# BioCode®

## CoV-2 Flu Plus Assay

**Package Insert**

Applied BioCode

Applied BioCode

Barcoded Magnetic Beads | Highly Multiplex Assays

# BioCode® CoV-2 Flu Plus Assay

## Instructions for Use

For Prescription Use Only
For *In Vitro* Diagnostic Use
For use under the Emergency Use Authorization (EUA) only

Catalog # 64-C0305

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IVD

For *In Vitro* Diagnostic Use.

## Table of Contents

# NAME AND INTENDED USE

## BioCode® CoV-2 Flu Plus Assay

The BioCode® CoV-2 Flu Plus Assay is a molecular assay based on reverse transcription polymerase chain reaction (RT-PCR) and end-point detection of amplified DNA sequences with analyte-specific probes that are coupled to barcoded magnetic beads (BMB) and is intended for the simultaneous qualitative detection and differentiation of RNA from SARS-CoV-2, influenza A (with H1 pdm09, H1 seasonal, H3 subtypes), influenza B and/or respiratory syncytial virus (RSV) in nasopharyngeal swab specimens collected from individuals suspected of respiratory viral infection, consistent with COVID-19, by their healthcare provider. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, influenza, and RSV can be similar. 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 identification and differentiation of SARS-CoV-2, influenza A (with H1 pdm09, H1 seasonal, H3 subtypes), influenza B and/or RSV. The BioCode CoV-2 Flu Plus Assay is not intended to detect the influenza C virus. RNA from influenza A, influenza B, RSV and SARS-CoV-2 are generally detectable in nasopharyngeal swab specimens during the acute phase of infection. Positive results are indicative of active infection but do not rule out bacterial infection or co-infection with other pathogens not detected by the test; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all SARS-CoV-2 results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2, influenza A, influenza B, and/or RSV, 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 BioCode® CoV-2 Flu Plus Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the operation of the BioCode MDx-3000and in vitro diagnostic procedures. The BioCode® CoV-2 Flu Plus Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

# SUMMARY AND EXPLANATION OF THE TEST

Acute respiratory infections (ARIs) are viral or bacterial infections that arise in the upper or lower respiratory systems. COVID-19 is caused by a novel coronavirus, SARS-CoV-2, which was initially known as 2019-nCoV. The original case cluster was observed in Wuhan, China and reported to World Health Organization (WHO) on December 31, 2019.[1] Since then the outbreak has spread to other regions of China, to neighboring countries, and subsequently to the rest of the world. WHO declared COVID-19 as a pandemic on March 11, 2020, with cases in numerous countries on 6 continents.[2] As of December 2021, over 267 million confirmed cases have been reported worldwide, and more than 5.2 million people, especially elderly and those with underlying medical conditions, have succumbed to COVID-19.[3]

The BioCode® CoV-2 Flu Plus Assay (CoV2P) is a multiplexed nucleic acid test intended for the qualitative detection and differentiation of SARS-CoV-2, influenza A (with subtypes), influenza B and RSV in nasopharyngeal swabs. The assay is designed to be used with the BioCode® MDx-3000 automated system, and test results from the BioCode® CoV-2P are available in about 5 hours.

**Coronaviruses**

Coronaviruses belong to the *Coronaviridae* virus family and contain positive-sense single-strand RNA. Human Coronaviruses OC43, HKU1, NL63, and 229E can all cause mild to moderate upper respiratory illness such as the common cold while infants, the elderly, and patients with weakened immune systems or cardiopulmonary disease are at a greater risk to develop lower respiratory infections including pneumonia and bronchitis.[4] Two other important coronaviruses, MERS-CoV and SARS-CoV, are responsible for more severe respiratory diseases and have caused major outbreaks in recent years. [4] As with other respiratory pathogens, coronaviruses are transmitted through droplets when an infected person coughs or sneezes. [4] In the US coronavirus infections can occur year-round but are most common during the fall and winter months. [4] The CDC tracks coronavirus cases with the National Respiratory and Enteric Virus Surveillance System (NREVSS) while the ECDC mainly tracks MERS-CoV and SARS-CoV. [4,5,6]
SARS-CoV-2 emerged as a respiratory virus outbreak in Wuhan, China in late 2019 and causes the disease COVID-19. During the initial outbreak in China, infection leads to respiratory distress or pneumonia in approximately 20% of confirmed COVID-19 cases, including critical illness in about 5% of cases requiring intubation or ventilators.[7] The infectious agent for COVID-19 is a β-coronavirus similar to SARS-CoV and is easily transmitted through aerosol droplets and contaminated surfaces.[8] In addition to transmission from symptomatic individuals, pre-symptomatic[9] and asymptomatic transmissions[10] have been reported. The CDC tracks COVID-19 cases within the United States using COVIDView.[11]

The BioCode® CoV-2 Flu Plus Assay includes an assay for detection of a conserved region of SARS-CoV-2 N gene. The BioCode® CoV-2 Flu Plus Assay does not detect common human coronaviruses (OC43, HKU1, NL63, and 229E), MERS-CoV, or SARS-CoV.

**Influenza A & B**

Influenza viruses are negative-sense single-strand RNA orthomyxoviruses. Influenza A and B are the two major subtypes that cause annual flu epidemics in humans. Common flu symptoms include fever, chills, cough, sore throat, nasal congestion and runny nose, body aches, headaches, and fatigue, with young children sometimes experiencing vomiting and diarrhea. Influenza A subtypes are identified based on antigenic differences in two glycoproteins: hemagglutinin and neuraminidase. Genetic reassortment is responsible for the emergence of novel influenza A strains; notably, a new strain of H1N1 was responsible for the 2009 "swine flu" pandemic. Influenza B has only one serotype and two known lineages – B/Yamagata and B/Victoria – with significant influenza B epidemics occurring about every 2-3 years.[12] The BioCode® Cov-2 Flu Plus Assay detects and differentiates flu A subtype H1, flu A subtype H1 2009 pandemic (pdm), flu A subtype H3, and flu B.

The flu is spread by droplets when an infected individual coughs, sneezes, or talks.[13] The virus may be present in the body for up to two days before a patient notices any symptoms, and most adults are able to infect others from the day before symptoms arise up to about a week after symptoms develop.[13] Importantly, some individuals infected with a flu virus may be asymptomatic; these people are still capable of spreading the virus to others.[13] A handful of antiviral drugs are available to treat the flu.[13] While the flu circulates year-round in the United States, cases spike in the fall and winter months, with flu season usually beginning in October and lasting as late as May.[13] The CDC closely tracks the number of respiratory specimens that test positive for influenza each flu season and publishes their findings online via FluView and FluView Interactive. Similarly, the European Centre for Disease Prevention and Control tracks flu cases in Europe and publishes weekly reports through the European Influenza Surveillance Network (EISN).[14, 15]

The WHO has tracked global data on flu cases and the predominant subtypes effecting different countries since the 1950s and uses their data to issue recommendations on flu vaccine compositions.[16] The WHO estimates that each year flu epidemics are responsible for between 3 and 5 million serious illnesses and up to 650,000 deaths worldwide, with most deaths in industrialized nations reported in adults over the age of 65 and the vast majority of deaths in children under the age of 5 occurring in developing countries.[17] The WHO recommends annual influenza vaccinations for young children, the elderly, pregnant women, healthcare workers, and individuals with chronic medical conditions.[17]

**Respiratory Syncytial Virus**

Human respiratory syncytial virus (RSV), also known as human orthopneumovirus, is a negative-sense single-strand RNA virus divided into subgroups A and B. The BioCode® CoV-2 Flu Plus Assay has one assay that detects both RSV A and RSV B. Like other respiratory viruses, RSV is spread through droplets expelled when an infected person coughs or sneezes.[18] The virus generally causes mild cold-like symptoms such as runny nose, sneezing, and coughing, but may cause serious illness in young children and the elderly.[18] RSV is the leading cause of severe respiratory illness in infants and young children, with 2.1 million outpatient visits and over 50,000 hospitalizations in children under five in the US annually.[18, 19] Worldwide it is estimated that between 66,000 and 199,000 children under the age of five die each year from RSV.[20] Among adults older than 65 in the US, RSV is responsible for an average of 177,000 hospitalizations and 14,000 deaths per year.[21] In the US RSV cases spike during the fall, winter, and spring, however different countries experience RSV seasons at varying times and durations based on climate and latitude.[18, 19] There is no RSV vaccine currently available, although the monoclonal antibody palivizumab can be administered in monthly doses to high-risk individuals such as infants born prematurely to prevent RSV infection during the peak season.[18, 19] The CDC monitors RSV cases in the US via the National Respiratory and Enteric Virus Surveillance System (NREVSS) while the European Influenza Surveillance Network (EISN) tracks data on RSV and other influenza-like illnesses in Europe.

# PRINCIPLE OF PROCEDURE

The BioCode® MDx-3000 is an automated system that integrates PCR amplification, target capture, signal generation and optical detection from nasopharyngeal swab (NPS) specimens, in either VTM or UTM. Nucleic acids from NPS are extracted with the NucliSENS® easyMAG® (bioMérieux) or MagNA Pure 96 (Roche) automated systems. Once the PCR plate is set up and sealed, the rest of the operations are automated on the MDx-3000.

## Nucleic Acid Extraction

Nucleic acids (both RNA and DNA) are captured by coated magnetic beads and eluted on either the NucliSENS® easyMAG® or MagNA Pure 96 automated systems according to the manufacturer provided protocol.

