CLIA Waiver by Application Approval Determination
Decision Summary

I. Document Number

CW250012

II. Parent Document Number

K252283

III. CLIA Waiver Type

Dual 510(k) and CLIA Waiver by Application (Dual Submission)

IV. Applicant

Nano-Ditech Corporation

V. Proprietary and Established Names

Nano-Check Influenza A+B Test

VI. Measurand (analyte)

Nucleoprotein antigens from Influenza A and B viruses

VII. Sample Type(s)

Anterior Nasal Swab (ANS) Samples

VIII. Type of Test

Qualitative lateral flow immunoassay

IX. Test System Description

A Overview

The Nano-Check Influenza A+B Test candidate device is a lateral flow immunoassay designed for the qualitative detection of influenza A and B antigens in anterior nasal swab (ANS) specimens from individuals with signs and symptoms of respiratory infection.

The assay kit consists of 25 test cassette devices, 25 reagent tubes, 25 ampules containing extraction buffer, 25 anterior nasal specimen collection swabs, one positive control swab

(contains noninfectious influenza A and B recombinant antigens), one negative control swab (without recombinant antigen), one Instructions for Use (IFU), and one Quick Reference Instruction (QRI).

Test strips are enclosed in a cassette housing 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 influenza A and influenza B. The complete strip assembly is fixed inside a plastic cassette. The schematic of the test cassette device is shown in Figure 1 below.

Figure 1. Test Device of Nano-Check Influenza A+B Test Strip

B Test System Components

The assay kit consists of 25 test cassette devices, 25 reagent tubes, 25 ampules containing extraction buffer, 25 anterior nasal specimen collection swabs, one positive control (contains noninfectious influenza A and influenza B recombinant antigen), one negative control swab (without recombinant antigen), one Instructions for Use (IFU), and one Quick Reference Instruction (QRI).

X. Specific Contents for CLIA Waiver

A Demonstrating "Simple":

The Nano-Check Influenza A+B Test is designed to be simple and easy to use incorporating the following key features:

- The test is self-contained.
- The test uses an unprocessed anterior nasal swab specimen directly.
- The test needs only basic, non-technique-dependent specimen manipulation and reagent handling.
- The supplied reagents are premeasured and provided in single-use vials.
- The test does not require any operator intervention during the analysis step.
- The test does not require technical or specialized training for troubleshooting or interpretation of multiple or complex error codes.

- The test does not require any electronic or mechanical maintenance beyond simple tasks.
- The test produces results that do not require operator calibration, interpretation, or calculation.
- The test produces easily determinable results, such as 'positive' or 'negative'.
- The test procedure within the QRI is written at a 7th-grade comprehension level, as determined by the Flesch-Kincaid Grade Level analysis.

B Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-Safe Mechanisms

1. Risk Analysis:

   The comprehensive risk analysis utilizing the Failure Modes and Effects Analysis (FMEA) method was performed in accordance with ISO 14971. The FMEAs included identification and addressing of potential risks or error sources, analyzing potential causes, effects and the existing measures or mitigation factors related to the Nano-Check Influenza A+B Test. The elements considered included operator error, environmental factors, specimen and reagent handling and storage and device calibration and external controls.

   Potential sources of errors that could adversely affect system performance were identified and mitigated first through system design verification and validation studies and then through additional precautions and warnings in the labeling. The identified risks which could result in erroneous test results were evaluated in flex studies that stressed the functional limits of the test system (see Item 3 below).

2. Fail-Safe and Failure Alert Mechanisms:
   The Nano-Check Influenza A+B Test was designed to include numerous features and fail-safe mechanisms built into the system to prevent erroneous results.

