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MobileDetect-BIO BCC19 Test Kit for SARS-CoV-2 Detection

Instructions for Use – Ver 2.0

October 6, 2020

For *In Vitro* Diagnostic Use Only. IVD
Prescription Use only. Rx ONLY
For Emergency Use Authorization only.

SUMMARY AND EXPLANATION

An outbreak of respiratory illness of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) on December 31, 2019. Chinese authorities identified a novel coronavirus (2019-nCoV) which has resulted in thousands of confirmed human infections in multiple provinces throughout China and exported cases in several Southeast Asian countries and more recently the United States. Cases of severe illness and some deaths have been reported. The International Committee for Taxonomy of Viruses (ICTV) renamed the virus SARS-CoV-2.

INTENDED USE

The MobileDetect Bio BCC19 Test Kit is a reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) test intended for the qualitative detection of RNA from the SARS-CoV-2 in nasopharyngeal (NP), oropharyngeal (OP), mid-turbinate (MT) and anterior nares (nasal) swab specimens 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 the requirements to perform high or moderate 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 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 MobileDetect Bio BCC19 Test Kit is intended for use by clinical laboratory personnel specifically instructed and trained in the techniques of in vitro diagnostic procedures. The MobileDetect Bio BCC19 Test Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

PRINCIPLES OF THE PROCEDURE

The MD-Bio BCC19 Test utilizes a loop mediated isothermal amplification polymerase reaction (LAMP) test intended for the detection of SAR-CoV-2 viral RNA. The test uses a set of specific primers designed to uniquely detect SARS-CoV-2 RNA.
The MD-Bio BCC19 Test utilizes an isothermal nucleic acid amplification technique wherein DNA amplification is carried out at a constant temperature of 65°C. The assay/test uses a set of primers, and a DNA polymerase with high strand displacement and replication activity. The assay primers are targeting the SARS-CoV-2 N and E gene. Also incorporated is a reverse transcriptase (RT) polymerase, which creates complimentary cDNA from RNA, and is in turn amplified by the DNA polymerase. These two polymerases work in tandem enabling the detection of viral DNA and RNA in the same reaction. The amplified DNA byproducts drive the pH of the reaction down to the point where it pushes the pH sensitive dyes to turn yellow, from their starting red color. Reactions displaying a visual color shift indicate that the target sequence is present.

MATERIALS PROVIDED

MD-Bio BCC19 Test Kit items can be seen in Table 1. All materials provided with the BCC19 Test Kit have been validated.

- 12 MD-Bio PCR Strips (the provided PCR strips have been validated with this assay)
- Optional: 1 MD-Bio Heater (other thermal cyclers from the approved list have been validated with this assay)
- 1 MD-Bio Preparation Station
- 1 1.25mL vial of BCC19 Reagent 1
- 1 1.2mL vial of BCC19 Reagent 2
- 1 MD-Bio Analysis Station
- 1 100uL vial of BCC19 Positive Control
    - Positive Control for this assay is diluted to 375 copies/µL (5X LoD) from Twist Bioscience, Synthetic SARS-CoV-2 RNA Control 1 (MT007544.1)) PN: 102019

Table 1: BCC19 Test Kit

Image	Quantity	Description
	12	**MD-Bio PCR Strip** 8 reactions each
	1	**1.25 mL BCC19 Reagent 1** 96 Rxn Total 12.5 µL/Rxn
	1	**1.2 mL BCC19 Reagent 2** 96 Rxn Total 11.5 µL/Rxn
	1	**MD-Bio Analysis Station**
	1	**MD-Bio Preparation Station**
	1	**100 µL BCC19 Positive Control** 100 Rxn Total 1 µL/Rxn

OPTIONAL MATERIALS NOT PROVIDED

- If not purchased as part of the MD-Bio BCC19 Test Kit, a lab must maintain one of the approved thermal cyclers that has been validated with this assay. Use of the test in moderate complexity laboratories requires use with the MD-Bio BCC19 Heater.
    - The approved thermal cyclers can be seen in Tables 2 and 4.
    - Thermal cyclers were validated by running 20 tests at LoD.
- If not purchased as part of the MD-Bio BCC19 Test Kit, a lab must maintain a form of Viral Transport Media (VTM) and one of following swabs: NP, OP, MT or nasal swab.

