



# Guava® SARS-CoV-2 Multi-Antigen Antibody Assay Package Insert



**RUO** For Research Use Only.  
Not for use in diagnostic procedures.  
89-30000-00-959 Rev A  
06/2021











**Technical Support**  
Telephone: 512-381-4397  
North America Toll Free: 1-877-785-2323  
International Toll Free: + 800-2939-4959  
Email: [support@luminexcorp.com](mailto:support@luminexcorp.com)  
[www.luminexcorp.com](http://www.luminexcorp.com)



Luminex Corporation  
12212 Technology Blvd  
Austin, TX 78727  
U.S.A.

## Symbols Glossary

You will encounter these symbols throughout this manual. They represent warnings, conditions, identifications, instructions, and regulatory agencies.

Symbol	Meaning	Symbol	Meaning
	Caution.		Use-by date.
	Temperature Limit.		Batch Code.
	Contains sufficient for <n> Tests.		Keep away from sunlight.
	For Research Use Only. Not for use in diagnostic procedures.		Catalog(ue) Number.
	Consult instructions for use.		Manufacturer.

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# Luminex Technical Support

Contact Luminex Technical Support by telephone in the U.S. and Canada by calling: 1-877-785-2323

Contact outside the U.S. and Canada by calling: +1 512-381-4397

International: + 800-2939-4959

Fax: 512-219-5114

Email: [support@luminexcorp.com](mailto:support@luminexcorp.com).

Additional information is available on the Luminex website. Search on the desired topic, navigate through menus. Also, review the website's FAQ section. Enter <http://www.luminexcorp.com> in your browser's address field.

This manual can be updated periodically. To ensure that you have a current version, contact Technical Support.

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## Description

The Guava® SARS-CoV-2 Multi-Antigen Antibody Assay is a serological, multiplexed microsphere-based assay designed to measure the presence of IgG, IgM, and IgA antibodies directed against the nucleocapsid protein (N), receptor-binding domain (RBD) of the spike protein, and the S1 subunit of the spike protein (S1) of SARS-CoV-2 in human serum or plasma<sup>[1-4]</sup>.

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## Principles of the Procedure

The Guava® SARS-CoV-2 Multi-Antigen Antibody Assay is a bead-based antigen capture and detection assay. Three bead populations are conjugated to antigens specific to SARS-CoV-2, namely nucleocapsid protein (N), the receptor-binding domain (RBD) of the spike protein, and the S1 subunit of the spike protein. Diluted human serum or plasma are incubated with the multiplexed microsphere mix, in combination with a negative reference bead to assist in gating. Bead-bound antibodies are subject to probing with phycoerythrin (PE) labeled antibodies against human IgG-, IgM-, or IgA-specific detection reagents. The microspheres are washed again to remove unbound detection reagent and suspended in wash buffer for analysis on a compatible instrument.

The Guava SARS-CoV-2 Multi-Antigen Antibody Assay is compatible with the Guava® Muse® Cell Analyzer, the Guava® easyCyte™ instrument, and any other flow cytometer equipped with either a 488 nm (blue) or 532 nm (green) laser for excitation and emission channels that detect yellow (583/26) and red (595/50) fluorescence. Positive beads are shifted to the right (analyte response) and easily detected by comparing the results with a negative reference bead. Median fluorescence intensity of each bead population is obtained. The presence or absence of SARS-CoV-2 specific antibodies can be interpreted by the user based on median fluorescent intensity information. Median fluorescent intensity data provides semi-quantitative information on the kit.

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## Materials Provided

The Guava® SARS-CoV-2 Multi-Antigen Antibody Assay Kit (Part No. FCPA100101) is sufficient for 100 tests and includes the following components:

Components	Volume	Part Number
Guava® SARS-CoV-2 Multi-Antigen Antibody Assay Microsphere Mix	5.5 mL	14-20327
PBS-TBN Wash Buffer	125.0 mL	14-20335
Guava® SARS-CoV-2 Multi-Antigen Antibody Assay IgG Detection Reagent	5.5 mL	14-20332
Guava® SARS-CoV-2 Multi-Antigen Antibody Assay IgM Detection Reagent	5.5 mL	14-20334
Guava® SARS-CoV-2 Multi-Antigen Antibody Assay IgA Detection Reagent	5.5 mL	14-20333

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## Materials Required but Not Provided

- One of the following instruments:
  - Guava® Muse® Cell Analyzer
  - Guava® easyCyte™ flow cytometer
  - Any flow cytometer with a 488 nm or 532 nm laser
- Magnetic base rack or base plate for manual washing
- Pipettes
- One of the following mixers:
  - (For tubes) Vortex mixer with adapter (Vortex-Genie® with 6" foam insert for 60 microcentrifuge tubes (Cat# 504-0233-00) or equivalent)
  - (For plates) Orbital plate shaker
- Timer
- Deionized (DI) water
- 10% bleach solution

**NOTE:** 10% bleach is defined as 0.6% sodium hypochlorite

- Pipette tips
- Polypropylene microcentrifuge tubes with screw caps, 1.5 mL, or 96-well plate (Corning 3789A or equivalent)
- Plate sealers
- Muse System Check Kit (Catalog No. MCH100101)
- Guava® easyCheck™ Kit (Catalog No. 4500-0025)
- Guava® Instrument Cleaning Fluid (ICF) (Catalog No. 4200-0140)

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## Warnings and Precautions

1. For Research Use Only. Not for use in diagnostic procedures.
2. Wear appropriate personal protective equipment (PPE), including a lab coat and disposable gloves, when performing procedures. Fresh, clean gloves must be worn in each area and must be changed before leaving that area. Wash your hands thoroughly after performing the test.
3. Do not eat, drink, smoke, or apply cosmetic products in the work areas.
4. Do not use the kit or any kit components past the expiration date indicated on the kit carton label. Do not interchange kit components from different kit lots. Lot numbers are identified on the kit label.
5. Do not pipette by mouth.
6. All aspirated fluids must be collected. All collection reservoirs must contain suitable disinfectants for inactivation of human pathogens. All reagents and materials, which come into contact with potentially infectious samples, must be treated with suitable disinfectants or be disposed of according to their hygiene requirements.
7. The concentration specifications and incubation times of the manufacturer must be followed carefully.

8. Only use the individual wells of the microtiter plate once.
9. Do not replace or mix the reagents with reagents from other manufacturers.
10. The reagents used in the Guava<sup>®</sup> SARS-CoV-2 Multi-Antigen Antibody Assay may contain sodium azide as a preservative, which is toxic if ingested. Reagents containing sodium azide should be considered a poison. If products containing sodium azide are swallowed, seek medical advice immediately and show product container or label. (Refer to NIOSH, National Institute for Occupational Safety and Health; CAS#: 2628-22-8; and also to GHS, The Globally Harmonized System of Classification and Labeling of Chemicals.) Aqueous solutions of sodium azide, when mixed with acids, may liberate toxic gas. Any reagents containing sodium azide should be evaluated for proper disposal. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent build-up in plumbing. Check with regulatory agencies to determine at what concentration sodium azide may cause a product to be regulated as hazardous.
11. Perform the procedure given in this package insert as described. Any deviation from the outlined protocols may result in assay failure or cause erroneous results.
12. Avoid contamination from positive controls and samples by following good laboratory practices.
13. Samples and waste fluid can contain biohazardous material. Where exposure to biohazardous material, including in an aerosol form, exists, follow appropriate biosafety procedures, use personal protective equipment (PPE), and use ventilation devices.
14. Train personnel who use, maintain, or clean the instrument in standard laboratory safety practices and follow those practices when handling the instrument.
15. Handle waste disposal in accordance with accepted medical practice and applicable regulations. If spillage occurs immediately disinfect following appropriate laboratory procedures. Refer to the applicable instrument user guide for additional information on adding bleach to the waste vial and waste disposal.
16. Take care when handling, storing, and disposing of potentially infectious materials (i.e. samples, specimens, waste fluid, etc). Suitable barrier protection against potential pathogens is recommended during all stages of use. Adherence to appropriate local biosafety and biohazard guidelines or regulations is recommended when working with potentially infectious materials that may be unknown.
17. Adhere to standard laboratory safety practices when handling hazardous, toxic, or flammable reagents and chemicals. Consult the package insert for the assay you are running and the Safety Data Sheet (SDS) for more information. Contact Luminex Technical Support when in doubt about compatibility of cleaning and decontamination agents or materials.
18. The fluorescent dyes in this reagent are light-sensitive. Store in the dark and shield from excessive exposure to light.
19. Follow your institution's safety procedures for working with chemicals and handling biological samples.
20. Safety Data Sheets (SDS) are available by contacting Luminex Corporation or visiting our website at [www.luminexcorp.com](http://www.luminexcorp.com).