   Design Features

   - Each test cassette is packaged in a foil pouch with desiccant to maintain the integrity of the test device and reagents.
   - The foil pouch is printed with the assay name/type, lot number, and expiration date to ensure clarity and appropriate use.
   - Each test cassette features distinct position marks within the results window to facilitate clear and accurate result interpretation. The control line is denoted as "CON", Flu A antigen line is denoted as "A", and Flu B antigen line is denoted as "B".
   - The test cassette is printed with "FLU A+B" to confirm the assay type being tested and to further ensure clarity and accuracy.
   - The reagent tube is marked with two lines on the side to serve as indicators of acceptable extraction buffer level. This marking ensures that the appropriate amount of extraction buffer is used, which in turn ensures the accuracy of the assay results. The instructions include the warning statement, "*Do not proceed with this test, if the liquid level is below the line 1, as this may result in false or invalid results.*" to ensure proper testing.

   Fail-safe Features

- *Internal Quality Control* – the test device contains a built-in procedural control. The internal procedural control "CON" line is designed to control for the flow of reagents, adequate sample migration, and integrity of the assay. A visible pink/red colored band must be present in the control "CON" region of the results window. If the control "CON" line does not develop within 15-30 minutes, the test result is considered invalid, and retesting with a new sample and new device is recommended.
- *External Quality Control* – two external control swabs are provided with the test device to ensure that the reagents and test cassette are functioning properly, and to demonstrate proper use and performance by the operator:
    - The Positive Control Swab contains non-infectious recombinant influenza A nucleoprotein, and recombinant influenza B nucleoprotein.
    - The Negative Control Swab does not contain recombinant protein.

  External control swabs are extracted and processed according to the test instructions for use. The Positive Control Swab is run first, followed by the Negative Control Swab. Each control swab should produce the expected positive or negative results to validate the test performance. Each control swab is individually packaged in a foil pouch with a barcode printed on the outside. The pouch is printed with information such as control swab type and the expiration date. Users are instructed not to use expired external controls.

  The manufacturer recommends that external controls minimally be run before using each new lot or shipment of test device, at regular intervals afterwards, or any time when the validity of the test results are questioned. If the controls do not perform as expected, users are instructed not to report patient results. Users are also instructed to follow local, state, and federation regulations regarding quality control procedures.

3. Flex Studies:

The operational limits of the candidate device were evaluated in a series of experiments of "stress", including conditions outside of those recommended in the instructions for use. The strains and concentrations of viruses (at 2X LoD) used in flex studies are shown in Table 1 below. The studies and data to support the CLIA Waiver Application for the Nano-Check Influenza A+B Test are listed in Table 2 followed by a summary of flex studies along with their respective mitigations are shown in Table 3 below.

Table 1: Strains and Concentrations of Viruses used in Flex Study

Virus Strains	Influenza A (A/Victoria/361/2011)	Influenza B (B/Hong Kong/330/2001)
Stock Conc.	$1.6x10^8$ $CEID_{50}$/mL	$1.8x10^7$ $CEID_{50}$/mL
2X LoD Conc.	$2.8x10^5$ $CEID_{50}$/mL	$4.5x10^5$ $CEID_{50}$/mL

Table 2: Summary Results of Flex Studies

Condition		Sample	Positive Replicates / Total Replicates	
			Flu A	Flu B
Environmental Tolerance - Temperature	Ambient at 23°C	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/10	0/10
	40°C	Influenza A Positive	10/10	0/10

Condition		Sample	Positive Replicates / Total Replicates	
			Flu A	Flu B
		Influenza B Positive	0/10	10/10
		Negative	0/10	0/10
	4°C	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/10	0/10
Environmental Tolerance - Humidity	35% RH	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/10	0/10
	>90% RH	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/10	0/10
	<20% RH	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/10	0/10
Sample Application Angle	Control - Vertical holding	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	45° tilted	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	Horizontal holding*	Influenza A Positive	N/A*	N/A*
		Influenza B Positive	N/A*	N/A*
		Negative	N/A*	N/A*
Non-level Surface study	Control – 0° (Surface level)	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	10° (Device heading upward)	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	10° (Device heading downward)	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	20° (Device heading upward)	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	20° (Device heading downward)	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	30° (Device heading upward) **	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	30° (Device heading downward)	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5

