Luminex®
complexity simplified.

xMAP® SARS-CoV-2 Multi-Antigen IgG Assay Package Insert

Rx Only

IVD For *In Vitro* Diagnostic Use.

For use under an Emergency Use Authorization (EUA) only.

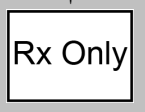
89-30000-00-872 Rev D
03/2022

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

Luminex Corporation
12212 Technology Blvd.
Austin, Texas 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
5.1.4* 	Use-by date. Indicates the date after which the medical device is not to be used.	5.4.3* 	Consult instructions for use. Indicates the need for the user to consult the instructions for use.
5.1.5* 	Batch Code. Indicates the manufacturer's batch code so that the batch or lot can be identified.	5.1.6* 	Catalog(ue) Number. Indicates the manufacturer's catalogue number so that the medical device can be identified.
5.1.7* 	Serial Number. Indicates the manufacturer's serial number so that a specific medical device can be identified.	5.4.4* 	Caution. Indicates the need for the user to consult the instructions for use for important cautionary information such as warnings and precautions that can-not, for a variety of reasons, be presented on the medical device itself.
5.5.5* 	Contains Sufficient for <n> Tests. Indicates the total number of IVD tests that can be performed with the IVD.	5.1.1* 	Manufacturer. Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.
5.3.7* 	Temperature Limit. Indicates the temperature limits to which the medical device can be safely exposed.	5.3.2* 	Keep away from sunlight. Indicates a medical device that needs protection from light sources.
5.5.1* 	*In vitro* diagnostic medical device Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.	† 	Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner (U.S. Only)

* ANSI/AAMI/ISO 15223-1:2016, Medical devices—Symbols to be used with medical device labels, labeling, and information to be supplied—Part 1: General requirements.

† 21 CFR 809 (FDA Code of Federal Regulations).

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*

Additional information is available on the 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.

Intended Use

The xMAP® SARS-CoV-2 Multi-Antigen IgG Assay is a multiplex, microsphere-based assay intended for qualitative detection of IgG antibodies to SARS-CoV-2 in human serum and plasma (dipotassium EDTA).

The xMAP SARS-CoV-2 Multi-Antigen IgG Assay is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity.

Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the detection of SARS-CoV-2 IgG antibodies. IgG antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

The sensitivity of xMAP SARS-CoV-2 Multi-Antigen IgG Assay early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

False positive results for xMAP SARS-CoV-2 Multi-Antigen IgG Assay may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

The xMAP SARS-CoV-2 Multi-Antigen IgG Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation of the Test

Background

The novel coronavirus, SARS-CoV-2 (the causative agent of COVID-19), has been responsible for the pandemic of pneumonia and associated deaths from late 2019 into 2020. The detection of the initial outbreak in the Hubei Province of China and the subsequent need for an effective diagnosis were quickly described (Li et al., 2020; Wu et al., 2020; Zhou et al., 2020). It has been reported that PCR-confirmed SARS-CoV-2 positive patients may seroconvert and develop antibodies against SARS-CoV-2 antigens anywhere from 6 to 21 days after the onset of clinical symptoms (Okba et al., 2020). The detection of human IgG antibodies to SARS-CoV-2 remains a key method, to effect contact tracing and serosurveillance (Okba et al., 2020).

The xMAP® SARS-CoV-2 Multi-Antigen IgG Assay is intended for qualitative detection of antibodies against the SARS-CoV-2 S1 subunit of the spike protein (S1), the Receptor-Binding Domain (RBD) of the spike protein, and the nucleocapsid protein (N) in serum or plasma samples from patients suspected of past COVID-19 infections by their healthcare provider.

Principles of the Procedure

The xMAP® SARS-CoV-2 Multi-Antigen IgG Assay is a serological, multiplexed microsphere-based assay to measure the presence of IgG 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.

The internal control microspheres include:

- IgG Detection Control monitors for sample addition and addition of IgG Detection Reagent,
- Background (BKGR) Control monitors for non-specific binding, and
- IgM and IgA Detection Controls monitor for isotype specificity.

The assay is compatible with the Luminex® xMAP technology and works with Luminex® 200™, MAGPIX®, and FLEXMAP 3D® Systems.

Diluted serum or plasma (dipotassium EDTA) sample is incubated with the multiplexed microspheres. The SARS-CoV-2 specific IgG antibodies present in the sample bind to the S1, RBD, and N antigen specific microspheres. The microspheres are washed and incubated with a fluorescently labeled, IgG specific Detection Reagent. The microspheres are washed again to remove unbound Detection Reagent and suspended in Wash Buffer for analysis on a Luminex xMAP compatible instrument. The presence or absence of SARS-CoV-2 specific IgG antibodies is determined by automated xMAP MULTI IgG CoV-2 Assay Software.

Assay Controls

Controls that are required but not provided with the test kit include:

Table 1. Assay Controls

Controls	Available Sources
External positive control – Human Serum or plasma sample containing SARS-CoV-2 IgG levels above the assay threshold	xMAP® SARS-CoV-2 IgG Positive Control – (included in the xMAP® SARS-CoV-2 IgG Control Kit, 30-00128) - 5% serum – requires 1:20 assay dilution to achieve expected results.
External negative control – Human Serum or plasma sample containing SARS-CoV-2 IgG levels below the assay threshold	xMAP® SARS-CoV-2 IgG Negative Control – (included in the xMAP® SARS-CoV-2 IgG Control Kit, 30-00128) - 5% serum – requires 1:20 assay dilution to achieve expected results.

