



Nano-Check™ Influenza A+B Test

- For *In Vitro* Diagnostic Use
- For Prescription Use Only
- For Use with Kit Provided Swabs
- CLIA Complexity-WAIVED for Use with Anterior Nasal Swab
- Certificate of Waiver is required to perform the test in a waived setting. To obtain CLIA waiver information and a Certificate of Waiver, contact your state health department. Additional information is available at www.cms.hhs.gov/CLIA.
- Laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test
- Failure to follow the instructions or any modification to the manufacturer's instructions will result in the test being classified as high complexity

1. INTENDED USE

The Nano-Check™ Influenza A+B Test is a lateral flow immunochromatographic assay intended for the qualitative detection of influenza A and influenza B nucleoprotein antigens directly from anterior nasal swab (ANS) samples from patients with signs and symptoms of respiratory infection. The test is intended for use as an aid in the differential diagnosis of influenza A and influenza B viral infections. The test is not intended for the detection of influenza C antigens. Negative test results are presumptive and should be confirmed by viral culture or an FDA-cleared influenza A and B molecular assay. Negative test results do not preclude influenza viral infection and should not be used as the sole basis for treatment or other patient management decisions.

Performance characteristics for influenza A and B were established during the 2022-2025 influenza seasons when influenza A/H1N1pdm09, influenza A/H3N2, and influenza B/Victoria lineage were the predominant influenza viruses in circulation. When other influenza viruses are emerging, performance characteristics may vary.

If infection with a novel influenza virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to the state or local health department for testing. Virus culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

2. SUMMARY AND EXPLANATION OF THE TEST

Influenza is an acute, highly contagious viral infection primarily affecting the respiratory tract, caused by immunologically diverse, single-stranded RNA viruses. There are three types of influenza viruses: 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.

A patient can be infected with a single virus or co-infected with 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.

The Nano-Check™ Influenza A+B Test provides a simple, rapid method

for the direct detection of the presence or absence of influenza A and/or influenza B antigens using ANS specimens. Its user-friendly format and rapid results allow for quick diagnosis and aid in treatment and hospitalization decisions.

3. PRINCIPLE

The Nano-Check™ Influenza A+B Test is designed to detect the extracted nucleoprotein antigen specific to influenza A and influenza B in ANS specimens directly collected from patients exhibiting signs or symptoms of a respiratory infection¹⁻³.

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

When the specimens are extracted and added to the sample well of the test device, any Flu A and/or Flu B nucleoprotein antigens present in the specimen will form complexes with the anti-Flu A/Flu B conjugate which are then captured by the specific anti-Flu A/Flu B mAb coated on the test line region (A/B line). The absence of the test line (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. Any result without this control line is invalid.

4. REAGENTS and MATERIALS

Provided with the test kit:

- 25 Test devices in sealed aluminum foil pouch with desiccant
- 25 Empty reagent tubes
- 25 Ampules containing extraction buffer (0.3mL)
- 25 Sample collection swabs (Anterior Nasal)
- 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. STORAGE AND STABILITY

- The test kit should be stored at 36~86°F (2~30°C) in the original sealed pouch. Do not freeze.
- Bring all test components to room temperature at least 30 minutes prior to use.
- The test is stable until the expiration date printed on the outside of the box. Do not use after the expiration date.

6. WARNINGS AND PRECAUTIONS

- Read all instructions carefully before performing the test. Failure to follow the instructions may result in inaccurate test results.
- Do not use this test for individuals who recently received nasally administered influenza A or influenza B vaccine, as they may have false positive test results after vaccination.
- Do not use if any of the test kit contents or packaging is damaged.
- Do not use any test component after the expiration date printed on the outer packaging.
- Do not interchange kit contents from different lots.
- Test components are single use. Do not re-use.

