CLIA Waiver by Application Approval Determination
Decision Summary

A. Document Number

CW240009

B. Parent Document Number

K231187

C. CLIA Waiver Type:

CLIA Waiver by Application

D. Applicant

Nano-Ditech Corporation

E. Proprietary and Established Names

Nano-Check COVID-19 Antigen Test

F. Measurand (analyte)

Nucleocapsid protein antigen from SARS-Coronavirus 2 (SARS-CoV-2)

G. Sample Type(s)

Direct anterior nasal swab specimens

H. Type of Test

Qualitative lateral flow immunoassay

I. Test System Description

1. Overview

The Nano-Check COVID-19 Antigen Test is an immunochromatographic lateral flow assay for detection of SARS-CoV-2 nucleoprotein antigens in human anterior nasal swab specimens without transport media from those who are suspected of COVID-19 within the first 4 days of symptom onset.

To initiate testing, a flocked swab is used to collect anterior nasal swab specimens from both nostrils. The patient sample is placed in the Reagent Tube, which is either provided pre-filled with buffer, or that the user pre-filled with buffer provided in the test kit's

ampule. The buffer disrupts the virus particles in the sample, exposing internal viral nucleoproteins. After disruption, the sample is dispensed into the Test Cassette sample well.

The Test Cassette is comprised of a plastic cassette with a nitrocellulose membrane and sample pads. Specifically, the test strip contains: (1) the Sample Pad, which receives the buffer and distributes the sample across the test strip; (2) the Biotin Pad, which contains biotinylated monoclonal antibodies specific to the SARS-CoV-2 nucleocapsid antigen; (3) the Dye Pad, which contains colloidal gold particles coupled with monoclonal antibodies specific to the SARS-CoV-2 nucleocapsid antigen; (4) the Test Line, which contains embedded streptavidin to capture the antibody-antigen immunocomplexes; and (5) the Absorbent Pad, which absorbs sample after it has migrated across the nitrocellulose membrane.

The sample will migrate up the test strip via capillary action. If SARS-CoV-2 nucleoprotein antigens are present, they will bind to the biotinylated monoclonal capture antibodies present on the biotin pad. As the sample passes through the dye pad, SARS-CoV-2 nucleoprotein antigen/biotin-antibody complexes will bind to specific monoclonal detection antibodies labelled with colloidal gold particles to form an immunocomplex with the biotinylated antibody, the nucleoprotein antigen, and the colloidal gold labelled antibody. At the test line, the immunocomplexes will be captured through interaction of the biotinylated antibody of the complexes with the streptavidin embedded into the test line, concentrating the colloidal gold-labeled antigen at the test line. This will form a visible pinkish-red line. Sample continues to flow through the test device which also contains a procedural control line to assess for sample presence and adequate sample flow. A visible pinkish-red line at the control region should always appear if the assay is performed correctly to verify proper liquid flow and gold conjugation. If no visible signal appears on C line, the test result is invalid, and this sample should be tested again with another test cassette. Test results are read between 15 and 30 minutes.

External positive control and negative control swabs are provided with each kit of Nano-Check COVID-19 Antigen Tests and should be processed according to the Instructions for Use upon receiving a new lot of test kits. The control swabs are intended to be used as quality control samples representative of positive and negative test samples to demonstrate that the reagents are functional, and the assay procedure is performed correctly.

2. Test System Components

The assay kit contains all materials needed to run the test, including external controls. For a 20-test kit, this includes:

- Individually packaged Test Cassettes (20): plastic housing with test strip containing monoclonal anti-SARS-CoV-2 antibodies sealed in an aluminum foil pouch with desiccant.
- Reagent: two test kit formats exist for the extraction reagent – all other components are the same between the test kit formats:

    - *Test Format A*: pre-filled reagent tubes (20) containing 300 µL buffer with detergents, reducing agents, and Proclin 300
    - *Test Format B*: ampules (20) containing 300 µL buffer with detergents, reducing agents, gentamicin, and Proclin 300 and empty reagent tubes (20)
- Sterile nasal swabs (20)
- External SARS-CoV-2 Positive Control Swab (1): swab is coated with non-infectious recombinant SARS-CoV-2 antigens.
- External Negative Control Swab (1): swab is coated with universal transport media
- Instructions for Use (1) and Quick Reference Instructions (1)

J. Demonstrating "Simple"

- *Is a fully automated instrument or a unitized or self-contained test.*

  The device is a unitized, self-contained test and only requires sample incubation in the extraction reagent prior to sample application.

- *Uses direct unprocessed specimens, such as capillary blood (fingerstick), venous whole blood, nasal swabs, throat swabs, or urine.*

  The test uses direct unprocessed anterior nasal swab specimens.

- *Needs only basic, non-technique-dependent specimen manipulation, including any for decontamination.*

  An untrained operator can conduct the test by performing seven to eight simple steps without sample manipulation: (1) collect the anterior nasal swab sample, (2a) for test kit Format B, first open the ampule and add the extraction buffer to the empty reagent tube, (2b) for both test kit formats, swirl the sample swab in the reagent tube with extraction buffer for 15 seconds, (3) express excess liquid from the swab by pinching the sides of the tube, (4) attach the dropper cap to the tube, (5) apply 2 drops of the sample to the cartridge, (6) wait 15 minutes, and (7) read the test results.

  No specialized equipment is needed for sample processing.