Impact of light source	**10-15 lux**	Influenza A Positive	3/5	0/5
		Influenza B Positive	0/5	2/5
		Negative	0/5	0/5
	100-500 lux	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	1000-2500 lux	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	>50,000 lux	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
Vibration effect	**Control – 0 rpm (No vibration)**	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	100 rpm (weak vibration)	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	300 rpm (mild vibration)	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	500 rpm (extreme vibration)	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
Extracted Sample Application Volume	**1 drop**	Influenza A Positive	3/10	0/10
		Influenza B Positive	0/10	3/10
		Negative	0/10	0/10
	Control – 2 drops	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/10	0/10
	3 drops	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/10	0/10
	4 drops	Influenza A Positive	8/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/10	0/10
Buffer Volume Variability	**25% volume dispensed (75µL)**	Influenza A Positive	2/5	0/5
		Influenza B Positive	0/5	2/5
		Negative	0/5	0/5
	50% volume dispensed (150µL)	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	75% volume dispensed (225µL)	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	100% volume dispensed (300µL)	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	125% volume dispensed (375µL)	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
Extraction Intensity – Depth of swab insertion	**Control - Swab head touched the bottom**	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	Swab head inserted without touching the bottom	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	Swab head not inserted into extraction buffer	Influenza A Positive	0/5	0/5
		Influenza B Positive	0/5	0/5
		Negative	0/5	0/5

Extraction Intensity – Depth of swab insertion	**No stirring**	Influenza A Positive	3/5	0/5
		Influenza B Positive	0/5	3/5
		Negative	0/5	0/5
	5 times stirring	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	Control - 15 times stirring	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	20 times stirring	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
Extraction Intensity – Squeezing swab head intensity	**Control (With Squeezing)**	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	Without Squeezing	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
Extraction Intensity – Reagent Tube holding position	**Control - Holding at the bottom of the tube tightly**	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	Holding at the top of the tube tightly	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	Without being held	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
Physical Impact of test device	**Control (No impact)**	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	Shaking immediately	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	Shaking 5 minutes later	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	Free fall from 3 ft immediately	Influenza A Positive	3/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	Free fall from 3 ft 5 minutes later	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
Sample Stability – Incubation of swab in the extraction buffer before sample application	**Control (no delay)**	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	30 min delay	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	60 min delay	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
Sample stability – Incubation of the extracted sample after removing the swab from the buffer	**Control (no delay)**	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	30 min delay	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	60 min delay	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5

Reading Time	**3 min**	Influenza A Positive	0/10	0/10
		Influenza B Positive	0/10	0/10
		Negative	0/10	0/10
	7 min	Influenza A Positive	6/10	0/10
		Influenza B Positive	0/10	6/10
		Negative	0/10	0/10
	10 min	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/10	0/10
	Control - 15 min	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/10	0/10
	30 min	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/10	0/10
	45 min	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/10	0/10
	60 min	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/10	0/10
Effect of Nasal discharge	**Control - Spiked swabs not coated with nasal discharge**	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	Spiked swabs coated with nasal discharge	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
Repeated Freeze-Thawing sample	**Control - Fresh Sample Swab**	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	1 F/T – Sample swab	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	2 F/T – Sample Swabs	Influenza A Positive	2/5	0/5
		Influenza B Positive	0/5	4/5
		Negative	0/5	0/5
	3 F/T - Sample Swabs	Influenza A Positive	2/5	0/5
		Influenza B Positive	0/5	2/5
		Negative	0/5	0/5
Bubbles in Reagent Tube	**Control – No bubbles**	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	Foaming Bubbles	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
Inadequate Temperature Equilibration of test kit	**Without Equilibration**	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	15 min of Equilibration at RT	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	Control - 30 min of Equilibration at RT	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5
	1 hr of Equilibration at RT	Influenza A Positive	5/5	0/5
		Influenza B Positive	0/5	5/5
		Negative	0/5	0/5

