EasyNAT Kit for Novel-Coronavirus (COVID-19) RNA (Isothermal Amplification-Real Time Fluorescence Assay)

For *in vitro* diagnostic use

For prescription use only

Validation of this test has not been reviewed by FDA. Review under the EUA program is pending.

Specifications

20 tests/Box, Product Code: U20223

Intended Use

The EasyNAT® Diagnostic Kit for Novel-Coronavirus (COVID-19) RNA (Isothermal Amplification-Real Time Fluorescence Assay) is a real-time RT-CPA in vitro diagnostic test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal and oropharyngeal swab specimens from individuals suspected of COVID-19 by their healthcare provider. The EasyNAT Diagnostic Kit for Novel Coronavirus is for use in conjunction with the EasyNAT® Nucleic Acid Amplification and Detection Analyzer[1]. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal and oropharyngeal swab specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude COVID-19 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the EasyNAT Diagnostic Kit for Novel-Coronavirus (COVID-19) RNA (Isothermal Amplification-Real Time Fluorescence Assay) is intended for use by trained laboratory personnel who are proficient in performing real-time RT-PCR assays. Validation of this test has not been reviewed by FDA. Review under the EUA program is pending.

Summary and Explanation

The EasyNAT Kit is a qualitative test run on the EasyNAT System for the detection of SARS-CoV-2 RNA in nasopharyngeal and oropharyngeal swabs in transport media. An internal control is included in the COVID-19 Cartridge to monitor the test process. The EasyNAT Kit includes positive and negative control material and may be used for quality control and laboratory verification.

Principle of the Procedure

The EasyNAT Kit contains the COVID-19 Cartridge (Figure 1 below) that is equipped with multiple hydrophobic separation layers to isolate the lysate, cleaning solution and reaction solution within the COVID-19 Cartridge. The COVID-19 Cartridge is pre-loaded with nucleic acid purification reagents (including magnetic beads, nucleic acid binding buffer and washing buffer in separate chambers), nucleic acid elution reagents, and Crossing Priming Amplification (CPA[2]) reaction reagents (including enzymes). The CPA reaction reagents detect ORF1ab and N gene sequences specific to COVID-19.

The COVID-19 Cartridge also includes an internal control (IC), which consists of a CPA system that specifically detects human Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA to monitor the effectiveness of sampling, extraction, purification, and amplification reactions.

[1] Throughout the IFU, the EasyNAT Diagnostic Kit for Novel-Coronavirus (COVID-19) RNA (Isothermal Amplification – Real Time Fluorescence Assay) will be referenced as the EasyNAT Kit and the EasyNAT Nucleic Acid Amplification and Detection Analyzer will be referenced as the EasyNAT System.

[2] Xu G, Hu L, Zhong H, etc. Cross priming amplification: mechanism and optimization for isothermal DNA amplification. Sci. Rep. 2012; 2:246.

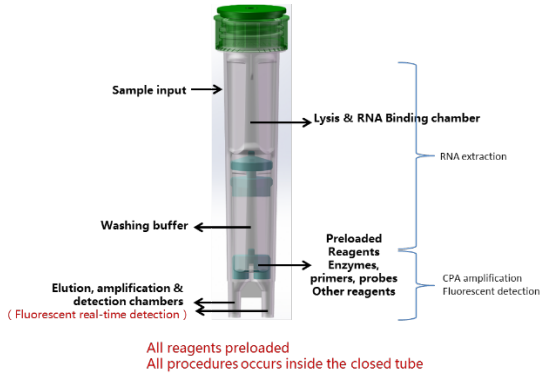

Figure 1: COVID-19 Cartridge Configuration

The reagent steps using the nucleic acid extraction solution, nucleic acid purification reagent and nucleic acid elution are automatically completed for the purification of nucleic acid in the sample in the COVID-19 Cartridge in the EasyNAT System. The purified nucleic acid and CPA reaction reagent are mixed and heated in the EasyNAT System and then subjected to constant temperature amplification. The detection system in the EasyNAT Kit together with the EasyNAT System automatically completes sample extraction, purification, amplification and detection of SARS-CoV-2 RNA gene fragments in a closed detection tube.

Controlled by the EasyNAT System, the extract is chemically cracked at high temperatures to release the nucleic acid and test the sample. At the same time as amplification, the fluorescent probe and the nucleic acid template in the primer amplification region specifically bind and amplify to generate a fluorescent signal. Through the magnetic permeability of the EasyNAT System, the nucleic acid of the specimen used for detection passes through different liquid layers and then nucleic acid is eluted in the COVID-19 Cartridge leg and the expansion reaction occurs, so as to realize the "one tube" full-automatic nucleic acid analysis, that is, the cleavage binding, cleaning, elution and amplification reaction are completed in the closed COVID-19 Cartridge. The EasyNAT System collects fluorescence signals in real time and automatic determination of inspection results by analyzing changes in fluorescence signals.

Through specific amplification primers, specific fluorescent probes and DNA polymerases with high activity of reverse transcriptase and chain displacement characteristic, the reaction system can complete the specific amplification process of SARS-CoV-2 fragments at a single time at a constant temperature; and the fluorescence signal is detected by EasyNAT System and a real-time fluorescence curve is automatically generated. At the same time as amplification, the fluorescent probe and the nucleic acid template in the primer amplification region specifically bind and amplify to generate a fluorescent signal. The EasyNAT System collects fluorescent signals and automatic determination of results by analyzing changes in fluorescent signals.

The user does not need to open the cover during the detection process, as an independent fully automatic detection of a single sample is completed by the EasyNAT System. This closed single-tube detection method minimizes the probability of cross-contamination between samples and improves the accuracy of the detection results.

Materials Provided

This product is composed of the components in **Table 1**:

Table 1: EasyNAT Kit Components

#	Name of Components	Specification	Quantity	Main components
1	COVID-19-Cartridge	1 test/cartridge	20 cartridges	Tris, MgSO4, 2019-nCoV specific primers and probe, human GAPDH specific primers and probes, deoxyribonucleoside triphosphate (dNTP), DNA polymerase
2	COVID-19-RNA Extraction Solution	1 mL/test/tube	20 tubes	Guanidine salt, Magnet beads
3	COVID-19-Positive Control	1.2 mL/tube	1 tube	Positive RNA Control nucleic acid is armored RNA containing SARS-CoV-2 specific fragments - ORF1ab and N gene target fragments
4	COVID-19-Negative Control	1.2 mL/tube	1 tube	RNA containing human GAPDH target fragment

Notes: The components in different kit lots cannot be used interchangeably.

Materials Required But Not Provided

The following components are required but not included with the test:

1. Ustar Biotechnologies EasyNAT Nucleic Acid Amplification Detection Analyzer (Model UC0102), EasyNAT System, with Software Version 1 or later.
2. Nasopharyngeal flocked swabs for collection of nasopharyngeal specimens.
3. Regular flocked swabs for collection of oropharyngeal specimens.
4. Transport media.

Warnings and Precautions

1. Validation of this test has not been reviewed by FDA. Review under the EUA program is pending.
2. Positive results are indicative of the presence of SARS-CoV-2 RNA.
3. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
4. Performance characteristics of this test have been established with the specimen types listed in the **Intended Use Section** only. The performance of this assay with other specimen types or samples has not been evaluated.
5. The EasyNAT Kit has been validated using the EasyNAT System Software Version 1, or later.
6. The COVID-19- RNA Extraction Solution contains insoluble particles. Please mix well before pipetting.
7. Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
8. Follow necessary precautions when handling specimens. User personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious sample.
9. Do not modify assay reagents, assay protocols, or instrumentation.
10. Do not use the kit after the indicated expiry date.
11. Do not mix components from kits with different lot numbers.
12. Do not use reagents from other manufacturers with this kit.
13. This product contains no human-derived materials.
14. Please read instructions carefully before use.
15. Before using the EasyNAT Kit with patient samples, run the COVID-19 Positive Control and COVID-19 Negative Control. Refer to Section 2.
16. This kit is disposable.
17. The work table and required items need to be disinfected regularly with 1% sodium hypochlorite, 75% alcohol or ultraviolet lamp.
18. Please ensure that the QR code of the COVID-19 (located above the cover of cartridge) is clean, clear and should not be written on or painted, so as not to affect the function of QR code.
19. COVID-19 Cartridge shall be amplified immediately once opening and feeding.
20. Do not open the lid of the sample chamber when running test.
21. The instrument will save test results automatically, please check in the “View” interface.
22. Do not squeeze the middle and lower part of the COVID-19 Cartridge when operating.
23. Please operate strictly in accordance with the instructions, the COVID-19 Cartridge may not be inserted before sample information is inputted onto the EasyNAT System.
24. Operators should use the product in a laboratory with biosafety protection and need to wear protective equipment.
25. Safety Data Sheets (SDS) are available upon request.