- *Needs only basic, non-technique-dependent reagent manipulation, such as "mix reagent A and reagent B."*

  The test requires only basic reagent handling to obtain accurate results.
    - For test kit Format A, the provided reagent is pre-measured and provided in single-use vials. No processing of reagents is needed prior to combining the sample and the reagent.
    - For test kit Format B, the provided reagent is pre-measured in a plastic ampule, which the user completely empties into a single-use empty vial. No measuring or additional processing of reagents is needed prior to combining the sample and the reagent.

  Both test kit formats are unitized and contain all the reagents required for analysis.

- *Needs no operator intervention during the analysis steps.*

  The test does not require any operator intervention during the analysis step. After application of two drops of the extracted sample to the test cassette, the test develops without user intervention for 15 minutes. Upon completion of the run time, the user interprets the test results by visual interpretation using the guidance provided in the Instructions for Use/Quick Reference Instructions.

- *Needs no technical or specialized training with respect to troubleshooting or interpretation of multiple or complex error codes.*

  No specialized instrumentation or machinery is required for this test. The Instructions for Use/Quick Reference Instructions contain information about result interpretation. No technical or specialized training is required for sample collection, sample processing, or result interpretation.

- *Needs no electronic or mechanical maintenance beyond simple tasks, e.g., changing a battery or power cord.*

  The Nano-Check COVID-19 Antigen Test does not require any instrumentation or machinery, and therefore does not need any electronic or mechanical maintenance.

- *Produces results that do not require operator calibration, interpretation, or calculation.*

  No operator calibration or calculation is required to interpret results from the Nano-Check COVID-19 Antigen Test. Operator visual interpretation of test results is required. The Instructions for Use/Quick Reference Instructions provided with the test kit include descriptions of how to interpret test results, including visual examples and indications of next steps to take based on the results.

- *Produces results that are easy to determine, such as 'positive' or 'negative,' a direct readout of numerical values, the clear presence or absence of a line, or obvious color gradations.*

  Nano-Check COVID-19 Antigen Test results are simple to determine as 'positive', 'negative', or 'invalid' based on the presence of absence of the pink-to-red colored test and control lines. The Instructions for Use/Quick Reference Instructions include descriptions and visual examples of test result interpretation.

- *Contains a quick reference instruction sheet that is written at no higher than a 7th grade reading level.*

  The Quick Reference Instructions are written at a 7th grade comprehension level.

K. Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-safe Mechanisms

1. Risk Analysis

Risk Management of the Nano-Check COVID-19 Antigen Test has been conducted in accordance with ISO 14971 and the FDA guidance, "*Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of In Vitro Diagnostic Devices*".

The sponsor utilized the Device Hazard Analysis and the Failure Mode Effects Analysis (FMEA) methods to assess the risks of failure that may occur during use or misuse of the device. The FMEA includes use-related (i.e., influenced by the user) failure modes associated with incorrect diagnosis, invalid results, unable to run test, and no result. The use related FMEA evaluates potential failures that may be caused by the user during use or misuse of the product. Elements considered include the intended user, environment (e.g., Physician Office, Lab, Hospital), human factors/potential human errors, and historical field data from similar devices.

Potential sources of errors that could adversely affect system performance were identified and mitigated first through system design and then through additional cautions in the labeling. The following safety risk control methods were used to eliminate or reduce the probability of occurrence of identified safety hazards:

- *Design* – incorporate design decisions to ensure a fail-safe architecture, including providing redundant hardware or selection of high reliability components to minimize potential safety risks.
- *Manufacturing Procedures* – procedures that are used in the manufacturing process are compliant with applicable quality system regulations to ensure product safety and efficacy.
- *Testing and Verifications* – specific device test or verification activities to verify product performance of in-process and final released product consistent with the cleared performance described in the labeling.
- *Labeling / Instructions for Use* – develop external labels for the device and instructions for use that define intended use and required operational use procedures and contra-indicate improper device use.

The identified risks which could result in erroneous test results were evaluated in flex studies that stressed the functional limits of the test system (Section K.3).

2. Fail-Safe and Failure Alert Mechanisms

The Nano-Check COVID-19 Antigen 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 "C" and the SARS-CoV-2 antigen line is denoted as "Ag".
- The test cassette is printed with "COVID-19" 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 for both test kit formats (kits with pre-filled tubes and kits with ampules and empty reagent tubes), which in turn ensures the accuracy of the assay results.

Fail-safe Features

- *Internal Quality Control* – the test device contains a built-in procedural control. The internal procedural control "C" 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 "C" region of the results window. If the control "C" 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 SARS-CoV-2 nucleocapsid protein with a preservative.
    - The Negative Control Swab contains only universal transport media (UTM)

  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 Nano-Check COVID-19 Antigen Test were evaluated in a series of experiments of "stress", including conditions outside of those recommended in the instructions for use. The studies to support the CLIA Waiver Application for the Nano-Check COVID-19 Antigen Test are listed in **Table 1.**

