# Luminex

## complexity simplified.

# NxTAG® CoV Extended Panel Assay Package Insert

**IVD** For *In Vitro* Diagnostic Use.

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

MLD-054-KPI-002 Rev C
03/2021

**REF** I054C0463

NxTAG CoV Extended Panel (IVD)  Σ  96 TESTS

# Symbols Glossary

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

| Symbol | Meaning | Symbol | Meaning |
|---|---|---|---|
| 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 cannot, for a variety of reasons, be presented on the medical device itself. | 5.1.4* | Use-by date. Indicates the date after which the medical device is not to be used. |
| 5.1.5* LOT | Batch Code. Indicates the manufacturer's batch code so that the batch or lot can be identified. | 5.1.1* | Manufacturer. Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC. |
| 5.5.5* | Contains Sufficient for <n> Tests. Indicates the total number of IVD tests that can be performed with the IVD. | 5.3.7* | Temperature Limit. Indicates the temperature limits to which the medical device can be safely exposed. |
| 5.4.3* | Consult instructions for use. Indicates the need for the user to consult the instructions for use. | 5.1.6* REF | Catalog(ue) Number. Indicates the manufacturer's catalogue number so that the medical device can be identified. |
| 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. | 5.3.4* | Keep dry. Indicates a medical device that needs to be protected from moisture. |
| 5.2.8* | Do not use if package is damaged. Indicates a medical device that should not be used if the package has been damaged or opened. | | |

* 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.

# 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

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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

NxTAG® CoV Extended Panel Assay for use on Luminex® MAGPIX® instrument is a RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal swab specimens from individuals suspected of COVID-19 by their healthcare provider.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal swab specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The NxTAG CoV Extended Panel is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of reverse transcriptase-PCR and *in vitro* diagnostic procedures.

For *In Vitro* Diagnostic Use. For Emergency Use Authorization (EUA) only.

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# Summary and Explanation of the Test

## Background

There is currently an outbreak of respiratory disease caused by a novel coronavirus that was first detected in Wuhan City, Hubei Province, China, which has now been designated a pandemic by the World Health Organization (WHO) and which has been detected internationally, including cases in the United States. The virus has been named "SARS-CoV-2" and the disease it causes has been named "Coronavirus Disease 2019" (COVID-19). SARS-CoV-2 has demonstrated the capability to spread rapidly, leading to significant impacts on healthcare systems and causing societal disruption. The potential public health threat posed by COVID-19 is high, both globally and to the United States. To respond effectively to the COVID-19 outbreak, rapid detection of cases and contacts, appropriate clinical management and infection control, and implementation of community mitigation efforts are critical. On February 4, 2020, the Secretary of Health and Human Services (HHS) determined that there is a public health emergency and that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of the novel coronavirus (2019-nCoV). Rapid detection of COVID-19 cases in the United States requires wide availability of diagnostic testing to control the emergence of this rapidly spreading, severe illness. [FDA - Policy for Diagnostic Tests for Coronavirus Disease-2019 during the Public Health Emergency, March 16, 2020].

The NxTAG® CoV Extended Panel Assay is intended for qualitative detection of SARS-CoV-2 RNA in nasopharyngeal swab specimens from individuals suspected of COVID-19 by their healthcare provider. The specimens can be collected in Universal Transport Medium (UTM-RT®, Copan Diagnostics, Inc, Catalogue 328C), Universal Viral Transport (UVT, BDTM Diagnostics, Catalogue 2200220) or equivalent. The collection media are not included in the test kit.

# Principles of the Procedure

The NxTAG® CoV Extended Panel (NxTAG CoV) incorporates multiplex Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) with the Luminex® proprietary universal tag sorting system on the Luminex platform to easily detect SARS-CoV-2s. Extracted total nucleic acid is added to pre-plated, Lyophilized Bead Reagents (LBRs) and mixed to resuspend the reaction reagents. The reaction is amplified via RT-PCR and the reaction product undergoes near simultaneous bead hybridization within the sealed reaction well. The hybridized, tagged beads are then sorted and read on the MAGPIX® instrument. The generated signals are analyzed using the NxTAG CoV Extended Panel Assay File for SYNCT™ Software, providing a reliable, qualitative call for SARS-CoV-2 viral target and internal controls within each reaction well.

The following genes are detected by the NxTAG CoV Extended Panel Assay.

| Virus Target | Gene Targets |
|---|---|
| Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) | ORF1ab |
| | N Gene |
| | E Gene |

## Figure 1: NxTAG® CoV Assay Workflow
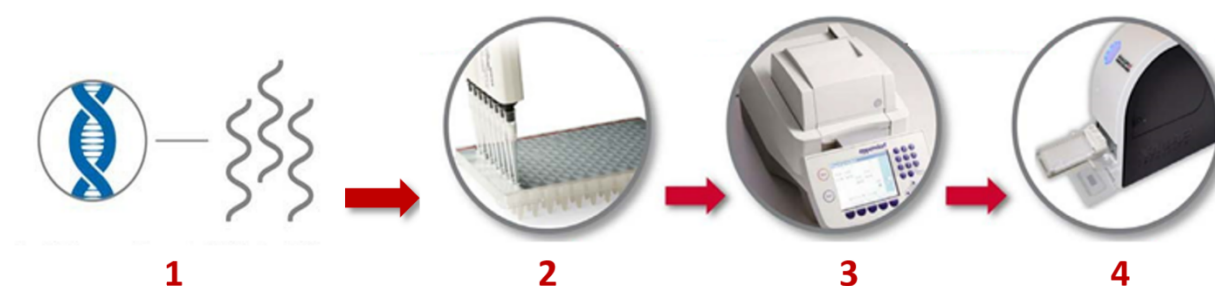


| Step 1 | Nucleic acid extraction |
|--------|--------------------------|
| Step 2 | Load extracted nucleic acid to pre-plated test wells |
| Step 3 | Multiplex RT-PCR and hybridization |
| Step 4 | Data acquisition on MAGPIX® instrument |

## Assay Controls

- **Internal Control** - Bacteriophage MS2 is included in the NxTAG® CoV Extended Panel Assay kit. This internal positive control is added to each sample prior to extraction. This internal control allows the user to ascertain whether the assay is functioning properly. Failure to detect the MS2 control indicates a failure at either the extraction step, or the reverse-transcription step, or the PCR step. Low or no MS2 signal may be indicative of the presence of amplification inhibitors, which can lead to false negative results.
- **Positive Controls** -  is not included in NxTAG CoV Extended Panel Assay kit but is recommended to be included in every run, as a good laboratory practice. External positive controls should be used in accordance with local, state, federal accrediting organizations, as applicable. Luminex has used purified RNA (BEI Resources, NR-52385) in NxTAG CoV Extended Panel Assay studies. The positive control is prepared by adding 1 µL (2.5E+03 GCE/µL) of purified RNA to 200 µL of NucliSENS® easyMag® lysis buffer containing 1 µL (10 µg/µL) of carrier RNA (Poly A single-stranded RNA homopolymer (GE Healthcare Life Sciences 27411001)) and 10 µL of Bac-teriophage MS2 and subjected to extraction procedure described above.
- **No Template Control (NTC)** - RNase-free water is used as negative amplification control.
- **Negative Extraction Control** – The negative extraction control is the collection media that has undergone the entire assay procedure, starting from extraction.

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# Materials Provided

The following table outlines reagents supplied in the kit and their storage conditions.

Table 1. Reagents Supplied with the NxTAG® CoV Extended Panel (NxTAG CoV) Kit

| Reagents | Volume for 96 Tests | Storage Conditions |
|---|---|---|
| NxTAG® CoV Extended Panel (NxTAG CoV) Plate | 1 - 96-well plate containing 2 Lyo-philized Bead Reagents per well | Store at 2°C to 8°C in the re-sealable pouch provided; avoid exposure to light and moisture. |
| MS2 Phage Internal Control | 1.5 mL x 1 vial | Store at -25°C to 8°C. |
| Foil Seals | 8 pieces x 1 case | Store at 2°C to 30°C. Store at 15°C to 30°C after first use. |

For a copy of the Safety Data Sheet (SDS), contact Luminex Technical Support.

