510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

K141775

B. Purpose for Submission:

To obtain a substantial equivalence determination for the Sofia® Strep A+ FIA.

C. Measurand:

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

D. Type of Test:

The Sofia® Strep A+ FIA is an immunofluorescence-based lateral flow *in vitro* diagnostic test for the qualitative detection of GAS antigens isolated from throat swab specimens obtained from symptomatic patients.

E. Applicant:

Quidel Corporation

F. Proprietary and Established Names:

Sofia® Strep A+ FIA and Sofia®

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3740 - *Streptococcus spp.* Serological Reagents

2. Classification:

   Class I

3. Product code:

   GTY – Antigens, *Streptococcus spp.*, All Groups

KHO – Fluorometer, for Clinical Use

4. Panel:

83- Microbiology

H. Intended Use:

1. Intended use(s):

The Sofia® Strep A+ FIA detects Group A Streptococcal antigens from throat swabs from patients with signs and symptoms of pharyngitis, such as sore throat. 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. The test is intended for professional and laboratory use as an aid in the diagnosis of Group A Streptococcal infection.

2. Indication(s) for use:

Same as intended use.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

Sofia®

I. Device Description:

The Sofia® Strep A+ FIA is a lateral flow immunofluorescence-based assay encased in a 2-D barcoded cassette. Antigen-extracted specimen is applied to the sample pad through a sample port, which is wicked through photoresistant europium chelate-impregnated polystyrene microparticles coated with polyclonal primary antibodies to *Streptococcus pyogenes* antigens, then through a localized stripe of secondary antibodies (Strep A Line), followed by a reference stripe that acts as a procedural control zone and reference line. The test is designed to reduce autofluorescence background by using a large Stokes shift from 365 to 618 nm. Once the barcode is scanned with the external barcode analyzer, the cassette is uni-directionally inserted into an analyzer drawer in the front of the apparatus. The Sofia® analyzer scans the region of the test strip containing the Strep A and Reference lines in the result window to yield three possible results: 1) Positive for Strep A, 2) Negative for Strep A, 3) Invalid. Europium chelate is detected by a scanning optics unit, exciting by a 365 nm filtered LED (Light Emitting Diode) and captured by a filtered photodiode, converted from analog RFU (Relative Fluorescence Units) data to digital.

A positive result for the analyte is determined by detection and analysis of the fluorescent signal at the test and reference lines, which are processed by an assay-specific algorithm. The algorithm employs a smoothing algorithm to the data, identifies peak maxima, minima and width, then calculates the RFU value based on peak height for the Strep A test line. The Limit of Blank (LoB) is 1948 RFU. When the test line value is ≥ 1948 RFU, the test result is positive; when the value is < 1948 RFU, the test results is negative. The procedural control zone cutoff for a valid versus invalid result is 30,000 RFU. If internal controls fail at any point, the result is invalid and an error code is presented. Results are presented on a screen and can be printed on an integrated printer (optional). The Sofia® analyzer has the capacity to operate in Read-Now Mode and Walk-Away Mode; however Read-Now Mode functionality has been disabled for this the Sofia® Strep A+ FIA.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Sofia® Strep A+ FIA with Sofia® (k123793)

2. Predicate 510(k) number(s):

   k123793

3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
	Sofia® Strep A+ FIA with Sofia® (k141775)	**Sofia® Strep A FIA with Sofia® (k123793)**

Similarities		
Item	**Device**	**Predicate**
	Sofia® Strep A+ FIA with Sofia® (k141775)	**Sofia® Strep A FIA with Sofia® (k123793)**
Intended Use	The Sofia® Strep A+ FIA detects Group A Streptococcal antigens from throat swabs from patients with signs and symptoms of pharyngitis, such as sore throat. 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. The test is intended for professional and laboratory use as an aid in the diagnosis of Group A Streptococcal infection.	The Sofia® Strep A FIA employs immunofluorescence technology to detect Group A Streptococcal antigens from throat swabs of symptomatic patients. 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. The test is intended for professional and laboratory use as an aid in the diagnosis of Group A Streptococcal infection.
Instrument	Sofia®	Same
Analyte	Group A Streptococcal antigen	Same
Qualitative	Yes	Same
Automated Analysis	Yes	Same
Read Results	Read results on instrument screen or print with optional printer	Same
Calibrator	Yes – Calibration Cassette and QC Card provided	Same
Read Result Time	5 Minutes	Same
Specimen Type	Throat swab	Same
Test Principle	Immunofluorescence Device	Same
Format	Lateral-flow Test Cassette	Same
Antibodies Used	Polyclonal rabbit antibodies that are specific to Group A Streptococcus carbohydrate antigen	Same
Detection Particle	Polystyrene microparticles dyed with Europium chelate	Same

