iStatis

COVID-19 Antigen Test

Single-use lateral flow immunoassay for the detection of nucleocapsid proteins from SARS-CoV-2

REF **90-1114**

For *in vitro* Diagnostic Use Only.

Read this Package Insert completely before using the product. Although the assay is designed to be simple to use, conformance with the test procedure is necessary to ensure accurate results.

INTENDED USE

The **iStatis COVID-19 Antigen Test** is a single use, visually read, lateral-flow *in vitro* qualitative immunoassay intended for the detection of nucleocapsid protein antigen from SARS-CoV-2 in anterior nasal swab specimens collected from individuals 18 years or older who are suspected of COVID-19 by their healthcare provider within the first seven days of symptom onset, or from individuals without symptoms or other epidemiological reasons to suspect COVID-19. The test is intended for serial testing of symptomatic individuals for use at least twice with 48 hours between tests or for serial testing of asymptomatic individuals for use at least three times with 48 hours between tests. iStatis COVID-19 Antigen Test is suitable for near-patient or point-of-care (POC) testing use only.

Results are based on the identification of the SARS-CoV-2 nucleocapsid protein antigen. The antigen is generally detectable in anterior nasal swab specimens during the acute phase of infection. Positive results indicate the presence of nucleocapsid protein, but the clinical correlation with patient history and other diagnostic information is necessary to determine the infection status. Positive results do not preclude bacterial infection or co-infection with other viruses. The detected agent may not be the definite cause of the disease.

Negative results do not preclude SARS-CoV-2 infection and should not be used as a primary source of data for treatment or patient management, including infection control. If an acute infection is suspected, direct testing for SARS-CoV-2 is necessary. A negative result should be followed up with repeat, or serial testing at least twice over three days with at least 48 hours between tests for symptomatic individuals and/or at least three times over five days with at least 48 hours between tests for asymptomatic individuals. A home test may be used for this additional testing.

Results should instead be considered within the context of a patient's recent exposures, history, and the presence of symptoms consistent with COVID-19, and confirmed with a molecular assay, if necessary, for any further decisions.

For serial testing programs, additional confirmatory testing with a molecular test for negative results may be necessary, if there is a high likelihood of SARS-CoV-2 infection, such as an individual with close contact with COVID-19 or with suspected exposure to COVID-19 or in communities with a high prevalence of infection. Additional confirmatory testing with a molecular test for positive results may also be necessary if there is a low likelihood of SARS-CoV-2 infection, such as in individuals without known exposures to SARS-CoV-2 or residing in communities with a low prevalence of infection.

The iStatis COVID-19 Antigen Test is intended for use by medical professionals or operators trained in performing tests in point of care settings.

SUMMARY AND EXPLANATION OF THE TEST

Coronaviruses (CoV) are a large family of viruses that can infect humans and animals.[1] In humans, coronaviruses cause illnesses ranging from the common cold to more severe diseases such as severe acute respiratory syndrome (SARS). SARS-CoV-2, is a new strain of coronavirus that was first identified during an outbreak in Wuhan, China in 2019 and causes Coronavirus Disease 2019 (COVID-19), a respiratory disease characterized by fever, cough, and shortness of breath.[2] In more severe cases, the infection can cause pneumonia, SARS, kidney failure, and death.[3]

The iStatis COVID-19 Antigen Test is a rapid lateral flow immunoassay for the qualitative detection and diagnosis of SARS-CoV-2 antigens directly from anterior nasal swabs specimens from patients with signs and symptoms of having COVID-19 within 15 minutes or less or taken from asymptomatic individuals being tested serially, as described in the authorized intended use. The test is intended to be interpreted visually in both laboratory and near-patient testing environments without an instrument.

PRINCIPLE OF THE TEST

The **iStatis COVID-19 Antigen Test** is a manual, visually read, lateral flow immunoassay for the qualitative detection of SARS-CoV-2 nucleocapsid proteins in anterior nasal swab specimens collected from individuals who are suspected of COVID-19 within the first seven days of symptom onset, or who are asymptomatic and undergoing serial testing, as described in the intended use. The assay is packaged as a kit containing a single-use cartridge unit along with a single-use buffer vial to be used with nasal swabs. The test consists of a set of sample absorption pads, a conjugate reagent pad with specific SARS-CoV-2 antibodies, and a test membrane where a secondary SARS-CoV-2 antibody, as well as a control antibody, have been striped on two distinct lines to form the entire test strip. Results are visualized in 15 minutes following the addition of the buffer solution to the test cartridge in the form of visible control and test lines. Results should not be read after 30 minutes.