Table 1. Summary of Flex Studies Performed

Risk Category	Failure Mode	Potential Failure Effect	Flex Study Result	Risk Control Measures
Environ-mental Factor	Temperature and Humidity Extremes	Altered assay performance	Assay performance was not affected by temperature (10-40° C) or humidity (<20% to >95% RH) extremes.	Labeling includes recommended test storage conditions.
	Poor Reading Conditions (Lighting Variability)	Inaccurate test processing, inaccurate result interpretation	Test use/result interpretation was not affected in most lighting conditions; difficulty with result interpretation was observed in low-light (dusk) conditions.	Labeling instructs user to ensure there is sufficient lighting for testing and interpretation.
Operator Errors/ Human Factors	Sample Application Variability	Altered assay performance	Assay performance was affected when dropper was held horizontally over the test when dispensing sample.	Labeling instructs user to hold the reagent tube vertically over the sample cassette.
	Sample Volume Variability	Altered assay performance	Assay performance was affected when too few or too many sample drops were added.	Labeling instructs the user to add 2 drop of sample and includes a warning statement about adding too few or too many drops.
	Extraction Buffer Volume Variability	Altered assay performance	Assay performance was affected with too little extraction buffer (75 µL, compared to the expected 300 µL).	Labeling instructs users to check the pre-marked volume lines on the side of the reagent tube to ensure sufficient buffer is present.
	Extraction Method Variability	Altered assay performance	Assay performance was affected when swabs were not mixed with extraction buffer.	Labeling instructs users to swirl swabs for 15 seconds and squeeze the swab head during removal.
	Test Kit Format B: Ampule Usability	Altered assay performance	Assay performance was not affected when using Test Kit Format B (with extraction buffer ampules that are emptied into empty reagent tubes)	Labeling includes instructions for how to use both test kit formats, including schematics.
	Disturbance Effect	Altered assay performance	Assay performance was affected when the device was dropped from 3 ft.	Labeling instructs users to conduct on testing on a flat surface.
	Reading Time Tolerance	Altered assay performance	Assay performance was affected when results were read very early (5 min) or late (45 minutes).	Labeling instructs user to read results at 15 minutes and warns of inaccurate

Risk Category	Failure Mode	Potential Failure Effect	Flex Study Result	Risk Control Measures
				results if read before or after 15-30 minutes.
	Extracted Sample Stability	Altered assay performance	Assay performance was not affected when samples were extracted and then tested after delays of up to 60 minutes.	Labeling instructs users to process extracted samples up to 30 minutes after collection.
	Open Pouch Stability	Altered assay performance	Assay performance was not affected when an open kit/test cassette pouch was used up to 4 hours after opening.	Labeling instructs users to use the test card within 90 minutes of opening.
Specimen Integrity and Handling	Specimen Stability	Altered assay performance	Assay performance was not affected when specimens were stored at room temperature (23-30°C) for 8 hours or 2-8°C for 48 hours.	Labeling instructs users to use freshly collected specimens, or specimens stored no more than 1 hour at RT and 48 hours at 2-8°C.
	Incorrect Specimen	Altered assay performance	Assay performance was not affected by saliva samples or nasal samples with discharge. Performance was affected by frozen nasal samples in VTM.	Labeling indicates the acceptable sample type (anterior nasal swab specimens) and warns VTM should not be used.
Reagent Integrity	Expired Test Kit Use	Altered assay performance	Assay performance was not affected when using kits up to 13 months past expiration date.	Labeling warns users not to use test kit beyond expiration date.
	External Control Repeatability	Altered assay performance	Assay performance with external controls was not affected by different lots or operators.	Labeling instructs users to contact technical support of external controls do not perform as expected.

Samples used for flex study testing were prepared in commercially available negative clinical matrix (NCM), which was prepared by pooling negative nasal cavity wash collected from healthy individuals in saline. Nasal wash was collected from a minimum of three healthy donors per batch. The pooled sample was confirmed COVID-19 negative by a highly sensitive SARS-CoV-2 RT-PCR assay. Matrix equivalency to nasal swab matrix was established in the parent submission K231187.

Contrived positive samples were prepared at 2x LoD by spiking NCM with heat-inactivated SARS-CoV-2 (isolate: hCoV-19/USA/MD-HP20874/2021) to obtain a concentration of 3.90×10^2 $TCID_{50}$/mL.

The negative and positive test samples (under the applicable flex conditions) were evaluated in replicates of five by three operators, for a total of 15 replicates for each condition. Samples were tested according to the Instructions for Use protocol, except for the noted deviations dictated by the flex parameter under evaluation. Unless otherwise specified, test kit Format A (with pre-filled reagent tubes) was used for flex testing. The effect of the following conditions on the performance of the test was evaluated, organized by the potential type of source error.

Operational Environment

a. Temperature and Humidity Variability

This study evaluated the impact of operating the test device at the extremes of the recommended temperature and humidity conditions. The Nano-Check COVID-19 Antigen Test is intended to be operated at room temperature between 15°C and 30°C at ambient humidity. This study simulated the variable operating conditions of temperature and humidity reasonably expected to occur indoors at near-patient and point-of-care facilities, including the extremes of recommended temperature. All materials and components were allowed to equilibrate to the tested operating environment temperature and humidity levels for at least one hour prior to testing. Samples were tested under the following conditions:

- low temperature (10°C)
- ambient room temperature (15-30°C)
- high temperature (40°C)
- low humidity (<20% relative humidity)
- ambient humidity (35% relative humidity)
- high humidity (>90% relative humidity).

For all replicates, there was 100% agreement with the expected negative and positive results in all environmental test conditions. This study supports the use of the test device in the environmental conditions expected in a near-patient setting.

b. Lighting Variability

This study evaluated the impact of lighting source on the user's ability to appropriately use the Nano-Check COVID-19 Antigen Test and interpret results. The following lighting conditions were tested:

- Dusk (10-15 lux)
- Shaded sunlight (500-1,000 lux)
- Incandescent lamp (2,000-2,500 lux)
- Direct sunlight (>50,000 lux).

There were three (3) false negative results (3/15) in the dusk (10-15 lux) condition. For all other replicates, there was 100% agreement with the expected negative and positive results in all environmental test conditions. This study supports the use of the test device in the lighting conditions expected in a near-patient setting. Mitigation for interpreting the test results in low lighting is included in the labeling.