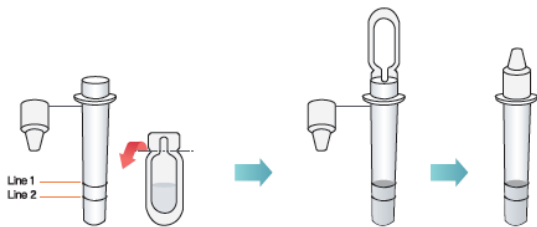
- Once opened, the test card should be used within 60 minutes.
- Ensure that testing and result interpretation are conducted in a well-lit space with sufficient lighting.
- These test results are shown as lines of color. Because these lines can be very faint, users with conditions affecting their vision 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).
- 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.
- Nitrile or latex gloves should be worn when performing this test.
- Dispose of used contents as biohazardous waste in accordance with federal, state, and local requirements.
- Handle all specimens as though they contain infectious agents.
- Inadequate or inappropriate sample collection, storage and transport may yield false test results.
- The Extraction Reagent contains potentially harmful chemicals (see table below). If the test solution contacts the skin or eye, flush with copious amounts of water. If irritation persists, seek medical advice: visit <https://www.poison.org/contact-us> or call 1-800-222-1222

Chemical Name	Harms (GHS Code) for each ingredient	Concentration
Sodium Azide	Acute Tox. 2 (Oral), H300 Acute Tox. 1 (Dermal), H310	0.09%
Gentamicin	Skin Sens. 1, H317	0.004%

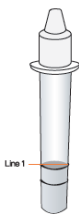
7. SAMPLE COLLECTION AND PREPARATION

Step 1: Fill the empty reagent tube with ampule solution.

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.



- 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.

Step 2: Collect Sample

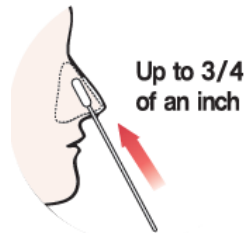
The acceptable specimen type for testing with the Nano-Check™ Influenza A+B Test is a direct anterior nasal swab specimen. It is essential that correct specimen collection must be followed. Inadequate specimen collection, improper specimen handling and/or transport may yield false results; therefore, specimen collection requires specific training and

guidance due to the importance of specimen quality to obtain accurate test results.

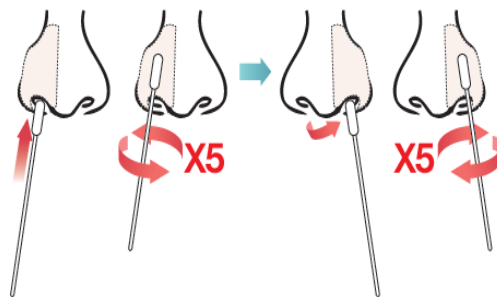
The freshly collected specimens should be processed immediately after collection.

To collect the anterior nasal swab specimen, tilt the patient's head back 70 degrees and insert the soft end of the swab into patient's nostril no more than 3/4 of an inch into the nose (no more than 1/2 inch if swabbing a child). Slowly rotate the swab, gently pressing against the inside of patient's nostril at least 5 times for a total of 15 seconds. Get as much nasal discharge as possible on the soft end of the swab. Gently remove the swab. Use the same end of the swab and repeat the same steps on the other nostril.

NOTE: When collecting a specimen, only use the swab provided in the kit.



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 hold the child's head while swabbing.



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

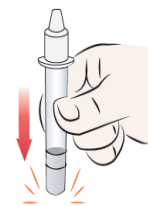
NOTE: Be careful not to touch the swab tip (soft end) with hand.

8. TEST PROCEDURE

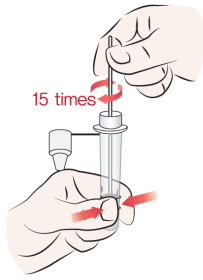
Collect specimens according to instructions in "Specimen Collection". Test device and specimen should be brought to room temperature 59~86°F (15~30°C) prior to testing.

Conduct all testing on a clean, level surface under ambient conditions. Carefully remove the test cassette from the sealed pouch and place it on the flat surface.

