CLIA Waiver by Application Approval Determination
Decision Summary

I. Document Number

CW250006

II. Parent Document Number

K251697

III. CLIA Waiver Type

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

IV. Applicant

ACON Laboratories, Inc.

V. Proprietary and Established Names

Flowflex® Plus Strep A Rapid Test Cassette; Flowflex® Plus Strep A Rapid Test Strip

VI. Measurand (analyte)

Group A β-hemolytic *Streptococcus* (GAS; *Streptococcus pyogenes*) antigens

VII. Sample Type(s)

Throat swab

VIII. Type of Test

Lateral flow chromatographic immunoassay

IX. Test System Description

A Overview

The ACON Flowflex Plus Strep A Rapid Test Strip and Flowflex Plus Strep A Rapid Test Cassette (also referred to here collectively as the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette) are rapid chromatographic immunoassays for the qualitative detection of Group A *Streptococcus* antigen from throat swab specimens from symptomatic patients (i.e., suspected of bacterial pharyngitis). For this test, a throat swab is collected from a patient, and the Strep A

antigen is extracted in an extraction tube. The test utilizes antibodies specific for whole cell Lancefield Group A *Streptococcus* to selectively detect Strep A antigen in a throat swab specimen. During testing, the extracted throat swab specimen reacts with an antibody to Strep A that is coated onto particles. This mixture migrates up the membrane to react with an antibody to Strep A on the membrane, and a colored line (pink-red) is generated at the test line region. The presence of this colored line in the test line region indicates a positive result for detection of Strep A antigen, while its absence indicates a negative result. To serve as a procedural control, a pink-red line will always appear at the control line region, indicating that a proper volume of specimen has been added and membrane wicking has occurred. If the pink-red control line does not appear, the test result is invalid.

B Test System Components

Table 1. Components of Each Cassette or Strip Kit

Cassette Kit Components	Strip Kit Components
• Test Cassettes • Test Tubes • Dropper Tips • Sterile Throat Swabs • Reagent A (2M Sodium Nitrite) • Reagent B (0.2M Acetic Acid) • Positive Control (Heat-inactivated Group A *Streptococcus*) • Negative Control (Heat-inactivated Group C *Streptococcus*) • Package Insert • Quick Reference Instructions	• Test Strips • Test Tubes • Sterile Throat Swabs • Reagent A (2M Sodium Nitrite) • Reagent B (0.2M Acetic Acid) • Positive Control (Heat-inactivated Group A *Streptococcus*) • Negative Control (Heat-inactivated Group C *Streptococcus*) • Package Insert • Quick Reference Instructions

X. Specific Contents for CLIA Waiver

A Demonstrating “Simple”:

Table 2. Demonstration of Simplicity for the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette

"Simple" Criteria	Device Characteristics
Is a fully automated instrument or a unitized or self-contained test?	The device is a unitized, self-contained test. The sample preparation requires mixing reagent A and B and placing the throat swab into the extraction tube before proceeding with next steps—all of which are manual. No special skills or tools are needed to perform the test.
Uses direct unprocessed specimens, such as capillary blood (fingerstick), venous whole blood, nasal swabs, throat swabs, or urine?	The test uses direct unprocessed throat swab specimens. No extra equipment or training is needed for sample processing. The sample collection process is a routine clinical procedure.
Needs only basic, non-technique-dependent specimen manipulation, including any for decontamination?	Only basic specimen manipulation is required, such as swirling, rotation, squeezing. No special skills or training are required. No extra tools are needed.

