INSTRUCTIONS FOR USE

Nano-Check™ Influenza+COVID-19 Dual Test

Under emergency use authorization (EUA) only
For *in vitro* diagnostic use
For Prescription Use Only
For use with anterior nasal swab specimens

1. INTENDED USE

The Nano-Check™ Influenza+COVID-19 Dual Test is a lateral flow immunochromatographic assay intended for in vitro rapid, simultaneous qualitative detection and differentiation of influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen directly from anterior nasal swab specimens of individuals with signs and symptoms of respiratory infection consistent with COVID-19 within the first five (5) days of symptom onset when tested at least twice over three days with at least 48 hours between tests. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar.

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 test 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.

Results are for the simultaneous in vitro detection and differentiation of SARS-CoV-2, influenza A virus, and influenza B virus protein antigens, but do not differentiate, between SARS-CoV and SARS-CoV-2 viruses and are not intended to detect influenza C antigens.

These viral antigens are generally detectable in anterior nasal 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 determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definitive cause of the disease.

All negative results are presumptive and should be confirmed with a molecular assay, if necessary, for patient management. Negative results do not rule out influenza or 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 each respiratory infection.

The Nano-Check™ Influenza+COVID-19 Dual Test is only for in vitro diagnostic use under the Food and Drug Administration's Emergency Use Authorization. This product has not been cleared or approved.

2. SUMMARY AND EXPLANATION OF THE TEST

COVID-19 and influenza are acute, highly contagious viral infections primarily affecting the respiratory tract. Immunologically diverse, single-stranded RNA viruses cause them: SARS-CoV-2 for COVID-19 and various influenza viruses (Types A, B, and C). Type A influenza is most prevalent and linked to severe illnesses, often causing epidemics. Type B causes milder illness and less frequent outbreaks, while Type C rarely causes widespread disease. COVID-19, caused by the novel coronavirus SARS-CoV-2, emerged in late 2019. Due to its rapid spread, the World Health Organization (WHO) declared a global pandemic on March 11, 2020.

A patient can be infected with a single virus or co-infected with SARS-CoV-2 and one or more types of influenza viruses. These viral infections occur more frequently during the respiratory illness season, which in the United States encompasses the fall and winter months. Symptoms typically manifest 3 to 7 days post-infection. Transmission of these viruses occurs readily through the coughing and sneezing of aerosolized droplets from infected individuals, who may be either symptomatic or asymptomatic. For symptomatic patients, the primary symptoms include fever, fatigue, dry cough, and loss of taste and smell. Additionally, nasal congestion, runny nose, sore throat, myalgia, and diarrhea are commonly associated symptoms.

3. PRINCIPLE

The Nano-Check™ Influenza+COVID-19 Dual Test is a rapid, immunochromatographic membrane assay that uses monoclonal antibodies to detect SARS-CoV-2 nucleocapsid protein and Influenza A and B proteins in anterior nasal swab specimens.

The test strip enclosed in a cassette housing is comprised of the following components: sample pad, reagent pad, biotin pad, reaction membrane, and absorbent pad. The reagent pad contains colloidal gold conjugated with monoclonal antibodies (mAb) specific for SARS-CoV-2, Influenza A, and Influenza B target proteins. The biotin pad contains biotin conjugated with mAb specific for SARS-CoV-2. The reaction membrane contains the secondary antibodies for the proteins of Flu A and Flu B, and streptavidin for the biotinylated SARS-CoV-2 antibody. The whole strip is fixed inside a plastic cassette.

When the sample extract is added into the sample well, conjugates dried onto the reagent/biotin pads are dissolved and migrate along with the sample. If Flu A, Flu B proteins and/or SARS-CoV-2 nucleocapsid antigen is present in the sample, a complex form between the anti-Flu A/Flu B/ SARS-CoV-2 conjugate and the viral antigen will be captured by the streptavidin or specific anti-Flu A/Flu B mAb coated on the test line region (C/A/B line). The absence of the test line (C/A/B line) suggests a negative result. To serve as a procedural control, a red line will always appear in the control line region (CON) indicating that proper volume of sample has been added and membrane wicking has occurred.

4. REAGENTS and MATERIALS

The Nano-Check™ Influenza+COVID-19 Dual Test kit contains reagents and materials for 20 tests. The following components are included in a kit.

Provided

- 20 Test devices in sealed aluminum foil pouch with desiccant
- 20 Empty reagent tubes
- 20 Ampules containing extraction buffer (0.3mL)
- 20 Sample collection swabs
- 1 Positive control swab
- 1 Negative control swab
- 1 Instructions for Use
- 1 Quick Reference Instruction

Required but not provided

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

5. WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use.

- 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, influenza A and influenza B, 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 SARS-CoV-2 negative results at least twice over three days (with 48 hours between tests) for symptomatic individuals. You may need to purchase additional tests to perform this serial (repeat) testing.**
- **Consistent with serial testing recommendations for SARS-CoV-2, for multi-analyte tests, symptomatic individuals who test positive for influenza A or B on the initial test but test negative for SARS-CoV-2 should be tested again in 48 hours to evaluate for co-infection with SARS-CoV-2 infection.**
- **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 results.**
- Do not use the test kit after its expiration date.
- Do not reuse the test cassette, processing solution, or swab.
- Do not use the test if the pouch is damaged or open.
- Do not interchange or mix components from different kit lots.
- Do not open the kit contents until ready for use. If the pouch is open for more than an hour, invalid test results may occur.
- Do not touch swab tip when handling the swab.
- Follow your clinical and/or laboratory safety guidelines and use appropriate precautions in the collection, handling, storage, and disposal of patient samples, all used kit contents, and all items exposed to patient samples.
- Use of nitrile or latex (or other equivalent) gloves and other personal protective equipment are recommended when handling patient samples.
- Inadequate or inappropriate sample collection, storage and transport may yield false test results.
- Only use the nasal swabs provided in the kit. Do not touch the swab tip prior to testing.
- Dispose of test kit contents in accordance with federal, state, and local regulations.
- Ensure that there is sufficient lighting for testing and interpretation.
- Do not use the kit to evaluate patient specimens if either the positive control swab or negative control swab fails to give the expected results.
- If any liquid spills from the buffer tube, discard test components and re-start the test using new test components.
- The Extraction Buffer vial contains only enough liquid for one test. Do not add a second buffer vial to the same reagent tube already containing the first extraction buffer as incorrect results may occur.
- **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: 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%
Gentamicin	Skin sensitization (H317)	0.004%

- 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: https://www.cdc.gov/COVID19

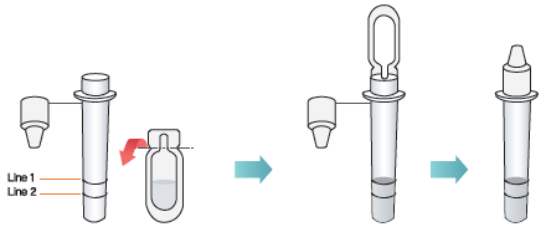
6. STORAGE AND STABILITY

- Store the test kit between 36~86°F (2~30°C) in a place out of direct sunlight.
- Reagents and materials must be used at room temperature (59~86°F/15~30°C).
- The unsealed cassette is stable for 1 hour. It is recommended to use the test kit immediately after opening.
- The expiration date is labeled on the package

7. SPECIMEN COLLECTION AND PREPARATION

NOTE: Do not open the test contents until ready for use. If the test cassette is open for an hour or longer, invalid test results may occur. When collecting a sample, only use the swab provided in the kit. Allow the test cassette, nasal swab and extraction buffer to come to room temperature [59~86°F/15~30°C] prior to testing.

