Bio-Plex Pro Human IgA, IgG, and IgM SARS-CoV-2 Serology Assay Performance in Freeze-Thawed and Refrigerated Serum, Plasma, and Saliva Samples

Deanna Woo and Vanitha Margan
Bio-Rad Laboratories, Inc., 6000 Alfred Nobel Drive, Hercules, CA 94547

Abstract

In this study, we compared the stability of Bio-Plex Pro Human SARS-CoV-2 IgA, IgG, and IgM Serology Assay results in serum, plasma, and saliva samples that were either freeze-thawed up to six times or refrigerated up to 6 days. We found that the median fluorescence intensity (MFI) results of freeze-thawed samples showed more consistency than those of the samples stored at 4°C, regardless of sample type. Thus, multiple freeze-thaw cycles had less impact on Bio-Plex Pro Human IgA, IgG, and IgM SARS-CoV-2 Serology Assay performance than extended refrigeration.

Introduction

The realities of the COVID-19 pandemic sometimes require that samples be stored and handled in less-than-ideal ways. When freezing samples, assay failure and/or retesting may require multiple freeze-thaw cycles. On the other hand, when refrigerating samples, workflow backlogs can result in extended refrigeration times. These nonideal storage and handling conditions can degrade samples and adversely impact serology assay performance. In this application note, we compared these nonideal storage and handling conditions using serum, plasma, and saliva samples to determine which conditions have the least impact on Bio-Plex Pro Human IgA, IgG, and IgM SARS-CoV-2 Serology Assay performance.

Materials and Methods

Serum, plasma, and saliva samples were tested for IgA, IgG, and IgM antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleocapsid (N), receptor binding domain (RBD), spike 1 (S1), and spike 2 (S2) antigens using the Bio-Plex Pro Human IgA, IgG, and IgM SARS-CoV-2 N/RBD/S1/S2 4-Plex Panels (Bio-Rad Laboratories Inc., catalog #12014665, 12014634, and 12014666). For each sample matrix, two SARS-CoV-2–positive samples and one healthy human (nonpositive) sample were run in duplicate 96-well plates. All assays were run on the Bio-Plex 200 System with HTF (Bio-Rad, #171000205) on the same day.

Prior to testing, SARS-CoV-2–positive and healthy human serum and plasma samples were each aliquoted into nine vials and stored at –80°C (Figure 1). Six aliquots of each sample were assayed after having undergone one to six freeze-thaw cycles. The remaining three aliquots of each sample were removed from –80°C and stored at 4°C, one each for 6 days, 3 days, and 1 day prior to testing.

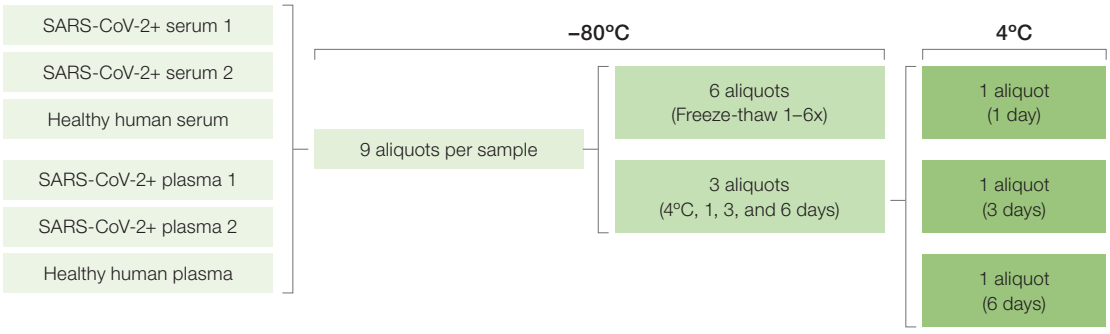

Fig. 1. Storage conditions for SARS-CoV-2-positive and healthy human serum and plasma samples. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

SARS-CoV-2–positive and healthy human saliva samples were each aliquoted into ten vials, nine of which were stored at –80°C and one of which was used directly for a pretest experiment (Figure 2). Six of the frozen SARS-CoV-2–positive saliva aliquots were subjected to one to six freeze-thaw cycles. The remaining three frozen aliquot vials were removed from the freezer and stored at 4°C, one each for 6 days, 3 days, and 1 day prior to testing.