"Simple" Criteria	Device Characteristics
	Operation process is clearly explained in labeling, and an untrained operator can conduct the test by performing the following simple steps without sample manipulation: 1) collect the throat swab sample; 2) remove the test strip or cassette from the sealed pouch; 3) prepare extraction buffer by mixing reagent A and B in an empty tube; 4) place the throat swab in the extraction buffer, mix 5x, and wait for 1 min. For strip: 5) remove the swab; 6) place the strip into the sample extraction tube; 7) wait for 5 min and then read the result from the strip. For cassette: 5) remove the swab, add the dropper tip to sample extraction tube, and mix; 6) add 3 drops of extracted material to the sample well of the cassette; 7) wait for 5 min and then read the result from the cassette.
Needs only basic, non-technique-dependent reagent manipulation, such as “mix reagent A and reagent B.”	The test only requires mixing reagent A and reagent B in an empty tube to prepare the extraction buffer. The kit is unitized and contains all tubes and reagents required for analysis. No extra equipment or tools are required for the reagent mixing. No extra skills or training are required, and all accessories are provided in the test kit.
Needs no operator intervention during the analysis steps.	The test does not require any operator intervention during the analysis step. After adding the test strip to the sample extraction tube or adding 3 drops of extracted material to the cassette sample well , the test develops without user intervention after 5 minutes. Upon completion of the run time, the user interprets the test results by visual interpretation. The kit is packaged with a Quick Reference Instructions (QRI) that outlines the test process and result interpretation in easy-to-follow steps with illustrations.
Needs no technical or specialized training with respect to troubleshooting or interpretation of multiple or complex error codes.	The result is read visually without assistance from an instrument, and no technical or specialized training is needed. No complex error codes are generated in the test result for interpretation or troubleshooting. No technical or specialized training is required for sample collection, sample processing, or result interpretation. Information regarding troubleshooting invalid test results (i.e., to not use test results if the controls do not yield the expected results) and contacting technical services or customer service is provided in the package insert and QRI.

"Simple" Criteria	Device Characteristics
Needs no electronic or mechanical maintenance beyond simple tasks, e.g., changing a battery or power cord.	There are no electrical or mechanical components in the device. The test does not require any instrumentation or machinery. No maintenance, calibration or calculation is required.
Produces results that require no operator calibration, interpretation, or calculation.	No operator calibration or calculation is required to interpret test results. Operator visual interpretation of test results is required. Test lines and control lines are clearly marked on the device and described in the package insert/QRI. Invalid results are clearly illustrated in the package insert/QRI. The package insert/QRI also includes 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.	The test provides a direct visual readout of test results via the presence or absence of a pink or red colored line at the "test" and "control" region of the strip or cassette. Results are simple to determine as positive, negative, or invalid for Strep A by following the QRI or package insert, which include descriptions and visual examples of result interpretations.
Contains a quick reference instruction sheet that is written at no higher than a 7th grade reading level.	The test procedure is written at a 7th grade comprehension level. The graph/drawings are presented in QRI to help user understand each operational step.

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

1. Risk Analysis:
   ACON Laboratories performed a comprehensive risk analysis to assess the safety risks associated with operation of the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette. Potential sources of errors that could adversely affect device performance were identified and mitigated by system design, as well as additional warnings and descriptive elements in the labeling. The identified risks that could result in erroneous test results were evaluated in flex studies that stressed the functional limits of the device (see below). All risks of harm to the patient or operator were mitigated to an acceptable level and were supported by flex studies and/or operator instructions.

2. Fail-Safe and Failure Alert Mechanisms:
   The ACON Flowflex Plus Strep A Rapid Test Strip and Cassette were designed to include features and fail-safe mechanisms built into the device to prevent erroneous results.

   Design Features
   - For those devices in pouches, each test strip/cassette is individually packaged to maintain the integrity of the test device. Strips packaged in a plastic canister contain desiccant.
   - The kit packaging is printed with the assay name and lot number to ensure clarity and

appropriate use.

- Adding the strip directly to the tube where the specimen is extracted eliminates the need to transfer the specimen.
- The strip is designed to be oriented for proper insertion into the extraction tube. An arrow is printed on the strip to indicate the proper orientation when adding the strip to the extracted specimen solution. For the cassette, the sample well is designated as *"S"* to show where to add the extracted sample.
- Each test device illustration includes distinct position marks on the test strip and cassette to facilitate clear and accurate result interpretation. The control line is denoted as *"C"*, and the Strep A antigen line is denoted as *"T"*.
- The tube for sample extraction is flexible to help squeeze the extracted analyte from the swab specimen.
- Swabs are supplied with the kit to facilitate optimal specimen collection from the throat and antigen release.

