510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K141757

B. Purpose for Submission:

To obtain a substantial equivalence determination for the Alere i Strep A performed on the Alere i Analyzer for the detection of *Streptococcus pyogenes* (Group A *Streptococcus*).

C. Measurand:

Group A *Streptococcus* DNA

D. Type of Test:

Isothermal DNA amplification assay for the qualitative detection of Group A *Streptococcus* DNA in throat swab specimens.

E. Applicant:

Alere Scarborough, Inc.

F. Proprietary and Established Names:

Alere™ i Strep A

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.2680 Streptococcus spp. Nucleic Acid-Based Assay

   21 CFR 862.2570 Real Time Nucleic Acid Amplification System

2. Classification:

   Class II

3. Product code:

   PGX: Groups A, C and G Beta-Hemolytic *Streptococcus* Nucleic Acid Amplification System

OOI: Real-Time Nucleic Acid Amplification System

4. Panel:

   83: Microbiology

H. Intended Use:

1. Intended use(s):

   Alere i Strep A is a rapid, instrument-based, molecular *in vitro* diagnostic test utilizing isothermal nucleic acid amplification technology for the qualitative detection of *Streptococcus pyogenes*, Group A *Streptococcus* bacterial nucleic acid in throat swab specimens obtained from patients with signs and symptoms of pharyngitis. It is intended to aid in the rapid diagnosis of Group A *Streptococcus* bacterial infections.

   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.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only

   False-negative results may occur in the presence of assay inhibitors when levels of *S. pyogenes* DNA are close to the limit of detection of the assay.

4. Special instrument requirements:

   Alere™ i Instrument

I. Device Description:

Alere i Strep A is a rapid, instrument-based isothermal assay for the qualitative detection of *Streptococcus pyogenes* (Group A *Streptococcus*) DNA in throat swab specimens from patients suspected of pharyngitis. The Alere i Strep A System comprises the Alere i Instrument and the following single-use disposables:

- Sample Receiver
  - Containing Elution Buffer for swab expression
- Transfer Cartridge
  - For transfer of the eluted sample to the Test Base

- Test Base
    - Comprised of two sealed reaction tubes, each containing a lyophilized reagent pellet;
    - One tube is for detection the *S. pyogenes* target, the other for the Internal Control

The reaction tubes in the Test Base contain the reagents required for lysis of *S. pyogenes*, and the subsequent amplification of the target nucleic acid and an Internal Control. Alere i Strep A uses a pair of templates (similar to primers) and a strand displacing DNA polymerase to amplify the *S. pyogenes* target DNA and Internal Control. Detection of the amplified products occurs through hybridization of fluorescently labeled Molecular Beacon probes. Alere i Strep A is performed within the confinement of the Test Base, and no other part of the Alere i Instrument is in contact with the sample during the amplification process, thereby reducing the risk of instrument contamination and sample carry-over between measurements.

To perform the assay, the Sample Receiver and Test Base are inserted into the Alere i Instrument. The sample is added to the Sample Receiver and transferred via the Transfer Cartridge to the Test Base to dissolve the lyophilized reagent pellets and conduct bacterial lysis and target amplification. Heating, mixing and fluorescent detection are performed by the instrument, and results are reported automatically.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Lyra™ Direct Strep Assay

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

   K133883

3. Comparison with predicate:

Similarities		
Item Name	**Alere i Strep A (K141757)**	**Lyra™ Direct Strep Assay (K133883)**
Intended Use	Alere i Strep A is a rapid, instrument-based, molecular *in vitro* diagnostic test utilizing isothermal nucleic acid amplification technology for the qualitative detection of *Streptococcus pyogenes*, Group A Strep bacterial nucleic acid in throat swab specimens obtained from patients with signs and symptoms of pharyngitis. It is intended to aid in the rapid diagnosis of Group	The Lyra Direct Strep Assay is a Real-Time PCR *in vitro* diagnostic test for the qualitative detection and differentiation of Group A β-hemolytic *Streptococcus* (*Streptococcus pyogenes*) and pyogenic Group C and G β-hemolytic *Streptococcus* nucleic acids isolated from throat swab specimens obtained from patients with signs and symptoms of pharyngitis,

