Nano-Check™ COVID-19 Antigen Test

For use under emergency use authorization (EUA) only
For *in vitro* diagnostic use
For prescription use only
For use with kit provided swabs

1. INTENDED USE

The Nano-Check™ COVID-19 Antigen Test is a lateral flow immunoassay intended for the qualitative detection of nucleocapsid protein antigen from SARS-CoV-2 in direct anterior nasal swab and direct nasopharyngeal swab specimens collected from individuals who are suspected of COVID-19 by their healthcare provider within the first five (5) days of symptoms onset when tested at least twice over three days with at least 48 hours between tests or from individuals without symptoms or other epidemiological reasons to suspect COVID-19 when tested at least three times over five days with at least 48 hours between tests. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform moderate, high or waived complexity tests. This product is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.

The Nano-Check™ COVID-19 Antigen Test does not differentiate between SARS-CoV or SARS-CoV-2 viruses.

Results are for the identification of SARS-CoV-2 nucleocapsid antigen. Antigen is generally detectable in direct anterior nasal swab and direct nasopharyngeal swab specimens during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to fully determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

All negative results are presumptive and may be confirmed with a molecular assay, if necessary, for patient management. 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. Negative results should be considered in the context of a patient's recent exposures, history and the presence of clinical signs and symptoms consistent with COVID-19.

The Nano-Check™ COVID-19 Antigen Test is intended for use by medical professionals or operators who are proficient in performing tests in point of care settings. The Nano-Check™ COVID-19 Antigen Test is only for *in vitro* diagnostic use under the Food and Drug Administration's Emergency Use Authorization. This product has not been FDA cleared or approved.

2. SUMMARY AND EXPLANATION OF THE TEST

The first case of the coronavirus disease 19 (COVID-19) was reported when an outbreak of unknown respiratory illnesses occurred in Wuhan, China on December 31, 2019. The COVID-19 Caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a respiratory illness, like influenza, with symptoms such as a cough, fever, fatigue, and in more severe cases, difficulty breathing or shortness of breath. The WHO officially declared COVID-19 a pandemic on March 11, 2020.

Nano-Check™ COVID-19 Antigen Test is a rapid chromatographic immunoassay intended for the direct detection of presence or absence SARS-CoV-2 antigen in 15 min using respiratory specimens collected from individuals suspected of COVID-19 by their healthcare provider within five days of symptom onset.

3. PRINCIPLE

The Nano-Check™ COVID-19 Antigen Test is designed to detect the extracted nucleocapsid protein antigen specific to SARS-CoV-2 in anterior nasal swab and nasopharyngeal swab specimens directly collected from individuals who are suspected of COVID-19 by their healthcare provider within the first five days of symptom onset.

When specimens are extracted and added to the sample well of test device, SARS-CoV-2 viral antigens present in the specimen bind to antibodies against SARS-CoV-2 nucleocapsid conjugated to gold colloidal particles and biotin in the test strip. The antigen-conjugate immunocomplexes migrate across the test strip and are captured at the test line of nitrocellulose membrane.

Test results are interpreted at 15-20 minutes visually. The presence of two pinkish red colored lines in the control line "C" and test line "Ag" indicates COVID-19 positive. The presence of one colored lines in the control line "C" indicates COVID-19 negative. The control line (C) must be present in the test window for self-procedure validation control. This colored control band always appears at the control line position (C) in valid test result. Any test result is not valid without appearance of the control line in the test window.

4. REAGENTS and MATERIALS

Provided

- 20 Test devices in sealed aluminum foil pouch with desiccant
- 20 Reagent tubes with extraction buffer (0.3 mL)
- 20 Sample collection swabs (Anterior nasal or Nasopharyngeal)
- 1 Positive control swab
- 1 Negative control swab
- 1 Instructions for Use/ Quick Reference Instruction

Required but not provided

- Timer
- Tube rack for specimens
- Any necessary personal protective equipment

5. STORAGE AND STABILITY

- The test kit should be stored at 2°C - 30°C in the original sealed pouch. Do not freeze and bring to room temperature at least 30 minutes prior to use. For the most current expiration dates of this test, please refer to: http//www.fda.gov.covid-tests.
- The freshly collected anterior nasal swab and nasopharyngeal swab specimen are recommended to be processed no later than one hour after specimen collection at room temperature (15°C - 30°C) or before 48 hours when stored at 2°C to 8°C.

6. WARNINGS AND PRECAUTIONS

- Read all instructions carefully before performing the test. Failure to follow the instructions may result in inaccurate test results.
- For *in-vitro* diagnostic use only.
- For prescription use only.
- For use with kit provided swabs. Use only swabs provided with the kit.