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.

NOTE: The kit is shipped at 2°C to 30°C. Upon receipt, store the kit at 2°C to 8°C.

NOTE: To avoid exposing the NxTAG CoV plate to moisture, do not discard the desiccants included in the resealable pouch. Ensure the pouch is completely sealed after each use.

## Software Supplied

- The NxTAG® CoV assay file for use in the SYNCT™ Software, the MAGPIX® data acquisition protocol file for use in xPONENT® Software, and the package insert is provided on a USB or can be requested from Luminex Technical Support.

# Materials Required but not Provided

## Recommended Extraction Agents

Choose an extraction system from the list below. The associated reagents and consumables are also required.

- bioMérieux® NucliSENS® easyMAG® System (Product No. 280140) with Generic protocol and associated reagents and consumables
- bioMérieux EMAG® System (Product No. 418591) with Generic protocol and associated reagents and consumables

# Equipment

- Computer with:
    - Operating System Microsoft® Windows® 7, 64-bit or Windows 10
    - PC Specifications as stated in the *SYNCT*™ *Release Notes*
    - SYNCT Software
- Luminex® instrument (MAGPIX®)
    - xPONENT® Software, calibrators, verifiers, controls, and Drive Fluid/Drive Fluid PLUS
- Multichannel pipette or single channel pipette (10 µL to 200 µL)
- Sonicator bath (Ultrasonic Cleaner, Cole-Parmer®, A-08849-00) or equivalent
- PCR cooler rack (Eppendorf® 022510509) or equivalent
- Micronic Pierceable TPE Capmat Black (Cat. No. MP53087) or equivalent for thermal cyclers without adjustable lids
- End-point Thermal Cycler

# Consumables

- Optional: EMAG® 1000 µL tips (bioMérieux® Ref. 418922)
- Isopropanol (70%)
- DNase/RNase-Free Water
- NxTAG® Probe Adjustment Strip (Cat # C000Z0452)
- 96-well Non-Skirted Plate in a clear frame (Cat # C000Z0453) for thermal cyclers that are not compatible with fully-skirted plate
- Skirted Plate (Cat # C000Z0455) (96-well in white frame)

# Replacement Materials (if needed)

NOTE: Full foil sheets can be purchased from 4titude, Catalog #: 4ti-0531.

- Foil Seals (Cat # C000Z0454) (8 pieces per case, each piece reseals 3 strips of 8-vessel/strip)

# Warnings and Precautions

1. For Use under the Emergency Use Authorization (EUA) only.
2. For *In Vitro* Diagnostic Use.
3. Do not eat, drink, smoke, or apply cosmetic products in the work areas.
4. Positive results are indicative of SARS-CoV-2 RNA.
5. Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free of DNases and RNases. Use only supplied or specified required consumables to ensure optimal test performance.

6. Care should be taken when handling, storing, and disposing of potentially infectious materials. Suitable barrier protection against potential pathogens is recommended during all stages of use. Gloves and laboratory coats should be worn at all times. Adherence to appropriate local biosafety and biohazard guidelines or regulations is recommended when working with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. Handle waste disposal in accordance with accepted medical practice and applicable regulations.

7. All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.

8. Fresh clean gloves must be worn in each area and must be changed before leaving that area.

9. Do not pipette by mouth.

10. For pre-analytical (sample extraction) steps, use the procedure that is provided with the sample extraction system.

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. Modifications to assay reagents, assay protocol, or instrumentation is not permitted, and are in violation of the product Emergency Use Authorization.

12. 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.

13. In the event of damage to the protective packaging, consult the Safety Data Sheet (SDS) for instructions.

14. Safety Data Sheets (SDS) are available by contacting Luminex Corporation or visiting our website at *www.luminexcorp.com*.

# Assay Procedure

## Sample Collection

**NOTE:** Standard precautions should be taken with regard to sample collection, handling, and storage prior to extraction (refer to the latest edition of the CLSI MM13-A Guideline[1]; and Farkas et al. (1996)[2]).

Collect and extract samples and external controls by either bioMérieux® NucliSENS® easyMAG® System or bioMérieux EMAG® System.

The recommended type for the NxTAG® CoV is a nasopharyngeal swab in Universal Transport Media (UTM™) or equivalent.

## Prepare Sample

### Extract Nucleic Acid

1. Briefly vortex to mix the sample.

2. Spike 10 µL of MS2 (internal control) into 200 µL of patient nasopharyngeal specimen.

3. Use one of the recommended extraction procedures (described below) for the NxTAG® CoV Extended Panel Assay.

   **NOTE:** Luminex recommends at least one negative extraction control per extraction batch.

## Extract Nucleic Acid using the bioMérieux® easyMAG® and EMAG® Systems

**NOTE:** The nucleic acid purification parameters for NxTAG® CoV are the same as the NxTAG Respiratory Pathogen Panel.

**NOTE:** Refer to the manufacturer's instructions for use of bioMérieux® easyMAG® and EMAG®.

To configure the easyMAG®, use these parameters:

Table 2.  Parameter for the bioMérieux® easyMAG® System with Generic Protocol

| Page Name | Parameters | Settings |
|---|---|---|
| Define Extraction Request | Sample ID | Enter Sample ID |
| | Protocol | Generic |
| | Matrix | Other |
| | Volume | 0.200 mL |
| | Eluate | 110 µL |
| | Type | Primary |
| | Priority | Normal or High |
| Create Run (New Run window) | Run | Enter run name |
| | Workflow | Select: On-board Lysis Incubation, On-board silica incubation |

Table 3. bioMérieux® easyMAG® System Silica Preparation and Addition

| Extraction Step | Instructions |
|---|---|
| Silica Preparation | Dilute easyMAG® silica 1:1 in DNAse/RNAse free water |
| Silica Addition | Add 100 µL of diluted silica after on-board lysis incubation is complete, pipette mix five times at 1000 µL |

To configure the EMAG® for use with NxTAG CoV, create an NxTAG CoV extraction protocol using the new protocol menu and the parameters provided below:

<u>Table 4. Parameter for the bioMérieux® EMAG® System with Generic Protocol</u>

| Tab Name | Parameters | Settings | | |
|---|---|---|---|---|
| General | Extraction Method Name | Name the protocol. Example: (NxTAG CoV) | | |
| | Description | Write a description of the protocol. Example: "Luminex protocol for NxTAG CoV sample extraction" | | |
| Input | Off-board Lysis | Off (Do not select) | | |
| | Matrices | Respiratory | | |
| | Valid input volumes | List volume: 210 µL<br>Default volume: 210 µL | | |
| Preparation | Add these items to the **Preparation protocol steps** table in the indicated order | **#** | **Preparation protocol steps** | **Details of selected step** |
| | | 1 | Samples already prepared: | Do nothing |
| | | 2 | Distribute reagent bottle to well: | Reagent Bottle: LB (lysis buffer)<br>Volume: 2000 µL |
| | | 3 | Incubate at room temperature: | Duration: 600 seconds |
| | | 4 | Transfer silica to well: | Silica Name: Silica<br>Volume: 50 µL |
| | | 5 | Incubate at room temperature: | Duration 600 seconds |
| Extraction | Extraction Protocol | Generic | | |
| | Valid Elution Volume | List volume: 110 µL, Default volume: 110 µL | | |
| Eluate Transfer | - | Select: Keep eluates in vessel | | |
| Status | - | Activated | | |

**NOTE:** If needed, store extracted nucleic acid at -70°C to -80 °C until testing or up to one month with NxTAG CoV Extended Panel Assay.

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# Program and Preheat Thermal Cycler

**NOTE:** Perform PCR setup in the pre-PCR area.

Program the following PCR protocol into the thermal cycler with a heated lid (105°C), and pre-heat the thermal cycler to 42°C prior to plate setup:

**NOTE:** The thermal cycling and data acquisition parameters are the same for NxTAG® CoV and NxTAG Respiratory Pathogen Panel, both assays can be run on the same thermal cycler and MAGPIX; however, care must be taken to properly identify each assay.

