



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY ONLY**

**I Background Information:**

**A 510(k) Number**

K251697

**B Applicant**

ACON Laboratories, Inc.

**C Proprietary and Established Names**

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

**D Regulatory Information**

Product Code(s)	Classification	Regulation Section	Panel
GTY	Class I	21 CFR 866.3740 - Streptococcus Spp. Serological Reagents	MI - Microbiology

**II Submission/Device Overview:**

**A Purpose for Submission:**

A Dual Submission to obtain 510(k) clearance and CLIA Waiver for the Flowflex Plus Strep A Rapid Test Cassette and Flowflex Plus Strep A Rapid Test Strip.

**B Measurand:**

Group A  $\beta$ -hemolytic *Streptococcus* (GAS; *Streptococcus pyogenes*) antigens in throat swab specimens.

**C Type of Test:**

Lateral flow chromatographic immunoassay

### **III Intended Use/Indications for Use:**

#### **A Intended Use(s):**

See Indications for Use below.

#### **B Indication(s) for Use:**

##### Flowflex Plus Strep A Rapid Test Cassette

The Flowflex Plus Strep A Rapid Test Cassette is a rapid chromatographic immunoassay for the qualitative detection of Group A Streptococcus antigen from throat swab specimens from symptomatic patients. The test is used to aid in the diagnosis of Group A Streptococcal infection. All negative test results should be confirmed by bacterial culture because negative results do not preclude infection with Group A Streptococcus and should not be used as the sole basis for treatment.

##### Flowflex Plus Strep A Rapid Test Strip

The Flowflex Plus Strep A Rapid Test Strip is a rapid chromatographic immunoassay for the qualitative detection of Group A Streptococcus antigen from throat swab specimens from symptomatic patients. The test is used to aid in the diagnosis of Group A Streptococcal infection. All negative test results should be confirmed by bacterial culture because negative results do not preclude infection with Group A Streptococcus and should not be used as the sole basis for treatment.

#### **C Special Conditions for Use Statement(s):**

- Rx - For Prescription Use Only
- For *in vitro* diagnostic use only
- A negative result must be confirmed by culture. A negative result may be obtained if the concentration of the Group A Streptococcus antigen present in the throat swab is not adequate or is below the detectable level of the test.

#### **D Special Instrument Requirements:**

N/A

### **IV Device/System Characteristics:**

#### **A Device Description:**

There are two formats of the test—cassette and strip. A test strip is included individually in the Flowflex Plus Strep A Rapid Test Strip format. In the cassette format, the test strip is already assembled in plastic housing to form the Flowflex Plus Strep A Rapid Test Cassette.

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). In 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.

For the Flowflex Plus Strep A Rapid Test Strip, the test strip is immediately placed in the extracted sample solution, which migrates up through the test strip. In the case of the Flowflex Plus Strep A Rapid Test Cassette, drops of the extracted sample are added to the sample well of the cassette. Each format contains a test line (T line) and a control line (C line). Results can be read after 5 minutes.

## B Principle of Operation:

The ACON Flowflex Plus Strep A Rapid Test Strip and Cassette are qualitative, lateral flow immunoassays for the detection of Strep A carbohydrate antigen in a throat swab. In this test, an antibody specific to Strep A carbohydrate antigen is coated on the test line region of the test strip. 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 the antibody to Strep A on the membrane, and a colored line (pink-red) is generated in 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 colored 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.

Each format of the Flowflex Plus Strep A Rapid Test (i.e., strip or cassette) has a unique Quick Reference Instruction (QRI) document and package insert and are provided as separate unique kits. Slight differences in the workflow between the strip and cassette formats are described below. **Table I** includes the components of Flowflex Plus Strep A Rapid Test Kits.

- Cassette Kit  
A dropper tip is attached firmly onto the tube containing the extracted sample, and the tube is mixed thoroughly by swirling or flicking the bottom. Three full drops of the extracted solution are added to the sample well (S) before starting the timer. Results are read at 5 minutes.
- Strip Kit  
With arrows pointing down, the test strip is inserted vertically into the extracted specimen solution and the timer is started. If the procedure is followed correctly, the solution should be below the maximum line (MAX) on the test strip. The strip is left in the tube for 5 minutes before reading results.