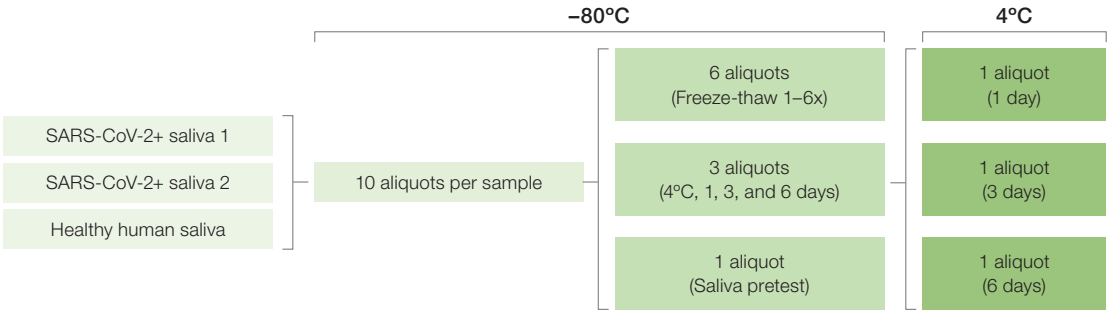

Fig. 2. Storage conditions for SARS-CoV-2–positive and healthy human saliva samples. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

The saliva pretest was done to determine the optimal dilution factor for testing saliva samples. The pretest aliquots for each saliva sample were diluted 2.5-fold, fivefold, and tenfold. Each dilution was assayed for IgA, IgG, and IgM. MFI values were distinctly higher for the 2.5-fold dilution for all three assays. Therefore, we determined 2.5-fold as the optimal dilution for the sample-storage study. Table 1 shows representative MFI data for the pretest experiment.

Table 1. MFI values for 1:2.5, 1:5, and 1:10 saliva sample dilutions assayed by the Bio-Plex Pro Human IgA SARS-CoV-2 N/RBD/S1/S2 4-Plex Panel.

	MFI Values											
	N (Bead Region 20)			RBD (Bead Region 36)			S1 (Bead Region 28)			S2 (Bead Region 42)		
Dilution	Saliva 1	Saliva 2	Healthy Saliva	Saliva 1	Saliva 2	Healthy Saliva	Saliva 1	Saliva 2	Healthy Saliva	Saliva 1	Saliva 2	Healthy Saliva
1:2.5	158.3	229.3		614.8	540.5		88.5	106.8		55.5	35.3	
1:5	55.3	68.8	28.3	163.8	170.0	70.5	30.8	41.0	10.0	21.3	17.5	10.0
1:10	27.8	36.5		74.8	62.0		15.5	18.3		11.5	10.0	

MFI, median fluorescence intensity; N, nucleocapsid; RBD, receptor binding domain; S1, spike 1; S2, spike 2.

Results

Sample degradation can impact overall fluorescence intensities in multiplex serology assays. For this reason, we used MFI values to evaluate the impact of sample storage conditions on the performance of Bio-Plex Pro Human IgA, IgG, and IgM SARS-CoV-2 Serology Assays.

Figures 3 and 4 show MFI results for IgA and IgG in SARS-CoV-2–positive serum, plasma, and saliva samples that went through up to six freeze-thaw cycles. The IgM data was similar and is not shown. All sample matrices showed some level of fluctuation in the MFI results across all three assays and were still considerably high. This indicates the SARS-CoV-2 antibodies were detectable after up to six freeze-thaw cycles. Variation between replicates was negligible (data not shown), indicating minimal sample degradation.

Both healthy human serum and plasma samples went through up to six freeze-thaw cycles and showed MFI results close to zero (data not shown). We expected the healthy human saliva sample to show similar results to the healthy human serum and plasma samples. Instead, it resulted in MFI values similar to SARS-CoV-2–positive saliva samples (data not shown), possibly due to sample degradation, cross-contamination, or an unknown factor. For this reason, we excluded the healthy human saliva sample for comparison with the SARS-CoV-2–positive saliva samples.