Table 2: Optional Materials to Purchase with MD-Bio BCC19 Kit

Image	Quantity	Description
	1	**Optional: MD-Bio BCC19 Heater**
	96	**Optional: VTM and NP Swab**

PRECAUTIONS – GENERAL

- For *in vitro* diagnostic use.
- For prescription use only.
- For Emergency Use Authorization only.
- The MD-Bio BCC19 test kit 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 or moderate complexity tests.
- This test has been validated only for the detection of nucleic acid from SARS CoV-2, not for any other viruses or pathogens.
- The MD-Bio BCC19 test kit 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.

- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Do not use the BCC19 Test Kit past the expiration date on the packaging label.

PRECAUTIONS – BCC19 TEST AND SAMPLE HANDLING

- Ensure that bench spaces used for all test steps have been properly cleaned with 10% bleach followed with 70% alcohol.
- Store the BCC19 reagents at the temperatures provided in the storage and testing conditions section below.
- For accurate results, pipette carefully using only calibrated equipment.
- Use only calibrated pipettes and filtered, sterile and PCR clean pipette tips.
- Do not mix reagents from kits with different lot numbers.
- Do not use reagents from other manufacturers with this kit.
- Avoid microbial and cross contamination of the kit reagents. Follow Good Laboratory Procedures.
- Perform sample collection, reagent preparation and sample addition in three different locations per the user manual.
- After the test is complete and analyzed, dispose of the PCR strip and its contents. Do not remove lid from the PCR strip after test.

PRECAUTIONS – USED TEST DISPOSAL

- Used PCR strips should be considered capable of transmitting infectious agent requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used PCR strips. If country or regional regulations do not provide clear direction on proper disposal, used PCR strips should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.
- Also consult your institution's environmental waste personnel on proper disposal of used PCR strips, which may contain amplified material. This material may exhibit characteristics of federal EPA Resource Conservation and Recovery Act (RCRA) hazardous waste requiring specific disposal requirements different from medical waste disposal. Check state and local regulations as they may differ from federal disposal regulations. Institutions should check the hazardous waste disposal requirements within their respective countries.

PRECAUTIONS –SAMPLE COLLECTION

- Treat all biological specimens as if capable of transmitting infectious agents. Wear PPE and clean lab coats and gloves. Change gloves between patients.
- Follow safety procedures set by your institution for handling biological specimens.
- Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention and the Clinical and Laboratory Standards Institute.

LIMITATIONS

- This assay is for in vitro diagnostic use under FDA Emergency Use Authorization only. 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 or moderate complexity tests.
- The performance of the MD-Bio BCC19 test has only been established with nasopharyngeal swab samples. Oropharyngeal, mid-turbinate and anterior nares swabs are also considered acceptable specimen types for use with the MD-Bio BCC19 test but performance has not been established.
- Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g., presence of symptoms), and/or stage of infection.
- A false negative result may arise from:
  -- Improper sample collection
  -- Degradation of the viral RNA during shipping/storage
  -- The presence of RT-PCR inhibitors
  -- Mutation(s) in the sequence of SARS-CoV-2 virus
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- False Positive results may arise from the contamination during specimen handling or preparation, or between patient samples.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined.
- Analyte targets (viral nucleic acid) may persist in vivo, independent of virus viability. Detection of analyte targets does not imply that the corresponding viruses are infectious or are the causative agents for clinical symptoms.
- Performance has not been established in asymptomatic individuals.
- This assay should not be used within 30 minutes of administering nasal or throat sprays.