- In the USA, this product has not been FDA cleared or approved, but has been authorized by FDA under an Emergency Use Authorization for use by authorized laboratories; use by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C §263a, that meet the requirements to perform moderate, high, or waived complexity tests. This product is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation. This product has been authorized only for the detection of proteins from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Serial testing should be performed in individuals with negative results at least twice over three days with at least 48 hours between tests for symptomatic individuals and three times over five days with at least 48 hours between tests for asymptomatic individuals. You may need to purchase additional tests to perform this serial (repeat) testing.
- Federal Law restricts this test to sale by or on the order of a licensed practitioner (U.S. only).
- If you have had symptoms longer than 5 days you should consider testing at least three times over five days with at least 48 hours between tests.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health laboratories.
- Do not use if any of the test kit contents or packaging is damaged.
- Do not use any test component after the expiration date which is printed on the outer packaging.
- Do not interchange kit contents from different lots.
- Test components are single-use. Do not re-use.
- Do not touch the swab tip.
- Once opened, the test card should be used within 90 minutes.
- Do not read test results before 15 minutes or after 20 minutes. Results read before 15 minutes or after 20 minutes may lead to a false positive, false negative, or invalid result.
- Do not eat, drink, or smoke in the area where the specimens and kit contents are handled.
- Do not use the kit to evaluate patient specimens if either the positive control swab or negative control swab fail to give expected results.
- As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
- Use appropriate precautions in the collection, handling, storage, and disposal of patient samples and used kit contents.
- Dispose of used contents as biohazardous wastes in accordance with federal, state, and local requirements.
- Nitrile or latex gloves should be worn when performing this test.
- Handle all specimens as though they contain infectious agents.
- Observe established precautions against microbiological hazards throughout the procedure and follow the standard procedures for proper disposal of specimens.
- The Extraction Reagent contains potentially harmful chemicals (see table below). If the test solution contacts the skin or eye, flush with copious amounts of water. If irritation persists, seek medical advice: visit https://www.poison.org/contact-us Or call 1-800-222-1222.

Chemical Name	Harms (GHS Code) for each ingredient	Concentration
Sodium Azide	Acute Tox. 2 (Oral), H300 Acute Tox. 1 (Dermal), H310	0.09%

- For more information on EUAs please visit: https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization
- For the most up to date information on COVID-19, please visit: www.cdc.gov/COVID19

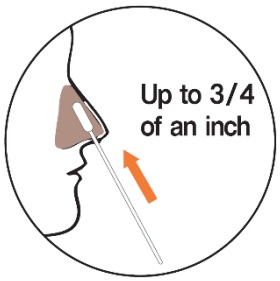
7. SPECIMEN COLLECTION AND PREPARATION

Acceptable specimen type for testing with the Nano-Check™ COVID-19 Antigen Test are direct anterior nasal swab and direct nasopharyngeal swab specimens. It is essential that correct specimen collection and preparation methods be followed. Inadequate specimen collection, improper specimen handling and/or transport may yield false results; therefore, specimen collection requires specific training and guidance due to the importance of specimen quality to obtain accurate test results.

Freshly collected specimens should be processed as soon as possible, but no later than one-hour at room temperature or up to 48 hours at 2-8ºC after specimen collection. Specimens in extraction buffer can be processed up to thirty minutes after collection when kept at room temperature.

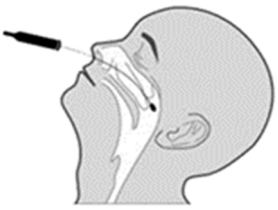
Anterior Nasal swab

To collect the anterior nasal swab sample, tilt the patient's head back 70 degrees and insert the soft end of the swab into patient's nostril no more than ¾ of an inch into the nose. Slowly rotate the swab, gently pressing against the inside of patient's nostril at least 5 times for a total of 15 seconds. Get as much nasal discharge as possible on the soft end of the swab. Gently remove the swab. Use the same end of the swab and repeat the same steps on the other nostril.



Nasopharyngeal swab

To collect the nasopharyngeal swab sample, tilt the patient's head back 70 degrees. Carefully insert the swab into the nostril that presents the most secretion under visual inspection. Slowly insert the swab parallel to the palate until resistance is encountered, or the distance is equivalent to that from the ear to the nostril of the patient. Rotate the swab several times, leave the swab in place for several seconds to absorb secretions and then remove it from the nasopharynx.



Refer to: Interim Guidelines for Collecting, Handling and Testing Clinical Specimens from persons for COVID-19 at https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html

8. TEST PROCEDURE AND PROTOCOL

Collect specimen according to instructions in "Specimen Collection and Preparation". Test device and sample should be brought to room temperature (20°C- 30°C) prior to testing. Remove the test device from the sealed pouch immediately before use. Label the device with patient or control identification. Conduct all testing on a level surface.

1.  Remove the cap from the Reagent tube.

2.  Insert the collected swab into the Reagent tube.

3. 

Swirl and plunge the swab up and down in the extraction buffer while squeezing the sides of the tube for 15 seconds.

4.  Remove the swab while squeezing the sides of the tube to the swab head for extracting the maximum amount of liquid from the swab. Properly discard the swab.

5.  Firmly close the dropper tip onto the Reagent tube containing the sample.

6. 

With the processed Reagent Tube hold vertically, squeeze gently to dispense 2 drops of the sample into the sample well of the test device.

Note: Too few drops can result in invalid results, and too many drops could produce incorrect results.