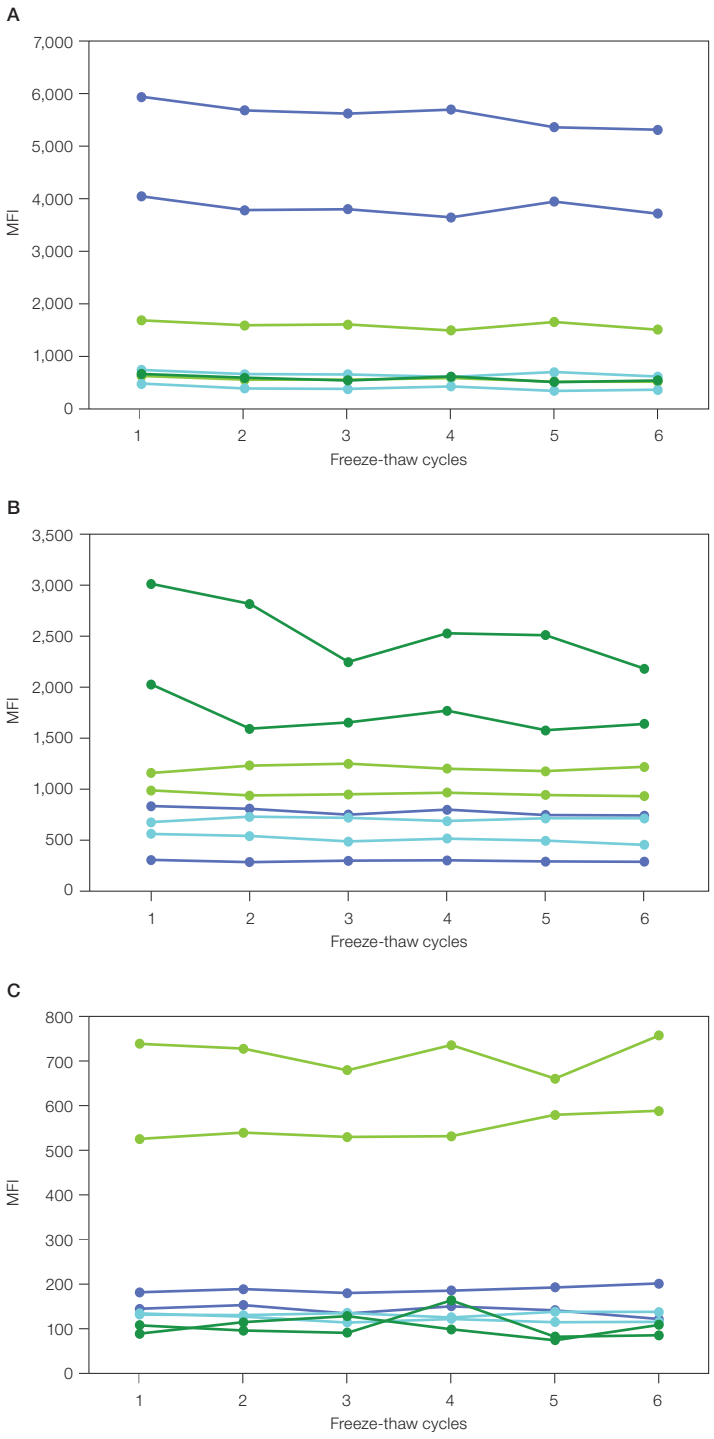

Fig. 3. IgA results for freeze-thawed SARS-CoV-2–positive samples. A, serum; **B**, plasma; **C**, saliva. Nucleocapsid (●), receptor binding domain (●), spike 1 (●), spike 2 (●). IgA, immunoglobulin A; MFI, median fluorescence intensity.

