BioCode®

SARS-CoV-2 Assay

Package Insert

Applied BioCode

Flexible and Affordable Syndromic Testing

Applied BioCode

Barcoded Magnetic Beads | Flexible Syndromic Tests

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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For *in vitro* Diagnostic Use.

Table of Contents

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 SARS-CoV-2 in upper respiratory specimens (nasopharyngeal swabs (NPS), oropharyngeal swabs (OPS), and nasal swabs) or bronchoalveolar lavage (BAL) from individuals suspected of COVID-19 by their healthcare provider (HCP).

This test is also for the qualitative detection of nucleic acid from SARS-CoV-2 in pooled samples containing up to 5 individual upper respiratory specimens (NPS, OPS, and nasal swabs) that are collected by an HCP using individual vials containing transport media, from individuals suspected of COVID-19 by their HCP. Negative results from pooled testing should not be treated as definitive. If patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive or invalid result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

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. SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. 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 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 Assay 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
bioMerieux 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 for emergency use 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. Emergency use of 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 Federal Food, Drug, and Cosmetic 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.
   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.
8. Contamination may occur if carryover of samples is not adequately controlled during sample pool preparation, handling, and processing.
9. Testing of pooled specimens may impact the detection capability of the BioCode® SARS-CoV-2 Assay and decrease sensitivity.

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

SPECIMEN POOLING - DETERMINING APPROPRIATE STRATEGY FOR IMPLEMENTATION AND MONITORING

When considering specimen pooling, laboratories should evaluate the appropriateness of a pooling strategy based on the positivity rate in the testing population and the efficiency of the pooling workflow. Refer to Appendix A of these Instructions for Use for additional information prior to implementation of specimen pooling.

Preparing Samples for Pooling - The following upper respiratory tract specimens authorized under the Emergency Use Authorization of the BioCode® SARS-CoV-2 assay may be tested with sample pooling - nasopharyngeal, oropharyngeal and nasal swab specimens. Only a single specimen type in a single type of collection media can be combined into a sample pool.

Determine the appropriate volume required from each individual specimen based on the pool size being implemented. Equal aliquots should be taken from the individual collection containers and combined in an appropriately sized container using aseptic techniques. The total volume of the pooled samples should be equivalent to the input volume used when testing individual samples, 200 µL. When performing testing using pooled samples an aliquot of at least 200 µL should be retained for individual sample 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 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 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

Interpretation of Results for Pooled Samples

Negative— When testing pooled samples and the result is negative, all samples that make up the pool are presumed to be negative. Results should be reported. If the patient's clinical signs and symptoms are inconsistent with a negative result and if results are necessary for patient management, then the patient should be considered for individual testing.

Positive—When testing pooled samples and the result is positive, the individual samples that make up the pool should be tested individually prior to reporting a result.

Invalid—When testing pooled samples and the result is invalid, specimens must be tested individually prior

to reporting a result. However, in instances of an invalid run, repeat testing of pooled specimens may be appropriate if sufficient volume is available.

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.
- Sample pooling has only been validated using nasopharyngeal swab specimens.
- Samples should only be pooled when testing demand exceeds laboratory capacity and/or when testing reagents are in short supply.
- 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 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 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.
- The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

CONDITIONS OF AUTHORIZATION FOR 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/in-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 laboratories[1] 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 using specimen pooling strategies when testing patient specimens with the BioCode® SARS-CoV-2 Assay will include with test result reports for specific patients whose specimen(s) were the subject of pooling, a notice that pooling was used during testing and that "Patient specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing".

f) Authorized laboratories implementing pooling strategies for testing patient specimens must use the "Specimen Pooling Implementation and Monitoring Guidelines for Laboratories" recommendations available in the authorized labeling to evaluate the appropriateness of continuing to use such strategies based on the recommendations in the protocol.

g) Authorized laboratories will keep records of specimen pooling strategies implemented including type of strategy, date implemented, and quantities tested, and test result data generated as part of the "Specimen Pooling Implementation and Monitoring Guidelines for Laboratories". For the first 12 months from the date of their creation, such records will be made available to FDA within 48 business hours for inspection upon request, and will be made available within a reasonable time after 12 months from the date of their creation.

h) 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) and TechSupport@ApBioCode.com if they become aware of any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics.

i) All laboratory personnel using the BioCode® SARS-CoV-2 Assay must be appropriately trained in RT-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.

