration and labeled as standard 1. Standards 2–7 for both IgA and IgM were prepared as threefold serial dilutions from standard 1.

The quantitative performances of the Bio-Plex Pro Human IgG, IgA, and IgM SARS-CoV-2 N/RBD/S1/S2 4-Plex Panels (Bio-Rad, #12014634, 12014665, and 12014666) were evaluated by determining the sensitivity, precision, accuracy, and working range for each assay (N, RBD, S1, and S2). To calculate these values, assays and blanks were run on three separate plates, each of which was run on a separate day. For each run/plate, standards and blanks were evaluated in duplicate wells. Assays were prepared and tested as described in the Bio-Plex Pro Human SARS-CoV-2 Serology Assays Instruction Manual (10000133853).

Results

To evaluate the quantitative performance of the Bio-Plex Pro Human IgG, IgA, and IgM SARS-CoV-2 N/RBD/S1/S2 4-Plex Panels, we determined the sensitivity, precision, accuracy, and working range for each assay (N, RBD, S1, and S2). We calculated these values using standard curves and blanks or only sample diluent for background data from three independent experiments for each IgG, IgA, and IgM panel. Figures 2–4 show the standard curves for each experiment and assay.

The sensitivity, or limit of detection (LOD), was determined for each assay by analyzing the background. More specifically, two standard deviations were added to the average MFI obtained from the background (across all three independent experiments for each IgG, IgA, and IgM panel). These values were then converted to concentrations (U/ml for IgG and ng/ml for IgA and IgM) by interpolating the standard curves. The IgG assay sensitivity ranged from 0.87 (RBD) to 10.16 U/ml (S1). The IgA sensitivity ranged from 0.23 (N) to 1.35 ng/ml (S2). The IgM sensitivity ranged from 0.22 (RBD) to 0.32 ng/ml (S1 and S2). The sensitivity for IgG, IgA, and IgM assays are summarized in Tables 1–3, respectively.

The precision of each assay was determined by analyzing the agreement of standard curve values within (intra-assay) and between (inter-assay) assays. All four assays (N, RBD, S1, and S2) demonstrated excellent inter- and intra-assay precision, as shown by the low-percentage coefficients of variation (%CV) for each assay (Tables 1–3).

Assay accuracy was determined by comparing the observed standard curve values to their expected values. Tables 1–3 present these data as the percentage of analyte recovered in each assay. All assays were found to be accurate within a recovery range of 87–111% for IgG (Table 1), 71–119% for IgA (Table 2), and 98–103% for IgM (Table 3). The working range for each assay, or the range in which the standard curve is precise and accurate, covers concentrations with an intra-assay %CV ≤10% and a recovery of at least 70–130%. Standard 1 in the IgG standard curve fell outside of the working range for all assays except for the N, S1, and S2 assays in experiment (plate) 2. The standard 1 in the IgA standard curve fell outside of the working range for RBD in experiment (plate) 3. Nevertheless, it provides an option to start the standard curve at the S2 concentration value.

Table 1. Quantitative IgG assay performance.

	Sensitivity	Assay Precision		Working Range		Accuracy
Analyte	LOD, U/ml	Mean Intra-Assay, %CV	Mean Inter-Assay, %CV	LLOQ, U/ml	ULOQ, U/ml	Recovery Range, %
Nucleocapsid	2.7	1.42	3.27	27	11,110	93–109
Receptor binding domain	0.87	2.97	2.02	27	6,670	87–107
Spike 1	10.16	1.47	2.85	27	11,110	95–111
Spike 2	3.08	1.35	1.22	27	11,110	97–103

CV, coefficient of variation; LLOQ, lower limit of quantitation; LOD, limit of detection; and ULOQ, upper limit of quantitation for serum and plasma in human serology diluent.

