U.S. FOOD & DRUG ADMINISTRATION

CLIA Waiver by Application Approval Determination
Decision Summary

I. Document Number

CW230020

II. Parent Document Number

K233358

III. CLIA Waiver Type

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

IV. Applicant

InBios International, Inc.

V. Proprietary and Established Names

SCoV-2 Ag Detect Rapid Test

VI. Measurand (analyte)

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

VII. Sample Type(s)

Direct anterior nasal swab samples

VIII. Type of Test

Qualitative lateral flow immunoassay

IX. Test System Description

A Overview

The SCoV-2 Ag Detect Rapid 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.

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
www.fda.gov

To initiate testing, a swab provided with the test kit is used to collect anterior nasal swab samples from both nostrils of the patient. The user places the head of the nasal swab directly into the sample port of the plastic test cassette so that it touches the sample pad and secures the swab in place by firmly pushing. The user then applies eight (8) drops of lysis buffer using the pre-filled dropper bottle provided with the test kit. The buffer disrupts the virus particles in the sample, exposing internal viral nucleoproteins, and washes the sample from the swab into the sample pad where it solubilizes the test reagents.

The test cassette also contains a membrane test strip that contacts with the sample pad. The test strip is pre-coated with anti-nucleoprotein antibody at the test line and an unrelated control antibody at the control line. The sample will migrate from the sample pad up the test strip via capillary action.

If SARS-CoV-2 nucleoprotein antigens are present in the sample, they will bind to the gold conjugated anti-nucleoprotein antibodies in the sample pad. Antigen-bound gold-conjugated immunocomplexes will travel up the test strip and be captured by the anti-nucleoprotein antibodies at the test line. This will form a visible pinkish-red line. Sample will continue to flow through the test strip which also contains a procedural control line to assess sample presence, adequate sample flow, and verify gold conjugation. A visible pinkish-red line at the control region should always appear if the assay is performed correctly. If no visible signal appears at the control line, the test result is invalid, and the sample should be tested again with a new test cassette. Test results are read between 20-25 minutes.

External positive control and negative control swabs are provided with each kit of SCoV-2 Ag Detect Rapid Test 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.

B Test System Components

The assay kit contains all materials needed to run the test, including external controls, and is sold in a 20- or 50-test format. Each kit includes:

- Individually packaged test cassettes: plastic housing with test strip containing monoclonal anti-SARS-CoV-2 antibodies sealed in a pouch
- Reagent: pre-filled dropper bottles with 6 mL of extraction reagent; multiple dropper bottles are included based on the kit size
- Sterile nasal swabs (one per test kit)
- External SARS-CoV-2 Positive Control Swab: one swab coated with non-infectious recombinant nucleoprotein antigen
- Instructions for Use and Quick Reference Instructions

X. Specific Contents for CLIA Waiver

A Demonstrating "Simple":

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

The SCoV-2 Ag Detect Rapid Test is a self-contained test and only requires sample insertion into the test cassette and extraction reagent addition to the cassette. No instrumentation is required, and the results are visually read based upon two lines (one test line and one control line).

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

  The test uses unprocessed anterior nasal swab specimens directly entered into the test device.

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

  An untrained operator can conduct the test by performing five simple steps without sample manipulation: (1) collect the anterior nasal swab sample, (2) insert the swab sample into the test cassette sample port, (3) add 8 drops of lysis buffer, (4) wait 20-25 minutes, and (5) 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. The provided extraction reagent does not require processing or preparation. The user applies 8 drops of the provided reagent directly to the test cassette. The kit contains all 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 insertion of the sample swab into the test cassette, the user applies eight drops of the extraction reagent, and the test develops without user intervention for 20 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 SCoV-2 Ag Detect Rapid 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 SCoV-2 Ag Detect Rapid 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.*

  SCoV-2 Ag Detect Rapid Test results are simple to determine as 'positive', 'negative', or 'invalid' based on the presence or 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. Prescription eyeglasses or contact lenses should be worn by the operator, as applicable.