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## Reagent Storage, Handling, and Stability

Store reagents away from light at 2°C to 8°C before and after use.

**NOTE:** Do not use the kit or any kit components past the expiration date indicated on the kit carton label. Do not interchange kit components from different kit lots. Lot numbers are identified on the kit label.

Return the Guava<sup>®</sup> SARS-CoV-2 Multi-Antigen Antibody Assay Kit components to their appropriate storage conditions immediately after use. Do not leave the kit out at higher temperatures for extended periods of time.

The Guava SARS-CoV-2 Multi-Antigen Antibody Assay Kit contains light-sensitive dyes. Protect the Guava SARS-CoV-2 Multi-Antigen Antibody Assay Kit from excessive exposure to light during storage and incubation.

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## Specimen Storage, Handling, and Transportation

The sample type is serum or plasma. Avoid microbial contamination of the sample. Remove non-soluble substances from the sample prior to incubation.

Do not use heat-inactivated, icteric, hemolytic, lipemic, or cloudy samples.

### Specimen Storage

Store serum and plasma at less than -20°C.

Luminex does not recommend repeated freezing and thawing of sample material due to the risk of false results.

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## Assay Procedure

### Dilute Samples

1. Remove serum, plasma samples, and/or controls (if applicable) from their appropriate storage conditions and allow them to come to room temperature.
2. Dilute the serum or plasma sample 1:400 by performing two 1:20 dilutions. 50 µL of diluted specimen is needed for each isotype evaluation.
  - a. Pipette 95 µL of PBS-TBN wash buffer into the dilution container (not provided).
  - b. Add 5 µL of non-diluted sample to the PBS-TBN wash buffer. Mix thoroughly.
  - c. Pipette 95 µL of PBS-TBN wash buffer into a second dilution container (not provided).
  - d. Add 5 µL of sample from the first dilution container to the PBS-TBN wash buffer in the second container. Mix thoroughly.

### Prepare Controls

Negative control samples can be the PBS-TBN Wash Buffer provided in the kit. Luminex recommends preparing a negative control sample to use in the Adjust Settings step for every data set run.

For positive controls, use external antibody standards against SARS-CoV-2 antigens, or positive SARS-CoV-2 serum or plasma. Compatible antibody controls are typically diluted to 0.125 to 0.5 µg/mL.

If positive SARS-CoV-2 serum or plasma is used, note if the controls are neat or pre-diluted. Typically, 50 µL of 0.25% (neat) serum or plasma is added to the assay.

**NOTE:** All vendor serum or plasma control samples are not positive for IgM and IgA response.

1. Dilute each control to a final 1:400 dilution of serum or plasma. Consider each control's starting serum concentration.

**NOTE:** The final concentration added to the plate well should be 0.25% serum or plasma.



## Prepare Samples

**NOTE:** Perform each step in the order described.

**NOTE:** Use a clean pipette tip whenever you add a sample or reagent to each tube or well.

1. Add 50  $\mu$ L of 1:400 diluted (0.25% serum or plasma) sample or control to the appropriate tube or well.
2. Vortex the Microsphere Mix for 30 seconds. Immediately after vortexing, add 50  $\mu$ L of Microsphere Mix to each well.

**NOTE:** Do not exceed 5 minutes between vortexing and adding the Microsphere Mix to the final well.

3. If using a sample tube, cap the tube, place it in the tube adapter of the mixer, and cover it with foil to protect from light. If using a plate, seal and place the plate on an orbital plate shaker and cover the plate with foil to protect it from light.
4. If using a tube, vortex the tube on low speed for 30 minutes at 25°C,  $\pm$ 5°C. If using a plate, shake the plate at 800-900 rpm for 30 minutes at 25°C,  $\pm$ 5°C.

## Wash the Tube or Plate

1. Once the tube or plate is done mixing, remove the tube from the mixer or the plate from the plate shaker.

**NOTE:** Make sure shaking is completely stopped prior to removing the plate or tube.

2. Place the tube or plate on a magnetic separator for 3 minutes to allow the microspheres to separate.
3. With the tube or plate still positioned on the magnetic separator, use a pipette to aspirate the supernatant from the tube or the sample wells.

**NOTE:** Take care not to disturb the microspheres.

4. Wash the tube or plate once.
  - a. Remove the tube or plate from the magnetic separator. Add 150  $\mu$ L of PBS-TBN wash buffer to the sample wells.
  - b. Place the tube or plate on the magnetic separator for 3 minutes to allow the microspheres to separate.
  - c. With the tube or plate still positioned on the magnetic separator, use a pipette to aspirate the supernatant from the tube or sample wells.

**NOTE:** Incomplete washing can result in false negative results.

## Add Detection Reagent

1. Vortex the detection reagent for 30 seconds.
2. Add 50  $\mu$ L of detection reagent to each sample well.
3. If using a sample tube, cap the tube, place it in the tube adapter of the vortex mixer, and cover it with foil to protect from light. If using a plate, seal and place the plate on an orbital plate shaker and cover the plate with foil to protect it from light.
4. If using a tube, vortex the tube on low speed for 30 minutes at 25°C,  $\pm$ 5°C. If using a plate, shake the plate at 800 rpm to 900 rpm for 30 minutes at 25°C,  $\pm$ 5°C.
5. Remove the supernatant by placing the tube or plate on the magnetic separator for 3 minutes to allow the microspheres to separate. With the tube or plate still positioned on the magnetic separator, use a pipette to aspirate the supernatant from the tube or wells.

**NOTE:** Incomplete removal of the supernatant can result in false negative results.

## Resuspend the Microspheres

1. Remove the tube or plate from the magnetic separator.
2. Add 200 µL of PBS-TBN wash buffer.

Samples are now ready for acquisition on the instrument.

## Set Up System Software

### Set Up and Acquire on Guava® Muse® Cell Analyzer

Run a System Check prior to performing the assay. For more information on Muse® System Check, refer to the *Guava® Muse Cell Analyzer User Guide*.

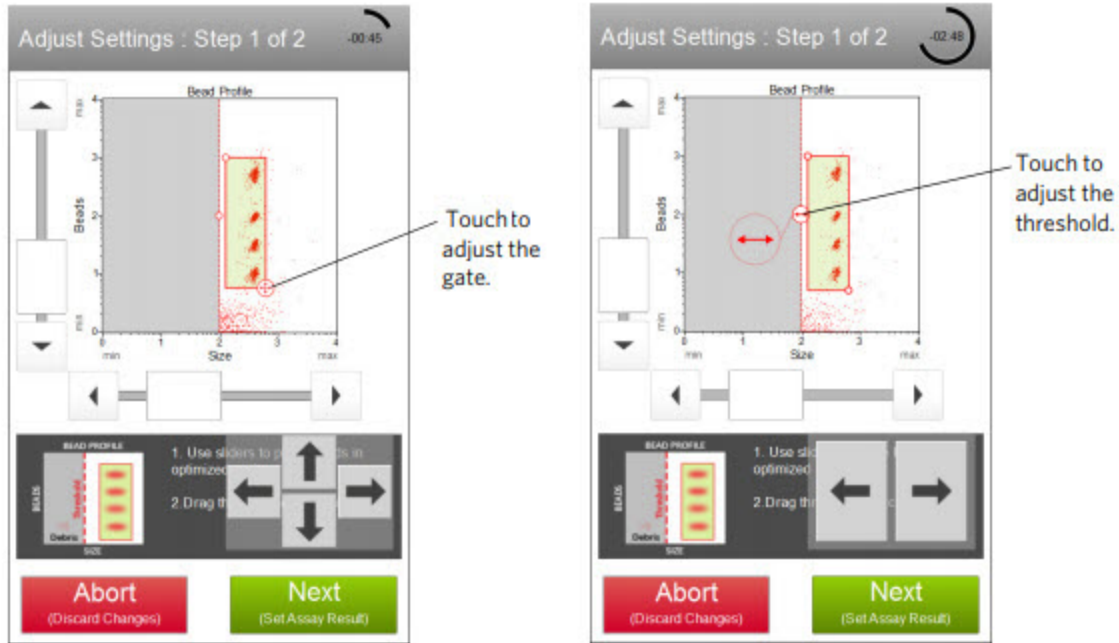
1. From the Muse Acquisition Software main menu, select **SARS-CoV-2 Antibody**.
2. Select **Run Assay**.
3. Load a negative control sample and select **Run (Adjust Settings)**. The **Adjust Settings** screen appears.