Materials Provided

USB (or other media) (CN-SW74-01) with the following contents:

- xMAP® SARS-CoV-2 Multi-Antigen IgG Assay Software (MULTI IgG CoV-2)
- xMAP® SARS-CoV-2 Multi-Antigen IgG Assay Software User Manual
- 3 - xPONENT® Software Data Acquisition Protocol Files (one for each xMAP system: MAGPIX®, Luminex® 200™, and FLEXMAP 3D®)
- MAGPIX Serology Post Clean Routine
- xMAP® SARS-CoV-2 Multi-Antigen IgG Assay Package Insert
- xMAP® SARS-CoV-2 IgG Control Kit Package Insert
- Product Fact Sheets

The xMAP® SARS-CoV-2 Multi-Antigen IgG Assay Kit (30-00124) is sufficient for 96 tests.

Table 2. Kit Components

Kit Components	Part Number
xMAP® SARS-CoV-2 Multi-Antigen IgG Assay Microsphere Mix, (6 mL)	14-20304
xMAP® SARS-CoV-2 Multi-Antigen IgG Assay IgG Detection Reagent, (6mL)	14-20305
xMAP® SARS-CoV-2 Multi-Antigen IgG Assay Wash Buffer, (125 mL)	14-20308
1 - 96-Well Plate, White, Round Bottom	11-00288
2 - Thermowell® 96-Well Plate, Mylar seal, Clr, 5.25 x 3.25	11-00586
Threshold Card	89-30000-00-870

For a copy of the Safety Data Sheets (SDS), contact Luminex Technical Support or visit our website at: *www.luminexcorp.com*.

NOTE: Do not use the kit or any kit components past the expiration date indicated on the kit carton label. Do not inter-change kit components from different kit lots. Kit lots are identified on the kit carton label.

Materials Required but not Provided

Equipment:

- Luminex® 200™, MAGPIX® (all configurations), or FLEXMAP 3D® (with MFI divider enabled), System
    - xPONENT® 4.3 Software: Calibrators, Verifiers, and Controls
- Computer with:
    - Windows® 10 Operating System
    - PC specifications as stated in the *xPONENT 4.3 Release Notes*

- ELISA washer with magnetic base plate, or a magnetic base plate for manual washing
- Pipettes
- Vortex mixer
- Orbital plate shaker
- Timer

Controls:

- xMAP SARS-CoV-2 IgG Positive Control – (included in the xMAP SARS-CoV-2 IgG Control Kit, 30-00128)
- xMAP SARS-CoV-2 IgG Negative Control – (included in the xMAP SARS-CoV-2 IgG Control Kit, 30-00128)

Consumables:

- Deionized (DI) water
- 0.1 N NaOH
- 10% Bleach solution

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

- Pipette tips
- Reaction containers for diluting samples, or equivalent

Warnings and Precautions

1. For use under the FDA Emergency Use Authorization (EUA) only.
2. For *In Vitro* Diagnostic Use.
3. For prescription use only.
4. Do not pipette by mouth.
5. Do not eat, drink, smoke, or apply cosmetic products in the work areas.
6. This test has not been FDA cleared or approved; this test has been authorized by FDA under an EUA for use by laboratories certified under CLIA, that meet requirements to perform high complexity tests.
7. This test has been authorized only for the presence of IgG antibodies against SARS-CoV-2, not for any other viruses or pathogens. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
8. 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. Modifications to assay reagents, assay protocol, or instrumentation is not permitted, and are in violation of the product Emergency Use Authorization.
9. 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. Kit lots are identified on the kit carton label.
10. The Luminex® 200™, MAGPIX®, and FLEXMAP 3D® Systems require system fluid from a reservoir and discharges fluid as waste after the reading. Clear separation of supply and waste container must be ensured, as the waste fluid must be regarded as potentially infectious and disposed of accordingly.
11. 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.
12. The concentration specifications and incubation times of the manufacturer must be followed carefully.

13. Only use the individual wells of the microtiter plate once.
14. Do not replace or mix the reagents with reagents from other manufacturers.
15. Handle all samples as if infectious, using safe laboratory procedures such as those outlined in CDC/ NIH *Biosafety in Microbiological and Biomedical Laboratories, and in the CLSI Document M29 Protection of Laboratory Workers from Occupationally Acquired Infections*.
16. Reagents contain sodium azide as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Refer to local guidelines and regulations for proper disposal of unused and used reagents, as well as other laboratory reagents containing this preservative.
17. Avoid contamination from positive controls and samples by following good laboratory practices.
18. Do not dilute the controls until ready to use.
19. Wear appropriate personal protective equipment (PPE), including a lab coat and disposable gloves, when performing procedures. Wash your hands thoroughly after performing the test.
20. Follow your institution's safety procedures for working with chemicals and handling biological samples.
21. Safety Data Sheets (SDS) are available by contacting Luminex Corporation or visiting our website at *www.luminexcorp.com*.

Reagent Storage, Handling, and Stability

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

Mix the reagents thoroughly before 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. Kit lots are identified on the kit carton label.

Specimen Storage, Handling, and Transportation

The sample material can be serum or plasma (dipotassium EDTA). Serum and plasma should be separated from the blood clot as quickly as possible after removal, to avoid hemolysis. Avoid microbial contamination of the sample. Non-soluble substances must be removed from the sample prior to incubation.

