

BioCode® SARS-CoV-2 Assay

Instructions for Use

For use under the Emergency Use Authorization (EUA) only

Catalog # 64-C0304

For *In Vitro* Diagnostic (IVD) Use Only

Rx Only

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NAME AND INTENDED USE

The BioCode® SARS-CoV-2 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) intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal swabs (NPS), oropharyngeal swabs (OPS), and nasal swabs) or bronchoalveolar lavage (BAL) from individuals suspected of COVID-19 by their healthcare provider. 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 of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of 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. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

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 BioCode® SARS-CoV-2 Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of PCR and *in vitro* diagnostic procedures. The BioCode® SARS-CoV-2 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 late April, 2020, over two million confirmed cases have been reported worldwide, and over 160,000 people, especially elderly and those with underlying medical conditions, have succumbed to COVID-19.³ 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.⁴ It is a β -coronavirus similar to SARS-CoV and is easily transmitted through aerosol droplets and contaminated surfaces.⁵ In addition to transmission from symptomatic individuals, pre-symptomatic transmission has been reported in an estimated 6.4 – 12.6% of cases.⁶ The CDC tracks COVID-19 cases within the United States using COVIDView.⁷

The BioCode® SARS-CoV-2 Assay is a multiplexed nucleic acid test intended for the qualitative detection of SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal swabs (NPS), oropharyngeal swabs (OPS), and nasal swabs) or bronchoalveolar lavage (BAL). The BioCode® SARS-CoV-2 Assay detects two different conserved regions of SARS-CoV-2 N gene. The BioCode® SARS-CoV-2 Assay does not detect common coronaviruses (OC43, HKU1, NL63, and 229E), MERS-CoV, or SARS-CoV. The assay is designed to be used with the BioCode® MDx-3000 automated system, and test results from the BioCode® SARS-CoV-2

Assays are available in about 5 hours.

PRINCIPLE OF PROCEDURE

The BioCode® MDx-3000 is an automated system that integrates PCR amplification, target capture, signal generation and optical detection for SARS-CoV-2 from upper respiratory specimens or from BAL specimens. Nucleic acids from upper respiratory specimens or BAL 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® SARS-CoV-2 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® SARS-CoV-2 Assay Kit (64-C0304)

Component Name	Part No.	Contents	Storage
BioCode® Master Mix A	24M0011	500 µL x 2	Store at -20°C, after thaw store at 4°C for up to 30 days
BioCode® SARS-CoV-2 Primer Mix	44-P0505	500 µL x 2	Store at -20°C, after thaw store at 4°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 4°C for up to 30 days
BioCode® SARS-CoV-2 BMB-Probe Mix	44-B0323	6000 µL x 1	Store at -20°C, after thaw vortex for 30 sec, store at 4°C up to 90 days

Materials Required (But Not Provided)

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

Component Name	Part No.	Contents	Storage
BioCode® SA-PE Mix	24-S0008	500 µL x 1	Single use; protect from light; store at 4°C. Do Not Freeze
BioCode® Buffer A	44-B0003	2 L x 1	Store at room temp

Table 3. BioCode MDx-3000 Consumables

Reagent	Source/Part No.	Quantity
Reagent Reservoirs	INTEGRA 4332	50 each x 4
Waste Bin and Lid	Applied BioCode 01-W0105	20 each
20 µL pipette tips	Beckman 717256	10 x 96 tips
250 µL pipette tips	Beckman 717252	10 x 96 tips
Bio-Rad 96-well hard shell plate 0.1 mL	Bio-Rad HSL9601	5 plates x 5 bags
PCR Adhesive Foil	Thermo Fisher Scientific AB-0626 or Eppendorf 0030127790	100 foils
Microtiter plate	Greiner bio-one 655101	10 plates x 10 bags
Microtiter plate lid	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

Extraction System	Part Name (Part No.)	Quantity
	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
bioMérieux NucliSENS® easyMAG®	EasyMAG® Magnetic Silica (280133)	24 x 1.2 mL
	EasyMAG® Lysis Buffer (280134)	4x 1000 mL
	EasyMAG® Buffer 1 (280130)	4x 1000 mL
	EasyMAG® Buffer 2 (280131)	4x 1000 mL
	EasyMAG® Buffer 3 (280132)	4x 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 (But 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. The BioCode® SARS-CoV-2 Assay has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
5. The BioCode® SARS-CoV-2 Assay has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
6. The BioCode® SARS-CoV-2 Assay is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. §360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
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® SARS-CoV-2 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® SARS-CoV-2 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.