Fail-safe Features

- *Line showing maximum immersion point*—There is a line labeled *"MAX"* in a figure within the Directions for Use section of the package insert and QRI to indicate the maximum immersion point of the strip for the extracted specimen.
- *Internal Procedural Control*— A red or pink line appearing in the control line region (C) is an internal procedural control. The appearance of the procedural control line indicates that a proper volume of specimen has been added, and capillary flow has occurred. If the procedural control line does not develop, the test result is considered invalid, and retesting with a new strip or cassette and sample is recommended.
- *External Quality Control Materials*—One Positive Control Solution (containing non-infectious heat-inactivated Strep A bacteria) and one Negative Control Solution (containing heat-inactivated Strep C bacteria) are included in each reagent kit. Each control is processed using a separate test strip or cassette. Both control solutions are ready-to-use and are tested following similar procedures used for patient samples. One drop of positive or negative control solution is added into a test tube. These controls monitor the entire assay and serve to detect product defects or reagent deterioration between the manufacturer's lot release date and the date of use. The controls also monitor the operator's use of the test and detect any errors in the procedure. In addition to the laboratory's standard quality control procedures, it is recommended that positive and negative controls be tested once for each untrained operator and once for each new shipment of kits.

3. <u>Flex Studies:</u>
   The operational limits of the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette were evaluated in a series of experiments of "stress", including conditions outside of those recommended in the instructions for use. Twelve studies are described below. Unless otherwise indicated, each study was conducted with negative samples (clinical matrix collected from volunteers determined to be negative for GAS) and contrived positive samples (2x LoD) prepared by spiking GAS into pooled negative clinical matrix. A 50 uL aliquot of sample was applied to swabs for testing in replicates using one or more lots of test kits. Flex studies to support the CLIA Waiver Application included the following:

   1. Reading Time Flex Study

2. Light Sources Impact Flex Study
3. Delay in Operational Steps Flex Study
4. Sample Volume Flex Study
5. Sample Extraction Flex Study
6. Disturbance Effect Flex Study
7. Cassette Orientation Effect Flex Study
8. Open Pouch Flex Study
9. Reagent Buffer Volume Flex Study
10. Control Volume Flex Study
11. Delay in Sample Testing Study
12. Sample in Swab Study (Additional Storage Times)

Reading Time

Recommended reading time: 5 minutes

To evaluate the potential for error due to an incorrect reading time after sample application, studies were conducted with reading times of 1, 2, 3, 5, 8, 10, 15, 30, and 60 minutes for both the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette using negative samples and positive samples (2× LoD). No false negative, false positive, or invalid results were obtained across a wide range of reading times from 2 to 60 minutes. While the cassette did not yield a result at 1 minute for either positive or negative samples (at 1 minute the sample flow had not fully reached the "C" line area yet), expected results were observed for the cassette format at all other time points tested. The strip generated expected results for both negative and positive samples across all time points. This study supports the result reading time specified in the labeling and demonstrates that reliable test results can be obtained with reading times from 2 to 60 minutes after sample application. However, labeling and QRI clearly indicate the recommended reading time.

Light Sources Impact Study

This study evaluated the impact of different light sources on the performance of the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette. Positive (2× LoD) and negative samples were prepared in negative clinical matrix and tested under seven different lighting conditions:1) indirect sunlight, 2) direct sunlight, 3) mercury vapor lamp, 4) sodium vapor lamp, 5) fluorescent lamp, 6) incandescent lamp, and 7) portable holder lamp. Each sample type was tested in five replicates under each lighting condition, and test results were read at 5 minutes according to package instructions. Observed test results were 100% in agreement with the expected result for all negative and positive samples for both the strip and cassette formats under each lighting condition tested. This study supports the use of both the strip and cassette formats of the ACON Flowflex Plus Strep A Rapid Test under various lighting conditions that may encountered in a near-patient setting.

Delay in Operational Steps Study

A study was conducted to examine the impact of delays in certain operational steps (e.g., sample exposure to extraction buffer at various temperatures and time periods) on performance of ACON Flowflex Plus Strep A Rapid Test Strip and Cassette. Negative samples and positive samples (2× LoD) were tested with five replicates under the following three conditions at 15°C and 30°C temperatures: 1) standard processing per package insert (control), 2) swab exposed to buffer for 1-3 hours before rotating swab, and 3) swab exposure to buffer 1-3 hours after rotating swab. All negative and positive samples produced

the expected results for both the strip and cassette formats under each condition tested. Leaving sample swabs in extraction buffer for up to 3 hours did not impact performance of the ACON Flowflex Plus Strep A Rapid Test Strip or Cassette at either 15°C or 30°C, demonstrating the robustness of the test even if operational timing variations may occur in the user setting.