SARS-CoV-2 Nucleocapsid Antigen Detection: The iStatis COVID-19 Antigen Test utilizes a set of monoclonal antibodies to SARS-CoV-2 nucleocapsid antigen. Upon addition of the swab sample to the buffer vial, the SARS-CoV-2 viral antigens bind to the antibodies conjugated to colloidal gold. This complex then migrates onto the test strip and is captured by capturing antibodies on the test line immobilized on the nitrocellulose membrane.

Test Complexity: The iStatis COVID-19 Antigen Test was designed to reduce protocol complexity. The iStatis COVID-19 Antigen Test is the extraction of a nasal sample from a swab into solution, and then the subsequent addition of solution to the cartridge. These requirements result in an assay that generates consistent results due to relative ease of use. Test results must be read no sooner than 15 minutes but no later than 30 minutes.

KIT COMPONENTS AND STORAGE

Store iStatis COVID-19 Antigen Test unopened at 2 to 30°C (35.6°to 86°F). 2°C–30°C

All kit components are packaged for single use only.

Each test kit contains the following materials:

1. **Test Cartridge**: individually packaged, prepared with control (antibody capture) and test (SARS-CoV-2 nucleocapsid antibody) lines. For single use with anterior nasal swab samples.
2. **Nasal Swab** STERILE: A single use sterile nasal swab for sample collection from the patient.
3. **Vial Holder:** Cardboard holder to store buffer vial during sample collection process.
4. **Buffer Vial:** A vial that contains buffer for extraction of the sample from the included nasal swab.
5. **Buffer Vial Cap:** A dispensing nozzle to dispense the collected sample onto the cartridge.

MATERIALS REQUIRED BUT NOT PROVIDED

- Personal protective equipment such as gloves, lab coat, or gown
- Biohazard waste containers
- Timer

WARNINGS

1. **Do not use test cartridges if the packaging has been damaged**
2. Do not use the kit beyond the expiry date
3. This product has been authorized only for the detection of proteins from SARS-CoV-2, not for any other viruses or pathogens.
4. Do not interchange kit contents from different lots.
5. Avoid microbial contamination and exercise care in handling the kit components.
6. Failure to use the provided buffer solution and vial cap may result in leakage and/or overflow of liquids from the test cartridge.
7. If the kit is refrigerated, ensure it is brought to room temperature before performing the test. If required use the iStatis COVID-19 Positive/Negative Controls to ensure proper kit performance.
8. ⚠ Sodium azide is present at 0.1% in the buffer solution. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. If products containing sodium azide are discarded into a drain, flush with large amounts of water to prevent azide build-up. Check with local regulatory agencies to determine at what concentration sodium azide may cause a product to be regulated as hazardous waste.
9. Individuals with color-impaired vision may not be able to adequately interpret test results.

SAFETY PRECAUTIONS

For *in vitro* diagnostic use only.

1. Wear disposable gloves while handling kit reagents or specimens. Change gloves and wash hands thoroughly after performing each test.
2. All specimens should be handled as if capable of transmitting infectious agents.
3. Avoid contact with skin and eyes. If contact occurs, wash affected areas with water.
4. Avoid touching any bleeding areas of the nostril area during specimen collection, as excess blood or mucus on the swab may interfere with test results.
5. Avoid forming aerosols.
6. Dispose of used contents as biohazardous wastes in accordance with federal, state, and local requirements.
7. Spills should be cleaned up and decontaminated in accordance with the user facility's established procedures for handling biohazardous spills.
8. Specific training or guidance is recommended if operators are not experienced with specimen collection and handling procedures.
9. Use appropriate precautions in the collection, handling, storage, and disposal of samples and used kit contents.

INSTRUCTIONS FOR USE

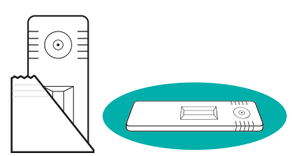
Workplace Preparations

- Gather the material you will need to perform the test.
- Allow the iStatis COVID-19 Antigen Test to come to room temperature before use.
- Testing should be performed under normal room lighting conditions.
- Refer to the Quality Control section in this Package Insert to determine when the Test Controls should be run.
- Test cartridge and swab should be used immediately upon opening; Do not remove Test Cartridge from the pouch until just before use.
- If the extraction buffer in the vial is spilled, discard the vial, and use a new test kit.