CONDITIONS OF AUTHORIZATION FOR THE LABORATORY

The MobileDetect Bio BCC19 Test Kit 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

However, to assist clinical laboratories using the MobileDetect Bio BCC19 Test Kit, the relevant Conditions of Authorization are listed below:

- Authorized laboratories[1] using the MobileDetect Bio BCC19 Test Kit will include with result reports of the test, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

- Authorized laboratories using the MobileDetect Bio BCC19 Test Kit will perform the MobileDetect Bio BCC19 test kit as outlined in the Instructions for Use. Deviations

from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform MobileDetect Bio BCC19 test kit are not permitted.

- Authorized laboratories that receive the MobileDetect Bio BCC19 Test Kit must notify relevant public health authorities of their intent to run the test prior to initiating testing.
- Authorized laboratories using the MobileDetect Bio BCC19 Test Kit will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Detectachem (Support@DetectaChem.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All laboratory personnel using the test must be appropriately trained in RT-LAMP techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
- Detectachem, its authorized distributor(s) and authorized laboratories using the MobileDetect Bio BCC19 Test Kit 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.

[1] For ease of reference, this will refer to, "Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified laboratories with FDA Emergency Use Authorization FDA for performing SARS-CoV-2 testing.

BCC19 TEST KIT STORAGE CONDITIONS

Store the unopened BCC19 reagents at -20ºC. Thaw reagents on ice for approximately 30 minutes after taking out of storage conditions. Do not use reagents until they are completely thawed. Keep reagents on ice in between tests. If there is a delay in between tests, store reagents back at -20ºC.

BCC19 TESTING CONDITIONS

Run a BCC19 Test at room temperature. This test should not be used outdoors and has not been tested at high temperatures or humidity.

QUALITY CONTROL – POSITIVE AND NEGATIVE CONTROLS

Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures.

Controls must be used to show that the BCC19 Test is working properly. The BCC19 Positive Control is provided with the test kit. The positive control is necessary to validate that the chemistry is still active. A positive control will consist of adding 1 µL of the provided BCC19 Positive Control to the PCR tube already containing the mixed reagents. The negative control is necessary to validate that the chemistry has not been contaminated. A negative control will consist of running a single test with 1µL of virgin VTM or 1µL of nuclease free water.

The BCC19 Positive Control must be stored at -20ºC. The positive control must be thawed on ice for at least 15 minutes before use. Do not use the positive control unless it is completely thawed. Keep the positive control stored at -20ºC anytime it is not being used.

MD-Bio recommends that a BCP19 Negative Control and Positive Control be run:

- Once for every 8 tube PCR strip or cluster of PCR tubes run in the same heat cycle
- When problems with testing are suspected or identified
- As deemed additionally necessary in order to conform with your internal quality control procedures, with local, state and/or federal regulations, or accrediting groups

If the positive or negative controls do not exhibit their expected reactivity, results of the assay should be considered invalid. In the event of an invalid negative control, the lab should redouble its sterilization procedures, use virgin pipettes and filtered tips, and use new chemistry. In the event of an invalid positive control, the lab should verify use of stable positive control material, as well as verify the expiration date of the reagents. If the control testing continues to fail, do not perform additional clinical specimen tests or report results. An example of what these controls are expected to look like is displayed in Figure 1.

Figure 1: Negative Control (Left) and Positive Control (Right) after amplification

SPECIMEN COLLECTION AND HANDLING

Adequate, appropriate specimen collection and storage are important in order to obtain sensitive and accurate test results. Training in correct specimen collection procedures is highly recommended to assure good quality specimens and results.

- VTM should be stored at room temperature prior to adding sample collection
- 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
- VTM with specimen samples should be stored at 2-8ºC for up to 72 hours (store at -80ºC for any time longer than 72 hours)

DIRECTIONS FOR RUNNING BCC19 TEST

Follow the step-by-step instructions provided below.

Step 1: Obtain Items Required but Not Provided in the BCC19 Test Kit

- If not purchasing a MD-Bio heater, obtain a thermal cycler from the approved list.