Open Pouch Stability	**Control - 0 min**	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/5	0/5
	30 min	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/5	0/5
	1 hr	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/5	0/5
	2 hrs	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/5	0/5
	3 hrs	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/5	0/5
	4 hrs	Influenza A Positive	10/10	0/10
		Influenza B Positive	0/10	10/10
		Negative	0/5	0/5
*N/A – Not available – In horizontal flat position, droplets were not produced, and assay could not be performed. **4 out of 20 replicates exhibited flooding with tilting of device at 30° upwards				

Table 3: Summary of Flex Studies Performed and Risk Mitigations

Category	Failure Mode	Results and Conclusion	Risk Control Measure
Operator Error	Sample Application Angle	Proper droplets could not be delivered in horizontal position preventing test performance. Accurate results produced when reagent tubes are held vertically and at a 45° tilt.	Instructions indicate to hold the reagent tubes vertically during sample application. Graphical instruction for users is provided in the QRI/IFU.
	Sample Drop Volume Tolerance	Assay performance with 2 or 3 drops of the extracted sample application was within the acceptable tolerance. Results failed with 1 or 4 drops due to insufficient sample volume or excess sample volume resulting in flooding.	Instructions indicate to apply 2 drops of the extracted sample to enhance user convenience accompanied by a cautionary note, "*Invalid or false results can occur if less than 2 drops are added to the sample well.*"
	Extraction Intensity – Depth of Swab Insertion	Acceptable performance was observed when the swab touched the tube bottom and when swab was properly immersed without touching the bottom.	Instructions indicate to follow the preset extraction procedure of inserting the swab head until it touches the bottom for consistent assay performance.
	Extraction Intensity – Stirring condition of Specimen Swab	Acceptable performance was observed when swabs were stirred 5, 15 or 20 times. False negative results were observed when swabs were not stirred.	Instructions indicate to follow the preset extraction procedure of stirring the swab at least 15 times in the reagent tube for consistent assay performance. Graphical instructions for swab insertion procedure are provided in the QRI/IFU.
	Extraction Intensity – Squeezing swab head intensity	Acceptable performance was observed whether the swab was removed with or without squeezing the swab head.	Graphical instructions for this procedure are provided in the QRI/IFU accompanied by a precautionary 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).*".

	Extraction Intensity – Incorrect holding position with Reagent tube	Acceptable performance was observed whether the reagent tube was held or not.	Instructions indicate to adhere to the established extraction procedure of holding the swab head tightly at the bottom of the tube by squeezing the tube to ensure maximum sample extraction intensity. Instructions for this process are provided in the QRI/IFU.
	Impact of the inclination angle	Acceptable performance was observed when the surface inclination is at or below 20° tilt.	QRI/IFU includes the statement, "*Conduct all testing on a level surface and ambient conditions.*"
	Physical Impact Resistance	Acceptable performance assay was observed to most physical impacts with the exception of very strong physical impact (fallen from above 3 ft) right after the sample application.	QRI/IFU includes the statement, "*Conduct all testing on a level surface and ambient conditions.*"
	Extraction Buffer Volume Variability	Acceptable performance was observed when the extraction buffer volume ranged from 50% to 125%.	QRI/IFU includes the warning statement, "*Do not proceed with this test, if the liquid level is below the line 1, as this may result in false or invalid results."*
	Extracted Sample Stability	Acceptable performance was observed across all time points tested (0, 30 and 60 minutes).	QRI/IFU includes the precautionary note, "*Specimen must be applied to the test cassette within 30 minutes of completing Step B.*"
	In-Use (Open-pouch) Stability	Acceptable performance was observed for cassettes for up to 4 hours after opening the pouch.	QRI/IFU includes the warning statement, "*Once opened, the test card should be used within 60 minutes.*"
	Tolerance Inadequate Temperature Equilibration	Acceptable performance was observed whether the test kit components were equilibrated to ambient temperature or not.	QRI/IFU includes the precautionary statement, "*Bring all test components to room temperature at least 30 minutes prior to use."*
	Reading time Tolerance	False negative results from positive samples were observed at 3 and 7 min of reading time after sample application. Acceptable performance was observed when results were read between 10 to 60 minutes.	QRI/IFU includes the warning statement, "*Do not read the test results before 15 minutes or after 20 minutes. Results read before 15 minutes or after 20 minutes may lead to false positive, false negative, or invalid results."*
	Bubbles in the reagent tube	Acceptable performance was observed in the presence of bubbles in the reagent tube after excessive shaking before sample application.	N/A
Environmental Factor	Temperature	Acceptable performance was observed when the temperature ranged from 10°C to 40°C.	QRI/IFU includes the precautionary statement, "*Bring all test components to room temperature at least 30 minutes prior to use.*"
	Humidity	Acceptable performance was observed when the humidity conditions ranged between 20% RH and 90% RH.	QRI/IFU includes the statement, "*Conduct all testing on a level surface and ambient conditions.*"
	Impact of light source	Acceptable performance was observed under conditions where light intensity was 100-500 lux or above. False negative results were obtained when the light intensity was 10-15 lux.	QRI/IFU includes the warning statement, "*Ensure that testing and result interpretation are conducted in a well-lit space with sufficient lighting.*"