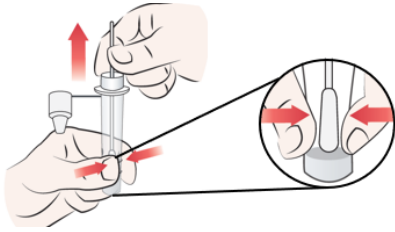
- Tap the tube vertically on the table and remove the dropper tip to open the tube.



- 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.

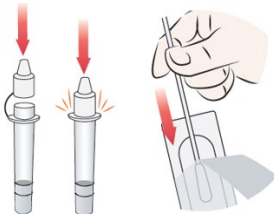


- C. Squeeze the sides of the tube to express as much liquid as possible from the swab head, and then remove the swab.

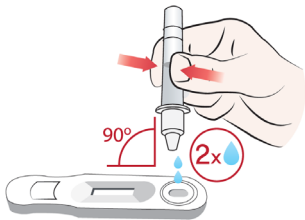


NOTE: If you don't squeeze the swab head, there may not be sufficient specimen material to perform the test properly (i.e., potentially resulting in a false negative result).

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



- E. 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: Specimen must be applied to the test cassette within 30 minutes of completing step B.

- F. Wait 15 minutes after adding the 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
A = Influenza A line
B = Influenza B line

Look for lines next to CON, 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. If the problem persists, please call +1- 855-297-7877 or info@nanoditech.com.

NEGATIVE RESULT



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

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

POSITIVE RESULT



Flu A Positive Flu B Positive Flu A & Flu B Positive

If the Control line (CON) 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 or influenza B lineage.

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 (A) or Flu B (B) region is considered a positive result. The intensity of the Control 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 and B. 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.

10. QUALITY CONTROL

Internal Quality Control: The presence of a pinkish-red colored band in the Control area of the window acts as an internal control to ensure adequate migration has occurred but does not determine if an adequate sample has been added. In the absence of this Control line, the test is invalid and must be repeated. If the control line does not develop in 15 minutes, the test result is considered invalid, and retesting with a new device is recommended. If the internal procedural control line is still absent in the retest, please contact 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 the clinical specimen swab, and conduct the assay as described in the Test Procedure section. Controls should minimally be run before using each new lot or shipment of Nano-Check™ Influenza A+B Test, before testing is conducted by each new operator, at regular intervals afterward or any time when the validity of the test results is 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 contents of this kit are to be used as a qualitative test and do not provide information on the viral concentration present in the specimen.
- 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 influenza as compared to a molecular test, especially in samples with low viral load.
- Test results must be evaluated in conjunction with other clinical data available to the physician.
- Viral transport media (VTM) should not be used with this test.
- The performance of this test has not been evaluated for use in patients without signs and symptoms of respiratory infection.
- This test device detects both viable (live) and non-viable influenza A and B. Test performance depends on the amount of virus (antigen) in 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 specimen is collected, handled or transported improperly.
- Negative test results are not intended to rule in other non-influenza viral or bacterial infections.
- Monoclonal antibodies may fail to detect, or detect with less sensitivity, influenza A viruses that have undergone minor amino acid changes in the target epitope region.
- The performance of this test has not been evaluated for monitoring antiviral treatment of influenza.
- Positive results do not rule out co-infection with other pathogens.
- Positive test results do not identify specific influenza A subtypes.
- If differentiation of specific influenza A subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- Children tend to shed virus for longer periods of time than adults, which may result in differences in sensitivity between adults and children.
- False positive test results are more likely when the prevalence of influenza A/B is low in the community.
- False positive results were observed when testing FluMist® at 15% (v/v) concentrations.

12. CLINICAL PERFORMANCE

The clinical performance of the Nano-Check™ Influenza A+B Test was established with 1,969 anterior nasal samples that were prospectively collected from subjects between November 2022 and February 2025 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 A+B 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 A+B 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.