TEST PROCEDURE

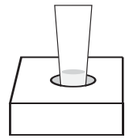
Test Set Up

1. Remove the test cartridge from its packaging and lay it flat on the table.



2. Tear off the foil seal of the Buffer Vial.

3. Place the Buffer Vial into the Vial Holder.

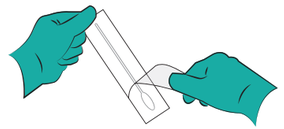


Sample Collection

Note: Freshly collected sample should be processed immediately after collection.

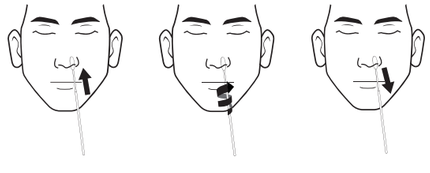
Step 1 - Opening

- Remove test swab from the sterile packaging, being mindful not to touch the soft end with your hand.



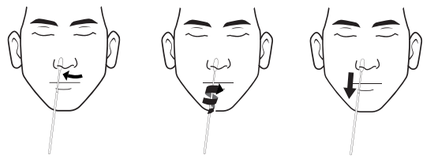
Step 2 - Nasal Swab Insertion

- Gently insert the swab no more than 3/4 inch into the LEFT nostril. Then, slowly rotate the swab at least 5 times in a circular path for a total of 15 seconds. If you have questions during the sample collection, refer to the CDC Guidelines for anterior nasal swab specimen collection. Once complete carefully remove from the LEFT nostril.



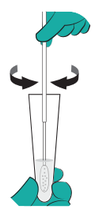
Step 3 - Repeat

- Place the swab directly into the RIGHT nostril, repeating the process of rotating at least 5 times in a circular path for a total of 15 seconds. Carefully remove the swab from the RIGHT nostril.



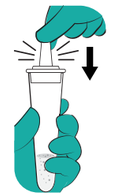
Testing of Nasal Sample

Step 1 - Place the swab into the Buffer Vial. Rotate the swab vigorously at least 5 times. Further rotate the swab another 5 times while squeezing the sides of the Buffer Vial.



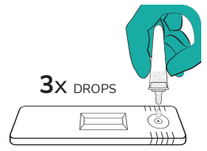
Step 2 - Remove the swab by rotating against the Buffer Vial while squeezing the sides of the vial to release the liquid from the swab. Discard the swab as per the Safety Precautions section of this package insert.

Step 3 - Insert the Buffer Vial Cap to the vial containing the sample and push firmly to close onto the vial.



Step 4 - While holding the top of the vial with one hand flick the bottom of the vial with the other to thoroughly mix the solution.

Step 5 - Slowly turn the vial vertically upside down, pinch the vial, and add 3 drops to the sample well on the cartridge. The first drop may contain bubbles, but this will not affect the test results.



⚠ IMPORTANT: Do not move or lift the cartridge during this step.

Step 6 - Start a Timer! The results of the test **CAN** be read at **15 minutes**. The test result should **NOT** be read after **30 minutes.**

QUALITY CONTROL

Kit Control:

The iStatis COVID-19 Antigen Test has a built-in procedural control that demonstrates assay validity and adequate sample addition. A red/purple color on the control line indicates that the assay procedure was performed correctly. (Refer to Interpretation of Results section of this Package Insert.)

iStatis COVID-19 Antigen Test Control swabs are available separately for use only with the iStatis COVID-19 Antigen Test. The controls are used to verify test performance and interpretation of results. Kit controls are recommended to be run under the following circumstances:

- Once for every new lot of kits and every new user operator verification prior to performing testing on patient specimens.
- When temperature during storage of the kit falls outside of 2° to 30°C (35.6°F to 86°F)
- When the temperature of the test area falls outside of 15° to 30°C (59°F to 86°F)
- At regular intervals as determined by the user facility

Refer to the iStatis COVID-19 Antigen Test Controls Package Insert for additional information on the use of these swabs. It is the responsibility of each laboratory using the iStatis COVID-19 Antigen Test to establish an adequate quality assurance program to ensure the performance under their specific locations and conditions of use.