7. 

Read the results at 15 minutes visually. Do not read result more than 20 minutes after the sample application.

Note: False negative or false positive results can occur if read before or after 15-20 minutes.

9. INTERPRETATION OF RESULTS

Positive

Appearance of pinkish red colored bands at both the control line and the test line indicates positive result. The colored test line depending on the concentration of SARS-CoV-2 virus in the test specimen will appear. The line in the control region (C) is the control line, which is used to indicate proper performance of the device. The color intensity of the test lines may be weaker or stronger than that of the control line.

Negative

A single pinkish red colored band at the control line without visual test line is a negative result. Negative result does not indicate the absolute absence of SARS-CoV-2 virus in specimen or rule out COVID-19; it only indicates that the specimen does not contain the virus concentration at above the detection limit of the level.

Note: Negative results are presumptive and may be confirmed with a molecular assay, if necessary, for patient management.

Invalid

If no lines are seen or no colored line appears in the control region(C), the test result is invalid. If the invalid result is obtained during initial testing, the assay should be repeated with a new test device.

10. QUALITY CONTROL

Internal Quality Control: The presence of a pinkish red colored band in the Control area of the window acts as an internal control to ensure adequate migration has occurred, but does not determine if an adequate sample has been added. In the absence of this Control line, the test is invalid and must be repeated. If the control line does not develop in 15 minutes, the test result is considered invalid and retesting with a new device is recommended. If the internal procedural control line is still absent in the retest, please contact the Technical Support at +1- 855-297-7877 or info@nanoditech.com.

External Control: Positive and negative control swabs are supplied with each kit. These controls provide additional quality control material to assess that the test kit reagents perform as expected. Process the controls in the same manner as clinical sample swab, and conduct the assay as described in Test Procedure section. Controls should minimally be run before using each new lot or shipment of Nano-Check™ COVID-19 Antigen Test, at regular intervals afterwards or any time when the validity of the test results are questioned. All users should follow local, state and federal regulations regarding quality control procedures. If the controls do not perform as expected, do not report patient results. Contact please the Technical Support at +1- 855-297-7877 or info@nanoditech.com.

Limitations:

- There is a higher chance of false negative results with antigen tests than with laboratory-based molecular tests due to the sensitivity of the test technology. This means that there is a higher chance this test will give a false negative result in an individual with COVID-19 as compared to a molecular test, especially in samples with low viral load.
- This test is not for use in at-home testing settings.
- Viral transport media (VTM) should not be used with this test.
- Negative test results are not intended to rule out other non-SARS viral or bacterial infections.
- Positive test results do not rule out co-infections with other bacterial or viral pathogens.
- Positive test results do not differentiate between SARS-CoV and SARS-CoV-2.
- All COVID-19 antigen test negative results are presumptive and confirmation with a molecular assay may be necessary.
- If the patient continues to have symptoms of COVID-19, and both the patient's first and second tests are negative, the patient may not have COVID-19, however additional follow-up may be needed.

- If the test is positive, then proteins from the virus that causes COVID-19 have been found in the sample and patient likely have COVID-19.
- This test is read visually and has not been validated for use by those with impaired vision or color-impaired vision.
- Incorrect test results may occur if a specimen is incorrectly collected or handled.
- Failure to follow the instructions for use may adversely affect test performance and/or invalidate the test result. Make sure to swirl and plunge the swab up and down in extraction buffer while squeezing the sides of the tube for 15 seconds; squeezing the swab head at least once or more in the reagent tube during the swab removal procedure. Insufficient swirling or squeezing of the swab head may produce false negative results.
- If the differentiation of specific SARS viruses and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- Results from antigen testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to determine infection status.
- This test detects both viable (live) and non-viable SARS-CoV-2 virus. Test performance depends on the amount of virus (antigens) in the sample and may or may not correlate with viral culture results performed on the same sample.
- Incorrect test results may occur if a specimen is incorrectly collected or handled.
- Results from the device should be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- A false-negative test result may occur if the level of viral antigen in a sample is below the detection limit of the test or if the sample was collected or transported improperly; therefore, a negative test result does not eliminate the possibility of SARS-CoV-2 infection.
- False negative results can occur if the cassette is not placed on a flat surface.
- False negative results may occur if testing is performed in conditions of low humidity and low temperatures (e.g., <5°C, <20% RH).
- This device is a qualitative test and does not provide information on the viral concentration present in the specimen.
- This device has been evaluated for use with human specimen material only.
- Monoclonal antibodies may fail to detect, or detect with less sensitivity, SARS-CoV-2 viruses that have undergone minor amino acid changes in the target epitope region.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens collected between March 19, 2021 and March 23, 2021. The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

CONDITIONS OF AUTNORIZATION FOR LABORATORIES

The Nano-Check™ COVID-19 Antigen Test Letter of Authorization[1], along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling will be available on the FDA website post authorization: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-useauthorizations-medical-devices/in-vitro-diagnostics-euas.