- *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.

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

1. Risk Analysis:

   Risk Management of the SCoV-2 Ag Detect Rapid Test has been conducted in accordance with ISO 14971 and the FDA guidance, "*Recommendation 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) to define and assess failure modes that may occur during normal use of the device. The FMEA analyzed hazardous situations that may result in false positive, false negative, or invalid test results. The FMEA included failures of the device (e.g., manufacturing defects) and failures of the user (e.g., applying too little Lysis Buffer). The probabilities of occurrence for specific failures were assigned during the design phase and later reassessed based on data obtained during performance evaluations, when testing was performed by operators meeting the intended use setting. All risks of harm from the device were mitigated to an acceptable level via modifications in design, manufacturing, or labeling.

   The efficacy of the risk mitigations was supported by flex studies that stressed the functional limits of the test system, described below in Section 3.

2. Fail-Safe and Failure Alert Mechanisms:

   The SCoV-2 Ag Detect Rapid Test was designed to include features and fail-safe mechanisms built into the system to prevent erroneous results.

Design Features

- Each test cassette is packaged in a sealed pouch to maintain the integrity of the device.
- The pouch is printed with the assay name/type, part number, lot number, expiration date, and storage conditions to ensure clarity and appropriate use.
- Each test cassette features distinct position marks within the results window to facilitate clear and accurate test result interpretation. The control line is denoted as "C" and the SARS-CoV-2 antigen test line is denoted as "T".
- The test cassette is printed with "InBios SCoV-2 Ag Detect" to confirm the assay type being tested and to further ensure clarity and accuracy.
- The test cassette includes a line for users to write the sample number, patient ID, or other identifier to ensure clarity and accuracy.
- The lysis buffer bottle is labeled with the assay name, part number, lot number, expiration date, and storage conditions to ensure clarity and appropriate use.

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 20-25 minutes, the test result is considered invalid, and retesting with a new sample and new device is recommended.
- *External Quality Control* – 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. This swab has a blue tint to distinguish it from other swabs in the kit.
    - The user is instructed to use a sterile swab provided in the kit as the Negative Control Swab for quality control testing.

  External control swabs are processed according to the test instructions for use. Each control swab should produce the expected positive or negative results to validate the test performance. The Positive Control Swab is individually packaged in a sealed pouch printed with information such as the lot number, expiration date, and storage conditions. The user can use an individually packaged sterile nasal swab provided in the kit for processing the Negative Control. 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 SCoV-2 Ag Detect Rapid Test were evaluated in a series of experiments under conditions of stress. Samples used for flex study testing were prepared in negative clinical matrix (NCM), which was prepared by pooling negative nasal swabs collected from healthy individuals and eluted in 1 mL of saline. The pooled NCM was confirmed negative by the SCoV-2 Ag Detect Rapid Test. Contrived positive samples were prepared at 2x LoD by spiking NCM with UV-inactivated SARS-CoV-2 (Omicron variant Lineage BA2.3) to obtain a concentration of $6.3x10^3$ $TCID_{50}$/mL.

The negative and positive test samples were evaluated under the flex conditions described below in replicates of five. Samples were tested according to the instructions for use protocol, except for the noted deviations dictated by the flex parameter under evaluation. 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, Humidity, and Pressure

This study evaluated the impact of variations in temperature, humidity, and pressure environmental conditions on test device performance. After sample addition, tests were incubated under the following conditions until results were read: ambient (control), high temperature and high humidity (43-47°C, 95% relative humidity (RH)), high temperature and low humidity (43-47°C, 5% RH), low temperature and high humidity (2-8°C, 95% RH), low temperature and low humidity (2-8°C, 5% RH), and ambient temperature (18-22°C) at 10.1 psi pressure. All positive and negative samples demonstrated 100% agreement with expected results.