	Extreme Vibration condition	Acceptable performance was observed under extreme vibration speeds up to 500 rpm after sample application.	QRI/IFU includes the statement, "*Conduct all testing on a level surface and ambient conditions.*"
Specimen Integrity	Effect of Repeated Freezing-Thawing Sample	Acceptable performance was observed with one time freeze thawing of positive and negative samples before extraction. However, false negative results were observed with 2-3 freeze/thaw cycles.	QRI/IFU includes the statement, "*The freshly collected specimens should be processed immediately after collection."*
	Specimen stability in high/low temperature and ambient conditions	Acceptable performance was observed for all contrived samples before extraction for up to 48 hours under all temperature conditions ranging from -20°C to 30°C.	
	Effect of Nasal discharge	Acceptable performance was observed in the presence of significant amount of nasal discharge.	N/A
Reagent Integrity	Short-term Stability at Low -20°C or high temperature +45°C	Acceptable performance was observed when test kits were stored at low temperature (-20°C) for 16 days and for 14 days at high temperature (45°C).	QRI/IFU recommends that the test kit be stored at 2°-30°C in the original sealed pouch.

The flex studies demonstrate that the test is robust in the claimed intended use condition with an insignificant risk of erroneous results.

C Demonstrating "Insignificant Risk of an Erroneous Result" - Accuracy

1. Comparison Study

The clinical performance of the Nano-Check Influenza A+B Test was evaluated in a multi-center, prospective clinical study in the U.S. between November 2022 and February 2025. The study only enrolled subjects who presented with symptoms of respiratory infection. A total of 2,223 subjects were initially enrolled. However, 254 samples were excluded due to subjects having symptoms longer than 4 days, invalid comparator results, or samples lost during shipment. The remaining 1,969 subjects were consecutively enrolled and tested by fourteen (14) operators who were representative of CLIA-waived users (i.e., not formally trained in a laboratory setting) across six (6) different clinical CLIA-waived sites. Two anterior nasal swabs (ANS) were collected from each study subject during the same visit. The first ANS specimen for the comparator method was collected by the operators from both sides of the nose. The comparator ANS specimens were stored in UVT media and packaged in dry ice for aero-transport, sent to a reference laboratory, and tested with an FDA cleared RT-PCR method as per the cleared instructions for use. The second ANS specimen was collected from both sides of the nose using the provided swab and was tested immediately using the Nano-Check Influenza A+B Test by each operator using the QRI only and with no training specifically with the candidate test.

The summary of demographics of the clinical subjects are shown in Table 4 below.