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

Specimen Storage

Serum and plasma can be stored at 2°C to 8°C for up to one week. For longer storage, sample material can be stored at -20°C or lower.

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

Assay Procedure

Dilute Samples

1. Dilute the serum or plasma sample 1:400 by performing two 1:20 dilutions.
   a. Pipette 190 µL of Wash Buffer into the dilution container (not provided).
   b. Add 10 µL of non-diluted sample to the Wash Buffer. Mix thoroughly.
   c. Pipette 190 µL of Wash Buffer into a second dilution container (not provided).
   d. Add 10 µL of sample from the first dilution container to the Wash Buffer. Mix thoroughly.

Dilute External Controls

1. Dilute each control to a final 1:400 dilution of serum or plasma (dipotassium EDTA). Consider each controls' starting serum concentration - Final concentration added to the plate well should be 0.25% serum or plasma.
   a. If you are using 5% serum controls from the xMAP® SARS-CoV-2 IgG Control Kit (30-00128), separately dilute each control 1:20 by adding 10 µL of the positive or negative control to 190 µL of Wash Buffer. Mix thoroughly.
   b. If using 100% serum controls, dilute the serum controls 1:400 by performing two 1:20 dilutions:
      i. Pipette 190 µL of Wash Buffer into the dilution container (not provided).
      ii. Add 10 µL of 100% serum control to the Wash Buffer. Mix thoroughly.
      iii. Pipette 190 µL of Wash Buffer into a second dilution container (not provided).
      iv. Add 10 µL of serum control from the first dilution container to the Wash Buffer in the second dilution container. Mix thoroughly.

Setup the Plate

NOTE: Each step must be performed in the order described.

NOTE: Use a clean pipette tip for each sample and reagent addition to each well.

1. Add 50 µL of 1:400 diluted (0.25% serum or plasma) sample or control to the appropriate well of the plate.
2. Vortex the Microsphere Mix for 30 seconds. Immediately after vortexing the Microsphere Mix, add 50 µL of Microsphere Mix to each well. Seal the plate with a Thermowell® seal.

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

3. Place the plate on an orbital plate shaker. Cover the plate with foil to protect from light. Shake the plate at 800 rpm for 60 minutes at 25°C (+/- 5°C).

Wash the Plate

1. Once the plate is done shaking, remove the plate from the plate shaker.

   NOTE: Ensure shaking is completely stopped prior to plate removal.

2. Place the plate on a magnetic separator for 120 seconds to allow the microspheres to separate. Remove the seal.
3. With the plate still positioned on the magnetic separator, use a pipette to aspirate the supernatant from the sample wells.

   NOTE: Take care not to disturb the microspheres.

4. Wash the plate two times.
   a. Remove the plate from the magnetic separator. Add 150 µL of Wash Buffer to the sample wells.
   b. Place the plate on the magnetic separator for 120 seconds to allow the microspheres to separate.
   c. With the plate still positioned on the magnetic separator, use a pipette to aspirate the supernatant from the sample wells.

      NOTE: Take care not to disturb the microspheres.

   d. Wash the plate for the second time.

Incomplete washing can result in erroneous results.

Add Detection Reagent

1. Vortex the Detection Reagent for 30 seconds.
2. Add 50 µL of Detection Reagent to each sample well. Seal plate with Thermowell® seal.
3. Place the plate on an orbital plate shaker. Cover the plate with foil to protect from light. Shake the plate at 800 rpm for 60 minutes at 25°C (+/- 5°C).

Wash the Plate

1. Once the plate is done shaking, remove the plate from the plate shaker.

   NOTE: Ensure shaking is completely stopped prior to plate removal.

2. Place the plate on a magnetic separator for 120 seconds to allow the microspheres to separate. Remove the seal.
3. With the plate still positioned on the magnetic separator, use a pipette to aspirate the supernatant from the sample wells.

   NOTE: Take care not to disturb the microspheres.

4. Wash the plate two times.
   a. Remove the plate from the magnetic separator. Add 150 µL of Wash Buffer to the sample wells.
   b. Place the plate on the magnetic separator for 120 seconds to allow the microspheres to separate.
   c. With the plate still positioned on the magnetic separator, use a pipette to aspirate the supernatant from the sample wells.

      NOTE: Take care not to disturb the microspheres.

   d. Wash the plate for the second time.

Incomplete washing can result in erroneous results.

Resuspend the Microspheres

1. Remove the plate from the magnetic separator.
2. Add 100 µL of Wash Buffer and mix the reactions by gently pipetting up and down to resuspend the microspheres.

Setup System Software

If you are using a FLEXMAP 3D® System, contact Luminex Technical Support to schedule input of the MFI divider.

Please ensure you are using a PC with Windows® 10 Operating System.

Import Data Acquisition Protocols into xPONENT® Software

NOTE: Please refer to the applicable user manual.

If the protocol is already installed on the computer that controls the Luminex® instrument where the assay is being run, skip the following steps.

1. Insert the USB with the protocol files into the PC.
2. On the PC desktop, double-click the Luminex **xPONENT** icon.
3. Enter your User ID on the **System Login** tab.
4. Enter your password if you are using a secure version of the software.
5. Click **Log In**. The **Home** page displays.
6. Navigate to the **Protocols** page > **Protocols** tab.
7. Click **Import**.
8. In the **Open** dialog box, navigate to the **Protocols for Luminex xPONENT** folder.
    - For Luminex® 200™: Double-click the **LXMultiCoV2[1].lxt2** file.
    - For MAGPIX®: Double-click the **MPMultiCoV2[1].lxt2** file.
    - For FLEXMAP 3D®: Double-click the **FMMultiCoV2[1].lxt2** file.
9. Click **Open**.
10. In the **Imported Protocol File** dialog box, click **OK**. The imported protocol displays in the **Installed Protocols** section.
11. Remove the USB from the PC.