## Overview of a BioCode® MDx-3000 Run

**Reverse Transcription and Multiplex PCR** – Since the target of the BioCode® CoV-2 Flu Plus Assay is an RNA virus, a reverse transcription (RT) step is performed to convert the viral RNA into cDNA prior to amplification. The purified nucleic acid solution is combined with a freshly prepared reaction mix for one-step RT-PCR to amplify the target nucleic acids present in the sample. One of the target-specific primers for each assay is biotinylated at the 5'-end to generate labeled PCR product for subsequent detection.
**Dispensing BMB-Probe Mix** – Towards the end of PCR amplification, the robotic head dispenses BMB-Probe mix into the designated reaction wells of the capture plate using disposable pipette tips.
**PCR Product Transfer** – After PCR amplification is completed, the robotic head pierces the foil seal with disposable pipette tips and transfers PCR products into corresponding wells of the capture plate.
**Target Capture** – Amplified PCR products labeled with biotin are captured at a defined temperature by target-specific probes that are covalently coupled to designated Barcoded Magnetic Beads (BMBs). During this step, BMBs are kept in suspension by gentle agitation. Differentiation of captured targets is achieved by assigning a unique barcode pattern (BMB) for each assay and the internal control.
**Signal Generation** – After washing off unbound PCR products and unused primers, a streptavidin-phycoerythrin (SA-PE) conjugate is automatically added to the reaction by the robot. High affinity binding between biotin and streptavidin ensures that captured PCR products with the biotin moiety are labeled with phycoerythrin in close proximity to the BMBs.
**Optical Detection** – Optical detection is performed for each reaction well of the capture plate, an optically clear, flat-bottom microtiter plate. After washing off unbound SA-PE, excitation of the fluorophore at the designated wavelength emits fluorescence signal from BMBs tagged with SA-PE conjugates. Each reaction well is imaged at the defined emission wavelength for fluorescent signal and under bright field for identifying the barcode patterns (decoding).
**Software -** The BioCode® MDx-3000 Software controls the operation of the instrument, collects and analyzes data, and automatically generates interpretation for test reports at the end of the run. Fluorescent signals from BMBs with the same barcode are sorted and calculated to generate median fluorescence index (MFI) for each analyte. The presence or absence of a pathogen is determined by MFI relative to the validated assay cutoff. The software also analyzes the results of external and internal controls to validate the run, and individual specimen results for reporting.

# MATERIALS REQUIRED

## Materials Required (Provided)

**Table 1.** Reagents provided in the BioCode® CoV-2 Flu Plus Assay kit (64-C0305)

| Component Name | Part No. | Contents | Storage |
|---|---|---|---|
| BioCode® Master Mix A | 24M0011 | 500 µL x 2 | Store at -20°C, after thaw store at 2 to 8°C for up to 30 days |
| BioCode® CoV-2 Flu Plus Primer Mix | 44-P0506 | 500 µL x 2 | Store at -20°C, after thaw store at 2 to 8°C for up to 30 days |
| BioCode® RT Mix | 24R0006 | 60 µL x 1 | Store at -20°C |
| BioCode® RNA-IC2 | 43-R0001 | 500 µL x 2 | Store at -20°C, after thaw store at 2 to 8°C for up to 30 days |
| BioCode® CoV-2 Flu Plus BMB-Probe Mix | 44-B0342 | 6000 µL x 1 | Store at -20°C, after thaw vortex for 30 sec, store at 2 to 8°C up to 90 days |

## Materials Required (Not Provided)

**Table 2.** General Reagents required for the BioCode MDx-3000

| Component Name | Part No. | Contents | Storage |
|---|---|---|---|
| BioCode® SA-PE Mix | 63-S0001 | 450 µL x 8 | Single use; protect from light; store at 2 to 8°C. **Do Not Freeze** |
| BioCode® Buffer A | 44-B0003 | 1 L x 2 | Store at room temp (15 to 25°C) |

**Table 3.** BioCode MDx-3000 Consumables

| Reagent | Part No. | Source/Part No. | Quantity |
|---|---|---|---|
| Reagent reservoirs | 01-R0005 | INTEGRA 4332 | 50 each x 4 |
| Waste lid | 01-W0104 | Applied BioCode | 25 lids |
| Waste bin | 01-W0105 | Applied BioCode | 25 bins |
| 20 µL pipette tips | 01-P0006 | Beckman 717256 | 10 x 96 tips |
| 250 µL pipette tips | 01-P0007 | Beckman 717252 | 10 x 96 tips |
| Bio-Rad 96-well hard-shell plate 0.1 mL | 01-P0011 | Bio-Rad HSL9601 | 5 plates x 5 bags |
| PCR adhesive foil | 01-P0012 | ThermoFisher Scientific AB-0626 or Eppendorf 0030127790 | 100 foils |
| Microtiter plate | 01-P0009 | Greiner bio-one 655101 | 10 plates x 10 bags |
| Microtiter plate lid | 01-P0010 | Nunc 5500 | 100 lids |

**Table 4.** Reagents and Consumables for Validated Extraction Systems

| Extraction System | Part Name (Part No.) | Quantity |
|---|---|---|
| Roche MagNA Pure 96 | DNA and Viral Small Volume Kit (06 374 913 001) | 576 extractions |
| | MagNA Pure 96 System Fluid (06640729001) | 5500 mL x 1 |
| | MagNA Pure 96 Processing Cartridge (06241603001) | 36 cartridges |
| | MagNA Pure 96 Output Plate (06241611001) | 60 plates |
| | MagNA Pure Tip 1000 µl (06241620001) | 40 x 96 tips |
| | MagNA Pure Sealing Foil (06241638001) | 100 foils |
| bioMerieux NucliSENS® easyMAG® | easyMAG® Magnetic Silica (280133) | 24 x 1.2 mL |
| | easyMAG® Lysis Buffer (280134) | 4 x 1000 mL |
| | easyMAG® Buffer 1 (280130) | 4 x 1000 mL |
| | easyMAG® Buffer 2 (280131) | 4 x 1000 mL |
| | easyMAG® Buffer 3 (280132) | 4 x 1000 mL |
| | easyMAG® Disposables (280135) | 16 x 3 vessels and aspirator disposables |
| | Biohit Pipette Tips (280146) | 10 x 96 tips |

**Equipment and Additional Consumables Required (Not Provided)**

- BioCode® MDx-3000
- NucliSENS® easyMAG® (bioMérieux) or MagNA Pure 96 (Roche) Extraction System
- Vortex
- Centrifuge
- Pipettes – single, multi-channel and/or repeater with accuracy range between 1-10 µL, 10-200 µL, and 100-1000 µL
- Sterile, RNase/DNase-free disposable aerosol-barrier micro pipettor tips
- 1.5 mL polypropylene micro centrifuge tubes and racks (RNase/DNase free recommended)
- Cooler racks for 1.5 mL tubes and 0.1 mL 96 well plate
- Biosafety cabinet (laminar flow hood) for extractions
- Freezer (manual defrost) at -10 to -30°C
- Freezer (manual defrost) at -60 to -90°C
- Refrigerator at 2 to 8°C

# WARNINGS AND PRECAUTIONS

## General Precautions

1. For Emergency Use Authorization (EUA) only.
2. For Prescription Use Only.
3. For *in vitro* diagnostic use.
4. This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
5. This product has been authorized only for the detection and differentiation of nucleic acid from SARS-CoV-2, influenza A, influenza B, and/or respiratory syncytial virus (RSV), not for any other virus or pathogen.
6. The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated, or authorization is revoked sooner.
7. Results should be interpreted in combination with the patient's signs and symptoms and results from other diagnostic tests by a trained healthcare professional.
8. The BioCode® CoV-2 Flu Plus Assay is to be used with the BioCode® MDx-3000 with MDx software, and easyMAG (bioMerieux) or MagNA Pure 96 (Roche) automated extraction instruments.

## Precaution Related to Public Health Reporting

Local, state, and federal rules and regulations for notification of reportable diseases are continually updated and include a number of organisms that are important for surveillance and outbreak investigations. Laboratories are responsible for following their state and/or local rules pertaining to reportable pathogens and should consult their local and/or state public health laboratories for isolate and/or clinical sample submission guidelines.

## Laboratory Precautions

1. Perform the protocol as described in this package insert. Deviations from this protocol may produce erroneous results.
2. The BioCode® CoV-2 Flu Plus Assay should be performed in clearly defined work areas moving in one direction from pre-amplification areas to the amplification/detection area to reduce potential for contamination.
   a. Begin with specimen preparation and reagent preparation before moving to amplification/detection.
   b. Use dedicated equipment and supplies for each area (including personal protective equipment, such as lab coats and disposable gloves).
   c. Clean work areas with 10% bleach or similar disinfectant followed by water before and after assay preparation.
3. A negative control must be tested for each run. If multiple lots are assayed at the same time, a negative control must be assayed for each lot.
4. Do not use reagents past the expiration date. Do not mix reagents or interchange kit components from different kit lots. Kit configurations are identified on the Kit outer carton and Kit Card.
5. Assay setup should be performed at room temperature. Keep Reaction Mix cold using a cooling block during formulation and loading of amplification plate.

### Safety Precautions

1. Follow universal safety procedures. All patient specimens should be considered potentially infectious and handled accordingly.
2. Dispose of unused kit reagents and specimens according to local, state and federal regulations.
3. Wear appropriate personal protective equipment including, but not limited to, lab coats, gloves, and protective eyewear. Change gloves often.
4. Do not pipette by mouth.
5. BioCode® RT Mix is classified as an irritant. See SDS for details.

## REAGENT STORAGE, HANDLING AND STABILITY

1. Store the kit components frozen (-20°C) prior to use.
2. Store RT Mix frozen (-20°C) except during use.
3. Once thawed, store Master Mix, Primer Mix, and RNA IC2 refrigerated (2 to 8°C) for up to 30 days.
4. Once thawed, store BMB-Probe Mix refrigerated (2 to 8°C) for up to 90 days.
5. SA-PE mix is for single use only. Store refrigerated (2 to 8°C). Protect from light. **DO NOT FREEZE.**
6. Store the Buffer A at room temperature (15 to 25°C).
7. Avoid storage of any materials near heating or cooling vents or in direct sunlight.
8. Always check the expiration date and do not use reagents beyond the expiration date printed.
9. Once RT-PCR reaction mix is prepared, the test run should be started as soon as possible (within 60 minutes).
10. Remove BMB-Probe Mix from MDx-3000 once the run is completed and store refrigerated (2 to 8°C).

## SAMPLE REQUIREMENTS

This section describes the requirements for specimen collection, preparation, and handling that will help ensure accurate test results.

**Nasopharyngeal Swab (NPS)** collected according to the standard procedure. Immediately place in 1 – 3 mL of transport medium (VTM or UTM). Samples should be tested as soon as possible. They may be stored at the following conditions:

- 2 to 8°C for 72 hours after collection

**Minimum Sample Volume** - 200 µL of sample is required for testing.

# PROCEDURE

Refer to the BioCode® MDx-3000 Operator's Manual for more detail and pictorial representations of the BioCode® MDx-3000 set up instructions.