The Instructions for Use and labeling instructs users to store the test at 15-30°C. It is assumed that the test will be conducted in ambient conditions. The study supports the intended environmental testing conditions. Variations in environmental conditions that may impact performance are adequately mitigated in the labeling.

b. Lighting

This study evaluated the impact of lighting source on the user's ability to appropriately use the test device and interpret results. The following lighting conditions were tested and both experienced and inexperienced readers were used to evaluate test results: fluorescent lighting, incandescent lighting, ambient light from a window on an overcast day, sunlight from a window on a sunny day, and low light (lit only by adjacent room/hallway light). All positive and negative samples demonstrated 100% agreement with expected results for both readers across all conditions.

The Instructions for Use and Quick Reference Instructions instruct users to perform the test interpretation in a well-lit area. The study supports the intended testing conditions. Variations in lighting conditions that may impact performance are adequately mitigated in the labeling.

c. *Lab Environment*

This study evaluated the impact of various lab environment testing conditions on device performance. Samples were tested and incubated in conditions that were ambient (control), drafty (near a fan), poorly ventilated (against a wall), and with vibration (near a centrifuge/vortex). All positive and negative samples demonstrated 100% agreement with expected results.

It is assumed that the test will be conducted in ambient conditions. The study supports device performance in conditions that may deviate from ambient conditions.

Operator Errors/Human Factors

d. *Result Reading Time*

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 20-25 minutes after sample application. The following reading times were assessed: 5, 10, 20, 25, 60, 75, 90, 105, and 125 minutes after buffer addition. Positive and negative samples yielded 100% agreement with expected results when results were read between 5 and 125 minutes. Positive external control swabs yielded 100% agreement with expected results when results were read between 10 and 125 minutes, with one false negative (1/5) result at 5 minutes; negative external control swabs yielded 100% agreement with expected results at all timepoints tested.

The Instructions for Use and Quick Reference Instructions instruct users to read the test results after 20 – 25 minutes and warn that results interpreted after 25 minutes may result in false positive, false negative, or invalid results. The study supports the result reading time specified in the labeling and demonstrates that reading times of 20-25 minutes do not adversely impact the performance of the SCoV-2 Ag Detect Rapid Test. Variations in read time that can impact performance are adequately mitigated in the labeling.

e. *Lysis Buffer Volume*

This study evaluated the impact of lysis buffer volume on device performance. Following swab sample insertion into the sample port, a total of 4, 6, 8, 14, or 16 drops of lysis buffer were added and results were read according to the Instructions for Use. All samples yielded invalid results with 4 drops of lysis buffer. With 6 drops of lysis buffer, one positive sample (1/5), two negative samples (2/5), three positive external control samples (3/5), and two negative external control samples (2/5) yielded invalid results. All samples demonstrated 100% agreement with expected results when 8 and 14 drops of lysis buffer were added. With 16 drops, all samples yielded expected results with the exception of two false negative results (2/5) for the positive samples.

The Instructions for Use and Quick Reference Instructions state: "*SLOWLY add eight (8) drops of Lysis buffer ON TOP of the swab head. ADD ONE (1) drop at a time.*" The warning "*Important! Adding less than 8 drops may produce invalid results*" is also included. The study supports the lysis buffer volume specified in the labeling. Variations in lysis buffer volume that can impact performance are adequately mitigated in the labeling.

f. Delay in Lysis Buffer Addition

This study evaluated the impact of delayed addition of lysis buffer on device performance. The lysis buffer was added 1, 3, 5, and 10 minutes after contrived samples were inserted into the sample port. All samples demonstrated 100% agreement with expected results.

