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

A. 510(k) Number:

K172126

B. Purpose for Submission:

To obtain a Substantial Equivalence Determination for the Xpress Strep A Assay

C. Measurand:

Conserved regions of the *Streptococcus pyogenes* bacterial genome

D. Type of Test:

Qualitative real-time Polymerase Chain Reaction (PCR)

E. Applicant:

Cepheid

F. Proprietary and Established Names:

Xpert Xpress Strep A

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.2680: *Streptococcus* spp. nucleic acid-based assay

2. Classification:

   Class II

3. Product code:

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

   OOI: Instrumentation for clinical multiplex test systems

4. Panel:

   83-Microbiology

H. Intended Use:

1. Intended use(s):

   The Xpert Xpress Strep A Assay, performed on the GeneXpert Instrument Systems, is a rapid, qualitative *in vitro* diagnostic test for the detection of *Streptococcus pyogenes* (Group A β-hemolytic *Streptococcus*, Strep A) in throat swab specimens from patients with signs and symptoms of pharyngitis.

   The Xpert Xpress Strep A Assay utilizes an automated real-time polymerase chain reaction (PCR) to detect *Streptococcus pyogenes* DNA.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only.

   Additional follow-up testing by culture is required if the Xpert Xpress Strep A assay result is negative and clinical symptoms persist, or in the event of an outbreak of acute rheumatic fever (ARF).

4. Special instrument requirements:

   The Xpert MRSA NxG assay is for use on the GeneXpert Dx, GeneXpert Infinity-48s and GeneXpert Infinity-80 instrument systems.

I. Device Description:

The Xpert Xpress Strep A Assay is an automated real-time polymerase chain reaction (PCR) *in vitro* diagnostic test for qualitative detection of *Streptococcus pyogenes* directly from throat swab specimens from patients with signs and symptoms of pharyngitis.

The Xpert Xpress Strep A Assay is performed on the Cepheid GeneXpert Instrument Systems (GeneXpert Dx, GeneXpert Infinity-48s, and GeneXpert Infinity-80 systems), that automate sample preparation, DNA amplification and real-time detection in single-use, disposable cartridges.

The Xpert Xpress Strep A Assay cartridge contains a pair of PCR primers and a hydrolysis probe that enable detection of a conserved DNA sequence within the *S. pyogenes* genome.

The assay also incorporates a Sample Processing Control (SPC) and a Probe Check Control (PCC) to monitor the integrity of the reagents and process workflow.

The GeneXpert Instrument Systems have between 1 and 80 randomly accessible modules, depending upon the instrument, that are each capable of performing separate sample preparation and real-time PCR assays. Each module contains a syringe drive for dispensing fluids, an ultrasonic horn for lysing cells, and a thermocycler unit for real-time PCR amplification and detection.

Once the instrument is loaded and the test initiated, all the steps associated with sample processing, PCR amplification/detection and result interpretation occur automatically. The final result report can be viewed on-screen and/or printed.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Liat Strep A Assay

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

   K141338

3. Comparison with predicate:

Similarities		
Item	**Device (K172126)**	**Predicate (K141338)**
	Xpert Xpress Strep A Assay	**Liat Strep A Assay**
Regulation	21 CFR 866.2680	Same
Product Code	PGX	Same
Device Class	Class II	Same
Intended Use	The Xpert Xpress Strep A Assay, performed on the GeneXpert Instrument Systems, is a rapid, qualitative *in vitro* diagnostic test for the detection of *Streptococcus pyogenes* (Group A β-hemolytic *Streptococcus*, Strep A) in throat swab specimens from patients with signs and symptoms of pharyngitis. The Xpert Xpress Strep A Assay utilizes an automated real-time polymerase chain reaction (PCR) to detect *Streptococcus pyogenes* DNA.	The Liat Strep A Assay, performed on the Liat Analyzer, is a qualitative *in vitro* diagnostic test for the detection of *Streptococcus pyogenes* (Group A β-hemolytic *Streptococcus*, Strep A) in throat swab specimens from patients with signs and symptoms of pharyngitis. The Liat Strep A Assay utilizes nucleic acid purification and polymerase chain reaction (PCR) technology to detect *Streptococcus pyogenes* by targeting a segment of the *Streptococcus pyogenes* genome.
Analyte	Group A *Streptococcus*	Same
Measurand	Conserved region of *S. pyogenes* DNA	Same
Specimen Type	Throat swab in liquid Amies medium	Same
Reagent Format	Unitized ready for use	Same
Assay Format	Automated DNA extraction, amplification and detection	Same
Process Control	Cell-based	Same
External Controls	Available	Same
Result Format	Qualitative	Same