6. BAL specimens should not be centrifuged, preprocessed, treated with any mucolytic or decontaminating agents (e.g. Mycoprep, Sputasol, Snap n' Diest, DTT, Trypsin, sodium hydroxide etc.) or placed into transport media before testing. Samples should be tested as soon as possible.

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 (2-8°C) for up to 30 days.
4. Once thawed, store BMB-Probe Mix refrigerated (2-8°C) for up to 90 days.
5. SA-PE mix is for single use only. Store refrigerated (2-8°C). Protect from light. **DO NOT FREEZE.**
6. Store the Buffer A at room temperature (15-25°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 (2-8°C).

SAMPLE REQUIREMENTS

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

For additional information, refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV).

<https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html>

Upper Respiratory Specimens collected according to the standard procedure. Immediately place in 1 – 3 mL of transport medium. Samples should be tested as soon as possible. They may be stored at the following conditions:

- 2 - 8°C for up to 72 hours after collection
- If delay in extraction is expected, store specimens at -70°C or lower
- Extracted nucleic acid should be stored at -70°C or lower

Bronchoalveolar lavage (BAL) collected according to the standard procedure. Collect 2-3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container. BAL specimens should not be centrifuged, preprocessed, treated with any mucolytic or decontaminating agents (e.g. Mycoprep, Sputasol, Snap n' Diest, DTT, Trypsin, sodium hydroxide etc.) or placed into transport media before testing. Samples should be tested as soon as possible. They may be stored at the following conditions:

- Room temperature for 8 hours
- 2 - 8°C for 3 days
- <-60°C for 90 days

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 External 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 External 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 2-8°C refrigerator if testing within 12 hours. Store at -60°C or below if testing cannot be completed within 12 hours of extraction. Extracted nucleic acids may be stored at -60°C or below for up to 90 days.

BioCode® SARS-CoV-2 Assay Set Up

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

1. Thaw Primer Mix, Master Mix A, and BMB-Probe Mix at room temperature. RT Mix is stored at -20°C and does not require thawing before use. 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® SARS-CoV-2 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 2-8°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 or external control into the wells.
6. Seal plate with pierceable foil. Store at 2-8°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).
7. Briefly centrifuge plate to collect samples at the bottom of the plate.
8. Load plate onto BioCode® MDx-3000.
9. Vortex thawed room temperature BMB-Probe Mix for 30 seconds at high speed and load the vial 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.)** Based on the number of specimens and controls required in the plate setup, the BioCode® MDx-3000 software calculates the bulk volume required and displays on the screen. The BioCode® MDx-3000 automatically distributes 50 µL of BMB-Probe Mix into each well of the optical detection plate, based on the plate setup.
10. Load reagents and consumables as prompted by graphic user interface.

INTERPRETATION OF RESULTS

The BioCode® MDx-3000 software will analyze 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 spiked with provided RNA IC2. 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	N Gene Targets	RNA-IC2	Description
Negative Control	Not Detected	Detected	Plate Status: Valid. Samples can be interpreted.
Negative Control	Detected	N/A	Plate Status: Invalid. Samples results cannot be interpreted. Results suppressed by software.
Negative Control	N/A	Not Detected	Plate Status: Invalid. 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.

Applied BioCode used the AccuPlex SARS-CoV-2 Reference Material Kit developed by SeraCare Life Sciences (MA, USA), during validation. This control, with a 200 µL total volume, was extracted neat per the BioCode SARS-CoV-2 Assay's instructions for use.

Table 7. Criteria for Valid Positive Control

Control	N Gene Targets	RNA IC	Recommendations
Positive Control	Detected	N/A	Report will indicate positive control is Valid. No user intervention required.
Positive Control	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 and negative control 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 wells with invalid RNA IC results (see table).

Table 8. Criteria for RNA Internal Control (RNA-IC2)

N Gene Targets	RNA IC	Recommendations
Detected	N/A	Sample status: Valid. Report all results.
Not Detected	Detected	Sample status: Valid. Report all results.
Not Detected	Not Detected	Sample status: Invalid. Not Detected results suppressed by software. Repeat/reflex testing.