Sample Volume Study (Cassette Only)
Recommended extracted sample volume: 3 drops of extracted sample

To evaluate the potential for error due to inappropriate sample volume application, a study was conducted by adding 1, 2, 3, 4, or 5 drops of extracted sample to the ACON Flowflex Plus Strep A Rapid Test Cassette sample well. Five replicates of negative samples and positive samples (2× LoD) were tested. When only 1 drop was applied, all samples yielded invalid results due to insufficient sample flow. Expected test results were obtained when extracted sample volume was tested with 2-5 drops in the ACON Flowflex Plus Strep A Rapid Test Cassette. Risks are further mitigated through labeling, which indicates 3 full drops of extracted sample should be added to the sample well.

Sample Extraction Study
Recommended swab process: Rotate swab 5 times while squeezing, keep swab in test tube for 1 minute, and remove swab while rotating and squeezing the tube before testing extracted material

A study was conducted to determine if variations in the swab extraction process affected the performance of the ACON Flowflex Plus Strep A Rapid Test Strip or Cassette. The study included nine different extraction conditions compared to the control condition. Three key variables were examined: 1) the number of swab rotations while squeezing the extraction tube (ranging from 0 to 8 times), 2) the duration of time the swab remains in the extraction tube (0 to 1.5 minutes), and 3) the method of swab removal (with or without rotation and squeezing). Five replicates of negative and positive samples (2x LoD) were tested using test strip and cassette formats. The study results demonstrated that most extraction process variations did not impact test performance, except for two conditions that generated false negative results for positive samples. The condition with no swab rotation achieved only a 20% positive detection rate (4 false negatives reported) using both the strip and cassette formats, and the condition with a 0-minute incubation time achieved a 60% positive detection rate (3 false negatives reported) for both strip and cassette formats. All other conditions yielded expected test results. Risks are further mitigated through labeling, which inform end-users of critical extraction steps and processes (i.e., swab incubation time and rotation).

Disturbance Effect Study
A study was conducted to examine the impact of physical disturbances during testing on performance of ACON Flowflex Plus Strep A Rapid Test Strip or Cassette. Five replicates of negative and positive (2x LoD) samples were tested under five different disturbance conditions: 1) dropping cassettes from bench height, 2) moving cassettes between surfaces (every minute), 3) placing cassettes on tilted surfaces at 15° angles, 4) moving test strips between tube holders, and 5) intermittently removing strips from test tubes. Results were read at the standard 5-minute timepoint. All negative and positive samples produced the expected results across all five disturbance scenarios. The study demonstrated that all tested

disturbance conditions do not adversely affect the ACON Flowflex Plus Strep A Rapid Test Strip or Cassette performance.

Cassette Orientation Effect Study

A study was conducted to determine if different cassette orientations could affect performance of the ACON Flowflex Plus Strep A Rapid Test Cassette. Five replicates of negative and positive (2x LoD) samples were tested using the cassette format across four orientation conditions: 1) flat surface (control), 2) upright with sample well at bottom, 3) upright with sample well at top, and 4) upside down. Cassettes were read at 5 minutes according to the package insert protocol. All negative and positive samples produced the expected results across all orientation conditions tested. This study showed that cassette orientation did not impact test performance for the ACON Flowflex Plus Strep A Rapid Test Cassette.

Open Pouch Study

Recommended: The test must remain sealed in the protective pouch until just prior to use. Remove the test cassette (or strip) from the sealed pouch and use it as soon as possible.

A study was conducted to evaluate the stability of the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette after exposure to ambient conditions following pouch opening. After opening the pouches, devices were exposed to two temperature conditions (15°C and 30°C) with 80% relative humidity for 0, 30, 60, and 75 minutes. Both negative and positive (2x LoD) samples were tested in five replicates under each condition. All negative and positive samples produced the expected results for both the strip and cassette formats across all time-points and environmental conditions tested. This study demonstrated that common environmental fluctuations do not adversely affect ACON Flowflex Plus Strep A Rapid Test Strip or Cassette performance after the device pouch has been opened. Risks are further mitigated through labeling, which instructs end users to test devices as soon as possible after opening the sealed pouch.

Reagent Buffer Volume Study

Recommended reagent volumes: 4 drops of Reagent A plus 4 drops of Reagent B

To determine the impact of errors associated with the mixing of extraction reagents on performance of the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette, a study was conducted with multiple combinations of different numbers of drops (2-6) of Reagent A and Reagent B (25 total combinations). Five replicates of negative and positive (2x LoD) samples were tested for each combination of Reagent A and Reagent B. Both cassettes and strips were read at 5 minutes. All reagent volume combinations tested [i.e., from 2+2 drops (4 drops total) up to 6+6 drops (12 drops total)] generated expected results with negative and positive samples using both test formats. Risks of erroneous results are further mitigated through labeling, which indicates a standard total of 8 drops (4 Reagent A and 4 Reagent B) should be added to the tube for extraction.