### Adjust Settings

1. In the **Adjust Settings** screen, fine-tune the settings for the Size vs. Beads plot, if necessary.
  - a. Adjust the **Size** slider to the left of the plot to move the bead population into the counting gate (the green region).
  - b. Touch and drag the corner of the red box to adjust the gate. Adjust the gate to exclude any debris.
  - c. Touch and drag the red vertical line to adjust the threshold. Adjust the threshold to exclude any debris.

- d. To make large changes, hold the threshold and drag. To make small changes, touch the arrow buttons located below the plot. The arrow buttons appear after you touch the threshold (the red vertical line).

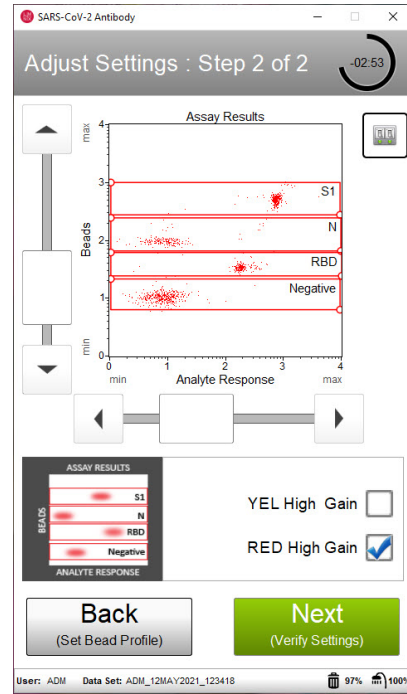
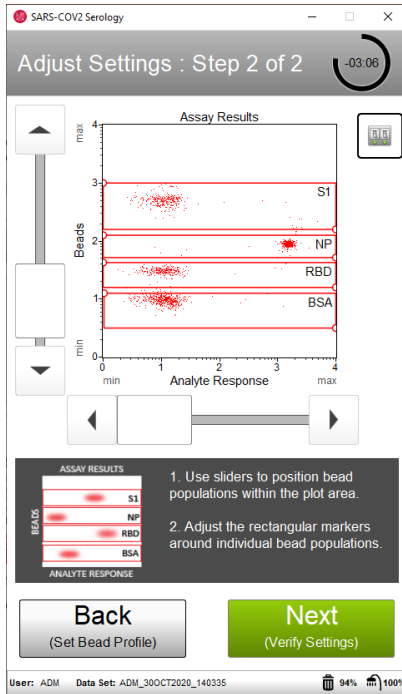
**NOTE:** If the acquisition times out after 2 minutes, select Abort (Discard Changes) to restart the Adjust Settings step, or select Next (Set Assay Result) to accept the settings and continue to the next step. Before restarting the Adjust Settings step, remove the tube and mix well before reloading and continuing.



- 2. Select **Next (Set Assay Result)** when the adjustments are complete.

3. Fine-tune the settings for the Analyte Response vs. Beads plot, if necessary. Contain each bead population within its individual gate.
  - a. Adjust the vertical and horizontal sliders on the **S1**, **N**, **RBD**, and **Negative** beads so that each bead population falls within its individual gate in the plot area.
  - b. Touch and drag the corner of a rectangular region marker to adjust a gate around a bead population.

**NOTE:** IgG and IgM samples have the YEL High Gain box deselected by default. Acquire IgA samples with the YEL High Gain box selected.



4. Select **Next** when the adjustments are complete and each bead population is in the appropriate region.
5. Verify the settings. If the settings are correct, select **Next**. The **Sample Info** screen appears.

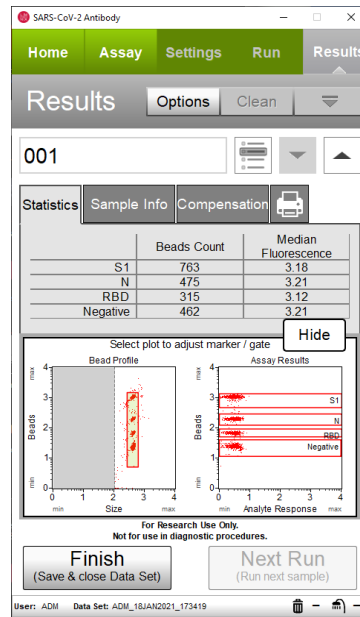
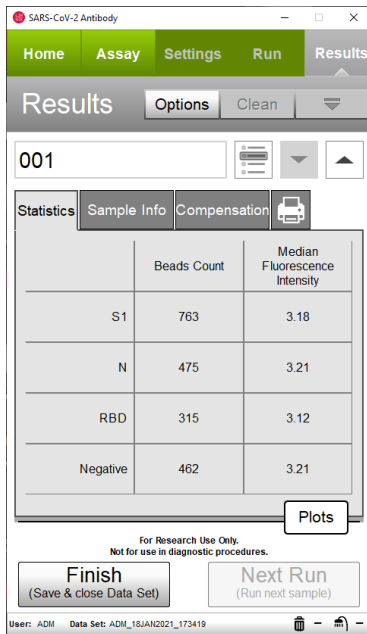
### Enter Sample Information

1. In the **Sample Info** screen, enter the **Sample ID**, **Reagent Lot #**, and the **Reagent Expiration Date** by touching the field, then using the keypad that appears on the screen to input the information.
2. Select **Done** after the Sample ID, lot number, and expiration date are entered.
3. Select **Next (Load Sample)**. The **Run Sample** screen appears.

### Acquire Sample and View Results

1. In the **Run Sample** screen, mix the first sample and load it on the instrument. Select **Run (Run Sample)** to run the sample.
2. When acquisition is complete, the results are displayed. If necessary, select **Plots** to display plots for the sample.
3. If necessary, select the **Sample Info** tab to view or change the **Sample ID** or add annotations for the current sample.

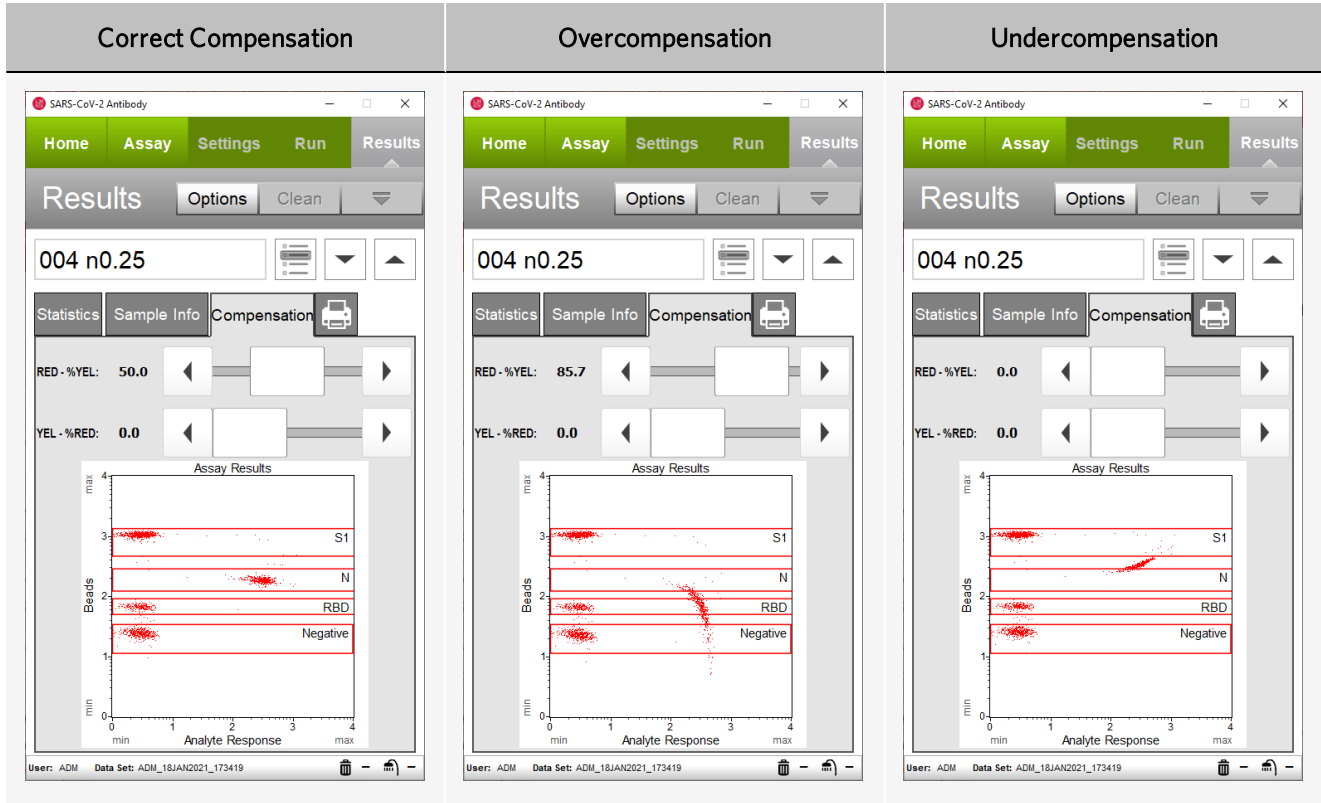
4. If necessary, select the **Printer** icon tab and print the results for the current sample.