j) Applied BioCode, Inc., authorized distributors, and authorized laboratories using the BioCode® SARS-CoV-2 Assay will ensure that any records associated with this EUA are maintained until

[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."

otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

PERFORMANCE CHARACTERISTICS

Limit of Detection

Sample type and extraction methods comparison study

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 (Zeptometrix) 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 $x10^{-2}$ $TCID_{50}$/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 $x10^{-2}$ $TCID_{50}$/mL	20/20
	MagNA Pure 96	1.72 $x10^{-2}$ $TCID_{50}$/mL	20/20
Bronchoalveolar lavage (BAL)	easyMAG	1.72 $x10^{-2}$ $TCID_{50}$/mL	20/20
	MagNA Pure 96	1.72 $x10^{-2}$ $TCID_{50}$/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.
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 10^6 CFU/mL for bacteria and 10^5 $TCID_{50}$/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	Zeptometrix	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

Organism	Vendor	Catalog #	Concentration tested (N X 10^n units/mL)	Cross-reactivity (Y/N)	Detected (n/3)
Human coronavirus NL63	Zeptometrix	0810228CF	3.16 x 10^4 $TCID_{50}$/mL	N	0/3
Human coronavirus OC43	Zeptometrix	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	Zeptometrix	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	Zeptometrix	0810070CF	3.16 x 10^4 $TCID_{50}$/mL	N	0/3
Enterovirus D68	Zeptometrix	0810300CF	1.26 x 10^4 $TCID_{50}$/mL	N	0/3
Human Metapneumovirus 16 (Type A1)	Zeptometrix	0810161CF	8.51 x 10^4 $TCID_{50}$/mL	N	0/3
Human Rhinovirus Type A1	Zeptometrix	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	Zeptometrix	0810036CF	1.15 x 10^5 $TCID_{50}$/mL	N	0/3
Influenza A H3N2 /Wisconsin/67/05a	Zeptometrix	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	Zeptometrix	0810165CF	3.80 x 10^4 $TCID_{50}$/mL	N	0/3
Influenza B/Florida/4/2006 (Yamagata)	Zeptometrix	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	Zeptometrix	0810016CF	3.39 x 10^5 $TCID_{50}$/mL	N	0/3
Parainfluenza Virus 4a	Zeptometrix	0810060CF	2.19 x 10^4 $TCID_{50}$/mL	N	0/3
Respiratory Syncytial Virus (Type A)	Zeptometrix	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)
Bordetella pertussis	Zeptometrix	801459	1.35 x 10^6 CFU/mL	N	0/3
Candida albicans	Zeptometrix	801504	1.96 x 10^6 CFU/mL	N	0/3
Chlamydia pneumoniae	ATCC	VR-1310	1.25 x 10^5 CFU/mL	N	0/3
Haemophilus influenzae	Zeptometrix	801679	2.40 x 10^6 CFU/mL	N	0/3

Organism[a]	Vendor	Catalog #	Titer tested (N X 10^n units/mL)	Cross-reactivity (Y/N)	Detected (n/3)
Legionella pneumophila	Zeptometrix	801645	3.17 x 10^6 CFU/mL	N	0/3
Mycobacterium tuberculosis	Zeptometrix	801660	7.23 x 10^6 CFU/mL	N	0/3
Mycoplasma pneumoniae	Zeptometrix	801579	2.47 x 10^5 CCU/mL	N	0/3
Pseudomonas aeruginosa	ATCC	39324	7.40 x 10^5 CFU/mL	N	0/3
Staphylococcus epidermidis	ATCC	14990	8.20 x 10^5 CFU/mL	N	0/3
Streptococcus salivarius	BEI	HM-121	1.12 x 10^6 CFU/mL	N	0/3
Streptococcus pneumoniae	Zeptometrix	801439	4.17 x 10^6 CFU/mL	N	0/3
Streptococcus pyogenes	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 (≤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-CoC-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)		
Primer/Probe Name	**Na F**	**Na R Bio**	**Na P**	**Nb F**	**Nb R Bio**	**Nb P**	**MS2 F**	**MS2 R Bio**	**MS2 P4**
Human coronavirus 229E	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Human coronavirus HKU1	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Human coronavirus NL63	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Human coronavirus OC43	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
MERS-coronavirus	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
SARS-coronavirus	≤80%	92%	92%	100%	≤80%	≤80%	≤80%	≤80%	≤80%
Adenovirus Species B Serotype 7A	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Adenovirus Species C Serotype 2	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Adenovirus Species E Serotype 4	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Enterovirus D68	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Human Metapneumovirus	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Human Rhinovirus Type A1	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Human Influenza A	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Influenza B	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Parainfluenza Virus 1	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Parainfluenza Virus 2	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Parainfluenza Virus 3	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Parainfluenza Virus 4a	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%