Step 2: Review All Information

- Read through the entire Information for Use
- Read through the entire Heater User Manual and Quick Reference Instructions
- Review all linked resources for specimen collection and handling

Step 3: Sample Collection and Preparation

A nasopharyngeal, oropharyngeal, mid-turbinate or anterior nares swab is used to collect the respiratory specimen from the patient. All samples should be collected in accordance with appropriate CDC guidelines and include appropriate reference: https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html. This swab is then placed in the provided VTM. 1µL of this solution is used as the analyte in the reaction.

Step 4: Reagent Preparation

Use the provided MD-Bio Preparation stand to help prepare the reagents as illustrated in Figure 2.

- Frozen reagents must be thawed. Mix thawed reagents by low speed vortex or by inverting 5 times. Mix reagents until they are visibly homogeneous.
- Place the thawed, homogenous reagents onto the MD-Bio preparation stand.
- Place MD-Bio PCR strip onto the other side of the MD-Bio preparation stand.
- Pipette reagents into the PCR strip.
    1. Pipette 12.5µL of Reagent 1 into each of the 8 wells of the provided MD-Bio PCR Strip
    2. Pipette 11.5µL of Reagent 2 into each of the 8 wells of the provided MD-Bio PCR Strip

For best practice, a separate pipette tip should be used for ***every single*** dispension. When running multiple tests, it is recommended that Reagent 1 is pipetted into all wells and then Reagent 2 is pipetted into all wells. This is generally more efficient. Keep reagents on ice in between tests. If there is a delay between test, store reagents at -20ºC.

Figure 2: MD-Bio Preparation Stand with Reagents and MD-Bio PCR Strip

Step 5: Sample Addition

Each well of the PCR strip will receive 1µL of VTM containing the patient's sample for investigation. If the VTM was frozen prior to testing, thaw completely before use. Mix by low speed vortex or by inverting 5 times. Mix thawed VTM until it is visibly homogeneous. Place the prepared PCR Strip in the MD-Bio BCC19 Heater (or approved Thermal Cycler).

Step 6: Heat Cycle

- For the MD-Bio BCC19 Heater:
  1. Power the MD-Bio BCC19 Heater on by plugging it into the wall using the provided power supply. The unit will perform a short self-test indicated by sequencing the lights thru red, green and amber, followed by a beep. At the conclusion of self-test, the unit will enter standby mode with only the red power light illuminated as illustrated in Figure 3.
     a. If the MD-Bio BCC19 Heater is not running a cycle and left untouched for 5 minutes or more, it will enter sleep mode. Wake the unit from sleep by pressing the 'Select' button once.

Figure 3: MD-Bio BCC19 Heater in Standby Mode

  2. To start the incubation heat cycle momentarily push the select button. The unit will beep and the green light will flash indicating that the unit is in its warm up cycle as illustrated in Figure 4. This cycle is approximately 1-2 minutes.
     a. If the heat cycle needs to be stopped at any moment once the incubation cycle has been started, hold down the select button for 5 seconds. When the unit beeps, release the button to cancel the test cycle.

Figure 4: MD-Bio BCC19 Heater in Warm Up Mode

3. Once the unit has reached the correct temperature, the green light will change from flashing to steady, and the amber testing light will flash, indicating that the unit has entered incubate mode as illustrated in Figure 5. The amber light will change its flash pattern to indicate progress during the test. During the first 10 minutes of the test it will flash once every three seconds, twice every three seconds during the second 10 minutes of test, and three times every three seconds during the final 10 minutes of the test.

Note: The unit will bypass warm up and go directly to the incubate portion of the heat cycle as indicated by steady green and flashing amber lights if it is already at the proper test temperature from a previous test.

Figure 5: MD-Bio BCC19 Heater in Incubation Mode

4. At the end of the 30-minute incubation cycle the MD-Bio BCC19 Heater will sound a short series of beeps and both green and amber lights will flash continuously. This indicates it is time to remove the PCR Strip as illustrated in Figure 6. Remove the PCR Strip from the heater and press the select button to return the unit to standby.

WARNING: If the PCR strip has not been removed within 5 minutes after the cycle is complete, the heater will go into a continuous beep mode alerting the user that the test is now invalid and the results should no longer be interpreted.