After evaluating the performance of the standard curves, we empirically determined the ideal sample dilution for IgG, IgA, and IgM quantitation of SARS-CoV-2–positive samples using the Bio-Plex Pro Human IgG, IgA, and IgM SARS-CoV-2 N/RBD/S1/S2 4-Plex Panels. To this end, we tested 61 SARS-CoV-2–positive samples at 500- and 1,000-fold dilutions for IgG. These samples were tested in the IgG experimental runs used to evaluate standard curve performance (Figure 2). Most samples (79–92%) fell within the working range for each assay. More specifically, 85, 89, 79, and 92% of samples produced values within the working range of the N, RBD, S1, and S2 assays, respectively. Only one to five samples had undetectable levels of anti–SARS-CoV-2 IgG at the high end of the standard curve in each assay; and only one sample was too dilute for detection of anti-RBD IgG. Although 500- and 1,000-fold sample dilutions were sufficient for quantitation of most SARS-CoV-2–positive samples across all four SARS-CoV-2 assays, 300-fold dilutions were used to successfully quantitate samples from convalescent outpatients with COVID-19 (data not shown). Despite these dilution factor recommendations, higher or lower sample dilutions may need to be generated in some cases to quantitate samples with exceptionally abundant or scarce antibodies, respectively.

We tested 70 SARS-CoV-2–positive samples at 100-fold dilution for IgA and IgM assay panels. The majority of samples (86–99% for IgA and 96–100% for IgM) fell within the working ranges for each assay. Six different samples had undetectable levels of anti–SARS-CoV-2 IgA across all four assays. Specifically, five samples for N and one sample for S2 at the high end of the IgA standard curves. All samples had detectable levels of anti–SARS-CoV-2 IgM for N. A total of eight samples had undetectable levels of anti–SARS-CoV-2 IgM for RBD, S1, and S2 at the high end of the IgM standard curves. We determined that a 1:100 sample dilution is sufficient to quantitate SARS-CoV-2–positive samples across all the assays using both the IgA and IgM panels. Based on the 1:100 sample dilution factor, we recommend using MFI cutoff values provided in the Product