1) Read all the instructions before you start the test.
2) Check the test's expiration date printed on the outer test packaging.
3) Open the package, take out the Test device in pouch, empty tube, ampule, and the swab.
4) **Fill the empty reagent tube with ampule solution.**



- Please look carefully, there are two lines on the empty tube.
- Flip over the TOP part of the ampule cap, then squeeze the ampule completely into the empty tube.

NOTE:
The level of liquid must be above line 1.

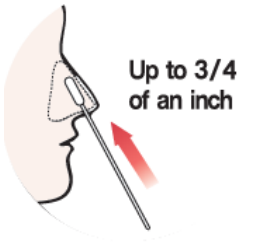
Do not proceed with this test, if the liquid level is below the line 1, as this may result in false or invalid results.

- Close the tube tightly with the dropper tip.

5) Remove the test cassette from the sealed pouch and lay it on a clean, flat surface.
6) Remove the swab from the pouch. Carefully insert the sterile swab no more than 3/4 inch (1.5 cm) into the nostril.

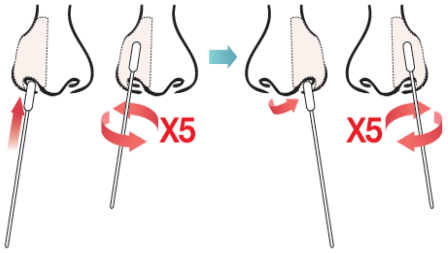
NOTE: Be careful not to touch the swab tip (soft end) with hand.

- Gently insert the entire absorbent tip of the swab (usually ½ to ¾ of an inch) into the patient's 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.

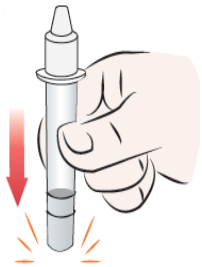
- Firmly and slowly rotate against insides of nostril in a circular motion against the nasal wall at least 5 times (Take at least 15 seconds to collect the specimen and be sure to collect any nasal drainage on the swab).
- Using the same swab, repeat the same sample collection procedure for the other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



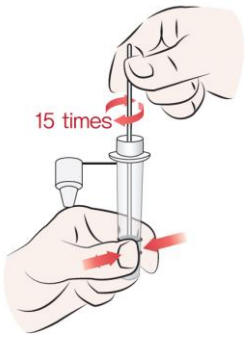
NOTE:
Failure to swab properly may cause false negative results.

8. TEST PROCEDURE

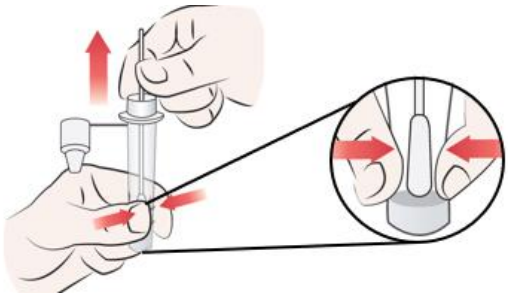
1) Tap the tube vertically on the table and remove the dropper tip to open the tube.



2) 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.

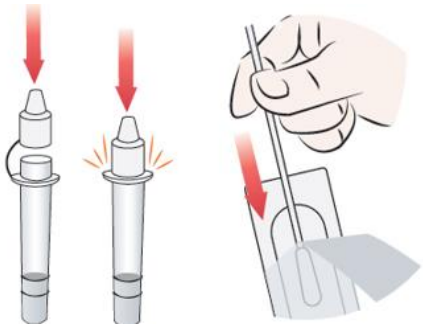


3) Squeeze the sides of the tube to express as much liquid as possible from the swab head, and then remove the swab.

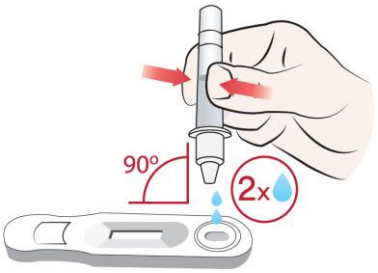


NOTE: If you don't squeeze the swab head, there may not be sufficient sample material to perform the test properly (i.e., potentially resulting in a false negative result).

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



5) Hold the tube vertically to dispense the sample. Add 2 drops of sample to the Sample loading well of the Test device



Caution: Invalid or false results can occur if less than 2 drops are added to the sample well.

NOTE: Sample must be applied to the test cassette within one hour of completing step 2).

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

NOTE: False results can occur if the test is read before 15 minutes or after 20 minutes

9. INTERPRETATION OF RESULTS

CON = Control line

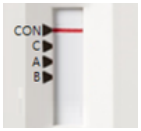
C = COVID-19 line
A = Influenza A line
B = Influenza B line

Look for lines next to CON, C, A, and B.

INVALID RESULT

A pinkish-red colored line should always appear at the Control line (Con) position. If a line does not form at the Control line position in 15 minutes, the test result is invalid and the test should be repeated with a new swab and a new test device.

NEGATIVE RESULT

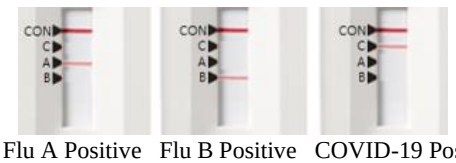


If the control line (CON) is visible, but no other lines appear the test is negative.

COVID-19 Negative (-) 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 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.

POSITIVE RESULT



Flu A Positive Flu B Positive COVID-19 Positive

COVID-19 & Flu A Positive

Flu A & Flu B Positive

If the Control line (C) is visible and one or more lines appear(s) for any of the viruses, the test is positive for that or those viruses.
A positive result does not rule out co-infections with other pathogens or identify any specific influenza A subtype, influenza B lineage, or SARS-CoV-2 variant.

NOTE: Positive test lines are usually very prominent but at times may vary in shade and intensity. A line of any intensity or thickness that appears in the Flu A, Flu B, or COV region is considered a positive result. The intensity of the C line should not be compared to that of the test line for the interpretation of the test result.
Take time to look at test lines very carefully. If you see a very light or faint test line appear, this is considered a POSITIVE result.

NOTE: It is possible to have more than one positive test line, which could indicate a co-infection with influenza A, B, and/or SARS-CoV-2. If more than one positive test line is observed, retest with a new patient sample and new test kit. Repeatable "dual positive" results should be confirmed by an FDA-cleared molecular assay before reporting results.

COVID-19 Positive (+) Result:
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™ Influenza+COVID-19 Dual 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.

Repeat Testing is needed to improve test accuracy for negative SARS-CoV-2 results. Please follow the table below when interpreting test results with symptoms. Serial (repeat) SARS-CoV-2 testing does not need to be performed if patients have a positive SARS-CoV-2 result.