Acquire Samples

NOTE: Please refer to the applicable user manual for software requirements, setup, calibration and verification, and troubleshooting.

When setting up xPONENT®, ensure that the Use US regionalization format only option is selected in Admin > CSV Options.

Before running samples, adjust the sample probe height (at least once a week), or as needed. For more information on adjusting the sample probe height, refer to the applicable user manual. The plate name MUST be saved as **Current 96-well plate**.

Ensure the FLEXMAP 3D®, Luminex® 200™, and MAGPIX® (all configurations) Systems are cleaned between every run. For the MAGPIX System, import and run the provided MAGPIX Serology Post Clean routine between every run.

Create a Batch in xPONENT® Software

1. Navigate to the **Batches** page > **Batches** tab > click **Create a New Batch from an Existing Protocol**.
2. Choose the protocol in the **Select a Protocol** list.
    - For Luminex® 200™: **LXMultiCoV2[1].lxt2**
    - For MAGPIX®: **MPMultiCoV2[1].lxt2**
    - For FLEXMAP 3D®: **FMMultiCoV2[1].lxt2**

3. Click **Next**. Select the appropriate wells where the samples will be analyzed and then click **Unknown**. The selected wells are highlighted.
4. Click **Import List** to import a sample list or enter the appropriate Sample ID for each well. Do not change the default **Dilution** settings.
5. Click **Save**. The batch is now saved as a pending batch and ready to run.

Run Batch in xPONENT® Software

1. Click **Eject** to eject the plate carrier. Place the plate on the plate carrier in the proper orientation. Click **Retract** to retract the plate carrier.
2. Navigate to the **Batches** page > **Batches** tab.
3. Choose the pending batch that you want to run, then click **Run** to start data acquisition.
4. Verify the information in the warning dialog boxes, and click **OK**.
5. After the last sample is read in xPONENT®, click **Eject** to eject the plate carrier with the plate. Then, click **Retract**.
6. Carefully discard the plate into a biohazard bag, sealing the bag to avoid aerosolization of any residual sample.
7. Ensure the FLEXMAP 3D®, Luminex® 200™, and MAGPIX® (all configurations) Systems are cleaned between every run.

   NOTE: For the MAGPIX System, import and run the provided **MAGPIX Serology Post Clean** routine between every run. Add the reagents to the off-plate reagent reservoirs of the plate:

   - **RA1**: deionized (DI) water
   - **RB1**: 0.1N NaOH
   - **RC1**: 10% Bleach solution

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

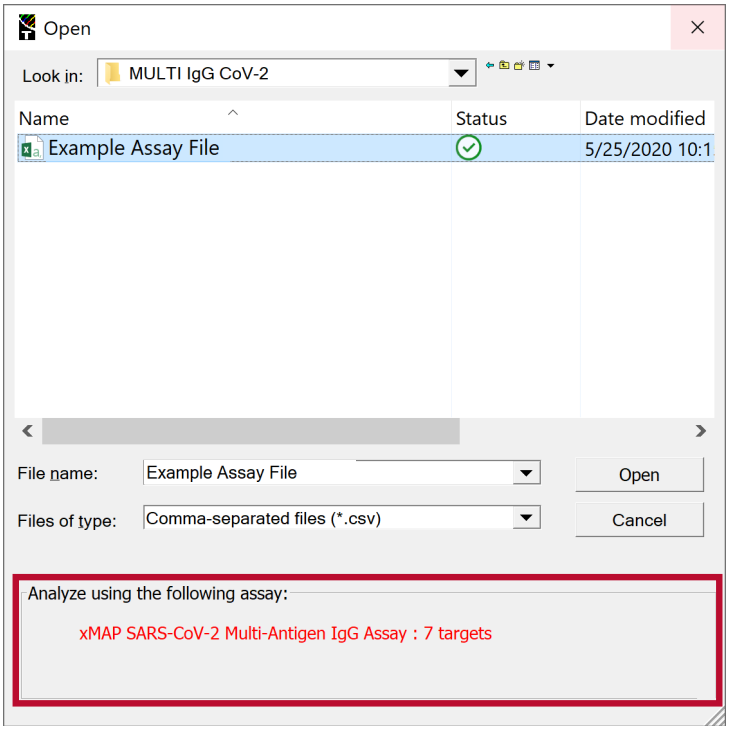
Analyze Data with xMAP® SARS-CoV-2 Multi-Antigen IgG Assay Software

NOTE: Install the xMAP® SARS-CoV-2 Multi-Antigen IgG Assay Software (Multi IgG CoV-2) according to the instructions in the user manual.

1. Navigate to the **File** menu, and choose **Open**.
2. In the **Open** dialog box, choose the **Files of type** from the drop-down menu. Choose to view either "Comma-separated files (*.csv)" or “All Files (*.*)” in the chosen directory.

   NOTE: Ensure that you choose only files created with the xPONENT® software, using the assay-specific data acquisition protocol specified in the kit package insert. If the chosen data file does not conform to the format required, the software will not analyze the data.
3. Browse to and choose the desired file.