Figure 2: PCR and Hybridization Conditions



| 1 Cycle<br>42°C<br>20 min | 1 Cycle<br>95°C<br>2 min 30 sec | 15 Cycles<br>95°C – 20 sec<br>65°C – 60 sec<br>72°C – 10 sec | 24 Cycles<br>95°C – 20 sec<br>56°C – 60 sec<br>72°C – 10 sec | 1 Cycle<br>37°C<br>45 min | End<br>37°C<br>HOLD |

The total thermal cycling run time for NxTAG CoV should range between 2 hours 15 minutes and 2 hours 25 minutes.

# Setup the NxTAG® CoV Reaction Plate

**NOTE:** Pre-heat the thermal cycler to 42°C prior to plate setup.

1. If frozen, thaw the extracted nucleic acid samples on ice. Briefly vortex the samples followed by a quick spin to collect the samples to the bottom.
2. Place samples on a chilled PCR cooler block or equivalent.
3. Remove the assay plate from its storage pouch. Place the required number of vessels into the appropriate PCR set-up plate (e.g., skirted plate for Eppendorf® and non-skirted plate for ABI Veriti thermal cycler).
   a. Firmly press down on the strips to snap into place, ensuring they are flush with the plate surface.
   b. Return unused vessels to the pouch, seal, and store at recommended storage conditions.
      **NOTE:** Protect the assay plate from prolonged light exposure.
4. Tap the plate on the benchtop to ensure the Lyophilized Bead Reagents (LBRs) are at the bottom of the vessel.
5. Place the plate on a chilled PCR cooler block or equivalent.
   **NOTE:** Keeping the PCR reaction cold during sample addition is important for limiting background signal.
6. Use the end-tabs to peel the clear release liner.
   **NOTE:** Do not touch the black adhesive.
7. Dispense 35 µL of sample or control to each PCR vessel, by using the pipette tip to pierce the foil at an angle.
   a. Insert the tip a third to halfway down into the vessel.
   b. Dispense the sample into the vessel and wait 1 to 2 seconds while maintaining the pipette tip inside the vessel.
   c. Push the tip all the way to the bottom of the vessel and pipette up and down at least three times to reconstitute the LBRs.
8. Reseal the plate after the sample addition using the precut strips of foil provided. Apply the foil(s) directly on top of the plate and press firmly on and around the wells to ensure a tight seal.
   **NOTE:** Ensure the foil covers the wells and surrounding black adhesive.
   **NOTE:** Do not vortex and spin down the plate.

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# Run Thermal Protocol

## Start the Thermal Program

1. Place the foil-sealed plate in the pre-heated thermal cycler and run the protocol.
2. If using a thermal cycler without an adjustable lid, place a micronic pierceable TPE Capmat black or equivalent on top of the sealed plate.

# Setup System Software

## Import the Data Acquisition Protocol into xPONENT® Software

**NOTE:** Please refer to the applicable user manual. Ensure the NxTAG® CoV Extended Panel (NxTAG CoV) data acquisition protocol is saved to a location that is accessible by the xPONENT® Software on the MAGPIX® computer.

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

1. Log into xPONENT Software.
2. Navigate to the **Protocols** page > **Protocols** tab.
3. Click **Import**.
4. In the **Open** dialog box, browse to the folder where the NxTAG CoV Extended Panel data acquisition protocol is located, and choose the **NxTAG CoV T-A[1].lxt2** protocol file. Click **Open**.
5. In the **Imported Protocol File** dialog box, click **OK**. The imported protocol displays in the **Installed Protocols** section.

# Acquire Samples

## Prepare the System

**NOTE:** Please refer to the applicable user manual for software requirements, setup, calibration and verification, and troubleshooting.
**NOTE:** When setting up xPONENT®, ensure that the Use US regionalization format only option is selected in Admin > CSV Options.
**NOTE:** Make sure you are using a NxTAG®-enabled MAGPIX® instrument.

1. Log into the xPONENT Software.
2. Perform the **Enhanced Startup Routine** at least once a week along with the required probe sonication.
3. Adjust the sample probe height at least once a week, or as needed.
   a. When adjusting the sample probe height, use the same plate type that will be used when running the assay plate. Use either the skirted plate or the non-skirted plate (if using an ABI thermal cycler) with the NxTAG Probe Adjustment Strip and one alignment sphere.

      **NOTE:** Probe height must be re-adjusted if changing between skirted and non-skirted plates.

   b. Save probe height adjustments as **NxTAG Assay Plate**. If prompted to over-write the existing results, click **Yes**.

      **NOTE:** For more information on adjusting the sample probe height, refer to the applicable user manual.

4. Navigate to the **Maintenance** page > **Probe & Heater** tab.

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5.  Select **ON** under **Plate Heater** and enter **37** in the **Set Temperature** field to heat the MAGPIX® heater plate to 37°C. Click **Apply**.

6.  Navigate to the **Maintenance** page > **Cmds & Routines** tab. Click **Eject**. Add the appropriate reagents to the off-plate reagent reservoirs, as specified by the **Post-Batch Routine** indicated in the software. Click **Retract**.

    **NOTE:** The Post-Batch Routine is included in the assay protocol.

## Create 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.

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.

    **NOTE:** The Sample ID name cannot be duplicated within a Run. Each sample MUST have a unique ID. If you are running replicates or running the same control sample more than once, please make sure you assign a unique Sample ID, for example, by assigning "-1" or "-2" to the end of the proposed Sample ID.

5.  Click **Save**. The batch is now saved as a pending batch and ready to run.

6.  If running multiple batches on the same plate, create a Multi-Batch.

## Create a Multi-Batch in xPONENT® Software

The Multi-batch feature automatically sets the batches side-by-side if space remains on the plate. Ensure that the batches fit on one plate. If space limitations create an overlap, an error message displays. Results for each batch are saved as individual batch files. Batches must be created first, before they can be combined on one plate to create a multi-batch.

**NOTE:** There is a limit of 96 batches in a multi-batch.

**NOTE:** You cannot add a batch that forces multiple plates to a multi-batch operation. All batches must use the same plate name.

1.  Navigate to the **Batches** page > **Batches** tab **>** click **Create a New Multi-Batch.** The **New Multi-Batch** subtab displays.

    a.  If the **Select Pending Batch** dialog box displays, choose the batch you want to add to the new multi-batch list.

    b.  Click **OK**.

2.  Click **Add** to add a batch. The **Select Pending Batch** dialog box displays.

3.  Choose a batch from the available options, including batches newly created.

4.  Click **OK**. The selected batch will then display on the plate layout.

    **NOTE:** After you add each batch, the software automatically adds the next batch to the first well of the next column or row (depending on the plate direction). You can also select a well first, which places the next batch in your chosen location.

    **NOTE:** If the batches chosen do not fit on the plate, a **Multi-Batch Error** dialog box opens, indicating you must edit one or more of the selected batches.

## Run Batch in xPONENT® Software

1. Navigate to the **Batches** page > **Batches** tab. Choose the pending batch that you want to run.
2. Upon completion of thermal cycling, click **Eject** to place the assay plate on the prepared MAGPIX heater block. Click **Retract** to retract the holder.

    **NOTE:** Be sure to leave the seal in place.

    **NOTE:** When placing the plate on the heater block, ensure that the numbers are on the left side and the letters are closest to you.



3. Click **Run** to start acquisition.
4. Verify the information in the warning dialog boxes and click **OK**.

## Complete Run in xPONENT® Software

1. After the last sample is read in xPONENT®, navigate to the **Home** page > **Probe and Heater** tab.
2. Select **OFF** to turn off the heater and click **Eject** to remove the plate from the heater block. Then, click **Retract**.
3. Carefully discard the test strip(s) into a biohazard bag, sealing the bag to avoid aerosolization of the amplicons.
4. If re-using the plate, clean by soaking in a 10% household bleach solution for 15 minutes.
5. Rinse the plate under running tap water to remove the bleach, and air dry on paper towels or wipe with a cloth soaked in 70% alcohol for fast drying, if necessary.