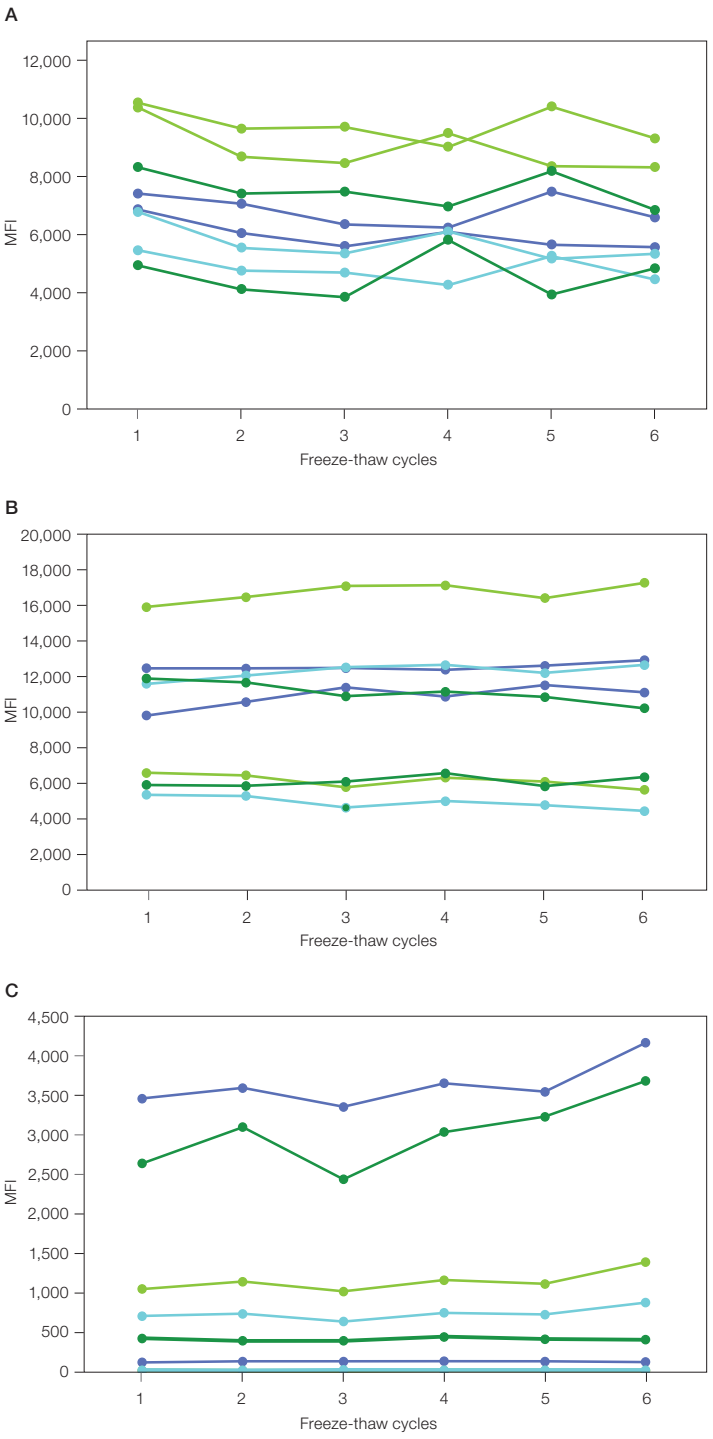

Fig. 4. IgG results for freeze-thawed SARS-CoV-2–positive samples. A, serum; **B**, plasma; **C**, saliva. Nucleocapsid (●), receptor binding domain (●), spike 1 (●), spike 2 (●). IgG, immunoglobulin G; MFI, median fluorescence intensity.

Figure 5 represents the trend observed across all three immunoglobulin assays for all four tested antigens (SARS-CoV-2 N, RBD, S1, and S2) when serum samples were stored at 4°C up to 6 days. The observed minor fluctuations in MFI values were similar to what we observed with freeze-thawed samples, and well-to-well variation was negligible. The same trend was seen among plasma and saliva matrices (data not shown). Despite the fluctuations, the MFI values were well within the measurement range, even after 6 days of 4°C refrigeration, indicating that high levels of antibodies are still measurable with extended refrigeration.