Organism	SARS-CoV-2 N gene (Target Na)			SARS-CoV-2 N gene (Target Nb)			RNA Internal Control (MS2)		
Primer/Probe Name	**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%
Bordetella pertussis	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Candida albicans	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Chlamydia pneumoniae	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Haemophilus influenzae	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Legionella pneumophila	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Mycobacterium tuberculosis	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Mycoplasma pneumoniae	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Pneumocystis jirovecii (PJP)	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Pseudomonas aeruginosa	85%	≤80%	91%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Staphylococcus epidermidis	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Streptococcus salivarius	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Streptococcus pneumoniae	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%	≤80%
Streptococcus pyogenes	≤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 highly sensitive 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. Around 20% (8/41) of positive samples were low positives. 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.

		EUA 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 – Five-Sample Pools

The performance of the BioCode SARS-CoV-2 Assay was assessed with 20 positive pools consisting of five samples (one positive sample and 4 negative samples) and 39 negative pools. A total of 108 unique negative samples from two geographic regions and 20 unique positive samples from two geographic regions were tested individually and in the pooled configurations. Greater than 25% of positive samples were determined to be weak positives by testing with an FDA EUA RT-PCR assay.

The pooled samples exhibited 100% positive and negative agreement to the expected non-pooled results.

Table 18. Individual and Pooled Specimens Agreement with a Pool Size of 5

Samples Tested in 5-Sample Pool	Individual Test Result (BioCode SARS-CoV-2 non-pooled)	
Pooled Test Result	**Positive**	**Negative**
Positive	20	0
Negative	0	39

Positive Percent Agreement = 100% (95% CIs: 83.16% – 100.00%)
Negative Percent Agreement = 100% (95% CIs: 90.97% – 100.00%)

Multi-Site Clinical Evaluation – Pooling Samples
The performance of the BioCode SARS-CoV-2 Assay was assessed with 725 samples consecutively collected from three geographically distinct locations. The sites tested each sample individually and pooled in parallel. Pools consisted of 5 consecutive individual samples in the order they were collected in the study until at least 15 consecutively collected individual positive samples were tested from each collection site. Some of the sample pools therefore contained more than one positive sample.

The result of the samples when tested in a pool was then compared to the individual test result for the sample and the positive percent agreement (PPA) and negative percent agreement (NPA) were calculated. Individually tested positive samples should still be detected as positive when tested in a 5-sample pool.

Table 19 – Summary from Multi-Site Pooled Sample Testing

Site	(n)	Positive Percent Agreement (compared to individual testing)		Negative Percent Agreement (compared to individual testing)	
		PPA (%)	95% CI	NPA (%)	95% CI
Site 1	76	24/24 (100%)	(86.2%, 100.0%)	52/52 (100%)	(93.1%, 100.0%)
Site 2*	36	16/17 (94.1%)	(71.7%, 98.9%)	16/19 (84.2%)	(62.4%, 94.85%)
Site 3	33	13/13 (100%)	(77.2%, 100.0%)	20/20 (100%)	(83.9%, 100.0%)
Total	**145**	**53/54 (98.1%)**	**(90.2%, 99.7%)**	**88/91 (96.7%)**	**(90.8%, 98.9%)**

*At site 2, 3 pools tested positive when the individual samples that make up the pool tested negative, 1 pool tested negative and included one individual sample in that pool tested positive. Upon repeat testing from a fresh extraction the three pools tested negative, the 4th pool also tested negative and still included one individual sample that tested positive. n = number of pools.

Positive percent agreement met and exceeded acceptance criteria of ≥85% for all three sites combined, supporting pooling of up to 5 samples.

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 20.

Table 20: 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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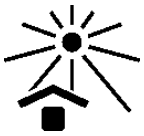
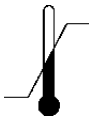
TABLE OF SYMBOLS

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

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		

APPENDIX A: SPECIMEN POOLING IMPLEMENTATION AND MONITORING GUIDELINES FOR LABORATORIES

Before Implementation of Pooling: Determine Appropriate Pool Size

Before a pooling strategy is implemented, a laboratory should determine the appropriate pool size based on percent positivity rate in the testing population and pooling testing efficiency (Table 21). The BioCode® SARS-CoV-2 Assay has been validated for n-sample pool sizes up to five samples per pool.