Various factors may cause performance changes during the storage, transportation, and use of reagents. For example, improper storage, transportation, sample collection, sample processing and testing procedures can potentially effect results. Please strictly follow the instructions. Due to the characteristics of the sample collection such as nasopharyngeal and oropharyngeal swabs specimens and virus infection, there may be false negative results caused by insufficient sample volume, or other factors.

Storage and Handling of Kit Reagents

1. Store the EasyNAT Kits at 2℃-8℃ until the expiration date listed on the outer kit box. Please refer to the label expiry date.
2. All kit components should be stored and used together. Do not use components from one kit with those of another kit. Discard any extra components from the kit after all COVID-19 Cartridges have been consumed.
3. The EasyNAT Kit may be transported at temperatures from -15℃ to 30℃ for up to 7 days.
4. The COVID-19 Cartridge is for single-use.

Sample Handling, Storage and Preparation

1. Following CDC recommendations, nasopharyngeal and oropharyngeal swab specimens should be collected and handled according to the manufacturer's recommended procedures. Recommended storage conditions for nasopharyngeal and oropharyngeal swab specimens resuspended in transport medium is 2°-8°C for up to 72 hours after collection. Samples may be stored at -70°C or below for transport on dry ice to the testing site.
2. Specimens should be sent to the laboratory as soon as possible after collection. Nasopharyngeal and oropharyngeal swab specimens should be transported in accordance with CDC guidelines or labeling provided with the swabs.
3. COVID-19 Cartridges with samples added may be stored at 2-8°C for up to 6 hours prior to running on the EasyNAT System.

Assay Procedure

Perform testing according to the requirements of this procedure.

1. Sample Loading & Test Procedure

1.1 Sample Loading

1.1.1 **Thoroughly mix the COVID-19 Extraction Solution, and then transfer all contents (1mL) of this solution to the COVID-19 Cartridge.**

1.1.2 Add 500 μL (nasopharyngeal or oropharyngeal swab specimen) to the COVID-19-Cartridge, screw the cap tightly, gently mix the lysis mixture (paraffin may float, continue to the next step) and the COVID-19 Cartridge is ready to be tested. It is recommended to start the test as soon as the sample has been added to the COVID-19 Cartridge. COVID-19 Cartridges with samples added may be stored at 2-8℃ for up to 6 hours prior to running on the EasyNAT System.

1.2 Running Test

Important Reminder: This section lists the basic steps for running the test. Refer to the EasyNAT System instruction of applicable instrument for details.

1.2.1 Input cartridge information: The EasyNAT System will automatically input the cartridge information and the amplification detection program CoV10 when scanning the QR code of the prepared COVID-19 cartridge (located on the cap of the cartridge). The cartridge information can also be entered manually by clicking the *Scan the QR Code on the cartridge* button on the touch screen of applicable instrument.

1.2.2 Input sample information: The EasyNAT System will automatically input the sample information when scanning the bar code of the sample according to step *1.2.1* on the EasyNAT System. The sample information can also be entered manually by clicking the *Scan the QR Code on the cartridge* button on the touch screen of applicable instrument.

1.2.3 Start Testing: The EasyNAT System has two test chambers that can be used to run two tests at the same time. To perform the test, load the COVID-19 Cartridge containing COVID-19-RNA Extraction Solution and test sample into one of the EasyNAT test chambers, then close the lid of the sample chamber and click the START button on the EasyNAT touch screen. The EasyNAT System will start the test procedure and the countdown will be displayed on the screen. The total test duration is 79 minutes, including: sample processing for 39 minutes (CoVtq – CoV Nucleic Acid), and amplification at 58 °C for 40 minutes (CoVfr – CoV Reconstitution and Amplification of Reagent).

1.3 Test Result

As soon as testing is complete, the test results are displayed on the EasyNAT System touch screen and saved automatically. For details, please refer to *Interpretation of the Test Results.*

2. Quality Control

2.1 The COVID-19 Cartridge includes an Internal Quality Control (IC) that is intended to monitor failures in sample collection, sample processing, amplification reagents, and malfunctions of applicable instruments. The Internal Control is Human GAPDH mRNA. GAPDH is a human house-keeping gene, and the mRNA comes from the patient cells in the specimen. The internal control will go through the entire testing procedure as the same as the target.

2.2 The EasyNAT Kit includes an external COVID-19-Positive Control and COVID-19-Negative Control control.

2.3 The COVID-19 Positive Control in the kit is armored RNA containing the target ORF1ab and N gene fragments of SARS-CoV-2. It is used to exclude the failure of the amplification reagents and the malfunction of applicable instruments.

2.4 The COVID-19 Negative Control is a human GADPH target fragment used to monitor whether the reagent or the environment is contaminated.

2.5 The external COVID-19-Positive Control and COVID-19-Negative Control are processed and outlined in the *Assay Procedure* section.

2.6 The external COVID-19-Positive Control and COVID-19-Negative Control should be run in the following situations:

a) The controls should be run for the first time use of instrument or assay.
b) Whenever the result is abnormal (suspicion of contamination or false negative etc.)
c) A new shipment of EasyNAT Kits.
d) Every week or as recommended by the FDA and/or CDC.

Interpretation of the Test Results

The EasyNAT System measures the fluorescent signals generated during the run and analyzes the change in fluorescent signals over time to determine the EasyNAT test results. The EasyNAT System uses the measurement of the change in fluorescent signals over time (Tt) to determine if a sample is positive or negative for the presence of SARS-CoV-2. The EasyNAT System performs the following steps automatically during sample processing:

- Determines the Baseline Value; the EasyNAT System determines the average of the fluorescent values from the first three (3) minutes.
- Calculates the Standard Deviation (SD) from the first three (3) minutes of fluorescent values.
- Determines the Threshold Value by multiplying the Baseline SD value by 10 and then adding this to the baseline value (Threshold Value = Baseline Value + 10x Baseline SD).
- The Tt value is amplification time (in minutes) of when the fluorescence value meets the threshold value.

The EasyNAT System displays the result for the sample and also includes the Tt values for the ORF1ab (CoV101), N gene (CoV10r) and GDAPH Internal Control (ICl and ICr) and reports positive or negative results by the following criteria:

- If the Tt value of gene ORF1ab is N/A, test result for gene ORF1ab will be 'Negative'. If the Tt value of gene ORF1ab is ≤40, test result for gene ORF1ab will be 'Positive'.
- If the Tt value of gene N is N/A, test result for gene N will be 'Negative'. If the Tt value of gene N is ≤40, test result for gene N will be 'Positive'.
- If the Tt value of the IC is N/A, test result for the corresponding IC will be 'Negative'. If the Tt value of an IC is ≤40, test result for the corresponding IC will be 'Positive'.

Table 2: Test Results and Interpretation

Test Result	Interpretation
Positive	Gene ORF1ab and/or N is 'Positive', which means SARS-CoV-2 RNA is detected in the sample. Remark: When the test result is positive, the test results of IC are not required.
Negative	Both gene ORF1ab and N are reported as 'Negative', and ICr and/or ICl is 'Positive', which means no SARS-CoV-2 RNA is detected in the sample.

Test Result	Interpretation
Invalid	ICr and ICl are 'Negative', which means the test fails in detecting 2019-nCoV RNA or human GAPDH mRNA.
No Results	The test system fails to collect sufficient data for analysis.

Conditions that Require a Retest

If any of the following situations occur, please retest with a new COVID-19-Cartridge.

- Invalid results. For example, invalid results may be caused by improper sample collection/processing procedures, inhibition of test reagent, or expired products.
- No results. For example, the test is aborted before the due time.
- Abnormal results of external COVID-19 Positive Control or COVID-19 Negative Control. For example, if the COVID-19 Negative Control reports a positive result, there may be contamination from the experimental environment.

Quality Control Results

This product contains Internal Quality Control and External Quality Control.

Internal Quality Control

The Internal Quality Control is intended to monitor failures in sample collection, sample processing, amplification reagents, and malfunctions of applicable instruments.

If a COVID-19 Positive result is reported, the test results of ICs are not required. However, the IC must be positive (IC Tt≤40), if a COVID-19 Negative result is reported, otherwise the test result is invalid.

External Quality Control

The COVID-19 Positive Control in the kit is armored RNA containing the target (ORF1ab and N gene) fragments of SARS-CoV-2. It is used to eliminate the failure of the amplification reagents, and the malfunctions of applicable instruments. The COVID-19 Negative Control is a human GAPDH target fragment used to monitor whether the reagent or the environment is contaminated.