Similarities		
Item	**Device**	**Predicate**
	Sofia® Strep A+ FIA with Sofia® (k141775)	**Sofia® Strep A FIA with Sofia® (k123793)**
Transfer Device	Fixed volume pipette used to transfer patient sample mixed with reagent into Test Cassette	Same
External Controls	Test kit contains Positive and Negative Control Swabs	Same
Quality Control Features	Built-in features include: • Built-in procedural control zone scanned by the analyzer to determine whether adequate flow occurred • Analyzer prevents used or expired cartridge from being read by the reader • Cassette properly inserted	Same
Storage	Room Temperature	Same

Differences		
Item	**Device**	**Predicate**
	Sofia® Strep A+ FIA with Sofia® (k141775)	**Sofia® Strep A FIA with Sofia® (k123793)**
Operational Modes	Walk-Away Mode only	Read-Now and Walk-Away Modes
Reagent	One reagent bottle containing sodium nitrite and hydrochloric acid in glass ampoule	One reagent bottle containing sodium nitrite and acetic acid in glass ampoule

Differences		
Item	**Device**	**Predicate**
	Sofia® Strep A+ FIA with Sofia® (k141775)	**Sofia® Strep A FIA with Sofia® (k123793)**
Performance Characteristics	Walk-Away Mode: Sensitivity: 93.7%[95% CI: 89.1% - 96.5%] Specificity: 94.4%[95% CI: 92.4% - 95.9%]	Read Now + Walk-Away Mode: Sensitivity: 90.6%[95% CI: 84.3% - 94.6%] Specificity: 96.1%[95% CI: 94.2% - 97.3%] Read-Now Mode: Sensitivity: 89.3%[95% CI: 82.2% - 93.8%] Specificity: 96.0%[95% CI: 94.0% - 97.3%] Walk-Away Mode: Sensitivity: 100.0%[95% CI: 80.6% - 100.0%] Specificity: 97.2%[95% CI: 85.8% - 99.5%]

K. Standard/Guidance Document Referenced (if applicable):

Not applicable.

L. Test Principle:

Analyte detection from the chemically lysed swab specimen with the lateral flow device begins with the pipetting of the extracted clinical sample into the test cassette's sample port and onto the sample pad, after which the sample is drawn by capillary action into and through the label pad, through the nitrocellulose strip and into the absorbent pad. The specimen interacts with distinct chemical environments as the fluid migrates along the course of the lateral flow device. Within the label pad, the specimen comes into contact with antibodies that have been coupled to the europium chelate-impregnated microbeads. During this interaction, the beads coated with anti-GAS polyclonal antibodies bind corresponding GAS carbohydrate antigens that are present in the specimen. The bead-coupled antigen-antibody complexes then begin to flow through the test strip. As they migrate on, if GAS antigens are present in the sample, they and the fluorescent beads to which they are bound will be subsequently captured on the surface of the nitrocellulose by the respective location-fixed, anti-GAS-specific capture antibodies. The flow and capture of the fluorescent microbeads coated with GAS antigens allows the accumulation of a fluorescent signal at the specific analyte line location on the test strip. Upon completion of the test, the Sofia® analyzer scans the test strip and objectively interprets the assay result. There are three possible results: (1) positive for Strep A; (2) negative for Strep A; and (3) invalid. It is

important to point out that the fluorescence signals obtained with this assay are invisible to the unaided eye. The test results can only be obtained with the proper use of the Sofia® analyzer. This ensures fully objective interpretation of the test result.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   All analytical studies were conducted in Read-Now Mode with the exception of the reproducibility studies, which were conducted with the Walk-Away mode. Although the Read-Now Mode is not available in this version of the device, this feature was unlocked in order to simplify testing. The primary difference between these modes is that in Read-Now Mode, the operator is responsible for the incubation time; in the Walk-Away Mode, the instrument will wait to read the strip until the specified development time has passed. A validation study was conducted to demonstrate that the Read-Now Mode does not impact the results of the analytical studies in the conditions tested. See section M.3.*c* below for more information and the results of this study.