Status on First Day of Testing: With Symptoms			
Day 0 (First Test)	Serial Testing	Day 2 (Second Test)	Final Interpretation
SARS-CoV-2 (+) Influenza A and B (-)	NO	Not needed	Positive for COVID-19 Presumptive Negative for Influenza
SARS-CoV-2 (+) Influenza A and/or B (+)	NO	Not needed	Positive for COVID-19 Positive for Influenza A and/or B

SARS-CoV-2 (-) Influenza A and/or B (-)	YES	SARS-CoV-2 (+) Influenza A and/or B (-)	Positive for COVID-19 Presumptive Negative for Influenza
SARS-CoV-2 (-) Influenza A and/or B (+)	YES	SARS-CoV-2 (+) Influenza A and/or B (+)	Positive for COVID-19 Positive for Influenza A and/or B
SARS-CoV-2 (-) Influenza A and/or B (-)	YES	SARS-CoV-2 (-) Influenza A and/or B (+)	Presumptive Negative for COVID-19 Positive for Influenza A and/or B
SARS-CoV-2 (-) Influenza A and/or B (-)	YES	SARS-CoV-2 (-) Influenza A and/or B (-)	Presumptive Negative for COVID-19 Presumptive Negative for Influenza
SARS-CoV-2 (-) Influenza A and/or B (-)	YES	SARS-CoV-2 (+) Influenza A and/or B (+)	Positive for COVID-19 Positive for Influenza A and/or B
SARS-CoV-2 (-) Influenza A and/or B (+)	YES	SARS-CoV-2 (-) Influenza A and/or B (-)	Presumptive Negative for COVID-19 Positive for Influenza A and/or B
SARS-CoV-2 (-) Influenza A and/or B (+)	YES	SARS-CoV-2 (-) Influenza A and/or B (+)	Presumptive Negative for COVID-19 Positive for Influenza A and/or B
SARS-CoV-2 (-) Influenza A and/or B (+)	YES	SARS-CoV-2 (+) Influenza A and/or B (+)	Positive for COVID-19 Positive for Influenza A and/or B

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 that an adequate volume of sample has been added. 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 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™ Influenza+ COVID-19 Dual 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. Please contact Technical Support at +1- 855-297-7877 or info@nanoditech.com.

11. LIMITATIONS

- The performance of this test was established based on the evaluation of a limited number of clinical specimens collected from November 2022 to February 2024. 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 SARS-CoV-2 as compared to a molecular test, especially in samples with low viral load.
- All antigen test negative results, for SARS-CoV-2 or influenza, 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 SARS-CoV-2 infection, however additional follow-up may be needed.
- If the test is positive, then proteins from the viruses that cause COVID-19 or influenza infection have been found in the sample and the individual likely has a respiratory infection with SARS-CoV-2 or influenza.
- Incorrect test results may occur if a specimen is incorrectly collected or handled.
- Use of Nano-Check™ Influenza+COVID-19 Dual Test is limited to laboratory personnel and CLIA waived users. Not for home use.
- The contents of this kit are to be used for the qualitative detection of influenza type A and B antigens as well as SARS-CoV-2 antigen from direct anterior nasal swab samples only.
- Negative test results are not indicative of the presence/absence of other viral or bacterial pathogens.
- This test detects both viable (live) and non-viable influenza A, influenza B, and 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 sample.
- A negative test result may occur if the level of antigen in the sample is below the detection limit of the test or if the sample is collected, handled or transported improperly.
- Failure to follow the instructions for use may adversely affect test performance and/or invalidate the test result.
- Test results must be evaluated in conjunction with other clinical data available.
- Positive test results do not exclude co-infections with other pathogens.
- Positive test results do not identify specific coronavirus, influenza A and B subtypes and strains.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods of low viral activity when prevalence is moderate to low.
- Individuals who recently received nasally administered influenza A or influenza B vaccine may have false positive test results after vaccination.
- Monoclonal antibodies may fail to detect, or detect with less sensitivity, influenza viruses that have undergone minor amino acid changes in the target epitope region.
- If differentiation of specific coronavirus or influenza A, influenza B subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- This test was not evaluated for use with the FluMist vaccine. Individuals who recently received nasally administered influenza A or influenza B vaccine may have false positive test results after vaccination.
- 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.

12. CONDITIONS OF AUTHORIZATION FOR THE LABORATORY AND PATIENT CARE SETTINGS

The Nano-Check™ Influenza+COVID-19 Dual Test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

However, to assist in using the Nano-Check™ Influenza+COVID-19 Dual Test ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

- Authorized laboratories* using your product must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating this labeling may be used, which may include mass media.
- Authorized laboratories using your product must use your product as outlined in Nano-Check™ Influenza+COVID-19 Dual Test Instructions for Use and Quick Reference Guide. Deviations from the authorized procedures, including authorized instruments, authorized clinical specimen types, authorized control materials, authorized ancillary reagents and authorized materials required to use your product are not permitted.
- Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- 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.
- Authorized laboratories must collect information on the performance of your product and report any significant deviations from the established performance characteristics of your product of which they become aware to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Nano-Ditech Corporation by contacting Technical Services (via email at info@nanoditech.com, via phone at 855-297-7877)
- 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.
- Nano-Ditech Corporation, authorized distributors, and authorized laboratories using your product must 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.

*The Letter of Authorization refers to "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet the requirements to perform moderate complexity, high complexity, or waived tests. This test 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".

13. CLINICAL PERFORMANCE

The clinical performance of the Nano-Check™ Influenza+COVID-19 Dual Test was established with 1589 anterior nasal samples that were prospectively collected from subjects between November 2022 and February 2024 at six clinical CLIA waived sites in the U.S. Samples were collected from sequentially enrolled subjects who presented with symptoms of respiratory infection. Two anterior nasal swabs were collected from each study subject during the same visit with the comparator collected first, followed by collection of the candidate test sample. Samples were tested with the Nano-Check™ Influenza+COVID-19 Dual Test. All subjects were confirmed as positive or negative by an FDA-cleared RT-PCR method, used as the comparator method for the study. Nano-Check™ Influenza+COVID-19 Dual Test was performed by operators who had no prior experience in the laboratory and were representative of the intended users in CLIA-waived settings. Operators used only the QRI to conduct testing without training provided. All testing was conducted by operators in a blinded fashion. Test results from the Nano-Check™ Influenza+COVID-19 Dual were compared to the results generated from comparator tests.

SARS-CoV-2: Nano-Check™ Influenza+COVID-19 Dual Test Results versus RT-PCR Comparator

Nano-Check™ Influenza+ COVID-19 Dual Test	Comparator RT-PCR		Total
	Positive	Negative	
Positive	192	1	193
Negative	34	1362	1396
Total	226	1363	1589

Positive Percent Agreement = (192/226) = 85.0% (95% CI: 79.7% - 89.0%)
Negative Percent Agreement = (1362/1363) = 99.9% (95% CI: 99.6% - 100.0%)

SARS-CoV-2: Nano-Check™ Influenza+COVID-19 Dual Test Results by Age Group versus RT-PCR Comparator

Age Group	Comparator RT-PCR method		
	# of Specimens Tested	# of Positive Specimens	Prevalence (%)
≤ 5 years	285	15	5.26
6 to 21 years	624	45	7.21
22 to 60 years	508	118	23.23
≥61 years	172	48	27.91
Total	1589	226*	14.22

**Nano-Check™ Influenza+COVID-19 Dual Test yielded positive results for 12 samples (80.0%) in the age group below 5 years old, 36 samples (90.0%) in the age group of 6 to 21 years, 99 samples (83.4%) in the age group of 22 to 60 years, and 45 samples (93.8%) in the age group more than 61 years.*

SARS-CoV-2: Positive Results Stratified by Days Post-Symptom Onset

Days Post Onset	RT-PCR Positive	Nano-Check™ Influenza+ COVID-19 Dual Test Positive	Positive Rate (%)	95% CI
1	72	64	88.9	79.6-94.3
2	74	62	83.8	73.8-90.5
3	51	43	84.3	72.0-91.8
4	17	14	82.4	59.0-93.8
5	12	9	75.0	46.8-91.1
Total	226	192	85.0	79.7-89.0