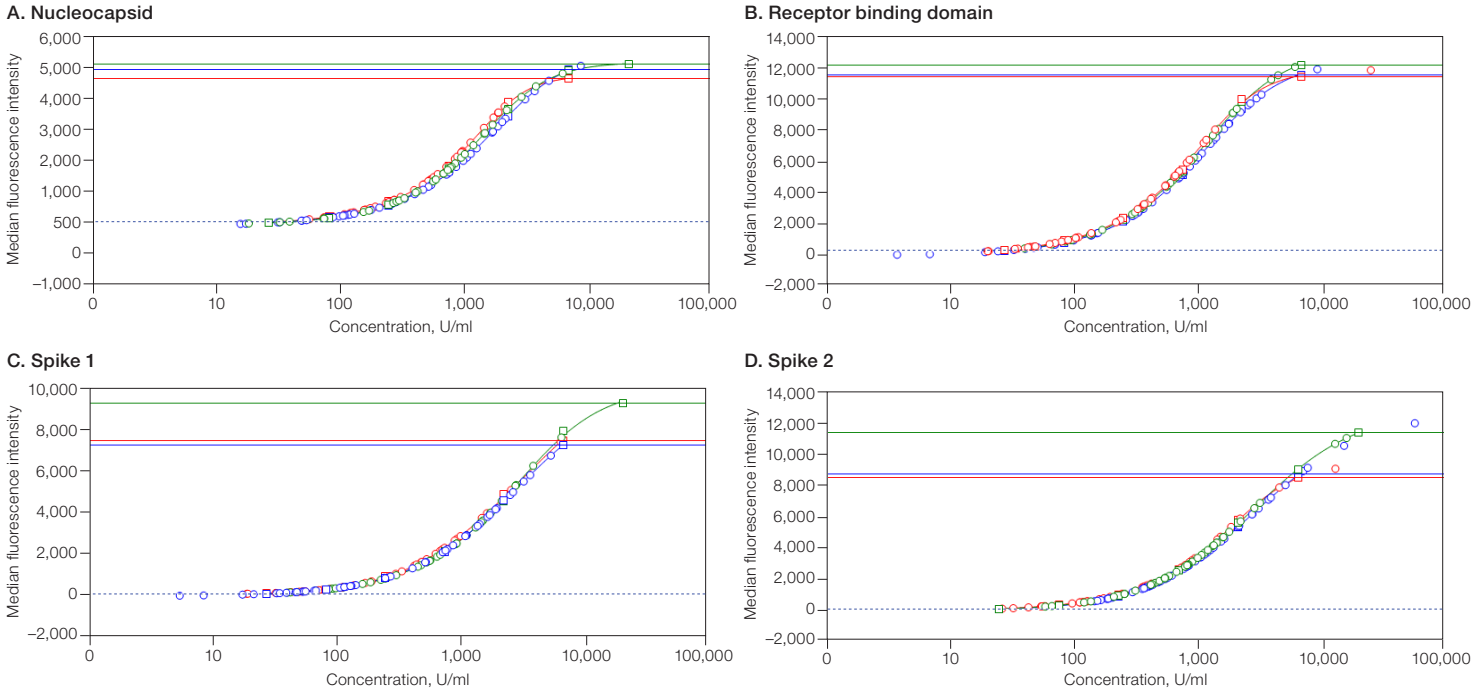

Fig. 2. IgG quantitation of samples and standards using the Bio-Plex Pro Human IgG SARS-CoV-2 N/RBD/S1/S2 4-Plex Panel. A, nucleocapsid; **B**, receptor binding domain; **C**, spike 1; and **D**, spike 2 antigens. Standard (□); unknown (○); upper limit of quantitation (═); lower limit of quantitation (----); plate 1 (□, ○); plate 2 (□, ○); plate 3 (□, ○). For the standard 1 curve value, the VIROTROL SARS-CoV-2 Single Level Control needs to be diluted fivefold before using it as standard 1.

Data Sheet as a first pass filter for results interpretation along with concentration values to ensure only samples above the MFI cutoff are considered positive for anti–SARS-CoV-2 IgA or IgM. Two hundred eighty-two SARS-CoV-2–negative samples were used to establish the MFI cutoff. Higher dilutions (300- and 500-fold) were successfully used to quantitate patient samples collected at 10 and 28 days after symptom onset, respectively (data not shown). Like the IgG assay, a higher or lower dilution may be necessary in some cases to quantitate SARS-CoV-2–positive samples with anticipated antibody response based on symptom onset and disease severity.

Table 2. Quantitative IgA assay performance.

	Sensitivity	Assay Precision		Working Range		Accuracy
Analyte	LOD, ng/ml	Mean Intra-Assay, %CV	Mean Inter-Assay, %CV	LLOQ, ng/ml	ULOQ, ng/ml	Recovery Range, %
Nucleocapsid	0.23	2.57	2.62	0.55	400.00	87–117
Receptor binding domain	0.25	1.33	2.09	0.41	233.33	71–119
Spike 1	0.28	1.62	1.85	0.41	300.00	89–113
Spike 2	1.35	2.42	1.24	1.10	800.00	90–106

CV, coefficient of variation; LLOQ, lower limit of quantitation; LOD, limit of detection; and ULOQ, upper limit of quantitation for serum and plasma in human serology diluent.