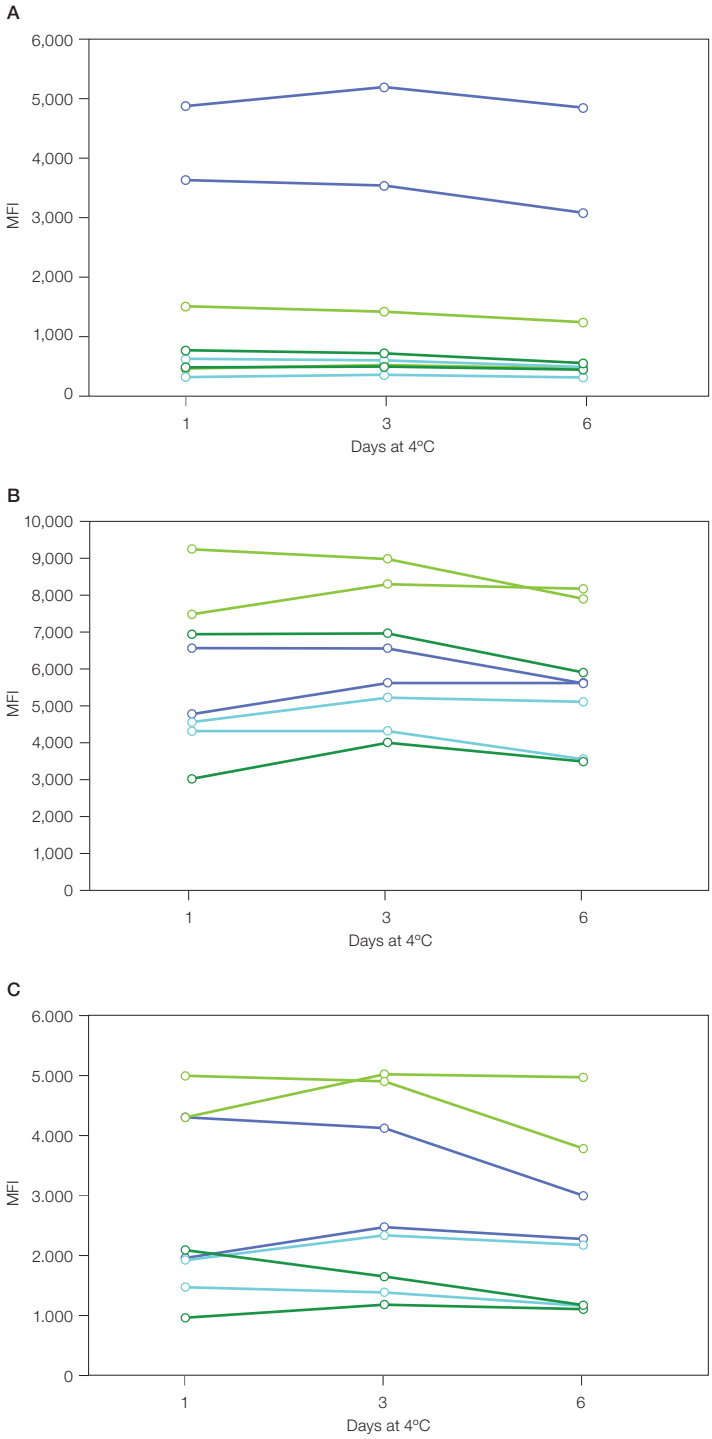

Fig. 5. Representation graphs showing MFI results for refrigerated SARS-CoV-2–positive serum samples. A, IgA; **B**, IgG; **C**, IgM. Nucleocapsid (—), receptor binding domain (—), spike 1 (—), spike 2 (—). IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; MFI, median fluorescence intensity.

Among all three sample matrices, plasma samples showed the most consistency between freeze-thaw cycles, with six freeze-thaw cycles yielding almost the same MFI results as one cycle. Plasma samples also exhibited relatively stable results compared to serum and saliva when stored at 4°C up to 6 days. Figure 6 shows an example comparison of IgM results for serum, plasma, and saliva matrices that were thawed up to six times and refrigerated up to 6 days at 4°C.