However, to assist clinical laboratories in using the Nano-Check™ COVID-19 Antigen Test, the relevant Conditions of Intended Authorization are listed below:

A. Authorized laboratories using your product must include with the test result reports, all Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
B. Authorized laboratories using your product must use the product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the product are not permitted.
C. Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run the product prior to initiating testing.
D. Authorized laboratories using your product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
E. Authorized laboratories must collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Nano-Ditech Corporation Product Support website: (www.nanoditech.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the product of which they become aware.
F. All operators using your product must be appropriately trained in performing and interpreting the results of your product, use appropriate personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.
G. Nano-Check™ COVID-19 Antigen Test, authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

11. PERFORMANCE CHARACTERISTICS

1) Clinical Performance

Nasopharyngeal Swab

The clinical performance of the Nano-Check™ COVID-19 Antigen Test with Nasopharyngeal Swab was evaluated in a prospective study in the U.S. in which patients were sequentially enrolled and tested in March 2021.
The performance of the Nano-Check™ COVID-19 Antigen Test was established with a total of 76 direct nasopharyngeal swabs collected from symptomatic patients within 5 days from onset. The samples were tested at a Point of Care (POC) CLIA waived clinical site. Test results were compared to the results of nasal swabs collected from the same patients and tested with a highly sensitive EUA Covid-19 RT-PCR test. Nano-Check™ COVID-19 Antigen Test was performed by operators with no laboratory experience and who were representative of the intended users. Operators were only using the QRI for the test without any training provided.

[1] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meets the requirements to perform high, moderate, or waived complexity tests. This product is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation." as "authorized laboratories."

Thirty-one (31) prospective samples were tested positive with the comparator RT-PCR tests while 28 samples were positive and the other 3 samples were negative using Nano-Check™ COVID-19 Antigen Test. All 45 samples tested negative with the comparator RT-PCR tests were negative on Nano-Check™ COVID-19 Antigen Test. The agreement between the Nano-Check™ COVID-19 Antigen Test and RT-PCR are presented below.

Table 1. Comparison Result with Comparator RT-PCR method

Nano-Check™ COVID-19 Antigen Test	Comparator RT-PCR		Total
	Positive	Negative	
Positive	28	0	28
Negative	3	45	48
Total	31	45	76
Positive Agreement: 28/31	90.32% (95% CI: 75.10% - 96.66%)		
Negative Agreement: 45/45	100.0% (95% CI: 92.14% - 100.0%)		

Table 2. Positive Results by Age Group

Age Group	Nano-Check™ COVID-19 Antigen Test		
	# of Specimen Tested	# of Positive Specimen	Prevalence (%)
≤ 5 years	0	0	N/A
6 to 21 years	4	0	0.00
22 to 60 years	63	23	36.51
≥61 years	9	5	55.56

Table 3. Positive Results Stratified by Days Post-Symptom Onset

Days Post Onset	RT-PCR Positive	Nano-Check™ COVID-19 Antigen Test Positive	Positive Rate (%)
0	5	5	100.00
1	9	9	100.00
2	20	18	90.00
3	24	21	87.50
4	28	25	89.29
5	31	28	90.32

Anterior Nasal Swab

The clinical performance of the Nano-Check™ COVID-19 Antigen Test was established with 135 anterior nasal samples prospectively collected from subjects between January and February 2022 at two clinical sites in the U.S. Samples were collected from sequentially enrolled subjects who presented with symptoms of COVID-19 and within 5 days of onset of symptoms. Samples were tested with Nano-Check™ COVID-19 Antigen Test. All subjects were confirmed as positive or negative by a reference high sensitivity extracted EUA RT-PCR method, used as comparator method for the study. Nano-Check™ COVID-19 Antigen Test was performed by operators with no laboratory experience and who were representative of the intended users. Operators were only using the QRI for the test without any training provided. All testing was conducted by operators blinded to the reference RT-PCR results.
Eighty (80) prospective samples were tested positive with the comparator RT-PCR tests while 66 samples were positive and the other 14 samples were negative using Nano-Check™ COVID-19 Antigen Test. All 55 samples tested negative with the comparator RT-PCR tests were negative on Nano-Check™ COVID-19 Antigen Test. The agreement between the Nano-Check™ COVID-19 Antigen Test and RT-PCR are presented below.

Table 4. Comparison Result with Comparator RT-PCR method

Nano-Check™ COVID-19 Antigen Test	Comparator RT-PCR		Total
	Positive	Negative	
Positive	66	0	66
Negative	14	55	69
Total	80	55	135
Positive Agreement: 66/80	82.50 % (95% CI: 72.74% - 89.28%)		
Negative Agreement: 55/55	100.0% (95% CI: 93.47% - 100.0%)		

Table 5. Positive Results by Age Group

Age Group	Nano-Check™ COVID-19 Antigen Test		
	# of Specimen Tested	# of Positive Specimen	Prevalence (%)
≤ 5 years	0	0	N/A
6 to 21 years	12	2	18.18
22 to 60 years	109	56	51.85
≥61 years	14	8	57.14