4. In the **Analyze using the following assay** panel, ensure the correct assay is displayed.



5. Click **Open**.

   NOTE: If the chosen CSV data file does not conform to the format required, then the data has been corrupted or has not been generated appropriately and the software will not analyze the data. This can occur, for example, if the bead names have been modified during or after the data acquisition stage, or if a sample name contains an unsupported character

6. Enter the lot specific values for the assay kit, if this is your first time opening the file. If the file has been previously opened, confirm or edit the lot specific values for the assay kit.
   a. Enter or confirm the **Lot Number**.
   b. Enter or confirm the **Expiration Date** (YYYY-MM-DD).

      NOTE: The Expiration Date can also be chosen from the calendar drop-down menu.

   c. Enter or confirm the **Nucleocapsid Value**.
   d. Enter or confirm the **S1 Value**.
   e. Enter or confirm the **RBD Value**.
   f. Enter or confirm the **BKGR Value**.
7. Review the lot specific values for the assay kit to ensure they are correct, then select the **Confirm values are correct for analysis** check box.
8. Click **OK**.

Interpretation of Results

For each specimen tested, the microsphere counts and median fluorescence intensity (MFI) of the controls are checked against pre-defined threshold values. The IgG control has coupled anti-human IgG antibody to monitor for sample addition and the addition of IgG Detection Reagent. A specific pre-determined ratio of IgG control signal is compared to IgA control signal and IgM control signal to monitor isotype specificity for each specimen tested. A background (BKGR) control ensures positive samples are not the result of non-specific binding. If there is insufficient microsphere counts or any control Fails, the sample is flagged and the result is NO CALL.

Next, a lot-specific threshold value is compared against the MFI from each of the antigen specific regions. A sample will be called SARS-CoV-2 IgG Positive if the nucleocapsid (N) target antigen control is above threshold and at least one other target antigen control (S1 subunit of the spike protein (S1) and/or the Receptor-Binding Domain (RBD) of the spike protein) is above threshold.

- If IgG control is above the defined threshold and the background control, IgA control, and IgM control are below defined thresholds, then the controls are valid.
    - The MFI of the IgG control must be above 2500 MFI to pass.
    - The MFI of the IgA control must be less than or equal to 0.8 when divided by the MFI of the IgG control to pass.
    - The MFI of the IgM control must be less than or equal to 0.5 when divided by the MFI of the IgG control to pass.
- The background (BKGR) control must be less than the lot-specific threshold, for a positive result to be reported.
- If the nucleocapsid (N) protein microsphere MFI and at least one other target antigen control MFI are not above the lot-specific thresholds, a SARS-CoV-2 IgG negative call will be reported. This negative call is still valid even when the background (BKGR) control has signal above the threshold.

Table 3. Interpretation of Results

Sample Result	N	RBD	S1	C IgG	C IgM	C IgA	BKGR
SARS-CoV-2 IgG Positive	+	+	-	+	-	-	-
SARS-CoV-2 IgG Positive	+	-	+	+	-	-	-
SARS-CoV-2 IgG Positive	+	+	+	+	-	-	-
SARS-CoV-2 IgG Negative	-	+	+	+	-	-	-
SARS-CoV-2 IgG Negative	+	-	-	+	-	-	-
SARS-CoV-2 IgG Negative	-	+	-	+	-	-	-
SARS-CoV-2 IgG Negative	-	-	+	+	-	-	-
SARS-CoV-2 IgG Negative	-	-	-	+	-	-	-
SARS-CoV-2 IgG Negative	-	-	-	+	-	-	+

Sample Result	N	RBD	S1	C IgG	C IgM	C IgA	BKGR
No Call	+/-	+/-	+/-		+/-	+/-	+/-
No Call	+/-	+/-	+/-	+/-		+/-	+/-
No Call	+/-	+/-	+/-	+/-	+/-		+/-
No Call	+	+	-	+	-	-	+
No Call	+	-	+	+	-	-	+
No Call	+	+	+	+	-	-	+
No Call	-	+	+	+	-	-	+
No Call	+	-	-	+	-	-	+
No Call	-	+	-	+	-	-	+
No Call	-	-	+	+	-	-	+

Target Calls

- Positive: SARS-CoV-2 specific IgG antibodies are detected.
- Negative: SARS-CoV-2 specific IgG antibodies are not detected.
- NO CALL: there is a failure in one or more assay parameters/controls.

Control Calls

- Pass: the recommended Internal Control pattern is as expected.
- FAIL: the recommended Internal Control pattern is not as expected.
- NO CALL: unable to determine presence or absence of the Internal Controls due to an assay-specific criterion not being met.

xMAP MULTI IgG CoV-2 Assay Software uses the notes in the "Notes and explanations" column and the "Warnings/Errors" section of the Luminex® data file to determine whether there was a problem encountered during the reading of the wells.

xMAP MULTI IgG CoV-2 Assay Software does not interpret the data of the wells for which the Luminex instrument either gives a "Sample Empty" or "User cancel."

DO NOT EDIT the Luminex data file before, during, or after the data reading step, otherwise xMAP MULTI IgG CoV-2 Assay Software cannot correctly interpret the data.

xMAP MULTI IgG CoV-2 Assay Software also displays a summary column, "Detected Targets", and a "Notes and explanations" column for each sample. For more information about the various calls and messages, please refer to the applicable software user manual or the help within the software.

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

Quality Control

Luminex recommends testing both positive and negative external controls once per assay run for the xMAP® SARS-CoV-2 Multi-Antigen IgG Assay.