Control Volume Study

Recommended: 1 full drop of positive or negative control solution into the test tube

A study was conducted to examine the effect of various external control volumes on ACON Flowflex Plus Strep A Rapid Test Strip and Cassette performance. A total of 1, 3, or 5 drops

of external positive or negative controls was tested with five replicates using the ACON Flowflex Plus Strep A Rapid Test Strip Kit and Cassette Kit, where the standard 1-drop procedure served as the control condition. All negative and positive samples produced the expected results for both the strip and cassette formants with all volume variations tested (1, 3, and 5 drops). Results demonstrated that minor variations in control volume application do not adversely affect test interpretation/results using the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette.

Delay in Sample Testing Study

Recommended: Testing should ideally be performed immediately after specimen collection

An initial study was conducted to determine whether delays in sample testing at various temperatures could impact the performance of the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette. Negative and positive (2× LoD) sample swabs were stored at different temperatures (e.g., 10°C, 15°C, 30°C, and 35°C) for varying durations (e.g., 0.5, 1, and 2 hours) before testing with both test formats. Five replicates were tested for each sample under all conditions. All negative and positive samples produced the expected results for both the strip and cassette formats under each condition tested. The study demonstrated that swabbed samples stored at temperatures ranging from 10°C to 35°C for up to 2 hours can produce consistent test results.

Sample in Swab Study (Additional Storage Times)

Recommended: Testing should ideally be performed immediately after specimen collection

An additional study was conducted to evaluate the effect of different temperatures on swab samples prior to analysis. Both negative and positive (contrived at 2×LoD) samples were applied to swabs, and swabs were stored at 4°C, room temperature, and 30°C. Five replicates of swab samples were stored at various time points (fresh, 24, 48, 72, and 96 hours) and tested with the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette. All negative and positive samples produced the expected results for both strip and cassette formats across all temperature and time conditions tested throughout the 96-hour storage period, regardless of whether the sample was stored at 4°C, room temperature, or 30°C. Labeling recommends testing immediately, when possible.

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

1. Clinical Study

   a. Study Design

   A clinical study was conducted to demonstrate performance of the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette in throat swab specimens obtained from symptomatic individuals in the hands of the intended users when performed in a CLIA waived setting. The clinical study data generated in this application was obtained to support this CLIA waiver application.

   Performance characteristics of the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette were established during a prospective clinical study conducted from August

2022 to March 2024. Subjects enrolled were patients with signs and symptoms of pharyngitis with completed informed consent prior to sample collection. Patient demographics included male and female patients across all ages. Three hundred and ninety-seven (397) fresh throat swab specimens were prospectively collected at five (5) sites across the United States. All testing was performed at sites holding CLIA certificates of waiver using untrained healthcare professionals. Three swab specimens were collected simultaneously from each patient using swabs provided with the assay—one swab for ACON Flowflex Plus Strep A Strip testing, one swab for ACON Flowflex Plus Strep A Cassette testing, and one swab for bacterial culture and organism ID confirmation. The swab collection order was randomized to avoid bias.

Bacterial culture was performed at CLIA-certified central clinical laboratories near the collection sites. Specimens were plated on blood agar plates and incubated following routine laboratory culture procedures for identifying *S. pyogenes*. Beta-hemolytic colonies from the blood agar plates were confirmed for Group A streptococci using streptococcal latex agglutination testing. Of the 397 specimens tested, 109 (27.5%) were culture-positive and 288 (72.5%) were culture-negative.

b. Clinical Performance of the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette

Tables 3-4 summarize the clinical performance of the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette. For the ACON Flowflex Plus Strep A Rapid Test Strip (**Table 3**), sensitivity and specificity performances were reported as 97.2% [(106/109) with 95% CI = 92.2% - 99.1%] and 95.8% [(276/288) with 95% CI = 92.9% - 97.6%], respectively, when compared to the reference culture method. The ACON Flowflex Plus Strep A Rapid Test Cassette had similar performance (**Table 4**) with a reported sensitivity and specificity of 98.2% [(107/109) with 95% CI = 93.6% - 99.5%] and 95.1% [(273/287) with 95% CI = 92.0% - 97.1%], respectively, when compared to the reference culture method. Both test formats met acceptance criteria of ≥95% sensitivity and specificity with lower bounds of the 95% confidence intervals exceeding 90%.