   a. Precision/Reproducibility:

   Specimens for precision and reproducibility studies were prepared by re-suspending each cultured strain in a contrived negative matrix, freezing until testing then diluting the strain to the indicated concentration in the table below. The rayon swab that is provided with the assay was inoculated with a fixed volume of the stock and run with the Sofia® Strep A+ FIA. The LoD values were based on the values obtained in the LoD study. See section M.2.*b* below for details on the matrix equivalency and freeze-thaw study.

   The reproducibility of the Sofia® Strep A+ FIA was evaluated at three (3) laboratory sites (two external, one in-house) on six (6) different analyzers. Reproducibility was assessed using a panel of four (4) simulated samples in negative clinical matrix that include negative, high negative, low positive and moderate positive GAS. The panels and controls were processed and tested on the Sofia® Strep A+ FIA at each site by 2 operators for 5 non-consecutive days over 2 weeks on 6 different instruments in Walk-Away Mode (2 operators x 3 replicates x 5 days x 3 sites = 90 results per concentration). The reproducibility study results are acceptable. The results are shown in the Table I below.

Table I: Reproducibility									
Panel ID		Negative (C_0)		High Negative (C_5)		Low Positive (C_{95})		Moderate Positive (C_{100})	
Site	Operator	Detected Pos/Total	%Pos	Detected Pos/Total	%Pos	Detected Pos/Total	%Pos	Detected Pos/Total	%Pos
Site 1	1	0/15	0%	2/15	13.3%	14/15	93.3%	15/15	100%
	2	0/15	0%	1/15	6.7%	13/15	86.7%	15/15	100%
Site 2	1	0/15	0%	0/15	0%	14/15	93.3%	15/15	100%
	2	0/15	0%	1/15	6.7%	9/15	60.0%	15/15	100%
Site 3	1	0/15	0%	3/15	20.0%	14/15	93.3%	15/15	100%
	2	0/15	0%	2/15	13.3%	14/15	93.3%	15/15	100%
Combined		9/90	0/90	0%	9/90	10.0%	78/90	86.7%	100%

b. Linearity/assay reportable range:

Not applicable.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

The Sofia® Strep A+ FIA has an internal a procedural control zone used to monitor sample processing and evaluate the presence of inhibitory substances or inadequate specimen preparation. External controls are used to confirm the integrity of assay reagents and cassette detection. The external quality controls used in these studies were positive control swabs spiked with heat inactivated, GAS cells and negative control swabs spiked with Group C Streptococcal antigen. The positive control serves as both a processing and extraction control. The positive and negative external control isolates were tested each day during the clinical studies.

Specimen stability studies demonstrated that contrived specimens were stable when stored at 2-8° C or 23° C for up to 96 hours. These studies were conducted with negative matrix and positive contrived specimens at 2.4 x LoD in replicates of six (6) per condition on a single instrument for 0, 6, 24, 48, 72 and 96 hours.

Additional temperature studies were conducted with negative matrix and positive contrived specimens at 1.6 x LoD in replicates of ten (10) per condition on ten (10) different instruments. These studies were conducted for 30 minutes at 2-8° C, 24° C and at 37° C.

Extracted specimens were demonstrated to be stable for up to 24 hours at 24° C. Studies were conducted with negative matrix and positive contrived specimens at 1.6 x LoD in replicates of 5 (five) per condition for 0, 1 and 30 minutes, 1, 2, 6 and 24 hours on six (6) different instruments.

Data was provided to demonstrate that the Sofia Strep A+ FIA can be operated at temperatures between 15° C -31° C. These studies were conducted with negative matrix and positive contrived specimens at 1.6 x LoD in replicates of ten (10) per condition on a single instrument at 15° C, 23° C and 31° C.

An inter-analyzer study demonstrated that results did not vary between instruments. These studies were conducted with negative matrix and positive contrived specimens at 1.6 x LoD using ten (10) analyzers in replicates of 20 for positive specimens and 10 for negative specimens.

The studies above meet or exceed the requirements in the labeling.

All controls performed as expected for all stability studies.