5. (Optional) If changes are needed to the gates, touch a plot to enlarge it, then adjust the bead profile gate and/or the assay results gates, as described in the Adjust Settings step. The size threshold cannot be adjusted after the sample has been acquired.

## Perform Compensation

- Adjust the compensation if beads are bleeding from one region to the next. Select the **Compensation** tab and adjust the slider for coarse adjustments, or use the arrows for fine adjustments.



- To apply gate and marker changes to other samples that have already been acquired, select the Apply Changes icon (  ) in the title bar.
- When no further adjustments are needed, select **Next Run**.

## Acquire Remaining Samples

- For each remaining sample, perform the Enter Sample Information and Acquire Sample and View Results procedures.
- (Optional) During the run, a message prompting you to load a tube of deionized (DI) water for a Quick Clean may appear. To perform the Quick Clean, load DI water, then select **Clean**. After the Quick Clean is complete, select **Next** to continue the run.

**NOTE:** The frequency of Quick Cleans is set by the system administrator. The administrator can also choose to allow users to skip the Quick Clean when the prompt appears. To perform a Quick Clean at any time during a run, select Clean in the title bar, then Quick Clean from the menu that appears.


- After the last sample has been acquired, select **Finish**.
- (Optional) To rename the data set, export the data set for analysis, save the current instrument settings, or view the event log, select **Options** from the title bar. Refer to the *Guava® Muse® Cell Analyzer User Guide* for more information.

## Set Up and Acquire on Guava® easyCyte™

**NOTE:** Run the Guava® easyCheck™ module before performing the Guava® SARS-CoV-2 Multi-Antigen Antibody Assay on the Guava® easyCyte™ instrument. Refer to the *Guava easyCheck Kit Package Insert* for more information.

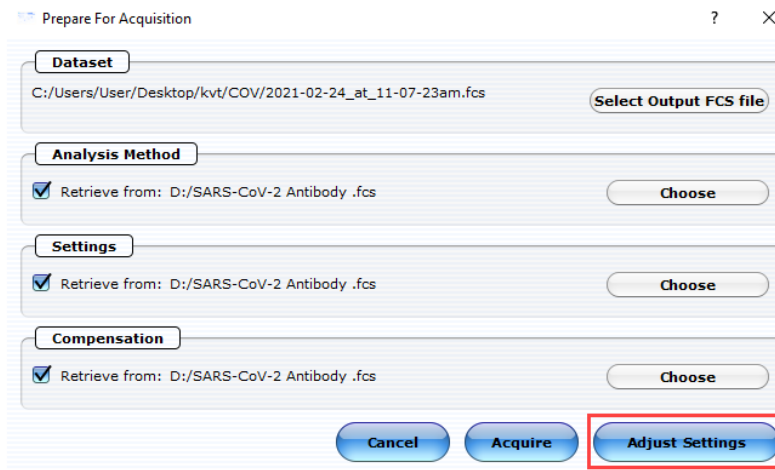
### Define a Worklist

**NOTE:** Worklists are only available when using the Guava® easyCyte™ HT instrument. If you are using an easyCyte SL instrument, skip to Prepare for Acquisition.

1. From the Guava easyCyte Acquisition Software main menu, select the **InCyte** module.
2. Click the **Edit Worklist** icon (  ).
3. Define the Worklist parameters and select **Run Worklist**.

### Prepare for Acquisition

1. The **Prepare for Acquisition** dialog box appears. Select a file name and location for the **Dataset**, then choose the **Analysis Method**, the instrument settings (the **Settings** section), and **Compensation** settings. Click **Adjust Settings** when finished.

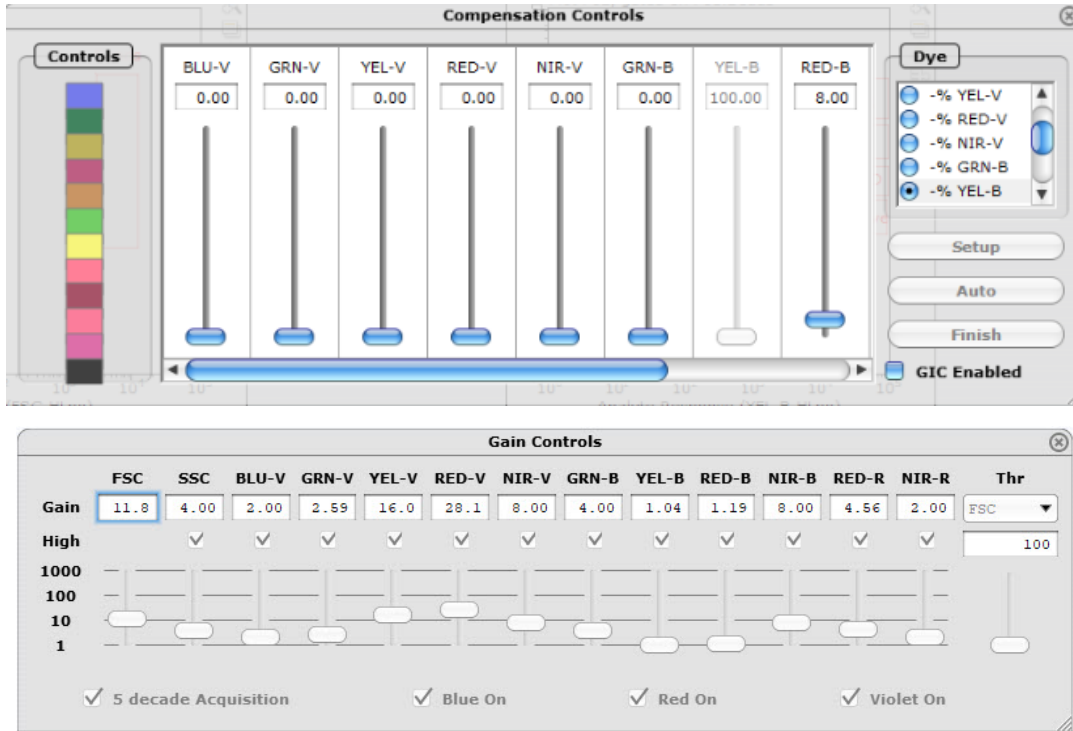


2. Place the plate or sample tubes in the tray.
3. Place the cleaning tubes in the tray.
4. After the samples and cleaning tubes are loaded, click **OK**. The **Adjust Settings** dialog box appears.