Operator Errors/Human Factors

c. Sample Application Position

This study evaluated the impact of how the reagent tube is held over the test cassette when adding sample to the cassette. The following conditions were tested:

- reagent tube held vertically over the cassette

- reagent tube held 45° over the cassette
- reagent tube held horizontally over the cassette.

In all conditions, two drops of sample (in accordance with the instructions for use) were added to the test cassette. Drops could not be properly delivered to the cassette when the reagent tube was held horizontally, leading to invalid results for all samples. For the other two test conditions, there was 100% agreement with the expected negative and positive results for all replicates. The Instructions for Use/Quick Reference Instructions instruct users to hold the reagent tube vertically when applying sample to the test cassette. This study supports the use of the test device as described in the labeling.

d. *Sample Volume Variability*

This study evaluated the impact of adding the incorrect number of drops of extracted sample to the test cassette. The Instructions for Use instructs users to add 2 drops into the test cassette sample well. This study simulated user error by flexing the number of drops of sample added to the sample well. The following number of drops were added:

- 1 drop
- 2 drops
- 3 drops
- 4 drops.

Invalid results were observed with 1 drop with both negative and positive samples. This sample volume (1 drop) appears to be near or below the minimum volume needed to achieve sufficient sample migration through the test strip. False negative results (3/15) and invalid results (1/15) were observed with positive samples when 4 drops of extracted sample were added. All other conditions yielded 100% agreement between observed and expected results for all replicates. The study supports the sample volume specified in the labeling and demonstrates that 2-3 sample drops do not adversely impact the performance of the Nano-Check COVID-19 Antigen Test. Additional mitigations to prevent an incorrect number of drops are also included in the labeling.

e. *Extraction Buffer Volume Variability*

This study evaluated the impact of extraction buffer volume on device performance. The Nano-Check COVID-19 Antigen Test is distributed in two formats: Format A includes reagent tubes pre-filled with 300 µL extraction buffer, and Format B includes ampules with extraction buffer that the user squeezes into an empty reagent tube. This study assesses device performance if extraction buffer volume is less than the expected 300 µL either due to manufacturing variability (Format A) or user error (e.g., spillage particularly for Format B). Samples were extracted in 75 µL, 150 µL, or 300 µL extraction buffer. Invalid results were observed for both negative and positive samples with the 75 µL condition due to insufficient sample volume being applied to the test. Positive and negative results in the 150 µL and 300 µL condition were in 100% agreement with expected agreements for all replicates. Processing

samples with too little extraction buffer volume is mitigated through the fill lines in the extraction buffer tube design and labeling instructions. This study supports the robustness of the device design.

f. Extraction Variability

This study evaluated the impact of variations in the swab sample extraction step on device performance. The Instructions for Use/Quick Reference Instructions instructs users to: "*Swirl and plunge the swab up and down in the extraction buffer while squeezing the sides of the tube for 15 seconds. Remove the swab while squeezing the sides of the tube to the swab head for extracting the maximum amount of liquid from the swab."* The following extraction conditions were tested:

- swab dipped in extraction reagent with no swirling or squeezing
- 5 second swirl in extraction reagent
- 15 second swirl in extraction reagent
- squeeze swab head during removal
- swab head not squeezed during removal
- reagent tube shaking (introduction of bubbles).

The condition in which swabs were only dipped in the extraction buffer without swirling or squeezing yielded 7 false negative results (7/15). One false negative result (1/15) was observed in the condition where the swab head wasn't squeezed. All other conditions yielded 100% agreement with expected results. This study supports the extraction procedure described in the labeling.

g. Disturbance Effect

This study evaluated the impact of physical disturbances to the device during testing. The following conditions were tested:

- device shaken immediately after sample application,
- device shaken 5 minutes after sample application
- device dropped from 3 feet immediately after sample application
- and device dropped from 3 feet 5 minutes after sample application.

The shaken devices yielded expected results for 100% of samples and replicates. The dropped devices yielded invalid results for positive samples both immediately after sample application (15/15 invalid replicates) and 5 minutes after sample application (10/15 invalid replicates). This level of physical impact is not expected to be common during use of the device. The instructions for use tell users to "*Conduct all testing on a level surface*." This study supports the use of the Nano-Check COVID-19 Test in the near-patient environment.

h. Reading Time Tolerance

This study evaluated the impact of reading results at various timepoints after adding sample to simulate user error in reading time. The Instructions for Use instructs users to read results 15 minutes after sample application. The following reading times were assessed: 5, 10, 15, 30, and 45 minutes. Positive and negative samples yielded 100%

agreement with expected results when results were read between 10 and 30 minutes. Positive samples yielded false negative results (14/15) when read at 5 minutes, likely due to insufficient time for test line development. Negative samples yielded false positive results (4/15) when read at 45 minutes, likely due to membrane drying. The Instructions for Use includes the following instructions: "*Read the results at 15 minutes visually. Do not read result more than 30 minutes after the sample application. Note: False negative or false positive results can occur if read before 15 or after 30 minutes*." The study supports the result reading time specified in the labeling and demonstrates that reading times of 10-30 minutes do not adversely impact the performance of the Nano-Check COVID-19 Antigen Test. Variations in read time that can impact performance are adequately mitigated in the instructions.

i. Extracted Sample Stability

This study evaluated the impact of delayed testing with extracted sample. Samples were extracted and then the reagent tube containing the extracted sample was left at room temperature (15-30°C) or high temperature (30°C). Samples were tested after 0, 30, and 60 minutes. All replicates yielded 100% agreement with expected results for all tested conditions. The instructions for use state the following: "*Samples in extraction buffer can be processed up to thirty minutes after collection when kept at room temperature."* This study supports the use of extracted samples for up to 30 minutes post-extraction with the Nano-Check COVID-19 Antigen Test.