Table 6. Positive Results Stratified by Days Post-Symptom Onset

Days Post Onset	RT-PCR Positive	Nano-Check™ COVID-19 Antigen Test Positive	Positive Rate (%)
Asymptomatic	10	7	70.00
0	2	0	0
1	11	7	63.63
2	27	20	74.07
3	48	40	83.33
4	67	57	85.07
5	80	66	82.50

2) Assay Sensitivity: Limit of Detection (LoD)

To verify analytical sensitivity of Nano-Check™ COVID-19 Antigen Test, Limit of Detection (LoD) was established using serial dilutions of gamma-irradiated SARS-CoV-2 isolate USA-WA1/2020 (NR-52287).
Contrived samples were prepared by spiking the strain into the pooled negative nasal wash solution collected before November 2019. A preliminary LoD was determined by spiking 50 µL of serially diluted sample onto swab heads and tested using the Nano-Check™ COVID-19 Antigen Test. The preliminary LoD initially determined by testing two-fold serial dilution series of 3 replicates was confirmed by testing in 20 replicates. Based on the testing procedure for this study the LoD of 7.0×10^{2} $TCID_{50}$/mL equates to 3.5×10^{1} $TCID_{50}$/swab.

3) NIH/RADx Variant Testing:

The performance of this test device in the detection of the Omicron variant of SARS-CoV-2 was evaluated in a dilution series of clinical specimens which were positive for the Omicron variant. This testing was conducted by the National Institutes of Health (NIH) as a component of the Rapid Acceleration of Diagnostics (RADx) initiative. Specimen pools were prepared by the RADx team using pooled clinical samples from currently

circulating Omicron strains and tested by RADx® to assess performance with the Omicron variant. Results from this dilution series cannot be compared to any devices tested with a different specimen pool and do not indicate that a test will have different clinical performance compared to other EUA authorized tests. Compared to an EUA authorized RT-PCR method, the Nano-Check COVID-19 Antigen Test detected 100% of live virus Omicron samples at a Ct-value of 26.0 (n=5). Testing was also compared to two additional EUA authorized antigen tests (Assay #1 and Assay #2). Omicron dilutions at lower viral concentrations (Ct-values higher than 26.0) were not detected by the Nano-Check COVID-19 Antigen Test in this study.

Table 7. Summary Performance of the Omicron Variant

Omicron Pool 1 – Live Omicron Clinical Samples	Average N2 Ct (n=9)	Nano-Check COVID-19 Antigen Test Percent Positive (n=5)	Assay #1 Percent Positive (n=5)	Assay #2 Percent Positive (n=5)
Dilution 1	20.6	100	100	100
Dilution 2	21.5	100	100	100
Dilution 3	22.7	100	100	100
Dilution 4	24.0	100	100	100
Dilution 5	25.3	100	100	100
Dilution 6	26.0	100	100	100
Dilution 7	27.3	0	0	60
Dilution 8	28.8	0	0	0
Dilution 9	29.2	0	0	0
Dilution 10	30.6	0	0	0
Dilution 11	31.7	0	0	0
Dilution 12	32.6	0	0	0

4) Assay Cross Reactivity and Microbial Interference

Cross-reactivity of the Nano-Check™ COVID-19 Antigen Test was evaluated by testing a panel of high prevalence respiratory pathogens that could potentially cross-react with the Nano-Check™ COVID-19 Antigen Test. The final concentration of each organism is described in the table below. The microbial interference was also performed with the same panel of microorganisms at the same concentrations in the samples that were spiked with SARS-CoV-2 at 3X LoD. The samples were tested in triplicates for both cross-reactivity and interference studies. No cross-reactivity and no microbial interference were observed. The results for cross-reactivity and microbial interference are presented in the table below.

Table 8. Cross-Reactivity/Microbial Interference of the Nano-Check™ COVID-19 Antigen Test