The Instructions for Use and Quick Reference Instructions instruct users to add lysis buffer following insertion of the swab into the test cassette and includes images depicting proper application. A warning statement is also included instructing users to ensure the swab head is in full contact with the sample pad if liquid does not start to move into the test window within approximately 1 minute, and to adjust the swab if needed. This study supports the sample processing workflow specified in the labeling. Variations in timing of lysis buffer addition that may impact performance are adequately mitigated in the labeling.

g. Incorrect Lysis Buffer Addition

This study evaluated the impact of incorrect lysis buffer addition on device performance. After contrived positive and negative samples were added to the sample port, lysis buffer was added to: the swab head only (8 drops, control condition specified in the IFU/QRI), reading window only (8 drops), reading window then swab head (4 drops each and 8 drops each), and the swab head then reading window (4 drops each and 8 drops each). Invalid and false negative results were variably observed for positive samples across all conditions other than the control swab head condition. Invalid results were variably observed for negative samples across all conditions other than the control swab head condition as well.

The Instructions for Use and Quick Reference Instructions instruct users to add lysis buffer on top of the swab head and includes an image of proper application. The study supports the sample processing workflow specified in the labeling. Variations in lysis buffer addition that may impact performance are adequately mitigated in the labeling.

h. Incorrect Order of Operational Steps

This study evaluated the impact of conducting the swab sample insertion and lysis buffer addition operational steps out of order. Contrived samples were prepared and processed by inserted the swab into the sample port followed by lysis buffer addition (in line with the Instructions for Use), by applying lysis buffer to the swab head prior to swab insertion into the sample port, and by adding lysis buffer to the sample port then inserting the swab. In the control condition (swab inserted followed by buffer addition) and in the condition where lysis buffer was added prior to swab insertion, all samples demonstrated 100% agreement with expected results. Samples processed by adding lysis buffer to the swab head prior to inserting the swab into the sample port yielded two (2/5) invalid results from positive samples and one (1/5) invalid result from negative samples tested.

The Instructions for Use and Quick Reference Instructions include descriptions and images of the correct sample collection and processing procedure and include a warning that failure to follow the instructions may lead to inaccurate test results. The study supports the sample processing workflow specified in the labeling. Variations in processing workflow that may impact performance are adequately mitigated in the labeling.

i. Disturbance Effect

This study evaluated the impact of physically disturbing the test after the sample is added. Contrived positive and negative and external control swabs were inserted into the sample port, lysis buffer was added, and the test was tested as follows:

- Level surface without movement (control condition)
- Moved to another surface >6 feet away
- Dropped from >3 feet
- Inclined 20-45°
- Declined 20-45°
- Inclined nearly upright (~90°C)
- Tested on its site
- Tested upside down

All samples demonstrated 100% agreement with expected results, with the exception of one invalid result observed when the test was run on its side.

The Instructions for Use and Quick Reference Instructions include descriptions and images of the correct sample collection and processing procedure. The internal control line provides assurance that the procedure was conducted properly and the test reagents are performing as expected. The study supports the testing conditions specified in the labeling. Variations in testing conditions that may impact performance are adequately mitigated by the device design and in the labeling.

j. Color Blind Reader

This study evaluated the impact of color blindness on the user's ability to read and interpret test results. The same test was run according to the IFU/QRI and read in ambient lighting by a reader with no color blindness and a reader that was color blind. Both readers were able to correctly read and interpret all positive and negative sample results.

The Instructions for Use include a statement that the test is read visually and has not been validated for use by those with impaired vision or color-impaired vision. The study supports the ability of a variety of users to read the test results. Variations in user color blindness that may impact performance are adequately mitigated in the labeling.

Specimen Integrity and Handling

k. Delay in Sample Testing

This study evaluated the impact of delayed testing after sample collection. Contrived positive and negative samples were prepared and then stored in an empty tube or opened paper pouch used to package the sterile swabs at room temperature. Samples were tested 0, 1-, 2-, 4-, and 8-hours post-collection. All samples demonstrated 100% agreement with expected results when tested 0 to 4 hours after preparation in both the tube and paper wrap storage conditions. One positive sample (1/5) stored in an empty tube yielded a false negative result after storage at 8 hours; all other samples tested in both conditions at this timepoint yielded expected results.