j. In-Use (Open Pouch) Stability

This study evaluated the impact of using an open test kit pouch on device performance. The foil pouch containing the test cassette was opened and allowed to sit at room temperature (23.5°C for 15 min., 30 min., 45 min., 1 hr., 1.5 hrs., 2 hrs., 3 hrs., or 4 hrs.) before use. Positive and negative samples yielded 100% agreement with expected results for all replicates across all conditions. The instructions for use include the following statement: "*Once opened, the test card should be used within 90 minutes.*" The study supports the stability of the opened device specified in the labeling and demonstrates that stability up to 4 hours does not impact performance.

k. *Specimen Integrity and Handling*

i. Specimen Stability

This study evaluated the impact of nasal swab specimen testing delays on device performance. Samples were prepared and tested at four analyte levels: negative (matrix only), high negative (0.3x LoD), low positive (1x LoD), and positive (3x LoD). Five replicates of each sample were stored at ambient temperature (23.5°C), high room temperature (30°C), and refrigerated (2-8°C) and tested after 1, 2, 4, 8, 24, 48, and 72 hours. Positive percent agreement was determined for each sample and condition. The positive (3x LoD) samples demonstrated 100% agreement for all tested conditions. The low positive (1x LoD) samples demonstrated 100% agreement for all 2-8°C storage conditions and 100% agreement for the room temperature conditions (23.5°C and 30°C) up to and including 24 hours. At 48 hours, positive percent agreement dropped to 60% (3/5) and 20% (1/5) for the 23.5°C and 30°C

conditions, respectively. The high negative (0.3x LoD) samples met the acceptance criteria (positivity ≤95%) under all storage conditions. The negative samples demonstrated expected results (0% positivity) in all conditions. Based on the study results, the following nasal swab specimen stability conditions were supported:

- 8 hours when stored at ambient room temperature (23.5°C)
- 8 hours when stored at high room temperature (30°C)
- 48 hours when stored refrigerated (2-8°C)

The Instructions for Use indicate the following: "*Process freshly collected anterior nasal swab samples immediately, but no later than one hour after collection. If needed, the swab may be stored at room temperature (15°C - 30°C) for 1 hour.*" The study supports the specimen stability and demonstrates the robustness of the Nano-Check COVID-19 Antigen Test when using samples that are not freshly tested.

ii. Use of Incorrect Specimen Type

The Nano-Check COVID-19 Antigen Test is intended for use with fresh anterior nasal swab specimens. This study evaluated the performance of the test device if incorrect or unvalidated sample types are used, specifically, nasal samples frozen overnight in viral transport media (VTM). Nasal swabs with excessive nasal discharge were also tested to assess the limitations of the assay with the intended sample type. For positive and negative samples, 100% agreement was observed with nasal samples coated with nasal discharge. For nasal samples frozen in VTM, false negative results were observed for 14 out of 15 total positive samples. The following labeling mitigations are included:

- *Acceptable specimen type for testing with the Nano-Check™ COVID-19 Antigen Test are anterior nasal swab specimens.*
- *Improper specimen handling and/or transport may yield false results.*
- *Use only swabs provided with the kit.*
- *Viral transport media (VTM) should not be used with this test.*
- *For use with human specimen material only.*

The study results demonstrate the robustness of the test device with incorrect sample types.

l. Reagent Integrity - Use of Expired Test Kit

This study evaluated the impact of using expired Nano-Check COVID-19 Antigen Test kits using two different lots of expired kits stored at room temperature and tested monthly over a three-month period. All results were as expected, demonstrating acceptable device performance with kits from 9 to 13 months past the expiration date. While the study supports the robustness of the test device use of expired reagents is generally not recommended, and the Instructions for Use/Quick Reference Instructions include the following warning statement: *"Do not use test kit beyond expiration date."*

m. External Control Repeatability

This study evaluated the repeatability of the external control swabs that are included with the Nano-Check COVID-19 Antigen Test. The external controls are intended to ensure the test device is operating appropriately as part of quality control. Three blinded operators tested ten replicates each of the external positive and negative controls. Each operator tested a unique lot of external controls on a unique lot of test devices. All results were as expected, with 100% agreement for positive external control samples and 100% agreement for negative external control samples. The Instructions for Use/Quick Reference Instructions state: *"If the controls do not perform as expected, do not report patient results. Contact please the Technical Support […]"* The study supports the robustness of the external control performance and adequately mitigates potential performance issues of the control upon regular use.

L. Demonstrating "Insignificant Risk of an Erroneous Result" –Accuracy

1. Comparison Study

a. Study Design

i. Study Sites and Duration

The CLIA Waiver Clinical Performance Study was conducted from January 2022 to February 2024 to demonstrate the clinical performance of the Nano-Check COVID-19 Antigen Test to detect SARS-CoV-2 viral nucleoprotein antigen in specimens obtained from nasal swabs. Omicron was the predominantly circulating variant during the study timeframe, and the performance estimates derived from the clinical study therefore reflect the performance expected for the device in real world testing scenarios at the time of granting this CLIA Waiver by Application request. In this study, nasal swabs were prospectively collected from symptomatic subjects by swabbing both sides of the nose and tested on the Nano-Check COVID-19 Antigen Test and the comparator device. This was a multi-center, prospective study performed at four clinical sites in the U.S.; comparator samples were tested at one reference laboratory (Error! Not a valid bookmark self-reference.). The first swab collected from subjects was shipped to a central reference laboratory for evaluation with the FDA-cleared comparator assay (Roche cobas SARS-CoV-2 RT-PCR assay). The second swab was tested on the Nano-Check COVID-19 Antigen Test by an untrained operator at the CLIA-Waived site.