The clinical performance provided for the CLIA Waiver by Application was acceptable. The sensitivity and specificity performance from the clinical study are summarized in **Table 3** and **Table 4** below.

Table 3. ACON Flowflex Plus Strep A Rapid Test Strip Clinical Performance vs. Reference Culture Method

Site	Total	TP	FN	TN	FP	Sensitivity [95% CI]	Specificity [95% CI]
Site 1	149	48	1	96	4	98.0% (48/49) [89.3%-99.6%]	96.0% (96/100) [90.2%-98.4%]
Site 2	144	31	2	107	4	93.9% (31/33) [80.4%-98.3%]	96.4% (107/111) [91.1%-98.6%]
Site 3	15	2	0	13	0	100% (2/2) [34.2%-100%]	100% (13/13) [77.2%-100%]
Site 4	66	21	0	42	3	100% (21/21) [84.5%-100%]	93.3% (42/45) [82.1%-97.7%]
Site 5	23	4	0	18	1	100% (4/4) [51.0%-100%]	94.7% (18/18) [75.4%-99.1%]
All Sites	397	106	3	276	12	97.2% (106/109) [92.2%-99.1%]	95.8% (276/288) [92.9%-97.6%]

Site	Total	TP	FN	TN	FP	Sensitivity [95% CI]	Specificity [95% CI]
						Prevalence (Reference Method): 27.5% (109/397)	

TP = true positive, FN = false negative, TN = true negative, FP = false positive, CI = confidence interval

Table 4. ACON Flowflex Plus Strep A Rapid Test Cassette Clinical Performance vs. Reference Culture Method

Site	Total	TP	FN	TN	FP	Sensitivity [95% CI]	Specificity [95% CI]
Site 1	149	49	0	95	5	100% (49/49) [92.7%-100%]	95.0% (95/100) [88.8%-97.8%]
Site 2	144	31	2	107	4	93.9% (31/33) [80.4%-98.3%]	96.4% (107/111) [91.1%-98.6%]
Site 3	15	2	0	12	1	100% (2/2) [34.2%-100%]	92.3% (12/13) [66.7%-98.6%]
Site 4	66	21	0	42	3	100% (21/21) [84.5%-100%]	93.3% (42/45) [82.1%-97.7%]
Site 5	22	4	0	17	1	100% (4/4) [51.0%-100%]	94.4% (17/18) [74.2%-99.0%]
All Sites	396	107	2	273	14	98.2% (107/109) [93.6%-99.5%]	95.1% (273/287) [92.0%-97.1%]
						Prevalence (Reference Method): 27.5% (109/396)	

TP = true positive, FN = false negative, TN = true negative, FP = false positive, CI = confidence interval

c. Operator Questionnaire

The 16 test operators who participated in the clinical study were untrained in the use of any ACON Flowflex Plus Strep A Rapid Test Strip or Cassette. No operators had prior clinical laboratory testing experience. Each study site provided the education level, employment status, years of employment, and job title of each operator. At the end 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. Based on the feedback, all of the operators either strongly agreed or agreed that the test procedures of the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette were easy to use, set-up, and operate. Operators indicated that the package insert and quick reference instructions (QRI) were easy to understand and follow.

d. Instructions for Use

The ACON Flowflex Plus Strep A Rapid Test Strip and Cassette were performed in accordance with the test procedure in the package insert and QRI. Each site was provided with copies of these materials. No other materials or instructions were supplied to operators, and the operators received no training in the use of the test.

2. Precision/Reproducibility Study

A study was conducted to evaluate the performance of ACON Flowflex Plus Strep A Rapid Test Strip and Cassette with weakly reactive samples when testing was performed by untrained users as part of Precision/Reproducibility testing in K251697. The precision/reproducibility of the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette were evaluated using contrived specimens prepared by spiking heat-inactivated Strep A bacteria (*Streptococcus pyogenes* ATCC 49399) into pooled negative clinical matrix from healthy volunteer throat swabs. Four specimen levels were prepared—negative, low negative (0.4× LoD), low positive (1× LoD), and medium positive (3× LoD)—with each specimen aliquoted into individual tubes and randomly coded for blinded testing.