**Table I. Cassette Kit and Strip Kit Components**

Cassette Kit Components	Strip Kit Components
<ul style="list-style-type: none"><li>• Test Cassettes</li><li>• Test Tubes</li><li>• Dropper Tips</li><li>• Sterile Throat Swabs</li><li>• Reagent A (2M Sodium Nitrite)</li><li>• Reagent B (0.2M Acetic Acid)</li><li>• Positive Control (Heat-inactivated Group A <i>Streptococcus</i>)</li></ul>	<ul style="list-style-type: none"><li>• Test Strips</li><li>• Test Tubes</li><li>• Sterile Throat Swabs</li><li>• Reagent A (2M Sodium Nitrite)</li><li>• Reagent B (0.2M Acetic Acid)</li><li>• Positive Control (Heat-inactivated Group A <i>Streptococcus</i>)</li></ul>

Cassette Kit Components	Strip Kit Components
<ul style="list-style-type: none"> <li>• Negative Control (Heat-inactivated Group C <i>Streptococcus</i>)</li> <li>• Package Insert</li> <li>• Quick Reference Instructions</li> </ul>	<ul style="list-style-type: none"> <li>• Negative Control (Heat-inactivated Group C <i>Streptococcus</i>)</li> <li>• Package Insert</li> <li>• Quick Reference Instructions</li> </ul>

**V Substantial Equivalence Information:**

**A Predicate Device Name(s):**

Wondfo Strep A Rapid Test

**B Predicate 510(k) Number(s):**

K133343

**C Comparison with Predicate(s):**

Device & Predicate Device(s):	<u>K251697</u> (subject device)	<u>K133343</u> (predicate device)
Device Trade Name	Flowflex Plus Strep A Rapid Test Cassette; Flowflex Plus Strep A Rapid Test Strip	Wondfo One Step Strep A Swab Test
General Device Characteristic Similarities		
Intended Use/Indications For Use	<p><b><u>Cassette:</u></b> The Flowflex Plus Strep A Rapid Test Cassette is a rapid chromatographic immunoassay for the qualitative detection of Group A Streptococcus antigen from throat swab specimens from symptomatic patients. The test is used to aid in the diagnosis of Group A Streptococcal infection. All negative test results should be confirmed by bacterial culture because negative results do not preclude infection with Group A Streptococcus and should not be used as the sole basis for treatment.</p> <p><b><u>Strip:</u></b> The Flowflex Plus Strep A Rapid Test Strip is a rapid chromatographic immunoassay for the qualitative detection of Group A Streptococcus antigen from throat swab</p>	<p>The Wondfo Strep A Rapid Test is a chromatographic immunoassay for the qualitative detection of Strep A antigen from throat swab specimens from symptomatic patients to aid in the diagnosis of Group A Streptococcal infection. All negative test results should be confirmed by bacterial culture because negative results do not preclude Group A Strep infection and should not be used as the sole basis for treatment. This test is intended for professional and laboratory use, only.</p>

Device & Predicate Device(s):	<u>K251697</u> (subject device)	<u>K133343</u> (predicate device)
	specimens from symptomatic patients. The test is used to aid in the diagnosis of Group A Streptococcal infection. All negative test results should be confirmed by bacterial culture because negative results do not preclude infection with Group A Streptococcus and should not be used as the sole basis for treatment.	
Specimen Type	Throat swab specimen	Same
Test Technology	Immunochromatographic lateral flow assay	Same
Indication for Use	Prescription Use	Same
Single Use	Yes	Same
Test Result	Qualitative	Same
Sample Collection Method	Use the throat swab supplied in the kit	Same
<b>General Device Characteristic Differences</b>		
Test Format	Cassette and Strip formats	Strip
Wait Time for Results Read	5 minutes	10 minutes
LoD	7.4 x 10 <sup>5</sup> CFU/mL (for contrived Strep A swab samples); 1.0 x 10 <sup>5</sup> CFU/mL (in extraction buffer)	1.5 x 10 <sup>5</sup> organisms/mL
Clinical Sensitivity/Specificity	<b><u>Cassette:</u></b> Sensitivity: 98.2% (95% CI: 93.6% - 99.5%) Specificity: 95.1% (95% CI: 92.0% - 97.1%)  <b><u>Strip:</u></b> Sensitivity: 97.2% (95% CI: 92.2% - 99.1%) Specificity: 95.8% (95% CI: 92.9% - 97.6%)	<b><u>Strip:</u></b> Sensitivity: 95% (95% CI: 88 - 98%) Specificity: 98% (95% CI: 96 - 99%)