INTERPRETATION OF RESULTS

- **Do not read the results if more than 30 minutes have elapsed following the addition of the sample to the test cartridge.**
- **If using the control swabs provided by bioLytical, all Positive Controls must be positive and all Negative Controls must be negative. Controls that produce incorrect or invalid results must be re-tested with a new iStatis test kit. If results are still incorrect or invalid, contact Technical Support at +1-866-674-6784 or customercare@biolytical.com.**
- **Control and test lines appear in red/purple color.**
- **Test line will appear close to the sample well and the control line appears further away from the sample well.**

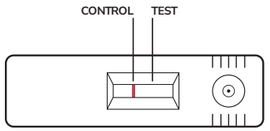
Table 1. Repeat testing is needed to improve test accuracy. Please follow the table below when interpreting test results.

Status on First Day of Testing	First Result Day 1	Second Result Day 3	Third Result Day 5	Interpretation
With Symptoms	Positive	NA	NA	Positive for COVID-19
	Negative	Positive	NA	Positive for COVID-19
	Negative	Negative	NA	Negative for COVID-19
Without Symptoms	Positive	NA	NA	Positive for COVID-19
	Negative	Positive	NA	Positive for COVID-19

	Negative	Negative	Positive	Positive for COVID-19
	Negative	Negative	Negative	Negative for COVID-19

Results should be considered in the context of an individual's recent exposures, history, and the presence of clinical signs and symptoms consistent with COVID-19.

NEGATIVE ► Control line is clearly visible the following development. Additionally, the absence of any test line indicates the specimen does not contain any SARS-CoV-2 nucleocapsid antigen and indicates a negative test result.



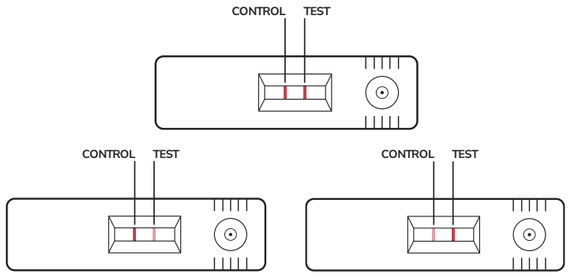
To increase the chance that the negative result for COVID-19 is accurate, you should:

- **Test again in 48 hours if the individual has symptoms on the first days of testing.**
- **Test 2 more times at least 48 hours apart if the individual does not have symptoms on the first day of testing.**

A negative test result indicates that the virus that causes COVID-19 was not detected in the sample. A negative result does not rule out COVID-19. There is a higher chance of false negative results with antigen tests compared to laboratory-based tests such as PCR tests. If the test is negative but COVID-19-like symptoms, e.g., fever, cough, and/or shortness of breath continue, follow-up testing for SARS-CoV-2 with a molecular test or testing for other respiratory diseases should be considered. If applicable, seek to follow-up care with the primary health care provider.

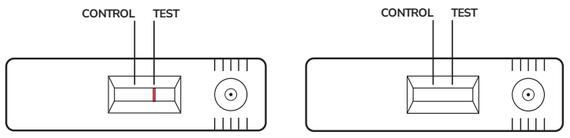
All negative results should be treated as presumptive and confirmation with a molecular assay may be necessary if there is a high likelihood of SARS-CoV-2 infection, such as in an individual with close contact with COVID-19 or with suspected exposure to COVID-19 or in communities with a high prevalence of infection. Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions.

POSITIVE ► Control line is clearly visible the following development. Additionally, any test line development, be it faint or distinct is present. This indicates the presence of SARS-CoV-2 nucleocapsid antigen and indicates a positive test result.



Note: **a)** The Test and Control Lines can be very faint. Any red/purple line visible here indicates a positive result.
b) Repeat testing does not need to be performed if the patient has a positive result at any time.

INVALID ► No control line is visible following development. An invalid test result means that the test was run incorrectly, or insufficient specimen was added. Invalid test results cannot be interpreted. Repeat the test with a new iStatis COVID-19 Antigen Test.



SERIAL TESTING RESULTS REPORTING

For serial testing programs, additional confirmatory testing with a molecular test for negative results may be necessary, if there is a high likelihood of COVID-19, such as an individual with close contact with COVID-19 or with suspected exposure to COVID-19 or in communities with a high prevalence of infection. Additional confirmatory testing with a molecular test for positive results may also be necessary if there is a low likelihood of COVID-19, such as in individuals without known exposures to COVID-19 or residing in communities with a low prevalence of infection.