These study results are acceptable.

d. Detection limit:

The limit of detection (LoD) of the Sofia® Strep A+ FIA was determined using contrived stocks of three (3) strains of GAS which were prepared per the protocol in M.1.*a*. The LoD was defined as the point at which at least 95% of all replicates tested positive (C_{95}). Preliminary testing was conducted to identify the approximate threshold for negative and positive detection for each strain. Low-positive (C_{50}) testing was then conducted with 60 replicates per strain. Dose-response curves were then generated to interpolate the expected LoD concentration for each strain. For each strain, the LoD was validated with 20 replicates. The LoD study results are shown in Table II below.

Table II: LoD for Group A β-hemolytic *Streptococcus*

Strain	Strain ID	CFU/ml	RFU
Group A Streptococcal strain 1 (S*treptococcus pyogenes*)	ATCC 19615	1.3×10^5	3357
Group A Streptococcal strain 2 (S*treptococcus pyogenes*)	ATCC 700942	2.7×10^5	3599
Group A Streptococcal strain 3 (S*treptococcus pyogenes*)	ATCC 700952	9.2×10^4	2706

These study results are acceptable.

e. Analytical Sensitivity:

Inclusivity studies were conducted with twenty-one (21) GAS strains in replicates of twenty (20) per strain at approximately 2 x LoD (5.8×10^5 CFU/ml) on with five (5) different instruments against three (3) different reagent lots. Each cultured strain was

prepared per the protocol described above in M.1.*a* above. The inclusivity study results and the final organism concentrations tested are shown in Table III below. All GAS strains were correctly detected by the assay (100% detected).

Table III: Group A β-hemolytic *Streptococcus* Inclusivity	
Strain	**Strain ID**
Group A Streptococcal strain 1	ATCC 19615
Group A Streptococcal strain 2	ATCC 700942
Group A Streptococcal strain 3	ATCC 700952
Group A Streptococcal strain 4	CI-52123
Group A Streptococcal strain 5	CI-52120
Group A Streptococcal strain 6	CI-62055
Group A Streptococcal strain 7	CI-52152
Group A Streptococcal strain 8	CI-62092
Group A Streptococcal strain 9	CI-52151
Group A Streptococcal strain 10	ATCC 700482
Group A Streptococcal strain 11	ATCC BAA 1315
Group A Streptococcal strain 12	ATCC 700459
Group A Streptococcal strain 13	ATCC 12203
Group A Streptococcal strain 14	ATCC 700944
Group A Streptococcal strain 15	CI-52154
Group A Streptococcal strain 16	CI-5036
Group A Streptococcal strain 17	CI-5095
Group A Streptococcal strain 18	CI-5017
Group A Streptococcal strain 19	CI-5060
Group A Streptococcal strain 20	CI-5112
Group A Streptococcal strain 21	CI-5008

These study results are acceptable.

f. Analytical specificity:

i. Microbial Cross-reactivity:

A study was performed to evaluate the performance of the Sofia® Strep A+ FIA in the presence of eighty-eight (88) other microorganisms commonly found in throat specimens. Each potentially cross-reacting microorganism was tested in replicates of five (5) or more with clinically relevant levels of viruses (1.0×10^5pfu/ml or the highest achievable concentration) and bacteria (1.0×10^6cfu/mL). All strain combinations were spiked into contrived negative matrix, prepared per the

protocol described above in M.1.*a* above. The strains included in the cross-reactivity study are shown in Table IV below.