Table 4: Subject Demographics

Age Group	Female		Male		Total	
	No. of Sample	%	No. of Sample	%	No. of Sample	%
≤ 5 years	201	19.8	215	22.5	416	21.1
6 to 21 years	401	39.5	462	48.5	863	43.8
22 to 60 years	313	30.8	204	21.4	517	26.3
≥ 61 years	101	9.9	72	7.6	173	8.8
Total	1016	100	953	100	1969	100
	Female: 51.6% (1016/1969)		Male: 48.4% (953/1969)			

The clinical performance of the Nano-Check Influenza A+B Test compared to the highly sensitive RT-PCR comparator is shown in Table 5 below.

Table 5: Clinical Performance of the Nano-Check Influenza A+B Test compared to the comparator.

Flu A			
Nano-Check Influenza A+B Test	**RT-PCR Comparator**		**Total**
	Positive	**Negative**	
Positive	417	6	423
Negative	63	1483	1546
Total	480	1489	1969
Positive Percent Agreement (PPA) = 86.9% (417/480, 95% CI: 83.6% - 89.6%)			
Negative Percent Agreement (NPA) = 99.6% (1483/1489, 95% CI: 99.1% - 99.8%)			
Flu B			
Nano-Check Influenza A+B Test	**RT-PCR Comparator**		**Total**
	Positive	**Negative**	
Positive	99	5	104
Negative	15	1850	1865
Total	114	1855	1969
Positive Percent Agreement (PPA) = 86.8% (99/114, 95% CI: 79.4% - 91.9%)			
Negative Percent Agreement (NPA) = 99.7% (1850/1855, 95% CI: 99.4% - 99.9%)			

2. Device Performance with Analyte Concentrations Near the Cutoff:

a. Lot-to-lot Precision Study:

A precision evaluation was conducted over a 12-day period to assess the lot-to-lot variability of test results across different production lots using contrived sample panel consisting of True Negative (TN), High Negative (0.1X LoD), C90 (concentration corresponding to 90% detection probability), and Moderate Positive (3X LoD) samples for influenza A and B. The study was conducted by three (3) on-site operators for over 12-days at one site. Each operator tested one (1) panel with 14 samples twice daily. This design yielded 28 samples per operator per day, or 1,008 samples across all operators over the course of the study. Samples were blinded and randomized across the seven (7) concentration levels; per-panel allocations varied by design, but study-level allocation was balanced to achieve 144 samples per level. Operators followed the candidate device IFU during testing. Results of the study are summarized in Table 6 below and demonstrates consistent performance across all operators and production lot numbers.

Table 6: Summary Lot-to-Lot Variability Study Results

Sample	Test line	No. of Positives / No. of Samples tested (%)			Total no. of positives / Total no. of samples (%)
		Lot 1	Lot 2	Lot 3	
True Negative	Flu A	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/144 (0%)
	Flu B	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/144 (0%)
Flu A (0.1X LoD)	Flu A	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/144 (0%)
	Flu B	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/144 (0%)
Flu A (C90)	Flu A	42/48 (87.5%)	41/48 (85.4%)	45/48 (93.8%)	128/144 (88.9%)
	Flu B	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/144 (0%)
Flu A (3X LoD)	Flu A	48/48 (100%)	48/48 (100%)	48/48 (100%)	144/144 (100%)
	Flu B	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/144 (0%)
Flu B (0.1X LoD)	Flu A	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/144 (0%)
	Flu B	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/144 (0%)
Flu B (C90)	Flu A	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/144 (0%)
	Flu B	44/48 (91.7%)	43/48 (89.6%)	45/48 (93.8%)	132/144 (91.7%)
Flu B (3X LoD)	Flu A	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/144 (0%)
	Flu B	48/48 100%	48/48 100%	48/48 100%	144/144 (100%)

b. Multi-site Reproducibility Study:

A multi-site reproducibility study was performed to assess the performance of the candidate device using a contrived sample panel comprised of a true negative (TN), a high negative sample (HN, 0.1X LoD), a low positive (LP, 1X LoD) and a moderate positive (MP, 5X LoD) sample for each analyte. The study was conducted by untrained operators in CLIA waived settings for over five non-consecutive days.