For influenza A detection, out of the 480 samples that tested positive with the comparator RT-PCR test, 417 were positive and 63 were negative using Nano-Check™ Influenza A+B Test. Additionally, 1,483 out of 1,489 samples that were negative on RT-PCR were also negative on Nano-Check™ Influenza A+B Test. The agreement between the Nano-Check™ Influenza A+B Test and RT-PCR are presented below.

Table 1.1: Nano-Check™ Influenza A+B Test-Results for Influenza A

Nano-Check™ Influenza A+B Test	Comparator RT-PCR		Total
	Positive	Negative	
Positive	417	6	423
Negative	63	1,483	1,546
Total	480	1,489	1,969

Positive Percent Agreement = $(417/480) = 86.9\%$ (95% CI: 83.6% - 89.6%)
 Negative Percent Agreement = $(1,483/1,489) = 99.6\%$ (95% CI: 99.1% - 99.8%)

For influenza B detection, out of the 114 samples that tested positive with the comparator RT-PCR test, 99 were positive and 15 were negative using Nano-Check™ Influenza A+B Test. Additionally, 1,850 out of 1,855 samples that were negative on RT-PCR were also negative on Nano-Check™ Influenza A+B Test. The agreement between the Nano-Check™ Influenza A+B Test and RT-PCR are presented below.

Table 1.2: Nano-Check™ Influenza A+B Test-Results for Influenza B

Nano-Check™ Influenza A+B Test	Comparator RT-PCR		Total
	Positive	Negative	
Positive	99	5	104
Negative	15	1,850	1,865
Total	114	1,855	1,969

Positive Percent Agreement = $(99/114) = 86.8\%$ (95% CI: 79.4% - 91.9%)
 Negative Percent Agreement = $(1,850/1,855) = 99.7\%$ (95% CI: 99.4% - 99.9%)

13. ASSAY SENSITIVITY: LIMIT OF DETECTION (LOD)

The limit of detection (LoD) for Nano-Check™ Influenza A+B Test was established using serial dilutions of 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 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 A+B 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.

Table 2. LoD Confirmation for Influenza A and Influenza B

Virus Strain	LoD	% Positive
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%

14. ANALYTICAL REACTIVITY/INCLUSIVITY

The analytical inclusivity (sensitivity) was established for a total of 27 strains of influenza A and 15 strains of influenza B, including most representing subtypes from past to recent.

Table 3: Summary of Analytical Reactivity (Inclusivity)

Virus	Virus Strains	Concentration Tested	Units
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
Flu A H1N2	A/Victoria/4897/2022(pdm09)	1.58E+07	EID ₅₀ /mL
	A/Victoria/2570/2019(pdm09)	9.98E+05	EID ₅₀ /mL
Flu A H3N2	A/Swine/Ohio/09SW1477/2009	2.30E+04	TCID ₅₀ /mL
	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/2576/2009	5.25E+05
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
Flu B	B/Florida/78/2015	2.80E+04	TCID ₅₀ /mL

Virus	Virus Strains	Concentration Tested	Units
(Yamagata Lineage)	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

15. ASSAY CROSS-REACTIVITY AND MICROBIAL INTERFERENCE

Cross-reactivity of the Nano-Check™ Influenza A+B Test was evaluated by testing 53 potential pathogens, including bacteria (19), fungi (2), viruses (30), and negative matrix (2) that could potentially cross-react with the Nano-Check™ Influenza A+B 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 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.