Gloves and other Personal Protective Equipment (PPE) should be used when handling specimens and reagents. Once PCR reagents are prepared and sample is added to PCR plate, it should be promptly transferred to the instrument to start the run. After the run is complete, the PCR plate and capture plate should be sealed and discarded.

## Extraction Methods

**Note: It is strongly recommended that sample preparation be performed in a biosafety cabinet with gloves and appropriate personal protective equipment (PPE).**

### easyMAG Extraction

1. Pipet 10 µL RNA-IC2 into each well of the easyMAG cartridge

2. Transfer 200 µL of specimen or Control into easyMAG cartridge and load into easyMAG

3. Perform Protocol: **Generic 2.0.1, volume 0.200 mL, Eluate: 50.0 µL, Sample Type: Primary, Matrix: Other**

   **3.1** Perform 10 min on-board incubation

   **3.2** When prompted add magnetic silica
   - 3.2.1 Combine 550 µL nuclease-free water and 550 µL magnetic silica mix in one 1.5 mL tube per easyMAG cartridge
   - 3.2.2 Mix thoroughly and dispense 125 µL into each well of an 8-well ELISA strip per easyMAG cartridge.
   - 3.2.3 Add 100 µL to each easyMAG cartridge well and mix thoroughly

   **3.3** Start remainder of run

### MagNA Pure 96 Extraction

1. Pipet 10 µL RNA IC2 into each well of the MagNA Pure 96 processing cartridge (Be careful to pipet directly to the bottom of each well in the cartridge and not produce bubbles. Liquid on the side of the well and bubbles will lead to incorrect volume sensing and the extraction will be aborted.)

2. Transfer 200 µL of specimen or Control into the MagNA Pure 96 processing cartridge (Be careful to pipet directly to the bottom of each well in the cartridge and not produce bubbles. Liquid on the side of the well and bubbles will lead to incorrect volume sensing and the extraction will be aborted.)

3. Perform Protocol: **Pathogen Universal 200 3.1 for MagNA Pure Kit: DNA/Viral NA SV 2.0. Volume: 200 µL, Eluate: 50 µL.**

## Nucleic Acid Storage Conditions

Transfer sample extracts from the cartridge into PCR grade micro-tubes, strips or plates and store samples in a 2 to 8°C refrigerator if testing within 12 hours. Store at -60 to -90°C if testing cannot be completed within 12 hours of extraction. Extracted nucleic acids may be stored at -60 to -90°C for up to 90 days.

## BioCode® CoV-2 Flu Plus Assay Set Up

**Note: Prepare the PCR Plate in a dedicated reaction mix prep area.**

1. Thaw Primer Mix, Master Mix and BMB-Probe Mix at room temperature. Perform a quick vortex (2-3 seconds) and centrifuge to collect reagents at the bottom of the tube.
2. Prepare the reaction mix in a polypropylene microcentrifuge tube as described below:

**Table 5.** Reaction Mix Formulation

| Component | Reaction Mix Volume (µL) per reaction | Reaction Mix Volume (µL) per 10 reactions |
|---|---|---|
| BioCode® Master Mix A | 10.0 µL | 100 µL |
| BioCode® CoV-2 Flu Plus Primer Mix | 9.5 µL | 95 µL |
| BioCode® RT Mix | 0.5 µL | 5 µL |
| **Reaction Mix Volume (µL)** | **20 µL** | **200 µL** |

3. Mix reaction mix by pipetting up and down 8 to 10 times and centrifuge to collect contents at the bottom of the tube. Store at 2 to 8°C or on a cooling block until ready to set up PCR (not to exceed one hour). Do NOT vortex reaction mix.
4. Pipette 20 µL of reaction mix into appropriate wells of a 96-well plate.
5. Pipette 5 µL of each extracted sample into the wells.
6. Pipette 5 µL extracted negative control into the NC well.
7. Seal plate with pierceable foil. Store at 2 to 8°C or on a cooling block until ready to load onto the BioCode® MDx-3000 (not to exceed one hour from the time the reaction mix is prepared).
8. Briefly centrifuge plate to collect samples at the bottom of the plate.
9. Load plate onto BioCode® MDx-3000.
10. Vortex thawed room temperature BMB-Probe Mix for 30 seconds at high speed and load onto the BioCode® MDx-3000. **(Note: Precipitates may appear at cold temperatures. If precipitates are present, allow the BMB-Probe Mix to warm to room temperature and vortex additional 30 seconds.)**
11. Load reagents and consumables as prompted by graphic user interface and run BioCode® CoV2P Protocol.

# INTERPRETATION OF RESULTS

The BioCode® MDx-3000 software analyzes the data based on plate validity, sample validity and Median Fluorescent Intensity (MFI) compared to an MFI threshold. The software will suppress results if Internal or Negative controls are invalid. The software will indicate if external positive controls are valid or invalid, but will not suppress results if the positive control is not valid.

## External Negative Controls

External negative controls can be transport media or well characterized negative specimens. The negative control should go through all processing steps (extractions, amplification, and detection). At least one negative control is required for each plate/kit lot. The BioCode® MDx-3000 software will suppress results for all samples if the Negative Control(s) are not valid (see table below).

**Table 6.** Criteria for Valid Negative Control

| Control | Targets | RNA IC | Description |
|---|---|---|---|
| Negative Control | Not Detected | Detected | Plate Status: Valid.<br>Samples can be interpreted. |
| Negative Control | Detected | N/A | Plate Status: Invalid.<br>Samples results cannot be interpreted. Results suppressed by software. |
| Negative Control | N/A | Not Detected | Plate Status: Invalid.<br>Samples results cannot be interpreted. Results suppressed by software. |

## External Positive Controls

Each laboratory should establish its own Quality Control ranges and frequency of QC testing based on applicable local laws, regulations and good laboratory practices.

External positive controls can be well characterized clinical samples, inactivated virus or synthetic constructs. The positive controls should go through all processing steps (extractions, amplification, and detection). It is recommended that at least one positive control be included for each assay run. Wells identified as Positive Controls will be trended by the BioCode® MDx-3000 software and the report will indicate a valid or invalid result on the report header (see table below). The software will not suppress results based on positive control results. If a positive control does not perform as expected, the user should review all samples in that batch to determine if results should be reported.

During validation, Applied BioCode used the AccuPlex SARS-CoV-2 Reference material (LGC SeraCare; 0505-0126) and the Influenza B, Influenza A H1, Influenza A H3, Respiratory Syncytial Virus A, and Influenza A H1N1pdm (Zeptometrix; NATRRPP-ABC) each at a 1:4 dilution, individually or combined in pairs extracted per IFU.

**Table 7**. Criteria for Valid Positive Control

| Control | Targets | RNA IC | Recommendations |
|---|---|---|---|
| Positive Control | Expected Target Detected | N/A | Report will indicate positive control is Valid. No user intervention required. |
| Positive Control | Expected Target Not Detected | N/A | Report will indicate positive control is Invalid. User should review results prior to release. |

## Internal Control

An RNA Internal Control (RNA IC2: bacteriophage MS2) is added to each sample during extraction. The internal control monitors the efficiency of the extraction, reverse transcription, amplification and detection stages of the assay. Positive results may be reported in the absence of RNA IC detection. However, the BioCode® MDx-3000 software will suppress negative results for any well with invalid RNA IC result. (see table).

**Table 8.** Criteria for RNA Internal Control (RNA IC)

| Targets | RNA IC | Recommendations |
|---|---|---|
| Detected | Detected | Well status: Valid.<br>Report all results. |
| Detected | Not Detected | Well status: Invalid.<br>Detected results may be reported. Repeat/reflex testing. |
| Not Detected | Not Detected | Well status: Invalid.<br>Not Detected results suppressed by software. Repeat/reflex testing. |

Lack of RNA IC signal may indicate sample-associated inhibition or reagent/instrumentation issues. Samples suspected of being inhibitory should be repeated from extraction. If reagent or instrument issues are suspected specimens may be repeated from stored nucleic acid extracts.

## Target Pathogen Interpretation

Fluorescent signals from BMBs with the same barcode are sorted and the median fluorescence intensity (MFI) is calculated for each analyte. The BMB assay is considered "Detected" by comparing the MFI to a validated assay cutoff. The SARS-CoV-2 and the Respiratory Syncytial Virus each have a single BMB assay in the multiplex. The software will report these as detected if the single BMB assay produces MFI above the threshold.

**Table 9.** Single BMB Assay names with corresponding results.

| Assay Name | Assay Result | Report Result |
|---|---|---|
| RSV | Detected | Respiratory Syncytial Virus Detected |
| CoV-2 | Detected | SARS-CoV-2 Detected |

In contrast, the test results for several targets rely on the combination of multiple BMB assays. These include the Influenza A, A subtyping and Influenza B assays. The tables below outline possible assay results for these targets and the corresponding test reports.

**Table 10.** Possible Assay results and corresponding report results for Influenza B (2 BMB assays).

| Assay Name/Result | Assay Name/Result | Report Result |
|---|---|---|
| FluB1 / Not Detected | FluB2 / Not Detected | Influenza B Not Detected |
| FluB1 / Detected | FluB2 / Any Result | Influenza B Detected |
| FluB1 / Any Result | FluB2 / Detected | Influenza B Detected |

**Table 11.** Possible Assay results and corresponding report results for Influenza A and HA subtyping assays

| Target Assay / BioCode® CoV2P Result | Flu A | Flu A/H1 | Flu A/H1 pdm09 | Flu A/H3 | Action |
|---|---|---|---|---|---|
| Influenza A Not Detected | Not Detected | Not Detected | Not Detected | Not Detected | Report result |
| Influenza A and A/H1 Detected | Detected | Detected | Not Detected | Not Detected | Report result |
| Influenza A/H1pdm09 Detected | Detected | Not Detected | Detected | Not Detected | Report result |
| Influenza A/H3 Detected | Detected | Not Detected | Not Detected | Detected | Report result |
| Influenza A/H1, and A/H1pdm09 Detected | Detected | Detected | Detected | Not Detected | Multiple infections are possible but rare, retest to confirm result [a] |
| Influenza A/H1, and A/H3 Detected | Detected | Detected | Not Detected | Detected | Multiple infections are possible but rare, retest to confirm result [a] |
| Influenza A/H1pdm09, and A/H3 Detected | Detected | Not Detected | Detected | Detected | Multiple infections are possible but rare, retest to confirm result [a] |
| Influenza A/H1, A/H1pdm09, and A/H3 Detected | Detected | Detected | Detected | Detected | Multiple infections are possible but rare, retest to confirm result [a] |
| Influenza A (no subtype detected) | Detected | Not Detected | Not Detected | Not Detected | Retest (see the section on Influenza A, no subtype detected below) |
| Influenza A Indeterminate | Not Detected | Detected | Detected or Not Detected | Detected or Not Detected | Retest [b] |
| | Not Detected | Detected or Not Detected | Detected | Detected or Not Detected | |
| | Not Detected | Detected or Not Detected | Detected or Not Detected | Detected | |

[a] Repeated multiple positive should be further confirmed by an FDA-cleared influenza subtyping assay.
[b] If the retest result confirms the original result, it is recommended that the sample be further investigated using an FDA-cleared influenza A subtyping assay and/or sending the residual sample to local public health laboratory for further testing.

