Nano-Check™ COVID-19 Antigen At-Home Test

Healthcare Provider Instructions for Use
For Use Under an Emergency Use Authorization (EUA) Only
For *In Vitro* Diagnostic Use
For Use with Kit Provided Swabs

1. INTENDED USE

The Nano-Check™ COVID-19 Antigen At-Home Test is a lateral flow immunoassay intended for the qualitative detection of nucleocapsid protein antigen from SARS-CoV-2.

This test is authorized for non-prescription home use with self-collected anterior nasal (nares) swab samples from individuals aged 14 years or older or adult collected anterior nasal (nares) swab samples from individuals aged two years or older. This test is authorized for individuals with symptoms of COVID-19 within the first 6 days of symptom onset when tested at least twice over three days with at least 48 hours between tests, and for 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.

The Nano-Check™ COVID-19 Antigen At-Home Test does not differentiate between SARS-CoV and SARS-CoV-2.

Results are for the identification of SARS-CoV-2 nucleocapsid protein antigen, which is generally detectable in anterior nasal (nares) samples during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with past medical history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses and the agent detected may not be the definitive cause of disease. Individuals who test positive with the Nano-Check™ COVID-19 Antigen At-Home Test should self-isolate and seek follow up care with their physician or healthcare provider as additional testing may be necessary.

All negative results are presumptive and confirmation with a molecular assay, if necessary for patient management, may be performed. 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 measures such as isolating from others and wearing masks. Negative 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.

Individuals who test negative and continue to experience COVID-19 like symptoms of fever, cough and/or shortness of breath may still have SARS-CoV-2 infection and should seek follow up care with their physician or healthcare provider.

Individuals should provide all results obtained with this product to their healthcare provider for public health reporting and to receive appropriate medical care. All healthcare providers will report all test results they receive from individuals who use the authorized product to relevant public health authorities in accordance with local, state, and federal requirements, using appropriate LOINC and SNOMED codes, as defined by the Laboratory In Vitro Diagnostics (LIVD) Test Code Mapping for SARS-CoV-2 Tests provided by CDC.

The Nano-Check™ COVID-19 Antigen At-Home Test is intended for non-prescription self-use and/or as applicable, for an adult lay user testing another person aged 2 years or older in a non-laboratory setting. The Nano-Check™ COVID-19 Antigen At-Home 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.

IMPORTANT! How To Use This Test

- *Serial testing should be performed in all individuals with negative results; individuals with symptoms of COVID-19 and initial negative results should be tested again after 48 hours. Individuals without symptoms of COVID-19, and with initial negative results, should be tested again after 48 hours and, if the 2nd test is also negative, a 3rd time after an additional 48 hours. You may need to purchase additional tests to perform this serial (repeat) testing.*

- *If you test negative but 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 your healthcare provider.*

- *If your test is positive, then proteins from the virus that causes COVID-19 have been found in your sample and you likely have COVID-19.*

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 At-Home Test is a rapid chromatographic immunoassay intended for the direct detection of presence or absence SARS-CoV-2 antigen in 15 min using self-collected or parent/guardian-collected nasal samples from individuals suspected of COVID-19.

3. PRINCIPLE

The Nano-Check™ COVID-19 Antigen At-Home Test is authorized for non-prescription home use with self-collected anterior nasal (nares) swab samples from individuals aged 14 years or older with symptoms of COVID-19 within the first six (6) days of symptom onset. This test is also authorized for non-prescription home use with adult-collected anterior nasal (nares) swab samples from individuals aged 2 years or older with symptoms of COVID-19 within the first six (6) days of symptom onset.