Table IV: Strains Included in Cross-Reactivity		
Strain		
Aracanobacterium haemolyticum	*Streptococcus mitis*	Streptococcus Group G Strain #3
Bacteroides fragilis	*Streptococcus oralis*	Streptococcus Group G Strain #4
Bordetella pertussis	*Streptococcus pneumoniae*	Streptococcus Group G Strain #5
Candida albicans	*Streptococcus salivaris*	Adenovirus Type 4*
Corynebacterium diphtheriae	*Streptococcus sanguinis*	Adenovirus Type 5
Enterococcus faecium	Streptococcus Group B Strain #1: *Streptococcus agalactiae*	Adenovirus Type 11
Escherichia coli	Streptococcus Group B Strain #2	Coronavirus 229E
Fusobacterium necrophorum	Streptococcus Group B Strain #3	Coxsackievirus
Haemophilus influenzae	Streptococcus Group B Strain #4	Cytomegalovirus
Haemophilus parahaemolyticus	Streptococcus Group B Strain #5	Echovirus
Klebsielle pneumoniae	Streptococcus Group C Strain #1: *Streptoccus dysgalactiae*	Herpes Simplex Virus 1
Moraxella catarrhalis	Streptococcus Group C Strain #2	Herpes Simplex Virus 2
Neisseria gonorrhoeae	Streptococcus Group C Strain #3	Influenza A/New Jersey/8/76 H1N1
Neisseria lactamica	Streptococcus Group C Strain #4	Influenza A/Victoria/3/75 H3N2
Neisseria meningitidis	Streptococcus Group C Strain #5	Influenza B/Hong Kong/5/72
Neisseria sicca	Streptococcus Group D Strain #1	Influenza B/Panama/45/90
Neisseria subflava	Streptococcus Group D Strain #2	Influenza C/Taylor/1233/47
Proteus vulgaris	Streptococcus Group D Strain #4	Adenovirus Type 3
Serratia marcescens	Streptococcus Group D Strain #5	Coronavirus OC43
Staphylococcus haemolyticus	Streptococcus Group F Strain #1	Epstein Barr Virus*
Staphylococcus intermedius	Streptococcus Group F Strain #2	Adenovirus Type 1
Staphylococcus saprophyticus	Streptococcus Group F Strain #3	Measles (Edmonston)
Streptococcus anginosus	*Streptococcus mutans*	Parainfluenza virus 1
Streptococcus gordonii	*Streptococcus parasanginis*	Parainfluenza virus 2
Corynebacterium pseudodiphtheriticum	Streptococcus Group D Strain #3	Parainfluenza virus 3
Enterococcus faecalis	Streptococcus Group F Strain #4	Parainfluenza virus 4A
Pseudomonas aeruginosa	Streptococcus Group F Strain #5	Rhinovirus Type 2

Staphylococcus aureus	Streptococcus Group G Strain #1: *Streptoccus dysgalactiae*	Rhinovirus Type 15
Staphylococcus epidermidis	Streptococcus Group G Strain #2	Mumps (Enders)

* Adenovirus Type 4 was tested at 4.0 x 10^3 PFU/ml; The Epstein Barr Virus was tested only with genomic copies at 4.5 x 10^7 copies/ml.

None of the eighty-eight (88) organisms cross-reacted at the concentrations tested.

ii. Microbial Interference:

Interference studies were conducted with each of the eighty-eight (88) microorganisms listed above in Table IV. Each organism was tested in replicates of five (5) at clinically relevant levels of viruses (1.0 x 10^5pfu/ml or the highest achievable concentration) and bacteria (1.0 x 10^6cfu/mL) in the presence of 1.6 x LoD Group A Streptococcus ATCC 19615. All strain combinations were spiked into contrived negative matrix, prepared per the protocol described above in M.1.*a* above.

Eleven (11) of the eighty-eight (88) tested microorganisms that might be found in throat specimens interfered with the assay. When interference was observed, follow-up tests were conducted to assess the level of interference at other organism concentration levels. Results are shown in Table V below:

Table V: Interfering Strains

Strains	Interference Frequency*
*Corynebacterium pseudodiphtheriticum***	1/5
*Enterococcus faecalis***	1/5
*Staphylococcus aureus***	1/5
*Streptococcus mutans***	4/15
*Streptococcus parasanginis***	1/5
Streptoccus dysgalactiae (Group C)**	1/25
Streptococcus Group D***	1/25
Streptococcus Group F***	1/25
Adenovirus Type 1**	1/10
Adenovirus Type 3*****	3/10
Epstein Barr Virus**	1/10
Mumps (Enders)****	1/10

* # interference results observed/total # tests

** No interference was observed in follow-up studies conducted at higher concentration (0/20).

*** Interference was observed in follow-up studies conducted at higher concentration for Group D (2/45) and Group F (6/90).