Similarities		
Item Name	**Alere i Strep A (K141757)**	**Lyra™ Direct Strep Assay (K133883)**
	A Strep bacterial infections. 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.	such as sore throat. The assay does not differentiate between pyogenic Groups C and G β-hemolytic *Streptococcus*. All negative test results should be confirmed by bacterial culture, because negative results do not preclude Group A, C or G Strep infection and should not be used as the sole basis for treatment. The assay is intended for use in hospital, reference, or state laboratory settings. The device is not intended for point-of-care use.
Regulation	21 CFR 866.2680	Same
Product Code	PGX, OOI	Same
Sample Type	Throat swab	Same
Internal Control	Yes	Same
Assay Method	Nucleic acid amplification and real-time fluorescent detection	Same
Extraction Method	None (crude lysate)	Same
Detection Technique	Fluorescent DNA probe	Same
Assay Result	Qualitative	Same

Differences		
Item Name	**Liat™ Strep A Assay (K141338)**	**Lyra™ Direct Strep Assay (K133883)**
Analyte	Group A *Streptococcus*	Group A, C and G *Streptococcus*
Assay Instrument	Alere i Instrument	Applied BioSystems 7500 Fast Dx Real-Time PCR Instrument
Cell lysis	Enzymatic	Heat
Internal Control	Oligonucleotide	Cellular
Amplification Technology	Isothermal	PCR
Time to result	Approximately 8 minutes	60-70 minutes

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

Not applicable.

L. Test Principle:

Alere i Strep A uses isothermal nucleic acid amplification for qualitative detection of *Streptococcus pyogenes* (Group A *Streptococcus*) DNA from throat swab specimens from patients with signs and symptoms of pharyngitis. Alere i Strep A for use on the Alere i Instrument consists of the following components:

a) Sample Receiver: containing Elution Buffer
b) Transfer Cartridge: for transfer of an aliquot of the eluted sample to the Test Base
c) Test Base: comprised of two sealed reaction tubes, each containing a lyophilized reagent pellet; one tube is for detection of the *S. pyogenes* target (if present), the other tube is for detection of the Internal Control.

The reaction tubes in the Test Base contain the reagents required for enzymatic lysis of *S. pyogenes* bacteria and amplification of the target nucleic acid and Internal Control. Isothermal amplification takes place using specific templates (similar to primers) that target a conserved region of the *S. pyogenes* genome. The same template primers are also used to amplify the oligonucleotide Internal Control. Detection of amplified products occurs through hybridization of fluorescently labeled Molecular Beacons that are specific to the intervening regions between the template primers on the Internal Control and *S. pyogenes* amplicons. Result interpretation is based upon the gradient and amplitude of the fluorescence curves for the *S. pyogenes* and Internal Control targets in their respective optical channels and comparison to the appropriate threshold values.

To perform the assay, the Sample Receiver and Test Base are inserted into the Alere i Instrument. A throat swab is then expressed in the Elution Buffer. The operator uses the Transfer Cartridge to transfer a portion of the Elution Buffer to the Test Base to dissolve the lyophilized reagent pellets and initiate cell lysis and DNA amplification. Heating, mixing and fluorescent detection are conducted automatically by the instrument. Results are displayed on the instrument screen and archived on-board together with the sample identification number and date and time of testing.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

a. Precision/Reproducibility:

A Reproducibility Study was conducted at three sites, with three operators per site. On each day of testing, each operator ran two replicates of the following: a true negative sample (diluent), a moderate positive sample (~19X LOD), a low positive sample (3X LOD) and high negative sample (<0.05X LOD), for a total of 90 data points per target level. Samples were prepared in phosphate buffered saline (PBS) from quantified stocks

of *S. pyogenes* ATCC 19615. The specific concentrations tested are shown in **Table 1**. To enable use of samples prepared in PBS rather than a clinical matrix, a study was performed to demonstrate that limit of detection of the assay was similar in the presence and absence of throat swab matrix (refer to **Section *2b***, Matrix Comparison).