LIMITATIONS OF THE TEST

- The iStatis COVID-19 Antigen Test must be used in accordance with the instructions in this package insert to obtain accurate results.
- Use iStatis Test in conjunction with the testing strategy outlined by public health authorities in your area.
- The performance of this device has not been assessed in a population vaccinated against COVID-19.
- The performance of this test has not yet been clinically validated for use in patients without signs and symptoms of respiratory infection, or for serial screening applications when tested twice over two or three days with at least 24 hours and no more than 48 hours between tests, and performance may differ in these populations.
- The clinical significance of the test results needs to be analyzed in combination with other test indicators and clinical manifestations.
- Results from antigen testing should not be used to diagnose or exclude SARS-COV-2 infection.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. If an acute infection is suspected, direct testing for SARS-CoV-2 is necessary.
- A negative result may be obtained if the specimen is inadequate, or antigen concentration is below the sensitivity/detection limit of the test. Therefore, it is recommended that all negative test results undergo confirmatory testing using other methods and/or qualified assays.
- Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
- Laboratories may be required to report all positive results in accordance with any country-specific or public health authority requirements.
- False negative results may occur if a specimen is improperly collected, transported, or handled.
- The test is designed for use with nasal swab samples only. Performance has not been established for use with other specimen types. Other specimen types have not been evaluated and should not be used with this assay.
- Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Serial Testing (Repeat Testing) information and Limitations

- Serial testing (i.e., testing every other day) is more likely to detect COVID-19, both when you do or do not have any symptoms.
- A negative result should be followed up with repeat, or serial testing at least twice over three days with at least 48 hours between tests for symptomatic individuals and/or at least three times over five days with at least 48 hours between tests for asymptomatic individuals. A home test may be used for this additional testing.
- The performance of this test was not clinically validated for serial testing. Serial testing recommendations are supported by the study conducted by the National Institutes of Health (NIH) and the University of Massachusetts Chan Medical School in collaboration with the US FDA.
- All COVID-19 antigen test negative results are presumptive and confirmation with a molecular assay may be necessary. If you continue to have symptoms of COVID-19, and both your first and second tests are negative, you may not have COVID-19, however you should follow-up with a healthcare provider.

PERFORMANCE CHARACTERISTICS

Assay Sensitivity: Limit of Detection (LoD):
Limit of Detection (Analytical Sensitivity): The iStatis COVID-19 Antigen Test Limit of detection (LoD) was determined by testing limiting dilutions of UV-inactivated SARS-CoV-2 virus (Delta Variant) in pooled human nasal matrix from confirmed negative donors. Each test concentration was inoculated onto iStatis kit-provided swabs and processed according to the test procedure. The LoD was determined by confirming the lowest detectable concentration of SARS-CoV-2 at which 95% of the 20 replicates analyzed resulted in a positive test result. The iStatis COVID-19 Antigen Test Limit of Detection (LoD) in the nasal matrix was confirmed to be 377.5 $TCID_{50}$ per swab.

Table 2. The iStatis COVID-19 Antigen Test Limit of Detection (LoD) in the nasal matrix

SARS-CoV-2 Concentration		Number of Positives/Total	% Detected
$TCID_{50}$/mL	$TCID_{50}$/Swab	20/20	100%
7.55×10^3	377.5		

Analytical Specificity (Cross Reactivity):
Cross reactivity with the following pathogens has been evaluated with the iStatis COVID-19 Antigen Test. Samples positive for the following organisms were found negative when tested with the iStatis COVID-19 Antigen Test. The final concentration of each organism is described in the table below. The samples were tested in triplicates and no cross-reactivity was observed.