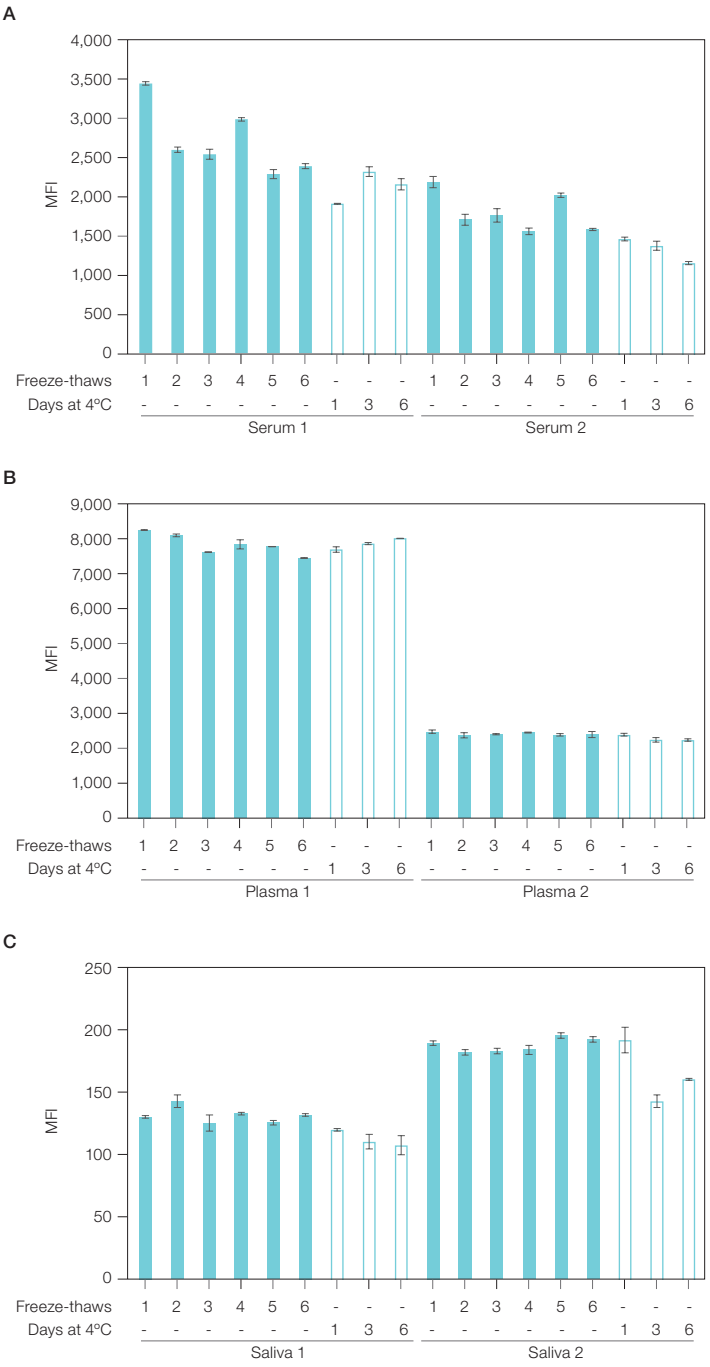

Fig. 6. Side-by-side comparison of IgM spike 1 results from freeze-thawed and refrigerated samples. A, serum; **B**, plasma; **C**, saliva. Error bars show standard deviation. IgM, immunoglobulin M; MFI, median fluorescence intensity.

Certain serum, plasma, and saliva samples stored at 4°C for up to 6 days showed a continued decrease in MFI values over time. This suggests that samples stored for more than one day at 4°C will experience minor degradation over time but can still produce measurable antibody levels (Figure 7).