Table 1. Panel Members included in the Reproducibility Study

Target Level	Multiple of LOD [1]	Genomic Copies/swab	Approximate CFU/swab
Negative	0	0	0
High Negative	<0.05X	32	<5
Low	3X	800	315
Moderate	~19X	5000	1964

[1] LOD = 267 copies of genomic DNA or 105 CFU/swab

Final test results for all three sites are summarized in **Table 2**. Over the course of the Reproducibility Study, 5 samples produced Invalid results (5/360; 1.4%), all of which were reported as positive upon retesting. At each target level, Alere i Strep A demonstrated acceptable reproducibility.

Table 2. Results from the Alere i Strep A Reproducibility Study by site and overall

Target Level	Number (%)							
	Site 1		Site 2		Site 3		Overall	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Negative	0	30 (100)	0	30 (100)	0	30 (100)	0	90 (100)
High Negative	0	30 (100)	4 (13.3)	26 (86.7)	1 (3.3)	29 (96.7)	5 (5.6)	85 (94.4)
Low Positive	27 (90.0)	3 (10.0)	28 [1] (93.3)	2 (6.7)	27 (90.0)	3 (10.0)	82 (91.1)	8 (8.9)
Moderate Positive	30 [2] (100)	0	30 (100)	0	30 [2] (100)	0	90 (100)	0

[1] 3 samples each gave an Invalid result on initial testing but resolved as Positive upon repeat testing
[2] 1 sample gave an Invalid result on initial testing but resolved as Positive upon repeat testing

b. Linearity/assay reportable range:

Not applicable.

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

Internal Control
Alere i Strep A contains an oligonucleotide Internal Control that is designed to monitor for inhibition of amplification and reagent function. The Internal Control is lyophilized in one of the two reaction tubes in the Test Base and is amplified and

detected separately from the *S. pyogenes* target (if present) utilizing a different optical channel of the Alere i Instrument. The Internal Control must be amplified and detected in order to report a negative assay result. For *S. pyogenes* positive samples in which target amplification occurs, the amplification of the Internal Control is ignored. If *S. pyogenes* DNA is not detected and the Internal Control fails to amplify the test result is reported as "Invalid".

Positive and Negative Control Swabs
The Alere i Strep A kit contains swabs for use as Positive and Negative Controls that must be tested once with each new shipment of reagents and once by each new operator. Additional controls may be tested as necessary to comply with Quality Control requirements. The Positive Control Swab is coated with inactivated Group A *Streptococcus* (3×10^6 organisms/swab). The Negative Control Swab is coated with inactivated Group C *Streptococcus* (1×10^7 organisms/swab). The high level of the Positive Control Swab is a potential source of contamination. This is noted in the device labeling.

Evaluation of Control Performance
The ability of the Alere i Strep A controls to monitor the performance of the assay was verified under various simulated failure modes (**Table 3**).

Table 3. Evaluation of Alere i Strep A control performance

Control(s) Tested	Failure Mode	Test Condition	Results
Internal Control Positive Control Negative Control	Substantial reagent failure	Removal of critical component of amplification mixture	All conditions tested produced "Invalid" results, demonstrating that the controls failed appropriately
Internal Control	Inhibition of amplification	Addition of known assay inhibitors	False-negative results were obtained in the presence of inhibitors and levels of *S. pyogenes* DNA close to the limit of detection of the assay. This is noted as the following limitation in the Package Insert: "False-negative results may occur in the presence of assay inhibitors when levels of *S. pyogenes* DNA are close to the limit of detection of the assay."
Negative Control	Contamination	Addition of low levels of *S. pyogenes* DNA	For each sample, the result was reported as positive "Positive", indicating that the control failed appropriately.