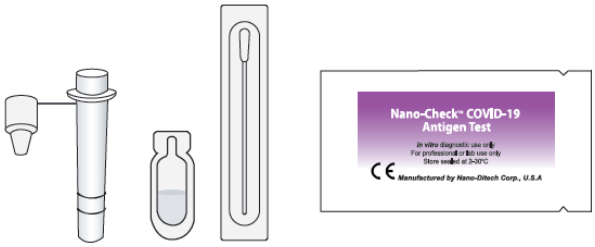
When specimens are lysed and added to the sample well of test device, SARS-CoV-2 viral antigens present in the specimen bind to antibodies against SARS-CoV-2 nucleocapsid conjugated to gold colloidal particles and biotin in the test strip. The antigen-conjugate immunocomplexes migrate across the test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

Test results are visually interpreted at 15-30 minutes (but no later than 30 minutes). 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 line 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

- 2 COVID-19 Test devices individually sealed aluminum foil pouch with desiccant
- 2 Empty tubes
- 2 Sealed ampules with lysis solution
- 2 Sample collection swabs
- 1 Quick Reference Instruction (Please see the company website: https://www.nanoditech.com)



Required but not provided

- Timer
- Any necessary personal protective equipment

5. WARNINGS, PRECAUTIONS, AND SAFETY INFORMATION

- 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 Emergency Use Authorization. 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 diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the 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 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.**
- An anterior nasal (nares) swab sample can be self-collected by an individual age 14 years and older. Children aged 2 to 13 years should be tested by an adult.
- Do not use on anyone under 2 years of age.
- Wear a safety mask or other face-covering when collecting a specimen from a child or another individual.
- Do not use if any of the test kit contents or packaging is damaged.
- 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 30 minutes. Results read before 15 minutes or after 30 minutes may lead to a false negative, false positive, or invalid result**.
- Ensure that there is sufficient lighting for testing and interpretation.
- When collecting an anterior nasal (nares) swab sample, only use the swab provided in the kit.
- Do not use kit past its expiration date. For information about current expiration dates for at-home OTC COVID-19 diagnostic tests, visit At-Home OTC COVID-19 Diagnostic Tests.
- If you have had symptoms longer than 6 days you should consider testing at least three times over five days with at least 48 hours between tests.
- Test samples immediately after collection. Samples are stable for up to 1 hour after swab placement into extraction buffer, if kept at room temperature.
- This product is only for the detection of proteins from SARS-CoV-2, not for any other viruses or pathogens.
- Testing should be performed in an area with good lighting and room temperature conditions.
- Dispose of all materials in household waste.
- Wash hands thoroughly or use hand sanitizer before and after the test.
- 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 times; 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.
- **Keep testing kit and kit components away from children and pets before and after use. Avoid contact with your skin, eyes, nose, or mouth. Do not ingest any kit components. The reagent solution contains harmful chemicals (see table below). If the solution contacts your skin, eyes, nose, or mouth, flush with large amounts of water. If irritation persists, seek medical advice: https://www.poisonhelp.org or 1-800-222-1222.**

Chemical Name	Harms (GHS Code) for each ingredient	Concentration
Gentamicin	Skin sensitization (H317)	0.004%
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

6. LIMITATIONS

- The performance of this test was established based on the evaluation of a limited number of clinical specimens collected between June 2022 and July 2022. The clinical performance has not been established for 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.
- 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.
- 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 the individual likely has COVID-19.
- These test results are shown as lines of color. Because these lines can be very faint, users with conditions affecting their vision - such as far-sightedness, glaucoma, or color blindness - are encouraged to seek assistance to interpret results accurately (e.g., reading glasses,

additional light source, or another person).

- Incorrect test results may occur if a specimen is incorrectly collected or handled.
- This test detects both viable (live) and nonviable SARS-CoV-2. 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.
- Test results must be evaluated in conjunction with other clinical data available to the physician.
- Positive test results do not exclude co-infection with other pathogens.
- Negative test results are not indicative of the presence/absence of other viral or bacterial pathogens.
- If the differentiation of specific coronaviruses and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- False negative results may occur in individuals who have indicated or whose clinical status or history would indicate they are currently taking high doses of biotin. There was no interference up to 3,500 ng/mL of biotin in the samples.