**** No interference was observed in follow-up studies (0/5).

***** Studies conducted at reduced concentration of the interfering organism did not demonstrate interference.

iii. Interfering Substances:

Twenty-five (25) chemical and biological substances were evaluated for potential to cross-react and interfere with the Sofia® Strep A+ FIA, including fresh whole human blood, bovine submaxillary mucin and some snack foods that contain specimen-thickening substances. Each substance was tested in replicates of five (5) or more using 1.6 x LoD Group A Streptococcus ATCC 19615, prepared per the protocol described above in M.1.*a*. Two (2) of the substances tested demonstrated a potential to cause false negative results, as shown in Table VI below:

Table VI: Interfering Substances

Strains	Interference Frequency*
Human Whole Blood 100µl/swab**	1/5
Bovine Submaxillary Mucin, 28.7 mg/ml	1/10

* #false negative results/total # tests at that concentration.

** Concentrations ≤ 75µl whole blood/swab did not interfere. 2/5 results were invalid.

No cross-reactivity was observed with any of the chemical or biological substances. Of the snack foods tested, Nacho Flavor Doritos at 250 mg/ml increased the viscosity of the specimens which reduced the flow and sample volume, leading to invalid results 4/10 times in cross-reactivity studies. No interference was observed at 125 mg/ml. Interferents were noted in the limitations section in labeling.

These study results are acceptable.

g. Assay cut-off:

The Limit of Blank (LoB) is 1948 RFU is the cutoff for this assay, which is the numerical threshold that is used to distinguish negative from positive. The procedural control zone cutoff for a valid versus invalid result is 30,000 RFU.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable.

b. Matrix comparison:

The analytical performance studies above were conducted in a contrived negative matrix that included bovine serum albumin and sodium azide with protocol that included a freeze-thaw step. In order to demonstrate that the results of analytical studies conducted in the contrived negative matrix are comparable to studies

conducted in the presence of a clinical matrix, a study was conducted to compare the following two conditions:

1) fresh bacteria spiked into a negative clinical matrix (pharyngeal swabs from patients)
2) spiked specimens prepared using a contrived negative the sample preparation protocol provided in the submission, which includes a freeze-thaw step

This study was conducted by testing twenty replicates each of four (4) different concentrations of *Streptococcus pyogenes* (Table VII) for each of the three (3) strains used to determine the LoD. All testing was conducted in Walk-Away Mode.

Table VII: Matrix Comparison Study

Panel ID		Pooled Negative Clinical Matrix		Contrived Negative Matrix	
		Detected	% Pos	Detected	% Pos
Group A *Streptococcus* (ATCC 19615)	2 x LoD	20/20	100.0%	20/20	100.0%
	1 x LoD	20/20	100.0%	20/20	100.0%
	0.5 x LoD	9/20	45.0%	15/20	75.0%
	0.25 x LoD	0/20	0.0%	4/20	20.0%
Group A *Streptococcus* (ATCC 700942)	2 x LoD	20/20	100.0%	20/20	100.0%
	1 x LoD	19/20	95.0%	19/20	95.0%
	0.5 x LoD	7/20	35.0%	4/20	20.0%
	0.25 x LoD	0/20	0.0%	0/20	0.0%
Group A *Streptococcus* (ATCC 700952)	2 x LoD	20/20	100.0%	20/20	100.0%
	1 x LoD	17/20	85.0%	20/20	100.0%
	0.5 x LoD	7/20	35.0%	7/20	35.0%
	0.25 x LoD	0/20	0.0%	0/20	0.0%

These studies demonstrate that the contrived negative matrix and freeze-thaw step does not alter the performance of the device in comparison to studies conducted in a clinical matrix. These study results are acceptable.

3. Clinical studies:

a. Clinical Sensitivity:

Performance characteristics of the Sofia® Strep A+ FIA were established during a prospective study conducted from April to May 2014. Eight hundred and sixty (860) fresh, throat swab specimens from female and male patients were prospectively collected and transported to each laboratory for testing with the Sofia® Strep A+ FIA

at seven (7) distinct geographical sites across the United States. All testing was done at laboratories holding a certificate of waiver. Three swab specimens were simultaneously collected were collected using the rayon throat swabs provided with the assay. One (1) was used for clinical testing, the second was used for Sofia® Strep A+ FIA testing and the third was shipped on ice in transport media to a central reference laboratory for culture and discrepant analysis using an FDA-cleared polymerase chain reaction (PCR) assay. All eight hundred and sixty (860) fresh throat specimens were cultured for Group A β-hemolytic Streptococcus (GAS) and tested with the Sofia® Strep A+ FIA. The specimen was considered positive if culture was positive for Group A β-hemolytic *Streptococcus*. Two (2) results were determined to be invalid in these studies. Seven (7) additional results were excluded due to the lack of valid external controls on the same day of running.