Influenza A: Nano-Check™ Influenza+COVID-19 Dual Test Results versus RT-PCR Comparator

Nano-Check™ Influenza+ COVID-19 Dual Test	Comparator RT-PCR		Total
	Positive	Negative	
Positive	297	5	302
Negative	49	1238	1287
Total	346	1243	1589

Positive Percent Agreement = (297/346) = 85.8% (95% CI: 81.8% - 89.1%)
Negative Percent Agreement = (1238/1243) = 99.6% (95% CI: 99.1% - 99.8%)

Influenza B: Nano-Check™ Influenza+COVID-19 Dual Test Results versus RT-PCR Comparator

Nano-Check™ Influenza+ COVID-19 Dual Test	Comparator RT-PCR		Total
	Positive	Negative	
Positive	115	5	120
Negative	15	1454	1469
Total	130	1459	1589

Positive Percent Agreement = (115/130) = 88.5% (95% CI: 81.8% - 92.9%)
Negative Percent Agreement = (1454/1459) = 99.7% (95% CI: 99.2% - 99.9%)

14. SERIAL TESTING

A prospective clinical study was conducted between January 2021 and May 2023 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 test 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.

Performance of the antigen test with serial testing in symptomatic individuals is described in the table below.

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	SYMPTOMATIC ON FIRST DAY OF TESTING		
	Ag Positive / PCR Positive (Antigen Test Performance % PPA)		
	1 Test	2 Tests	3 Tests
0	34/57 (59.6%)	47/51 (92.2%)	44/47 (93.6%)
2	58/62 (93.5%)	59/60 (98.3%)	43/43 (100%)
4	55/58 (94.8%)	53/54 (98.1%)	39/40 (97.5%)
6	27/34 (79.4%)	26/33 (78.8%)	22/27 (81.5%)
8	12/17 (70.6%)	12/17 (70.6%)	7/11 (63.6%)
10	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 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.
3Tests = 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.

15. ASSAY SENSITIVITY: LIMIT OF DETECTION (LOD)

The limit of detection (LoD) for Nano-Check™ Influenza+COVID-19 Dual Test was established using serial dilutions of two SARS-CoV-2 Omicron variant strains (USA/MD-HP20874/2021, USA/COR-22-0631 13/2022, two influenza A strains (Influenza A H1N1: A/California/04 /2009, Influenza A H3N2: A/Victoria/361/2011) and two influenza B strains (Influenza B/Hong Kong/330/2001 and Influenza B/Phuket/3073 /13) in a negative clinical matrix. Contrived samples were prepared by spiking the isolate/strain into the pooled negative nasal fluid solution confirmed negative for SARS-CoV-2, influenza A, and influenza B by RT-PCR. A preliminary LoD was determined by spiking 50 µL of serially diluted sample onto swab heads and tested using the Nano-Check™ Influenza+COVID-19 Dual Test. A preliminary LoD test was performed by spiking 50 µL of each diluted sample onto the sample collection swab head. The confirmatory LoD test was performed at the selected preliminary LoD concentration and at concentrations above and below the preliminary LoD with an additional 20 replicates. Based on the testing procedure for this study, the results of LoD are presented in the table below.

Virus Strain	LoD	% Positive
SARS-CoV-2: USA/MD-HP20874/2021, Heat Inactivated	1.95×10^2 $TCID_{50}$/mL (9.75×10^0 $TCID_{50}$/swab)	95%
SARS-CoV-2: USA/COR-22-063113/2022, Heat Inactivated)	1.27×10^4 $TCID_{50}$/mL (6.35×10^2 $TCID_{50}$/swab)	100%
Influenza A H1N1: A/California/04/2009	2.8×10^3 $TCID_{50}$/mL (1.4×10^2 $TCID_{50}$/swab)	100%
Influenza A H3N2: A/Victoria/361/2011	1.4×10^5 $CEID_{50}$/mL (7.0×10^3 $CEID_{50}$/swab)	95%
Influenza B Victoria: B/Hong Kong/330/2001	2.25×10^5 $CEID_{50}$/mL (1.13×10^4 $CEID_{50}$/swab)	95%
Influenza B Yamagata: B/Phuket/3073/13	1.04×10^2 $TCID_{50}$/mL (5.2×10^0 $TCID_{50}$/swab)	100%

Furthermore, the LoD studies with the 1st WHO International Standard for SARS-CoV-2 Antigen (NIBSC 21/368) were determined and the results are presented below.

Description	Source	NIBSC. No.	Concentration (IU/mL)	IU/Swab	% Positive
WHO Standard	NIBSC	21/368	667	33.5	95

16. ANALYTICAL REACTIVITY/INCLUSIVITY

The analytical inclusivity (sensitivity) was established for a total of 14 strains of SARS-CoV-2, 31 strains of Influenza A, and 16 strains of Influenza B, including most representing subtypes from past to recent.

Virus	Virus Strains	Concentration	Units
SARS-CoV-2 (B.1.1.7, Alpha)	USA/CA/CDC/5574/2020[1)]	6.77E+06	GE /mL
	USA/CA/CDC/5574/2020[2)]	2.39E+04	$TCID_{50}$/mL
	England/204820464/2020	7.19E+03	$TCID_{50}$/mL
SARS-CoV-2 (B.1.351, Beta)	USA/MD-HP01542/2021[3)]	7.20E+04	GC/mL
	USA/MD-HP01542/2021[4)]	3.80E+06	GC/mL
	South Africa/KRISP-K0053 25/2020	1.90E+04	$TCID_{50}$/mL
SARS-CoV-2 (B.1.617.2, Delta)	USA/MD-HP05285/2021[5)]	7.20E+07	GC/mL
	USA/MD-HP05285/2021[6)]	5.00E+06	GC/mL
	USA/PHC658/2021	5.21E+02	$TCID_{50}$/mL
SARS-CoV-2 (P.1, Gamma)	Japan/TY7-503/2021	1.58E+04	$TCID_{50}$/mL
	USA/NY-Wadsworth-21033 899-01/ 2021	7.85E+03	$TCID_{50}$/mL
SARS-CoV-2 (B.1.617.1, Kappa)	USA/CA-Stanford-15_S02/ 2021	8.48E+04	$TCID_{50}$/mL
SARS-CoV-2 (B.1.1.529, Omicron)	USA/GA-EHC-2811C/2021	2.11E+07	GC/mL
SARS-CoV-2 (JN.1, Omicron)	USA/New York/PV96109/2023	3.14E+03	$TCID_{50}$/mL