7. STORAGE CONDITIONS

Store the Nano-Check™ COVID-19 Antigen At-Home Test between 36-86°F (2-30°C) in a place out of direct sunlight and out of reach of children. Reagents and devices must be stored at room temperature 65-86 °F (18-30 °C) before use. It is recommended to use the test kit immediately after opening. Unsealed cassettes that are not used within 90 minutes should be discarded. The expiration date for the unopened kit assigned at manufacturing is on the package. For information about current expiration dates for Nano-Check™ COVID-19 Antigen At-Home Test, visit At-Home OTC COVID-19 Diagnostic Tests.

8. QUALITY CONTROL

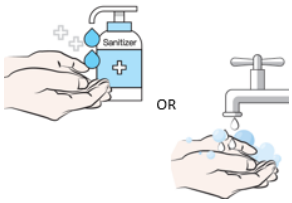
Internal Quality Control: The presence of a pinkish red colored band in the Control area of the window acts as an internal control to ensure adequate migration has occurred, but does not determine if an adequate sample has been added. In the absence of this Control line, the test is invalid and must 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.

9. TEST PROCEDURE

Scan the QR code to watch and follow "Nano-Check COVID-19 Antigen At-Home Test" instruction video on smart phone. Each step has a corresponding instructional video on the website. Watch the video and perform the test according to the instructions.

Note:
Freshly collected specimens should be processed as soon as possible.

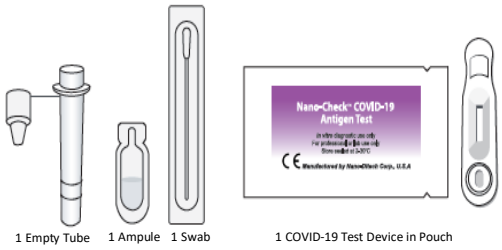
Wash hands with soap and water or use hand sanitizer before starting the test.



Remove the test device from the sealed pouch immediately before use. Conduct all testing on a level surface.

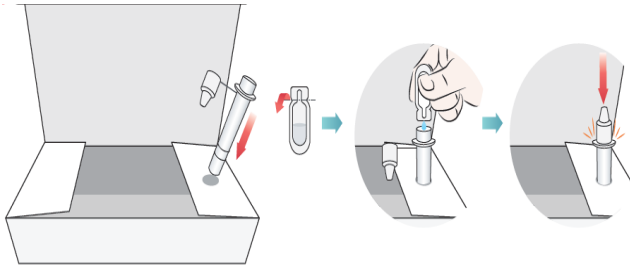
Step 1. Prepare Materials

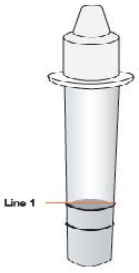
Open the package, take out the COVID-19 Test device in Pouch, empty tube, ampule, and the Swab. When you are ready to proceed with the test, open the foil pouch of the COVID-19 Test Device.



Please look carefully, there are two lines on the empty tube.
Insert the empty tube through the circle hole on the side flap of kit box to form a tube holder. Flip over the TOP part of the ampule cap, then squeeze the ampule completely into the empty tube. Close the tube tightly with the dropper tip.

Please confirm the liquid level is at or above line 1, then go to Step 2 Collect Sample.



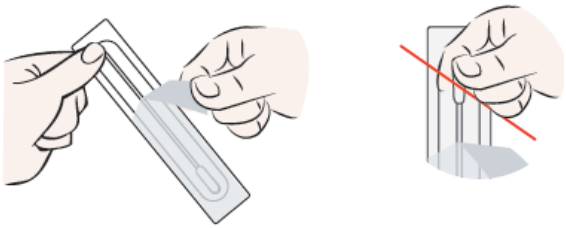


Note:
It is acceptable if the liquid level is above the line 1.

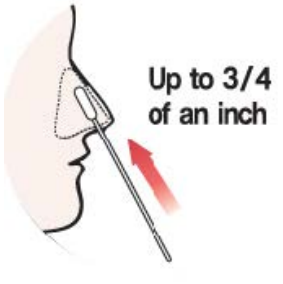
However, please do not proceed with this test, if the liquid level is below the line 1, as this may result in false or invalid results.