During the prospective Clinical Study (refer to **Section 3**), Positive and Negative Control Swabs were run on each Alere i instrument on each day that clinical samples were tested. The expected results were obtained from 158/163 (96.9%) Positive Control Swabs and 157/163 (96.3%) Negative Control Swabs.

Reagent Stability
Alere i Strep A kits should be stored at 2-30°C until the expiration date shown on the outer packaging.

Sample Stability:
During the Clinical Study that was conducted to validate the performance of Alere i Strep A, all the throat samples were collected using the BD BBL CultureSwab EZ II device and were tested within 1 day of collection. Those samples not tested within 2 hours of collection were held at 2-8°C.

Additional sample stability was demonstrated analytically using alternative types of collection swab that were seeded with clinical matrix either with or without *S. pyogenes*, and stored for different durations prior to testing. Three *S. pyogenes* positive (~3X LOD) and 3 *S. pyogenes* negative samples were tested at each time point. All samples produced the expected results. The sample storage conditions that were validated for each swab type are summarized in **Table 4**.

Table 4. Sample storage conditions demonstrated with alternative swab types

Collection & Transport Device	**Maximum Storage Time**	
	2-8°C	**~20 °C**
Puritan Foam Swab (in sheath)	5 days	24 hours
BBL CultureSwab Liquid Stuart	24 hours	24 hours
BBL CultureSwab Liquid Amies	24 hours	24 hours
Eswab Liquid Amies, Flocked Swab	24 hours	24 hours

Note: The Intended Use of Alere i Strep A requires that all negative test results are confirmed by culture. The risk for occurrence of false-negative test results due to inappropriate sample storage is therefore mitigated.

d. Detection limit:

The limit of detection (LOD) for Alere i Strep A, defined as the concentration of *S. pyogenes* that produces positive results approximately 95% of the time, was identified by evaluating different concentrations of *S. pyogenes* in throat swab matrix that was diluted in Elution Buffer. Testing was performed with two strains of *S. pyogenes* as shown in **Table 5**. Target levels were estimated by plate counts and by quantitative PCR to determine genomic equivalents (Morozumi *et al*. *J Clin Microbiol* 2006 44: 1440-1446).

Table 5. Limits of detection for Alere i Strep A

Strain of *S. pyogenes*	**Per mL of Elution Buffer**	
	CFU	**Genomic copies**
ATCC 12344	4.2	924
ATCC 19615	42	2672

Thirteen additional strains of *S. pyogenes* were diluted in phosphate buffered saline and tested at target levels close to the LOD of the assay. The lowest dilution of each strain at which each of 3 assay replicates produced a positive result is shown in **Table 6**.

Because the LOD and Analytical Reactivity Studies were not performed in naturally occurring throat swab matrix, additional testing was conducted to demonstrate that limit of detection of the assay is similar in the presence and absence of throat swab matrix (refer to **Section *2b***, Matrix Comparison). The results showed that the analytical sensitivity of the assay is not affected by the presence of throat swab matrix and therefore that the data from the LOD and Analytical Reactivity Studies can be considered representative of the performance observed in clinical matrix.

Table 6. Reactivity titers of different strains of *S. pyogenes*

Strain	Per mL Elution Buffer	
	CFU	Genomic copies
ATCC 8135	21	504
ATCC 12384	39	2276
ATCC 12202	9	389
ATCC 12203	6	267
ATCC 12204	32	1124
ATCC 12365	55	1112
ATCC 14289	2	234
ATCC 49399	126	5840
ATCC 51339	37	1296
ATCC 700294	31	548
ATCC 12357	3	384
ATCC 12385 Loomis	5	296
ATCC 12385 Type 4	8	672