Contrived swab samples were prepared by spiking pooled human nasal wash solution (confirmed Influenza A and Influenza B negative by RT-PCR) with Influenza A (H1N1) and Influenza B (Victoria Lineage) to concentrations of 5X LoD, 1X LoD or 0.1X LoD. Each diluted sample (50 µL) was directly applied onto the sample collection swab head. True negative swab samples were prepared by applying fifty (50) µL of negative pooled human nasal wash directly onto the sample collection swab head.

The contrived sample swabs were randomized and blinded to each operator at three (3) discrete CLIA-waived Point-of-Care (PoC) sites and an in-house site. Eight (8) operators across three (3) CLIA Waived sites and three (3) additional trained operators at the internal site conducted testing. Each operator tested 105 encoded samples, consisting of 15 samples each for various analyte levels.

The results in Table 7 showed high concordance among the eleven (11) operators across most sample types. Complete agreement (100%) was observed for TN samples, MP samples for both Flu A and Flu B, and LP-Flu B samples. For HN-Flu B samples, overall agreement was

99.4% (164/165 samples) with one false positive result at Site 3. For LP-Flu A samples, overall agreement was 99.4% (164/165 samples), with one false negative result at Site 3. These outcomes met the predefined acceptance criteria. While Site 3 showed minor variations (one false positive for HN-FluB and one false negative for LP-Flu A), no statistically significant differences were observed between sites.

Table 7: Summary Results of Multi-site Reproducibility Study

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)	
True Negative NCM	0/30 (0%)	0/45 (0%)	0/45 (0%)	0/45 (0%)	0/165 (0%)
0.1X LoD Flu A	0/30 (0%)	0/45 (0%)	0/45 (0%)	0/45 (0%)	0/165 (0%)
0.1X LoD Flu B	0/30 (0%)	0/45 (0%)	1/45 (2.2%)	0/45 (0%)	164/165 (99.4%)
1X LoD Flu A	30/30 (100%)	45/45 (100%)	44/45 (97.8%)	45/45 (100%)	164/165 (99.4%)
1X LoD Flu B	30/30 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	165/165 (100%)
5X LoD Flu A	30/30 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	165/165 (100%)
5X LoD Flu B	30/30 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)	165/165 (100%)

3. Operator Questionnaire:
   At the end of the study, each operator included in the CLIA waiver Clinical Evaluation and Reproducibility studies was given a questionnaire to provide feedback on the ease of use of the Nano-Check Influenza A+B Test. The questionnaire had 12 questions that evaluated the following general topics:

   - Ease of use of the test
   - Ability to follow the test instructions
   - Ability to properly interpret test results
   - Ability to test the control materials

   The operators performing the testing at each site also filled out a questionnaire about their professional training and background. Based on the operators' feedback, the overall Nano-Check Influenza A+B Test was found to be easy to use without the help of an expert. Processing the sample, applying it to the cassette correctly, and seeing and understanding the results were easy. Operators also found the written instructions for the Nano-Check Influenza A+B Test were clear and easy to follow.

D Labeling for Waived Devices

The labeling submitted for the Nano-Check Influenza A+B Test consists of:

1. Quick Reference Instructions (QRI)
2. Instructions for Use (IFU)
3. Package Labeling – kit box labels, device pouch label, sample collection swabs, empty reagent tubes, ampules containing extraction buffer

4. Package Labeling of the control swabs – positive control swab label and negative control swab label

The following elements are appropriately present:

- The test procedure within the QRI is written at 7th grade comprehension level.
- The QRI and the IFU identify the test as CLIA waived.
- The IFU contains a statement that a Certificate of Waiver is required to perform the test in a waived setting.
- The QRI and the IFU contain a statement that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test.
- The IFU contains a statement that any modification to the test or the manufacturer's instructions will result in the test being classified as high complexity.
- The IFU and QRI provide instructions for conducting quality control procedures.
- The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

XI. Conclusion

The submitted information in this CLIA waiver application supports a CLIA waiver approval decision.