Table 21. Efficiency of pooling based on the positivity of SARS-CoV-2 RNA in individual samples (as an example)

P, percent of positive subjects in the tested population	$n_{maxefficiency}$ (n corresponding to the maximal efficiency)	Efficiency of n-sample pooling corresponding to $n_{maxefficiency}$ (a maximum increase in the number of tested patients when Dorfman n-pooling strategy used)
5%	5	2.35
6%	5	2.15
7%	4	1.99
8%	4	1.87
9%	4	1.77
10%	4	1.68
11%	4	1.61
12%	4	1.54
13%	3	1.48
14%	3	1.43
15%	3	1.39
16%	3	1.35
17%	3	1.31
18%	3	1.28
19%	3	1.25
20%	3	1.22
21%	3	1.19
22%	3	1.16
23%	3	1.14
24%	3	1.12
25%	3	1.10

If Historical Data for Individual Specimens is Available

Positivity Rate of Individual Testing

- Estimate positivity rate ($P_{individual}$) in the laboratory based on individual sample testing. For this, consider the 7-10 previous days and calculate the number of patients tested during those days. $P_{individual}$ is the number of positive results divided by the total number of tested patients during these 7-10 days.

Selection of test developer validated size of sample pools, n

- Use $P_{individual}$ and Table 21 to choose an appropriate validated pool size. Table 21 presents the pool size with the maximum efficiency for the validated pool sizes and positivity rates. If the positivity rate ($P_{individual}$) is in Table 21, choose n from Table 21 which corresponds to the maximum efficiency (F).
- If $P_{individual}$ in your laboratory does not correspond to the largest validated pool size in Table 21, the pool size with maximum efficiency for this positivity rate was not validated and you should choose the maximum n which was validated. For example, for the calculation of efficiency of 5-sample pooling, using formula $F=1/(1+1/5-(1-P)^5)$, when $P_{individual}$ is 1%, the efficiency F is 3.46 for n=5. It means that 1,000 tests can cover testing of 3,460 patients on average.
- If $P_{individual}$ is greater than 25%, then pooling patient samples is not efficient and should not be implemented.

If Historical Individual Data for Individual Specimens is Unavailable

If historical data from the previous 7-10 days is unavailable, the maximum pool size validated in the EUA and any smaller pool sizes can still be implemented, as the EUA test has been validated for the maximum pool size-specimen pooling. However, note that without $P_{individual}$, the laboratory may choose a pooling size that does not maximize pooling efficiency.

Recommended Pooled Testing Monitoring Plan for Laboratories Testing Pooled Samples

After implementing a n-sample pooling strategy, calculate the percent positivity rate (P_{pool}) based on n sample pooling strategy periodically using the data from pooled samples from the previous 7-10 days*.

If Historical Individual Data for Individual Specimens is Available

If historical data for individual specimens is available, compare P_{pool} to $P_{individual}$ periodically. If P_{pool} is less than 85% of $P_{individual}$ ($P_{pool} < 0.85 \times P_{individual}$), it is recommended that:

- The n-samples pooling should be re-assessed by conducting a re-assessment study as described in "Sample Pooling Re-assessment" below.
- If P_{pool} is greater than 25%, pooling of patient samples is not efficient and should be discontinued until the percent positivity rate decreases.

If Historical Data for Individual Specimens is Unavailable

- After implementing a n-sample pooling strategy, first calculate the positivity rate ($P_{pool\text{-}initial}$) based on n-sample pool size using the data from testing pooled samples from the first 7-10 days*.
  - If $P_{pool\text{-}initial}$ is greater than 25%, pooling of patient specimens is not efficient and should be discontinued until the percent positivity rate decreases.
  - If $P_{pool\text{-}initial}$ is less than or equal to 25%, pooling of patient specimens can be continued.

- Continue to monitor n-sample pooling strategy by calculating the positivity rate among patient samples during n-sample pooling ($P_{pools\text{-}x}$) for subsequent 7-10* day period based on n-sample pool testing. ($P_{pool\text{-}x}$) should be updated daily using a moving average.