The Instructions for Use and Quick Reference Instructions instruct users to process freshly collected samples immediately and if delays are unavoidable, to store samples in a sterile container for up to 1 hour at room temperature (20-25°C) or 48 hours refrigerated

(2-8°C). The study supports the post-collection sample storage conditions specified in the labeling. Variations in sample storage after collection that can impact performance are adequately mitigated in the labeling.

l. Incorrect Sample Storage

This study evaluated the impact of samples incorrectly stored on test device performance. Contrived positive and negative samples were prepared and then stored overnight in a refrigerator (2-8°C) or freezer (15-25°C). Samples were removed from the refrigerator/ freezer and then tested immediately or brought to room temperature before testing. All samples demonstrated 100% agreement with expected results.

The Instructions for Use and Quick Reference Instructions instruct users to process freshly collected samples immediately or stored for no longer than 1 hour at room temperature (20-25°C) or 48 hours refrigerated (2-8°C). The study supports the post-collection sample storage conditions specified in the labeling. Variations in sample storage after collection that may impact performance are adequately mitigated in the labeling.

m. Incorrect Swab Insertion

This study evaluated the impact of incompletely inserting the swab sample into the test device sample port. Contrived positive and negative samples and external control samples were either fully inserted into the sample port (control condition in line with the IFU/QRI) or partially inserted so that the swab was not fully in contact with the sample pad. All control condition samples demonstrated expected results. Swabs not fully inserted yielded inconsistent results: 2/5 positive samples were invalid, 1/5 negative samples were invalid, 4/5 external negative control samples were invalid, and external positive control samples yielded 1/5 invalid results and 2/5 false negative results.

The Instructions for Use and Quick Reference Instructions include images of the sample processing workflow and statements/warnings instructing users to ensure the head of the nasal swab is touching the sample pad. Variations in sample insertion that may impact performance are adequately mitigated in the labeling.

n. Improper Test Handling

This study evaluated the impact of users mis-handling test kit components during processing. During testing, users mis-handled the test cassette in the following ways prior to processing the sample:

- Touching the cassette membrane with gloves, without gloves, without gloves and with lotion on the hands, and without gloves with hand sanitizer on the hands
- Touching the lysis buffer tip with gloves, with gloves with lotion, and with gloves with hand sanitizer
- Touching the lysis buffer tip with the positive control swab
- Using gloved fingers to push the swab head into the port
- Touching the swab head with ungloved fingers prior to adding the sample
- Removing the cassette from the pouch and dropping it >5ft onto the floor
- Shaking the lysis buffer bottle vigorously prior to use

All positive and negative samples demonstrated 100% agreement with expected results.

The Instructions for Use and Quick Reference Instructions include descriptions and images of the correct sample collection and processing procedure. The study supports the sample processing workflow specified in the labeling.

Reagent Integrity

o. *Frozen and Refrigerated Kit Storage*

This study evaluated the impact of storing test kits frozen or refrigerated with or without equilibration to room temperature prior to testing. Test kits were stored at room temperature (RT, control), frozen (-20°C) for 8 hours, frozen (-20°C) for 8 hours and brought to RT, frozen (-20°C) for 24 hours, and frozen (-20°C) for 24 hours and brought to RT. All positive and negative samples and external positive and negative control swabs demonstrated 100% agreement with expected results.

The Instructions for Use, Quick Reference Instructions, and outer package labeling instruct users to store kits at 15-30°C. Variations in test kit storage that may impact performance are adequately mitigated in the labeling.

p. *Un-pouched Cassette Storage*

This study evaluated the impact of storing un-pouched test cassettes and other kit components in various conditions prior to testing. The test cassette was removed from the sealed pouch provided in the kit and stored with other kit components (lysis buffer, sample collection swabs in their paper pouch, and external control swabs) in hot and humid conditions (represented by a 30°C covered water bath) or cold and dry conditions (2-8°C with 0-10% RH). Kits were stored for 1, 8, or 24 hours prior to testing. Un-pouched cassettes and kit components were also tested at ambient temperature/ humidity after being un-pouched for <5 min to represent conditions described in the IFU/QRI. All positive, negative, and external control samples demonstrated 100% agreement with expected results across all tested conditions.