Pathogen	Concentration Tested	Cross-Reactivity/ Microbial Interference
Bordetella pertussis, 5	1.0 x 10^6 cfu/ mL	No
Candida albicans, Z006	1.0 x 10^6 cfu/ mL	No
Chlamydophila pneumoniae	1.0 x 10^6 IFU/mL	No
Haemophilus influenzae	1.0 x 10^6 cfu/ mL	No
Legionella pneumophila	1.0 x 10^6 cfu/ mL	No
Mycoplasma pneumoniae	1.0 x 10^6 cfu/ mL	No
Streptococcus pneumoniae, Z022/Serotype 19F	1.0 x 10^6 cfu/ mL	No
Streptococcus pyogenes. Bruno	1.0 x 10^6 cfu/ mL	No
Staphylococcus aureus, MASA, COL	1.0 x 10^6 cfu/ mL	No
Staphylococcus epidermidis, MRSE, RP62A	1.0 x 10^6 cfu/ mL	No
Pneumocystis jiroveci,W303-Pji	1.0 x 10^6 cfu/ mL	No
Coronavirus, NL63	7.0 x 10^4 $TCID_{50}$/ mL	No
Enterovirus 71, MP4	1.0 x 10^5 $TCID_{50}$/ mL	No
Adenovirus type 2, C	1.0 x 10^5 $TCID_{50}$/ mL	No
Coronavirus, 229E	1.0 x 10^5 $TCID_{50}$/ mL	No
Coronavirus, OC43	4.5 x 10^4 $TCID_{50}$/ mL	No
Metapneumovirus, TN/83-1211	1.0 x 10^5 $TCID_{50}$/ mL	No
Parainfluenza Virus 1/FRA/ 29221106/2009	1.0 x 10^5 $TCID_{50}$/ mL	No
Parainfluenza Virus 2, Greer	1.0 x 10^5 $TCID_{50}$/ mL	No
Parainfluenza Virus 3, NIH 47885	1.0 x 10^5 $TCID_{50}$/ mL	No
Parainfluenza Virus 4B, 19503	1.0 x 10^5 $TCID_{50}$/ mL	No
RSV, A1998/12-21	1.0 x 10^5 $TCID_{50}$/ mL	No
MERS-CoV, EMC/2012	1.0 x 10^5 $TCID_{50}$/ mL	No
SARS-CoV, Urbani	1.0 x 10^5 pfu/ mL	No
Rhinovirus 20, 15-CV19	5.0 x 10^5 $TCID_{50}$/ mL	No
Influenza A/New Caledonia/ 20/ 1999 (H1N1)	1.0 x 10^5 $CEID_{50}$/ mL	No
Influenza A/San Diego/1/2009 (H1N1) pdm09	1.0 x 10^5 $TCID_{50}$/ mL	No
Influenza A/Victoria/361/2011 (H3N2)	1.0 x 10^5 $CEID_{50}$/ mL	No
Influenza A/Wisconsin/67/ 2005 (H3N2)	1.0 x 10^5 $CEID_{50}$/ mL	No
Influenza B/Brisbane/60/2008	1.0 x 10^5 $CEID_{50}$/ mL	No
Influenza B/Texas/06/2011	1.0 x 10^5 $CEID_{50}$/ mL	No
Influenza B/GL/1739/54	1.0 x 10^5 $CEID_{50}$/mL	No

To estimate the likelihood of cross-reactivity with SARS-CoV-2 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.

- *The comparison between SARS-CoV-2 nucleocapsid protein and human coronavirus HKU1 revealed that the only potential for homology was with the HKU1 nucleocapsid phosphoprotein. Although homology was relatively low, at 36.7% across 82% of sequences, but cross-reactivity cannot be ruled out.*
- *No protein sequence homology was found between M. tuberculosis, however, cross-reactivity cannot be ruled out.*

5) Endogenous Interference

To assess endogenous interference with the performance of the Nano-Check™ 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 sixteen (16) potentially interfering substances do not cross-react nor interfere with the detection of SARS-CoV-2 in Nano-Check™ COVID-19 Antigen Test.

Table 9. Endogenous Interference

Potential Interfering Substances	Concentration
Nasal Spray 1 - Afrin	15% v/v
Nasal Spray 2 - NasalCrom	15% v/v
Nasal Spray 3 - FLONASE	15% v/v
Sore Throat 1 - Oral Pain Reliver Spray	15% v/v
Sore Throat 2 - Lozenges	15% w/v
Nasal Drops	15% v/v
NasoGel (Gel Spray)	15% v/v
Nasal Allergy Relief	15% v/v
Homeopathic Allergy Nasal Spray	15% v/v

Zinc Lozenges	5% w/v
Mucin	0.5%
Tobramycin	4 µg/mL
Mupirocin	10 mg/mL
Tamiflu (Oseltamivir Phosphate)	5 mg/mL
Whole Blood	4%
Biotin	3500 ng/mL

6) High-Dose Hook Effect

The Nano-Check™ COVID-19 Antigen Test was tested up to 2.8 x 10^6 $TCID_{50}$/mL of gamma-irradiated SARS-CoV-2 and no high-dose hook effect was observed.

7) Point of Care Use

The Nano-Check™ COVID-19 Antigen Test demonstrated at near patient or Point of Care (POC) testing that non-laboratory personnel can perform the test accurately in the intended use environment. In addition, the robust use of the Nano-Check™ COVID-19 Antigen Test for near patient or Point of Care (POC) testing was verified by twelve (12) Flex studies.

12. REFERENCES

1. IFCC Information Guide on COVID-19 (https://www.ifcc.org/ ifcc-news/2020-03-26-ifcc-information-guide-on-covid-19/).
2. Li Z, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. J Med Virol 2020; DOI: 10.1002/jmv.25727.
3. Neeraja R, et al. Diagnostics for SARS-CoV-2 detection: A comprehensive review of the FDA-EUA COVID-19 testing landscape. Biosensors and Bioelectronics 165 (2020) 112454.
4. Gimenez LG, et al. Development of an Enzyme-Linked Immunosorbent Assay-Based Test with a Cocktail of Nucleocapsid and Spike Proteins for Detection of Severe Acute Respiratory Syndrome-Associated Coronavirus-Specific Antibody. Clin Vaccine Immunol 2009;16(2); 241–245.
5. Diao B, et al. Diagnosis of Acute Respiratory Syndrome Coronavirus 2 Infection by Detection of Nucleocapsid Protein. medRxiv doi:https://doi.org/10.1101/2020.03.07.20032524.
6. Liotti FM, et al. Performance of a novel diagnostic assay for rapid SARS-CoV-2 antigen detection in nasopharynx samples. Clinical Microbiology and Infection, https://doi.org/10.10 16/j.cmi. 2020.09.030.