**Influenza A (no subtype detected):**
If the FluA assay is positive, but none of the hemagglutinin (HA) subtyping assays are positive, then the interpretation is Influenza A (no subtype detected). This result could occur when the titer of the virus in the specimen is low and not detected by the subtyping assays. This result could also indicate the presence of a novel Influenza A strain or a seasonal Influenza A/H3 or A/H1pdm09 strain with critical sequence mismatches to the primers and/or probes of the BioCode CoV2P influenza A HA subtyping assays. In both

cases, the sample in question should be retested. If the retest provides a different result, test the sample a third time to ensure the accuracy of the result. If the retest provides the same result, then the function of the BioCode CoV2P should be verified by testing with appropriate external control materials (known positive samples for Influenza A/H1, Influenza A/H3 and Influenza A/H1pdm09), and a negative control should also be run to test for PCR-product contamination. If the BioCode CoV2P accurately identifies the external positive and negative controls, contact the appropriate public health authorities for confirmatory testing.

## BioCode® CoV-2 Flu Plus Assay Test Report

The analyzed BioCode® MDx-3000 results are displayed in two report formats: Run Report for the entire run including multiple specimens, or Sample Report for individual specimens. Both reports can be exported as a PDF or CSV file. Each report includes fully analyzed and interpreted results for specimens and/or controls but is formatted differently. Refer to operator manual for more details and examples of the BioCode® MDx-3000 reports.

The Run Report displays analyzed results in a tabular format for all wells (specimens/controls) in a run from a specific Kit lot. If more than one lot is run together, separate Run Reports will be generated by the software for each lot. Possible results by target are: Detected, Not detected, Indeterminate (for Influenza A only), Invalid or N/A (if not ordered and result is masked).

**Note:** Masking of SARS-CoV-2 results is not appropriate for an assay under EUA. Results for SARS-CoV-2 must be reported for all specimens.

The Sample report displays results for a single well (specimen/control). In addition to results for each target, the Sample Reports include a results summary section which allows positive results to be reviewed at a glance. The Sample Report results summary will also indicate well validity based on BMB counts, background MFI, and external and internal controls. Sample reports also include any samples specific comments entered during setup.

Both report headers provide traceability information for: Run name, Run start and finish time, User ID, Software version, Instrument ID, Kit Name, and Reagent lots and expiration dates. The headers also include sections for Run Status and External Controls status. The Run Status section will specify if the run is Incomplete, Valid or Invalid based on the Negative Control results for the specific run/kit lot. The External Controls section indicates the results for the negative controls (Valid or Invalid) and Positive Controls (Valid, Invalid, or N/A if not assayed). The Run Status and Controls sections should be reviewed prior to review of target results. In addition to these summaries, the software will also mask results in the detailed tabular sections based on plate and well validity requirements (see interpretation of results for details).

Completed reports can be electronically reviewed. Reviewer comments will be added to the report footer for traceability under the review section. In addition, MFI (Median Florescence Intensity) reports are available for information only for administrator level users.

## LIMITATIONS OF THE PROCEDURE

- The use of this assay as an *in vitro* diagnostic under FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, and meet requirements to perform high complexity tests.
- The BioCode® CoV-2 Flu Plus Assay is to be used with the BioCode® MDx-3000 with MDx software, and easyMAG (bioMerieux) or MagNA Pure 96 (Roche) automated extraction instruments. Results of this test should be interpreted by a trained clinician in conjunction with clinical history, epidemiological data and any other laboratory data.
- This assay is qualitative and does not provide a quantitative value for the pathogen(s) present in the sample.
- The performance of the BioCode® CoV-2 Flu Plus Assay has been validated with nasopharyngeal swab (NPS) specimens in VTM or UTM. It has not been validated for other specimen types.
- The performance of this test has not been established for patients without signs of symptoms of respiratory infection.
- The performance of the BioCode® CoV-2 Flu Plus Assay is dependent upon proper sample collection, handling, transportation, storage, and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false positive and false negative results caused by improperly collected, transported, or handled specimens. The internal control (RNA IC2) will not indicate whether or not nucleic acid has been lost due to inadequate collection, transport or storage of specimens. Particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. The 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-CoV2 and their prevalence, which change over time.
- Negative results do not exclude the possibility of infection. Negative test results may occur from sequence variants in the region targeted by the assay, the presence of inhibitors, technical error, sample mix-up, or an infection caused by a pathogen not detected by the panel. Test results may also be affected by concurrent antiviral therapy or viral load in the sample that are below the limit of detection for the test. Negative results should not be used as the sole basis for diagnosis, treatment, or other management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test or lower respiratory tract infection that is not detected by a nasopharyngeal swab specimen.
- Nucleic acids may persist independently of transmissibility or it may be asymptomatically carried. Therefore, a positive result does not necessarily indicate the presence of transmissible virus level.
- There is a risk of false positive results due to cross-contamination with other samples, viral nucleic acids or amplified product. Particular attention should be given to the Laboratory Precautions noted under the *Warnings and Precautions* section above.
- There is a risk of false positive results due to non-specific amplification. Erroneous results due to cross-reactivity with organisms found in respiratory tract that were not evaluated or new variant sequences that emerge are possible.

- The performance of this test has not been established for monitoring treatment of COVID-19 infection.
- The performance of this device has not been assessed in a population vaccinated against COVID-19.
- The performance of this test has not been evaluated for immunocompromised individuals.
- The effect of antibiotic treatment on test performance has not been evaluated.
- The performance of this test has not been established for screening of blood or blood products.

## CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The BioCode® CoV-2 Flu Plus 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

To assist clinical laboratories using the BioCode® CoV-2 Flu Plus Assay, the relevant Conditions of Authorization are listed verbatim below and are required to be met by laboratories performing the EUA test.

a) Authorized laboratories using your product[1] must 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.
b) Authorized laboratories using your product must use your product as outlined in the authorized labeling. Deviation from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required are not permitted.
c) Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.
d) Authorized laboratories using your product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
e) Authorized laboratories must collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH_EUA-Reporting@fda.hhs.gov) and you (TechSupport@ApBioCode.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
f) All laboratory personnel using your product must be appropriately trained in PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use this product in accordance with the authorized labeling.
g) Applied BioCode, authorized distributors, and authorized laboratories using your product must 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.

[1] "Your product" refers to the BioCode CoV-2 Flu Plus Assay. The letter of authorization refers 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" as "authorized laboratories".

## PERFORMANCE CHARACTERISTICS

The BioCode® CoV-2 Flu Plus Assay was developed by combining a set of primers and probes from the BioCode® SARS-CoV-2 EUA assay with a subset of primers and probes from the FDA-cleared BioCode® Respiratory Pathogen Panel (RPP). For all assays shared between the two panels, the sequences of primers and probes were unchanged. In addition, the BioCode® CoV-2 Flu Plus Assay also includes an additional reverse primer with a single base variation for influenza A H3 to broaden sequence variation coverage. Studies were performed to demonstrate that the performance characteristics of the assays were unaffected by the panel modification. The original studies of the BioCode® Respiratory Pathogen Panel for the included targets and the BioCode® SARS-CoV-2 assay are presented below as well as these additional studies.

### Clinical Performance

#### Clinical comparison study of BioCode® CoV-2 Flu Plus Assay– Retrospective samples

The BioCode® CoV-2 Flu Plus Assay was evaluated with 115 frozen de-identified nasopharyngeal swab samples in UTM/VTM. The samples were collected by qualified personnel and stored in compliance with conditions stated in the IFU. As influenza H1 positive samples were unavailable, an additional 10 unique negative NPS samples in VTM were spiked with influenza A H1. This data with contrived samples is presented separately. Randomized samples were tested in a blinded fashion and compared to the FDA-cleared molecular assay (for Flu A including subtypes, Flu B and RSV) and FDA authorized SARS-CoV-2 molecular test (for SARS-CoV-2) after extraction per instructions for use. The BioCode CoV-2 Flu Plus Assay was extracted and assayed per instructions for use with both the easyMAG and MagNA Pure 96 system. Separate extractions for each sample were performed prior to testing with the subject assay or the comparison assays. A summary of the demographic information of the tested samples is provided in Table 12.

**Table 12.** Demographics of retrospective clinical specimens

| Retrospective Clinical Specimens | |
|---|---|
| Total Specimens | 115 |
| Gender | n/N (%) |
| Male | 54 (47%) |
| Female | 61 (53%) |
| Age Category | n/N (%) |
| 0-5 years | 8/115 (7%) |
| 6-21 years | 14/115 (12%) |
| 22-64 years | 76/115 (66%) |
| 65+ years | 17/115 (15%) |

There were two samples that were invalid with the BioCode CoV2P assay after initial extraction with the easyMAG. Both were valid after repeat extraction. There were no invalid samples for the BioCode CoV2P MP96 extractions or either reference assay. One sample was indeterminate for flu A and one was flu A no subtype by the reference method. One sample was inconclusive for SARS-CoV-2 by the

comparator assay on initial testing and was detected upon repeat testing.

The Retrospective study results stratified by target and BioCode CoV2P using both the MagNA Pure and easyMAG extraction systems are presented in the tables below.