Lack of RNA-IC2 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

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be acceptable. If the internal control is not valid, the patient results cannot be interpreted. Fluorescent signals from BMBs with the same barcode are sorted and the median fluorescence intensity (MFI) is calculated for each analyte. BioCode® MDx software interprets the results based on MFI values and pre-determined cutoff for each assay together with the algorithm for interpretation. The end user is not required to review MFI values for interpretation or determine indeterminate/invalid results.

Table 9. Assessment of Test Results

Interpretation/ Follow-Up Testing	N gene Target a	N gene Target b	RNA-IC
Positive	Detected	Detected	N/A
Positive	Detected	Not detected	N/A
Positive	Not detected	Detected	N/A
Negative	Not detected	Not detected	Detected
Invalid/Repeat [^]	Not detected	Not detected	Not detected

N/A = Not Applicable; ^ - Repeat the specimen

BioCode® SARS-CoV-2 Assay 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, or Invalid.

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 BioCode® SARS-CoV-2 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 BioCode® SARS-CoV-2 Assay can be used with the specimens listed in the Intended Use statement. It has not been validated for other specimen types.
- The performance of the BioCode® SARS-CoV-2 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.
- 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 SARS-CoV-2 transmissibility or it may be asymptotically 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 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® SARS-CoV-2 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/vitro-diagnostics-euas>

To assist clinical laboratories using the BioCode® SARS-CoV-2 Assay the relevant Conditions of Authorization are listed verbatim below.

- a) Authorized laboratories¹ using the BioCode® SARS-CoV-2 Assay will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods of disseminating these Fact Sheets may be used, which may include mass media.
- b) Authorized laboratories using the BioCode® SARS-CoV-2 Assay will use the BioCode® SARS-CoV-2 Assay as outlined in the Instructions for Use. Deviation from the authorized procedures, such as the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control material, authorized other ancillary reagents, and authorized materials required are not permitted.
- c) Authorized laboratories that receive the BioCode® SARS-CoV-2 Assay will notify the relevant public health authorities of their intent to run the BioCode® SARS-CoV-2 Assay prior to initiating testing.
- d) Authorized laboratories using the BioCode® SARS-CoV-2 Assay will have a process in place for reporting test results to healthcare providers and relevant public health authorities.
- e) Authorized laboratories will collect information on the performance of the BioCode® SARS-CoV-2 Assay and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: [CDRH EUA-Reporting@fda.hhs.gov](mailto:CDRH_EUA-Reporting@fda.hhs.gov)) and TechSupport@ApBioCode.com if they become aware of any

¹ 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."

suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics.

- f) All laboratory personnel using the BioCode® SARS-CoV-2 Assay must be appropriately trained in PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use the BioCode® SARS-CoV-2 Assay in accordance with the authorized labeling.
- g) Applied BioCode, Inc. and authorized laboratories using the BioCode® SARS-CoV-2 Assay will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

PERFORMANCE CHARACTERISTICS

Limit of Detection

A study was performed to assess the performance of the BioCode® SARS-CoV-2 Assay on the BioCode® MDx-3000 at the Limit of Detection (LoD) for specimens. In this study, the BioCode® SARS-CoV-2 Assay was tested with quantified inactivated virus stocks (Zeptomatrix) spiked into SARS-CoV-2 negative NPS and BAL. For preliminary LoD testing, four replicates of each concentration of 3-fold dilution series were extracted on the easyMAG and MagNA Pure 96 Systems and tested singly with the BioCode® SARS-CoV-2 Assay on the BioCode® MDx-3000 system to estimate LoD. The LoD was confirmed by extracting 20 replicates of each specimen type at or near the preliminary LoD. LoD of the BioCode® SARS-CoV-2 Assay for each extraction was defined as the lowest concentration with ≥95% detection of 20 replicates (19 out of 20) and is 1.72×10^{-2} TCID₅₀/mL.

Table 10. Limit of Detection Stratified by Extraction System and Specimen Type.