Bioinformatic analysis:
To determine the level of sequence conservation within the targeted region, the NCBI Nucleotide Database (http://www.ncbi.nlm.nih.gov/nuccore) and Genome Database (http://www.ncbi.nlm.nih.gov/genome?db=genome) were searched by organism and gene name to collect all relevant *S. pyogenes* sequences available in the public domain. BLAST analysis was also performed with the Alere i Strep A amplicon sequence against the NCBI nucleotide database using MEGABLAST with the "*S. pyogenes* (taxid: 1314)" option selected to restrict the results of the search to *S. pyogenes* sequences. All the sequences identified in this manner exhibited 100% homology to the *S. pyogenes* target sequence, and no other regions of the *S. pyogenes* genome showed a significant level of identity.

e. Analytical specificity:

Cross-Reactivity Study

The analytical specificity of Alere i Strep A was evaluated by testing a variety of commensal and pathogenic species of bacteria and yeast (**Table 7**). Suspensions of each organism were diluted in phosphate buffered saline and tested in triplicate at a final concentration of 10^6-10^9 cells/mL of Elution Buffer. One false-positive result was obtained with *Moraxella catarrhalis* at $>10^8$ cells/mL. *M. catarrhalis* and two other species also produced Invalid results when tested at $>10^6$ cells/mL. For each of these species, negative results were obtained at lower organism concentrations. The potential for cross-reaction and/or false-positive results with certain species is included as a limitation in the Package Insert.

Table 7. Species tested for cross-reaction in Alere i Strep A

Arcanobacterium haemolyticum [1]	*Pseudomonas aeruginosa*
Bacillus cereus	*Staphylococcus aureus*
Bordetella pertussis	*Staphylococcus epidermidis*
Burkholderia cepacia	*Streptococcus agalactiae*
Camplyobacter rectus	*Streptococcus anginosus*
Candida albicans	*Streptococcus canis*
Corynebacterium diphtheriae	*Streptococcus dysgalactiae subsp. equisimilis*
Enterococcus faecalis	*Streptococcus gallolyticus*
Escherichia coli	*Streptococcus intermedius*
Fusobacterium necrophorum	*Streptococcus mitis*
Haemophilis influenza	*Streptococcus mutans*
Klebsiella pneumonia	*Streptococcus pneumoniae*
Lactobacillus acidophilis	*Streptococcus salivarius*
Moraxella catarrhalis [1, 2]	*Streptococcus sanguinus*
Neisseria gonorrhoeae	*Treponema denticola*
Peptostreptococcus micros	*Veillonella parvula*
Prevotella (Bacteroides) oralis [1]	

[1] Invalid results obtained at $\geq 10^6$ cells/mL of Elution Buffer

[2] False positive result obtained at $>10^8$ cells/mL of Elution Buffer

Bioinformatic Analysis:

MEGABLAST analyses were performed using the NCBI Nucleotide collection (nr/nt) database and with the organism exclude option selected for "*S. pyogenes* taxid: 1314" to restrict the results to non-*S. pyogenes* sequences. No sequences of significant homology were identified. Additional MEGABLAST analyses of the NCBI Nucleotide collection were performed with each of the individual species shown in **Table 8**. None of the searches showed evidence of homology with the Alere i Strep A target sequence that is likely to result in cross-reaction and generation of false-positive results.

Table 8. Organisms and viruses analyzed *in silico* for potential cross-reaction/interference in Alere i Strep A

Candida spp.	Adenovirus Type 1
Enterococcus spp.	Adenovirus Type 7
Klebsiella spp.	Human influenza virus A
Lactococcus lactis	Human influenza virus B
Legionella spp.	Human parainfluenza
Mycoplasma pneumoniae	Human metapneumovirus
Pseudomonas spp.	Respiratory syncytial virus Type B
Saccharomyces cerevisiae	Rhinovirus
Stenotrophomonas maltophilia	