# Analyze Results in SYNCT™ Software

## Import the Assay File into SYNCT™ Software

**NOTE:** Ensure the NxTAG® CoV assay file is saved to a location that is accessible by the SYNCT™ Software.

If you have already imported the correct version of the NxTAG CoV assay file into SYNCT (Assay Code: NCEC, Assay Version: A), skip the following steps:

1. Start the SYNCT Software and login with the appropriate SYNCT user.
2. Click ≡ in the upper left-hand corner of the screen and navigate to **Assay Management** > **Assay Management** page.
3. Click **Import Assay** from the Page Action bar at bottom of the page. The **Import File** window displays.

   NOTE: Do NOT double click. SYNCT™ Software requires one click when navigating to the correct file location.

   a. Choose the location under **Devices**.
   a. Choose the **Devices** and the **Files**.
   b. Choose the location under **Files** to locate **NxTAG CoV_NCEC_A. assay** to import, the file name will populate in the **File Name** field.
   c. Click **OK**.

## Define a Negative Amplification Control (No Template Control) in SYNCT™ Software

To define a negative amplification control in SYNCT™ Software, complete the following:

1. Click ≡ in the upper left-hand corner of the screen and navigate to **Assay Management** > **Controls** page.
2. Click **New Control** from the Page Action bar at bottom of the page.
3. In the window that displays, complete the following:
   a. Enter the control **Name** (Required) and **Manufacturer** (Optional) information.
   b. Choose the **NxTAG CoV** assay in the **Assay** field with the corresponding assay code and version.
   c. Click in the **Expected Results** (Required) field. The **Expected Results** window displays.
      i. Set the expected result for all tests to **Negative** by selecting the **All Negative** check box.
      ii. Click **Close**.
   d. Click **Save**. The newly defined control displays in the **Controls** window.

## Define a Negative Extraction Control in SYNCT™ Software

To define a negative extraction control in SYNCT™ Software, complete the following:

1. Click ≡ in the upper left-hand corner of the screen and navigate to **Assay Management** > **Controls** page.
2. Click **New Control** from the Page Action bar at bottom of the page.
3. In the window that displays, complete the following:
   a. Enter the control **Name** (Required) and **Manufacturer** (Optional) information.
   b. Choose the **NxTAG CoV** assay in the **Assay** field with the corresponding assay code and version.
   c. Click in the **Expected Results** (Required) field. The **Expected Results** window displays.
      i. Set the expected result for all tests to **Negative** by selecting the **All Negative** check box.

         NOTE: If the internal control was added to the negative, select Positive as the expected result for the internal control.

      ii. Click **Close**.
   d. Click **Save**. The newly defined control displays in the **Controls** window.

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## Define a External Positive Control in SYNCT™ Software

**NOTE:** Name the controls the same exact name as the controls in xPONENT®, so the control will be automatically defined in the SYNCT™ Software.

To define a external positive control in SYNCT Software, complete the following:

1. Click ☰ in the upper left-hand corner of the screen and navigate to **Assay Management** > **Controls** page.
2. Click **New Control** from the Page Action bar at bottom of the page.
3. In the window that displays, complete the following:

    a. Enter the control **Name** (Required) and **Manufacturer** (Optional) information.

    b. Choose the **NxTAG CoV** assay in the **Assay** field with the corresponding assay code and version.

    c. Click in the **Expected Results** (Required) field. The **Expected Results** window displays.

       i. For tests that are known to be positive in the sample, set the expected result to **Positive**.

       ii. For tests that are known to be negative in the sample, set the expected result to **Negative**.

       iii. If the expected result is unknown for a particular test, select **NA (No Analysis)**.

       iv. Click **Close**.

    d. Click **Save**. The newly defined control displays in the **Controls** window.

## Create Run from Imported Raw Data in SYNCT™ Software

The Import Raw Data function allows a raw data (CSV) file from xPONENT® Software to be imported.

> ⚠ Modified output csv data files cannot be used for diagnostics purposes. The integrity of the xPONENT ® CSV file will be checked when the file is imported into SYNCT™. The user will be notified if the file has been modified outside of the system.

To manually import the xPONENT raw data into the SYNCT Software, complete the following:

1. Click ☰ in the upper left-hand corner of the screen and navigate to **NxTAG** > **Runs** page.
2. Click **Import Raw Data** from the Page Action bar at bottom of the page. The **Import xPONENT Data** window displays.

    **NOTE:** Do NOT double click. SYNCT Software requires a single click when navigating to the correct file location.

    a. Choose the **Location** and the **Files**.

    b. Choose the batch file. The **Run Name** field is automatically populated with the Batch name from the xPONENT file.

       **NOTE:** By default, the Run Name is the same as the batch name imported from the xPONENT file.

    c. Click **OK**. Orders are created for all samples within the imported batch file and can then be edited in SYNCT.

## Edit and Review Orders in SYNCT™ Software

After the batch data is imported, an Order is created for each of the samples in the batch file. Review and edit the Orders prior to analyzing the Run.

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**NOTE:** The Sample ID name cannot be duplicated within a Run. Each sample MUST have a unique ID. If you are running replicates or running the same control sample more than once, please make sure you assign a unique Sample ID, for example, by assigning "-1" or "-2" to the end of the proposed Sample ID.

Select multiple orders of the same sample type (sample or control) and edit them at the same time. This is useful when entering kit lot information for all sample orders at the same time, or for applying a Test Panel to multiple orders at the same time. Complete the following in SYNCT™ Software:

1. Click ☰ in the upper left-hand corner of the screen and navigate to **NxTAG** > **Runs** page.
2. Click the "+" sign next to the Run that contains the samples to edit.
3. Select the sample(s) to edit.
4. Click **Edit Orders** from the Page Action bar at bottom of the page.
5. In the window that displays, edit the following information:

   - For **Samples**:

     i. From the **Sample Type** drop-down menu, choose **Sample**.
     ii. If allowed, from the **Test Panels** drop-down menu, choose the appropriate Test Panel OR customize any of the tests listed by clicking **Selected** or **Masked**.
     iii. Update the name of the sample in the **Sample ID** field. (Available if a single Order is selected for editing.)
     iv. Optionally, include any necessary information in the **Accession ID** and **Requisition Number** fields.

       **NOTE:** Depending on the SYNCT settings, the Accession ID and Requisition Number may not be visible or you may not have to enter any information within those fields.

     v. Optionally, enter the kit lot number in the **Kit Lot Number** field.

       **NOTE:** Kit lot numbers are 11 digits separated by a dash. Do not omit the dash when entering the number.

       **NOTE:** If you enter a Kit Lot Number, you will be required to enter a Lot Expiration date.

     vi. Optionally, click the calendar icon in the **Kit Lot Expiration** field to set the lot expiration date.

       **NOTE:** Use information provided with your kit for the Kit Lot Number and Kit Lot Expiration.

     vii. Click **OK**.

   - For **Control**:

     i. From the **Sample Type** drop-down menu, choose **Control**.
     ii. Click to choose a pre-defined control to be applied.
     iii. Enter the name of the control in the **Sample ID** field. (Available if a single Order is selected for editing.)
     iv. Optionally, enter the kit lot information in the **Kit Lot Number** field.
     v. Optionally, click the calendar icon in the **Kit Lot Expiration** field to set the lot expiration date.

       **NOTE:** Use information provided with your kit for the Kit Lot Number and Kit Lot Expiration.

     vi. Click **OK.**

## Process Run in SYNCT™ Software

To process the Run in SYNCT™ Software, complete the following:

1. Click ☰ in the upper left-hand corner of the screen and navigate to **NxTAG > Runs** page.
2. Select the Sample ID (Run) to process.

3. Click **Process Run** from the Page Action bar at bottom of the page. A dialog box displays, "**Confirm all orders are correct before proceeding. Do you want to continue?**".

4. Click **Yes** to proceed with processing the Run.

5. Once the Run has completed processing, the Run is removed from the **NxTAG Run** view. The results of the Run can be found by clicking the **Results** icon from the **System Navigation Menu** and locating the processed Run from the list.

## Result Call Definitions

For a general description of Results page functionality, please refer to the *SYNCT™ Software User Manual*.

1. Click ≡ in the upper left-hand corner of the screen and navigate to **Results** > **Results** page.

2. Click the "+" sign next to the Run Results you want to see a Status for.

   - The **Status** column indicates whether there are Errors, Warnings, Info messages, or user comments for a sample. Click the ▷ in the **Status** column to display the messages in the sample row. The **Status** column will display a ❌ if a sample has an error. If there are no messages for the sample, the ▷ will not appear.