Differences		
Item	**Device (K172126)**	**Predicate (K141338)**
	Xpert Xpress Strep A Assay	**Liat Strep A Assay**
Instrument System	GeneXpert Dx, GeneXpert Infinity-48s or GeneXpert Infinity-80 instrument systems	Liat Analyzer
Bacterial Lysis	Mechanical (sonication)	Chaotrope and enzymatic digestion

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

1. Guidance for Industry, FDA Reviewers and Compliance on: Off-the-Shelf Software Use in Medical Devices; September 9, 1999.
2. General Principles of Software Validation; Final Guidance for Industry and FDA Staff; January 11, 2002.
3. Guidance for Industry: Cybersecurity for Networked Medical Devices Containing Off-the-Shelf (OTS) Software; January 14, 2005.
4. Guidance for Industry and FDA Staff: Class II Special Controls Guidance Document - Instrumentation for Clinical Multiplex Test Systems; March 10, 2005.
5. Guidance for Industry and FDA Staff: Guidance for the Content of Pre-market Submissions for Software Contained in Medical Devices; May 11, 2005.
6. Guidance for Industry and FDA Staff: Format for Traditional and Abbreviated 510(k)s; August 12, 2005.
7. Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff: Informed Consent for *In Vitro* Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable; April 25, 2006.
8. Guidance for Industry and FDA Staff: Content of Premarket Submissions for Management of Cybersecurity in Medical Devices; October 2, 2014.
9. CLSI. *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - 3rd Edition.* CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
10. CLSI. *Interference Testing in Clinical Chemistry; Approved Guideline - 2nd Edition.* CLSI document EP07-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
11. CLSI. *Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - 3rd Edition.* CLSI document EP09-A3. Wayne, PA: Clinical and laboratory Standards Institute; 2013.
12. CLSI. *User Verification of Precision and Estimation of Bias; Approved Guideline - 3rd Edition*. CLSI document EP15-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
13. CLSI. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline - 2nd Edition.* CLSI document EP17-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
14. CLSI. *Molecular Diagnostic Methods for Infectious Diseases - 3rd Edition*. CLSI report MM03. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
15. CLSI. *Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline.* CLSI document MM13-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
16. CLSI. *Verification and Validation of Multiplex Nucleic Acid Assays; Approved Guideline.* CLSI document MM17-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
17. ASTM D4169-14, *Standard Practice for Performance Testing of Shipping Containers and Systems*, 2014.
18. Federal Communication Commission Part 15, Subparts A and B.
19. Federal Communication Commission Part 18.

20. BS EN ISO 23640: 2011. *In vitro* diagnostic medical devices – Evaluation of stability of *in vitro* diagnostic reagents.
21. IEC CISPR-11 Industrial, Scientific, and Medical Equipment Radio Disturbance Characteristics - Limits and Methods of Measurement; 2004.
22. IEC CISPR-22 Information technology equipment - Radio Disturbance Characteristics - Limits and Methods of Measurement; 2006.
23. IEC 61010-1 Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1, General Requirements; 2001.
24. EN 61010-1 Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1, General Requirements; 2001.
25. UL 61010-1 Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1, General Requirements; 2004.
26. IEC 61010-2-101 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use – Part 2-101: Particular requirements for *In-Vitro* Diagnostic (IVD) Medical Equipment; 2002.
27. EN 61010-2-101 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use – Part 2-101: Particular requirements for *In-Vitro* Diagnostic (IVD) Medical Equipment; 2002.
28. EN 61326-1 Electrical Equipment for Measurement, Control, and Laboratory Use – EMC Requirements: General Requirements; 2006.
29. EN 61326-2-6 Electrical Equipment for Measurement, Control, and Laboratory Use – EMC Requirements – Part 2-6; 2006.
30. EN 55011 Industrial, Scientific and Medical (ISM) Radio-Frequency Equipment – Electromagnetic Disturbance Characteristics; 2010.
31. CAN/CSA C22.2 No. 61010-1 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use – Part 1: General Requirements; 2004.
32. CAN/CSA C22.2 No. 61010-2-101 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use – Part 2-101: Particular Requirements for *In-Vitro* Diagnostic (IVD) Medical Equipment; 2004.
33. WEEE Directive 2002/96/EC; 2002.
34. EMC (Electromagnetic Compatibility) Directive, 2004/108/EC; 2004.
35. LVD (Low Voltage Directive) 2014/35/EU; 2014.