**Table 13.** Summary of Clinical Study results compared to the BioCode CoV2P with MagNA Pure 96 extraction system: Retrospective specimens stratified by target

| Target | (n) | Positive Agreement | | Negative Agreement | |
|---|---|---|---|---|---|
| | | PPA (%) | 95% CI | NPA (%) | 95% CI |
| Influenza A | 115 | 23/23 (100%) | 85.7%,100.0% | 91/91[d] (100%) | 95.9%,100.0% |
| Influenza A H1[a] | 115 | N/A | N/A | 114/114[d] (100%) | 96.7%,100.0% |
| Influenza A H1 2009pdm | 115 | 10/10 (100%) | 72.2%,100.0% | 104/104[d] (100%) | 96.4%,100.0% |
| Influenza A H3 | 115 | 12/12 (100%) | 75.8%,100.0% | 101/102[bd] (99.0%) | 94.7%,99.8% |
| Influenza B | 115 | 10/10 (100%) | 72.2%,100.0% | 105/105 (100%) | 96.5%,100.0% |
| Respiratory Syncytial Virus | 115 | 10/10 (100%) | 72.2%,100.0% | 103/105[c] (98.1%) | 93.3%,99.5% |
| SARS-CoV-2 | 115 | 30/30 (100%) | 88.6%,100.0% | 85/85 (100%) | 95.7%,100.0% |

a. Archived specimens were not available, therefore spiked negative NPS was used. See Table 15 for breakdown of organisms and concentrations.
b. One specimen was detected as Influenza A/H3 by BioCode CoV2P and only as flu A (no subtype) by the comparator assay. Bi-directional sequencing of this sample confirmed as H3 with sequence similarity to the A/Kansas/14/2017 (H3N2) strain.
c. Two specimens were detected as RSV by BioCode CoV2P and negative by the comparator assay. Repeat of these specimens were negative by both methods. Placement on the plate suggests a pipetting error for the initial false positive.
d. One specimen was indeterminate for flu A with the reference method. These were not included in the flu A agreement calculations.

**Table 14.** Summary of Clinical Study results compared to the BioCode CoV2P with easyMAG extraction system: Retrospective specimens stratified by target

| Target | (n) | Positive Agreement | | Negative Agreement | |
|---|---|---|---|---|---|
| | | PPA (%) | 95% CI | NPA (%) | 95% CI |
| Influenza A | 115 | 23/23 (100%) | 85.7%,100.0% | 90/91 (98.9%) [c, d] | 94.0%,99.8% |
| Influenza A H1[a] | 115 | N/A | N/A | 114/114 (100%) [d] | 96.7%,100.0% |
| Influenza A H1 2009pdm | 115 | 10/10 (100%) | 72.2%,100.0% | 103/104 (99.0%) [c, d] | 94.7%,99.8% |
| Influenza A H3[b] | 115 | 12/12 (100%) | 75.8%,100.0% | 101/102 (99.0%) [b, d] | 94.7%,99.8% |
| Influenza B | 115 | 10/10 (100%) | 72.2%,100.0% | 104/105 (99.0%%) [c] | 94.8%,99.8% |
| Respiratory Syncytial Virus | 115 | 10/10 (100%) | 72.2%,100.0% | 105/105 (100%) | 96.5%,100.0% |

| Target | (n) | Positive Agreement | | Negative Agreement | |
|---|---|---|---|---|---|
| | | PPA (%) | 95% CI | NPA (%) | 95% CI |
| SARS-CoV-2[e] | 115 | 29/30 (96.7%) | 83.3%,99.4% | 84/85 (98.8%) [c] | 93.6%,99.8% |

a. Archived specimens were not available, therefore spiked negative NPS was used. See Table 15 for breakdown of organisms and concentrations.
b. One specimen was detected as Influenza A/H3 by BioCode CoV2P and only as flu A (no subtype) by the comparator assay. Bi-directional sequencing of this sample confirmed as H3 with sequence similarity to the A/Kansas/14/2017 (H3N2) strain.
c. Three specimens (one each for influenza A/H1N1pdm, influenza B and SARS-CoV-2) were positive by BioCode CoV2P and negative by the comparator assay. Two of the three were negative on repeat by both methods.
d. One specimen was indeterminate for flu A with the comparator assay. It was not included in the flu A agreement calculations.
e. One specimen was detected as SARS-CoV-2 with the reference method and not detected by the BioCode CoV2P. The sample had undergone 2 freeze thaws before extraction with the easyMAG. It was detected by BioCode CoV2P on the initial testing prior to freeze thaw (see MP96 result).

**Clinical comparison study of BioCode® CoV-2 Flu Plus Assay– Contrived Influenza A H1 specimens**

Due to the rare occurrence of influenza A H1, archived positive specimens could not be obtained. To compare performance with the comparator assay, contrived clinical specimens were prepared using 10 unique prescreened natural NPS specimens UTM. Contrived specimens were spiked at concentrations of 1.3X LOD or greater using 2 different strains of influenza A H1. The spiked samples were blinded and compared to the FDA-cleared molecular assay and after extraction with the MagNA Pure 96 system in parallel with the retrospective samples. The results and breakdown of the strains and concentrations tested are presented in Table 15.

**Table 15.** Results from Influenza A H1 spiked NPS specimens

| Target | Source | Strain/Isolate | Fold LoD [a] | Concentration | PPA (%) | 95% CI |
|---|---|---|---|---|---|---|
| **Influenza A H1N1** | **Zeptometrix 0810036CF** | **A/New Caledonia/20/99** | **1.3** | **20 $TCID_{50}$/ mL** | 2/2 (100%) | 34.2%, 100% |
| | | | **4** | **60 $TCID_{50}$/ mL** | 2/2 (100%) | 34.2%, 100% |
| | | | **12** | **180 $TCID_{50}$/ mL** | 2/2 (100%) | 34.2%, 100% |
| | **Zeptometrix 0810244CF** | **A/Brisbane/59/07** | **1.3** | **20 $TCID_{50}$/ mL** | 2/2 (100%) | 34.2%, 100% |
| | | | **4** | **60 $TCID_{50}$/ mL** | 1/1 (100%) | 20.7%, 100% |
| | | | **12** | **180 $TCID_{50}$/ mL** | 1/1 (100%) | 20.7%, 100% |
| | **Combined** | | | | **10/10 (100%)** | **72.2%, 100%** |

a. LoD as presented in the comparator assay Package Insert

## Limit of Detection

### Comparison Testing Near Limit of Detection for BioCode® CoV-2 Flu Plus Assay

A study was performed to compare the performance of the BioCode® CoV-2 Flu Plus Assay on the BioCode® MDX-3000 near the Limit of Detection compared to the BioCode® RPP or BioCode® SARS-CoV-2. Quantified stocks were serially diluted and extracted in quadruplicate with both the easyMAG and MagNA Pure 96 extraction systems and assayed in parallel with BioCode® CoV2P and BioCode® RPP or BioCode SARS-CoV-2 assays to confirm equivalent LoDs. All targets were detected equivalently (within 3-fold) or better with BioCode® CoV2P (Table 16). Additionally, the Influenza A/Kansas/14/2017(H3N2) was detected as flu A (no subtype) by BioCode® RPP and Influenza A H3 by BioCode® CoV2P which contains the additional primer for improved detection.

**Table 16.** Results from LoD comparison study for BioCode® CoV-2 Flu Plus Assay and BioCode® RPP or BioCode® SARS-CoV-2 Assays.

| | | | Concentration (easyMAG) | | Concentration (MagNA Pure 96) | |
|---|---|---|---|---|---|---|
| Target | Species/Strain/Isolate | Source | RPP or SARS-CoV-2 | CoV2P | RPP or SARS-CoV-2 | CoV2P |
| Influenza A H1N1 | A/New Caledonia/20/99 | Zeptometrix 0810036CF | $5.0x10^{0}$ $TCID_{50}$/mL | $1.7x10^{0}$ $TCID_{50}$/mL | $5.6x10^{-1}$ $TCID_{50}$/mL | $5.6x10^{-1}$ $TCID_{50}$/mL |
| | A/Brisbane/59/2007 | Zeptometrix 0810244CF | $4.5x10^{1}$ $TCID_{50}$/mL | $5.0x10^{0}$ $TCID_{50}$/mL | $4.5x10^{1}$ $TCID_{50}$/mL | $1.5x10^{1}$ $TCID_{50}$/mL |
| Influenza A H1N1 pdm09 | A/California/07/09 | Zeptometrix 0810165CF | $1.3x10^{-1}$ $TCID_{50}$/mL | $1.3x10^{-1}$ $TCID_{50}$/mL | $1.3x10^{-1}$ $TCID_{50}$/mL | $4.0x10^{-1}$ $TCID_{50}$/mL |
| | A/Mexico/4108/09 | Zeptometrix 0810166CF | $7.3x10^{-1}$ $TCID_{50}$/mL | $2.4x10^{-1}$ $TCID_{50}$/mL | $2.2x10^{0}$ $TCID_{50}$/mL | $2.2x10^{0}$ $TCID_{50}$/mL |
| Influenza A H3N2 | A/Wisconsin/67/05 | Zeptometrix 0810252CF | $1.3x10^{0}$ $TCID_{50}$/mL | $3.9x10^{0}$ $TCID_{50}$/mL | $1.3x10^{0}$ $TCID_{50}$/mL | $1.3x10^{0}$ $TCID_{50}$/mL |
| | A/Kansas/14/2017 | CDC | >$4.5x10^{3}$ $EID_{50}$/mL [a] | $1.7x10^{2}$ $EID_{50}$/mL | >$4.5x10^{3}$ $EID_{50}$/mL [a] | $1.7x10^{2}$ $EID_{50}$/mL |
| Influenza B | B/Wisconsin/1/10 (Yamagata) | Zeptometrix 0810241CF | $2.7x10^{-1}$ $TCID_{50}$/mL | $2.7x10^{-1}$ $TCID_{50}$/mL | $9.0x10^{-2}$ $TCID_{50}$/mL | $9.0x10^{-2}$ $TCID_{50}$/mL |
| | B/Malaysia/2560/2004 (Victoria) | Zeptometrix 0810258CF | $1.1x10^{-2}$ $TCID_{50}$/mL | $3.3x10^{-2}$ $TCID_{50}$/mL | $3.3x10^{-2}$ $TCID_{50}$/mL | $3.3x10^{-2}$ $TCID_{50}$/mL |
| Respiratory Syncytial Virus | Type A | Zeptometrix 0810040ACF | $1.1x10^{-1}$ $TCID_{50}$/mL | $3.3x10^{-1}$ $TCID_{50}$/mL | $1.1x10^{-1}$ $TCID_{50}$/mL | $1.1x10^{-1}$ $TCID_{50}$/mL |
| | B WV/14617/85 | ATCC VR-1400 | $6.0x10^{-2}$ $TCID_{50}$/mL | $6.0x10^{-2}$ $TCID_{50}$/mL | $2.0x10^{-2}$ $TCID_{50}$/mL | $2.0x10^{-2}$ $TCID_{50}$/mL |
| SARS-CoV-2 | USA-WA1/2020 | Zeptometrix 0810587CFHI | $1.7x10^{-2}$ $TCID_{50}$/mL | $1.7x10^{-2}$ $TCID_{50}$/mL | $5.7x10^{-3}$ $TCID_{50}$/mL | $5.7x10^{-3}$ $TCID_{50}$/mL |

a. Influenza A H3N2 (A/Kansas/14/2017) was detected as flu A (No Subtype) with BioCode RPP at $1.7x10^{2}$ $EID_{50}$/mL.