### Adjust Settings

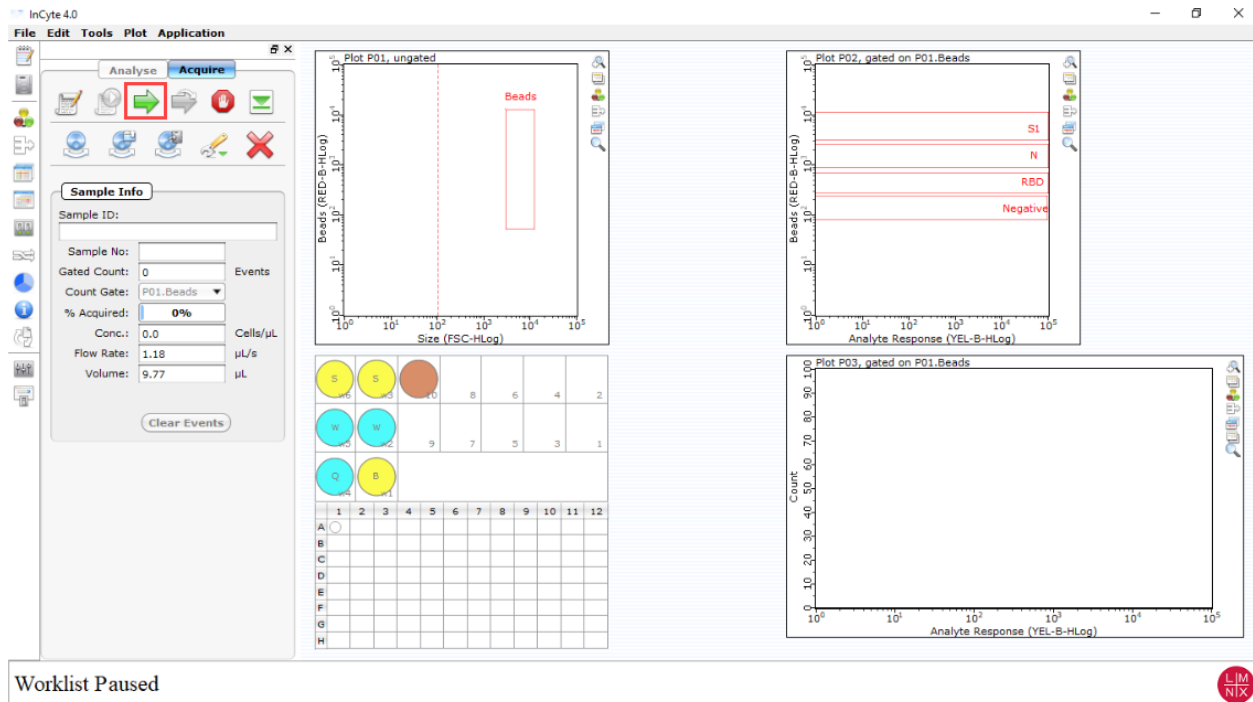
1. In the **Adjust Settings** dialog box, select a sample location to adjust settings for and click **OK**.

2. Fine-tune the settings using the **Gain Controls** and **Compensation Controls** windows.



3. Click the **Next Step** icon to finish adjusting settings

## Acquire Samples



1. Click **Resume Worklist** (easyCyte™ HT) or **Run Sample** (easyCyte SL). The system begins acquiring the first sample.



- When the Worklist or sample acquisition is complete, a copy of the Data, Method, and Analyzed Group are automatically loaded to the **Analysis** panel.

**NOTE:** For further analysis, export the files to a USB drive.

Refer to the *Guava® easyCyte™ HT System User Guide* for more information on acquiring multiple samples on the Guava easyCyte HT instrument. Refer to the *Guava easyCyte SL System User Guide* for more information on acquiring samples on the Guava easyCyte SL instrument.

## Interpretation of Results

The Guava® Muse® Acquisition Software and the Guava® easyCyte™ Acquisition Software display results on the screen after each sample is acquired. Results displayed include sample information, test results for the presence or absence (positive or negative) of each SARS-CoV-2 antigen, and the number of beads for each population. Results display with or without dot plots. You can also export a report as a PDF file.

**Figure 1: Sample Results in Guava Muse Acquisition Software**

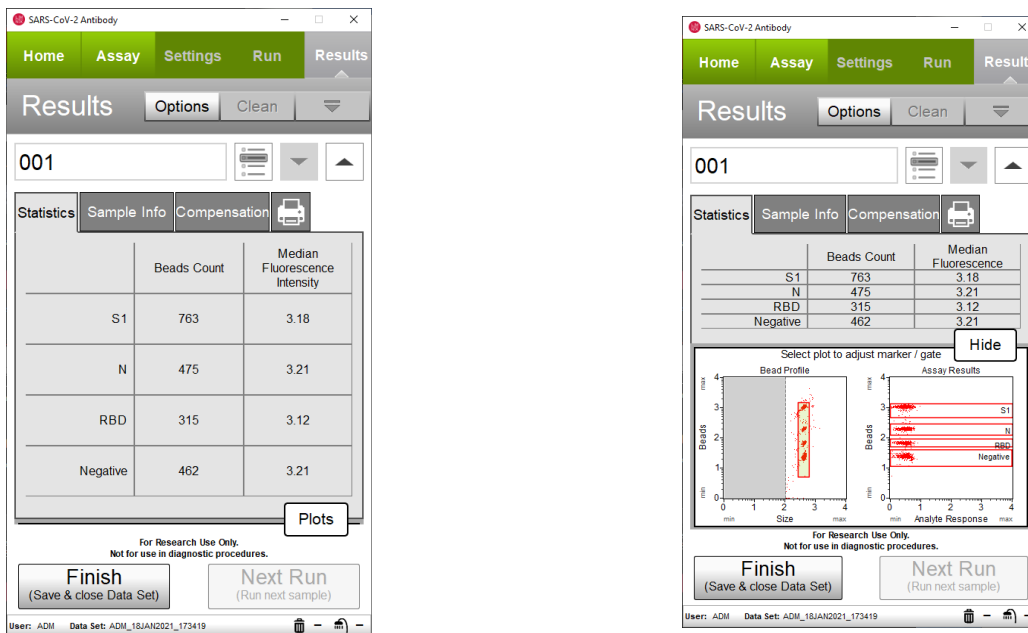
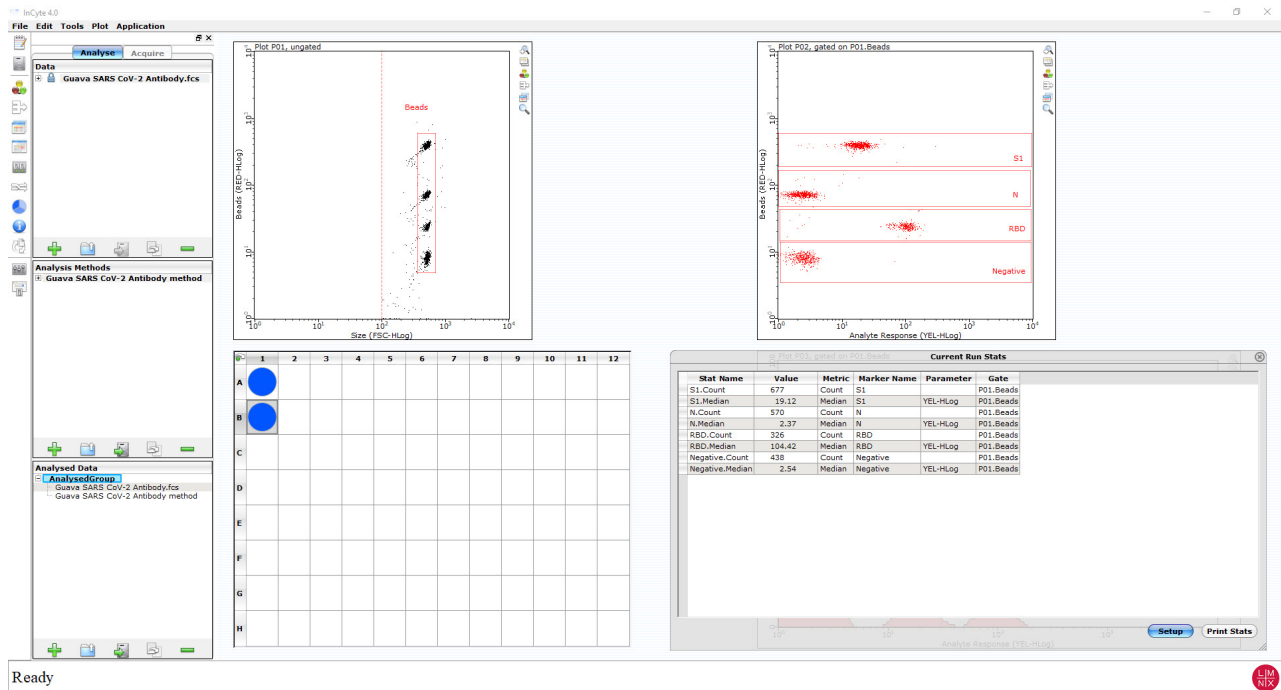