The Instructions for Use, Quick Reference Instructions, and outer package labeling instruct users to store kits including test cassettes at 15-30°C. The test procedure also instructs users to remove the cassette from its pouch at the start of the testing procedure. Variations in test kit storage that may impact performance are adequately mitigated in the labeling.

q. *Use of Expired Kits*

This study evaluated the impact of using expired SCoV-2 Ag Detect Rapid Test kits. Two lots of test kits 1-3 months beyond the expiration date were tested. All positive and negative samples demonstrated 100% agreement with expected results. The kit external positive control swabs were also tested and yielded expected results. The negative control swab for one test kit was tested and yielded expected results.

While the study supports the robustness of the test device, use of expired reagents is generally not recommended. The Instructions for Use and Quick Reference Instructions instruct users to not use the test device or any test components after the printed expiration date. Variations in test kit usage that may impact performance are adequately mitigated in the labeling.

Hardware, Software, and Electronics Integrity

Not applicable, as the test device does not require instrumentation.

Stability of Calibration and Internal Controls

Not applicable. The test device is not calibrated. Internal control stability was assessed in the real-time stability study included in K233358 and deemed acceptable.

Based on the assessment of the design features, robustness of the test system, and device labeling, all the hazards and sources of potential errors have been mitigated to reasonably acceptable levels.

C 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 April 2021 to April 2024 as part of the clinical evaluation of the test device to demonstrate the clinical performance of the SCoV-2 Ag Detect Rapid Test to detect SARS-CoV-2 viral nucleoprotein antigen in specimens obtained from nasal swabs. The predominantly circulating variant varied during the study timeframe. Omicron (the current predominant circulating variant at the time of this CLIA Waiver granting) was the predominantly circulating variant during collection of 47% (604/1285) of the total evaluable samples. In this study, nasal swabs were prospectively collected from symptomatic subjects by swabbing both sides of the nose. Samples were tested with the SCoV-2 Ag Detect Rapid Test and the comparator device. This was a multi-center, prospective study performed at three clinical sites in the U.S. One swab was used for comparator testing internally at InBios using the Quidel Lyra SARS-CoV-2 RT-PCR assay. The other swab was tested with the SCoV-2 Ag Detect Rapid Test by an untrained operator at the CLIA-Waived site.

ii. Operators

The study was conducted with 12 operators, representative of CLIA Waived users, across the three clinical testing sites. Operators consisted of administrative personnel, medical assistants, physicians, and other patient care providers. All operators were assigned to subject enrollment and/or sample collection and testing for the CLIA Waiver clinical performance study. No operators had any prior experience in high-moderate complexity laboratory settings. No operators were previously trained in the use of the SCoV-2 Ag Detect Rapid Test and relied solely 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.

iii. Instructions for Use

The SCoV-2 Ag Detect Rapid Test was performed in accordance with the assay procedure described in the draft Quick Reference Instructions. No other materials or instructions were provided, and the operators received no training on the use of the test.

iv. Subjects (Patients)

Performance characteristics of the SCoV-2 Ag Detect Rapid Test were established in samples from symptomatic study subjects prospectively enrolled from April 2021 to April 2024. A total of 1362 subjects were enrolled in the study.

Inclusion Criteria

- Male and female subjects at least 18 years of age, or at least 2 years of age and accompanied by a parent or legal guardian
- Subject (or parent/guardian) able and willing to provide informed consent.
- Each subject within 5 days of enrollment will have had signs or symptoms of possible SARSCoV-2 infection including: fever, chills, cough, shortness of breath, difficulty breathing, fatigue, muscle or body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, diarrhea.