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For Use Under an Emergency Use Authorization (EUA) Only.
For *in vitro* diagnostic use, For prescription use only, For use with kit provided swabs

Rx ONLY IVD

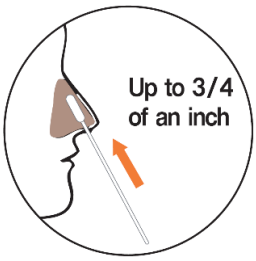
The Nano-Check™ COVID-19 Antigen Test is a lateral flow immunoassay intended for the qualitative detection of nucleocapsid antigen from SARS-CoV-2 in direct anterior nasal swab and direct nasopharyngeal swab specimens collected from individuals who are suspected of COVID-19 by their healthcare provider within the first five (5) days of symptoms when tested at least twice over three days with at least 48 hours between tests or from individuals without symptoms or other epidemiological reasons to suspect COVID-19 when tested at least three times over five days with at least 48 hours between tests.

IMPORTANT:

- Refer to Product Insert, including QC section, for complete use instructions, warnings, precautions, and limitations.
- The test kit should be stored at 2°C - 30°C in the original sealed pouch. Do not freeze and bring to room temperature at least 30 minutes prior to use. For the most current expiration dates of this test, please refer to: http//www.fda.gov.covid-tests.
- The freshly collected anterior nasal swab and nasopharyngeal swab specimen are recommended to be processed no later than one hour after specimen collection at room temperature (15°C - 30°C) or before 48 hours when stored at 2°C to 8°C.
- Refer to Interim Guidelines for Collecting, Handling and Testing Clinical Specimens from persons for COVID-19 at https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html

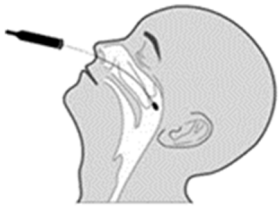
Sample Collection Method

Anterior Nasal Swab



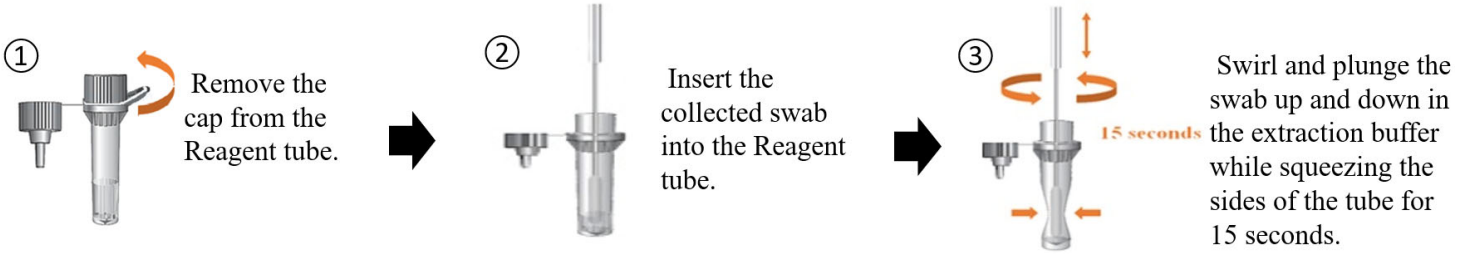
To collect the anterior nasal swab sample, tilt the patient's head back 70 degrees and insert the soft end of the swab into patient's nostril no more than ¾ of an inch into the nose. Slowly rotate the swab, gently pressing against the inside of patient's nostril at least 5 times for a total of 15 seconds. Get as much nasal discharge as possible on the soft end of the swab. Gently remove the swab. Use the same end of the swab and repeat the same steps on the other nostril.