   - The **Alert** column indicates if any test has a positive result. If the result is positive the **Alert** column will display a ➕ for that sample.

   - The **Alert** column indicates if a control has failed. If the control failed, the **Alert** column will display a red exclamation mark for that control.

   - The **Result** column displays the summary result for the sample. To see individual results for each test, click ▷ next to the summary results in the **Result** column. The results are shown grouped by result type in the sample row.

## Report Type Definitions

The following reports are available for the assay:

| Report Title | Summary of Contents |
|---|---|
| Clinical Summary | Shows the result for each target for a sample. |
| Sample Details | Shows the result, calculated signal value, and threshold used to determine the result for each target for a sample. |
| Control Summary | Shows the expected result, and pass or fail result, for each target for a control. |
| Control Details | Shows the expected result, pass or fail result, and calculated signal for each target for a control. |
| Run Report | Shows a summary result for each sample that includes all positive tests. |
| Run Details | Contains a Run summary, Sample Details for each sample (with optional graph), and details for each selected target (with optional graph). |

## View Results in SYNCT™ Software

1. Click ☰ in the upper left-hand corner of the screen and navigate to **Results** > **Results** page.
2. When there are multiple pages of results in SYNCT™ Software, page arrows and numbers will display at the bottom of the screen. Click on the left and right arrows to scroll through the pages of results or, if you know what page the results are on, click on the page number.
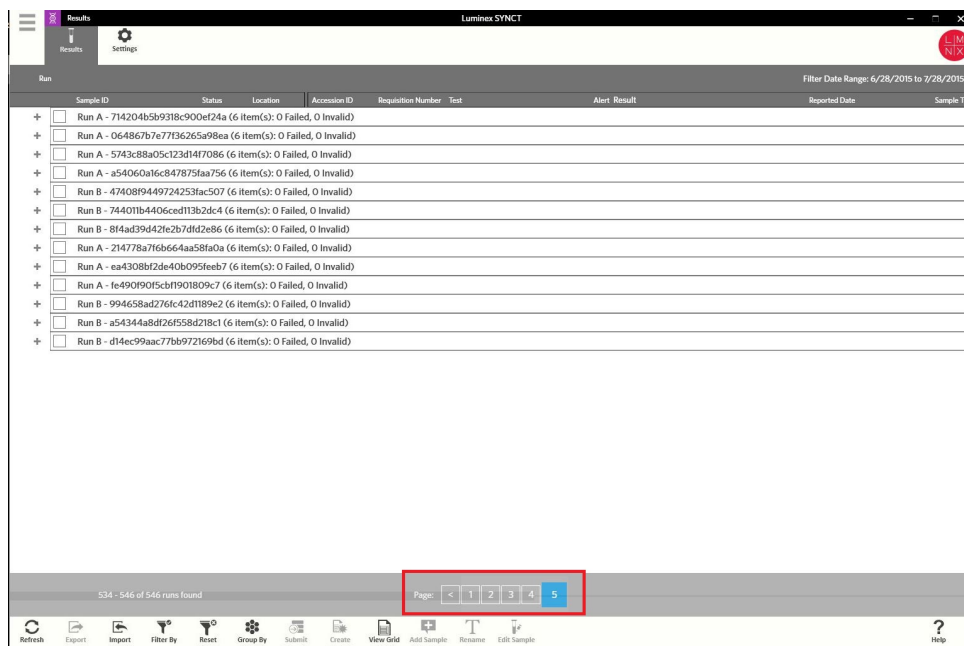


## Create and Print a Report in SYNCT™ Software

To create a report, complete the following:

1. Click ☰ in the upper left-hand corner of the screen and navigate to **Results** > **Results** page.
2. Select the Run or samples that the report is to be generated for.
3. Click **Create Report** from the Page Action bar at bottom of the page. The **Generate Reports** window displays.

    **NOTE:** You can select one sample in order to view the report, however the report may have results from other samples in it. You can also export to a chosen location and print the report.

4. Choose the type of report to be created from the options provided. The report displays in a separate window.

    **NOTE:** Reports generated can have a customized header.

5. In the Report window, click **Print Report** to print the report. The **Print** dialog box will display.

    a.  Choose the printer and print settings, then click **Print**.

## Interpretation of Results

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. A NxTAG® CoV Extended Panel assay file will be provided to obtain the analysis result. It will report one Target call SARS-CoV-2, which contains 3 analytes (SARS-CoV-2 - ORF1ab, SARS-CoV-2 – N and SARS-CoV-2 – E). The target will have a positive call if any one or more analytes is detected.

The following results will appear for samples:

| Result Column | Meaning |
|---|---|
| Invalid | Sample failed or SARS-CoV-2 result is Invalid. See Troubleshoot section, *Resolve Invalid Results*. |
| SARS-CoV-2 Positive | At least one of the SARS-CoV-2 targets is identified. |
| Negative | Internal Control has Pass call and SARS-CoV-2 result is Negative. |

The following results will appear for controls:

| Result Column | Meaning |
|---|---|
| Pass | All target results match the expected results. |
| Fail | Any target result does not match the expected result. |

# Troubleshoot

# Re-Test Recommendations Prior to Data Acquisition

Thermal Cycler Error: If an error in the thermal cycler program is noticed after a particular step is initiated, re-test the samples.

## Resolve Invalid Results

Under certain circumstances, data analysis software will generate a target call of "Invalid" with associated error message(s) for one or more samples in a plate. These scenarios are summarized (with re-test recommendations) in the table below.

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Table 5. Invalid Results

| Software Result and Messages | Problem | Possible Cause(s) | Recommendation(s) |
|---|---|---|---|
| *Result:* Invalid<br><br>*Message:* "Internal Control failed." | MS2 specific signal is below the positive call cutoff. | Extraction failure, or no MS2 was spiked into that sample.<br><br>Insufficient sample was added during setup.<br><br>Failed to fully re-suspend Lyophilized Bead Reagents. | Re-extract the sample, making sure that MS2 is spiked into the sample.<br><br>Ensure the correct sample volume was added to the extraction system.<br><br>Ensure the Lyophilized Bead Reagents were fully re-suspended. |
| *Result*: Invalid<br><br>*Message*: "<Target Name>: non- specific signal detected in control sample" | An unexpected target was detected in a control sample. | Contamination may have occurred during extraction, with extraction reagents, during sample addition, or the internal control was added to the negative extraction control. | Re-extract the samples, including the negative extraction control with new (un-used) reagents. Clean all work surfaces and equipment with a 10% household bleach solution or other products (such as peroxide wipes) that are capable of degrading DNA amplicon contamination. Use fresh gloves and lab coats and follow a direction workflow to avoid introducing post-PCR amplicon contamination. |
| *Result:* Invalid<br><br>*Message:* "Run failed. All negative control samples have failed" | An instrument error occurred and all samples identified as negative controls are invalid. | Refer to the applicable user manual for possible causes. | Re-run the sample. |
| *Result:* Invalid<br><br>*Message:* "<Target Name>: invalid value encountered"<br>OR<br>"<Target Name>: low bead count" | The sample probe failed to acquire enough of the sample. | Low sample volume; sample probe height adjustment was not completed successfully.<br><br>Failed to fully re-suspend Lyophilized Bead Reagents. | 1. Repeat sample probe height adjustment procedure. Re-run the sample.<br>2. Ensure the Lyophilized Bead Reagents were fully re-suspended. |

| Software Result and Messages | Problem | Possible Cause(s) | Recommendation(s) |
|---|---|---|---|
| *Result*: Invalid

*Message*: "<Target Name>: invalid negative control value" | Failed to acquire enough of target signal within all negative control samples. | Sample probe height was not completed successfully.