  Note that based on performance, samples collected at 5 DPSO were excluded from the final DPSO claim and performance analysis

- Subject (or parent/guardian) is able to speak, read, and comprehend English.

Exclusion Criteria

- Subject is an inmate or deprived of freedom by court order.
- Received COVID-19 vaccine (only excluded for samples collected on or before Dec. 8, 2021).

v. Samples

Two nasal samples were collected from each study subject during the same visit. One swab was collected for testing with the comparator test, placed into a tube with viral transport media (VTM), stored frozen, and send to InBios for comparator testing. The other swab was tested on the SCoV-2 Ag Detect Rapid Test by a CLIA-waived test operator at the site.

A total of 1362 paired nasal swab specimens were enrolled in the study. Of those, 58 specimens were excluded from the final analysis:

- A total of 58 samples were excluded because they were collected from patients who were over 4 days post symptom onset
- A total of 19 samples were excluded due to issues associated with comparator sample testing

A total of 1285 nasal swab specimens were considered evaluable for the purpose of analysis in the clinical performance study.

vi. Comparative Method

The sponsor identified a RT-PCR molecular test with high sensitivity as their comparator that 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 the CLIA Waiver Clinical Evaluation study demonstrate acceptable performance in a total of 1285 evaluable subjects, comprising 355 positives and 930 negatives, with an overall sensitivity as estimated by PPA of 84.2% (299/255, 95% CI: 80.1% - 87.6%) and specificity as estimated by NPA of 99.8% (928/930, 95% CI: 99.2% - 99.9%) (**Table 1**).

Table 1. Clinical performance of the SCoV-2 Ag Detect Rapid Test

		RT-PCR Comparator		
		Pos	**Neg**	*Total*
SCoV-2 Ag Detect Rapid Test	**Pos**	299	2	*301*
	Neg	56	928	*984*
	Total	*355*	*930*	*1285*
PPA: 84.2% (299/355) [95% CI: 80.1% - 87.6%]				
NPA: 99.8% (928/930) [95% CI: 99.2% - 99.9%]				

2. Operator Questionnaire

At the end of the clinical evaluation study, 10 of the 12 point-of-care operators completed a questionnaire to provide feedback on the ease of the use of the SCoV-2 Ag Detect Rapid Test. The questionnaire had 12 questions the evaluated the following general topics:

- Ease of use of the test and test kit components
- Ability to follow the Quick Reference Instructions
- Ability to properly interpret results

Based on the feedback from the 10 operators, the SCoV-2 Ag Detect Rapid 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. Device Performance with Analyte Concentrations Near the Cutoff

The sponsor conducted a reproducibility study with samples near the cutoff (LoD) to evaluate the inter-site reproducibility of SCoV-2 Ag Detect Rapid Test when tested by the intended user. The study was conducted at three distinct CLIA waived point-of-care clinical sites using a blinded panel of contrived samples to demonstrate that personnel at these sites, who are representative of the intended user, could perform the test consistently and correctly. Three untrained operators representative of the intended user were included at each site.

The study panel included contrived positive samples prepared in negative clinical matrix at three levels: negative (Neg), high negative (HN, ~0.1xLoD = $3.9x10^2$ $TCID_{50}$/mL), and low positive (LP, 1x LoD = $3.1x10^3$ $TCID_{50}$/mL). HN and LP samples were prepared with SARS-

CoV-2 (Omicron variant lineage BA2.3) prepared in negative clinical matrix. Each panel consisted of one LP aliquot and two aliquots randomly selected as either Neg or HN, for a total of three samples per panel. The sequence of samples was randomized so that each untrained operator tested a total of 10 LP samples, 12 HN samples, and 8 Neg samples.

Panels were tested over five non-consecutive days by a total of nine untrained operators across the three external point-of-care sites. Testing was performed in accordance with the SCoV-2 Ag Detect Rapid Test Quick Reference Instructions.