Sample Type	Extraction System	Concentration	Detected (n of 20)
Nasopharyngeal Swab (NPS)	easyMAG	1.72×10^{-2} TCID ₅₀ /mL	20/20
	MagNA Pure 96	1.72×10^{-2} TCID ₅₀ /mL	20/20
Bronchoalveolar lavage (BAL)	easyMAG	1.72×10^{-2} TCID ₅₀ /mL	20/20
	MagNA Pure 96	1.72×10^{-2} TCID ₅₀ /mL	20/20

Analytical Reactivity (Inclusivity)

Applied BioCode conducted an *in silico* analysis of 5000 publicly available SARS-CoV-2 nucleic acid sequences in GenBank as of June 11, 2020 with primers and probes for the BioCode® SARS-CoV-2 Assay. Over 99.9% sequence match was demonstrated for all primers and probes. Most mismatches were single occurrences. There was an G to A mismatch in the 6th base from the 3' end of the Nb reverse primer in 5 of the 5000 sequences queried. There was a T to C mismatch in the 10th base from the 3' end of the Na Forward primer for 2 of 5000 sequences. There was a G to T mismatch in the 12th base from the 3' end of the Na Probe for 2 of 5000 sequences. Based on the high percentage of analyzed sequences having no mismatches and that the few sequences with mismatches have only a single mismatched base, the likelihood of a false negative is very low.

Table 11. Summary of *In Silico* Inclusivity Analysis of the BioCode SARS-CoV-2 Primers and Probes

Primer Probe Sequence ID	Blast Results
Na F	Out of 5000 SARS-CoV-2 specific sequences, primer sequence demonstrates 99.9% perfect match.
Na R Bio	Out of 5000 SARS-CoV-2 specific sequences, primer sequence demonstrates over 99.9% perfect match.
Na P	Out of 4627 SARS-CoV-2 specific sequences, probe sequence demonstrates over 99.9% perfect match.

Primer Probe Sequence ID	Blast Results
Nb F	Out of 4627 SARS-CoV-2 specific sequences, primer sequence demonstrates over 99.9% perfect match.
Nb R Bio	Out of 5000 SARS-CoV-2 specific sequences, primer sequence demonstrates over 99.9% perfect match.
Nb P	Out of 5000 SARS-CoV-2 specific sequences, probe sequence demonstrates over 99.9% perfect match.

Analytical Specificity (Cross-Reactivity)

A study was performed to verify that the BioCode® SARS-CoV-2 Assay does not detect DNA or RNA from other organisms commonly found in respiratory specimens or from organisms and viruses that can cause similar clinical symptoms. Organisms and viruses that were not available for wet testing were analyzed *in silico* comparing the whole genome against all primers to assess potential for cross reactivity.

Microorganisms were tested at 10⁶ CFU/mL for bacteria and 10⁵ TCID₅₀/mL for viruses or higher when possible at Applied BioCode, Inc. Stock organisms were diluted and combined with simulated NPS in UTM matrix prior to extraction. Each organism was extracted in triplicate on the easyMAG and assayed singly on the BioCode® MDx-3000 system according to the instructions for use. For each concentration tested, the number of replicates that gave valid results (per the Interpretation Algorithm) was determined.

No cross-reactivity was observed for all 3 replicates (all 3 replicates should be negative for both N gene sets) for all organisms assessed in this study (see tables below).

Table 12. Coronavirus Types Analyzed for Analytical Specificity (Cross-Reactivity)

Organism	Vendor	Catalog #	Concentration tested (N X 10 ⁿ units/mL)	Cross-reactivity (Y/N)	Detected (n/3)
Human coronavirus 229E	Zeptomatrix	0810229CF	3.80 x 10 ⁴ TCID ₅₀ /mL	N	0/3
Human coronavirus HKU1	Clinical sample RESP_5016	N/A	2.40 x 10 ⁵ TCID ₅₀ /mL	N	0/3
Human coronavirus NL63	Zeptomatrix	0810228CF	3.16 x 10 ⁴ TCID ₅₀ /mL	N	0/3
Human coronavirus OC43	Zeptomatrix	0810024CF	1.26 x 10 ⁴ TCID ₅₀ /mL	N	0/3
MERS-coronavirus gRNA	BEI	NR-45843	2.53 x 10 ⁸ copies/mL	N	0/3
SARS-CoV, formaldehyde- and UV-inactivated, purified(vaccine) vaccine 1% ^a	BEI	NR-3883	1:100 dilution (0.1 µg/mL)	N	0/3
SARS-CoV, formaldehyde- and UV-inactivated, purified(vaccine) vaccine 0.1%	BEI	NR-3883	1:1000 dilution (0.01 µg/mL)	N	0/3

^a – No signal was detected including the RNA-IC2, most likely due to formaldehyde which is a known PCR inhibitor. A 1:10 dilution of formaldehyde inactivated vaccine was tested that generated an invalid result, most likely due to formaldehyde which is a known PCR inhibitor.