Table 3. Final concentration of each organism for Cross-reactivity testing

Organism Tested	Concentration Tested for Cross-Reactivity
Human coronavirus 229E	$>1.0x10^5$ $TCID_{50}$/mL
Human coronavirus OC43	$>1.0x10^5$ $TCID_{50}$/mL
Human coronavirus NL63	$>1.0x10^5$ $TCID_{50}$/mL
Human coronavirus MERS-CoV	$1.0x10^5$ $TCID_{50}$/mL
Human coronavirus SARS-CoV	1/2 Dilution of CT27.6
Adenovirus	$>1.0x10^5$ $TCID_{50}$/mL
Human Metapneumovirus	$>1.0x10^5$ $TCID_{50}$/mL
Parainfluenza Virus type 1	$1.0x10^5$ $TCID_{50}$/mL
Parainfluenza Virus type 2	$1.0x10^5$ $TCID_{50}$/mL
Parainfluenza Virus type 3	$1.0x10^5$ $TCID_{50}$/mL
Parainfluenza Virus type 4	$>1.0x10^5$ $TCID_{50}$/mL
Influenza A	$>1.0x10^5$ $TCID_{50}$/mL
Influenza B	$>1.0x10^5$ $TCID_{50}$/mL
Enterovirus	$1.0x10^5$ $TCID_{50}$/mL
Respiratory syncytial virus	$1.0x10^5$ $TCID_{50}$/mL
Rhinovirus	$1.0x10^5$ $TCID_{50}$/mL
Haemophilus influenzae	$>1.0x10^6$ CFU/mL
Streptococcus pneumoniae	$>1.0x10^6$ CFU/mL
Streptococcus pyogenes	$>1.0x10^6$ CFU/mL
Candida albicans	$>1.0x10^6$ CFU/mL
Pooled human nasal wash	N/A
Bordetella pertussis	$>1.0x10^6$ CFU/mL
Mycoplasma pneumoniae	$>1.0x10^6$ CFU/mL
Chlamydophila pneumoniae	$>1.0x10^6$ CFU/mL
Legionella pneumophila	$>1.0x10^6$ CFU/mL
Staphylococcus aureus	$>1.0x10^6$ CFU/mL
Staphylococcus epidermis	$>1.0x10^6$ CFU/mL

To estimate the likelihood of cross-reactivity with SARS-CoV-2 virus in the presence of organisms that were not available for wet testing, In silico analysis using the Basic Local Alignment Search Tool (BLAST) managed by the National Center for Biotechnology Information (NCBI) was used to assess the degree of protein sequence homology.

- For P. jirovecii one area of sequence similarity shows 45% homology across 18% of the sequence, making cross-reactivity in the iStatis COVID-19 Antigen Test highly unlikely.
- No protein sequence homology was found between M. tuberculosis, and thus homology based cross-reactivity can be ruled out.
- The comparison between SARS-CoV-2 nucleocapsid protein, MERS-CoV and human coronavirus HKU1 revealed that cross-reactivity cannot be ruled out. Homology for KHU1 and MERS-CoV is relatively low, at 37.8% across 95% of the sequence and 57.14% across 87% of the sequence, respectively.

Endogenous and Exogenous Substances (Interference):
To assess endogenous and exogenous substances' interference with the performance of the iStatis COVID-19 Antigen Test, positive and negative samples were tested with potentially interfering substances that may be found in the upper respiratory tract. This study was performed to demonstrate that fourteen (14) potentially interfering substances do not cross-react nor interfere with the detection of SARS-CoV-2 in iStatis COVID-19 Antigen Test.

Table 4. Potential Interfering substances tested with correlated concentrations

Potential Interfering Substances	Concentration Tested
Whole Blood	4%
Mucin	0.50%
Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL
Naso GEL (NeilMed)	5% v/v
Phenylephrine Hydrochloride (Phenylephrine Nasal Drops)	15% v/v
Oxymetazoline (Nasal Drops)	15% v/v
Cromolyn Sodium Salt (Cromolyn Nasal Spray)	15% v/v
Homeopathic (Zicam)	5% v/v
Homeopathic (Alkalol)	1:10 dilution
Sore Throat Phenol Spray	15% v/v
Tobramycin (antibiotic)	4 µg/mL
Mupirocin (antibacterial)	10 mg/mL
Fluticasone Propionate (Flonase)	5% v/v
Oseltamivir Phosphate (Tamiflu) (antiviral)	5 mg/mL

High-Dose Hook Effect:
The iStatis COVID-19 Antigen Test was tested up to 1.51×10^6 $TCID_{50}$/mL, app. 200xLoD of UV inactivated SARS-CoV-2 virus and no high-dose hook effect was observed.

Clinical Performance
Note: This clinical performance data reflects the accuracy of the test when testing once, this test was not clinically validated for serial testing. The serial testing recommendations are supported by the study conducted by the National Institutes of Health (NIH) and the University of Massachusetts Chan Medical School in collaboration with the US FDA.