Failed to fully re-suspend Lyophilized Bead Reagents. | Re-extract and re-run samples since you cannot rule out contamination for this target. |
| *Result*: Invalid

*Message*: "Inconclusive results based on abnormal signals" | Background cannot be calculated as multiple targets have abnormal signals. | Contamination may have occurred during extraction, during sample addition, or instrument failure. | Re-extract and re-run the sample. |
| *Result*: Invalid

*Message*: "This well was not read by the Luminex instrument." | No signal is detected. | A sample specific error may have occurred. | Re-extract and re-run the sample. |
| *Result*: Fail

*Message*: "Control failed: <Target name> result did not match expected result"
OR
"<Target Name>: non-specific signal detected" | Unexpected target call in the control. | Wrong control samples were used or Extraction failure or error occurred during extraction or sample addition. | Re-extract and re-run the sample. |

## Resolve Low Bead Count

<u>Table 6. Low Bead Count</u>

| Software Result and Messages | Problem | Possible Cause(s) | Recommendations |
|---|---|---|---|
| *Result*: Invalid

*Message*: "Internal Control failed." | Low Bead Count | An insufficient number of beads were aspirated by the MAGPIX® instrument or the beads aggregated in the instrument, preventing an accurate count. | Sonicate and clean the MAGPIX sample probe. Ensure the enhanced startup routine and post-batch cleaning routines are being performed. |

# Limitations

1. This device may not be able to differentiate newly emerging SARS–CoV-2 subtypes.
2. Performance of the NxTAG® CoV Extended Panel (NxTAG CoV) has only been established in nasopharyngeal swab specimens.
3. Analyte targets (viral sequences) may persist *in vivo*, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious, or are the causative agents for clinical symptoms.
4. All results from this and other tests must be considered in conjunction with the clinical history, epidemiological data and other data available to the clinician evaluating the patient.
5. The detection of pathogen nucleic acids is dependent upon proper specimen collection, handling, transportation, storage and preparation (including extraction). Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false negative values resulting from improperly collected, transported, or handled specimens.
6. The performance of Luminex® NxTAG CoV Extended Panel was established using nasopharyngeal swabs. Nasal swabs and mid-turbinate nasal swabs are also considered acceptable specimen types for use with the NxTAG CoV Extended Panel but performance has not been established. Testing of nasal and mid-turbinate nasal swab (self collected or collected by a healthcare provider) is limited to patients with symptoms of COVID-19.
7. This test is a qualitative test and does not provide the quantitative value of detected organisms present.
8. There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.
9. There is a risk of false negative values due to the presence of sequence variants in the pathogen targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms for amplification.
10. A specimen yielding a negative result may contain respiratory pathogens not probed by the assay.
11. The performance of this assay was not established in immunocompromised patients.
12. The performance for some viruses and subtypes may vary depending on the prevalence and population tested.
13. The performance of this test has not been established for screening of blood or blood products.
14. This test cannot rule out infections caused by other viral or bacterial pathogens not present on this panel.
15. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.
16. This device has been evaluated for use with human specimen material only.
17. The performance of this device has not been evaluated for patients without signs and symptoms of infection.
18. The performance of this device has not been evaluated for monitoring treatment of infection.
19. The performance of this device has not been assessed in a population vaccinated against COVID-19.

# Performance Characteristics

# Limit of Detection (LoD)

The Limit of Detection (LoD) study established the lowest SARS-CoV-2 viral concentration (Genomic Copy Equivalents or GCE) that can be detected by the NxTAG® CoV Extended Panel Assay at least 95% of the time using viral genomic RNA. Negative nasopharyngeal specimens (Negative Clinical Matrix, NCM) collected from 6/29/2019 to 7/14/2019 from a US site were pooled and used for this study. Purified SARS-CoV-2 viral genomic RNA (USA_WA1/2020 strain obtained from World Reference Center for Emerging Viruses and Arboviruses (WRCEVA)) was spiked into 200 μL of pooled NCM containing 10 μL MS2 internal control that was pre-mixed witheasyMAG® lysis buffer to bring the total volume to 1 mL to help stabilize the viral RNA and subjected to,easyMAG extraction generic protocol set for pre-lysed samples. Note that only the easyMAG extraction method was used to determine the LoD of the NxTAG CoV Extended Panel. This was considered acceptable as the easyMAG and EMAG® extraction kits were shown to be equivalent when used with the Luminex® NxTAG RPP assay (K193167).A two-phase approach was used to determine the LoD of the NxTAG CoV Extended Panel Assay. In phase I, the preliminary LoD was established by testing 3 replicates at each of 6 serially diluted concentrations (3-fold dilutions).  The two lowest concentrations to detect 3 out of 3 replicates were further evaluated in the second phase by testing 20 replicates. The lowest concentration that achieved greater than 95% positivity was confirmed to be 5.00E+03.

For each analyte in a sample, the multi-dimension detection (MDD) value is a measure resulting from the subtraction of the sample's noise signal from the analyte's signal. The result is a measure that has been adjusted for the noise within the sample.

**Table 7. NxTAG® CoV Extended Panel Assay LoD Results Summary (Phase II)**

| Concentration (GCE/mL) | Replicate | SARS-CoV-2 - ORF1ab | | SARS-CoV-2 - N | | SARS-Cov-2 - E | | Overall SARS-Cov-2 - 2 Result* | Overall % Positivity |
|---|---|---|---|---|---|---|---|---|---|
| | | MDD Value | Result | MDD Value | Result | MDD Value | Result | | |
| 5.00E+03 | 1 | 46 | Positive | 92 | Negative | 49 | Positive | Positive | 100.0% |
| 5.00E+03 | 2 | 82 | Positive | 117 | Positive | 27.5 | Positive | Positive | 100.0% |
| 5.00E+03 | 3 | 225 | Positive | 9 | Negative | 114 | Positive | Positive | 100.0% |
| 5.00E+03 | 4 | 67 | Positive | 104 | Positive | 130 | Positive | Positive | 100.0% |
| 5.00E+03 | 5 | 64 | Positive | 134 | Positive | 38 | Positive | Positive | 100.0% |
| 5.00E+03 | 6 | 161 | Positive | 189 | Positive | 87 | Positive | Positive | 100.0% |
| 5.00E+03 | 7 | 69 | Positive | 101 | Positive | 77 | Positive | Positive | 100.0% |
| 5.00E+03 | 8 | 204 | Positive | 159 | Positive | 140 | Positive | Positive | 100.0% |
| 5.00E+03 | 9 | 82 | Positive | 182 | Positive | 109.5 | Positive | Positive | 100.0% |
| 5.00E+03 | 10 | 55 | Positive | 20 | Negative | 22 | Negative | Positive | 100.0% |
| 5.00E+03 | 11 | 1112 | Positive | 181 | Positive | 123 | Positive | Positive | 100.0% |

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| Concentration (GCE/mL) | Replicate | SARS-CoV-2 - ORF1ab | | SARS-CoV-2 - N | | SARS-Cov-2 - E | | Overall SARS-Cov-2 - 2 Result* | Overall % Positivity |
|---|---|---|---|---|---|---|---|---|---|
| | | MDD Value | Result | MDD Value | Result | MDD Value | Result | | |
| 5.00E+03 | 12 | 79 | Positive | 171.5 | Positive | 163 | Positive | Positive | 100.0% |
| 5.00E+03 | 13 | 114 | Positive | 83 | Negative | 93 | Positive | Positive | 100.0% |
| 5.00E+03 | 14 | 54 | Positive | 162 | Positive | 109 | Positive | Positive | 100.0% |
| 5.00E+03 | 15 | 25 | Negative | 62 | Negative | 27 | Positive | Positive | 100.0% |
| 5.00E+03 | 16 | 131.5 | Positive | 351 | Positive | 167.5 | Positive | Positive | 100.0% |
| 5.00E+03 | 17 | 78.5 | Positive | 61 | Negative | 59 | Positive | Positive | 100.0% |
| 5.00E+03 | 18 | 35 | Positive | 58 | Negative | 27 | Positive | Positive | 100.0% |
| 5.00E+03 | 19 | 57 | Positive | 93 | Negative | 46 | Positive | Positive | 100.0% |
| 5.00E+03 | 20 | 169 | Positive | 166 | Positive | 139 | Positive | Positive | 100.0% |
| 1.67E+03 | 1 | 57 | Positive | 15 | Negative | 32 | Positive | Positive | 80.0% |
| 1.67E+03 | 2 | 22 | Negative | 75.5 | Negative | 38 | Positive | Positive | 80.0% |
| 1.67E+03 | 3 | 20 | Negative | 29 | Negative | 43 | Positive | Positive | 80.0% |
| 1.67E+03 | 4 | 72 | Positive | 79 | Negative | 31 | Positive | Positive | 80.0% |
| 1.67E+03 | 5 | 25 | Negative | 145 | Positive | 60 | Positive | Positive | 80.0% |
| 1.67E+03 | 6 | 33 | Negative | 23 | Negative | 5 | Negative | Negative | 80.0% |
| 1.67E+03 | 7 | 24 | Negative | 61 | Negative | 28 | Positive | Positive | 80.0% |
| 1.67E+03 | 8 | 76 | Positive | 0 | Negative | 21 | Negative | Positive | 80.0% |
| 1.67E+03 | 9 | 35 | Positive | 32 | Negative | 57 | Positive | Positive | 80.0% |
| 1.67E+03 | 10 | 1 | Negative | 25 | Negative | 14 | Negative | Negative | 80.0% |
| 1.67E+03 | 11 | 22 | Negative | 19 | Negative | 43.5 | Positive | Positive | 80.0% |
| 1.67E+03 | 12 | 42.5 | Positive | 72 | Negative | 34 | Positive | Positive | 80.0% |
| 1.67E+03 | 13 | 15 | Negative | 70 | Negative | 8 | Negative | Negative | 80.0% |
| 1.67E+03 | 14 | 18.5 | Negative | 58.5 | Negative | 65.5 | Positive | Positive | 80.0% |