Limitations

1. The assay has only been validated using dipotassium Ethylenediaminetetraacetic acid (EDTA) plasma and serum.
2. Performance has not been established using alternate plasma collection devices.
3. Do not use heat-inactivated, icteric, hemolytic, lipemic, or cloudy samples.
4. This test is for clinical laboratory use only. It is not for point of care or home use.
5. A positive result may not indicate previous SARS-CoV-2 infection. Consider other information including clinical history and local disease prevalence, in assessing the need for a second but different serology test to confirm an immune response.
6. Patient specimens may be nonreactive if collected during the early (pre-seroconversion) phase of illness or due to a decline in titer over time. In addition, the immune response may be depressed in elderly, immunocompromised, or immunosuppressed patients.
7. Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Testing with a molecular diagnostic should be performed to evaluate for active infection in symptomatic individuals.
8. The assay should not be used to diagnose or exclude acute infection. Results are not intended to be used as the sole basis for patient management decisions.
9. This test is for qualitative detection of anti-COVID-19 antibody in human serum or plasma and does not measure the quantity of the antibodies
10. The assay performance characteristics have not been established for matrices other than serum or plasma.
11. This test should not be used for donor screening.
12. It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to reinfection.
13. The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Conditions of Authorization for Laboratory

The xMAP® SARS-CoV-2 Multi-Antigen IgG Assay Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas

Authorized laboratories using the xMAP SARS-CoV-2 Multi-Antigen IgG Assay must adhere to the Conditions of Authorization indicated in the Letter of Authorization as listed below:

1. Authorized laboratories* using the xMAP SARS-CoV-2 Multi-Antigen IgG Assay will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
2. Authorized laboratories using the xMAP SARS-CoV-2 Multi-Antigen IgG Assay will use the product as outlined in the authorized Instructions for Use. Deviations from the authorized procedures, including the authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the xMAP SARS-CoV-2 Multi-Antigen IgG Assay are not permitted.
3. Authorized laboratories that receive the xMAP SARS-CoV-2 Multi-Antigen IgG Assay will notify the relevant public health authorities of their intent to run the assay prior to initiating testing.
4. Authorized laboratories using the xMAP SARS-CoV-2 Multi-Antigen IgG Assay will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
5. Authorized laboratories will collect information on the performance of the xMAP SARS-CoV-2 Multi-Antigen IgG Assay and report to Division of Microbiology (DMD)/Office of Health Technology 7 (OHT7)-Office of In Vitro Diagnostics and Radiological Health (OIR)/Office of Product Evaluation and Quality (OPEQ)/Center for Devices and Radiological Health (CDRH) (via email: CDRH EUA Reporting@fda.hhs.gov) and Luminex Technical Support (www.luminexcorp.com) any suspected occurrence of false reactive or false non-reactive results and significant deviations from the established performance characteristics of the assay of which they become aware.
6. All laboratory personnel using the xMAP SARS-CoV-2 Multi-Antigen IgG Assay must be appropriately trained in immunoassay techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the xMAP SARS-CoV-2 Multi-Antigen IgG Assay in accordance with the authorized labeling. All laboratory personnel using the assay must also be trained in and be familiar with the interpretation of results of the xMAP SARS-CoV-2 Multi-Antigen IgG Assay.
7. Luminex Corporation, authorized distributors, and authorized laboratories using the xMAP SARS-CoV-2 Multi-Antigen IgG Assay will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

*The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform high complexity tests" as "authorized laboratories".

Performance Characteristics

Analytical Sensitivity and Specificity

Cross Reactivity

A total of 308 serum specimens collected in the US prior to December 2019 were tested and specificity of 100% was observed for each Luminex instrument. A total of 132 plasma specimens collected in the US prior to December 2019 were tested and specificity of 99.2% was observed for each Luminex instrument. The high specificity observed in both serum and plasma samples, demonstrates the assay is not expected to cross-react with commonly acquired illnesses.

Class Specificity

Class specificity was evaluated for each sample through internal controls designed to monitor for class specific detection. Three internal control microspheres coupled individually with anti-human IgG, anti-human IgM, and anti-human IgA monitor each sample tested for isotype specificity. When the anti-human IgG detection antibody is present with human serum and/or plasma, only the anti-human IgG microsphere is expected to cross the threshold ("Present"). Each sample is monitored to ensure the anti-human IgG microsphere provides signals above the control threshold, while the anti-human IgM and anti-human IgA microspheres are expected to provide signal below the control thresholds ("Absent"). Any sample that does not meet the expected control pattern is considered invalid and results in a "No Call".

Of the 420 serum specimens tested to define clinical agreement, >99.5% (418/420) demonstrated the expected isotype control pattern (IgG+, IgM-, IgA-). The two specimens that did not demonstrate the expected isotype control pattern resulted in a No Call.

Of the 183 plasma specimens tested to define clinical agreement, >98.9% (181/183) demonstrated the expected isotype control pattern (IgG+, IgM-, IgA-). The two specimens that did not demonstrate the expected isotype control pattern resulted in a No Call.

Clinical Agreement Study

Clinical agreement was evaluated using human serum and plasma (dipotassium EDTA).

Serum specimens collected from patients with known molecular positive results from an EUA PCR method were tested with the xMAP® SARS-CoV-2 Multi-Antigen IgG Assay . Positive percent agreement in serum was determined by using 53 serum specimens collected > 14 days from onset of symptoms, or when date of symptom onset is unknown, collected > 14 days from SARS-CoV-2 molecular positive result. In addition, 21 serum specimens collected from day 8 through 14, and 38 serum specimens collected through day 7 were also tested and positive percent agreement was calculated for each subset.