Table 13. Viruses Analyzed for Analytical Specificity (Cross-Reactivity)

Organism	Vendor	Catalog #	Titer tested (N X 10 ⁿ units/mL)	Cross-reactivity (Y/N)	Detected (n/3)
Adenovirus Species B Serotype 7A	Zeptomatrix	0810021CF	1.02 x 10 ⁵ TCID ₅₀ /mL	N	0/3
Adenovirus Species C Serotype 2	ATCC	VR-846	2.81 x 10 ⁵ TCID ₅₀ /mL	N	0/3
Adenovirus Species E Serotype 4	Zeptomatrix	0810070CF	3.16 x 10 ⁴ TCID ₅₀ /mL	N	0/3

Organism	Vendor	Catalog #	Titer tested (N X 10 ⁿ units/mL)	Cross-reactivity (Y/N)	Detected (n/3)
Enterovirus D68	Zeptomatrix	0810300CF	1.26 x 10 ⁴ TCID ₅₀ /mL	N	0/3
Human Metapneumovirus 16 (Type A1)	Zeptomatrix	0810161CF	8.51 x 10 ⁴ TCID ₅₀ /mL	N	0/3
Human Rhinovirus Type A1	Zeptomatrix	0810012CFN	1.05 x 10 ⁴ TCID ₅₀ /mL	N	0/3
Influenza A H1N1 /NWS/33	ATCC	VR-219	7.40 x 10 ⁵ TCID ₅₀ /mL	N	0/3
Influenza A H1N1/New Caledonia/20/99	Zeptomatrix	0810036CF	1.15 x 10 ⁵ TCID ₅₀ /mL	N	0/3
Influenza A H3N2 /Wisconsin/67/05a	Zeptomatrix	0810252CF	1.26 x 10 ⁴ TCID ₅₀ /mL	N	0/3
Influenza A H3N2/Alice	ATCC	VR-776	5.00 x 10 ⁵ TCID ₅₀ /mL	N	0/3
Influenza A (H1N1 pdm09)/California/07/09	Zeptomatrix	0810165CF	3.80 x 10 ⁴ TCID ₅₀ /mL	N	0/3
Influenza B/Florida/4/2006 (Yamagata)	Zeptomatrix	0810255CF	1.26 x 10 ⁴ TCID ₅₀ /mL	N	0/3
Influenza B/Hong Kong/S/1972 (Victoria)	ATCC	VR-823	1.00 x 10 ⁵ TCID ₅₀ /mL	N	0/3
Parainfluenza Virus 1	ATCC	VR-94	1.60 x 10 ⁵ TCID ₅₀ /mL	N	0/3
Parainfluenza Virus 2	ATCC	VR-92	5.90 x 10 ⁴ TCID ₅₀ /mL	N	0/3
Parainfluenza Virus 3	Zeptomatrix	0810016CF	3.39 x 10 ⁵ TCID ₅₀ /mL	N	0/3
Parainfluenza Virus 4a	Zeptomatrix	0810060CF	2.19 x 10 ⁴ TCID ₅₀ /mL	N	0/3
Respiratory Syncytial Virus (Type A)	Zeptomatrix	0810040ACF	1.26 x 10 ⁴ TCID ₅₀ /mL	N	0/3

Table 14. Bacteria Analyzed for Analytical Specificity (Cross Reactivity)

Organism ^a	Vendor	Catalog #	Titer tested (N X 10 ⁿ units/mL)	Cross-reactivity (Y/N)	Detected (n/3)
<i>Bordetella pertussis</i>	Zeptomatrix	801459	1.35 x 10 ⁶ CFU/mL	N	0/3
<i>Candida albicans</i>	Zeptomatrix	801504	1.96 x 10 ⁶ CFU/mL	N	0/3
<i>Chlamydia pneumoniae</i>	ATCC	VR-1310	1.25 x 10 ⁵ CFU/mL	N	0/3
<i>Haemophilus influenzae</i>	Zeptomatrix	801679	2.40 x 10 ⁶ CFU/mL	N	0/3
<i>Legionella pneumophila</i>	Zeptomatrix	801645	3.17 x 10 ⁶ CFU/mL	N	0/3
<i>Mycobacterium tuberculosis</i>	Zeptomatrix	801660	7.23 x 10 ⁶ CFU/mL	N	0/3
<i>Mycoplasma pneumoniae</i>	Zeptomatrix	801579	2.47 x 10 ⁵ CCU/mL	N	0/3
<i>Pseudomonas aeruginosa</i>	ATCC	39324	7.40 x 10 ⁵ CFU/mL	N	0/3
<i>Staphylococcus epidermidis</i>	ATCC	14990	8.20 x 10 ⁵ CFU/mL	N	0/3
<i>Streptococcus salivarius</i>	BEI	HM-121	1.12 x 10 ⁶ CFU/mL	N	0/3
<i>Streptococcus pneumoniae</i>	Zeptomatrix	801439	4.17 x 10 ⁶ CFU/mL	N	0/3
<i>Streptococcus pyogenes</i>	BEI	NR-15274	2.90 x 10 ⁵ CFU/mL	N	0/3