Step 2. Collect Sample

a. Remove the swab from its package, being careful not to touch the tip of the swab. Please keep the swab package for later use.

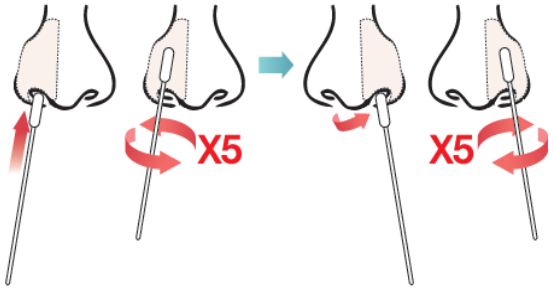


b. Gently insert the entire absorbent tip of the swab (usually ½ to ¾ of an inch) into your nostril.



Note: With children, the maximum depth of insertion into the nostril may be less than ¾ of an inch, and you may need to have a second person hold the child's head while swabbing.

c. Firmly and slowly brush against insides of nostril in a circular motion against the nasal wall at least 5 times (Take at least 15 seconds to collect the specimen and be sure to collect any nasal drainage on the swab). Using the same swab, repeat the same sample collection procedure for the other nostril. Be sure to brush **BOTH** nostrils with the **SAME SWAB.**

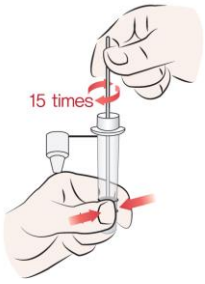


Note: Failure to swab properly may cause false negative results.

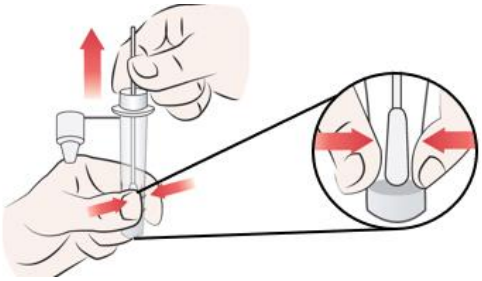
Step 3. Process Sample

a. Tap the tube vertically on the table and remove the dropper tip to open the tube.

b. Insert the swab into the tube until the swab head touches the bottom of the tube. Hold the swab head at the bottom of the tube tightly by squeezing the tube. Then stir the swab at least 15 times.

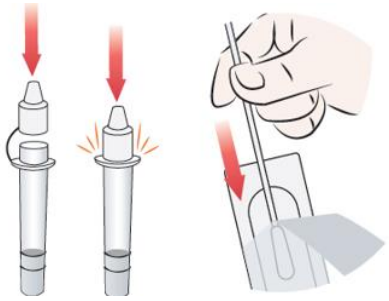


c. Squeeze the sides of the tube to express as much liquid as possible from the swab head, and then remove the swab.



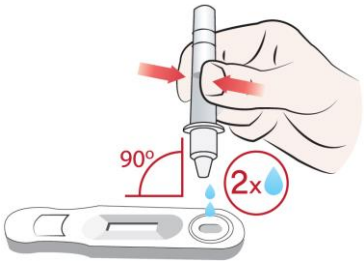
Note: If you don't squeeze the swab head, there may not be sufficient sample material to perform the test properly. (i.e., potentially resulting in a false negative result).

d. Firmly close the dropper tip, put the swab back into the package. Safely dispose of the swab and the package.



Step 4. Add Sample

Hold the tube vertically to dispense the sample. Add 2 drops of sample to the Sample loading hole of the COVID-19 Test device.



Note: A false negative or invalid result may occur if too little solution is added to the test device.

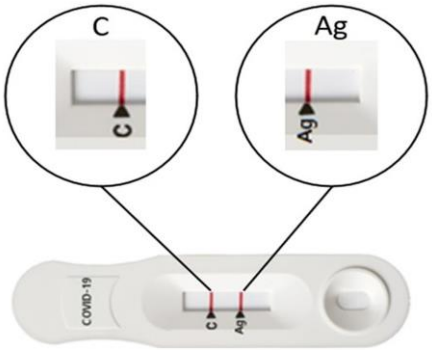
Step 5. Wait 15 Minutes

Wait 15 minutes after adding sample to the Sample loading hole and read the results at 15 minutes visually.