**Table 17.** Results of comparison at or near LoD for BioCode® CoV-2 Flu Plus Assay and BioCode® RPP or BioCode® SARS-CoV-2 Assays with easyMAG extraction.

| Organism | Source | Concentration | Target Probe | Number Detected (n of n) | Replicate 1 | Replicate 2 | Replicate 3 | Replicate 4 |
|---|---|---|---|---|---|---|---|---|
| Influenza A H1N1 (A/New Caledonia/20/99) | Zeptometrix 0810036CF | 5.0 x $10^0$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 1.7 x $10^0$ $TCID_{50}$/mL | BioCode RPP | 3/4 | Flu A (No subtype) | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 5.6 x $10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 0/4 | Indeterminate | Flu A (No subtype) | Not Detected | Not Detected |
| | | | BioCode CoV2P | 0/4 | Not Detected | Flu A (No subtype) | Not Detected | Flu A (No subtype) |
| Influenza A H1N1 (A/Brisbane/59/2007) | Zeptometrix 0810244CF | 4.5 x $10^1$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 1.5 x $10^1$ $TCID_{50}$/mL | BioCode RPP | 3/4 | Detected | Detected | Detected | Flu A (No subtype) |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 5.0 x $10^0$ $TCID_{50}$/mL | BioCode RPP | 1/4 | Flu A (No subtype) | Flu A (No subtype) | Flu A (No subtype) | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 1.7 x $10^0$ $TCID_{50}$/mL | BioCode RPP | 1/4 | Flu A (No subtype) | Flu A (No subtype) | Flu A (No subtype) | Detected |
| | | | BioCode CoV2P | 2/4 | Detected | Flu A (No subtype) | Detected | Flu A (No subtype) |
| Influenza A H1N1 pdm09 (A(H1N1)/California/07/09) | Zeptometrix 0810165CF | 4.0 x $10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 1.3 x $10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |

| Organism | Source | Concentration | Target Probe | Number Detected (n of n) | Replicate 1 | Replicate 2 | Replicate 3 | Replicate 4 |
|---|---|---|---|---|---|---|---|---|
| | | $4.4 \times 10^{-2}$ $TCID_{50}$/mL | BioCode RPP | 1/4 | Indeterminate | Flu A (No subtype) | Detected | Flu A (No subtype) |
| | | | BioCode CoV2P | 0/4 | Not Detected | Indeterminate | Indeterminate | Indeterminate |
| Influenza A H1N1 pdm09 (A/Mexico/4108/09) | Zeptometrix 0810166CF | $7.3 \times 10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | $2.4 \times 10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 3/4 | Detected | Detected | Detected | Indeterminate |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | $8.2 \times 10^{-2}$ $TCID_{50}$/mL | BioCode RPP | 0/4 | Indeterminate | Indeterminate | Indeterminate | Flu A (No subtype) |
| | | | BioCode CoV2P | 1/4 | Not Detected | Flu A (No subtype) | Detected | Not Detected |
| Influenza A H3N2 (A/Wisconsin/67/05) | Zeptometrix 0810252CF | $3.9 \times 10^{0}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | $1.3 \times 10^{0}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 3/4 | Detected | Detected | Flu A (No subtype) | Detected |
| | | $4.3 \times 10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 3/4 | Flu A (No subtype) | Detected | Detected | Detected |
| | | | BioCode CoV2P | 3/4 | Detected | Detected | Detected | Indeterminate |
| Influenza H3N2 (A/Kansas/14/2017) | CDC VP2019 | $5.0 \times 10^{2}$ $EID_{50}$/mL | BioCode RPP | 0/4 | Flu A (No subtype) | Flu A (No subtype) | Flu A (No subtype) | Flu A (No subtype) |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | $1.7 \times 10^{2}$ $EID_{50}$/mL | BioCode RPP | 0/4 | Flu A (No subtype) | Flu A (No subtype) | Flu A (No subtype) | Flu A (No subtype) |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | $5.6 \times 10^{1}$ $EID_{50}$/mL | BioCode RPP | 0/4 | Not Detected | Flu A (No subtype) | Flu A (No subtype) | Flu A (No subtype) |
| | | | BioCode CoV2P | 0/4 | Flu A (No subtype) | Not Detected | Flu A (No subtype) | Flu A (No subtype) |

| Organism | Source | Concentration | Target Probe | Number Detected (n of n) | Replicate 1 | Replicate 2 | Replicate 3 | Replicate 4 |
|---|---|---|---|---|---|---|---|---|
| Influenza B (Wisconsin/1/10) Yamagata | Zeptometrix 0810241CF | 2.7 x $10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 9.0 x $10^{-2}$ $TCID_{50}$/mL | BioCode RPP | 3/4 | Detected | Detected | Detected | Not Detected |
| | | | BioCode CoV2P | 3/4 | Not Detected | Detected | Detected | Detected |
| | | 3.0 x $10^{-2}$ $TCID_{50}$/mL | BioCode RPP | 1/4 | Not Detected | Not Detected | Detected | Not Detected |
| | | | BioCode CoV2P | 2/4 | Detected | Not Detected | Detected | Not Detected |
| Influenza B (Malaysia/2560/2004) Victoria | Zeptometrix 0810258CF | 3.3 x $10^{-2}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 1.1 x $10^{-2}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 2/4 | Not Detected | Detected | Detected | Not Detected |
| | | 3.7 x $10^{-3}$ $TCID_{50}$/mL | BioCode RPP | 3/4 | Detected | Detected | Detected | Not Detected |
| | | | BioCode CoV2P | 0/4 | Not Detected | Not Detected | Not Detected | Not Detected |
| Respiratory Syncytial Virus (Type A) | Zeptometrix 0810040ACF | 3.3 x $10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 1.1 x $10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 3/4 | Detected | Detected | Not Detected | Detected |
| | | 3.7 x $10^{-2}$ $TCID_{50}$/mL | BioCode RPP | 2/4 | Detected | Detected | Not Detected | Not Detected |
| | | | BioCode CoV2P | 3/4 | Detected | Detected | Detected | Not Detected |
| Respiratory Syncytial Virus (Type B WV/14617/85) | ATCC VR-1400 | 1.8 x $10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 6.0 x $10^{-2}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |

| Organism | Source | Concentration | Target Probe | Number Detected (n of n) | Replicate 1 | Replicate 2 | Replicate 3 | Replicate 4 |
|---|---|---|---|---|---|---|---|---|
| | | 2.0 x $10^{-2}$ $TCID_{50}$/mL | BioCode RPP | 3/4 | Detected | Detected | Not Detected | Detected |
| | | | BioCode CoV2P | 2/4 | Detected | Not Detected | Not Detected | Detected |
| SARS-CoV-2 (USA-WA1/2020) | Zeptometrix 0810587CFHI | 5.1 x $10^{-2}$ $TCID_{50}$/mL | BioCode CoV2 | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 1.7 x $10^{-2}$ $TCID_{50}$/mL | BioCode CoV2 | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 5.7 x $10^{-3}$ $TCID_{50}$/mL | BioCode CoV2 | 2/4 | Detected | Not Detected | Detected | Not Detected |
| | | | BioCode CoV2P | 2/4 | Detected | Detected | Not Detected | Not Detected |

**Table 18.** Results of comparison at or near LoD for BioCode® CoV-2 Flu Plus Assay and BioCode® RPP or BioCode® SARS-CoV-2 Assays with MagNA Pure 96 extraction.

| Organism | Source | Concentration | Target Probe | Number Detected (n of n) | Replicate 1 | Replicate 2 | Replicate 3 | Replicate 4 |
|---|---|---|---|---|---|---|---|---|
| Influenza A H1N1 (A/New Caledonia/20/99) | Zeptometrix 0810036CF | 1.7 x $10^{0}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 5.6 x $10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 1.9 x $10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 0/4 | Flu A (No subtype) | Flu A (No subtype) | Not Detected | Indeterminate |
| | | | BioCode CoV2P | 2/4 | Flu A (No subtype) | Not Detected | Detected | Detected |
| Influenza A H1N1 (A/Brisbane/59/2007) | Zeptometrix 0810244CF | 4.5 x $10^{1}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 1.5 x $10^{1}$ $TCID_{50}$/mL | BioCode RPP | 3/4 | Flu A (No subtype) | Detected | Detected | Detected |

| Organism | Source | Concentration | Target Probe | Number Detected (n of n) | Replicate 1 | Replicate 2 | Replicate 3 | Replicate 4 |
|---|---|---|---|---|---|---|---|---|
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 5.0 x $10^0$ $TCID_{50}$/mL | BioCode RPP | 3/4 | Flu A (No subtype) | Detected | Detected | Detected |
| | | | BioCode CoV2P | 3/4 | Detected | Detected | Flu A (No subtype) | Detected |
| Influenza A H1N1 pdm09 (A(H1N1)/California/07/09) | Zeptometrix 0810165CF | 4.0 x $10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 1.3 x $10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 2/4 | Detected | Indeterminate | Detected | Indeterminate |
| | | 4.4 x $10^{-2}$ $TCID_{50}$/mL | BioCode RPP | 3/4 | Not Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 1/4 | Flu A (No subtype) | Indeterminate | Detected | Not Detected |
| Influenza A H1N1 pdm09 (A/Mexico/4108/09) | Zeptometrix 0810166CF | 6.6 x $10^0$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 2.2 x $10^0$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 7.3 x $10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 3/4 | Detected | Detected | Detected | Indeterminate |
| | | | BioCode CoV2P | 3/4 | Detected | Detected | Indeterminate | Detected |
| Influenza A H3N2 (A/Wisconsin/67/05) | Zeptometrix 0810252CF | 3.9 x $10^0$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 1.3 x $10^0$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 4.3 x $10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 3/4 | Indeterminate | Detected | Detected | Detected |
| | | | BioCode CoV2P | 3/4 | Detected | Flu A (No subtype) | Detected | Detected |