ii. Operators

There were a total of 12 operators representative of CLIA-waived users across the four clinical testing sits (**Table 2**), consisting of administrative personnel, medical assistants, nurses, physicians, and other patient care providers. All operators were assigned to subject enrollment and/or sample collection, testing, and/or shipping for the CLIA Waiver clinical performance study. All operators employed in this CLIA Waiver clinical performance study had prior experience in CLIA-waived settings. No operators had any prior experience in high-moderate complexity

laboratory settings. No operators were previously trained in the use of the Nano-Check COVID-19 Antigen Test and relied soley on the Quick Reference Instructions. Upon completion of the study, the operators at each site were asked to complete an Operator Questionnaire that asked them to rate the ease of use of the test procedure (Section L.2).

Table 2. Clinical Study Operators

Site	Operator	Occupation	CLIA Waived
1	1	Nurse	Yes
	2	Nurse	Yes
2	3	Medical Assistant	Yes
	4	Medical Assistant	Yes
3	5	Nurse	Yes
	6	Nurse	Yes
4	7	Medical Assistant	Yes
	8	Physician	Yes
	9	Medical Assistant	Yes
	10	Medical Assistant	Yes
	11	Physician	Yes
	12	Physician	Yes

iii. Instructions for Use

The Nano-Check COVID-19 Antigen Test was performed in accordance with the assay procedure in the draft Quick Reference Instructions. No other materials or instructions were provided and the operators received no training in the use of the test.

iv. Subjects (Patients)

Performance characteristics of the Nano-Check COVID-19 Antigen Test were established with samples from symptomatic study subjects, prospectively enrolled from January 2022 to February 2024. A total of 972 subjects were enrolled in the study.

Inclusion Criteria

1. Any patients that visit the clinical site through reservation or as a walk-in.
2. Any patient presenting with clinical symptoms of COVID-19
3. Individuals aged 18 years or older who are the legal responsibility of themselves.
4. Individuals aged 17 years or less who have an accompanying parent that can give permission to participate.

Exclusion Criteria

1. Any individual who is currently receiving or has ever received approved or experimental treatment for COVID-19.

v. Samples

Two nasal samples were collected from each study subject during the same visit. The first swab was collected for testing with the comparator test, placed into a BD Universal Viral Transport (UVT) System tube, stored on dry ice, and shipped to the reference laboratory. Upon receipt by the reference laboratory, the nasal swab was tested with the chosen comparator method. The second swab was tested on the Nano-Check COVID-19 Antigen Test by a CLIA-waived test operator at the site.

A total of 972 paired nasal swab specimens were enrolled in the study. Of those, 148 specimens were excluded from the final analysis:

- 133 samples were collected from patients who were over 4 days post symptom onset.
- 4 samples were collected from patients without symptoms.
- 11 samples were lost during shipment to the reference lab for comparator testing.

A total of 824 nasal swab specimens were considered evaluable for the purpose of analysis in the clinical performance study.

vi. Comparative Method (CM)

The sponsor identified the Roche cobas SARS-CoV-2 on Roche cobas 6800 System (Roche Molecular Systems Inc.; K213804) as the comparator method. The chosen comparator is a RT-PCR molecular test with high sensitivity, employs an RNA extraction and purification steps, was validated with clinical samples that contained an acceptable number of low positive samples and demonstrated acceptable performance.

b. Results and Analysis

i. Statistical Analysis of Comparison Study Results

The results from this CLIA Waiver Clinical Evaluation study demonstrate acceptable performance in a total of 901 evaluable subjects, comprising 184 positives and 640 negatives, with an overall sensitivity of 84.78% and a specificity of 99.69% (**Table 3**).

Table 3. Nano-Check COVID-19 Antigen Test Performance

	Comparator Positive	**Comparator Negative**	**Total**
Nano-Check Positive	156	2	158
Nano-Check Negative	28	638	666
Total	184	640	824

PPA: 84.78% (156/184) [95% CI: 78.9 – 89.3%]
NPA: 99.69% (638/640) [95% CI: 98.9 – 99.9%]

Clinical performance was also stratified by each Operator (**Table 4**) and by each Site (**Table 5**).

Table 4. Clinical Performance Stratified by Study Operator

Site	Operator	n	TP	FP	FN	TN	PPA	NPA
1	1	104	23	1	2	78	92.00%	98.73%
	2	124	14	0	7	103	66.67%	100.00%
2	3	108	19	1	4	84	82.61%	98.82%
	4	125	24	0	4	97	85.71%	100.00%
3	5	39	6	0	1	32	85.71%	100.00%
	6	58	5	0	1	52	83.33%	100.00%
4	7	67	19	0	4	44	82.61%	100.00%
	8	55	15	0	2	38	88.24%	100.00%
	9	27	4	0	2	21	66.67%	100.00%
	10	24	4	0	1	19	80.00%	100.00%
	11	60	13	0	0	47	100.00%	100.00%
	12	33	10	0	0	23	100.00%	100.00%

Table 5. Clinical Performance Stratified by Study Site

Site	N	TP	FP	FN	TN	PPA	NPA
1	228	37	1	9	181	80.44%	99.45%
2	233	43	1	8	181	84.31%	99.45%
3	97	11	0	2	84	84.62%	100.0%
4	266	65	0	9	192	87.84%	100.0%

ii. Device Performance with Analyte Concentrations Near the Cutoff

The sponsor conducted a Reproducibility study to evaluate the inter-site reproducibility of the Nano-Check COVID-19 Antigen Test when tested by the intended user. This study was conducted at three distinct CLIA waiver sites using a blinded panel of contrived samples to demonstrate that the personnel at these sites, who are representative of the intended user, could perform the test consistently and correctly. At site 1, three operators were included. At sites 2 and 3, two operators were included.