Flu A H1N1	A/Puerto Rico/8/34	8.00E+06	$CEID_{50}$/mL
	A/Brisbane/59/2007	4.45E+06	$CEID_{50}$/mL
	A/Denver/1/57	4.00E+05	$CEID_{50}$/mL
	A/San Diego/1/2009 pdm09	2.80E+04	$TCID_{50}$/mL
	A/Tijuana/4/09	2.45E+01	$TCID_{50}$/mL
	A/Solomon Islands/3/2006	4.45E+05	$CEID_{50}$/mL
	A/NWS/33	1.23E+05	$CEID_{50}$/mL
	A/FM/1/47	4.25E+05	$CEID_{50}$/mL
	A/New Jersey/8/76	1.70E+04	$CEID_{50}$/mL
	A/New Caledonia/20/1999	4.00E+05	$CEID_{50}$/mL
	A/Hawaii/66/2019	1.28	HA
	A/Hawaii/66/2019 X-345A	1.28	HA
	A/Guangdong-Maonan/1536/ 2019	1.28	HA
	A/Guangdong-Maonan/1536/ 2019 CNIC-1909	0.64	HA
	A/Victoria/4897/2022(pdm09)	1.58E+07	EID_{50}/mL
	A/Victoria/2570/2019 (pdm09)	9.98E+05	EID_{50}/mL
Flu A H1N2	A/Swine/Ohio/09SW1477/2009	2.30E+04	$TCID_{50}$/mL
Flu A H3N2	A/Hong Kong/8/1968	1.40E+05	$CEID_{50}$/mL
	A/Aichi/2/1968	4.00E+05	$CEID_{50}$/mL
	A/Wisconsin/67/2005	7.00E+05	$CEID_{50}$/mL
	A/Hong Kong/4801/2014	9.60E+05	$CEID_{50}$/mL
	A/Netherlands/22/2003	8.00E+02	$TCID_{50}$/mL
	A/Netherlands/823/1992	1.44E+01	$TCID_{50}$/mL
	A/Brisbane/10/2007	1.38E+05	$CEID_{50}$/mL
	A/Wisconsin/15/2009	5.00E+03	$CEID_{50}$/mL
	A/Sydney/5/97	4.45E+04	$CEID_{50}$/mL
	A/Port Chalmers/1/73	2.00E+05	$CEID_{50}$/mL
	A/Victoria/3/75	4.00E+05	$CEID_{50}$/mL
	A/Perth/16/2009 x A/Puerto Rico/8/19 34, NIB-64	2.80E+06	$CEID_{50}$/mL
	A/Singapore/INFIMH-16-0019 /16	2.51E+03	$TCID_{50}$/mL
Flu A H5N1	A/mallard/Wisconsin/2576/200 9	5.25E+05	GE/mL
Flu B (Victoria Lineage)	B/Brisbane/60/2008	9.00E+04	$CEID_{50}$/mL
	B/Malaysia/2506/2004	1.12E+06	$CEID_{50}$/mL
	B/New York/1056/2003	3.20E+03	$TCID_{50}$/mL
	B/Washington/02/2019	1.28	HA
Flu B (Yamagata Lineage)	B/Florida/78/2015	2.80E+04	$TCID_{50}$/mL
	B/Texas/06/2011	4.45E+08	$CEID_{50}$/mL
	B/New York/1061/2004	8.00E+02	$TCID_{50}$/mL
	B/Christchurch/33/2004	8.00E+02	$TCID_{50}$/mL
	B/Sydney/507/2006	1.60E+05	$TCID_{50}$/mL
	B/Wisconsin/1/2010	1.80E+06	$CEID_{50}$/mL
	B/Florida/4/2006	3.50E+05	$CEID_{50}$/mL
Flu B (Non-Victoria/ Yamagata)	B/Colorado/6/17	1.78E+02	$TCID_{50}$/mL
	B/Taiwan/2/1962	4.45E+03	$CEID_{50}$/mL
	B/Lee/1940	9.00E+04	$CEID_{50}$/mL
	B/GL/1739/54	5.00E+04	$CEID_{50}$/mL
	B/Great Lakes/1739/1954	3.20E+04	$CEID_{50}$/mL

1) Source: Bei Resources (Cat. #: NR-55245, Lot#:70043111), 2) Source: ZeptoMetrix (Cat. #: 0810612CFHI, Lot#:328055), 3) Source: Bei Resources (Cat. #: NR-553651, Lot#:70045299), 4) Source: Bei Resources (Cat. #: NR-55350, Lot#:70045608), 5) Source: Bei Resources (Cat. #: NR-56128, Lot#:70048021), 6) Source: ATCC (Cat. #: VR-3342HK, Lot#:70048932)

17. ANALYTICAL SPECIFICITY: ASSAY CROSS-REACTIVITY and MICROBIAL INTERFERENCE

Cross-reactivity of Nano-Check™ Influenza+COVID-19 Dual Test was evaluated by 50 potential pathogens of bacteria (19), fungi (1), virus (28), and negative matrix (2) that could potentially cross-react with the Nano-Check™ Influenza+COVID-19 Dual 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, Influenza A, and Influenza B viruses at 2 x LoD. The samples were tested in triplicate 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.

Pathogens	Concentration Tested	Cross-Reactivity/ Microbial Interference
Bordetella pertussis	1.0×10^6 cfu/mL	No
Candida albicans	1.0×10^6 cfu/mL	No
Chlamydophila pneumoniae	1.0×10^6 IFU/mL	No
Corynebacterium diphtheriae	1.0×10^6 cfu/mL	No
Escherichia coli	1.0×10^6 IFU/mL	No
Haemophilus influenzae, B	1.0×10^6 cfu/mL	No
Lactobacillus acidophilus	1.0×10^6 cfu/mL	No
Legionella Pneumophila subsp. *Pneumophila*	1.0×10^6 cfu/mL	No
Moraxella catarrhalis	1.0×10^6 cfu/mL	No
Mycobacterium tuberculosis	1.0×10^6 cfu/mL	No
Mycoplasma pneumoniae	1.0×10^6 cfu/mL	No
Neisseria meningitidis	1.0×10^6 cfu/mL	No
Neisseria mucosa	1.0×10^6 cfu/mL	No
Neisseria subflava	1.0×10^6 cfu/mL	No
Pseudomonas aeruginosa	1.0×10^6 cfu/mL	No
Staphylococcus aureus	1.0×10^6 cfu/mL	No
Staphylococcus epidermidis	1.0×10^6 cfu/mL	No
Streptococcus pneumoniae	1.0×10^6 cfu/mL	No
Streptococcus pyogenes	1.0×10^6 cfu/mL	No
Streptococcus salivarius salivarius	1.0×10^6 cfu/mL	No
Epstein-Barr Virus	1.0×10^5 cp/mL	No
Enterovirus 71	1.0×10^5 $TCID_{50}$/mL	No
Enterovirus D 68	1.0×10^5 $TCID_{50}$/mL	No
Human Herpesvirus	8.0×10^4 $TCID_{50}$/mL	No
Human Adenovirus 1	1.0×10^5 $TCID_{50}$/mL	No
Human Adenovirus 2	$.0\times10^5$ $TCID_{50}$/mL	No
Human Adenovirus 7, Gomen	1.0×10^5 $TCID_{50}$/mL	No
Human Coronavirus, 229E	1.0×10^5 $TCID_{50}$/mL	No
Human Coronavirus, NL63	7.0×10^4 $TCID_{50}$/mL	No
Human Coronavirus, OC43	4.5×10^4 $TCID_{50}$/mL	No
Human Metapneumovirus 3, B1, Peru2-2002	1.95×10^4 $TCID_{50}$/mL	No
Human Metapneumovirus, TN/83-1211	1.0×10^5 $TCID_{50}$/mL	No
Human Parainfluenza Virus 1	1.0×10^5 $TCID_{50}$/mL	No
Human Parainfluenza Virus 2	1.0×10^5 $TCID_{50}$/mL	No
Human Parainfluenza Virus 3	1.0×10^5 $TCID_{50}$/mL	No
Human Parainfluenza Virus 4B	1.0×10^5 $TCID_{50}$/mL	No
Human RSV, A Long	1.0×10^5 $TCID_{50}$/mL	No
Human RSV, A 9320	1.0×10^5 $TCID_{50}$/mL	No
Human RSV, A2	1.0×10^5 $TCID_{50}$/mL	No
Human RSV, B 18537	1.0×10^5 $TCID_{50}$/mL	No
Human RSV, B WV/14617/85	1.0×10^5 $TCID_{50}$/mL	No
Human RSV, B1	1.0×10^5 $TCID_{50}$/mL	No
Human Rhinovirus 1A, 2060	1.0×10^5 PFU/mL	No
Measles Virus, Edmonston	1.7×10^4 $TCID_{50}$/mL	No
MERS-CoV, EMC/2012	1.0×10^5 $TCID_{50}$/mL	No
Mumps Virus, MuV/Iowa.US/2006	1.0×10^5 $TCID_{50}$/mL	No
Rhinovirus 20, 15-CV19	1.0×10^5 $TCID_{50}$/mL	No
SARS-CoV	1.0×10^5 PFU/mL	No
Pooled Human Nasal Wash	N/A	No
Pooled Human Nasal Fluid	N/A	No
Coronavirus HKU1 was not tested for cross-reactivity due to a lack of availability. 20 clinical samples containing Coronavirus HKU1 were tested and all resulted as negative, however, the viral load/concentration of each sample is unknown.		