Negative percent agreement in serum was determined by using 308 presumed SARS-CoV-2 IgG antibody negative specimens from serum specimens collected in the US prior to December 2019.

Plasma specimens collected from patients with known molecular positive results from an EUA PCR method were tested with the xMAP SARS-CoV-2 Multi-Antigen IgG Assay. Positive percent agreement in plasma was determined by using 30 plasma specimens collected > 14 days from onset of symptoms, or when date of symptom onset is unknown, collected > 14 days from SARS-CoV-2 molecular positive result. In addition, 11 plasma specimens collected from day 8 through 14, and 9 plasma specimens collected through day 7 were also tested and positive percent agreement was calculated for each subset.

Negative percent agreement in plasma was determined by using 133 presumed SARS-CoV-2 IgG antibody negative specimens from plasma specimens collected in the US prior to December 2019.

The performance was determined separately for each instrument (Luminex® 200™, MAGPIX® NxTAG enabled, MAGPIX, and FLEXMAP 3D® Systems) and each matrix (serum and plasma). Any differences between the number of samples tested and the number of samples included in the performance tables are a result of No Calls.

Table 4. Clinical Agreement of xMAP® SARS-CoV-2 Multi-Antigen IgG Assay on the MAGPIX® NxTAG®-enabled System

	Days from symptom onset (or days from molecular positive)	TP	FN	PPA	95% CI	TN	FP	NPA	95% CI	Total
Serum	≤7	27	11	71.1%	55.0% - 83.0%	307	0	100.0%	99.0% - 100.0%	419
	8-14	15	6	71.4%	50.0% - 86.0%					
	>14	51	2	96.2%	87.0% - 99.0%					
Plasma	≤7	9	0	100.0%	70.0% - 100.0%	132	1	99.2%	96.0%- 100.0%	182
	8-14	9	2	81.8%	52.0%- 95.0%					
	>14	28	1	96.6%	83.0%- 99.0%					

Table 5. Clinical Agreement of xMAP® SARS-CoV-2 Multi-Antigen IgG Assay on the MAGPIX® System

	Days from symptom onset (or days from molecular positive)	TP	FN	PPA	95% CI	TN	FP	NPA	95% CI	Tot-al
Serum	≤7	27	11	71.1%	55.0%-83.0%	307	0	100.0%	99.0%-100.0%	417
	8-14	16	4	80.0%	58.0%-92.0%					
	>14	51	1	98.1%	90.0%-100.0%					
Plasma	≤7	9	0	100.0%	70.0%-100.0%	131	1	99.2%	96.0%-100.0%	180
	8-14	9	1	90.0%	60.0%-98.0%					
	>14	28	1	96.6%	83.0%-99.0%					

Table 6. Clinical Agreement of xMAP® SARS-CoV-2 Multi-Antigen IgG Assay on the FLEXMAP 3D® System

	Days from symptom onset (or days from molecular positive)	TP	FN	PPA	95% CI	TN	FP	NPA	95% CI	Tot-al
Serum	≤7	27	11	71.1%	55.0%-83.0%	307	0	100.0%	99.0%-100.0%	418
	8-14	16	4	80.0%	58.0%-92.0%					
	>14	52	1	98.1%	90.0%-100.0%					
Plasma	≤7	9	0	100.0%	70.0%-100.0%	132	1	99.2%	96.0%-100.0%	180
	8-14	9	1	90.0%	60.0%-98.0%					
	>14	27	1	96.4%	82.0%-99.0%					

Table 7. Clinical Agreement of xMAP® SARS-CoV-2 Multi-Antigen IgG Assay on the Luminex® 200™ System

	Days from symptom onset (or days from molecular positive)	TP	FN	PPA	95% CI	TN	FP	NPA	95% CI	Tot-al
Serum	≤7	28	10	73.7%	58.0%-85.0%	307	0	100.0%	99.0%-100.0%	417
	8-14	16	4	80.0%	58.0%-92.0%					
	>14	52	1	98.1%	90.0%-100.0%					
Plasma	≤7	9	0	100.0%	70.0%-100.0%	130	1	99.2%	96.0%-100.0%	178
	8-14	9	1	90.0%	60.0%-98.0%					
	>14	27	1	96.4%	82.0%-99.0%					

Luminex Corporation (Luminexreserves the right to modify its products and services at any time. Notifications will be sent to end users regarding changes that impact the use, performance and /or safety and effectiveness of the device. Any modifications to the device will be made in accordance with applicable regulatory requirements. Luminex assumes no liability for any damages resulting from the off-label application or misuse of this information.

This product, or use thereof, is covered, in whole or in part, or made by processes covered by one or more patents: *www.luminexcorp.com/patents*.

Luminex®
complexity simplified.

xMAP® SARS-CoV-2 IgG Control Kit Package Insert

Rx Only

IVD For *In Vitro* Diagnostic Use.

For use under an Emergency Use Authorization (EUA) only.