The test result of COVID-19 Positive Control should be COVID-19 Positive (Tt≤40 for ORF1ab or N), while the test result of COVID-19 Negative Control should be COVID-19 Negative (Tt=N/A for ORF1ab and N, and Tt≤40 for IC).

Limitations of the Procedure

1. Validation of this test has not been reviewed by FDA. Review under the EUA program is pending.
2. Laboratories should include the following statement in test reports to healthcare providers "The test has been validated but FDA's independent review of this validation is pending."
3. Do not use reagents past their expiration date.
4. This assay cannot rule out diseases caused by other bacterial or viral pathogens.
5. Negative results do not preclude COVID-19 infection and should not be the sole basis for treatment of patient management decisions.
6. The performance of the EasyNAT Kit has been validated for use on specimens obtained from nasopharyngeal or oropharyngeal swabs.
7. Test results from this product are only for reference for clinical physicians. For patients' clinical diagnosis and treatment, it should be considered in combination with their symptoms, signs, medical history, other laboratory tests, and therapeutic effects.
8. False negative results may come from improper sample collection, transportation, and processing procedures, or samples with low viral load.

9. Other unverified interfering substances or amplification inhibitors may cause false negative results.

Product Performance Characteristics

1. Limit of Detection (LoD)

The analytical limit of detection was assessed for the EasyNAT Kit to determine the lowest concentration of SARS-CoV-2 RNA that can be detected by the EasyNAT Kit at least 95% of the time. The preliminary LoD of the EasyNAT Kit was determined using three (3) simulated clinical samples positive for SARS-CoV-2 diluted in oropharyngeal matrix over five (5) dilutions to achieve 500 copies/mL, 1,000 copies/mL, 2,500 copies/mL, 5,000 copies/mL and 10,000 copies/mL. The preliminary LoD was determined by testing 20 replicates of the three (3) samples at each dilution with three (3) lots of the EasyNAT Kit. The LoD was determined as the lowest dilution giving a sample detection ≥ 95%. The preliminary LoD for the EasyNAT Kit is 1,000 copies/mL.

Table 3: Preliminary LoD Results

Target Level	Sample 1		Sample 2		Sample 3	
	n	Detection Rate	n	Detection Rate	n	Detection Rate
10,000 copies/ml	60	100%	60	100%	60	100%
5,000 copies/mL	60	100%	60	100%	60	100%
2,500 copies/mL	60	100%	60	100%	60	100%
1,000 copies/mL	60	95%	60	95%	60	95%
500 copies/mL	60	88%	60	88%	60	90%

The LoD of 1,000 copies/mL was confirmed with nasopharyngeal and oropharyngeal swab specimens spiked with SARS-CoV-2 RNA. A total of 180 individually spiked nasopharyngeal swab specimens and 180 individually spiked oropharyngeal swab specimens were tested across three (3) different batches of nucleic acids (included in the cartridges), and 60 COVID-19 cartridges from each of three (3) lots for a total of 180 COVID-19 cartridges. Nasopharyngeal and oropharyngeal swab specimens spiked with 1,000 copies/mL had a 100% positive detection rate.

2. Analytical Sensitivity - Inclusivity (*in silico* analysis)

In Silico analysis was conducted to confirm the detection of the SARS-CoV-2 strains for the ORF1ab and N gene assay included in the EasyNAT Kit. *In Silico* analysis was performed on all sequences available in the Global Initiative on Sharing All Influenza Data (GISAID) databases. 62,528 sequences for ORF1ab and 63,424 sequences for N gene were aligned against the EasyNAT Kit's primers and probes.

ORF1ab Assay

For the ORF1ab primer and probe set, there were 1461 strains (out of 62528) that exhibited mismatches (2.3%). Of the 1461 strains, 1454 had a mismatch in only one sequence (primer or probe). For the remaining 7 strains, mismatches occurred in two sequences (3 are in both the WHCoV-2K-1-RB and WHCoV-2K-1-CPR, 2 are in both the probe and WHCoV-2K-1-CPR, 1 is all in WHCoV-2K-1-CPR, 1 is in both WHCoV-2K-1-CPF and WHCoV-2K-1-IP). Annealing temperature calculations indicate that these mismatches do not significantly impact the annealing temperature and therefore, all 1461 strains are predicted to anneal to the ORF1ab oligonucleotides. Overall, in silico testing confirmed that the ORF1ab primers and probes will bind to and amplify all available SARS-CoV-2 partial and complete genomes published by GISAID.

N Assay

For the N primer and probe set, there were 584 strains (out of 63424) that exhibited mismatches (0.9%). Of the 584 strains, all had a mismatch in only one sequence (primer or probe). Annealing temperature calculations indicate that these mismatches do not significantly impact the annealing temperature and therefore, all 584 strains are predicted to anneal to the ORF1ab oligonucleotides. Overall, *in silico* testing confirmed that the N primers and probes will bind to and amplify all available SARS-CoV-2 partial and complete genomes published by GISAID.

3. Cross Reactivity – Analytical Specificity

In silico

To evaluate the analytical specificity (cross-reactivity) of the EasyNAT Kit, *in silico* analysis of RefSeq genomes of other common respiratory viral, bacterial and yeast pathogens in the table below were performed. Each ORF1ab and N gene primers and probes were compared against all available genome sequences. Analyzed organisms are listed in **Tables 4** and **5**.

Table 4: *in silico* analysis ORF1ab Primer and Probe Sequences

Pathogen	Strain	GenBank Acc#	% Homology Test WHCoV-2K-1-FB	% Homology Test WHCoV-2K-1-RB	% Homology Test WHCoV-2K-1-CPF	% Homology Test WHCoV-2K-1-CPR	% Homology Test WHCoV-2K-1-IP	% Homology Test WHCoV-2K-1-Amp2
Human coronavirus 229E	HCoV-229E/USA/UAMS_DID_0108/2017	MT438700.1	68.42%	45.45%	59.09%	40.00%	45.45%	60.00%
Human coronavirus 229E	HCoV-229E/USA/ACRI_0246/2017	MT438699.1	68.42%	45.45%	45.45%	40.00%	45.45%	60.00%
Human coronavirus OC43	HCoV_OC43/Seattle/USA/SC0776/2019	MN310478.1	52.63%	45.45%	40.91%	50.00%	50.00%	50.00%
Human coronavirus OC43	HCoV_OC43/Seattle/USA/SC9428/2018	MN310476.1	52.63%	45.45%	40.91%	50.00%	50.00%	50.00%
Human coronavirus HKU1	SI17244	MH940245.1	47.37%	50.00%	40.91%	45.00%	40.91%	50.00%
Human coronavirus HKU1	SC2521	MK167038.1	47.37%	45.45%	40.91%	45.00%	40.91%	50.00%
Human coronavirus NL63	ChinaGD03	MK334044.1	52.63%	45.45%	40.91%	45.00%	50.00%	55.00%
Human coronavirus NL63	HCoV_NL63/Seattle/USA/SC0768/2019	MN306040.1	47.37%	45.45%	40.91%	45.00%	50.00%	55.00%
MERS	NL140422	MG021452.1	63.16%	50.00%	40.91%	45.00%	45.45%	45.00%
MERS	Bat-CoV/P.khulii/Italy/206645-63/2011	MG596803.1	63.16%	45.45%	40.91%	70.00%	54.55%	50.00%
Adenovirus 71	human/DEU/HEIM_00085/1987/71[P9H20F71]	KF268207.1	47.37%	45.45%	36.36%	40.00%	40.91%	55.00%
Human Metapneumovirus (hMPV)	HMPV/02/KEN/2012	MK588637.1	52.63%	40.91%	40.91%	40.00%	40.91%	55.00%
Human Metapneumovirus (hMPV)	HMPV/05/ZAM/2012	MK588635.1	52.63%	40.91%	54.55%	40.00%	50.00%	45.00%
Parainfluenza virus 4a	HPIV4a/Seattle/USA/SC9717/2018	MN369047.1	47.37%	40.91%	40.91%	45.00%	45.45%	45.00%
Parainfluenza virus 4a	HPIV4a/Seattle/USA/SC9537/2019	MN306056.1	47.37%	40.91%	40.91%	45.00%	40.91%	45.00%
Parainfluenza virus 4b	HPIV4b/Seattle/USA/SC9597/2019	MN306058.1	47.37%	50.00%	45.45%	45.00%	45.45%	45.00%
Parainfluenza virus 4b	HPIV4b/Seattle/USA/SC0496/2019	MN306032.1	47.37%	50.00%	40.91%	45.00%	45.45%	45.00%
Enterovirus D68	USA/2018-23272	MT081368.1	63.16%	40.91%	0.00%	45.00%	40.91%	45.00%
Enterovirus D68	USA/SC/2016-23288	MN259117.1	63.16%	45.45%	40.91%	40.00%	40.91%	45.00%
Respiratory syncytial virus	Respiratory syncytial virus, complete genome	U39661.1	57.89%	59.09%	59.09%	50.00%	45.45%	50.00%
Respiratory syncytial virus	RSV Memphis-37, complete genome	KM360090.1	52.63%	59.09%	59.09%	45.00%	45.45%	50.00%