## VI Standards/Guidance Documents Referenced:

N/A

## VII Performance Characteristics (if/when applicable):

### A Analytical Performance:

#### 1. Reproducibility/Precision:

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. Performance of the Flowflex Plus Strep A Rapid Test Strip and Cassette with weakly reactive samples was also performed by untrained users as part of reproducibility testing. Four specimen levels were prepared—negative, low negative ( $0.4 \times \text{LoD}$ ), low positive ( $1 \times \text{LoD}$ ), and medium positive ( $3 \times \text{LoD}$ )—with each specimen aliquoted into individual tubes and randomly coded for blinded testing.

The Reproducibility Study 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 formats. A total of 135 data points were collected per sample level per test format ( $3 \text{ operators} \times 3 \text{ sites} \times 5 \text{ days} \times 3 \text{ replicates} = 135 \text{ results per concentration}$ ). 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 II-Table IV).

**Table II. 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 III. 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%

**Table IV. Summary of the External Controls Testing with  
ACON Flowflex Plus Strep A Rapid Test Strip/Cassette from Different Sites**

Control	Flowflex Plus Strep A Rapid Test Strip (Observed results/Expected results)		Flowflex Plus Strep A Rapid Test Cassette (Observed results/Expected results)	
	Negative Control	Positive Control	Negative Control	Positive Control
Site 1	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)
Site 2	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)
Site 3	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)
Overall	45/45 (100%)	45/45 (100%)	45/45 (100%)	45/45 (100%)

2. Linearity:

N/A

3. Analytical Specificity/Interference:

To prepare positive samples for the remaining analytical studies, all microorganisms were spiked into pooled negative clinical matrix prepared from healthy volunteer throat swabs that was confirmed negative by testing with a commercial Strep A test and bacterial culture.

Cross-Reactivity Study

A Cross-Reactivity Study was conducted to evaluate the performance of the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette in the presence of fifty-five (55) microorganisms commonly found in throat specimens that could potentially interfere with test results. The microorganisms tested included various streptococcal species (Groups B, C, D, F, and G), respiratory viruses (influenza, RSV, rhinovirus), bacterial pathogens (*Staphylococcus*, *Neisseria*, *Haemophilus*), and other throat flora. Each potentially cross-reacting microorganism was tested in replicates of five (5) at clinically relevant levels of viruses (from  $4.86 \times 10^4$  TCID<sub>50</sub>/mL to  $1.0 \times 10^6$  TCID<sub>50</sub>/mL, or at the highest achievable concentration) and bacteria ( $1.0 \times 10^7$  CFU/mL). Samples were randomized, blinded, and tested according to the package insert instructions. There were no false positive reactions with any of the tested microorganisms at the specified concentrations, confirming that the ACON Flowflex Plus Strep A tests do not cross-react with the certain bacteria and viruses that may be present in throat specimens. See **Table V** below for the cross-reactivity panel tested.

Table V. Organism Panel for Cross-Reactivity Study	
Adeno virus Type 1	Neisseria sicca Z043, T
Arcanobacterium haemolyticum	Neisseria Subflava Biovar Flava Z119
Bordetella pertussis A639	Proteus vulgaris Z129
Candida albicans Z006	Pseudomonas aeruginosa Z139
Corynebacterium diphtheriae Z116	Respiratory syncytial virus Type A (RSV-A) (1/2015 isolate)
Cytomegalo-virus	Rhinovirus Type 1A culture fluid
Echovirus Type 09	Serratia marcescens Z053
Enterococcus faecalis Z346 VSE	Staphylococcus aureus MRSA
Enterococcus faecium Z265	Staphylococcus epidermidis MRSE, RP62A