^a – *Pneumocystis jirovecii* was not available for bench testing, however, no reactivity is predicted by *in silico* analysis.

In Silico Analysis

In silico analysis was performed for each primer and probe using NCBI BLAST. In summary, no significant homology ($\leq 80\%$) 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 Na target and 100% homology with the forward primer for the Nb target. Detection of SARS-CoV-1 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. Additionally, no evidence of cross-reactivity with SARS-CoV-1 was observed in wet testing. 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 15. *In Silico* Cross-Reactivity Data

Organism	SARS-CoV-2 N gene (Target Na)			SARS-CoV-2 N gene (Target Nb)			RNA Internal Control (MS2)		
	Na F	Na R Bio	Na P	Nb F	Nb R Bio	Nb P	MS2 F	MS2 R Bio	MS2 P4
Human coronavirus 229E	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$
Human coronavirus HKU1	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$
Human coronavirus NL63	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$
Human coronavirus OC43	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$
MERS-coronavirus	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$
SARS-coronavirus	$\leq 80\%$	92%	92%	100%	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$
Adenovirus Species B Serotype 7A	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$
Adenovirus Species C Serotype 2	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$
Adenovirus Species E Serotype 4	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$
Enterovirus D68	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$
Human Metapneumovirus	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$
Human Rhinovirus Type A1	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$
Human Influenza A	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$
Influenza B	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$
Parainfluenza Virus 1	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$
Parainfluenza Virus 2	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$
Parainfluenza Virus 3	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$
Parainfluenza Virus 4a	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$	$\leq 80\%$

Organism	SARS-CoV-2 N gene (Target Na)			SARS-CoV-2 N gene (Target Nb)			RNA Internal Control (MS2)		
	Na F	Na R Bio	Na P	Nb F	Nb R Bio	Nb P	MS2 F	MS2 R Bio	MS2 P4
Respiratory Syncytial Virus (Type A)	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
<i>Bordetella pertussis</i>	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
<i>Candida albicans</i>	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
<i>Chlamydia pneumoniae</i>	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
<i>Haemophilus influenzae</i>	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
<i>Legionella pneumophila</i>	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
<i>Mycobacterium tuberculosis</i>	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
<i>Mycoplasma pneumoniae</i>	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
<i>Pneumocystis jirovecii (PJP)</i>	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
<i>Pseudomonas aeruginosa</i>	85%	≤80%	91%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
<i>Staphylococcus epidermidis</i>	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
<i>Streptococcus salivarius</i>	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
<i>Streptococcus pneumoniae</i>	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
<i>Streptococcus pyogenes</i>	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%

Clinical Performance

Clinical Evaluation - Clinical Samples

The BioCode® SARS-CoV-2 Assay was evaluated with 93 frozen de-identified nasopharyngeal swab samples. The samples were collected by qualified personnel from patients suspected of SARS-CoV-2 infection and stored in compliance with conditions stated in the IFU. Samples were tested in a blinded fashion as per IFU for the BioCode® SARS-CoV-2 and a RT-PCR comparator assay with Emergency Use Authorization. A summary of the demographic information of the tested samples is provided in Table 16.