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| Concentration (GCE/mL) | Replicate | SARS-CoV-2 - ORF1ab | | SARS-CoV-2 - N | | SARS-Cov-2 - E | | Overall SARS-Cov-2 - 2 Result* | Overall % Positivity |
|---|---|---|---|---|---|---|---|---|---|
| | | MDD Value | Result | MDD Value | Result | MDD Value | Result | | |
| 1.67E+03 | 15 | 49 | Positive | 40 | Negative | 37 | Positive | Positive | 80.0% |
| 1.67E+03 | 16 | 23 | Negative | 63 | Negative | 18 | Negative | Negative | 80.0% |
| 1.67E+03 | 17 | 17 | Negative | -2 | Negative | 25 | Positive | Positive | 80.0% |
| 1.67E+03 | 18 | 37 | Positive | 29 | Negative | 22.5 | Negative | Positive | 80.0% |
| 1.67E+03 | 19 | 24 | Negative | 39 | Negative | 26 | Positive | Positive | 80.0% |
| 1.67E+03 | 20 | 47.5 | Positive | -1 | Negative | 39 | Positive | Positive | 80.0% |

* Positive - At least one of the three targets was positive; Negative - all three targets were negative.

# Clinical Evaluation

## Contrived Samples

The performance of the NxTAG® CoV Extended Panel Assay was established using leftover nasopharyngeal swab clinical specimens collected from patients with signs and symptoms of upper respiratory tract infection. Individual negative nasopharyngeal clinical specimens (Negative Clinical Matrix, NCM) were collected from 01/03/2019 to 04/03/2019 from a U.S. site, by qualified personnel according to the package insert of the collection device. Specimens were handled as described in the package insert of the collection device, aliquoted, and stored frozen until use.

All specimens were shown to be negative for common upper respiratory tract infection pathogens by testing with a commercially available nucleic acid test or an in-house PCR followed by bi-directional sequencing. All samples were also shown to be negative for SARS-CoV-2 by testing with the Luminex NxTAG CoV Extended Panel.

Thirty (30) contrived positives were generated for testing, of which twenty (20) were prepared at 2X LoD, five at 3X LoD, and five at 5X LoD. Purified SARS-CoV-2 viral genomic RNA (isolate USA_WA1/2020 strain obtained from World Reference Center for Emerging Viruses and Arboviruses (WRCEVA)) was spiked into 200 µL of individual NCM containing 10 µL MS2 internal control that was pre-mixed with easyMAG® lysis buffer to bring the total volume to 1 mL, to help stabilize the viral RNA and subjected to easyMAG extraction generic protocol set for pre-lysed samples.

Thirty (30) clinical negative specimens were extracted and tested as described above. The positive and negative clinical specimens were tested in a blinded and randomized fashion. The results are summarized in the table below and demonstrated a PPA and NPA of 100%.

For each analyte in a sample, the multi-dimension detection (MDD) value is a measure resulting from the subtraction of the sample's noise signal from the analyte's signal. The result is a measure that has been adjusted for the noise within the sample.

<u>Table 8. Clinical Performance of NxTAG® CoV</u>

| Target RNA Concentration | Number of Samples Tested | SARS-CoV-2 - ORF1ab | | SARS-CoV-2 - N | | SARS-CoV-2 - E | | Overall SARS-CoV-2 Result* |
|---|---|---|---|---|---|---|---|---|
| | | Mean MDD Value | % Agree-ment (#Pos or Neg) / Total | Mean MDD Value | % Agree-ment (#Pos or Neg) / Total | Mean MDD Value | % Agree-ment (#Pos or Neg) / Total | |
| SARS-CoV-2 - 2x LoD | 20 | 181 | 95% 19/20 | 217 | 100% 20/20 | 119 | 100% 20/20 | Positive |
| SARS-CoV-2 - 3x LoD | 5 | 212 | 100% 5/5 | 273 | 100% 5/5 | 175.3 | 100% 5/5 | Positive |
| SARS-CoV-2 - 5x LoD | 5 | 223 | 100% 5/5 | 247 | 100% 5/5 | 167 | 100% 5/5 | Positive |
| SARS-CoV-2 - Total | 30 | 1 | 100% 30/30 | -2 | 100% 30/30 | -1 | 100% 30/30 | Negative |

* Positive result assignment if at least one of three gene targets was detected.
A single replicate was negative for ORF1ab, however other gene targets were detected for this replicate, and it was assigned a positive result.

# Reactivity/Inclusivity

Based on *in silico* analysis, it is predicted that NxTAG® CoV will detect all analyzed SARS-CoV-2 sequences in NCBI and in GISAID databases.

*In silico* inclusivity analyses of the oligonucleotide (oligo) sequences for the SARS-CoV-2 ORF1ab, N, and E sets were initially initially performed against all SARS-CoV-2 sequences available in the GISAID database as of March 5, 2020, as well as the SARS-CoV-2 reference sequence from GenBank. The analysis included 177 sequences in the ORF1ab gene region, 188 sequences in the N gene region, and 176 sequences in the E gene region. Oligo sets from both ORF1ab and E gene have >95% homology to all analyzed sequences. Oligo set from N gene has >95% homology to all but six recently submitted sequences to the GISAID database. These six sequences have homology of 88.5% with the N gene forward primer due to mismatches near the 5' end of the primer sequence. These mismatches are not expected to affect assay performance.

An additional *in silico* analysis was performed to assess the coverage of the NxTAG CoV Extended Panel against the rapidly emerging SARS-CoV-2 strains from the United Kingdom and South Africa. The UK SARS-CoV-2 strains are defined by the B.1.1.7 lineage (SARS-CoV-2 VUI 202012/01) and the South Africa strains are defined by the 501Y.V2 variant. The analysis consisted of sequences of the B.1.1.7 and 501Y.V2 variants that were available in the GISAID database as of January 19, 2021. Only sequences with full coverage of all oligo-binding regions for the ORF1ab, N gene and E gene oligo sets were included in the analysis. Based on sequence homology of each oligo to its intended binding region, it is predicted that the NxTAG CoV Extended Panel will detect 97 of 97 sequences of the B.1.1.7 lineage and 90 of 90 sequences of the 501Y.V2 variant assessed in this analysis.

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# Cross-Reactivity

An *in silico* cross-reactivity analysis was performed with all primer and probe sequences in NxTAG® CoV against sequences from GenBank nt database available as of March 3, 2020 for organisms listed in *Table 9*. Based on *in silico* analysis, it is predicted that the assay will not cross-react with any organisms listed in *Table 9*.