Table V. Organism Panel for Cross-Reactivity Study	
Epstein-Barr virus	Staphylococcus haemolyticus
Escherichia coli clinical isolate,	Strep B
Fusobacterium necrophorum Z239	Strep C
Haemophilus parainfluenzae Z492	Strep D
Human metapneumo-virus (hMPV) 16 Type A1	Strep F
Influenza A	Strep G
Influenza A H3N2	Streptococcus agalactiae Z019
Klebsiella pneumoniae KPC-2	Streptococcus anginosus Z199
Klebsiella pneumoniae Z026	Streptococcus aureus CoNS
Lactobacillus salivarius	Streptococcus bovis Z167
Measles virus culture fluid	Streptococcus constellatus Z124
Moraxella catarrhalis Ne 11	Streptococcus dysgalactiae subso. Equisimilis Z068
Mumps virus	Streptococcus intermedius Z216
Mycobacterium tuberculosis	Streptococcus mitis Clinical Isolate
Mycoplasma pneumoniae	Streptococcus mutans Z072
Neisseria gonorrhoeae Z017	Streptococcus oralis Z307
Neisseria lactamica Z041	Streptococcus salivarius Z127
Neisseria Meningitidis Serogroup A	Streptococcus sanguinis Z089
Neisseria mucosa	

#### Microbial Interference Study

A Microbial Interference Study was conducted to evaluate the performance of the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette in the presence of microorganisms listed in **Table V** above. Each organism was tested in replicates of five (5) with clinically relevant levels of viruses ( $\geq 1.0 \times 10^5$  TCID<sub>50</sub>/mL or the highest achievable concentration) and bacteria ( $\geq 1.0 \times 10^6$  CFU/mL) in the presence of 2× LoD of *S. pyogenes* ATCC 49399 ( $1.48 \times 10^6$  CFU/mL). All strain combinations were spiked into pooled negative clinical matrix prepared from healthy volunteer throat swabs that was confirmed negative by commercial Strep A testing and blood culture. No microbial interference was observed for any of the organisms at the concentrations tested and the results are acceptable.

#### Interfering Substances Study

Thirty-two (32) endogenous and exogenous substances were evaluated for potential to interfere with the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette, including fresh whole human blood, bovine submaxillary mucin, over-the-counter mouthwashes, throat sprays, cough syrups, throat lozenges, and active pharmaceutical ingredients commonly found in cold and allergy medications. Each substance was tested in replicates of five (5) both in the absence and presence of GAS [2× LoD of *S. pyogenes* ATCC 49399 ( $1.48 \times 10^6$  CFU/mL)]. Samples were prepared in pooled negative clinical matrix from healthy volunteer throat swabs. None of the thirty-two (32) substances tested demonstrated a potential to cause false positive, false negative, or invalid results at the concentrations tested. All samples without GAS generated negative results, and all samples with GAS generated positive results for both the strip and cassette formats. Results confirmed that the tested endogenous and exogenous substances did not interfere with test performance. See **Table VI** below for a list of substances and concentrations tested.



**Table VI. List of Endogenous and Exogenous Substances Tested**

Substance	Concentration
Mucin (Bovine Submaxillary Gland, type I-S)	0.4 mg/ml
Ethanol	99.5%
Blood (human), EDTA anticoagulated	20% (v/v)
Group A $\beta$ -Strep Blood Agar	N/A
<b>OTC Mouthwashes:</b>	
Listerine Antiseptic	25% (v/v)
Listerine Cool Mint	25% (v/v)
Crest Pro-Health Clean Night Mint	25% (v/v)
<b>OTC Throat Sprays:</b>	
Chloraseptic Max	25% (v/v)
<b>OTC Cough Syrups:</b>	
Tylenol Cold, Cough and Sore Throat	25% (v/v)
Rite Aid Tussin CF	25% (v/v)
Children's Dimetapp Cold & Cough	25% (v/v)
Children's Dimetapp Cold & Allergy	25% (v/v)
Children's Dimetapp Nighttime Cold & Congestion	25% (v/v)
Children's Wal-Tap Elixir Cold & Allergy	25% (v/v)
Robitussin Cough & Chest Congestion DM	25% (v/v)
Robitussin Nighttime Cough DM	25% (v/v)
Robitussin Cough & Cold-CF Max	25% (v/v)
Halls Cherry Mentholyptus	20% (w/v)
Halls Plus Mentholyptus	20% (w/v)
Ricola LemonMint Herb Throat Drops-Sugar Free	20% (w/v)
Cepacol Cherry Sore Throat	20% (w/v)
BreathSavers 3 Hour Mint-Spearmint	20% (w/v)
Tic Tac wintergreen	20% (w/v)
<b>Active Ingredients:</b>	
Acetaminophen (Tylenol)	10 mg/ml
Brompheniramine Maleate	5 mg/ml
Chlorpheniramine Maleate	5 mg/ml
Dextromethorphan HBr	5 mg/ml
Diphenhydramine HCl	5 mg/ml
Doxylamine Succinate	5 mg/ml
Guaifenesin (GuaicolGlyceryl)	20 mg/ml
Ibuprofen (Advil)	10 mg/ml
Phenylephrine HCl	5 mg/ml