Pathogen	Strain	GenBank Acc#	% Homology Test WHCoV-2K-1-FB	% Homology Test WHCoV-2K-1-RB	% Homology Test WHCoV-2K-1-CPF	% Homology Test WHCoV-2K-1-CPR	% Homology Test WHCoV-2K-1-IP	% Homology Test WHCoV-2K-1-Amp2
Chlamydia pneumoniae	assembly YK41, chromosome : 1	LN849040.1	47.37%	54.55%	59.09%	50.00%	50.00%	50.00%
Chlamydia pneumoniae	assembly Wien3, chromosome : 1	LN847257.1	63.16%	54.55%	59.09%	60.00%	54.55%	55.00%
Haemophilus influenzae	strain NCTC12699 genome assembly, chromosome: 1	LR134171.1	63.16%	63.64%	59.09%	60.00%	59.09%	60.00%
Haemophilus influenzae	strain P676-2514 chromosome, complete genome	CP031679.1	63.16%	63.64%	59.09%	55.00%	59.09%	60.00%
Legionella pneumophila	strain AUSMDU00010536 isolate SBT211 chromosome, complete genome	CP045974.1	68.42%	63.64%	77.27%	65.00%	59.09%	65.00%
Legionella pneumophila	strain FDAARGOS_779 chromosome, complete genome	CP040987.1	68.42%	63.64%	77.27%	60.00%	59.09%	65.00%
Mycobacterium tuberculosis	strain FDAARGOS_756 chromosome, complete genome	CP054014.1	63.16%	0.00%	59.09%	55.00%	0.00%	55.00%
Mycobacterium tuberculosis	strain 4860 chromosome, complete genome	CP053092.1	63.16%	0.00%	59.09%	55.00%	0.00%	55.00%
Streptococcus pneumoniae	strain 6A-10 chromosome, complete genome	CP053210.1	73.68%	59.09%	54.55%	70.00%	63.64%	60.00%
Streptococcus pneumoniae	strain PZ900701590 chromosome, complete genome	CP050175.1	73.68%	59.09%	54.55%	70.00%	63.64%	60.00%
Streptococcus pyogenes	strain 4063-05 chromosome, complete genome	CP051138.1	68.42%	59.09%	63.64%	60.00%	72.73%	65.00%
Streptococcus pyogenes	strain MGAS2221 chromosome, complete genome	CP043530.1	68.42%	59.09%	63.64%	60.00%	72.73%	70.00%
Bordetella pertussis	No significant similarity found.	None	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Mycoplasma pneumoniae	strain 16-734 chromosome, complete genome	CP039761.1	63.16%	54.55%	50.00%	65.00%	68.18%	65.00%
Mycoplasma pneumoniae	strain 16-710 chromosome, complete genome	CP039762.1	63.16%	54.55%	50.00%	65.00%	68.18%	65.00%
Pneumocystis jirovecii	SW7_full mitochondrion, complete genome	MH010446.1	63.16%	50.00%	40.91%	55.00%	45.45%	45.00%
Pneumocystis jirovecii	SW1_full mitochondrion, complete genome	MH010444.1	63.16%	50.00%	40.91%	55.00%	45.45%	45.00%
Escherichia coli	strain EcPF5 chromosome, complete genome	CP054236.1	78.95%	59.09%	59.09%	0.00%	59.09%	65.00%
Escherichia coli	strain EcPF7 chromosome, complete genome	CP054232.1	78.95%	59.09%	59.09%	0.00%	59.09%	0.00%
Acinetobacter baumannii	strain FDAARGOS_533 chromosome, complete genome	CP033768.1	73.68%	63.64%	63.64%	65.00%	59.09%	65.00%
Acinetobacter baumannii	strain AB042, complete genome	CP019034.1	73.68%	63.64%	63.64%	80.00%	63.64%	80.00%

Pathogen	Strain	GenBank Acc#	% Homology Test WHCoV-2K-1-FB	% Homology Test WHCoV-2K-1-RB	% Homology Test WHCoV-2K-1-CPF	% Homology Test WHCoV-2K-1-CPR	% Homology Test WHCoV-2K-1-IP	% Homology Test WHCoV-2K-1-Amp2
Streptococcus salivarius	strain NCTC7366 genome assembly, chromosome: 1	LS483366.1	73.68%	59.09%	59.09%	65.00%	54.55%	65.00%
Streptococcus salivarius	strain FDAARGOS_771 chromosome, complete genome	CP053998.1	63.16%	59.09%	59.09%	65.00%	54.55%	60.00%
Klebsiella pneumoniae	strain MS14393 chromosome, complete genome	CP054303.1	68.42%	54.55%	59.09%	75.00%	54.55%	60.00%
Klebsiella pneumoniae	strain WSHvKP chromosome, complete genome	CP054063.1	68.42%	54.55%	59.09%	75.00%	54.55%	60.00%
Influenza C virus	(C/Yamagata/10/89) P3 gene for polymerase 3, complete cds	LC123514.1	52.63%	40.91%	36.36%	40.00%	31.82%	40.00%
Influenza C virus	(C/Seoul/APD462/2015) segment 3 polymerase P3 (P3) gene, complete cds	MK050102.1	47.37%	45.45%	40.91%	40.00%	31.82%	40.00%
Parechovirus	Human parechovirus 5, complete genome	KY067444.1	57.89%	40.91%	40.91%	45.00%	40.91%	55.00%
Parechovirus	Human parechovirus 1 strain BJ-37359, complete genome	KJ659491.1	57.89%	40.91%	40.91%	45.00%	36.36%	45.00%
Candida albicans	strain TIMM 1768 chromosome 2	CP032013.1	78.95%	59.09%	68.18%	50.00%	54.55%	70.00%
Candida albicans	strain SC5314-P0 chromosome 2B	CP025159.1	78.95%	59.09%	68.18%	60.00%	54.55%	70.00%
Corynebacterium diphtheriae	strain TH1526 chromosome, complete genome	CP038504.1	57.89%	59.09%	54.55%	60.00%	59.09%	65.00%
Corynebacterium diphtheriae	Corynebacterium diphtheriae gravis NCTC13129, complete genome; segment 6/8	BX248359.1	57.89%	45.45%	50.00%	60.00%	59.09%	50.00%
Bacillus anthracis	strain FDAARGOS_704 chromosome, complete genome	CP047111.1	63.16%	63.64%	72.73%	65.00%	63.64%	70.00%
Bacillus anthracis	strain FDAARGOS_705 chromosome, complete genome	CP047107.1	63.16%	63.64%	59.09%	65.00%	63.64%	70.00%
Moraxella catarrhalis	strain 46P58B1 chromosome, complete genome	CP034662.1	73.68%	59.09%	50.00%	60.00%	59.09%	60.00%
Moraxella catarrhalis	strain 5P47B2 chromosome, complete genome	CP034666.1	73.68%	59.09%	50.00%	60.00%	54.55%	60.00%
Neisseria elongata	strain M15910 chromosome, complete genome	CP031255.1	63.16%	54.55%	54.55%	60.00%	59.09%	55.00%
Neisseria elongata	strain M15911 chromosome, complete genome	CP031252.1	63.16%	54.55%	50.00%	55.00%	63.64%	55.00%
Neisseria meningitidis	strain M24705, complete genome	CP016682.1	68.42%	54.55%	63.64%	60.00%	54.55%	55.00%
Neisseria meningitidis	strain M22822, complete genome	CP016680.1	68.42%	54.55%	50.00%	60.00%	54.55%	55.00%
Pseudomonas aeruginosa	strain PA59 chromosome, complete genome	CP024630.1	63.16%	54.55%	54.55%	0.00%	0.00%	0.00%