Table 4: Summary of Cross-reactivity and Microbial Interference

Pathogens	Concentration Tested	Cross-Reactivity/ Microbial Interference*
<i>Bordetella pertussis</i>	1.0×10 ⁶ cfu/mL	No
<i>Candida albicans</i>	1.0×10 ⁶ cfu/mL	No
<i>Chlamydomypha pneumoniae</i>	1.0×10 ⁶ IFU/mL	No
<i>Corynebacterium diphtheriae</i>	1.0×10 ⁶ cfu/mL	No
<i>Escherichia coli</i>	1.0×10 ⁶ IFU/mL	No
<i>Haemophilus influenzae, B</i>	1.0×10 ⁶ cfu/mL	No
<i>Lactobacillus acidophilus</i>	1.0×10 ⁶ cfu/mL	No
<i>Legionella Pneumophila</i> subsp. <i>Pneumophila</i>	1.0×10 ⁶ cfu/mL	No
<i>Moraxella catarrhalis</i>	1.0×10 ⁶ cfu/mL	No
<i>Mycobacterium tuberculosis</i>	1.0×10 ⁶ cfu/mL	No
<i>Mycoplasma pneumoniae</i>	1.0×10 ⁶ cfu/mL	No
<i>Neisseria meningitidis</i>	1.0×10 ⁶ cfu/mL	No
<i>Neisseria mucosa</i>	1.0×10 ⁶ cfu/mL	No
<i>Neisseria subflava</i>	1.0×10 ⁶ cfu/mL	No
<i>Pseudomonas aeruginosa</i>	1.0×10 ⁶ cfu/mL	No
<i>Pneumocystis jirovecii</i> - <i>S. cerevisiae</i>	1.0×10 ⁶ cfu/mL	No
<i>Staphylococcus aureus</i>	1.0×10 ⁶ cfu/mL	No
<i>Staphylococcus epidermidis</i>	1.0×10 ⁶ cfu/mL	No
<i>Streptococcus pneumoniae</i>	1.0×10 ⁶ cfu/mL	No
<i>Streptococcus pyogenes</i>	1.0×10 ⁶ cfu/mL	No
<i>Streptococcus salivarius</i>	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

Pathogens	Concentration Tested	Cross-Reactivity/ Microbial Interference*
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	1.0×10 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
SARS-CoV-2, USA/MD-HP20874/2021	1.95×10 ⁴ TCID ₅₀ /mL	No
SARS-CoV-2, USA/COR-22-063113/2022	1.0×10 ⁵ TCID ₅₀ /mL	No
Pooled Human Nasal Wash	N/A	No
Pooled Human Nasal Fluid	N/A	No

Note: 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.

*Results represent positive and negative results for both influenza A and B (i.e., 3 replicates for each).

16. ENDOGENOUS/EXOGENOUS INTERFERENCE

To assess endogenous/exogenous interference with the performance of Nano-Check™ Influenza A+B Test, positive and negative samples were tested with potentially interfering substances that may be found in the upper respiratory tract.

With the exception of the FluMist® trivalent live attenuated influenza vaccine, none of the substances caused a false positive result in the unspiked samples. While the presence of the FluMist® vaccine at 15% v/v concentration did not interfere with the detection of true positive results of the 3 x LoD co-spiked samples, the vaccine also resulted in false positive results for Flu A (as expected based on the composition of the vaccine) at 15% v/v. When diluted down to 1.5% v/v, the results of the unspiked samples were negative for Flu A. Hand sanitizer lotion at 15% v/v showed false negative results for Flu B, but detected all analytes at 10% v/v.