A prospective clinical study was conducted from 14 January 2022 to 21 January 2022 at Point of Care (POC)/CLIA waivered sites in the United States to evaluate the performance of the iStatis COVID-19 Ag test for direct anterior nasal swab specimens compared to an FDA Emergency Use Authorized (EUA) RT-PCR test. A total of seven (7) operators from two (2) CLIA waiver sites and (1) satellite/mobile site that is part of one of the CLIA waiver sites were involved in the study. Patients were prospectively and sequentially enrolled at each site. Samples were collected from patients of ages greater than 18 who visited the doctor with signs and symptoms of suspected COVID-19 infection. The performance of iStatis COVID-19 Ag test was established with anterior nasal swab samples collected from 96 patients within 7 days of symptoms onset of COVID-19. Two nasal swabs were collected from each patient. Clinical studies in asymptomatic patients undergoing serial testing are ongoing to establish the clinical performance.

One anterior nasal swab was tested directly using the iStatis COVID-19 Ag according to the Package Insert by the untrained operators. The other nasal/nasopharyngeal swab was collected, transported in the VTM and/or PBS buffer medium and tested as per the FDA EUA RT-PCR's Package Insert. Swabs were randomly assigned to test with the iStatis COVID-19 Ag test or the RT-PCR.

Table 5. iStatis COVID-19 Antigen Test Performance within '7' days of symptom onset against the Comparator Method

iStatis COVID-19 Antigen Test	Comparator Method (RT-PCR)		
	Positive	Negative	Total
Positive	32	0	32
Negative	3[a]	61	64
Total	35	61	96
Positive Agreement	91.43%, 95% CI (76.94- 98.20)		
Negative Agreement	100%, 95% CI (94.04-100.00)		

[a]COVID-19 was not detected in 3 specimens using an alternative FDA/Health Canada authorized RT-PCR assay.

The performance of this test has not yet been clinically validated for use in patients without signs and symptoms of respiratory infection or for serial screening applications, and performance may differ in these populations.

Serial-testing Clinical Performance

A prospective clinical study was conducted between January 2021 and May 2022 as a component of the Rapid Acceleration of Diagnostics (RADx) initiative from the National Institutes of Health (NIH). A total of 7,361 individuals were enrolled via a decentralized clinical study design, with a broad geographical representation of the United States. Per inclusion criteria, all individuals were asymptomatic upon enrollment in the study and at least 14 days prior to it and did not have a SARS-CoV-2 infection in the three months prior to enrollment. Participants were assigned to one of three EUA-authorized SARS-CoV-2 OTC rapid antigen tests to conduct serial testing (every 48 hours) for 15 days. If an antigen test was positive, the serial-antigen testing result was considered positive.

At each rapid antigen testing time point, study subjects also collected a nasal swab for comparator testing using a home collection kit (using a 15-minute normalization window between swabs). SARS-CoV-2 infection status was determined by a composite comparator method on the day of the first antigen test, using at least two highly sensitive EUA RT-PCRs. If the results of the first two molecular tests were discordant a third highly sensitive EUA RT-PCR test was performed, and the final test result was based upon the majority rule.

Study participants reported symptom status throughout the study using the MyDataHelps app. Two-day serial antigen testing is defined as performing two antigen tests 36 – 48 hours apart. Three-day serial antigen testing is defined as performing three antigen tests over five days with at least 48 hours between each test.

Out of the 7,361 participants enrolled in the study, 5,609 were eligible for analysis. Among eligible participants, 154 tested positive for SARS-CoV-2 infection based on RT-PCR, of which 97 (62%) were asymptomatic on the first day of their infection, whereas 57 (39%) reported symptoms on the first day of infection. Pre-symptomatic subjects were included in the positive percent agreement (PPA) of asymptomatic individuals, if they were asymptomatic on the first day of antigen testing, regardless of whether they developed symptoms at any time after the first day of testing.

Performance of the antigen test with serial testing in individuals is described in Table 6..

Table 6. Data establishing PPA of COVID-19 antigen serial testing compared to the molecular comparator single-day testing throughout the course of infection with serial testing. Data is from all antigen tests in the study combined.