Figure 2: Sample Results in the Guava® InCyte™ Module Using a Guava easyCyte Instrument

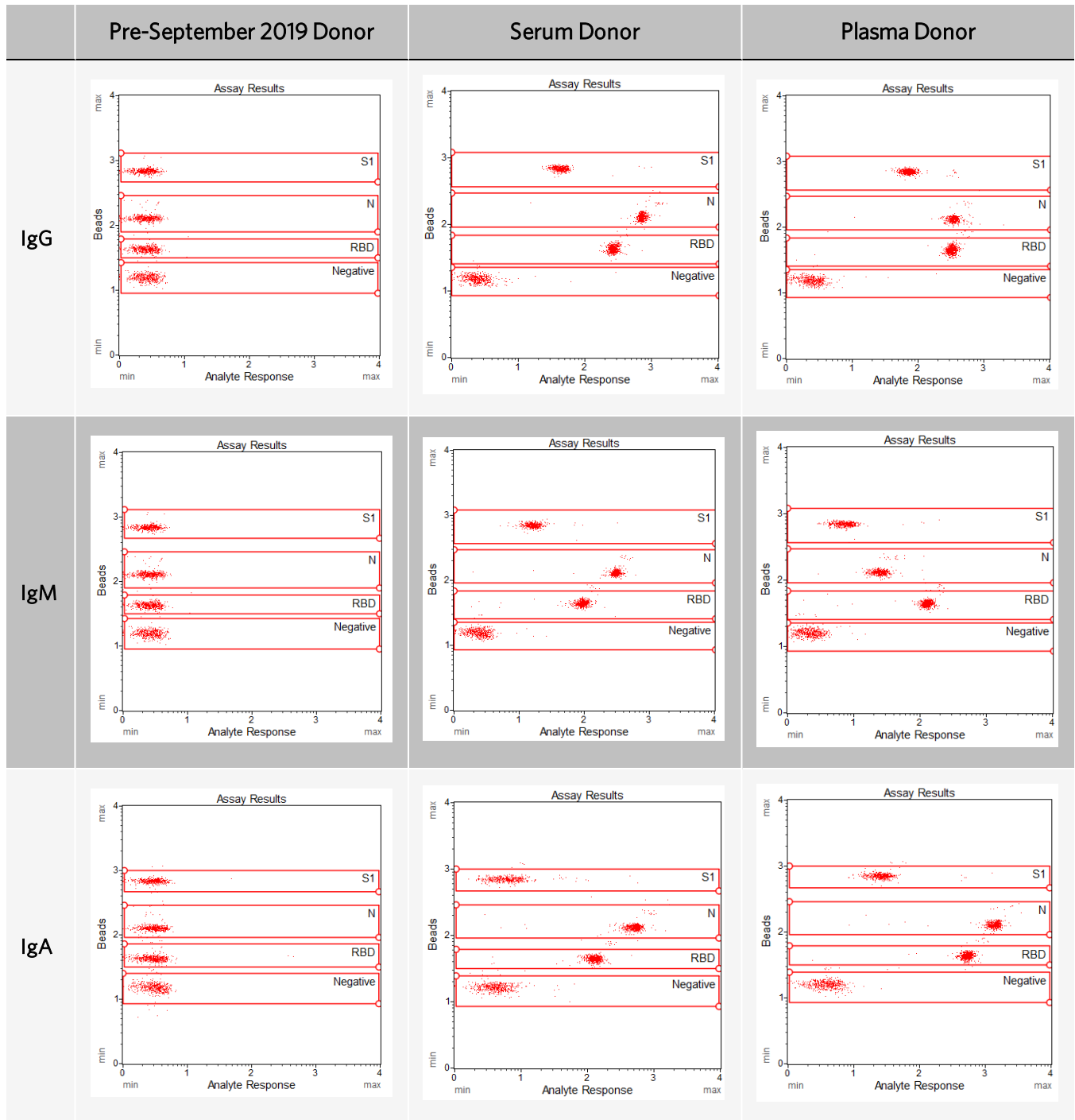


Use the S/N calculated from the median fluorescent intensity information to evaluate antibody response to each antigen. S/N is the median fluorescent intensity of each individual bead (e.g. RBD, S1, or N-bead), divided by the median fluorescent intensity of the Negative beads in the same sample.

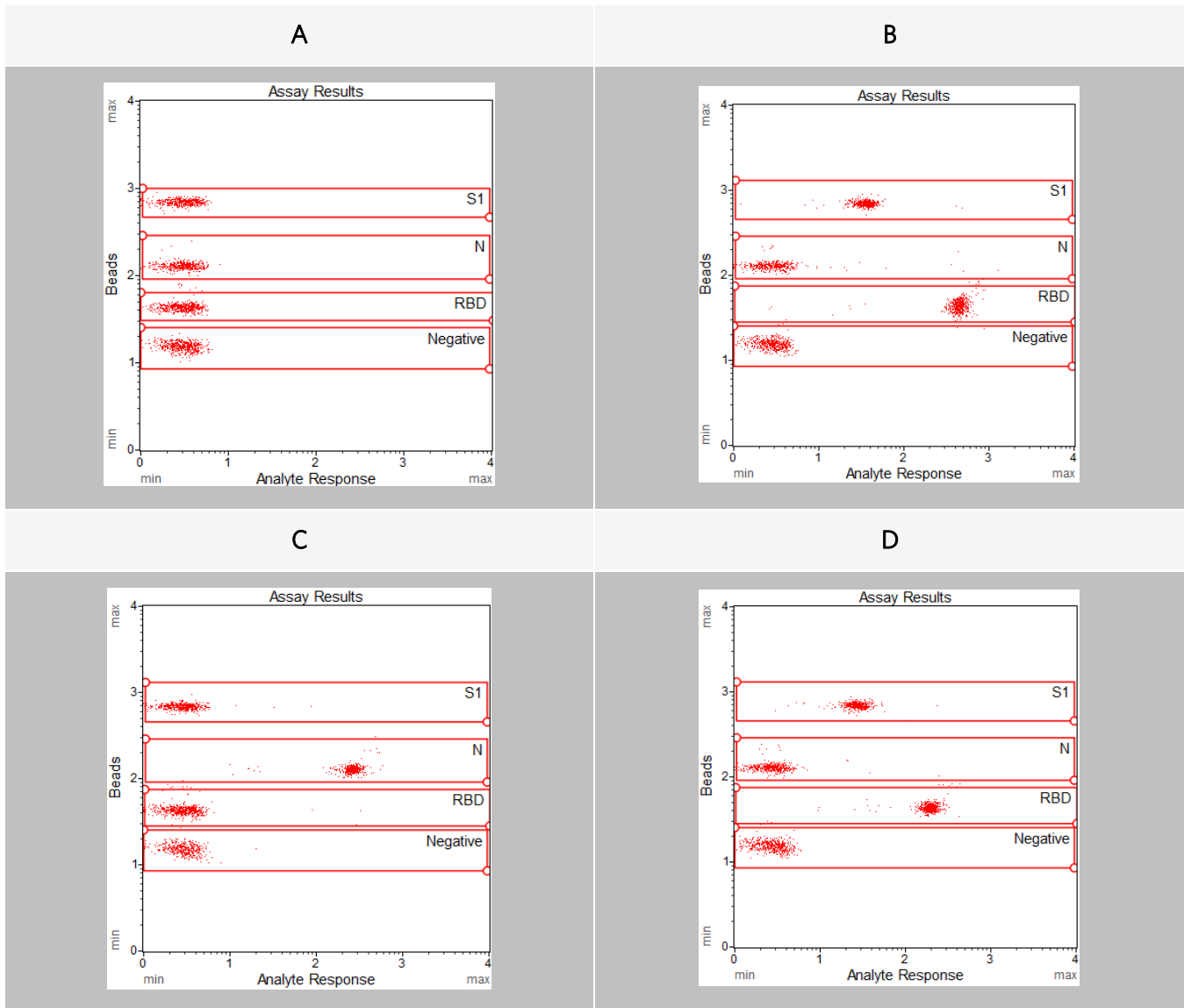
In datasets studied, an  $S/N > 2$  was typically observed in SARS-CoV-2 positive samples for IgG and IgA isotypes and distinguished them from negative samples. For IgM analysis, an  $S/N > 3$  was typically observed in SARS-CoV-2 positive samples studied.