Bacterial culture was performed at the central reference laboratory where specimens were plated on GAS selective agar with 5% sheep's blood. Plates were cultured at 37° C with 5% CO_2 and examined for the presence of β-hemolytic colonies at twenty-four (24) hours. If no β-hemolytic colonies were observed, the plates were placed at room temperature and cultured aerobically for another twenty-four (24) hours. All β-hemolytic colonies positive culture plates were tested for catalase and confirmed for the presence of GAS using the FDA-cleared Streptex® Rapid Agglutination Test for identification of Lancefield groups A, B, C, D, F and G streptococci.

The clinical performance of the Sofia® Strep A+ FIA was demonstrated with eight hundred and fifty-one (851) prospectively collected fresh throat specimens at seven sites across the United States. The breakdown of performance for the Sofia® Strep A+ FIA is summarized in Table VIII below:

Table VIII: Clinical Performance Data for the Sofia® Strep A+ FIA vs. Swab Culture for Group A β-hemolytic *Streptococcus*

All Sites			
Sofia® Strep A+ FIA	**Swab Culture**		
	Positive	**Negative**	**Total**
Positive	164	38*	202
Negative	11**	638	649
Total	175	676	851
Sensitivity: 93.7% (164/175) 95% CI (89.1%-96.5%) **Specificity:** 94.4% (638/676) 95% CI (92.4%-95.9%)			
* Of the 38 discordant specimens, 24 of these specimens were positive for GAS when tested with an FDA-cleared molecular device, 14 were negative. ** Of the 11 discordant specimens, 3 were negative when tested with an FDA-cleared molecular device, 8 were positive.			

Site 1			
Sofia® Strep A+ FIA	**Swab Culture**		
	Positive	**Negative**	**Total**
Positive	30	15*	45
Negative	1**	132	133
Total	31	147	178
Sensitivity: 96.8% (30/31) 95% CI (83.1%-99.4%) **Specificity:** 89.8% (132/147) 95% CI (83.9%-93.7%)			
* Of the 15 discordant specimens, 10 of these specimens were positive for GAS when tested with an FDA-cleared molecular device, 5 were negative. ** Of the one discordant specimen, one was positive when tested with an FDA-cleared molecular device.			
Site 2			
Sofia® Strep A+ FIA	**Swab Culture**		
	Positive	**Negative**	**Total**
Positive	4	4*	8
Negative	0	31	31
Total	4	35	39
Sensitivity: 100.0% (4/4) 95% CI (51.0%-100.0%) **Specificity:** 88.6% (31/35) 95% CI (74.1%-95.5%)			
* Of the 4 discordant specimens, 3 of these specimens were positive for GAS when tested with an FDA-cleared molecular device, 1 was negative.			
Site 3			
Sofia® Strep A+ FIA	**Swab Culture**		
	Positive	**Negative**	**Total**
Positive	2	0	2
Negative	1**	29	30
Total	3	29	32
Sensitivity: 66.7% (2/3) 95% CI (20.8%-93.9%) **Specificity:** 100.0% (29/29) 95% CI (88.3%-100.0%)			
** Of the one discordant specimen, one was positive when tested with an FDA-cleared molecular device.			