Figure 6: MD-Bio BCC19 Heater when Test is Completed

5. Please power the MD-BIO Heater off by unplugging from the wall when not in use.

- For an approved Thermal Cycler:

  Utilize the operating manual and instructions of the chosen thermal cycler. Set the well plate temperature to 65°C and the lid temperature to 95°C. Set the incubation time to 30 minutes, set solution volume to 25µL, and incubate the prepared MD-Bio PCR Strip. After the cycle is complete, remove the PCR strip from the thermal cycler.

Step 7: Cool Down

Place the PCR Strip in the MD-Bio Analysis Station, as illustrated in Figure 7, and allow it to cool for approximately 1 minute before color interpretation. This will allow the chemistry to come to thermal equilibrium and more clearly show the yellow positive and red negative results.

Figure 7: MD-Bio Analysis Station

Step 8: Results Interpretation

NOTE: Results can only be interpreted after controls have shown to have valid results.

The results are meant to be interpreted once the test cycle is complete and the PCR strip has cooled for 1 minute. After 8 hours, the results are no longer valid for interpretation. Use visual color recognition per the user manual to identify whether the result is positive (yellow), negative (red) or invalid (any other color) based on color change. An example of this can be seen in Figure 8. Edge cases for positive and negative results can be seen in Figure 9. Any color variance stronger than the edge cases should be deemed inconclusive and the sample should be re-tested.

Figure 8: Example of Test Results (4 Left Negative, 4 Right Positive

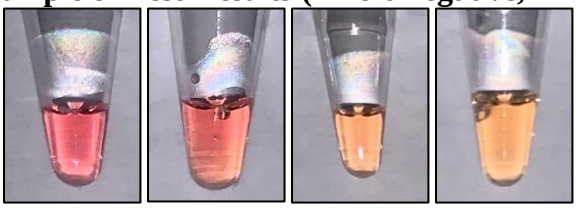

Figure 9: Example of Edge Case Test Results (2 Left Negative, 2 Right Positive)

LIMIT OF DETECTION

A Limit of Detection Determination test series was conducted to determine the lower limit of detection using the MD-Bio Heater. The positive control used to determine LoD was Twist Bioscience, Synthetic SARS-CoV-2 RNA Control 1 (MT007544.1 PN: 102019) which contains the entire viral genome. This analyte was spiked into Viral Transport Media (VTM) from a NP (nasal pharyngeal) negative patient sample at the following concentrations: 500 copies/µL, 375 copies/µL, 250 copies/µL, 125 copies/µL, 100 copies/µL, 75 copies/µL, 50 copies/µL, and 25 copies/µL. Then 1µL of each of the contrived analytes was used to yield the total number of copies per reaction below. All measurements were performed using 3 individual samples. The conclusion of the LoD determination test series is that 75 copies/µL is the LoD for this assay. This value was the lowest analyte value attempted that correctly agreed with all 3 samples yielding positive results. For concentrations near the LoD, additional samples were performed to determine the concentration at which at least 95% of the samples (19/20) were positive. All results from the LoD determination test series can be viewed in Table 3.

Table 3: LoD Testing for MD-Bio Reagents

Concentration (copies/µL)	Positive Samples Detected
500	100% (3/3)
375	100% (3/3)
250	100% (3/3)
125	100% (3/3)
100	100% (20/20)
75	100% (20/20)
50	60% (12/20)
25	30% (6/20)

To validate the list of approved thermal cyclers with this assay, 20 tests at LoD were run on all thermal cyclers. The results of these validation studies can be seen below in Table 4.