Contamination Study

The risk of contamination between samples run on the same Alere i instrument was assessed by testing an alternating series of *S. pyogenes* Positive and Negative Control Swabs. In total, 30 Positive and 31 Negative Control Swabs were tested. The Positive Control Swabs contained a target level that is equivalent to ~2400X LOD. All the controls produced the expected results and no false positive results were observed, indicating that the risk of contamination between runs is acceptably low.

f. Assay cut-off:

S. pyogenes Group A *Streptococcus* Threshold

The gradient threshold for fluorescence amplification curve of the *S. pyogenes* target was established through analysis of Alere i Strep A results from negative samples as well as samples containing known copy numbers of targets. The threshold for the *S. pyogenes* channel was set at 1.5X the highest value observed among the *S. pyogenes* negative samples. This threshold enabled all samples estimated to contain ≥25 copies of target to be distinguished from negative samples and was verified and validated in subsequent analytical and clinical studies.

Internal Control Threshold

The threshold for the Internal Control was established though evaluation of 134 samples that were presumed to be negative for *S. pyogenes*. These consisted of a mixture of throat swabs and analytical samples that were spiked with known quantities of genomic DNA. The lowest gradient values were observed among samples that were spiked with 2µg genomic DNA. Analysis of approximately 100 throat swabs obtained from *S. pyogene*s negative subjects showed that the majority of clinical throat swabs are likely to contain significantly less than 2µg genomic DNA. The gradient threshold for the Internal Control was therefore set just below the measured peak gradient for the sample that had the lowest gradient value.

g. Assay Interference:

Alere i Strep A was evaluated with 21 endogenous and exogenous substances that may be present in throat swab samples. Each substance was tested in triplicate in the presence and absence of *S. pyogenes* ATCC 19615 at 3X LOD for this strain. No interference was observed with any of the substances when tested at the concentrations listed in **Table 9**. Toothpaste and mucin were both shown to interfere with the assay at high concentration but the effect was alleviated by dilution to lower levels. This is reflected in the Package Insert in a footnote to the list of potential interfering substances that were evaluated.

Table 9. Substances tested for potential interference with Alere i Strep A

Substance	Concentration Tested
Acetaminophen	19.4mg/mL
Acetylsalicylic Acid	12.4mg/mL
ACT® Total Care – Fresh Mint	20% (v/v)
Albuterol	0.5mg/mL
Cepacol® Mouthwash	20% (v/v)
Cepacol® Sore Throat Lozenges – cherry	20% (w/v)
Children's Dimetapp® Cough & Cold (Repeat)	20% (v/v)
Chloraseptic® Max Sore Throat Relief + Coating Action – wild berry	20% (v/v)
Contact Cold & Flu Tablets – Night	20% (w/v)
Crest® Complete Multi-Benefit Whitening + Deep Clean Toothpaste	0.16% (w/v) [1]
Diphenhydramine HCL	2.7mg/mL
Halls Plus® – Honey Lemon	20% (w/v)
Human Saliva	10% (v/v)
Ibuprophen	15.4mg/mL
Listerine® Antiseptic Mouthwash – Original	20% (v/v)
Mucin	0.016% (w/v) [2]
Robitussin® Maximum Strength Nighttime Cough DM	20% (v/v)
Sucrets® Sore Throat & Cough – cherry (Repeat)	20% (w/v)
Tylenol® Cold Multi-Symptom Liquid	20% (v/v)
Whole Blood	5% (v/v)
Zicam® Oral Mist – Artic Mint	20% (v/v)

[1] Crest® Complete Multi-Benefit Whitening + Deep Clean Toothpaste control solution generated Invalid results at 20% and 4% (w/v) and false negative results at 0.8% (w/v).
[2] Mucin generated false negative results at 2%, 0.4%, and 0.08% w/v.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable.

b. Matrix comparison:

Several of the studies that were conducted to establish the performance characteristics of Alere i Strep A were performed in the absence of clinical matrix or in matrix that was diluted in Elution Buffer. Additional testing was therefore conducted to determine whether the data from these studies accurately reflect the performance of Alere i Strep A in the presence of clinical matrix.