18. ENDOGENOUS/EXOGENOUS INTERFERENCE

To assess endogenous interference with the performance of Nano-Check™ Influenza+COVID-19 Dual 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 thirty-five (35) potentially interfering substances do not cross-react nor interfere with the detection of SARS-CoV-2, Influenza A, or Influenza B in Nano-Check™ Influenza+COVID-19 Dual Test.

Interfering Substances	Active ingredients	Concentration tested	Interference (Yes/No)
Nasal Spray 1	Fluticasone propionate	15% v/v	No
Nasal Spray 2	Phenylephrine	15% v/v	No
Nasal Spray 3	Cardiospermum Galphimia glauca Luffa operculata Sabadilla	15% v/v	No
Nasal Spray 4	Oxymetazoline	15% v/v	No
Budesonide Nasal Spray	Budesonide	15% v/v	No
Nasonex 24 hr Allergy	Mometasone furoate monohydrate	15% v/v	No
Nasacort Allergy 24HR	Triamcinolone acetonide	15% v/v	No
Sore Throat (Oral Pain Reliever spay)	Phenol, Menthol	15% v/v	No
ZICAM® Oral mist	Zincum aceticum, Zincum gluconicum	15% v/v	No
Sore Throat Lozenges	Benzocaine,Menthol	15% w/v	No
Zinc Cold Therapy	Zincum Gluconicum	15% w/v	No
Homeopathic Allergy Nasal Spray	N/A	15% v/v	No
NasoGEL (Gel Spray)	Sodium Hyaluronate, Allantoin, Sodium chloride, Methylparaben, Propylparaben	15% v/v	No
Nasalcrom® Nasal Allergy spray	Cromolyn sodium	15% v/v	No
Histaminum 30C	Histaminum hydrochloricum	15% w/v	No
Skin relief hand cream	Dimethicone	1% w/v	No
Hand Soap Fresh Breeze Scent	N/A	1% w/v	No
Antibacterial liquid Hand Soap	Benzalkonium Chloride	1% w/v	No
Hand Sanitizer Gel	Ethyl alcohol	1% w/v	No
Disinfectant Spray	Alkyl -dimethyl benzyl ammonium saccharinate, Ethanol	1% v/v	No
Mucin (Bovine submaxillary Glands, Type I-S)	Mucin protein	5 mg/mL	No
Human Neutrophils	N/A	5×10^6 cells/mL	No
Whole Blood	N/A	2.50%	No
Acetylsalicylic acid	Acetylsalicylic acid	3.00×10^1 µg/mL	No
Dexamethasone	Dexamethasone	1.20×10^1 µg/mL	No
Mometasone furoate	Mometasone furoate	4.50×10^{-4} µg/mL	No
Mupirocin	Mupirocin	1.50×10^0 µg/mL	No
Oseltamivir phosphate	Oseltamivir phosphate	3.99×10^{-1} µg/mL	No
Tobramycin	Tobramycin	3.30×10^1 µg/mL	No
Beclomethasone dipropionate	Beclomethasone dipropionate	5.04 µg/mL	No
Flunisolide	Flunisolide	870 µg/mL	No
Molnupiravir	Molnupiravir	3.29 mg/mL	No
Remdesivir	Remdesivir	240 µg/mL	No
Zanamivir	Zanamivir	30 mg/mL	No
Biotin	N/A	3500 ng/mL	No

19. HIGH-DOSE HOOK EFFECT

No high-dose hook effect was observed with Nano-Check™ Influenza+ COVID-19 Dual Test when testing high concentrations of SARS-CoV-2, Influenza A, or Influenza B strains.

Virus Strain Tested	Concentration
SARS-CoV-2, USA/MD-HP20874/2021	3.89×10^4 $TCID_{50}$/mL
SARS-CoV-2, USA/COR-22-063113/2022	2.53×10^6 $TCID_{50}$/mL
Influenza A/California/04/2009	2.80×10^6 $TCID_{50}$/mL
Influenza A/Victoria/361/2011	2.80×10^8 $CEID_{50}$/mL
Influenza B/Hong Kong/330/2001	1.80×10^7 $TCID_{50}$/mL
Influenza B/Phuket/3073/13	4.17×10^5 $TCID_{50}$/mL

20. COMPETITIVE INHIBITION

For co-infection, SARS-CoV-2 at levels near LoD was tested in the presence of high levels of influenza A or influenza B and near LoD influenza A and influenza B in the presence of high levels of SARS-CoV-2. Additionally, the performance of Nano-Check™ Influenza+ COVID-19 Dual Test was evaluated in the presence of high levels of influenza A and influenza B. Contrived high and low titer influenza A (H1N1 and H3N2) and B positive samples were. No competitive interference was observed between SARS-CoV-2 and influenza A and B as listed in the table below.

High Titer Target		Low Titer Target		Low Titer Target Percent Positivity
Virus Name	Concentration	Virus Name	Concentration	
Flu A (H1N1)	2.8×10^5	SARS-CoV-2	3.81×10^4	100%
Flu A (H1N1)	2.8×10^5	Flu B (Victoria)	6.75×10^5	100%
Flu A (H1N1)	2.8×10^5	Flu B (Yamagata)	3.12×10^2	100%
Flu A (H3N2)	2.8×10^6	SARS-CoV-2	3.81×10^4	100%
Flu A (H3N2)	2.8×10^6	Flu B (Victoria)	6.75×10^5	100%
Flu A (H3N2)	2.8×10^6	Flu B (Yamagata)	3.12×10^2	100%
Flu B (Victoria)	1.8×10^6	SARS-CoV-2	3.81×10^4	100%
Flu B (Victoria)	1.8×10^6	Flu A (H1N1)	8.4×10^3	100%
Flu B (Victoria)	1.8×10^6	Flu A (H3N2)	4.2×10^5	100%
Flu B (Yamagata)	4.17×10^5	SARS-CoV-2	3.81×10^4	100%
Flu B (Yamagata)	4.17×10^5	Flu A (H1N1)	8.4×10^3	100%
Flu B (Yamagata)	4.17×10^5	Flu A (H3N2)	4.2×10^5	100%
SARS-CoV-2	2.53×10^5	Flu A (H1N1)	8.4×10^3	100%
SARS-CoV-2	2.53×10^5	Flu A (H3N2)	4.2×10^5	100%
SARS-CoV-2	2.53×10^5	Flu B (Victoria)	6.75×10^5	100%
SARS-CoV-2	2.53×10^5	Flu B (Yamagata)	3.12×10^2	100%
Flu A (H1N1)	2.8×10^5	Flu B (Yamagata)	3.12×10^2	100%
		SARS-CoV-2	3.81×10^4	100%
Flu A (H1N1)	2.8×10^5	Flu B (Victoria)	6.75×10^5	100%
		SARS-CoV-2	3.81×10^4	100%
Flu A (H3N2)	2.8×10^6	Flu B (Yamagata)	3.12×10^2	100%
		SARS-CoV-2	3.81×10^4	100%
Flu A (H3N2)	2.8×10^6	Flu B (Victoria)	6.75×10^5	100%
		SARS-CoV-2	3.81×10^4	100%
Flu B (Victoria)	1.8×10^6	Flu A (H1N1)	8.4×10^3	100%
		SARS-CoV-2	3.81×10^4	100%
Flu B (Victoria)	1.8×10^6	Flu A (H3N2)	4.2×10^5	100%
		SARS-CoV-2	3.81×10^4	100%
Flu B (Yamagata)	4.17×10^5	Flu A (H1N1)	8.4×10^3	100%
		SARS-CoV-2	3.81×10^4	100%