A positive response to more than one analyte can also be combined for optimal evaluation of sample status. Users are recommended to evaluate S/N responses from known negative donors in their studies.

**Figure 3: Example Profile from the Application of the Guava® SARS-CoV-2 Multi-Antigen Antibody Assay to Negative Serum Samples, SARS-CoV-2-Positive Serum Samples, and SARS-CoV-2-Positive Plasma Samples**



The negative serum sample is a sample from a pre-September 2019 donor, and the SARS-CoV-2 samples are from donors who were positive based on molecular test results.

**Figure 4: Example of Controls Used in the Guava SARS-CoV-2 Multi-Antigen Antibody Assay**

**A:** Negative control sample using the PBS-TBN wash buffer provided in the Guava SARS-CoV-2 Multi-Antigen Antibody Assay Kit; this has a similar profile to the IgG, IgA, or IgM PE Detection Reagent included in the kit.

**B:** Commercial Human Anti-SARS-CoV-2 Spike RBD Neutralizing Monoclonal Antibody, Human IgG1, at 0.125 µg/mL, used with the Anti-Human IgG detection reagent provided in the Guava SARS-CoV-2 Multi-Antigen Antibody Assay Kit to evaluate RBD and S1 bead response.

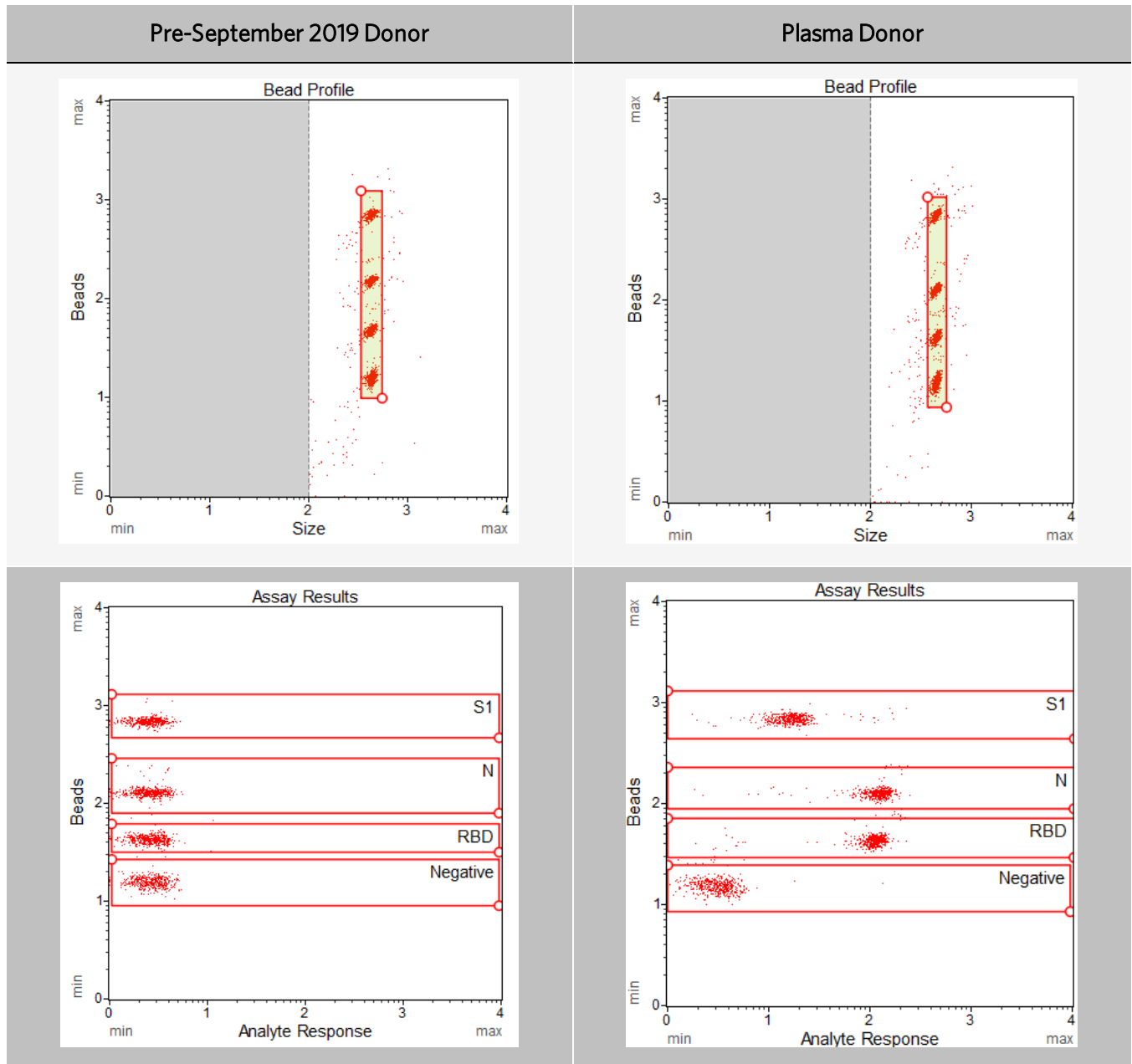
**C:** Human IgG Anti-SARS-CoV-2 Nucleoprotein Monoclonal Antibody at 0.25 µg/mL, used with the Guava SARS-CoV-2 Multi-Antigen Antibody Assay and IgG detection to evaluate N bead response.

**D:** Human IgM Anti-SARS-CoV-2 Spike (S1) Antibody at 0.125 µg/mL, used as a control with the Anti-Human IgM Detection Reagent provided in the Guava SARS-CoV-2 Multi-Antigen Antibody Assay Kit.

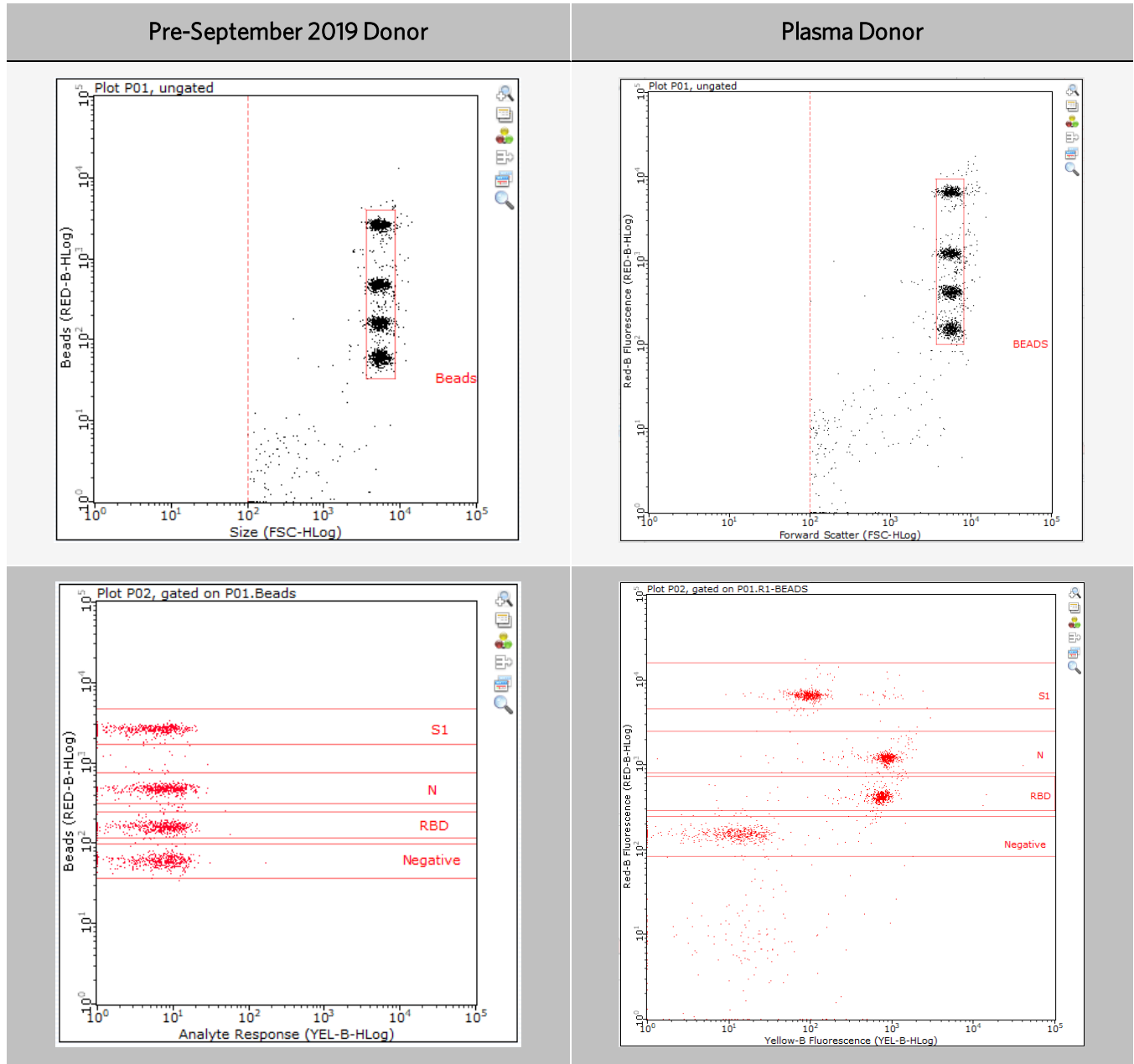
All evaluations in this figure were conducted using the Guava® Muse® Cell Analyzer.