The study panel included contrived positive samples prepared in negative clinical matrix at four levels: negative (N), high negative (HN), low positive (LP), and moderate positive (MP) (**Table 6**).

Table 6. Reproducibility Study Sample Panel

Sample Level	Concentration
Negative (N)	N/A – NCM only
High Negative (HN)	0.1x LoD (0.7 x 10^2 $TCID_{50}$/mL)

Sample Level	Concentration
Low Positive (LP)	1x LoD (7.0 x 10^2 $TCID_{50}$/mL)
Moderate Positive (MP)	3x LoD (2.1 x 10^3 $TCID_{50}$/mL)

Panels were tested over five non-consecutive days by a total of seven operators across the three external point-of-care sites. Operators were untrained and reflective of the intended users of the device. Testing was performed in accordance with the Nano-Check COVID-19 Antigen Test Quick Reference Instructions.

Overall, the reproducibility study generated a total of 420 results, with each operator testing 60 samples (15 at each sample level). The overall qualitative and quantitative results are summarized below in **Table 7**.

Table 7. Reproducibility Performance

Site	Sample	Operator	Total Samples Tested	# of Invalid Results	# of Negative Results	# of Positive Results	Agreement (%)*	95%CI
1	True Negative	1	15	0	15	0	100.0	76.6-100.0
		2	15	0	15	0	100.0	76.6-100.0
		3	15	0	15	0	100.0	76.6-100.0
		All	45	0	45	0	100.0	92.1-100.0
	High Negative	1	15	0	15	0	100.0	76.6-100.0
		2	15	0	15	0	100.0	76.6-100.0
		3	15	0	15	0	100.0	76.6-100.0
		All	45	0	45	0	100.0	92.1-100.0
	Low Positive	1	15	0	2	13	86.7	62.1-96.3
		2	15	0	1	14	93.3	70.2-98.8
		3	15	0	0	15	100.0	76.6-100.0
		All	45	0	3	41	93.2	81.8-97.6
	Moderate Positive	1	15	0	0	15	100.0	76.6-100.0
		2	15	0	0	15	100.0	76.6-100.0
		3	15	0	0	15	100.0	76.6-100.0
		All	45	0	0	45	100.0	92.1-100.0
	Total		*180*	*0*	*93*	*87*	*96.7*	*90.7-98.9*
2	True Negative	1	15	0	15	0	100.0	76.6-100.0
		2	15	0	15	0	100.0	76.6-100.0
		All	30	0	30	0	100.0	88.7-100.0
	High Negative	1	15	0	15	0	100.0	76.6-100.0
		2	15	0	15	0	100.0	76.6-100.0
		All	30	0	30	0	100.0	88.7-100.0
	Low Positive	1	15	0	0	15	100.0	76.6-100.0
		2	15	0	0	15	100.0	76.6-100.0
		All	30	0	0	30	100.0	88.7-100.0
	Moderate Positive	1	15	0	0	15	100.0	76.6-100.0
		2	15	0	0	15	100.0	76.6-100.0

Site	Sample	Operator	Total Samples Tested	# of Invalid Results	# of Negative Results	# of Positive Results	Agreement (%)*	95%CI
		All	30	0	0	30	100.0	88.7-100.0
	Total		*120*	*0*	*60*	*60*	*100.0*	*93.9-100.0*
3	True Negative	1	15	0	15	0	100.0	76.6-100.0
		2	15	0	15	0	100.0	76.6-100.0
		All	30	0	30	0	100.0	88.7-100.0
	High Negative	1	15	0	15	0	100.0	76.6-100.0
		2	15	0	15	0	100.0	76.6-100.0
		All	30	0	30	0	100.0	88.7-100.0
	Low Positive	1	15	0	0	15	100.0	76.6-100.0
		2	15	0	0	15	100.0	76.6-100.0
		All	30	0	0	30	100.0	88.7-100.0
	Moderate Positive	1	15	0	0	15	100.0	76.6-100.0
		2	15	0	0	15	100.0	76.6-100.0
		All	30	0	0	30	100.0	88.7-100.0
	Total		*120*	*0*	*60*	*60*	*100.0*	*93.9-100.0*

* Agreement is defined as negative percent agreement for negative samples and positive percent agreement for positive samples.

2. 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 COVID-19 Antigen Test. The questionnaire had 12 questions and was dived into the following sections:

1) Assay Procedure and Instructions for Use (10 questions)
2) External Controls (1 question)
3) Overall Test (1 question)

The operators performing the testing at each site also filled out a questionnaire about their professional training and background (see Table 2 above). Based on the feedback from the 7 operators, the overall Nano-Check COVID-19 Antigen Test was found to be easy to set up, operate, and interpret results. Operators also found the Instructions for Use easy to use and understand.