| Organism | Source | Concentration | Target Probe | Number Detected (n of n) | Replicate 1 | Replicate 2 | Replicate 3 | Replicate 4 |
|---|---|---|---|---|---|---|---|---|
| Influenza A H3N2 (A/Kansas/14/2017) | CDC VP2019 | 5.0 x $10^2$ $EID_{50}$/mL | BioCode RPP | 1/4 | Detected | Flu A (No subtype) | Flu A (No subtype) | Flu A (No subtype) |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 1.7 x $10^2$ $EID_{50}$/mL | BioCode RPP | 0/4 | Flu A (No subtype) | Flu A (No subtype) | Flu A (No subtype) | Flu A (No subtype) |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 5.6 x $10^1$ $TCID_{50}$/mL | BioCode RPP | 0/4 | Flu A (No subtype) | Flu A (No subtype) | Flu A (No subtype) | Flu A (No subtype) |
| | | | BioCode CoV2P | 2/4 | Flu A (No subtype) | Flu A (No subtype) | Detected | Detected |
| Influenza B (Wisconsin/1/10) Yamagata | Zeptometrix 0810241CF | 2.7 x $10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 9.0 x $10^{-2}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 3.0 x $10^{-2}$ $TCID_{50}$/mL | BioCode RPP | 1/4 | Detected | Not Detected | Not Detected | Not Detected |
| | | | BioCode CoV2P | 1/4 | Detected | Not Detected | Not Detected | Not Detected |
| Influenza B (Malaysia/2560/2004) Victoria | Zeptometrix 0810258CF | 1.0 x $10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 3.3 x $10^{-2}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 1.1 x $10^{-2}$ $TCID_{50}$/mL | BioCode RPP | 3/4 | Detected | Detected | Detected | Not Detected |
| | | | BioCode CoV2P | 3/4 | Detected | Detected | Not Detected | Detected |
| Respiratory Syncytial Virus (Type A) | Zeptometrix 0810040ACF | 3.3 x $10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | 1.1 x $10^{-1}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |

| Organism | Source | Concentration | Target Probe | Number Detected (n of n) | Replicate 1 | Replicate 2 | Replicate 3 | Replicate 4 |
|---|---|---|---|---|---|---|---|---|
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | $3.7 \times 10^{-2}$ $TCID_{50}$/mL | BioCode RPP | 2/4 | Detected | Detected | Not Detected | Not Detected |
| | | | BioCode CoV2P | 1/4 | Detected | Not Detected | Not Detected | Not Detected |
| Respiratory Syncytial Virus (Type B WV/14617/85) | ATCC VR-1400 | $6.0 \times 10^{-2}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | $2.0 \times 10^{-2}$ $TCID_{50}$/mL | BioCode RPP | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | $6.7 \times 10^{-3}$ $TCID_{50}$/mL | BioCode RPP | 2/4 | Not Detected | Not Detected | Detected | Detected |
| | | | BioCode CoV2P | 1/4 | Not Detected | Not Detected | Not Detected | Detected |
| SARS-CoV-2 (USA-WA1/2020) | Zeptometrix 0810587CFHI | $1.7 \times 10^{-2}$ $TCID_{50}$/mL | BioCode CoV2 | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | $5.7 \times 10^{-3}$ $TCID_{50}$/mL | BioCode CoV2 | 4/4 | Detected | Detected | Detected | Detected |
| | | | BioCode CoV2P | 4/4 | Detected | Detected | Detected | Detected |
| | | $1.9 \times 10^{-3}$ $TCID_{50}$/mL | BioCode CoV2 | 3/4 | Detected | Not Detected | Detected | Detected |
| | | | BioCode CoV2P | 0/4 | Not Detected | Not Detected | Not Detected | Not Detected |

**Limit of Detection for BioCode® CoV-2 Flu Plus Assay – Contemporary influenza A and influenza B strains**

A study was performed to determine the Limit of Detection for the BioCode® CoV-2 Flu Plus Assay with contemporary strains of influenza A and B. For this study quantified viral stocks were spiked into prescreened negative nasopharyngeal swab matrix, then extracted using both the easyMAG (EM) and MagNA Pure (MP96) extraction systems. Preliminary LoD was determined with 4 replicates of 3-fold serial dilutions then confirmed with 20 replicates. LoD was defined as the lowest concentration with ≥95% detection of 20 replicates (at least 19 out of 20).

**Table 19.** Summary of LoD study results for BioCode® CoV-2 Flu Plus Assay – contemporary influenza A and influenza B strains by extraction system.

| Organism | Type/Origin/Strain/Year | Source | easyMag | MagNA Pure 96 |
|---|---|---|---|---|
| Influenza A H1N1 | A/Brisbane/59/07 | Zeptometrix 0810244CF | $5.0x10^{0}$ $TCID_{50}$/mL | $1.5x10^{1}$ $TCID_{50}$/mL |
| Influenza A H1N1 pdm09 | A/Brisbane/02/18 | Zeptometrix 0810585CF | $3.6x10^{0}$ $TCID_{50}$/mL | $3.6x10^{0}$ $TCID_{50}$/mL |
| Influenza A H3N2 | A/Hong Kong/2671/19 | Zeptometrix 0810609CF | $1.0x10^{1}$ $TCID_{50}$/mL | $1.0x10^{1}$ $TCID_{50}$/mL |
| Influenza B | B/Utah/9/14 (Yamagata) | Zeptometrix 0810516CF | $2.7x10^{-1}$ $TCID_{50}$/mL | $2.7x10^{-1}$ $TCID_{50}$/mL |
| | B/Brisbane/46/15 (Victoria) | Zeptometrix 0810528CF | $1.1x10^{-2}$ $TCID_{50}$/mL | $3.3x10^{-2}$ $TCID_{50}$/mL |

## Analytical Reactivity (Inclusivity)

**Analytical Reactivity (Inclusivity) – BioCode® CoV-2 Flu Plus Assay**

***In silico* Inclusivity analysis for the SARS-CoV-2 assay**

*In silico* analysis for the SARS-CoV-2 assay was performed against US sequences in the GISAID database as of September 20th, 2021. The analysis included 1,033,881 sequences. Sequences were evaluated for percent homology and predicted binding efficiency for identified mismatches. Oligo binding is predicted for >99.9% of all sequences evaluated.

*A second in silico* analysis for the SARS-CoV-2 assay was performed against additional US sequences in the GISAID database as of March 22nd, 2022. The analysis included an additional 544,885 sequences (added between 09/20/2021-03/22/2022). Sequences were evaluated for percent homology and predicted binding efficiency for identified mismatches. Oligo binding for forward and reverse primers is predicted for >99.8% of all sequences evaluated. When the probe is included, oligo binding is predicted for >96% for the evaluated sequences.

***In silico* Inclusivity analysis for Flu A, Flu B and RSV Assays**

*In silico* inclusivity analysis was performed to evaluate the primers and probes for each assay based on percent sequence homology and review of predicted binding efficiency for identified mismatches. For the Flu A and Flu B primers and probe sequences for the BioCode CoV-2 Flu Plus Assay was performed against the US sequences in the GISAID database as of March 11th, 2021. The Flu A general assay analysis included 27,922 sequences including sequences for Flu A/H1, A/H1pdm09 and A/H3N2, oligo binding is predicted for all evaluated sequences. For the Flu A/H1pdm09 assay, 12,048 sequences were evaluated. Oligo binding is predicted for 99.97% of the evaluated sequences. For the Flu A/H1 seasonal assay 1392 sequences were evaluated. Of these, oligo binding is predicted for 99.0%. For the Flu A/H3 assay, 16502 sequences since January 2015 were evaluated. Oligo binding is predicted for 99.6% of the evaluated sequences. For the Flu B1 and Flu B2 assays, 11090 and 9927 sequences were evaluated (respectively). Oligo binding is predicted for all sequences for the Flu B1 assay and 99.1% for the Flu B2 assay. The RSV

assay was evaluated with 2560 sequences from the NCBI database, oligo binding is predicted for 99.3% of the evaluated sequences.

## Analytical Specificity (Cross-Reactivity)

### *In Silico* Analysis – BioCode® CoV-2 Flu Plus Assay

*In silico* analysis was performed for each primer and probe using NCBI BLAST. In summary, no significant homology (≤80%) was found between primers and probes and analyzed organisms with the exception of SARS-CoV and *Pseudomonas aeruginosa*. For SARS-CoV, 92% homology was observed for the reverse primer and probe for the N target. Detection of SARS-CoV is not expected because of limited homology in each reaction for at least one primer/probe needed for the detection and amplification of an analyte. For *Pseudomonas aeruginosa* 85% homology was observed with the forward primer and 91% homology with the probe. Detection of *Pseudomonas aeruginosa* is not expected because of limited homology with the reverse primer, which is required for the detection and amplification of an analyte Additionally, no evidence of cross-reactivity with *Pseudomonas aeruginosa* was observed in wet testing.