The following figures are an analysis of negative (Pre-September 2019) and SARS-CoV-2-positive plasma samples on multiple cytometry platforms.

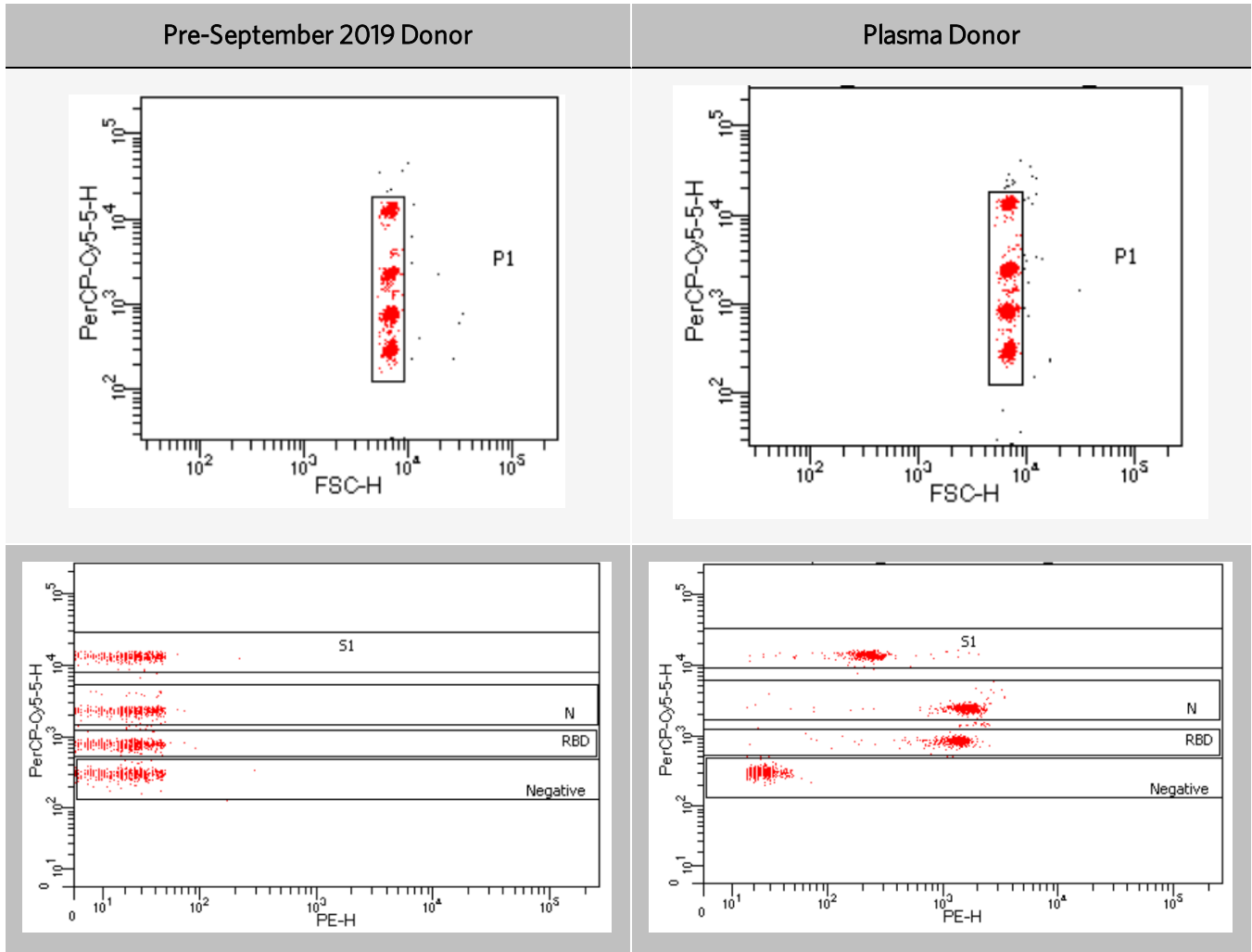
**Figure 5: Analysis of Negative (Pre-September 2019) and SARS-CoV-2-Positive Plasma Samples on the GuavaMuse Cell Analyzer**



**Figure 6: Analysis of Negative (Pre-September 2019) and SARS-CoV-2-Positive Plasma Samples on the Guava® easyCyte™ HT Flow Cytometer**



**Figure 7: Analysis of Negative (Pre-September 2019) and SARS-CoV-2-Positive Plasma Samples on a Third-Party Flow Cytometer**



Precision studies indicate that %CVs of <10% were obtainable for tube-based sample preparation and  $\leq 15\%$  for plate-based sample preparation for 10 replicates.

## Troubleshooting

Possible Problem(s)	Recommendations
Acquisition is taking longer than expected or progress bar stops during acquisition	Ensure the System Check procedure was run and passed. If the progress bar stops during acquisition, the fluid system may be clogged. Run a Quick Clean procedure.
Instrument clogging; too many events	Run a Quick Clean to clean out the capillary. This procedure can be performed during or after an assay.
"Low Bead Concentration" warning during acquisition	Handle samples carefully during separation steps to minimize bead loss during sample preparation. Insufficient beads will result in the software miscalling the population. Repeat sample preparation and testing. Vortex samples prior to running them on the Guava® Muse® Cell Analyzer, as beads can settle quickly and impact the count.
High background is seen in samples	Ensure the samples are acquired at the right setting for each isotype. High background may also result from incomplete removal of sample in the wash steps. Prepare the sample again, making sure the instructions for wash steps are followed carefully.
Low level of staining	To ensure the procedure is working properly, Luminex recommends using positive and negative controls. If control staining is low, ensure the correct volume of each reagent is added and that mixing is performed at each step when you are preparing samples. Poor staining on the Guava® easyCyte™ HT system may be due to the use of non-compatible microplates in the assay.
Variability in day-to-day experiments	If there appears to be day-to-day variation in the staining pattern, ensure the acquisition instrument is working properly. Run the Muse System Check or Guava® easyCheck™ procedure to verify proper instrument function and accuracy.
One or more bead populations appears to be missing	Confirm the beads were compensated properly and evaluate if the population can be seen after adjusting compensation. Make sure the beads are acquired using the right settings for each isotype.



Possible Problem(s)	Recommendations
High variation in samples	<p>Pipettes and/or multi-channel pipettes may not be calibrated. Calibrate the pipettes.</p> <p>Plate or tube washing was not uniform. Confirm all reagents are removed completely in all wash steps.</p> <p>Agitation was insufficient. Agitate the tube or plate during all incubation steps at a speed where the beads are in constant motion.</p>

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## Limitations of the Procedure

1. The results are dependent upon proper use of reagents, products, and instruments.
2. This assay has only been validated using Ethylenediaminetetraacetic acid (EDTA) plasma, and performance has not been established using alternate plasma collection devices.
3. Any changes to defined wash steps shall be validated.
4. Do not use heat-inactivated, icteric, hemolytic, lipemic, or cloudy samples.

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## References

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