Table 5: Summary of Endogenous/Exogenous Interfering Substances

Interfering Substances	Concentration Tested	Interference (Yes/No)
Nasal Spray 1	15% v/v	No
Nasal Spray 2	15% v/v	No
Nasal Spray 3	15% v/v	No
Nasal Spray 4	15% v/v	No
Budesonide Nasal Spray	15% v/v	No
Nasonex 24 hr Allergy	15% v/v	No
Nasacort Allergy 24HR	15% v/v	No
Sore Throat (Oral Pain Reliever spray)	15% v/v	No
ZICAM® Oral mist	15% v/v	No
Sore Throat Lozenges	15% w/v	No
Zinc Cold Therapy	15% w/v	No
Homeopathic Allergy Nasal Spray	15% v/v	No
NasoGEL (Gel Spray)	15% v/v	No
NasalCrom Nasal Allergy spray	15% v/v	No
Histaminum 30C	15% w/v	No
Skin relief hand cream	1% w/v	No
Hand Soap Fresh Breeze Scent	1% w/v	No
Antibacterial liquid Hand Soap	15% w/v	No
Hand sanitizer gel	15% w/v	No
Hand sanitizer lotion	15% w/v	Yes
	10% w/v	No
Disinfectant Spray	1% v/v	No
Acetylsalicylic acid	3.00×10 ¹ µg/mL	No
Dexamethasone	1.20×10 ¹ µg/mL	No
Mometasone furoate	4.50×10 ⁻⁴ µg/mL	No
Mupirocin	1.50×10 ⁰ µg/mL	No
Oseltamivir phosphate	3.99×10 ⁻¹ µg/mL	No
Tobramycin	3.30×10 ⁻¹ µg/mL	No
Beclomethasone dipropionate	5.04 µg/mL	No
Flunisolide	870 µg/mL	No
Molnupiravir	3.29 mg/mL	No
Remdesivir	240 µg/mL	No
Zanamivir	30 mg/mL	No
Human Neutrophils	5×10 ⁶ cells/mL	No
Mucin -Bovine submaxillary Glands (Type I-S)	5 mg/mL	No
Whole Blood	2.5%	No
FluMist® (Trivalent/Live)	15% v/v*	Yes
	1.5% v/v	No
Biotin	3500 ng/mL	No

*False positive results were also observed at 3% and 6% v/v.

17. HIGH-DOSE HOOK EFFECT

No high-dose hook effect was observed with Nano-Check™ Influenza A+B Test when testing high concentrations of influenza A or influenza B strains.

Table 6: Summary of High-dose Hook Effect

Virus Strain Tested	Concentration Tested
Influenza A/California/04/2009	2.80×10 ⁶ TCID ₅₀ /mL
Influenza A/Victoria/361/2011	2.80×10 ⁸ CEID ₅₀ /mL

Virus Strain Tested	Concentration Tested
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

18. COMPETITIVE INHIBITION

For co-infection, influenza A at levels near LoD was tested in the presence of high levels of influenza B, and influenza B at levels near LoD was tested in the presence of high levels of influenza A. Contrived samples with both high and low titers of influenza A (H1N1 and H3N2) and influenza B were evaluated. No competitive interference was observed between influenza A and B as listed in the table below.

Table 7: Summary of Competitive Interference Results

High Titer Target		Low Titer Target		Low Titer Target Percent Positivity
Virus Name	Concentration Tested	Virus Name	Concentration Tested	
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	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	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	Flu A (H1N1)	8.4 × 10 ³	100%
Flu B (Yamagata)	4.17 × 10 ⁵	Flu A (H3N2)	4.2 × 10 ⁵	100%

19. 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.

Table 8: Summary of Reproducibility Results

Sample	No. of Positives / No. of Samples Tested (%)				Total No. of Positives / Total No. of Samples (%)
	CLIA-Waived Site 1 (2 operators)	CLIA-Waived Site 2 (3 operators)	CLIA-Waived Site 3 (3 operators)	In-house Site 4 (3 operators)	
TN	0/30 (0%)	0/45 (0%)	0/45 (0%)	0/45 (0%)	0/165 (0%)
HN	0/30 (0%)	0/45 (0%)	0/45 (0%)	0/45 (0%)	0/165 (0%)
Flu A	0/30 (0%)	0/45 (0%)	1/45 (2.2%)	0/45 (0%)	1/165 (0.6%)
LP	30/30 (100%)	45/45 (100%)	44/45 (97.8%)	45/45 (100%)	164/165 (99.4%)
Flu B	30/30 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	165/165 (100%)
MP	30/30 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	165/165 (100%)
Flu A	30/30 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	165/165 (100%)
Flu B	30/30 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	165/165 (100%)

20. FLEX STUDIES

Using risk analysis as a guide, analytical flex studies were conducted. The studies demonstrated that the test was not sensitive to the environmental stresses or potential user errors.