Pathogen	Strain	GenBank Acc#	% Homology Test WHCoV-2K-1-FB	% Homology Test WHCoV-2K-1-RB	% Homology Test WHCoV-2K-1-CPF	% Homology Test WHCoV-2K-1-CPR	% Homology Test WHCoV-2K-1-IP	% Homology Test WHCoV-2K-1-Amp2
Pseudomonas aeruginosa	strain Y31 chromosome, complete genome	CP030910.1	63.16%	54.55%	54.55%	75.00%	68.18%	0.00%
Staphylococcus epidermidis	strain NCCP 16828 chromosome, complete genome	CP043847.1	73.68%	59.09%	63.64%	70.00%	63.64%	75.00%
Staphylococcus epidermidis	strain O47 chromosome, complete genome	CP040883.1	68.42%	72.73%	63.64%	60.00%	63.64%	75.00%
Leptospira	strain R12 chromosome 1	CP047508.1	63.16%	72.73%	54.55%	60.00%	63.64%	70.00%
Leptospira	strain R19 chromosome 1	CP047514.1	63.16%	72.73%	54.55%	60.00%	63.64%	70.00%
Chlamydia psittaci	strain AMK chromosome, complete genome	CP047319.1	68.42%	72.73%	54.55%	55.00%	54.55%	60.00%
Chlamydia psittaci	strain Rostinovo-70 chromosome, complete genome	CP041038.1	68.42%	72.73%	54.55%	55.00%	60.00%	60.00%
Coxiella burnetii	strain RSA439 chromosome, complete genome	CP040059.1	73.68%	63.64%	54.55%	60.00%	50.00%	65.00%
Coxiella burnetii	strain nine mile phase II chromosome, complete genome	CP035112.1	73.68%	63.64%	54.55%	60.00%	50.00%	65.00%
Influenza A H3N2	Influenza A virus H3N2 A/Fukushima/114/96 nucleoprotein (NP) gene, complete cds	AF038257.1	42.11%	31.82%	31.82%	40.00%	36.37%	35.00%
Influenza A H3N2	Influenza A virus H3N2 A/Niigata/137/96 nucleoprotein (NP) gene, complete cds	AF038256.1	42.11%	31.82%	31.82%	40.00%	36.36%	35.00%
Influenza B Yamagata	Influenza B virus (B/Yamagata/16/1988) segment 8, complete sequence	CY018769.1	42.11%	31.82%	40.91%	40.00%	31.82%	40.00%
Influenza B Yamagata	Influenza B virus (B/Yamagata/16/1988) segment 2, complete sequence	CY018772.1	36.84%	31.82%	50.00%	40.00%	36.36%	40.00%

With the exception of *Acinetobacter baumannii* for WHCoV-ORF1ab-Amp2 and WHCoV-ORF1ab-CPR, none of the pathogen sequences displayed greater than 80% homology with the ORF1ab gene primers and probes sequences.

Table 5: in silico Analysis N primer and Probe Sequences

Pathogen	Strain	GenBank Acc#	% Homology Test WHCoV-N-6-FB	% Homology Test WHCoV-N-6-RB	% Homology Test WHCoV-N-6-CPF	% Homology Test WHCoV-N-6-CPR	% Homology Test WHCoV-N-6-IP	% Homology Test WHCoV-N-6-Amp2
Human coronavirus 229E	strain HCoV_229E/Seattle/USA/SC9724/2018, complete genome	MN369046.1	69.57%	55.56%	50.00%	50.00%	40.00%	61.90%
Human coronavirus 229E	strain HCoV_229E/Seattle/USA/SC0865/2019, complete genome	MN306046.1	69.57%	55.56%	50.00%	50.00%	40.00%	61.90%
Human coronavirus OC43	strain WZ-522, complete genome	MG197717.1	47.83%	55.56%	50.00%	65.00%	45.00%	42.86%
Human coronavirus OC43	strain HCoV_OC43/Seattle/USA/SC0776/2019, complete genome	MN310478.1	43.48%	55.56%	50.00%	65.00%	45.00%	42.86%
Human coronavirus HKU1	strain N09-1605B, complete genome	KY674943.1	43.48%	44.44%	50.00%	50.00%	50.00%	47.62%

Pathogen	Strain	GenBank Acc#	% Homology Test WHCoV-N-6-FB	% Homology Test WHCoV-N-6-RB	% Homology Test WHCoV-N-6-CPF	% Homology Test WHCoV-N-6-CPR	% Homology Test WHCoV-N-6-IP	% Homology Test WHCoV-N-6-Amp2
Human coronavirus HKU1	strain N09-1627B, complete genome	KY674942.1	43.48%	44.44%	50.00%	50.00%	50.00%	47.62%
Human coronavirus NL63	strain HCoV_NL63/Seattle/USA/SC0768/2019, complete genome	MN306040.1	43.48%	44.44%	50.00%	70.00%	40.00%	47.62%
Human coronavirus NL63	strain ChinaGD04, complete genome	MK334047.1	43.48%	50.00%	50.00%	70.00%	40.00%	52.38%
MERS-coronavirus	Middle East respiratory syndrome-related coronavirus strain Hu/Riyadh-KSA-19003852/2019, complete genome	MN365233.1	47.83%	55.56%	65.00%	45.00%	45.00%	42.86%
MERS-coronavirus	Middle East respiratory syndrome-related coronavirus strain Hu/Albaha-KSA-0800H/2018, complete genome	MK483839.1	47.83%	55.56%	65.00%	45.00%	45.00%	42.86%
Adenovirus (e.g. C1 Ad. 71)	Human adenovirus 7 strain HAdV-B/USA_WI/5539/2018/P7H7F7, complete genome	MN307161.1	43.48%	55.56%	50.00%	45.00%	50.00%	47.62%
Adenovirus (e.g. C1 Ad. 71)	Human adenovirus 7 strain 163, complete genome	MN135993.1	43.48%	55.56%	50.00%	45.00%	50.00%	47.62%
Human Metapneumovirus (hMPV)	strain BJ-1610, complete genome	KU821121.1	39.13%	55.56%	50.00%	55.00%	45.00%	0.00%
Human Metapneumovirus (hMPV)	strain HMPV/AUS/144834728/2003/A	KC562241.1	43.48%	55.56%	50.00%	55.00%	45.00%	0.00%
Human Parainfluenza virus 1 (C39)	No significant similarity found.	None	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Human parainfluenza virus 2	No significant similarity found.	None	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Human parainfluenza virus 4a	strain HPIV4a/Seattle/USA/SC9717/2018, complete genome	MN369047.1	43.48%	44.44%	40.00%	55.00%	40.00%	47.62%
Human parainfluenza virus 4a	strain HPIV4a/Seattle/USA/SC9971/2018, complete genome	MN369032.1	43.48%	44.44%	40.00%	55.00%	40.00%	47.62%
Human parainfluenza virus 4b	strain HPIV4b/Seattle/USA/SC9597/2019, complete genome	MN306058.1	43.48%	55.56%	50.00%	65.00%	45.00%	42.86%
Human parainfluenza virus 4b	strain HPIV4b/Seattle/USA/SC0496/2019, complete genome	MN306032.1	43.48%	55.56%	50.00%	50.00%	60.00%	42.86%
Enterovirus (e.g. EV68)	strain USA/AK/2008-23112, complete genome	MN240494.1	43.48%	61.11%	45.00%	40.00%	50.00%	38.10%
Enterovirus (e.g. EV68)	strain EVD68/Homo sapiens/USA/MO60/2014, complete genome	KT347261.1	43.48%	61.11%	45.00%	45.00%	40.00%	38.10%
Respiratory syncytial virus	strain B/WI/629-Q0190/10, complete genome	JN032120.1	43.48%	0.00%	50.00%	50.00%	50.00%	42.86%
Respiratory syncytial virus	strain B/WI/629-DC1/08-09, complete genome	JN032119.1	43.48%	0.00%	50.00%	50.00%	50.00%	42.86%
Chlamydia pneumonia	assembly YK41, chromosome : 1	LN849039.1	47.83%	66.67%	60.00%	65.00%	60.00%	57.14%
Chlamydia pneumonia	assembly Wien3, chromosome : 1	LN847257.1	47.83%	66.67%	60.00%	65.00%	60.00%	57.14%