Compare $P_{pool\text{-}initial}$ to $P_{pool\text{-}x}$ periodically. If $P_{pool\text{-}x}$ is less than 90% of $P_{pool\text{-}initial}$ ($P_{pool\text{-}x} < 0.90 \times P_{pool\text{-}initial}$), it is recommended that:

- The n-samples pooling should be re-assessed by conducting a re-assessment study as described in "Sample Pooling Re-assessment" below.
- If P_{pool} is greater than 25%, pooling of patient samples is not efficient and should be discontinued until the percent positivity rate decreases.

* It is recommended that $P_{individual}$ be calculated from the previous 7-10 days, while P_{pool} and $P_{pool\text{-}x}$ are calculated from data collected during a 7-10 day time frame. However, when determining if 7-10 days is appropriate, take into consideration the laboratory testing volume and percent positivity, among other factors. Note that if the number of individual or pooled positive results collected during a given time frame is less than 10, $P_{individual}$, P_{pools}, and $P_{pool\text{-}x}$ may not be representative of the percent positivity in the testing population and the laboratory may want to consider extending the testing time period to increase the chance of capturing positives.

Sample Pooling Re-assessment for Laboratories Testing Pooled Samples

Option 1: Stop n-sample pooling and return to individual testing

- Patient samples should be tested individually until 10 consecutive positive samples have been collected. The total number of samples, tested individually, depends on the positivity rate.
- Using these samples, 10 pools should be created and tested with 1 positive and (n-1) negative samples and the PPA between testing sample pools and individual samples should be calculated.

Option 2: Continue n-sample pooling

- Re-assessment study should start from time T0 and should consist of individual sample testing in parallel with the pooled testing. However, since all non-negative sample pools require individual testing of all individual samples included in the pool as a part of the n-sample pooling and deconvoluting workflow, the re-assessment study essentially consists of testing individual samples from the negative n-sample pools.
- Re-assessment study may pause at time T1 when a minimum of 10 consecutive positive individual results are obtained, including both positive individual results generated from individual testing of samples from the non-negative sample pools following the n-sample pooling and deconvoluting workflow, and positive individual results obtained from individual testing of samples from the negative sample pools for the time period from T0 to T1 [T0, T1].
- Considering that number of positive individual sample results among negative pools is K, PPA between testing n-sample pools and assaying single specimens using the candidate test should be calculated as PPA (EUA Test $_{pool}$ vs. EUA Test $_{individual}$) = 100% x (10-K)/10. It is critical that all consecutive positive samples from time period [T0, T1] are included in the PPA calculations. With regard to calculating the PPA, all non-negative results testing pooled samples should be counted as in agreement with positive individually tested results.

Re-assessment Acceptance Criteria for Option 1 and Option 2

- If the PPA (EUA Test $_{pool}$ vs. EUA Test $_{individual}$) is ≥ 90% (9 out of 10 or 10 out of 10), then implementation of testing using n-sample pooling is acceptable.
- If the PPA between pooled-testing results and individual-testing results is less than 90%:
  - If PPA ≤70% (7 out of 10), reduce the pool size (consider a new n as n-1)
  - If PPA is 80% (8 out of 10), collect an additional 10 consecutive individually positive samples. Then, calculate the PPA from the combined data of 20 samples, between pooled testing results and individual testing results. If the PPA is ≥ 85%, then implementation of testing using n-sample pooling is acceptable. Or, to compensate for lost sensitivity, reduce the pool size (consider a new n as n-1) and continue with the re-assessment testing until PPA of pooled compared to individual testing is ≥ 90%.
- If PPA of at least 85% cannot be reached for any pool size evaluated in the re-assessment, cease pooling patient specimens.

If n-sample pooling is acceptable based on re-assessment, re-establish P $_{individual}$ in your laboratory by estimating the positivity rate from individual testing in the population from which the 10 (or 20) consecutive individual positive samples were collected. If the total number of samples (N*) that needed to be tested to obtain the 10 (or 20) consecutive positive samples is stopped at the 10th (or 20th) positive sample, then the positivity rate of 10/N* (or 20/N*) is overestimated. The positivity rate should be corrected by the following corresponding multiplier:

- Positivity rate for 10 samples is (10/N*) × (10/11)
- Positivity rate for 20 samples is (20/N*) × (20/21).

This updated new positivity rate should be used as P $_{individual}$ in the future laboratory monitoring (return to section “Recommended Pooled Testing Monitoring Plan for Laboratories Testing Pooled Samples”**)**.