Table 4: Thermal cycler LoD Verification

Thermal cycler	Concentration (copies/µL)	% Replicates Detected
Bio-Rad T100	75	100% (20/20)
Detectachem MD-Bio Heater	75	100% (20/20)
Benchmark Scientific T5005-3205	75	100% (20/20)
Techne 5Prime/02	75	100% (20/20)
EVDOtek EDVOcycler 2	75	100% (20/20)
EVDOtek EDVOcycler Jr.	75	100% (20/20)
Applied Biosystems Veriti	75	100% (20/20)
Applied Biosystems MiniAmp	75	100% (20/20)

ANALYTICAL REACTIVITY/INCLUSIVITY

The assay targets specific genomic regions of the SARS-CoV-2 nucleocapsid (N) gene and small envelope (E) gene. Inclusivity was demonstrated by mapping the primers and probes to the complete SARS-CoV-2 genomes that were available in GISAID (Global Initiative on Sharing All Influenza Data) database as of August 25, 2020. For all primers, there was a 99.41% overall match rate for the N-gene, and 91.42% match rate for the E-gene. The rate of 1 mismatch was below 1.0% for all individual N-Gene and E-Gene primers with the exception of the E-gene FIP. While the E-gene FIP had a mismatch rate of 50.57% due to a recent E-gene mutation, an overwhelming majority of these are 1 mismatch. These mismatches were located at the extreme 5' end of the respective primers and are not expected to result in amplification failure. Previous work on MERS-CoV has demonstrated that a single nucleotide mismatch in one primer set had a negligible impact on the detection using LAMP assays (PMID 25103205). Additionally, the assay only requires one of the primer sets to match to cause amplification and therefore detection, meaning that the N-gene primers can detect SARS-CoV-2 presence in a sample alone. This data is displayed in Table 5a and 5b.

Table 5a: In silico Inclusivity Analysis N-gene

	N-gene Primers					
Primer	**F3**	**B3**	**FIP**	**BIP**	**Loop-F**	**Loop-B**
Total Primer Length (nt)	21	25	42	40	21	20
Total # of Strains Evaluated	14949	14977	14948	14842	17681	17278
100% Match	99.90%	99.81%	99.44%	99.99%	98.82%	98.51%
1 Mismatch	14	27	81	1	207	256
2 Mismatches	1	1	2	0	2	1
3 Mismatches	0	0	0	0	0	0
>3 Mismatches	0	0	0	0	0	0

Table 5b: In silico Inclusivity Analysis E-gene

	E-gene Primers					
Primer	**F3**	**B3**	**FIP**	**BIP**	**Loop-F**	**Loop-B**
Total Primer Length (nt)	21	25	42	40	21	20
Total # of Strains Evaluated	15375	14895	14957	14908	14922	14966
100% Match	99.96%	99.53%	49.43%	99.74%	99.87%	99.99%
1 Mismatch	6	69	7537	39	18	2
2 Mismatches	0	0	23	0	2	0
3 Mismatches	0	1	4	0	0	0
>3 Mismatches	0	0	1	0	0	0

ANALYTICAL SPECIFICITY – CROSS REACTIVITY

In silico cross-reactivity analysis was performed by aligning the N-gene and E-gene LAMP primer sequences against sequences of coronaviruses related to SARS-Cov-2, as well as common respiratory pathogens.

SARS-CoV was the only organism analyzed that showed a higher than 80% overall match for the F3, LF, and LB primer sets from the N gene assay target. Per FDA recommendation, wet testing was performed to test cross-reactivity of SARS-CoV with the MD-Bio BCC19 test as described below.

Wet testing was performed for SARS-CoV due to its higher than 80% match rate to the N-gene and E-gene primer sets. Wet testing was also performed on RSV, Flu, Human Metapneumovirus, and Streptococcus salivarius because they are common respiratory flora and viral pathogens. Samples were prepared by spiking SARS-CoV RNA (BEI Resources, Catalog: 011N-03), RSV (BEI Resources, Catalog: NR-43976), Influenza B (BEI Resources, Catalog: NR-45848), Human Metapneumovirus (BEI Resources, Catalog: NR-49122), and Streptococcus salivarius (BEI Resources, Catalog: HM-121) into NP negative clinical matrix. SARS-CoV RNA samples were diluted at 200,000 copies/µL. RSV, Influenza B, and Metapneumovirus samples were diluted at 10^5 pfu/ml. Streptococcus salivarius samples were diluted at 10^6 CFU/ml. All wet testing results showed no Cross-Reactivity with the assay, as seen in Table 6.