Note: Do NOT interpret your result until after your 15-min timer has completed, as the Ag line may take as long as 15 minutes to appear.

Step 6. Read Result
Result should not be read after 30 minutes (Result shown at 2x magnification).

Note: A false negative or false positive result may occur if the test result is read before 15 minutes or after 30 minutes.



Note: The Ag line can be extremely faint.

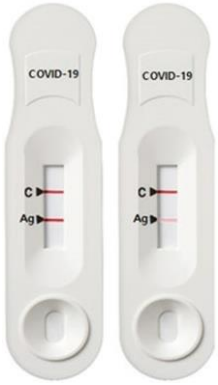
Step 7. Test Result Interpretation

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	N/A	N/A	Positive for COVID-19
	Negative	Positive	N/A	Positive for COVID-19
	Negative	Negative	N/A	Negative for COVID-19
Without Symptoms	Positive	N/A	N/A	Positive for COVID-19
	Negative	Positive	N/A	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.

COVID-19 Positive (+) Result



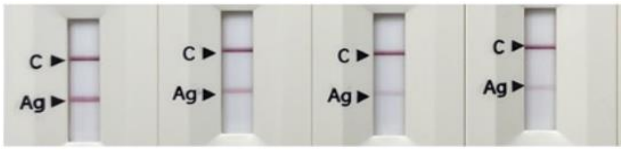
If the Control (C) line and the Test (Ag) line are visible, the test is positive. Any faint visible colored Test (Ag) line with the control line (C) should be read as positive.

Repeat testing does not need to be performed if patients have a positive result at any time.

A positive test result means that the virus that causes COVID-19 was detected in the sample, and it is very likely the individual has COVID-19 and is contagious. Please contact the patient's doctor/primary care physician (if applicable) and the local health authority immediately and instruct your patient to adhere to the local guidelines regarding self-isolation. There is a very small chance that this test can give a positive result that is incorrect (a false positive).

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. Individuals who test positive with the Nano-Check™ COVID-19 Antigen At-Home Test should self-isolate and seek follow up care with their physician or healthcare provider as 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 low prevalence of infection.

Below are photos of actual positive tests. Please note that the Ag line may be faint.



COVID-19 Negative (-) Result

If the Control (C) line is visible, but the Test (Ag) line is not visible, the test is negative.

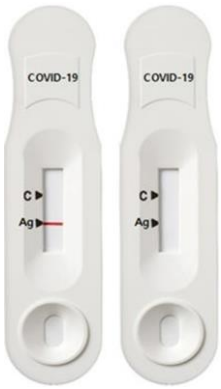
To increase the chance that the negative result for COVID-19 is accurate, you should:

- **Test again in 48 hours if the individual has symptoms on the first day 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 disease should be considered. If applicable, seek 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 a close contact with COVID-19 or with suspected exposure to COVID-19 or in communities with 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.

Invalid Result



If the control (C) line is not visible, the test is invalid. Re-test with a new swab and new test device.

Invalid result means that the test did not function correctly. **You will need to retest with a fresh sample, new ampule, and new test device.**

If upon retesting, the test result is still invalid, contact our technical support.

Step 8. Dispose the Test Kit

After test is completed, dispose of all kit components in the trash and wash your hands.

Step 9. Report Test Result

Report your test result(s) at MakeMyTestCount.Org – this voluntary and anonymous reporting helps public health teams understand COVID-19 spread in your area and across the country and informs public health decisions.