Testing was performed to determine the effect of specimen matrix on the analytical sensitivity of Alere i Strep A. *S. pyogenes* ATCC 19615 cells were tested at four different levels around the estimated LOD for this strain in the presence and absence of throat swab matrix (**Table 10**). A similar proportion of positive results was observed at each target level indicating that the presence of throat swab matrix did not have a noticeable affect the analytical sensitivity of the assay. There were also no important differences between the two conditions in the gradient values for the fluorescence curves for either the *S. pyogenes* target or Internal Control. These data therefore substantiate the results obtained in analytical studies that were performed in the absence of throat swab matrix or matrix that was diluted in Elution Buffer.

Table 10. Results from matrix vs no matrix comparison (n = 20 per condition)

	Number (%) Positive			
	2X LOD	**1X LOD**	**0.5X LOD**	**0.25X LOD**
No Matrix	20 (100)	20 (100)	16 (80)	4 (20)
Matrix	20 (100)	20 (100)	15 (75)	3 (15) [1]

LOD: Limit of Detection (267 copies of genomic DNA/swab)
[1] 1 sample gave repeat Invalid results; an additional sample was therefore tested to bring the total number of valid results to 20

3. Clinical studies:

a. Clinical Sensitivity:

The clinical performance of Alere i Strep A was established in a multi-center, prospective clinical study conducted at 8 US trial sites between January and March of 2014 among patients who presented with a sore throat and symptoms congruent with pharyngitis. A total of 505 subjects were enrolled in the study of whom 10 were excluded for the following reasons: failure to meet the eligibility criteria (1), lack of a reference result (5) or protocol violation in sample handling for Alere i Strep A or

reference testing (4). Of the remaining 495 samples, 24 (4.8%) generated an Invalid result on initial testing, of which 10 resolved upon repeat analysis. The final rate of Invalid results was therefore 14/495 (2.8%) and 481 subjects were considered evaluable for the purposes of data analysis, of whom 182 (38%) were male and 299 (62%) were female.

Two throat swabs were collected from each subject using the BD BBL CultureSwab EZ II device. One swab was used for the reference bacterial culture according to standard procedures (inoculation of sheep blood agar followed by identification of beta-hemolytic colonies by latex agglutination). The other swab was tested with Alere i Strep A.

For the reference method, seven of the eight sites shipped the swabs in dry transport tubes to a central laboratory for culture. The remaining site performed the reference culture in a local laboratory. All reference cultures were started within 2 days of specimen collection. The majority (431/481, 90%) of samples were tested with Alere i Strep A on the same day that the specimen was collected and all were tested within 24 hours.

The performance of Alere i Strep A for the detection of *S. pyogenes* relative to bacterial culture is shown in **Tables 11** and **12**. Most of the positive specimens were obtained from three geographically diverse sites (9A, 10A and 33A).

Table 11. Overall clinical performance of Alere i Strep A versus bacterial culture

		Reference Culture		
		Positive	**Negative**	**Total**
Alere i Strep A	**Positive**	141	18 [1]	159
	Negative	6 [1]	316	322
	Total	147	334	481
Sensitivity		141/147 = 95.9% (95% CI: 91.4 – 98.1%)		
Specificity		316/334 = 94.6% (95% CI: 91.6 – 96.6%)		
Positive Predictive Value		141/159 = 88.7% (95% CI: 82.8 – 92.7%)		
Negative Predictive Value		316/322 = 98.1% (95% CI: 96.0 – 99.1%)		

[1] 13/18 Alere i Strep A false-positive samples gave positive results by a laboratory developed PCR method, as did 2/6 Alere i Strep A false-negative samples