21. REFERENCES

- Zhang, P. et al. A Highly Sensitive Europium Nanoparticle-Based Immunoassay for Detection of Influenza A+B Virus Antigen in Clinical Specimens. *J Clin Microbiol.* 2014; 52(12): 4385-4387.
- Kim, W. S. et al. Development and diagnostic application/ evaluation of pandemic (H1N1) 2009 influenza virus-specific monoclonal antibodies. *Microbiol Immunol.* 2012; 56(6): 372-377.
- 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.



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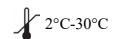
Glossary



Prescription use only



For in vitro diagnostic use



Temperature limitation 2°C-30°C



Catalog number



Positive control



Negative control



Batch code



Use by



Do not re-use



Manufacturer



Consult instructions for use



Contains sufficient components for 25 tests

Cat. No. ND-MD8153

P/N EP-3454 T0 (December 16, 2025)



Nano-Check™ Influenza A+B Test

Rx ONLY IVD i

CLIA WAIVED

For *In Vitro* Diagnostic Use
For Prescription Use Only
For Use with Kit Provided Swab

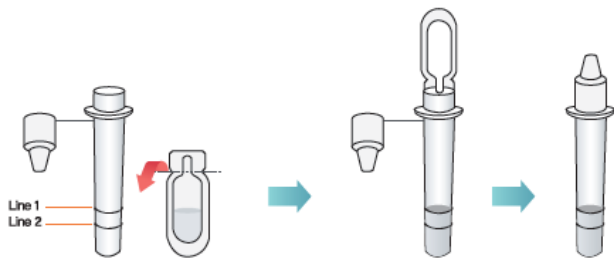
- CLIA waived for use with anterior nasal swabs.
- Laboratories with Certificate of Waiver must follow the manufacturer's instructions for performing the test.
- Failure to follow the instructions or any modification to the manufacturer's instructions will result in the test being classified as high complexity.

For more information, refer to the Product Instructions for Use, test procedure, warnings, precautions, limitations, and the QC section.

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).

Sample Collection and Preparation

Step 1: 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.
- Close the tube tightly with the dropper tip.

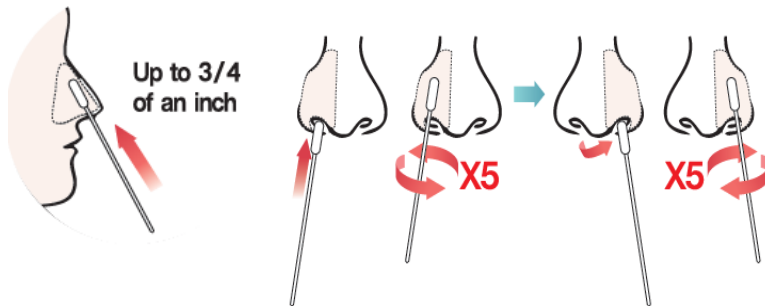


Please confirm that the liquid level is at or above line 1, then go to Step 2 Collect Sample.

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



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

- To collect the anterior nasal swab sample, tilt the patient's head back 70 degrees and insert the soft end of the swab into the patient's nostril no more than 3/4 of an inch.
- Slowly brush the swab 5 times for a total of 15 seconds, gently pressing against the inside of the patient's nostril.
- Remove the swab and repeat the same steps on the other nostril with the same swab.

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 hold the child's head while swabbing.
- Failure to swab properly may cause false negative results.
- The freshly collected specimens should be processed immediately after collection.
- Do not freeze swab specimens before testing.