DAYS AFTER THE FIRST PCR	ASYMPTOMATIC ON THE FIRST DAY OF TESTING	SYMPTOMATIC ON THE FIRST DAY OF TESTING
	Ag Positive/PCR Positive (Antigen Test Performance % PPA)	

POSITIVE TEST RESULT	1 Test	2 Tests	3 Tests	1 Test	2 Tests	3 Tests
0	9/97 (9.3%)	35/89 (39.3%)	44/78 (56.4%)	34/57 (59.6%)	47/51 (92.2%)	44/47 (93.6%)
2	17/34 (50.0%)	23/34 (67.6%)	25/32 (78.1%)	58/62 (93.5%)	59/60 (98.3%)	43/43 (100%)
4	16/21 (76.2%)	15/20 (75.0%)	13/15 (86.7%)	55/58 (94.8%)	53/54 (98.1%)	39/40 (97.5%)
6	20/28 (71.4%)	21/27 (77.8%)	16/18 (88.9%)	27/34 (79.4%)	26/33 (78.8%)	22/27 (81.5%)
8	13/23 (56.5%)	13/22 (59.1%)	4/11 (36.4%)	12/17 (70.6%)	12/17 (70.6%)	7/11 (63.6%)
10	5/9 (55.6%)	5/8 (62.5%)		4/9 (44.4%)	3/7 (42.9%)	

1 Test = one (1) test performed o the noted days after first PCR positive result. Day 0 is the first day of documented infection with SARS-CoV-2.
2 Tests = two (2) tests performed an average of 48 hours apart. The first test performed on the indicated day and the second test performed 48 hours later.
3 Tests = three (3) tests performance an average of 48 hours apart. The first test performed on the indicated day, the second test performed 48 hours later, and a final test performed 48 hours after the second test.

Table 7. Patient Demographics

Age (years)	iStatis COVID-19 Antigen Test		
	Total	Positive	Prevalence
20-40	38	14	36.84%
41-60	34	7	20.59%
60 +	23	11	47.83%
Total	95	32	33.68%

Table 8. Positive results broken down by days since symptom onset

Days since symptom onset	Cumulative Comparator Method Positive (+)	Cumulative iStatis COVID-19 Antigen Test Positive (+)	PPA
0	1	1	100.00%
1	4	4	100.00%
2	10	10	100.00%
3	19	18	94.74%
4	25	23	92.00%
5	29	27	93.10%
6	33	31	93.93%
7	35	32	91.43%
Total	35	32	91.43%

A retrospective study was conducted in Germany to assess the diagnostic sensitivity and specificity of the iStatis COVID-19 Antigen Test for the detection of SARS-CoV-2 Antigens. A total of 503 samples were tested in this study, where 103 were confirmed COVID-19 positive and 300 were confirmed COVID-19 Negative. In addition, 100 nasal swab samples collected from COVID-19 negative hospitalized patients were also included in the study. All these 503 samples' COVID-19 status was confirmed with FDA EUA authorized SARS-CoV-2 RT-PCR assay.
Out of 103 RT-PCR confirmed positive samples, iStatis COVID-19 Antigen Test was able to accurately detect all 103 samples with a sensitivity of 100% (95% CI: 96.4 – 100.0). iStatis COVID-19 Antigen Test was able to detect 397 negatives (including the hospitalized samples) from 400 RT-PCR confirmed negative cases accurately, with a specificity of 99.85% (95% CI: 99.55 – 99.95).

Table 9. iStatis COVID-19 Antigen Test Performance using retrospective samples against the Comparator Method

iStatis COVID-19 Antigen Test	Comparator Method (RT-PCR)		
	Positive	Negative	Total
Positive	103	3	106
Negative	0	397	397
Total	103	400[a]	503
Positive Agreement	100%, 95% CI (96.4 – 100.0)		
Negative Agreement	99.85%, 95% CI (99.55 – 99.95)		

[a]Out of 400 Negative samples, 100 nasal swab specimens were collected from COVID-19 negative hospitalized patients.

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TECHNICAL INFORMATION

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GLOSSARY OF SYMBOLS

2°C 30°C	Store at 2°C to 30°C		Manufacturer
IVD	*In Vitro* diagnostic medical device		Caution Harmful if swallowed
	Consult Package Insert	STERILE EO	Sterilization by Ethylene Oxide

Do not reuse	Contains sufficient for “N” tests
Do not use if damaged	Keep dry
Catalogue Number	Keep away from direct sunlight
Use By Date	This side up
Lot number	

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