3. Kit Format B Usability

The Nano-Check COVID-19 Antigen Test has two kit formats: Format A contains a pre-filled reagent tube, and Format B contains an empty reagent tube and ampule containing extraction buffer. A usability study was conducted to evaluate the usability of the procedure with test kit Format B. In this study, blinded operators evaluated negative and contrived positive samples prepared with heat-inactivated SARS-CoV-2 in negative clinical matrix (NCM) at four levels: true negative ("TN", composed of NCM only), high negative ("HN", 0.1x LoD), low positive ("LP", 1x LoD), and moderate positive ("MP", 3x LoD). A total of four sites and eight operators were used: three external point-of-care sites with two untrained operators at each site and one internal site with two trained

operators. Operators tested samples for five consecutive days in accordance with the package insert. Three lots of buffer-containing ampule were included and one lot of pre-filled reagent tube was included.

Results were compared between kit Format A and kit Format B. No differences in the detection of any of the sample levels were observed, and there was 100% agreement in results between test kit Format A and Format B. No differences between operator or ampule lot were observed. Additionally, users were able to squeeze the buffer from the ampule into the empty reagent tube up to or exceeding the volume line equivalent to pre-filled tubes 100% of the time. The study and results demonstrate that results generated from test kit Format A and Format B are equivalent and there is insignificant risk of erroneous results when using test kit Format B.

Additionally, the sponsor conducted a comprehensive risk analysis using failure mode and effect analysis (FMEA) to identify potential risks associated with the proposed alternative testing method using test kit Format B (ampule containing reagent solution).

- No risks related to the manufacturing process or environmental hazards were identified.
- The following risks related to assay performance were identified: (1) lower sensitivity due to increased volume in the ampule and therefore the reagent tube; (2) interference testing with the ampule reagent which contains the additional component of gentamicin; (3) microbial cross-reactivity testing with the new ampule containing the additional component of gentamicin.
    - These risks were addressed and deemed to be insignificant through the analytical validation studies (LoD, interference, cross-reactivity) included in the parent submission (K231187).
- Risk related to operator error was identified.
    - This risk was assessed and deemed to be insignificant through the usability study above that assessed the ampule usage instructions as described in the QRI and ampule squeezing volume study above.

Based on the risk analysis above and the ampule usability study, the alternative test kit Format B is sufficiently robust.

M. Labeling for Waived Devices

The labeling submitted for the Nano-Check COVID-19 Antigen Test consists of:

1. Instructions for Use: Nano-Check COVID-19 Antigen Test Package Insert
2. Quick Reference Instructions: Nano-Check COVID-19 Antigen Test QRI

The following elements are appropriately present:

- The Quick Reference Guide and the Instructions for Use are written at no higher than a 7th grade reading level.
- The Instructions for Use and Quick Reference Guide identify the test as CLIA waived.
- The Instructions for Use and test cartridge package insert contain a statement that a Certificate of Waiver is required to perform the test in a waived setting.
- The Instructions for Use and Quick Reference Guide contain a statement that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test. 42 CFR 493.15(e)(1).
- The Instructions for Use and Quick Reference Guide provide instructions for conducting quality control procedures.
- The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

N. Benefit-Risk Considerations

The evidence provided in this submission indicates that this assay will appropriately diagnose SARS-CoV-2 within the intended use population. This assay was validated more vigorously as compared to an EUA device to support a full authorization and classification as a Class II device. The CLIA Waiver Clinical Evaluation study demonstrated an acceptable sensitivity of 84.78% (156/184) and an acceptable specificity of 99.69% (638/640). The clinical study took place when Omicron was the predominant circulating variant. The derived performance estimates therefore reflect the expected device performance under current real-world use at the time of granting this CLIA Waiver Request by Application.

The risks associated with the device, when used as intended, are those related to the risk of false test results, failure to correctly interpret the test results, and failure to correctly operate the device. The clinical benefits outweigh the probable risk of erroneous results for the proposed assay, considering the product labeling, special controls, and general controls. The clinical benefits of the assay include ease of use for the healthcare provider. The results of the CLIA Waiver Clinical Evaluation study, Reproducibility study, and Operator Questionnaire suggest that errors will be uncommon and are mitigated by the device labeling, which will facilitate accurate assay implementation and interpretation of results.

We acknowledge that the device sensitivity falls below that of the Sofia 2 SARS Antigen + FIA test (DEN220039), which has been cleared for use in near-patient settings. That is, the Sofia device demonstrated a PPA of 89.0% against an FDA EUA-authorized highly sensitive RT-PCR comparator, whereas the Nano-Check COVID-19 Antigen Test, with similar specificity, demonstrated a PPA of 84.8% against an equivalent molecular comparator test. The observed differences in sensitivity are not specific to the Nano-Check COVID-19 Antigen Test but have been observed by the Agency for many similar tests. They are associated with a change in circulating SARS-CoV-2 variants, the vaccine and/or infection induced immunity of the population, and the related shift in viral load peaks with a narrowing

viremic phase during current SARS-CoV-2 infections.[1] FDA determined that these changes in the virus strains and course of infection when weighed against the need for additional simple to use COVID-19 tests in CLIA Waived settings justify a PPA of 84.8% as long as the performance is reflective of both the currently relevant circulating strains and the course of disease currently observed in infected patients (including current peaks and distributions of viral loads).

Thus, granting of a CLIA Waiver for the Nano-Check COVID-19 Antigen Test will provide substantial benefits to patients and healthcare providers as an aid in the diagnosis of SARS-CoV-2 when used in conjunction with other laboratory results and clinical information, and will be a benefit to public health.

O. Conclusion:

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

[1] JK Frediani et al. The new normal: delayed peak SARS-CoV-2 viral loads relative to symptom onset and implications for COVID-19 testing programs. 2024. *Clinical Infectious Diseases.* 78(2):301-307.