OTC = over the counter

#### High Dose Hook Effect

A High-Dose Hook Effect Study was conducted to evaluate the performance of the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette when exposed to high concentrations of GAS. Contrived samples were prepared by spiking heat-inactivated *S. pyogenes* (ATCC 49399) into pooled negative clinical matrix at various levels—negative sample (0 CFU/mL), intermediate concentrations ( $3.7 \times 10^5$  CFU/mL and  $7.4 \times 10^5$  CFU/mL), and very high concentrations ( $1.5 \times 10^8$  CFU/mL). Testing was performed on both strip and cassette formats of the ACON Flowflex Plus Strep A Rapid Test, where each sample was tested in five replicates following the manufacturer's package insert instructions. Results demonstrated

that no hook effect (false negative results) was observed at the highest tested concentration,  $1.5 \times 10^8$  CFU/mL, using both the strip and cassette formats. While concentrations at/above  $7.4 \times 10^5$  CFU/mL showed 100% detection of the target, the lowest concentration of GAS spiked,  $3.7 \times 10^5$  CFU/mL, showed variable results (2/5 positive for strips, 3/5 positive for cassettes). This was acceptable since the lower concentration tested in the study was below the reported LoD for the test with that strain.

4. Assay Reportable Range:

N/A

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Quality Control

The ACON Flowflex Plus Strep A Rapid Test Strip and Cassette formats incorporate three controls:

- Internal Positive 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 proper volume of sample has been added and capillary flow occurred. If the procedural control line does not develop, the test result is considered invalid, and retesting with a new strip/cassette and sample is recommended.
- External Controls: Positive and Negative control solutions are supplied with each kit. The positive control solution is non-infectious heat-inactivated Strep A bacteria with buffer and stabilizer solution. The negative control solution is heat-inactivated Strep C bacteria with buffer solution. The instructions for use (package insert) recommends that a positive and negative external control be tested once for each untrained operator and once for each new shipment of kits—provided that each different lot in the shipment is tested. These controls help to ensure that the test procedure is performed correctly.

Evaluation of External Control Performance

During the Clinical Study, each operator tested one positive control and one negative control according to the instructions recommended in the package insert and quick reference instructions of the product. All positive controls [100% (14/14) each format] and negative controls [100% (14/14) each format] were detected accurately with the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette.

In the analytical studies, External Quality Controls (Negative Control and Positive Control) were tested prior to performing each analytical study and results were recorded. All controls gave expected results. Quality Control performance during the Reproducibility Study is presented above in **Table IV**. The data from both clinical and analytical studies demonstrate acceptable results when testing positive and negative external controls.

Sample Stability

- 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 each swab sample 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.

## 6. Detection Limit:

### Limit of Detection

The limit of detection (LoD) of the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette was determined using contrived stocks of heat-inactivated *S. pyogenes* (ATCC 49399) prepared in pooled negative clinical matrix from healthy volunteer throat swabs. The LoD was defined as the lowest Strep A concentration detected ≥95% of the time (at least 19 out of 20 replicates testing positive). Preliminary testing was conducted with concentrations ranging from  $1.98 \times 10^4$  to  $1.98 \times 10^7$  CFU/mL using five replicates per concentration across three lots of each test format. Confirmation studies were then performed with concentrations around the preliminary LoD using 20 replicates per concentration across three lots. Based on the confirmation study results, the limit of detection was established as  $7.4 \times 10^5$  CFU/mL for contrived Strep A samples [equivalent to  $1.0 \times 10^5$  CFU/mL in extraction buffer or  $3.7 \times 10^4$  CFU/swab for both the strip and cassette kit formats].