Table 16. Demographics of retrospective clinical specimens

Retrospective Clinical Specimens	
Total Specimens	93
Gender	n/N (%)
Male	39/93 (42%)
Female	54/93 (58%)
Age Category	n/N (%)
0-5 years	2/93 (2%)
6-21 years	16/93 (17%)
22-64 years	66/93 (71%)
65+ years	9/93 (10%)

By the comparator method, 41 were positive, 51 were negative and 1 was inconclusive. The inconclusive sample was excluded from the performance analysis. There were no invalid samples for either assay. The BioCode® SARS-CoV-2 Assay exhibited 97.6% positive agreement (40/41) and 96.1% negative agreement (49/51) to the comparator.

Table 17. Summary from Clinical Study.

		SARS-CoV-2 RT-PCR Comparator Test		
		Positive	Negative	Total
BioCode® SARS-CoV-2 Assay	Positive	40	2 ^a	42
	Negative	1	49	50
	Total	41	51	92
Positive Agreement		97.6% (40/41); 87.4-99.6%		
Negative Agreement		96.1% (49/51); 86.8-98.9%		

a – One of the 2 samples detected by BioCode® SARS-CoV-2 and not detected by the comparator was detected by an alternative EUA assay. The other was negative by the comparator and alternative EUA assay.

Clinical Evaluation - Contrived Samples

Clinical performance of BioCode® SARS-CoV-2 Assay was evaluated using a contrived clinical study. The contrived clinical specimens were prepared by spiking 30 unique natural NPS and 30 unique BAL specimens that were previously tested negative for SARS-CoV-2 with quantitated inactivated virus from Zeptomatrix (catalog #: 0810587CFHI USA-WA1/2020 NCBI ACC #: MN985325). Twenty contrived specimens were spiked at concentrations of 1-2X LOD and ten specimens at higher concentration covering the range of detection. The 30 positive samples were prepared, interspersed with negative samples, and randomized before testing. A total of 120 samples, including 30 positives each for NPS and BAL, were tested. The

results of the BioCode® SARS-CoV-2 Assay testing are presented in the following table:

Table 18. Results from Contrived Samples Tested by the BioCode® SARS-CoV-2 Assay

Sample Type	Fold LoD	Concentration*	Agreement (%)	95% CI	
Nasopharyngeal Swab (NPS)	1	1.72 x10 ⁻² TCID ₅₀ /mL	20/20 (100%)	83.9 - 100.0%	
	10	1.72 x10 ⁻¹ TCID ₅₀ /mL	2/2 (100%)	72.2 - 100%	
	100	1.72 x10 ⁰ TCID ₅₀ /mL	2/2 (100%)		
	1,000	1.72 x10 ¹ TCID ₅₀ /mL	2/2 (100%)		
	10,000	1.72 x10 ² TCID ₅₀ /mL	2/2 (100%)		
	100,000	1.72 x10 ³ TCID ₅₀ /mL	2/2 (100%)		
	Combined Positive				30/30 (100%)
	Negative			30/30 (100%)	88.6% - 100.0%
Bronchoalveolar lavage (BAL)	1	1.72 x10 ⁻² TCID ₅₀ /mL	20/20 (100%)	83.9% - 100.0%	
	10	1.72 x10 ⁻¹ TCID ₅₀ /mL	2/2 (100%)	72.2% - 100%	
	100	1.72 x10 ⁰ TCID ₅₀ /mL	2/2 (100%)		
	1,000	1.72 x10 ¹ TCID ₅₀ /mL	2/2 (100%)		
	10,000	1.72 x10 ² TCID ₅₀ /mL	2/2 (100%)		
	100,000	1.72 x10 ³ TCID ₅₀ /mL	2/2 (100%)		
	Combined Positive				30/30 (100%)
	Negative			30/30 (100%)	88.6% - 100.0%

* Samples were contrived with quantitated inactivated virus from Zeptomatrix (catalog #: 0810587CFHI USA-WA1/2020 NCBI ACC #: MN985325)

No invalid results were observed with either NPS or BAL specimens.

FDA SARS-COV-2 REFERENCE PANEL TESTING

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method and instrument used were the easyMag and BioCode MDx-3000, respectively. The results are summarized in Table 19.

Table 19: Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab (NPS)	5.4x10 ³ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL

N/A: Not applicable



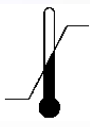








ND: Not detected

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TABLE OF SYMBOLS

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

	Batch code		Keep away from sunlight		Temperature limitations
	Catalog number		Contains sufficient for <n> tests		Consult instructions for use
	Use by YYYY-MM-DD		Do Not Reuse		Manufacturer
	Emergency Use Authorization		For Prescription Use Only		

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