Pathogen	Strain	GenBank Acc#	% Homology Test WHCoV-N-6-FB	% Homology Test WHCoV-N-6-RB	% Homology Test WHCoV-N-6-CPF	% Homology Test WHCoV-N-6-CPR	% Homology Test WHCoV-N-6-IP	% Homology Test WHCoV-N-6-Amp2
Haemophilus influenzae	strain P662-7189 chromosome, complete genome	CP031683.1	60.87%	61.11%	70.00%	60.00%	60.00%	66.67%
Haemophilus influenzae	strain P641-4342 chromosome, complete genome	CP031687.1	60.87%	61.11%	65.00%	60.00%	60.00%	66.67%
Legionella pneumophila	strain ERS1305867 chromosome, complete genome	CP048618.1	69.57%	66.67%	75.00%	70.00%	70.00%	66.67%
Legionella pneumophila	strain D-4040 chromosome, complete genome	CP021274.1	69.57%	66.67%	85.00%	70.00%	65.00%	66.67%
Mycobacterium tuberculosis	strain FDAARGOS_756 chromosome, complete genome	CP054014.1	56.52%	72.22%	55.00%	60.00%	55.00%	61.90%
Mycobacterium tuberculosis	strain 4860 chromosome, complete genome	CP053092.1	56.52%	72.22%	55.00%	60.00%	55.00%	61.90%
Streptococus pneumoniae	strain SWU02, complete genome	CP018347.1	60.87%	66.67%	65.00%	60.00%	80.00%	66.67%
Streptococus pneumoniae	strain SP64, complete genome	CP018138.1	60.87%	66.67%	65.00%	75.00%	80.00%	66.67%
Streptococcus pyrogenes	strain STAB10048 chromosome, complete genome	CP036531.1	69.57%	72.22%	65.00%	60.00%	70.00%	61.90%
Streptococcus pyrogenes	strain AUSMDU00010539 chromosome, complete genome	CP045930.1	69.57%	72.22%	65.00%	60.00%	70.00%	61.90%
Bordetella pertussis	strain J029 chromosome, complete genome	CP046995.1	0.00%	72.22%	0.00%	0.00%	0.00%	61.90%
Bordetella pertussis	strain A639 chromosome, complete genome	CP046993.1	0.00%	72.22%	0.00%	0.00%	0.00%	61.90%
Mycoplasma pneumoniae	strain 16-734 chromosome, complete genome	CP039761.1	73.91%	61.11%	65.00%	55.00%	65.00%	61.90%
Mycoplasma pneumoniae	strain 16-710 chromosome, complete genome	CP039762.1	73.91%	61.11%	65.00%	55.00%	65.00%	61.90%
Pneumocystis jirovecii (PJP)	isolate SW7_full mitochondrion, complete genome	MH010446.1	43.48%	44.44%	45.00%	50.00%	0.00%	47.62%
Pneumocystis jirovecii (PJP)	isolate SW1_full mitochondrion, complete genome	MH010444.1	43.48%	44.44%	45.00%	50.00%	0.00%	47.62%
Influenza C	virus (C/Yamagata/3/2005) M1, CM2 genes for matrix protein, CM2 protein, complete cds	LC123842.1	47.83%	0.00%	0.00%	40.00%	40.00%	52.38%
Influenza C	virus (C/Yamagata/1/2005) M1, CM2 genes for matrix protein, CM2 protein, complete cds	LC123840.1	47.83%	0.00%	0.00%	40.00%	40.00%	52.38%
Parechovirus	Human parechovirus 4 strain VEN/2018-23123B, complete genome	MK652145.1	47.83%	44.44%	45.00%	50.00%	45.00%	52.38%
Parechovirus	Human parechovirus 1 strain VEN/2018-23121B, complete genome	MK652142.1	34.78%	44.44%	45.00%	70.00%	45.00%	42.86%
Candida albicans	strain SC5314-P0 chromosome 4B	CP025161.1	69.57%	55.56%	60.00%	60.00%	70.00%	66.67%

Pathogen	Strain	GenBank Acc#	% Homology Test WHCoV-N-6-FB	% Homology Test WHCoV-N-6-RB	% Homology Test WHCoV-N-6-CPF	% Homology Test WHCoV-N-6-CPR	% Homology Test WHCoV-N-6-IP	% Homology Test WHCoV-N-6-Amp2
Candida albicans	strain SC5314-P0 chromosome 7A	CP025156.1	69.57%	61.11%	70.00%	70.00%	60.00%	61.90%
Corynebacterium diphtheriae	strain TH1526 chromosome, complete genome	CP038504.1	65.22%	72.22%	65.00%	60.00%	55.00%	61.90%
Corynebacterium diphtheriae	strain BQ11 chromosome, complete genome	CP029644.1	65.22%	66.67%	65.00%	60.00%	55.00%	61.90%
Legionella pneumophila	strain ERS1305867 chromosome, complete genome	CP048618.1	69.57%	66.67%	75.00%	75.00%	70.00%	66.67%
Legionella pneumophila	strain D-4040 chromosome, complete genome	CP021274.1	69.57%	66.67%	85.00%	75.00%	65.00%	66.67%
Bacillus anthracis	strain FDAARGOS_704 chromosome, complete genome	CP047111.1	78.26%	61.11%	65.00%	70.00%	75.00%	66.67%
Bacillus anthracis	strain FDAARGOS_705 chromosome, complete genome	CP047107.1	78.26%	61.11%	65.00%	70.00%	75.00%	66.67%
Moraxella catarrhalis	strain 46P58B1 chromosome, complete genome	CP034662.1	56.52%	66.67%	80.00%	65.00%	65.00%	57.14%
Moraxella catarrhalis	strain 5P47B2 chromosome, complete genome	CP034666.1	56.52%	66.67%	80.00%	65.00%	65.00%	57.14%
Neisseria elongate	strain M15910 chromosome, complete genome	CP031255.1	56.52%	61.11%	65.00%	65.00%	60.00%	61.90%
Neisseria elongate	strain M15911 chromosome, complete genome	CP031252.1	56.52%	61.11%	65.00%	65.00%	60.00%	61.90%
Neisseria miningitidis	strain AUSMDU00005726 chromosome, complete genome	CP045960.1	60.87%	55.56%	60.00%	60.00%	60.00%	61.90%
Neisseria miningitidis	strain 95-134 chromosome, complete genome	CP021725.1	60.87%	55.56%	60.00%	60.00%	60.00%	61.90%
Pseudomonas aeruginosa	strain AR441 chromosome, complete genome	CP029093.1	52.17%	66.67%	70.00%	65.00%	0.00%	66.67%
Pseudomonas aeruginosa	strain AR_0356 chromosome, complete genome	CP027169.1	52.17%	66.67%	70.00%	65.00%	0.00%	66.67%
Staphylococcus epidermis	strain FDAARGOS_529 chromosome, complete genome	CP033782.1	65.22%	66.67%	65.00%	65.00%	65.00%	57.14%
Staphylococcus epidermis	strain ATCC 12228, complete genome	CP022247.1	65.22%	66.67%	65.00%	65.00%	65.00%	66.67%
Leptospira	strain R12 chromosome 1	CP047508.1	56.52%	61.11%	65.00%	70.00%	75.00%	76.19%
Leptospira	strain R19 chromosome 1	CP047514.1	56.52%	61.11%	65.00%	70.00%	75.00%	76.19%
Chlamydia psittaci	strain AMK chromosome, complete genome	CP047319.1	56.52%	66.67%	65.00%	65.00%	60.00%	61.90%
Chlamydia psittaci	strain Rostinovo-70 chromosome, complete genome	CP041038.1	56.52%	66.67%	65.00%	65.00%	60.00%	61.90%
Coxiella burneti	strain RSA439 chromosome, complete genome	CP040059.1	60.87%	72.22%	75.00%	65.00%	70.00%	61.90%
Coxiella burneti	strain nine mile phase II chromosome, complete genome	CP035112.1	60.87%	72.22%	75.00%	65.00%	70.00%	61.90%

Pathogen	Strain	GenBank Acc#	% Homology Test WHCoV-N-6-FB	% Homology Test WHCoV-N-6-RB	% Homology Test WHCoV-N-6-CPF	% Homology Test WHCoV-N-6-CPR	% Homology Test WHCoV-N-6-IP	% Homology Test WHCoV-N-6-Amp2
Coxiella burnetii Q321	No significant similarity found.		0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Escherichia coli	strain EcPF15 chromosome, complete genome	CP054227.1	60.87%	72.22%	0.00%	0.00%	65.00%	71.43%
Escherichia coli	strain SCU-321 chromosome, complete genome	CP055158.1	60.87%	72.22%	0.00%	70.00%	65.00%	71.43%
Acinetobacter baumannii	strain VB473 chromosome, complete genome	CP050388.1	60.87%	66.67%	65.00%	75.00%	60.00%	66.67%
Acinetobacter baumannii	strain VB11737 chromosome, complete genome	CP050400.1	60.87%	66.67%	65.00%	75.00%	65.00%	66.67%
Klebsiella pneumoniae	strain D17KP0018 chromosome, complete genome	CP052336.1	69.57%	77.78%	70.00%	65.00%	65.00%	76.19%
Klebsiella pneumoniae	strain KPN41053 chromosome, complete genome	CP052036.1	69.57%	77.78%	60.00%	65.00%	65.00%	76.19%
Influenza A H3N2	Influenza A virus H3N2 A/Shiga/25/97 PB1 polymerase subunit (PB1) gene, complete cds	AF037423.1	34.78%	44.44%	40.00%	45.00%	40.00%	38.10%
Influenza A H3N2	Influenza A virus H3N2 A/Akita/1/95 PB1 polymerase subunit (PB1) gene, complete cds	U71129.1	34.78%	44.44%	40.00%	45.00%	40.00%	38.10%
Influenza B Yamagata	Influenza B virus (B/Yamagata/16/1988) segment 3, complete sequence	CY018770.1	34.78%	44.44%	40.00%	45.00%	55.00%	33.33%
Influenza B Yamagata	Influenza B virus (B/Yamagata/16/1988) segment 1, complete sequence	CY018771.1	34.78%	44.44%	55.00%	40.00%	40.00%	42.86%

With the exception of *streptococcus pneumoniae* for WHCoV-B-6-IP, none of the pathogen sequences displayed greater than 80% homology with the N gene primers and probes sequences.