Table 12. Alere i Strep A Assay performance stratified by site

Site	Samples (%)	Culture Positive (% Prevalence)	Percent (95% CI)			
			Sensitivity	Specificity	PPV	NPV
008A [1]	3 (0.6)	0 (0)	NA	100 (43.9-100)	NA	100 (96.0-99.1)
009A [2]	196 (40.7)	56 (28.6)	96.4 (87.9-99.0)	97.9 (93.9-99.3)	94.7 (82.8-92.7)	98.6 (96.0-99.1)
010A	189 (39.3)	70 (37.0)	94.3 (86.2-97.8)	91.6 (85.2-95.4)	86.8 (82.8-92.7)	96.5 (96.0-99.1)
017A	14 (2.9)	0 (0)	NA	92.9 (68.5-98.7)	NA	100 (96.0-99.1)
017B	22 (4.6)	5 (22.7)	100 (56.6-100)	88.2 (65.7-96.7)	71.4 (82.8-92.7)	100 (96.0-99.1)
017C	25 (5.2)	3 (12.0)	100 (43.9-100)	95.5 (78.2-99.2)	75.0 (82.8-92.7)	100 (96.0-99.1)
021A [1]	2 (0.4)	0 (0)	NA	100 (34.2-100)	NA	100 (96.0-99.1)
033A	30 (6.2)	13 (43.3)	100 (77.2-100)	94.1 (73.0-99.0)	92.9 (82.8-92.7)	100 (96.0-99.1)
Total	**481 (100)**	**147 (30.6)**	**95.9 (91.4-98.1)**	**94.6 (91.6-96.6)**	**88.7 (82.8-92.7)**	**98.1 (96.0-99.1)**

NA: Not applicable

[1] Enrollment at Sites 008A and 021A was low but the samples were collected according to the study protocol and are therefore included in the analysis

[2] Site 009A performed reference culture at a local laboratory; all other sites sent samples to a central reference laboratory for culture

*b. Clinical specificity**:***

Refer to **Tables 11** and **12** above.

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

Not applicable.

4. Clinical cut-off**:**

Not applicable.

5. Expected values/Reference range:

The clinical study included 481 specimens from 8 U.S. sites that were collected between January and March of 2014. The overall prevalence of *S. pyogenes* as determined by culture was 30.6% (147/481), and as determined by Alere i Strep A it was 33.1% (159/481). The prevalence by age and gender of the subjects as determined by Alere i Strep A is shown in **Table 13**.

Table 13. Prevalence of *S. pyogenes* positive subjects by age and gender

Age/Gender	Total	Alere i Strep A Positive	% Prevalence [1]
0 to 2 years	12	1	8.3
3-12 years	265	125	47.2
13-20 years	92	12	13.0
>20 years	112	21	18.8
Male	182	59	32.4
Female	299	100	33.4
Total	**481**	**159**	**33.1**

[1] As determined by Alere i Strep A

N. Instrument Name

Alere i Instrument

O. System Description

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 _____

   The software reviewed under this submission was v3.1.0.2 (release date January 15th, 2014).

0. Specimen Identification:

   Specimens are identified by scanning or typing in the sample identifier.

3. Specimen Sampling and Handling:

   Swab specimens are expressed in the Elution Buffer contained within the Sample Receiver. The operator transfers 2 x 100µL of the expressed specimen to the Test Base using the Transfer Cartridge to rehydrate the reagents for target and Internal Control amplification/detection. Refer to **Section I** above for more information.

4. Calibration:

   End-user calibration for the Alere i Instrument is not required. Calibration of the optical and thermal is performed during the manufacturing process. The instrument is maintenance-free and has no serviceable parts. However, if the instrument is transported or moved, a performance check using Alere i Positive and Negative Controls is recommended by the manufacture to ensure proper functionality.

5. Quality Control:

   Refer to **Section M.1.*c*** for details regarding the performance of the Positive and Negative Control Swabs and Internal Control.

P. Proposed Labeling:

The labeling is sufficient and 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.