10. PERFORMANCE CHARACTERISTICS

1) Assay Sensitivity: Limit of Detection (LoD)

The analytical sensitivity of Nano-Check™ COVID-19 Antigen At-Home Test, Limit of Detection (LoD) was established using serial dilutions of gamma-irradiated SARS-CoV-2 isolate USA-WA1/2020 (NR-52287) and heat-inactivated SARS-CoV2 Omicron (hCoV/USA/MD-HO 20874/2021. Contrived samples were prepared by spiking the strain into pooled negative nasal wash matrix. 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 At-Home 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 was determined to be 7.0×10^{2}TCID$_{50}$/mL (USA-WA1/2020) and 1.95×10^{2}TCID$_{50}$/mL (hCoV/USA/MD-HO20874/2021), equating to 3.5×10^{1} TCID$_{50}$/swab and 9.75×10^{0} TCID$_{50}$/swab, respectively.

2) Assay Cross Reactivity and Microbial Interference

Cross-reactivity of the Nano-Check™ COVID-19 Antigen At-Home Test was evaluated by testing a panel of high prevalence respiratory pathogens that could potentially cross-react with the Nano-Check™ COVID-19 Antigen At-Home 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 with the tested organism. The results for cross-reactivity and microbial interference are presented in the table below.

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.*

3) Endogenous Interference

To assess endogenous interference with the performance of the Nano-Check™ COVID-19 Antigen At-Home 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 twenty-three (23) potentially interfering substances do not cross-react nor interfere with the detection of SARS-CoV-2 in Nano-Check™ COVID-19 Antigen At-Home Test.

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
Nasal Spray 4 - CVS	15% v/v
Sore Throat 1 - Oral Pain Reliver Spray	15% v/v
Sore Throat 2 - Lozenges	15% w/v
Nasal Drops - Orrivine	15% v/v
NasoGel (Gel Spray) - NeIlMed®	15% v/v
Nasal Allergy Relief -Similasan	15% v/v
Homeopathic Allergy Nasal Spray - Alkalol	15% v/v
Zinc Lozenges - Walgreen	5% w/v
Healing Ointment - Cetaphil	0.5% w/v
Daily Moisturizing Lotion - Aveeno	0.5% w/v
Hand Soap Fresh Breeze Scent - Softsoap	10% v/v
Antibacterial Liquid Hand Soap- Dial Complete	1% v/v
Hand Sanitizer Gel- Ethyl alcohol 70%	1% v/v
Disinfectant Spray - Lysol	1% v/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

4) High-Dose Hook Effect

The Nano-Check™ COVID-19 Antigen At-Home 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.

5) Clinical Performance

A prospective study was completed at three (3) sites between June and July 2022 in the United States for clinical validation of the Nano-Check™ COVID-19 Antigen At-Home Test for the detection of the SARS-CoV-2 in subject-collected anterior nasal (AN) swab samples. The study evaluated the investigational test's performance in symptomatic individuals (those suspected of COVID-19).

A total of 131 symptomatic subjects were enrolled and each were currently experiencing symptoms associated with COVID-19, within 6 days of symptom onset. Each enrolled subject either self-collected one sample from their anterior nasal passages (from both nostrils), or had one sample collected from him/her by another individual. Each subject then had a second AN swab sample (from both nostrils) collected from him/her by one of the study personnel. Test results from the Nano-Check™ COVID-19 Antigen At-Home Test (candidate test) were compared to highly sensitive molecular FDA EUA Authorized SARS-CoV-2 assays to determine test performance.

As shown, the positive percent agreement (PPA) is 83.64% and the negative percent agreement (NPA) is 98.68% with the 95% confidence interval bounds of 71.74% to 91.13% for the PPA and 92.92% to 99.77% for the NPA, respectively.