The *in silico* analysis for the ORF1ab and N demonstrated < 80% homology with all organisms except for the exceptions stated above.

Wet Testing

Cross-reactivity and potential interference of the EasyNAT Kit was evaluated by testing various microorganism and other respiratory infection pathogens and negative oropharyngeal swab matrix with the EasyNAT Kit. Each microorganism and pathogen were tested in triplicate (3) across three (3) EasyNAT Kit lots. The results are summarized in **Table 6**. None of the tested pathogens were reactive.

Table 6: EasyNAT Kit Wet Testing Cross Reactivity Results

Virus/Bacteria/Parasite	Strain	Source	Sample type	Concentration	Result (3/3 Replicates)
Mycoplasma pneumoniae	M129	Institute of pathogenic biology, Chinese Academy of Medical Sciences	Clinical sample	10^6 bacteria/mL	Negative
Chlamydia pneumoniae	CWL029		Clinical sample	10^6 bacteria/mL	Negative
Legionella	Legionella pneumophila subsp. pneumophila str. Philadelphia 1		Clinical sample	10^6 bacteria/mL	Negative
Bordetella pertussis	Tohama I		Culture	10^6 bacteria/mL	Negative
Haemophilus influenzae	Rd KW20		Culture	10^6 bacteria/mL	Negative

Virus/Bacteria/Parasite	Strain	Source	Sample type	Concentration	Result (3/3 Replicates)
Staphylococcus aureus	NCTC 8325		Culture	10^6 bacteria/mL	Negative
Streptococcus pneumonia	R6		Culture	10^6 bacteria/mL	Negative
Streptococcus pyrogenes	Streptococcus pyogenes M1 GAS		Culture	10^6 bacteria/mL	Negative
Streptococcus salivarius	NCTC 8618		Clinical sample	10^6 bacteria/mL	Negative
klebsiella pneumoniae	subsp. pneumoniae HS11286		Culture	10^6 bacteria/mL	Negative
Mycobacterium tuberculosis	H37Rv		Culture	10^6 bacteria/mL	Negative
Aspergillus fumigatus	Af293		Culture	10^6 bacteria/mL	Negative
Candida albicans	SC5314		Culture	10^6 bacteria/mL	Negative
Candida glabrata	CBS 138		Culture	10^6 bacteria/mL	Negative
Cryptococcus neoformans	Cryptococcus neoformans var. grubii H99		Culture	10^6 bacteria/mL	Negative
Pneumocystis jirovecii (PJP)	Pneumocystis jirovecii RU7		Clinical sample	10^6 bacteria/mL	Negative
Human coronavirus HKU1	Human coronavirus HKU1		Culture	10^4 copies/mL	Negative
Human coronavirus OC43	OC43		Culture	10^4 copies/mL	Negative
Human coronavirus NL63	NL63		Culture	10^4 copies/mL	Negative
Human coronavirus 229E	229E		Culture	10^4 copies/mL	Negative
SARS coronavirus	Tor2		Pseudovirus	10^4 copies/mL	Negative
MERS coronavirus	2c EMC/2012		Pseudovirus	10^4 copies/mL	Negative
New influenza A (H1N1) virus (2009)	H1N1		Culture	10^4 copies/mL	Negative
Influenza B Victoria	Rd KW20		Culture	10^4 copies/mL	Negative
Influenza B Yamagata	Rd KW20		Culture	10^4 copies/mL	Negative
Respiratory syncytial virus A	S2 ts1C		Culture	10^4 copies/mL	Negative
Respiratory syncytial virus B	CBSH-349		Culture	10^4 copies/mL	Negative
Parainfluenza virus Type 1	Washington 1964		Clinical sample	10^4 copies/mL	Negative
Parainfluenza virus Type 2	Toshiba		Clinical sample	10^4 copies/mL	Negative
Parainfluenza virus Type 3	JSHA2016		Clinical sample	10^4 copies/mL	Negative
Parainfluenza virus Type 4	M-25		Clinical sample	10^4 copies/mL	Negative
Rhinovirus A	HRV-A20		Clinical sample	10^4 copies/mL	Negative
Rhinovirus B	RV-B/Homo sapiens/USA/SSENT34/2014		Clinical sample	10^4 copies/mL	Negative
Rhinovirus C	NIV1722289		Clinical sample	10^4 copies/mL	Negative
Adenovirus 2	CAU257/AdV/KOR/2016		Clinical sample	10^4 copies/mL	Negative
Adenovirus 3	HLJ0955		Clinical sample	10^4 copies/mL	Negative
Adenovirus 4	NHRC-36401		Clinical sample	10^4 copies/mL	Negative
Adenovirus 5	NHRC Ad5FS 7151		Clinical sample	10^4 copies/mL	Negative
Adenovirus 7	HAdV-B/USA_WI/5539/2018/P7H7F7		Clinical sample	10^4 copies/mL	Negative
Adenovirus type55	TY26		Clinical sample	10^4 copies/mL	Negative
Enterovirus A	10-2879-1		Clinical sample	10^4 copies/mL	Negative

Virus/Bacteria/Parasite	Strain	Source	Sample type	Concentration	Result (3/3 Replicates)
Enterovirus B	BU290a		Clinical sample	10^4 copies/mL	Negative
Enterovirus C	03-0100		Clinical sample	10^4 copies/mL	Negative
Enterovirus D	EV-D68/Homo sapiens/USA/SSENT14/2014		Clinical sample	10^4 copies/mL	Negative
Human lung virus	Not Applicable		Clinical sample	10^4 copies/mL	Negative
Human metapneumovirus	TN982-42		Clinical sample	10^4 copies/mL	Negative
EB virus	HNNPC8		Culture	10^4 copies/mL	Negative
Measles virus	Ichinose-B95a		Culture	10^4 copies/mL	Negative
Human cytomegalovirus	Towne		Clinical sample	10^4 copies/mL	Negative
Rotavirus	RVB/Pig-wt/USA/KS2/2012/NSP1 or RVA/Cow-tc/VEN/BRV033/1990/G6P6		Clinical sample	10^4 copies/mL	Negative
Norovirus	Hu/GII.P21-GII.3/RUS/Novosibirsk/NS16-C32/2016		Clinical sample	10^4 copies/mL	Negative
Mumps virus	Miyahara		Clinical sample	10^4 copies/mL	Negative
Varicella-zoster virus	Dumas		Clinical sample	10^4 copies/mL	Negative
Adenovirus 1	SH2016		Culture	10^4 copies/mL	Negative
Human genome DNA	Not Applicable		Clinical sample	10^4copies/test	Negative
Escherichia coli	K-12		Culture	10^6 bacteria/mL	Negative
Acinetobacter baumannii	AB30		Culture	10^6 bacteria/mL	Negative
Pseudomonas aeruginosa	PAO1		Culture	10^6 bacteria/mL	Negative
Seasonal H1N1 influenza virus	H1N1		Culture	10^4 copies/mL	Negative
H3N2	H3N2		Culture	10^4 copies/mL	Negative
H5N1	H5N1		Culture	10^4 copies/mL	Negative
H7N9	(A/Shanghai/02/2013(H7N9))		Culture	10^4 copies/mL	Negative
Staphylococcus epidermis	ATCC 12228		Clinical sample	10^6 bacteria/mL	Negative

4. Exogenous and Endogenous Interfering Substances

Potential endogenous (blood and mucin) and common exogenous substances that may be found in respiratory specimens were tested by spiking the potential interfering substances into simulated COVID-19 oropharyngeal swab specimens and No Template Control (NTC) matrix with the EasyNAT Kit. Each potential interfering substance was tested in triplicate across three (3) EasyNAT Kit Lots. The results are summarized in **Table 7** for known positive COVID-19 samples and **Table 8** for known negative COVID-19 samples.