Table 6: Cross-Reactivity/Exclusivity Wet Testing Results

Organism	Strain	N-gene Detected Replicates	E-gene Detected Replicates
SARS-CoV	N/A	0/8	0/8
RSV	A1997/12-35	0/3	0/3
FLU	B/Nevada/03/2011	0/3	0/3
Human Metapneumovirus	TN/83-1211	0/3	0/3
Streptococcus salivarius	SK126	0/3	0/3

ANALYTICAL SPECIFICITY – INTERFERING SUBSTANCES

Exogenous and endogenous potential interfering substances were tested to evaluate any potential effects on the assay. SARS-COV-2 negative nasopharyngeal swabs were collected in triplicate and spiked with interferent. Additional negative nasopharyngeal swabs were collected and spiked with synthetic SARS-CoV-2 RNA (Twist Synthetic SARS-CoV-2 RNA Control) at 5X LoD in addition to the potential interfering substances. None of these potential interferents were found to inhibit the performance of the assay, as all swabs with and without the potential interferents were found to have expected results. These results can be seen in Table 7.

Table 7. Endogenous and Exogenous Substances Evaluated for Potential Assay Interference

Substance	Active Ingredient	Final Concentration	% Agreement with Expected Results	
			Positive Control (3X LoD, 225 copies/µL)	Negative Control
Whole Blood	N/A	10% v/v	100% (3/3)	100% (3/3)
Mucin	N/A	0.5% w/v	100% (3/3)	100% (3/3)
Substance	**Active Ingredient**	**Final Concentration**	**% Agreement with Expected Results**	
			Positive Control (5X LoD, 375 copies/µL)	**Negative Control**
Alcohol	Ethanol	10% v/v	100% (3/3)	100% (3/3)
Chloraseptic spray	Phenol, Glycerin	20% v/v	100% (3/3)	100% (3/3)
Mouthwash	Eucalyptol, menthol, Methyl Salicylate, Thymol	10% v/v	100% (3/3)	100% (3/3)
Nasal allergy spray	Triamcinolone acetonide	15% v/v	100% (3/3)	100% (3/3)
Nasal congestion spray	Oxymetazoline HCl	20% v/v	100% (3/3)	100% (3/3)
Nyquil	Acetaminophen, Doxylamine succinate, Dextromethorphan HBr	10% v/v	100% (3/3)	100% (3/3)
Saline nasal spray	NaCL, Phenylcarbinol, Nemalkonium Chloride	20% v/v	100% (3/3)	100% (3/3)

Substance	Active Ingredient	Final Concentration	% Agreement with Expected Results	
			Positive Control (5X LoD, 375 copies/µL)	Negative Control
Sore throat and cough lozenges	Benzocaine, Dextromethorphan HBr	10% w/v	100% (3/3)	100% (3/3)
Tobacco	Nicotine, Tobacco	10% w/v	100% (3/3)	100% (3/3)
THC	N/A	5% w/v	100% (3/3)	100% (3/3)
Zinc	Zinc	2% w/v	100% (3/3)	100% (3/3)

CLINICAL EVALUATION

Performance of the DetectaChem MobileDetect Bio BCC19 Test Kit was evaluated using confirmed clinical nasopharyngeal samples.

Clinical Study with Confirmed Positive and Negative Samples

Real patient nasopharyngeal samples were randomly selected and tested simultaneously alongside an EUA authorized RT-PCR comparator assay. The clinical study had 1 false negative occur. Table 8 shows the summation of the Patient Sample Test Report. The clinical study included a control positive and control negative with every heat cycle. All controls performed as expected.

Table 8: Clinical Performance of the MD-Bio BCC19 Test

Detectachem Result	EUA Authorized Comparator Positive	EUA Authorized Comparator Negative	Total
Positive	43	0	43
Negative	1	63	64
Total	44	63	107
Positive Agreement	97.7 % (43/44) 95% CI: 88.0-100%		
Negative Agreement	100% (63/63) 95% CI: 94.3-100%		

CUSTOMER SUPPORT

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