Overall, the reproducibility study generated a total of 260 results, with each untrained operator testing 30 samples. The overall qualitative and quantitative results are summarized below in **Table 2**.

Table 2. Near-cutoff Reproducibility Performance

Site	Sample	Operator	Total Samples Tested	# Invalid Results	# Negative Results	# Positive Results	% Agreement*
1	Negative	A	8	0	8	0	100.0
		B	8	0	8	0	100.0
		C	8	0	8	0	100.0
		All	24	0	24	0	100.0
	High Negative	A	12	0	5	7	41.7
		B	12	0	9	3	75.0
		C	12	0	12	0	100.0
		All	36	0	26	10	72.2
	Low Positive	A	10	0	0	10	100.0
		B	10	0	0	10	100.0
		C	10	0	0	10	100.0
		All	30	0	0	30	100.0
2	Negative	A	8	0	8	0	100.0
		B	8	0	8	0	100.0
		C	8	0	8	0	100.0
		All	24	0	24	0	100.0
	High Negative	A	12	0	12	0	100.0
		B	12	0	12	0	100.0
		C	12	0	12	0	100.0
		All	36	0	36	0	100.0
	Low Positive	A	10	0	1	9	90.0
		B	10	0	0	10	100.0
		C	10	0	0	10	100.0
		All	30	0	1	29	96.7
3	Negative	A	8	0	8	0	100.0
		B	8	0	8	0	100.0
		C	8	0	8	0	100.0
		All	24	0	24	0	100.0
	High Negative	A	12	0	12	0	100.0
		B	12	0	12	0	100.0
		C	12	0	10	2	83.3
		All	36	0	34	2	94.4
	Low	A	10	0	2	8	80.0

Site	Sample	Operator	Total Samples Tested	# Invalid Results	# Negative Results	# Positive Results	% Agreement*
	Positive	B	10	0	1	9	90.0
		C	10	0	0	10	100.0
		All	30	0	27	30	90.0
Combined Sites and Operators	Negative		72	0	72	72	100.0 (95% CI: 94.9-100.0)
	High Negative		108	0	96	12	88.9 (95% CI: 81.6-93.5)
	Low Positive		90	0	4	86	95.6 (95% CI: 89.1-98.3)

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

D Labeling for Waived Devices

The labeling submitted for the SCoV-2 Ag Detect Rapid Test consists of:

1. Instructions for Use: SCoV-2 Ag Detect Rapid Test Package Insert
2. Quick Reference Instructions: SCoV-2 Ag Detect Rapid Test QRI
3. External Control Labeling: SCoV-2 Ag Detect Rapid Test Control Kit Product Information Card

The following elements are appropriately present:

- The Quick Reference Guide 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.

XI. Benefit-Risk Considerations

The evidence provided in this submission indicates that this assay will appropriately diagnose SARS-CoV-2 within the intended use population when used with follow up testing of negative results as intended.

The CLIA Waiver Clinical Evaluation study demonstrated an acceptable sensitivity of 84.2% (299/355) and an acceptable specificity of 99.8% (928/930). The clinical study took place when both non-Omicron variants and Omicron circulated in the U.S. population. The derived performance estimates therefore are likely to 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 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 Predicate (DEN220039), which has been cleared for use in near-patient settings with a PPA of 89.0%. The SCoV-2 Ag Detect Rapid Test has a PPA of 84.2%. The observed differences in sensitivity are not specific to the SCoV-2 Ag Detect Rapid Test but have been observed by the Agency for many similar tests. They are associated with a change in circulating SARS-CoV-2 variants, 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. 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 outweighs the risk associated with a PPA of 84.2% when the test is used as intended, including confirmation of negative results as described in the intended use.

Thus, granting of a CLIA Waiver for the SCoV-2 Ag Detect Rapid Test will provide 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.

XII. Conclusion

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