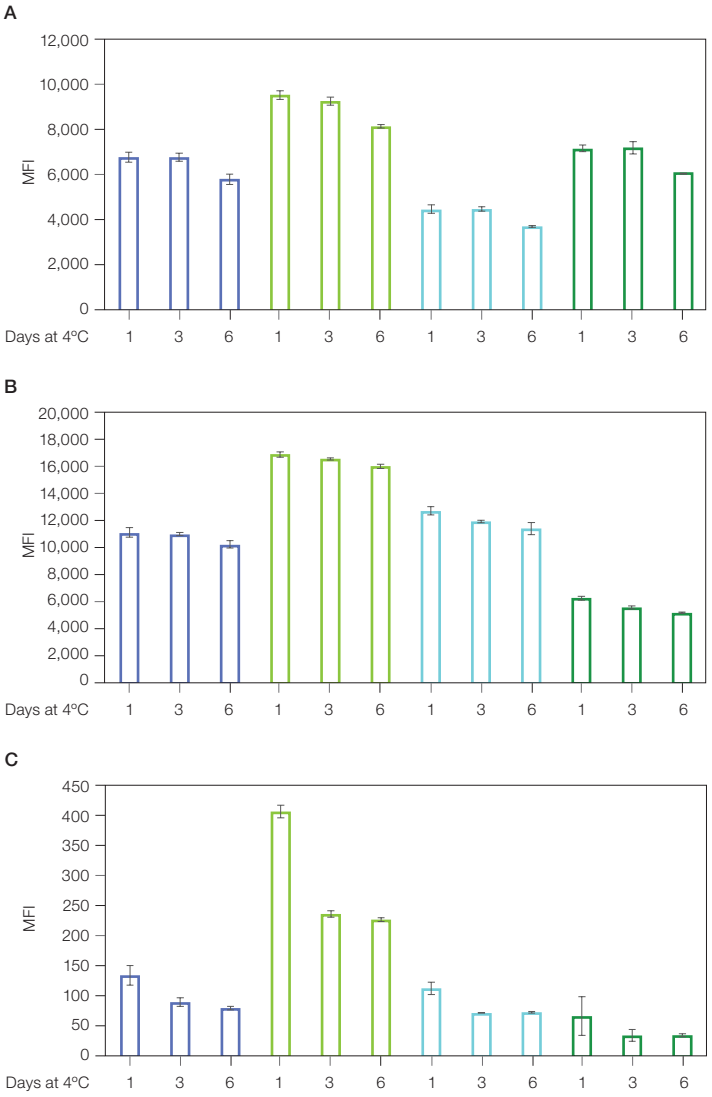

Fig. 7. Charts showing a continuous decrease in serum IgG, plasma IgG, and saliva IgA MFI values with increasing refrigeration time. A, serum IgG; **B**, plasma IgG; **C**, saliva IgA. Error bars show standard deviation. Nucleocapsid ( ), receptor binding domain ( ), spike 1 ( ), spike 2 ( ). IgG, immunoglobulin G; MFI, median fluorescence intensity.

Conclusion

The MFI values across Bio-Plex Pro Human IgA, IgG, and IgM SARS-CoV-2 Serology Assays and across serum, plasma, and saliva sample matrices show measurable antibody levels when the samples were freeze-thawed up to six times or stored at 4°C for up to 6 days. In general, the MFI results of freeze-thawed samples showed more consistency compared to the samples stored at 4°C. All three sample matrices (serum, plasma, and saliva) showed various levels of degradation when stored at 4°C. However, any well-to-well differences in MFI values among all sample matrices were negligible and were within the variability of the assays for both freeze-thawed and refrigerated storage conditions.

In this study, we have demonstrated that freeze-thawed serum, plasma, and saliva samples produced more stable results than refrigerated samples when assayed with Bio-Plex Pro Human IgA, IgG, and IgM SARS-CoV-2 Serology Assays.

Visit **bio-rad.com/SARSCoV2Serology** for more information.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Website *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *00 800 00 24 67 23* ***Belgium*** *00 800 00 24 67 23* ***Brazil*** *4003 0399*
Canada *1 905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *00 800 00 24 67 23* ***Denmark*** *00 800 00 24 67 23* ***Finland*** *00 800 00 24 67 23*
France *00 800 00 24 67 23* ***Germany*** *00 800 00 24 67 23* ***Hong Kong*** *852 2789 3300* ***Hungary*** *00 800 00 24 67 23* ***India*** *91 124 4029300* ***Israel*** *0 3 9636050*
Italy *00 800 00 24 67 23* ***Japan*** *81 3 6361 7000* ***Korea*** *82 2 3473 4460* ***Luxembourg*** *00 800 00 24 67 23* ***Mexico*** *52 555 488 7670*
The Netherlands *00 800 00 24 67 23* ***New Zealand*** *64 9 415 2280* ***Norway*** *00 800 00 24 67 23* ***Poland*** *00 800 00 24 67 23* ***Portugal*** *00 800 00 24 67 23*
Russian Federation *00 800 00 24 67 23* ***Singapore*** *65 6415 3188* ***South Africa*** *00 800 00 24 67 23* ***Spain*** *00 800 00 24 67 23* ***Sweden*** *00 800 00 24 67 23*
Switzerland *00 800 00 24 67 23* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 651 8311* ***United Arab Emirates*** *36 1 459 6150* ***United Kingdom*** *00 800 00 24 67 23*

Bulletin 3303 Ver A US/EG *22-0120 0522 Sig 0122*