Testing was conducted at three CLIA-waived sites across different geographic locations using a total of nine untrained operators with diverse educational backgrounds. Each operator tested negative and positive controls daily with each kit format. One panel of samples was tested every day for five days using three different lots per strip and cassette format. A total of 135 data points were collected per sample level per test format (3 operators × 3 sites × 5 days × 3 replicates = 135 results per concentration). The study showed that sample results met predefined acceptance criteria for each concentration level tested, and both positive and negative controls were in 100% agreement with expected results across all operators and sites. Results demonstrated that users untrained in the Flowflex Plus Strep A Rapid Test Strip and Cassette test procedure were able to perform the test correctly. The study results were acceptable (**Table 5-Table 6**).

Table 5. Summary of Precision/Reproducibility of ACON Flowflex Plus Strep A Rapid Test Strip from all Sites

Sample Type	Positives / Total (Positive agreement %) -Strip				
	Site-1	Site-2	Site-3	All Sites	Overall; 95% CI
Negative	0/45 (0.0 %)	0/45 (0.0 %)	0/45 (0.0 %)	0/135 (0.0%)	0%-2.8%
Low negative	22/45 (48.9 %)	21/45 (46.7 %)	21/45 (46.7 %)	64/135 (47.4 %)	39.2%-55.8%
Low Positive	44/45 (97.8 %)	44/45 (97.8 %)	43/45 (95.6 %)	131/135 (97.0%)	92.6%-98.8%
Medium Positive	45/45 (100.0 %)	45/45 (100.0 %)	45/45 (100.0 %)	135/135 (100.0%)	97.2%-100%

Table 6. Summary of Precision/Reproducibility of ACON Flowflex Plus Strep A Rapid Test Cassette from all Sites

Sample Type	Positives / Total (Positive agreement %) - Cassette				
	Site-1	Site-2	Site-3	All Sites	Overall; 95% CI
Negative	0/45 (0.0 %)	0/45 (0.0 %)	0/45 (0.0 %)	0/135(0.0%)	0%-2.8%
Low negative	22/45 (48.9 %)	22/45 (48.9 %)	21/45 (46.7 %)	65/135(48.1%)	39.9%-56.5%
Low positive	43/45 (95.6 %)	44/45 (97.8 %)	45/45 (100.0%)	132/135(97.8%)	93.7%-99.2%
Medium positive	45/45 (100.0 %)	45/45 (100.0 %)	45/45 (100.0 %)	135/135(100.0%)	97.2%-100%

D Labeling for Waived Devices

The labeling associated with each kit of the ACON Flowflex Plus Strep A Rapid Test Strip and the ACON Flowflex Plus Strep A Rapid Test Cassette consists of:

1. Package Insert
2. Quick Reference Instructions (QRI)

The following elements are present in the labeling:

- The QRI is written at no higher than a 7th grade reading level. It contains illustrations of the device components and procedure steps.
- The package insert and the QRI identify the test as CLIA-waived.
- The package insert contains a statement that a Certificate of Waiver is required to perform the test in a waived setting.
- Per 42 CFR 493.15(e)(1), the package insert contains a statement that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test.
- Instructions for conducting quality control (QC) procedures are integrated with procedural instructions for performing the test in both the package insert and the QRI.
- Appropriate cautions have been added to the package insert and QRI to ensure safe use of the product.
- The results of a Clinical Study that support the determination of eligibility for CLIA Waiver are included in the package insert.
- The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

XI. Benefit-Risk Considerations

For the ACON Flowflex Plus Strep A Rapid Test Strip, sensitivity and specificity performances were reported as 97.2% [(106/109) with 95% CI = 92.2% - 99.1%] and 95.8% [(276/288) with 95% CI = 92.9% - 97.6%], respectively, when compared to the reference culture method. The ACON Flowflex Plus Strep A Rapid Test Cassette had similar performance with a reported sensitivity and specificity of 98.2% [(107/109) with 95% CI = 93.6% - 99.5%] and 95.1% [(273/287) with 95% CI = 92.0% - 97.1%], respectively, when compared to the reference culture method. While acceptable (>95%), this level of sensitivity warrants the requirement for culture confirmation of negative test results. This requirement is sufficient to mitigate the risks associated with a false-negative result.

XII. Conclusion

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