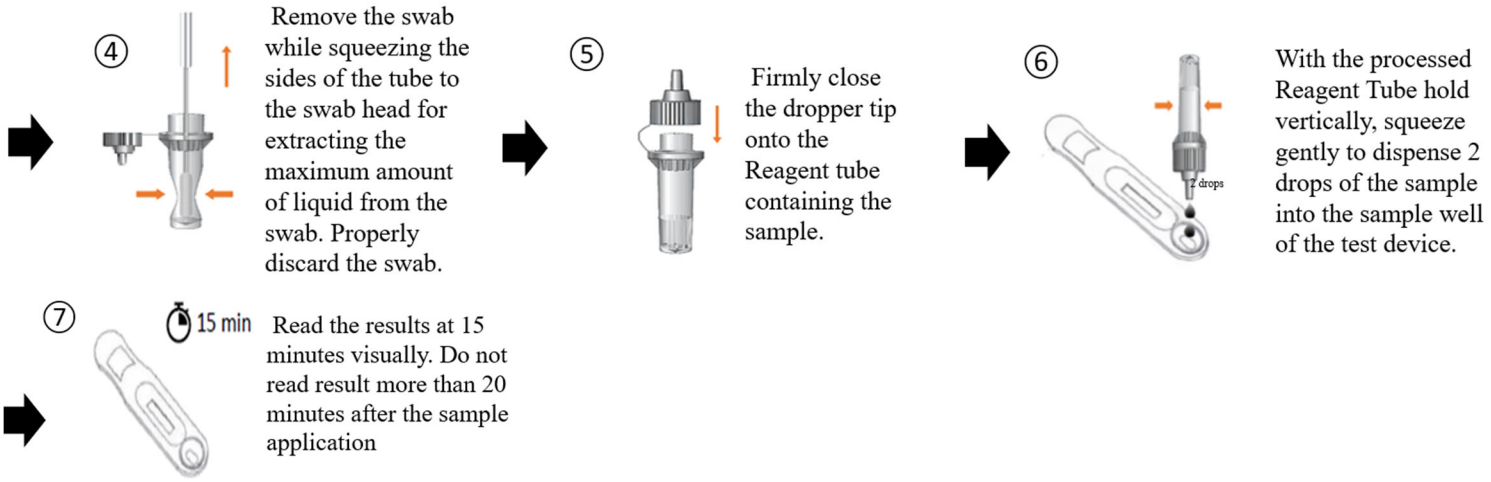
Nasopharyngeal Swab



To collect the nasopharyngeal swab sample, tilt the patient's head back 70 degrees. Carefully insert the swab into the nostril that presents the most secretion under visual inspection. Slowly insert the swab parallel to the palate until resistance is encountered, or the distance is equivalent to that from the ear to the nostril of the patient. Rotate the swab several times, leave the swab in place for several seconds to absorb secretions and then remove it from the nasopharynx.

Sample Testing Method





Positive

Appearance of pinkish red colored bands at both the control line and the test line indicates positive result. The colored test line depending on the concentration of SARS-CoV-2 virus in the test specimen will appear. The line in the control region (C) is the control line, which is used to indicate proper performance of the device. The color intensity of the test lines may be weaker or stronger than that of the control line.

Negative

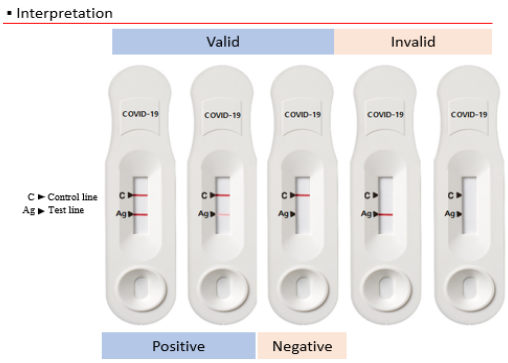
A single pinkish red colored band at the control line without visual test line is a negative result. Negative result does not indicate the absolute absence of SARS-CoV-2 virus in specimen or rule out COVID-19; it only indicates that the specimen does not contain the virus concentration at above the detection limit of the level.

Note: All negative results are presumptive and may be confirmed with a molecular assay, if necessary, for patient management.

Note: Serial testing should be performed in individuals with negative results at least twice over three days (with 48 hours between tests) for symptomatic individuals and three times over five days (with at least 48 hours between tests) for asymptomatic individuals. You may need to purchase additional tests to perform this serial (repeat) testing.

Invalid

If no lines are seen or no colored line appears in the control region(C), the test result is invalid. If the invalid result is obtained during initial testing, the assay should be repeated with a new test device



External Quality Control Test Step Instructions

External positive and negative control swabs are supplied with each kit. These controls provide additional quality control material to assess that the test kit reagents perform as expected. Process the controls in the same manner as clinical sample swab, and conduct the assay as described in Test Procedure section. It is recommended that positive and negative external control swabs are run once with every new lot, shipment, and each new user.

- Read all instructions carefully before performing the test. Failure to follow the instructions may result in inaccurate test results.
- In the USA, this product has not been FDA cleared or approved, but has been authorized by FDA under an EUA for use by authorized laboratories; use by laboratories certified under the CLIA, 42 U.S.C. §263a, that meet requirements to perform moderate complexity, high complexity, or waived tests. This product is authorized for use at the Point of Care (POC), i.e., in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation.
- This product has been authorized only for the detection of proteins from SARS-CoV-2, not for any other viruses or pathogens.
- In the USA, the emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of the virus that causes COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or the authorization is revoked sooner.

Glossary

℞ ONLY Prescription use only	IVD For In Vitro use only	Manufacturer	CE CE mark of conformity	EC REP Authorized Representative in the European Community
LOT Batch code	Use by	Temperature limitation	Consult instructions for use	Σ 20 Contains sufficient for 20 determinations
REF Catalog number	CONTROL + Positive control	CONTROL − Negative control		