Results from the *in silico* cross-reactivity analysis showed the only organisms in *Table 9* with oligo-hit sequence homology ≥80% areSARS-coronavirus, *Mycobacterium tuberculosis*, *Legionella pneumonophila*, and *Pseudomonas aeruginosa*. For these non-target organisms, only a single oligo component (forward primer, reverse primer, or probe) has ≥80% homology. Despite having ≥80% homology of one oligo component, there is no anticipated amplification or detection of these non-target organisms because hybridization and extension of three oligo components are required to generate a detection signal.

**Table 9. Organisms Assessed in *In Silico* Cross-Reactivity Analysis**

| | |
|---|---|
| Human coronavirus 229E | Respiratory syncytial virus A |
| Human coronavirus OC43 | Respiratory syncytial virus B |
| Human coronavirus HKU1 | Rhinovirus |
| Human coronavirus NL63 | *Chlamydia pneumoniae* |
| SARS-coronavirus | *Haemophilus influenzae* |
| MERS-coronavirus | *Legionella pneumophila* |
| Adenovirus | *Mycobacterium tuberculosis* |
| Human Metapneumovirus (hMPV) | *Streptococcus pneumoniae* |
| Parainfluenza virus 1 | *Streptococcus pyogenes* |
| Parainfluenza virus 2 | *Bordetella pertussis* |
| Parainfluenza virus 3 | *Mycoplasma pneumoniae* |
| Parainfluenza virus 4 | *Pneumocystis jirovecii* (PJP) |
| Influenza A | *Candida albicans* |
| Influenza B | *Pseudomonas aeruginosa* |
| Enterovirus | *Staphylococcus epidermis* |
| | *Streptococcus salivarius* |

In addition to *in silico* analysis, cross-reactivity was evaluated by testing the whole organisms listed in High concentration of the potentially cross-reactive organisms were spiked into nasopharyngeal swab matrix and tested with the NxTAG CoV Extended Panel. No cross reactivity was observed.

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Table 10. Organisms Tested for Potential Cross-Reactivity

| Organism | Concentration Tested | Results |
|---|---|---|
| Human coronavirus - HKU1 | 2.14E+06   Copies/mL | Negative |
| Human coronavirus - NL63 | 1.00E+05  Copies/mL | Negative |
| Human Coronavirus - 229E | 1.00E+05  Copies/mL | Negative |
| Human coronavirus - OC43 (Betacoronavirus 1) | 1.00E+05 Copies/mL | Negative |
| Adenovirus C1 | $1.00E+05\ TCID_{50}/mL$ | Negative |
| Cytomegalovirus | $1.00E+05\ TCID_{50}/mL$ | Negative |
| Enterovirus D68 | $1.00E+05\ TCID_{50}/mL$ | Negative |
| Rhinovirus Type A1 | $1.70E+04\ TCID_{50}/mL$ | Negative |
| Epstein-Barr virus | $1.00E+06\ TCID_{50}/mL$ | Negative |
| Human metapneumovirus (hMPV) | $1.00E+05\ TCID_{50}/mL$ | Negative |
| Human parainfluenza virus 1 | $3.95E+02\ TCID_{50}/mL$ | Negative |
| Human parainfluenza virus 2 | $2.81E+05\ TCID_{50}/mL$ | Negative |
| Human parainfluenza virus 3 | $9.88E+04\ TCID_{50}/mL$ | Negative |
| Human parainfluenza virus 4A | $1.00E+05\ TCID_{50}/mL$ | Negative |
| Human parainfluenza virus 4B | $9.98E+03\ TCID_{50}/mL$ | Negative |
| Human respiratory syncytial virus A | $2.81E+05\ TCID_{50}/mL$ | Negative |
| Human respiratory syncytial virus B | $8.89E+04\ TCID_{50}/mL$ | Negative |
| Influenza A H1N1 (New Cal/20/99) | $1.00E+05\ TCID_{50}/mL$ | Negative |
| Influenza H1N1pdm (NY/02/09) | $2.45E+04\ TCID_{50}/mL$ | Negative |
| Influenza A H3N2 (Brisbane/10/07) | $6.14E+03\ TCID_{50}/mL$ | Negative |
| Influenza A H3N2 (A/Victoria/3/75) | $1.00E+05\ TCID_{50}/mL$ | Negative |

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| Organism | Concentration Tested | Results |
|---|---|---|
| Influenza B (Florida/02/06) | 4.57E+05 TCID$_{50}$/mL | Negative |
| Measles virus (Rubeola) | 1.00E+05 TCID$_{50}$/mL | Negative |
| Mumps virus | 3.03E+05 TCID$_{50}$/mL | Negative |
| Acholeplasma laidlawii | 1.00E+06 CFU/mL | Negative |
| Bordetella bronchiseptica | 1.00E+06 CFU/mL | Negative |
| Bordetella holmesii | 1.00E+06 CFU/mL | Negative |
| Bordetella parapertussis | 1.00E+06 CFU/mL | Negative |
| Bordetella pertussis | 1.00E+06 CFU/mL | Negative |
| Burkholderia cepacia | 1.00E+06 CFU/mL | Negative |
| Candida albicans | 1.00E+06 CFU/mL | Negative |
| Candida glabrata | 1.00E+06 CFU/mL | Negative |
| Chlamydia trachomatis | 1.00E+05 CFU/mL | Negative |
| Chlamydophila pneumoniae | 1.58E+03 CFU/mL | Negative |
| Corynebacterium genitalium | 1.00E+06 CFU/mL | Negative |
| Corynebacterium glutamicum | 1.00E+06 CFU/mL | Negative |
| Haemophilus influenzae | 1.00E+06 CFU/mL | Negative |
| Klebsiella pneumoniae | 1.00E+06 CFU/mL | Negative |
| Lactobacillus plantarum | 1.00E+06 CFU/mL | Negative |
| Lactobacillus reuteri | 1.00E+06 CFU/mL | Negative |
| Legionella birminghamensis | 1.00E+06 CFU/mL | Negative |
| Legionella cincinnatiensis | 1.00E+06 CFU/mL | Negative |
| Legionella pneumophila | 1.31E+09 CFU/mL | Negative |
| Moraxella catarrhalis | 1.00E+06 CFU/mL | Negative |
| Mycobacterium avium | 1.00E+06 CFU/mL | Negative |
| Mycobacterium tuberculosis H37Ra-1 | 1.00E+06 CFU/mL | Negative |

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| Organism | Concentration Tested | Results |
|---|---|---|
| *Mycoplasma arginini* | 1.00E+06 CFU/mL | Negative |
| *Mycoplasma gallisepticum* | 1.00E+06 CFU/mL | Negative |
| Mycoplasma hominis | 1.00E+06 CFU/mL | Negative |
| *Mycoplasma hyorhinis* | 1.00E+06 CFU/mL | Negative |
| Mycoplasma pneumoniae | 4.64E+06 CCU/mL | Negative |
| *Mycoplasma salivarium* | 1.00E+06 CFU/mL | Negative |
| Mycoplasma synoviae | 1.00E+06 CFU/mL | Negative |
| *Neisseria elongata* | 1.00E+06 CFU/mL | Negative |
| *Neisseria sicca* | 1.00E+06 CFU/mL | Negative |
| *Proteus vulgaris* | 1.00E+06 CFU/mL | Negative |
| Pseudomonas aeruginosa | 1.00E+06 CFU/mL | Negative |
| *Staphylococcus aureus* subsp. *aureus* (protein A producer) | 1.00E+06 CFU/mL | Negative |
| *Staphylococcus epidermidis* (FDA strain PCI 1200) | 1.00E+06 CFU/mL | Negative |
| *Staphylococcus epidermidis* (MSSE; HER 1292) | 1.00E+06 CFU/mL | Negative |
| Streptococcus pneumoniae (z022;19F) | 1.00E+06 CFU/mL | Negative |
| *Streptococcus pyogenes* | 1.00E+06 CFU/mL | Negative |
| *Streptococcus salivarius* | 1.00E+06 CFU/mL | Negative |

# References

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2. Specimen collection and storage for diagnostic molecular pathology investigation. Farkas DH, Kaul KL, Wiedbrauk DL, Kiechle FL Arch Pathol Lab Med. 1996 Jun;120(6):591-6.

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