**Nano-Check™
Influenza A+B Test**

Rx ONLY IVD i

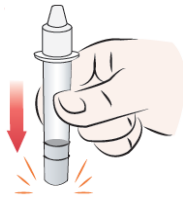


For *In Vitro* Diagnostic Use
For Prescription Use Only
For Use with Kit Provided Swab

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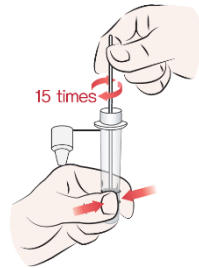
Test Procedure

A



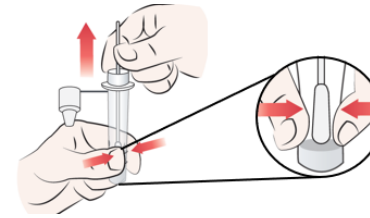
Tap the tube vertically on the table and remove the dropper tip to open the tube

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.

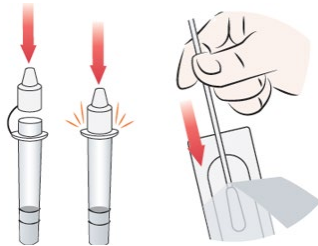
C



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

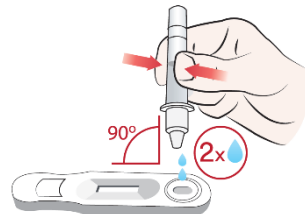
Note: If you don't squeeze the swab head, there may not be sufficient sample material to perform the test properly (i.e., potentially resulting in a false negative result).

D



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

E



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

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

Note: Sample must be applied to the test cassette within 30 minutes of completing step B.

F



Wait 15 minutes after adding sample to the Sample loading hole 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.



Nano-Check™ Influenza A+B Test



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For Use with Kit Provided Swab

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Interpretation of Results



CON = Control line
A = Influenza A line
B = Influenza B line

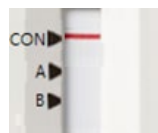
Look for lines next to CON, A, and B.

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 test device. If the problem persists, please call +1- 855-297-7877 or info@nanoditech.com

NEGATIVE RESULT



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

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

POSITIVE RESULT



Flu A Positive Flu B Positive Flu A & Flu B Positive

If the Control line (CON) 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 or influenza B lineage

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 (A) or Flu B (B) region is considered a positive result. The intensity of the Control 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 and/or B. 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.

External Quality Control Test Step Instructions

External positive and negative control swabs are supplied with each kit. These controls provide additional quality control material to assess that the test kit reagents perform as expected. Process the provided control swabs in the same manner as clinical sample swab and follow the steps in the "Sample Testing Procedure" section. The positive and negative external control swabs must be run once for each new kit lot; each new shipment of the test kits; each new operator; or as required by internal quality control procedures and in accordance with local, state, and federal regulations or accreditation requirements.



Nano-Check™ Influenza A+B Test



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INTENDED USE:

The Nano-Check™ Influenza A+B Test is a lateral flow immuno-chromatographic assay intended for the qualitative detection of influenza A and influenza B nucleoprotein antigens directly from anterior nasal swab (ANS) samples from patients with signs and symptoms of respiratory infection. The test is intended for use as an aid in the differential diagnosis of influenza A and influenza B viral infections. The test is not intended for the detection of influenza C antigens. Negative test results are presumptive and should be confirmed by viral culture or an FDA-cleared influenza A and B molecular assay. Negative test results do not preclude influenza viral infection and should not be used as the sole basis for treatment or other patient management decisions.

Performance characteristics for influenza A and B were established during the 2022-2025 influenza seasons when influenza A/H1N1pdm09, influenza A/H3N2, and influenza B/Victoria lineage were the predominant influenza viruses in circulation. When other influenza viruses are emerging, performance characteristics may vary.

If infection with a novel influenza virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to the state or local health department for testing. Virus culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

Assistance:

For questions or technical support, please contact the Technical Service and Support at +1- 855-297-7877 or info@nanoditech.com



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Glossary

Prescription use only	For in vitro diagnostic use	2°C-30°C Temperature limitation	Catalog number	Positive control	Negative control
Batch code	Use by	Do not re-use	Manufacturer	Consult instructions for use	Contains sufficient for 25 determinations

Cat. No. ND-MD8153
P/N EP-3454 QRI R0 (December 16, 2025)