Site 4			
Sofia® Strep A+ FIA	**Swab Culture**		
	Positive	**Negative**	**Total**
Positive	55	3*	58
Negative	2**	175	177
Total	57	178	235
Sensitivity: 96.5% (55/57) 95% CI (88.1%-99.0%) **Specificity:** 98.3% (175/178) 95% CI (95.2%-99.4%)			
* Of the 3 discordant specimens, 2 of these specimens were positive for GAS when tested with an FDA-cleared molecular device, 1 was negative. ** Of the 2 discordant specimens, 1 was positive when tested with an FDA-cleared molecular device, 1 was negative.			
Site 5			
Sofia® Strep A+ FIA	**Swab Culture**		
	Positive	**Negative**	**Total**
Positive	39	9*	48
Negative	4**	121	125
Total	43	130	173
Sensitivity: 90.7% (39/43) 95% CI (78.4%-96.3%) **Specificity:** 93.1% (121/130) 95% CI (87.4%-96.3%)			
* Of the 9 discordant specimens, 7 of these specimens were positive for GAS when tested with an FDA-cleared molecular device, 2 were negative. ** Of the 4 discordant specimens, 4 were positive when tested with an FDA-cleared molecular device.			
Site 6			
Sofia® Strep A+ FIA	**Swab Culture**		
	Positive	**Negative**	**Total**
Positive	22	4*	26
Negative	2**	62	64
Total	24	66	90
Sensitivity: 91.7% (22/24) 95% CI (74.2%-97.7%) **Specificity:** 93.9% (62/66) 95% CI (84.4%-97.6%)			
* Of the 4 discordant specimens, 2 of these specimens were positive for GAS when tested with an FDA-cleared molecular device, 2 were negative. ** Of the 2 discordant specimens, 1 was negative when tested with an additional FDA-cleared molecular device, 1 was positive.			

Site 7			
Sofia® Strep A+ FIA	**Swab Culture**		
	Positive	**Negative**	**Total**
Positive	12	3*	15
Negative	1**	88	89
Total	13	91	104
Sensitivity: 92.3% (12/13) 95% CI (66.7%-98.6%) **Specificity:** 96.7% (88/91) 95% CI (90.8%-98.9%)			
* Of the 3 discordant specimens, 3 of these specimens were negative for GAS when tested with an FDA-cleared molecular device. ** Of the 1 discordant specimen, 1 was negative when tested with an FDA-cleared molecular device.			

The external quality controls used in these studies were: 1) Positive control consisting of swabs spiked with heat inactivated, Group A Streptococcus cells and 2) Negative control consisting of swabs spiked with Group C Streptococcal antigen. The positive control serves as both a processing and extraction control. Positive and negative external controls were tested each day during the clinical studies. Of 253 Group A Streptococcus positive controls tested, 250 (98.8%) were detected accurately. Of the three (3) incorrect results, two (2) were positive upon retesting and one (1) was not retested. Of the 253 Group C Streptococcus negative controls tested, 253 (98.8%) were detected accurately. Of the three (3) incorrect results, all three (3) were negative upon retesting. Results from clinical specimens were included for evaluation of study performance only if valid positive and negative external control results were attained on the same day.

These study results are acceptable.

b. Clinical specificity:

See table above.

c. Other clinical supportive data (when a. and b. are not applicable):

See section M.1 above for more information about why this study was conducted.

To demonstrate that performance of the Sofia® Strep A+ FIA is not influenced by operating the device in Read-Now Mode under the conditions used for analytical studies, a comparison study was conducted with ten (10) replicates of one (1) strain for each of three (3) concentrations of GAS on five (5) Sofia® analyzers (5 analyzers x 10 replicates = 50 tests/concentration).

Table IX: Read-Now versus Walk Away Mode Comparison Study					
Panel ID		Read-Now Mode		Walk-Away Mode	
		Detected	% Pos	Detected	% Pos
Group A *Streptococcus* (ATCC 19615)	1.6 x LoD	50/50	100.0%	50/50	100.0%
	1 x LoD	49/50	98.0%	48/50	96.0%
	Negative	0/50	0.0%	0/50	0.0%

These results demonstrate that there is no notable difference in performance between Read-Now and Walk-Away Modes in the analytical testing setting.

These study results are acceptable.

4. Clinical cut-off**:**

Not Applicable.

5. Expected values/Reference range:

The overall prevalence of Group A β-hemolytic *Streptococcus* in patients tested during this study was 20.6% (177/858) based on bacterial culture. All clinical specimens collected during this study were collected between April, 2014 and May 2014.

N. Instrument Name:

Sofia®

O. System Descriptions:

1. Modes of Operation:

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes _____ or No ___X____

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes _____ or No ___X____

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ___X____ or No _____

3. Specimen Identification:

   Not applicable.

4. Specimen Sampling and Handling:

   One specimen is used per test. Specimens are not sampled. See section M.1.*c* for information on the studies provided to validate the conditions for specimen handling.

5. Calibration:

   A calibration cassette is provided with the assay. Calibration is to be conducted within two weeks. See section M.1.*c* for more information.

6. Quality Control:

   See section M.1.*c* for information on internal and external controls.

P. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

Q. Conclusion:

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