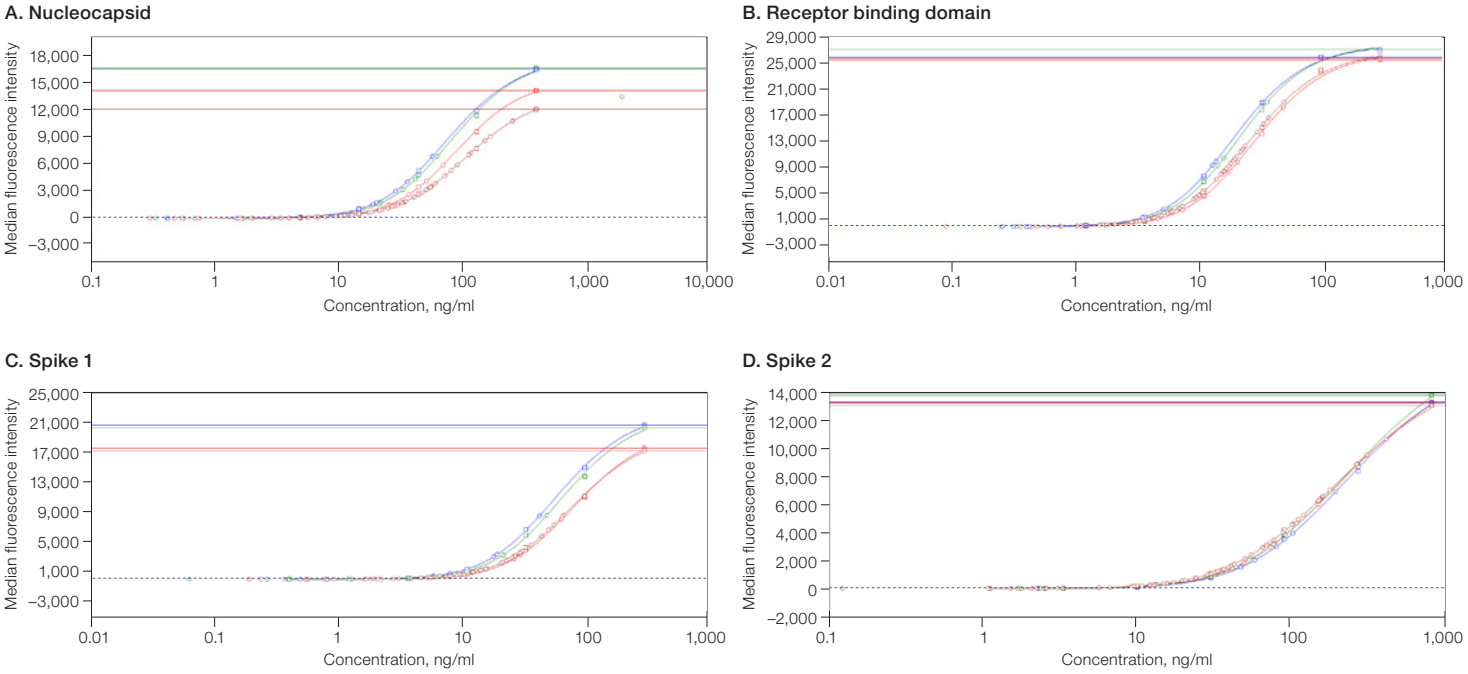

Fig.3. IgA quantitation of samples and standards using the Bio-Plex Pro Human IgA SARS-CoV-2 N/RBD/S1/S1 4-Plex Panel. A, nucleocapsid; **B**, receptor binding domain; **C**, spike 1; and **D**, spike 2 antigens. Standard (□); unknown (○); upper limit of quantitation (≡); lower limit of quantitation (----); plate 1 (□, ○); plate 2 (□, ○); plate 3 (□, ○); plate 4 (□, ○). For the standard 1 curve value, the IgA positive control is ready to use as standard 1.

Table 3. Quantitative IgM assay performance.

	Sensitivity	Assay Precision		Working Range		Accuracy
Analyte	LOD, ng/ml	Mean Intra-Assay, %CV	Mean Inter-Assay, %CV	LLOQ, ng/ml	ULOQ, ng/ml	Recovery Range, %
Nucleocapsid	0.25	1.33	0.73	1.37	1,000	99–102
Receptor binding domain	0.22	1.60	1.00	1.37	1,000	98–103
Spike 1	0.32	1.83	0.55	1.37	1,000	99–102
Spike 2	0.32	2.62	0.78	1.37	1,000	98–102

CV, coefficient of variation; LLOQ, lower limit of quantitation; LOD, limit of detection; and ULOQ, upper limit of quantitation for serum and plasma in human serology diluent.