Flu B (Yamagata)	4.17×10^5	Flu A (H3N2)	4.2×10^5	100%
		SARS-CoV-2	3.81×10^4	100%
SARS-CoV-2	2.53×10^5	Flu A (H1N1)	8.4×10^3	100%
		Flu B (Victoria)	6.75×10^5	100%
SARS-CoV-2	2.53×10^5	Flu A (H1N1)	8.4×10^3	100%
		Flu B (Yamagata)	3.12×10^2	100%
SARS-CoV-2	2.53×10^5	Flu A (H3N2)	4.2×10^5	100%
		Flu B (Victoria)	6.75×10^5	100%
SARS-CoV-2	2.53×10^5	Flu A (H3N2)	4.2×10^5	100%
		Flu B (Yamagata)	3.12×10^2	100%

21. REPRODUCIBILITY

The reproducibility was evaluated at three external CLIA-waived sites with a total of eight untrained operators and one internal site with three trained operators. The reproducibility panel was composed of a panel consisting of true negative (TN), a high negative sample (HN, 0.1x LoD), a low positive (LP, 1 x LoD), and a moderate positive (MP, 5x LoD) sample for each analyte. This resulted in 165 total tests per sample level. The results are shown in the table below.

Sample	No of Positive Result/No of Total Tested (% Positive Rate)				Agreement	
	Site 1 (2Operators)	Site 2 (3Operators)	Site 3 (3Operators)	Site 4 (3Operators)	Total	95% CI
TN	0/30 (0%)	0/45 (0%)	0/45 (0%)	0/45 (0%)	165/165 (100%)	97.7-100.0
HN COVID	0/30 (0%)	0/45 (0%)	0/45 (0%)	0/45 (0%)	165/165 (100%)	97.7-100.0
HN Flu A	0/30 (0%)	0/45 (0%)	0/45 (0%)	0/45 (0%)	165/165 (100%)	97.7-100.0
HN Flu B	0/30 (0%)	0/45 (0%)	1/45 (2.2%)	0/45 (0%)	164/165 (99.4%)	96.7-99.9
LP COVID	30/30 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	165/165 (100%)	97.7-100.0
LP Flu A	30/30 (100%)	45/45 (100%)	44/45 (97.8%)	45/45 (100%)	164/165 (99.4%)	96.7-99.9
LP Flu B	30/30 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	165/165 (100%)	97.7-100.0
MP COVID	30/30 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	165/165 (100%)	97.7-100.0
MP Flu A	30/30 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	165/165 (100%)	97.7-100.0
MP Flu B	30/30 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	165/165 (100%)	97.7-100.0

22. REFERENCES

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10. Cazacu, A. C. et al, Comparison of lateral-flow immunoassay and enzyme immunoassay with viral culture for rapid detection of influenza virus in nasal wash specimens from children. J. Clin. Microbiol. 2003; 41:2132-2134

For more information or any questions about this product, please contact customer service at:

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Info@nanoditech.com
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Glossary

Rx ONLY — Prescription use only
IVD — For In Vitro use only
2°C-30°C — Temperature limitation
REF — Catalog number
CONTROL + — Positive control
CONTROL − — Negative control
LOT — Batch code
Use by
Do not re-use
Manufacturer
Consult instructions for use
Σ 20 — Contains sufficient components for 20 tests

Nano-Check™ Influenza + COVID-19 Dual Test
Quick Reference Instruction

For Use Under an Emergency Use Authorization (EUA) Only
For *in vitro* diagnostic use
For Prescription Use Only
For use with anterior nasal swab specimens

Rx Only IVD

INTENDED USE

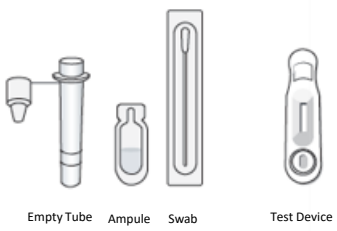
The Nano-Check™ Influenza+COVID-19 Dual Test is a lateral flow immunochromatographic assay intended for in vitro rapid, simultaneous qualitative detection and differentiation of influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen directly from anterior nasal swab specimens of individuals with signs and symptoms of respiratory infection consistent with COVID-19 within the first five (5) days of symptom onset when tested at least twice over three days with at least 48 hours between tests. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar.

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 test 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.

Please see the Instructions for Use for the full intended use.

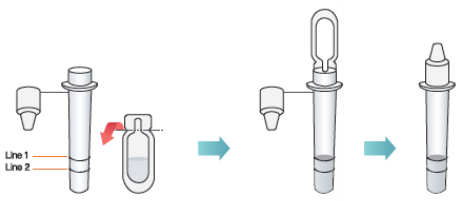
1 Prepare Materials

Open the package, take out the Nano-Check™ Influenza+COVID-19 Dual 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 Test Device.

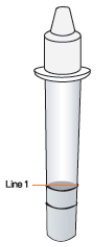


2 Filling Reagent Tube

Please look carefully, there are two lines on the empty tube.
Flip over the TOP part of the ampule cap, then squeeze the ampule solution completely into the empty tube.
Close the tube tightly with the dropper tip.



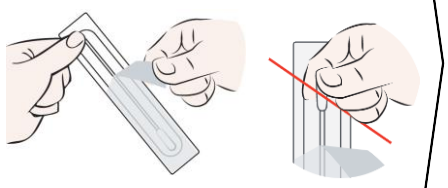
Please confirm that the liquid level is at or above line 1, then go to Step 3 Collect Sample.



Note:
The level of liquid must be above line 1.
Do not proceed with this test, if the liquid level is below the line 1, as this may result in false or invalid results.

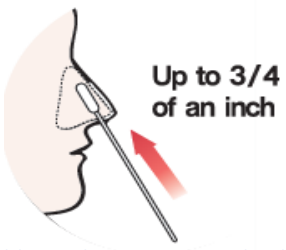
3 Collect Sample

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.



Note: Be careful not to touch the swab tip (soft end) with hand

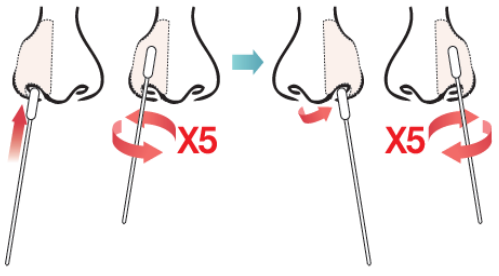
B. Gently insert the entire absorbent tip of the swab (about 1 /2 to 3/4 of an inch) into nostril.