**Table 20. Results from *in silico* cross reactivity analysis for influenza A assays in the BioCode CoV2P.**

| Organism | Influenza A Matrix | | | Influenza A H1 (seasonal) | | | | Influenza A H1N1 pdm09 | | | Influenza A H3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | INFA 1 Pb | Flu A F | Flu A R Bio | Flu A H1 P1 | Flu A H1 F | Flu A H1 R(A) Bio | Flu A H1 R(B) Bio | CDC H1N1 probe | 2009 H1N1 F | 2009 H1N1 R bio | Flu A H3 P | Flu A H3 F | Flu A H3 R Bio | Flu A H3 Rc Bio |
| Human coronavirus 229E | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| Human coronavirus HKU1 | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| Human coronavirus NL63 | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| Human coronavirus OC43 | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| MERS-coronavirus | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| SARS-coronavirus | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| Adenovirus Species B Serotype 7A | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| Adenovirus Species C Serotype 2 | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| Adenovirus Species E Serotype 4 | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| Enterovirus D68 | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| Human Metapneumovirus | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| Human Rhinovirus Type A1 | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| Human Influenza A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Influenza B | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| Parainfluenza Virus 1 | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| Parainfluenza Virus 2 | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| Parainfluenza Virus 3 | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| Parainfluenza Virus 4a | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| Respiratory Syncytial Virus (Type A) | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| *Bordetella pertussis* | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| *Candida albicans* | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| *Chlamydia pneumoniae* | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| *Haemophilus influenzae* | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| *Legionella pneumophila* | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| *Mycobacterium tuberculosis* | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| *Mycoplasma pneumoniae* | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| *Pneumocystis jirovecii (PJP)* | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| *Pseudomonas aeruginosa* | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| *Staphylococcus epidermidis* | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| *Streptococcus salivarius* | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |
| *Streptococcus pneumoniae* | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |

| Organism | Influenza A Matrix | | | Influenza A H1 (seasonal) | | | | Influenza A H1N1 pdm09 | | | Influenza A H3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | INFA 1 Pb | Flu A F | Flu A R Bio | Flu A H1 P1 | Flu A H1 F | Flu A H1 R(A) Bio | Flu A H1 R(B) Bio | CDC H1N1 probe | 2009 H1N1 F | 2009 H1N1 R bio | Flu A H3 P | Flu A H3 F | Flu A H3 R Bio | Flu A H3 Rc Bio |
| *Streptococcus pyogenes* | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% | <80% |

**Table 21. Results from *in silico* cross reactivity analysis for the influenza B, RSV, SARS-CoV-2 and Internal Control assays in the BioCode CoV2P.**

| Organism | Influenza B | | | | | | Respiratory Syncytial Virus | | | SARS-CoV-2 N gene (Assay A) | | | RNA Internal Control (MS2) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flu B F (A) | Flu B R Bio (A) | Flu B 1P | Flu B F (B) | Flu B R Bio (B) | Flu B 2P | RSVA F | RSVA R | RSVP | Na F | Na R Bio | Na P | MS2 F | MS2 R Bio | MS2 P4 |
| Human coronavirus 229E | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| Human coronavirus HKU1 | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| Human coronavirus NL63 | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| Human coronavirus OC43 | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| MERS-coronavirus | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| SARS-coronavirus | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | 92% | 92% | ≤80% | ≤80% | ≤80% |
| Adenovirus Species B Serotype 7A | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| Adenovirus Species C Serotype 2 | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| Adenovirus Species E Serotype 4 | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| Enterovirus D68 | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| Human Metapneumovirus | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| Human Rhinovirus Type A1 | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| Human Influenza A | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| Influenza B | N/A | N/A | N/A | N/A | N/A | N/A | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| Parainfluenza Virus 1 | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| Parainfluenza Virus 2 | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| Parainfluenza Virus 3 | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| Parainfluenza Virus 4a | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| Respiratory Syncytial Virus (Type A) | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | N/A | N/A | N/A | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| *Bordetella pertussis* | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| *Candida albicans* | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| *Chlamydia pneumoniae* | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| *Haemophilus influenzae* | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| *Legionella pneumophila* | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| *Mycobacterium tuberculosis* | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| *Mycoplasma pneumoniae* | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| *Pneumocystis jirovecii (PJP)* | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| *Pseudomonas aeruginosa* | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | 85% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| *Staphylococcus epidermidis* | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| *Streptococcus salivarius* | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |

| Organism | Influenza B | | | | | | Respiratory Syncytial Virus | | | SARS-CoV-2 N gene (Assay A) | | | RNA Internal Control (MS2) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Flu B F (A) | Flu B R Bio (A) | Flu B 1P | Flu B F (B) | Flu B R Bio (B) | Flu B 2P | RSVA F | RSVA R | RSVP | Na F | Na R Bio | Na P | MS2 F | MS2 R Bio | MS2 P4 |
| *Streptococcus pneumoniae* | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |
| *Streptococcus pyogenes* | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% | ≤80% |

## Competitive Inhibition BioCode® CoV-2 Flu Plus Assay

A study was performed to evaluate the potential for inhibition in samples with mixed infections with the BioCode® CoV-2 Flu Plus Assay on the MDx-3000. Samples were prepared by spiking one target at high concentration (≥$10^5$ units/mL) and one target at low-positive concentration (≤3x LoD) into prescreened negative nasopharyngeal swab matrix. All replicates for each target were detected.

**Table 22.** Results from Competitive inhibition study for CoV-2 Flu Plus Assay

| Panel Designation | Type/Origin/Strain/Year | Level | Titer Tested | Result (n of 3 Detected) |
|---|---|---|---|---|
| CI-1 | Influenza A H1N1 pdm09 A/Brisbane/02/18 | High (≥$10^5$ units/mL) | 1.0x$10^5$ $TCID_{50}$/mL | 3/3 |
| | Influenza B/Utah/9/14 (Yamagata) | Low-positive (3X LoD) | 8.1x$10^{-1}$ $TCID_{50}$/mL | 3/3 |
| CI-2 | Influenza B/Utah/9/14 (Yamagata) | High (≥$10^5$ units/mL) | 1.0x$10^5$ $TCID_{50}$/mL | 3/3 |
| | Influenza A H1N1 pdm09 A/Brisbane/02/18 | Low-positive (3X LoD) | 1.1x$10^1$ $TCID_{50}$/mL | 3/3 |
| CI-3 | Influenza A H1N1 pdm09 A/Brisbane/02/18 | High (≥$10^5$ units/mL) | 1.0x$10^5$ $TCID_{50}$/mL | 3/3 |
| | Respiratory Syncytial Virus | Low-positive (3X LoD) | 9.9x$10^{-1}$ $TCID_{50}$/mL | 3/3 |
| CI-4 | Respiratory Syncytial Virus | High (≥$10^5$ units/mL) | 1.0x$10^5$ $TCID_{50}$/mL | 3/3 |
| | Influenza A H1N1 pdm09 A/Brisbane/02/18 | Low-positive (3X LoD) | 1.1x$10^1$ $TCID_{50}$/mL | 3/3 |
| CI-5 | Influenza A H1N1 pdm09 A/Brisbane/02/18 | High (≥$10^5$ units/mL) | 1.0x$10^5$ $TCID_{50}$/mL | 3/3 |
| | SARS-CoV-2 Hong Kong/VM20001061/20 | Low-positive (3X LoD) | 4.6x$10^{-1}$ $TCID_{50}$/mL | 3/3 |
| CI-6 | SARS-CoV-2 Hong Kong/VM20001061/20 | High (≥$10^5$ units/mL) | 1.0x$10^5$ $TCID_{50}$/mL | 3/3 |
| | Influenza A H1N1 pdm09 A/Brisbane/02/18 | Low-positive (3X LoD) | 1.1x$10^1$ $TCID_{50}$/mL | 3/3 |
| CI-7 | Influenza B/Utah/9/14 (Yamagata) | High (≥$10^5$ units/mL) | 1.0x$10^5$ $TCID_{50}$/mL | 3/3 |
| | Respiratory Syncytial Virus | Low-positive (3X LoD) | 9.9x$10^{-1}$ $TCID_{50}$/mL | 3/3 |
| CI-8 | Respiratory Syncytial Virus | High (≥$10^5$ units/mL) | 1.0x$10^5$ $TCID_{50}$/mL | 3/3 |
| | Influenza B/Utah/9/14 (Yamagata) | Low-positive (3X LoD) | 8.1x$10^{-1}$ $TCID_{50}$/mL | 3/3 |
| CI-9 | Influenza B/Utah/9/14 (Yamagata) | High (≥$10^5$ units/mL) | 1.0x$10^5$ $TCID_{50}$/mL | 3/3 |
| | SARS-CoV-2 Hong Kong/VM20001061/20 | Low-positive (3X LoD) | 4.6x$10^{-1}$ $TCID_{50}$/mL | 3/3 |

| Panel Designation | Type/Origin/Strain/Year | Level | Titer Tested | Result (n of 3 Detected) |
|---|---|---|---|---|
| CI-10 | SARS-CoV-2 Hong Kong/VM20001061/20 | High ($\geq 10^5$ units/mL) | $1.0x10^5$ $TCID_{50}$/mL | 3/3 |
| | Influenza B/Utah/9/14 (Yamagata) | Low-positive (3X LoD) | $8.1x10^{-1}$ $TCID_{50}$/mL | 3/3 |
| CI-11 | Respiratory Syncytial Virus | High ($\geq 10^5$ units/mL) | $1.0x10^5$ $TCID_{50}$/mL | 3/3 |
| | SARS-CoV-2 Hong Kong/VM20001061/20 | Low-positive (3X LoD) | $4.6x10^{-1}$ $TCID_{50}$/mL | 3/3 |
| CI-12 | SARS-CoV-2 Hong Kong/VM20001061/20 | High ($\geq 10^5$ units/mL) | $1.0x10^5$ $TCID_{50}$/mL | 3/3 |
| | Respiratory Syncytial Virus | Low-positive (3X LoD) | $9.9x10^{-1}$ $TCID_{50}$/mL | 3/3 |
| CI-13 | NPS (negative control) | N/A | N/A | 3/3 |

## Cross-Contamination and Carryover

Carry-over contamination studies have been performed for the BioCode® MDx-3000 system in conjunction with the easyMAG® (K180041) and MagNA Pure 96 systems (K190585). Since this study is not assay-specific, no additional testing was performed for BioCode® CoV2P.

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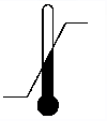
## TABLE OF SYMBOLS

The following symbols are used on the BioCode® CoV-2 Flu Plus Assay kit components and/or in this package insert.

| | | | | | |
|---|---|---|---|---|---|
| LOT | Batch code |  | Keep away from sunlight |  | Temperature limitations |
| REF | Catalog number |  | Contains sufficient for <n> tests |  | Consult instructions for use |
|  | Use by YYYY-MM-DD |  | Do Not Reuse |  | Manufacturer |
| EUA | Emergency Use Authorization |  | For Prescription Use Only | IVD | *In vitro* diagnostic device |