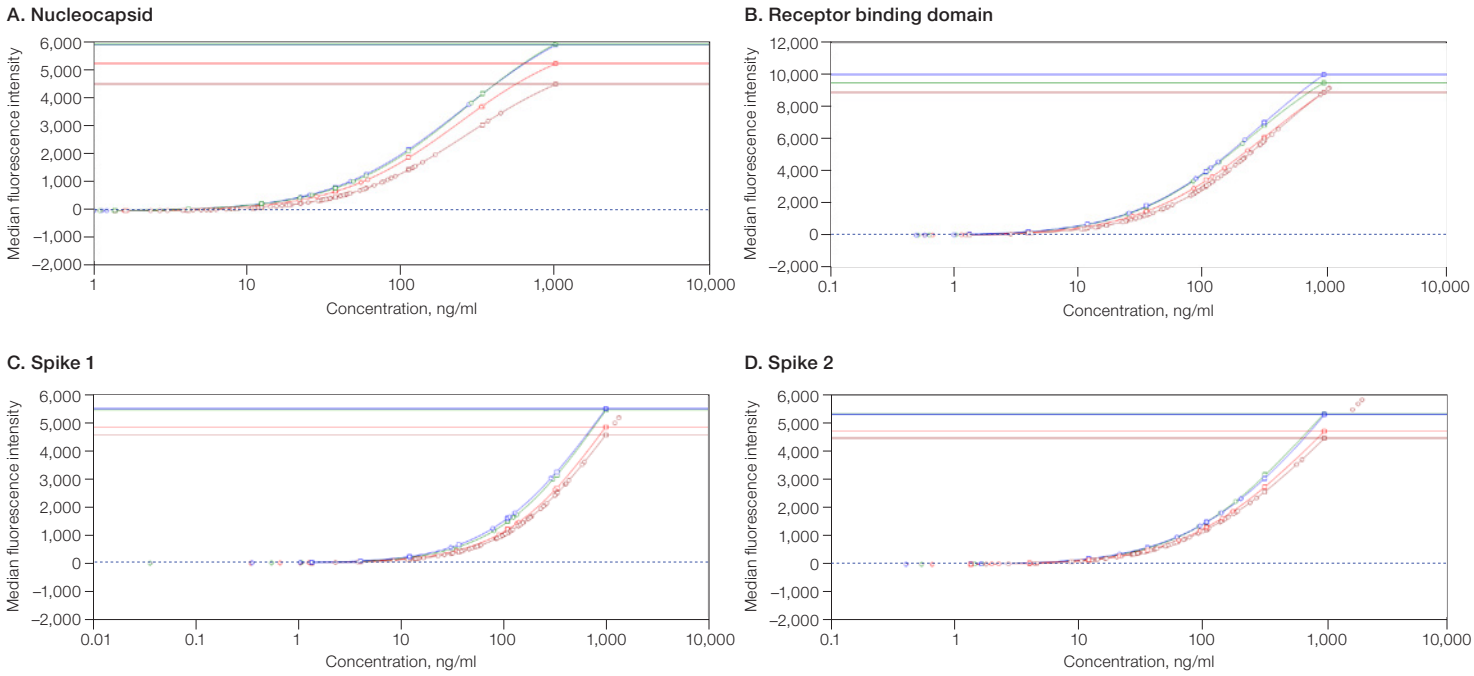

Fig. 4. IgM quantitation of samples and standards using Bio-Plex Pro Human IgM SARS-CoV-2 N/RBD/S1/S1 4-Plex Panel. A, nucleocapsid; **B**, receptor binding domain; **C**, spike 1; and **D**, spike 2 antigens. Standard (□); unknown (○); upper limit of quantitation (≡); lower limit of quantitation (-----); plate 1 (□, ○); plate 2 (□, ○); plate 3 (□, ○); plate 4 (□, ○). For the standard 1 curve value, the IgM positive control is ready to use as standard 1.

Conclusions

The Bio-Plex Pro Human IgG, IgA, and IgM SARS-CoV-2 N/RBD/S1/S2 4-Plex Panels can provide qualitative and quantitative results for the measurement of human IgG, IgA, and IgM antibodies specific to SARS-CoV-2. Using the VIROTROL SARS-CoV-2 Single Level Control for IgG and kit positive controls for IgA and IgM as reference standards, the IgG, IgA, and IgM quantitative assays achieve accurate and precise quantitation across a wide dynamic range of SARS-CoV-2 antibody concentrations. These quantitative assays will be useful for longitudinal studies that test samples spanning a wide range of antibody responses as a result of differences in COVID-19 disease severity or vaccine immunogenicity.

References

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