Note:
With children, the maximum depth of insertion into the nostril may be less than 3/4 of an inch, and you may need to have a second person to hold the child's head while swabbing.

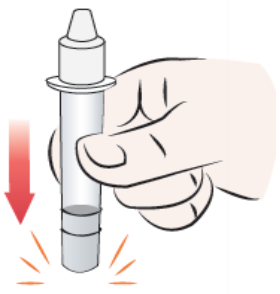
C. Firmly and slowly rotate against insides of nostril in a circular motion against the nasal wall at least 5 times (Take at least 15 seconds to collect the specimen and be sure to collect any nasal drainage on the swab).

Using the same swab, repeat the same sample collection procedure for the other nostril. Be sure to brush **BOTH** nostrils with the **SAME SWAB**.

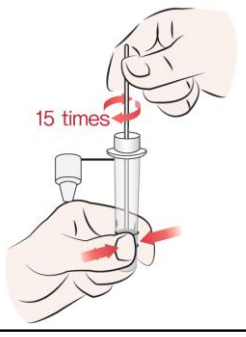


Note:
Failure to swab properly may cause false negative results.

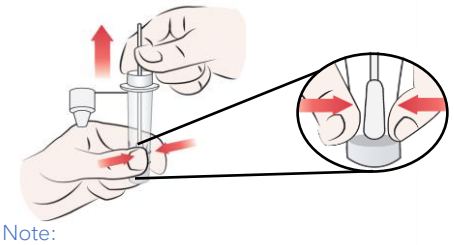
4 Tap the tube vertically on the table and remove the dropper tip to open the tube.



5 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.

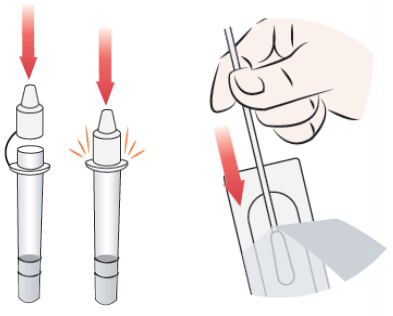


6 Squeeze the sides of the tube to express as much liquid as possible from the swab head, and then remove the swab.

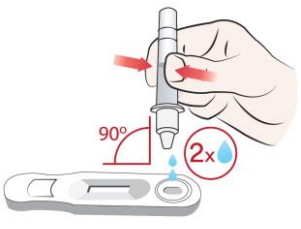


Note:
If you don't squeeze the swab head, there may not be sufficient sample material to perform the test properly (i.e., potentially resulting in a false negative result).

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



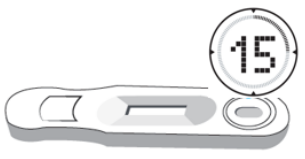
8 Hold the tube vertically to dispense the sample. Add 2 drops of sample to the Sample loading well of the Test device.



CAUTION: Invalid or false results can occur if less than 2 drops are added to the sample well.

Note: Sample must be applied to the test cassette within one hour of completing step 5).

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



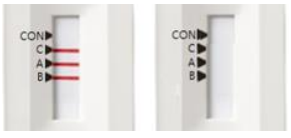
Note:
False results can occur if the test is read before 15 minutes or after 20 minutes

TEST RESULT INTERPRETATION

CON = Control line
C = COVID-19 line
A = Influenza A line
B = Influenza B line

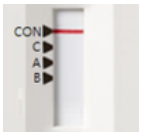
Look for lines next to CON, C, A, and B.

INVALID RESULTS



If a control line does not form in 15 minutes, the test result is invalid and the test should be repeated with a new swab and a new test device.

NEGATIVE RESULT



If the control line (CON) is visible, but no other lines appear the test is **negative**. Negative results should be reported as a presumptive negative for the presence of influenza and/or SARS-CoV-2 antigen.

POSITIVE RESULTS

Flu A Positive | Flu B Positive | COVID-19 Positive | Flu A & Flu B Positive | COVID-19 & Flu A Positive

If the control line (CON) is visible and any other line or multiple lines on 'C', 'A', and/or 'B' appear, the test is **positive.**

It is possible to have more than one positive test line, which could indicate a co-infection with influenza A, B, and/or SARS-CoV-2. If more than one positive test line is observed, retest with a new patient sample and new test kit. Repeatable "dual positive" results should be confirmed by an FDA-cleared molecular assay before reporting results.

SERIAL TESTING

Repeat Testing is needed to improve test accuracy for negative SARS-CoV-2 results. Please follow the table below when interpreting test results with symptoms. Serial (repeat) SARS-CoV-2 testing does not need to be performed if patients have a positive SARS-CoV-2 result

Status on First Day of Testing: With Symptoms			
Day 0 (First Test)	Serial Testing	Day 2 (Second Test)	Final Interpretation
SARS-CoV-2 (+) Influenza A and B (-)	NO	Not Needed	Positive for COVID-19; Presumptive Negative for Influenza
SARS-CoV-2 (+) Influenza A and/or B (+)	NO	Not needed	Positive for COVID-19; Positive for Influenza A and/or B
SARS-CoV-2 (-) Influenza A and/or B (-)	YES	SARS-CoV-2 (+) Influenza A and/or B (-)	Positive for COVID-19; Presumptive Negative for Influenza
SARS-CoV-2 (-) Influenza A and/or B (+)	YES	SARS-CoV-2 (+) Influenza A and/or B (+)	Positive for COVID-19; Positive for Influenza A and/or B
SARS-CoV-2 (-) Influenza A and/or B (-)	YES	SARS-CoV-2 (-) Influenza A and/or B (+)	Presumptive Negative for COVID-19; Positive for Influenza A and/or B
SARS-CoV-2 (-) Influenza A and/or B (-)	YES	SARS-CoV-2 (-) Influenza A and/or B (-)	Presumptive Negative for COVID-19; Presumptive Negative for Influenza
SARS-CoV-2 (-) Influenza A and/or B (-)	YES	SARS-CoV-2 (+) Influenza A and/or B (+)	Positive for COVID-19; Positive for Influenza A and/or B
SARS-CoV-2 (-) Influenza A and/or B (+)	YES	SARS-CoV-2 (-) Influenza A and/or B (-)	Presumptive Negative for COVID-19; Positive for Influenza A and/or B
SARS-CoV-2 (-) Influenza A and/or B (+)	YES	SARS-CoV-2 (-) Influenza A and/or B (+)	Presumptive Negative for COVID-19; Positive for Influenza A and/or B
SARS-CoV-2 (-) Influenza A and/or B (+)	YES	SARS-CoV-2 (+) Influenza A and/or B (+)	Positive for COVID-19; Positive for Influenza A and/or B

QUALITY CONTROL TEST STEP INSTRUCTION

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.

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 that an adequate volume of sample has been added. 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 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 Influenza+ COVID-19 Dual 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. Please contact Technical Support at +1- 855-297-7877 or info@nanoditech.com.

WARNING AND PRECAUTIONS

Read the instructions fully and carefully before performing the procedure.- Failure to follow the instructions may result in inaccurate 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, Influenza A, and Influenza B, 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 SARS-CoV-2 negative results at least twice over three days (with 48 hours between tests) for symptomatic individuals. You may need to purchase additional tests to perform this serial (repeat) testing.
- Do not read test results before 15 minutes or after 20 minutes. Results read before 15 minutes or after 20 minutes may result in false positive, false negative or invalid results.
- Individuals who recently received nasally administered influenza A or influenza B vaccine may have false positive test results after vaccination.

TECHNICAL SUPPORT

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