Table 7: Interfering Substances Results for Simulated COVID-19 Positive Samples

Potential Interfering Substance		Concentration	Results (3/3 Replicates)
			2000 copies/ml (2*LOD) (Sample: nucleic acid extract from positive COVID-19 sample)
Mucin: bovine submaxillary gland, type I-S	mucin	25mg/mL	Positive
	bovine submaxillary gland, type I-S	25mg/mL	Positive

Potential Interfering Substance		Concentration	Results (3/3 Replicates) 2000 copies/ml (2*LOD) (Sample: nucleic acid extract from positive COVID-19 sample)
Blood (human)	Blood (human)	2% (v/v)	Positive
Nasal sprays or drops	Oxymetazoline	0.5mg/ ml	Positive
	sodium chloride	90mg/ml	Positive
	Fluticasone	200ug/ ml	Positive
	Albuterol Sulfate	0.83mg/mL	Positive
Nasal corticosteroids	phenylephrine	5mg/ ml	Positive
	Beclomethasone	0.1mg/mL	Positive
	Dexamethasone	5mg/ ml	Positive
	flunisolide	1mg/ ml	Positive
	triamcinolone acetonide	220ug/ml	Positive
	Budesonide	0.5mg/mL	Positive
Nasal gel	mometasone	0.1mg/mL	Positive
	Interferon alpha	50g/ml	Positive
	Nasal ointment	10 mg/mL	Positive
	Zicam Nasal Gel	15% (v/v)	Positive
Flu Mist	Saline Nasal Spray	15% (v/v)	Positive
	PHNY Nasal Drops	15% (v/v)	Positive
Homeopathic allergy relief medicine	Histamine hydrochloride	13mg/ml	Positive
Throat lozenges, oral anesthetic and analgesic	Benzocaine	1.7mg/mL	Positive
	Menthol	1.7mg/mL	Positive
Anti-viral drugs	ribavirin	6ug/ml	Positive
	Oseltamivir	75mg/ml	Positive
	peramivir	30mg/ml	Positive
	lopinavir	0.36ug/ml	Positive
	Arbidol	0.2g/ml	Positive
Antibiotic, nasal ointment	Levofloxacin	17μg/ ml	Positive
	Azithromycin	0.56mg/L	Positive
	Ceftriaxone sodium	8mg/ml	Positive
	Meropenem	8mg/ml	Positive
Antibacterial, systemic	Ritonavir	0.09ug/ml	Positive
	Tobramycin	15mg/L	Positive
	zanamivir	142ng /ml	Positive

*Taken from the recently-updated 3rd edition of CLSI guideline EP07, Interference Testing in Clinical Chemistry (Section 3.4.2) which now defers to CLSI EP37, Supplemental Tables for Interference Testing in Clinical Chemistry for the recommended concentrations for testing common endogenous substances (Table 2) and have subsequently updated a number of the recommended testing concentrations.

Table 8: Interfering Substances Results for Simulated COVID-19 Negative Samples

Potential Interfering Substance		Concentration	Results (3/3 Replicates) 2000 copies/ml (2*LOD) (Sample: nucleic acid extract from positive COVID-19 sample)
Mucin: bovine submaxillary gland, type I-S	mucin	25mg/mL	Negative
	bovine submaxillary gland, type I-S	25mg/mL	Negative
Blood (human)	Blood (human)	2% (v/v)	Negative
Nasal sprays or drops	Oxymetazoline	0.5mg/ ml	Negative

Potential Interfering Substance		Concentration	Results (3/3 Replicates) 2000 copies/ml (2*LOD) (Sample: nucleic acid extract from positive COVID-19 sample)
	sodium chloride	90mg/ml	Negative
	Fluticasone	200ug/ ml	Negative
	Albuterol Sulfate	0.83mg/mL	Negative
Nasal corticosteroids	phenylephrine	5mg/ ml	Negative
	Beclomethasone	0.1mg/mL	Negative
	Dexamethasone	5mg/ ml	Negative
	flunisolide	1mg/ ml	Negative
	triamcinolone acetonide	220ug/ml	Negative
	Budesonide	0.5mg/mL	Negative
Nasal gel	mometasone	0.1mg/mL	Negative
	Interferon alpha	50g/ml	Negative
	Nasal ointment	10 mg/mL	Negative
	Zicam Nasal Gel	15% (v/v)	Negative
Flu Mist	Saline Nasal Spray	15% (v/v)	Negative
	PHNY Nasal Drops	15% (v/v)	Negative
Homeopathic allergy relief medicine	Histamine hydrochloride	13mg/ml	Negative
Throat lozenges, oral anesthetic and analgesic	Benzocaine	1.7mg/mL	Negative
	Menthol	1.7mg/mL	Negative
Anti-viral drugs	ribavirin	6ug/ml	Negative
	Oseltamivir	75mg/ml	Negative
	peramivir	30mg/ml	Negative
	lopinavir	0.36ug/ml	Negative
	Arbidol	0.2g/ml	Negative
Antibiotic, nasal ointment	Levofloxacin	17μg/ ml	Negative
	Azithromycin	0.56mg/L	Negative
	Ceftriaxone sodium	8mg/ml	Negative
	Meropenem	8mg/ml	Negative
Antibacterial, systemic	Ritonavir	0.09ug/ml	Negative
	Tobramycin	15mg/L	Negative
	zanamivir	142ng /ml	Negative

*Taken from the recently-updated 3rd edition of CLSI guideline EP07, Interference Testing in Clinical Chemistry (Section 3.4.2) which now defers to CLSI EP37, Supplemental Tables for Interference Testing in Clinical Chemistry for the recommended concentrations for testing common endogenous substances (Table 2) and have subsequently updated a number of the recommended testing concentrations

5. Clinical Evaluation

A clinical evaluation of the EasyNAT Kit was performed to demonstrate the positive and negative percent agreements of the EasyNAT Kit test when compared to an EUA authorized RT-PCR test using at least 30 negative and 30 positive nasopharyngeal swab clinical specimens.

Positive Percent Agreement (PPA)

Of the 30 specimen s tested using the EasyNAT Kit, initial results demonstrated PPA of 28/30, or 93.3% agreement, missing the 95% acceptance criteria by one (1) specimen result. In reviewing the raw data from the two (2) discordant samples, there was no identifiable cause for the discordant results. The two (2) discordant samples were retested on the same day by the same

operator. For repeat testing new cartridges were prepared from the initial test samples, a second 500µL of nasopharyngeal swab specimen was transferred to the EasyNAT Kit COVID-19-Cartridges. After repeat testing, the PPA for EasyNAT Kit was 30/30, or 100% agreement, meeting the 95% acceptance criteria for PPA.

Negative Percent Agreement (NPA)

Of the 30 specimens tested using the EasyNAT Kit, initial results demonstrated NPA of 30/30, or 100.0% agreement, meeting the 95% acceptance criteria for NPA.

Combined Agreement

- Combining initial results from 60 specimens tested using the EasyNAT Diagnostic Kit, initial results demonstrated a combined agreement of 58/60, or 96.7% agreement.
- Combining results from repeat testing of positive specimens with negative specimens using the EasyNAT Diagnostic Kit, repeat results demonstrated a combined agreement of 60/60, or 100.0% agreement.

The results are summarized in **Table 9**.

Table 9: EasyNAT Kit Clinical Performance Estimates

EasyNAT Diagnostic Kit	Comparator Result		
	Positive	Negative	Total
Positive	28	0	28
Negative	2*	30	30
Total	30	30	60

*Initial EasyNAT Diagnostic Kit was negative; repeat tests were positive.

Bibliography

1. Xu G, Hu L, Zhong H, etc. Cross priming amplification: mechanism and optimization for isothermal DNA amplification. Sci. Rep. 2012; 2:246.

Manufacturer information

Manufacturer
Ustar Biotechnologies (Hangzhou) Ltd.

Registered address: Rooms 801-808, No.3766 Nanhuan Rd, Binjiang District, Hangzhou, Zhejiang Province, China 310053

Telephone: +86571 88939388 Fax: +86571 88939378

Company website: http://www.bioustar.com/en/

Sale Service: Ustar Biotechnologies (Hangzhou) Ltd.

Technical support: 86-4008707025
Address of manufacturing: Bldg C, Fl 1-2 and Bldg A, Fl 5, No.399 Qiuyi Rd, Changhe Street, Binjiang District, Hangzhou, China 310052

Production license number: Zhejiang Food and Drug Supervisory Production/Production No. 20120044

US Distributor
eHealthcare, Inc
250 East Gish Road
San Jose, CA 95070
Company website: http://www.ehealthcareinc.com
Phone No. 408 487 1700