### Inclusivity Study

Inclusivity studies were conducted with three *S. pyogenes* strains (ATCC 12344, 12370, and 19615) in replicates of five per strain using both the ACON Flowflex Plus Strep A Rapid Test Strip and Cassette formats. Each cultured strain was prepared by serial dilution into pooled negative clinical matrix from healthy volunteer throat swabs. Preliminary LoDs were determined followed by confirmation testing at concentrations two-fold above and below the preliminary LoD. The study results demonstrated that all three GAS strains were correctly

detected by both test formats with confirmed LoD concentrations ranging from  $2.71 \times 10^5$  to  $3.68 \times 10^5$  CFU/mL for contrived samples. See **Table VII** below.

**Table VII. Inclusivity Study Report**

Item No.	Pathogens	ATCC #	LoD Conc. of prepared sample (CFU/mL)	Conc. in extraction buffer (CFU/mL)
1	<i>Streptococcus pyogenes</i> typing strain T1 [NCIB 11841, SF 130]	12344	$2.85 \times 10^5$	$3.85 \times 10^4$
2	<i>Streptococcus pyogenes</i> typing strain C94	12370	$3.68 \times 10^5$	$4.97 \times 10^4$
3	<i>Streptococcus pyogenes</i> strain Bruno [CIP 104226]	19615	$2.71 \times 10^5$	$3.66 \times 10^4$

7. Assay Cut-Off:  
N/A

## **B Comparison Studies:**

1. Method Comparison with Predicate Device:  
N/A
2. Matrix Comparison:  
N/A

## **C Clinical Studies:**

1. Clinical Sensitivity:  
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 throat 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.

**Tables VIII-IX** 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 VIII**), 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 IX**) 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  $\geq 95\%$  sensitivity and specificity with lower bounds of the 95% confidence intervals exceeding 90%.

Sixteen (16) untrained healthcare professional operators from diverse educational backgrounds participated in the study. No operators had prior clinical laboratory testing experience. Post-study questionnaire results supported usability with 100% of operators agreeing that the overall instructions and test procedures were easy to follow and sample processing was easy to perform correctly. The study supports that untrained operators could successfully perform both test formats following QRI and package insert instructions.

The clinical performance provided was used for both the 510(k) and CLIA Waiver by Application. The sensitivity and specificity performance from the clinical study are summarized in **Table VIII-Table IX** below.

**Table VIII. 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%]
Prevalence (Reference Method): 27.5% (109/397)							

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

**Table IX. 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

2. Clinical Specificity:

See above.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

N/A

**D Clinical Cut-Off:**

N/A

**E Expected Values/Reference Range:**

In the multi-center clinical study conducted by ACON from August 2022 to March 2024, 27.5% (Table X) of patients presenting with pharyngitis were found to be culture positive for Strep A in the study to evaluate ACON Flowflex Plus Strep A Rapid Test Strip and Cassette performance.

**Table X. Clinical Performance Stratified by Age**

Test Format	Age	Number Samples per Age Range (% of Total Samples)	Sensitivity [95% CI]	Specificity [95% CI]	Number of Positives by Reference Method (% Prevalence by Age Group)
Strip	1-5	64 (16.1%)	100% [77.2%-100%]	96.1% [86.8%-98.9%]	13 (20.3%)
	6-20	176 (44.3%)	95.9% [86.3%-98.9%]	95.3% [90.1%-97.8%]	49 (27.8%)
	21+	157 (39.5%)	97.9% [88.9%-99.6%]	96.4% [91.0%-98.6%]	47 (29.9%)
	All Ages	397	97.2% [92.2%-99.1%]	95.8% [92.9%-97.6%]	109 (27.5%)
Cassette	1-5	64 (16.2%)	100% [77.2%-100%]	96.1% [86.8%-99.0%]	See numbers above
	6-20	175 (44.2%)	95.9% [86.3%-98.9%]	95.2% [90.0%-97.8%]	
	21+	157 (39.6%)	100% [92.5%-100%]	94.5% [88.6%-97.5%]	
	All Ages	396	98.2% [93.6%-99.5%]	95.1% [92.0%-97.1%]	

**VIII Proposed Labeling:**

The labeling supports the finding of substantial equivalence for this device.

## **IX Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.