89-30000-00-885 Rev 7
07/2020

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

Luminex Corporation
12212 Technology Blvd.
Austin, Texas 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
5.1.4*	Use-by date. Indicates the date after which the medical device is not to be used.	5.4.3*	Consult instructions for use. Indicates the need for the user to consult the instructions for use.
5.1.5* LOT	Batch Code. Indicates the manufacturer's batch code so that the batch or lot can be identified.	5.1.6* REF	Catalog(ue) Number. Indicates the manufacturer's catalogue number so that the medical device can be identified.
5.1.7* SN	Serial Number. Indicates the manufacturer's serial number so that a specific medical device can be identified.	5.4.4*	Caution. Indicates the need for the user to consult the instructions for use for important cautionary information such as warnings and precautions that can-not, for a variety of reasons, be presented on the medical device itself.
5.5.5* Σ	Contains Sufficient for <n> Tests. Indicates the total number of IVD tests that can be performed with the IVD.	5.1.1*	Manufacturer. Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.
5.3.7*	Temperature Limit. Indicates the temperature limits to which the medical device can be safely exposed.	5.3.2*	Keep away from sunlight. Indicates a medical device that needs protection from light sources.
5.5.1* IVD	*In vitro* diagnostic medical device Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.	† Rx Only	Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner (U.S. Only)

* ANSI/AAMI/ISO 15223-1:2016, Medical devices—Symbols to be used with medical device labels, labeling, and information to be supplied—Part 1: General requirements.

† 21 CFR 809 (FDA Code of Federal Regulations).

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*

Additional information is available on the 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.

For Use Under Emergency Authorization Only.

For *in vitro* diagnostic use.

For Professional Use.

Intended Use

The xMAP® SARS-CoV-2 IgG Control Kit is for the verification of test performance and the detection of systematic performance deviations when run in conjunction with the xMAP SARS-CoV-2 Multi-Antigen IgG Assay for the qualitative detection of IgG antibodies to SARS-CoV-2 in human serum or dipotassium (K_2) EDTA.

The xMAP SARS-CoV-2 Multi-Antigen IgG Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

For additional information, refer to the xMAP SARS-CoV-2 Multi-Antigen IgG Assay Package Insert.

Materials Provided

The xMAP® SARS-CoV-2 IgG Control Kit, IVD 30-00128 includes:

Table 1. Kit Components

Kit Components	Amount
Positive Control (5% serum)	50 µL
Negative Control (5% serum)	50 µL

NOTE: Do not use the kit or any kit components past the expiration date indicated on the kit carton label.

Reagent Storage and Handling

xMAP® SARS-CoV-2 IgG Control Kits are shipped frozen. Store at -25°C to -15°C, frozen, once received. Once thawed, store at 2°C to 8°C, refrigerated, for up to one week. Do not re-freeze.

NOTE: Do not use the kit or any kit components past the expiration date indicated on the kit carton label.

Assay Controls

Positive and negative controls contain inactivated human sera reactive for anti-SARS-CoV-2 IgG spiked into human sera non-reactive for anti-SARS-CoV-2 IgG found to be non-reactive for HIV-1 Ag, HIV-2 Ag, anti-HIV-1/HIV-2, HBsAg, and anti-HCV.

Negative and positive controls are provided pre-diluted 1:20 in a buffer solution containing stabilizers and preservatives (resulting in a final concentration of 5% serum).

Warnings and Precautions

1. For use under the FDA Emergency Use Authorization (EUA) only.
2. For *In Vitro* Diagnostic Use.
3. For prescription use only.
4. Do not pipette by mouth.
5. Do not eat, drink, smoke, or apply cosmetic products in the work areas.
6. This test has not been FDA cleared or approved; this test has been authorized by FDA under an EUA for use by laboratories certified under CLIA, that meet requirements to perform high complexity tests.
7. This test has been authorized only for the presence of IgG antibodies against SARS-CoV-2, not for any other viruses or pathogens. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
8. 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. Modifications to assay reagents, assay protocol, or instrumentation is not permitted, and are in violation of the product Emergency Use Authorization.
9. Do not use the kit or any kit components past the expiration date indicated on the kit carton label.
10. Only use the individual wells of the microtiter plate once.
11. Do not replace or mix the reagents with reagents from other manufacturers.
12. Handle all samples as if infectious, using safe laboratory procedures such as those outlined in CDC/ NIH *Biosafety in Microbiological and Biomedical Laboratories, and in the CLSI Document M29 Protection of Laboratory Workers from Occupationally Acquired Infections*.
13. Reagents contain sodium azide as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Refer to local guidelines and regulations for proper disposal of unused and used reagents, as well as other laboratory reagents containing this preservative.
14. Avoid contamination from positive controls and samples by following good laboratory practices.
15. Do not dilute the controls until ready to use.
16. Wear appropriate personal protective equipment (PPE), including a lab coat and disposable gloves, when performing procedures. Wash your hands thoroughly after performing the test.
17. Follow your institution's safety procedures for working with chemicals and handling biological samples.
18. Safety Data Sheets (SDS) are available by contacting Luminex Corporation or visiting our website at www.luminexcorp.com.

Prepare Controls for Use

1. Thaw and bring to room temperature before use.
2. Quick spin the tube to ensure the entire volume is at the bottom.
3. Prior to each use, mix by pipetting up and down twice.
4. Dilute each control to the intended final serum concentration. Refer to the appropriate assay package insert for specific instructions.

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

Quality Control

Luminex recommends testing both positive and negative controls once per assay run for the xMAP® SARS-CoV-2 Multi-Antigen IgG Assay.

Luminex Corporation (Luminex) reserves the right to modify its products and services at any time. Notifications will be sent to end users regarding changes that impact the use, performance and /or safety and effectiveness of the device. Any modifications to the device will be made in accordance with applicable regulatory requirements. Luminex assumes no liability for any damages resulting from the off-label application or misuse of this information.