Nano-Check™ COVID-19 Antigen At-Home Test	Comparator RT-PCR		Total
	Positive	Negative	
Positive	46	1[1)]	47
Negative	9[2)]	75	84
Total	55	76	131
PPA[3)] = (46/55) x 100% = 83.64% (95% CI: 71.74-91.13%)			
NPA[4)] = (75/76) x 100% = 98.68% (95% CI: 92.92-99.77%)			

1) One discrepant sample was negative by 2nd FDA EUA authorized RT-PCR method.
2) Of 9 discrepant results, a sample was negative by both Nano-Check™ COVID-19 Antigen At-Home Test and 2nd FDA EUA authorized RT-PCR method.
3) PPA: Positive Percent Agreement; 4) NPA: Negative Percent Agreement

Subject Age	Female	Male	Positives	% Positivity Rate
< 14 years of age	13	20	11	33.33
14 - 23 years of age	10	7	8	47.06
24 - 64 years of age	36	25	24	39.34
≥65 years of age	10	10	12	60.00
Total	69	62	55	41.98

Days Post Onset	Number of Specimens Tested	RT-PCR Positive	Confirmed Positives	PPA
0-1	23	13	9	69.23
0-2	73	34	29	85.29
0-3	104	43	35	81.40
0-4	119	49	40	81.63
0-5	126	53	44	83.02
0-6	131	55	46	83.64

6) Serial Testing

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 is 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 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 1.

Table 1: 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 study combined.

DAYS AFTER FIRST PCR POSITIVE TEST RESULT	ASYMPTOMATIC ON FIRST DAY OF TESTING			SYMPTOMATIC ON FIRST DAY OF TESTING		
	Ag Positive / PCR Positive (Antigen Test Performance % PPA)					
	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 on the noted days after first PCR positive test result. Day 0 is the first day of documented infection with SARS-CoV-2.
2 Test = 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 Test = 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.

7) 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 2: 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

11. FREQUENTELYY ASKED QUESTIONS (FAQ):

1) WHAT ARE THE KNOWN AND POTENTIAL RISKS AND BENEFITS OF THE TEST?

Potential risks include:
- Possible discomfort during sample collection.
- Possible incorrect test result (see Warnings and Result Interpretation sections for more information).

Potential benefits include:
- The results, along with other information, can help you and your healthcare provider make informed recommendations about your care.
- The results of this test may help limit the potential spread of COVID-19 to your family and others in your community.

For more information on EUAs go here: https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization

2) WHAT IS THE DIFFERENCE BETWEEN AN ANTIGEN AND MOLECULAR TEST?

There are different kinds of tests for the SARS-CoV-2 virus that causes COVID-19. Molecular tests detect genetic material from the virus. Antigen tests, such as the Nano-Check™ COVID-19 Antigen At-Home Test, detect proteins from the virus. Due to the lower sensitivity of antigen tests, there is a higher chance this test will give you a false negative result when you have COVID-19 than a molecular test would.

3) HOW ACCURATE IS THIS TEST?

Clinical studies have shown that antigen tests more accurately determine whether you are infected with the virus that causes COVID-19 when taken multiple times across several days. Repeat testing improves test accuracy. This serial testing approach is recommended to minimize the risk of incorrect results.

4) WHAT IF I HAVE A POSITIVE TEST RESULT?

A positive result means that it is very likely you have COVID-19 because proteins from the virus that causes COVID-19 were found in your sample. You should self-isolate from others and contact a healthcare provider for medical advice about your positive result.

5) WHAT IF I HAVE A NEGATIVE TEST RESULT?

A negative test result indicates that antigens from the virus that causes COVID-19 were not detected in your sample. However, if you have symptoms of COVID-19, and your first test is negative, you should test again in 48 hours since antigen tests are not as sensitive as molecular tests. If you do not have symptoms and received a negative result, you should test at least two more times with 48 hours in between tests for a total of three tests. If you have a negative result, it does not rule out SARSCoV-2 infection; you may still be infected and you may still infect others. It is important that you work with your healthcare provider to help you understand the next steps you should take.

6) WHAT DOES AN INVALID TEST RESULT MEAN?

An invalid result means the test was not able to tell if you have COVID-19 or not. If the test is invalid, a new swab should be used to collect a new nasal specimen and you should test again with a new test.

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.

Nano-Ditech Corp.
259 Prospect Plains Road, Bldg. K
Cranbury, NJ 08512 USA
Tel: 1-855-297-7877
Info@nanoditech.com
www.nanoditech.com