L. Test Principle:

The Xpert Xpress Strep A Assay is performed on the GeneXpert Instrument Systems (GeneXpert Dx, GeneXpert Infinity-48s, and GeneXpert Infinity-80 systems) that automate nucleic acid extraction, amplification and detection in single-use, disposable cartridges. Each cartridge contains primers and probes for detection of the targeted region of the *S. pyogenes* chromosome (if present) and a Sample Processing Control (SPC). The SPC and a separate Probe Check Control (PCC) are used by the GeneXpert systems to monitor reagent and process integrity.

Throat swab specimens for testing with the Xpert Xpress Strep A Assay are collected using the Copan Liquid Amies Elution Swab (ESwab) Collection and Transport System. Upon receipt in the testing laboratory, the ESwab tube is mixed by shaking and 300µL of the

transport medium is added to an Xpert Xpress Strep A Assay cartridge using a disposable transfer pipette. The operator then initiates the test from the user interface and loads the cartridge into the GeneXpert instrument, after which all process steps are performed automatically.

Once complete, the final result report can be viewed on-screen and/or printed. Results are reported as *Strep A DETECTED, Strep A NOT DETECTED, INVALID* (SPC failure)*, ERROR* (SPC and PCC failure) or *NO RESULT* (insufficient data collected). Instructions for retesting are provided for samples with indeterminate results.

The Xpert Xpress Strep A Assay has an Early Termination Feature whereby results for samples that are strongly positive for *S. pyogenes* are reported prior to completion of the full number of PCR cycles designated in the assay definition file.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   To estimate the components of variance associated with the Xpert Xpress Strep A Assay a Reproducibility Study was conducted at 3 sites, with 2 operators per site. Each operator tested a 3 member panel of samples in duplicate twice per day over 6 days using 3 lots of reagents (3 sites x 2 operators/site x 2 panels/day x 2 replicates/panel member x 6 days = 144 results/panel member). Two sites conducted testing using the GeneXpert Dx and one site used the Infinity-80. Samples were prepared using simulated throat swab matrix in Liquid Amies transport medium and stored at 2-8°C until testing. Separate studies were conducted to demonstrate equivalent assay performance with simulated and natural throat swab matrix (**Section M(2)(b)**). The panel members used in the Reproducibility Study are summarized in **Table 1**.

Table 1. Summary of Reproducibility Study panel members

S. pyogenes **Strain**	**Panel Member**	**Multiple of LoD**	**CFU/mL [1]**
Not applicable	Negative	Not applicable	0
ATCC BAA-946	Low Positive	1X	~10
	Moderate Positive	3X	~30

ATCC: American Type Culture Collection; LoD: Limit of Detection

[1] CFU per mL of ESwab transport medium

When the study was initially performed, there was an unexpectedly high rate of indeterminate results (47/432 = 10.8%), although no false positive or false negative results were observed (i.e., there was 100% agreement at all target levels among samples with valid results). An investigation showed that the high rate of indeterminate results was due to a combination of the duration of storage of the

simulated samples prior to testing, in combination with the specific reagent lots used in the study. Even though the results of the Reproducibility Study were acceptable, the study was repeated using new panel members that were stored for a shorter duration prior to testing, as well as new reagent lots. The results of the repeat study are described below.

Eleven (11) indeterminate results were obtained over the course of the repeat study (ERROR: 5; INVALID: 4 and NO RESULT: 2) for an initial indeterminate rate of 2.5% (11/432). In all cases, the expected results were obtained upon retesting.

After retesting, percent agreement with the expected results for Negative and Moderate Positive target levels was 100% (144/144). For Low Positive samples, the percent agreement was 98.6% (142/144). ANOVA (Analysis Of Variance) was performed to assess the variance components of the Ct values for the *S. pyogenes* and SPC targets (**Table 2**). The total variation for all targets was between 0.9 and 2.3 Ct (2.5-6.6%).

The Xpert Xpress Strep A Assay demonstrated acceptable reproducibility across sites, GeneXpert systems, operators, panel members and reagent lots.

Table 2. Summary of Ct variance components observed in the Reproducibility Study

Sample	Target	N	Ct											
			Mean	Between Site		Between Lot		Between Day		Between Operator		Within Assay		Total	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative	SPC	144	34.7	0.0	0.0	1.9	5.3	0.3	1.0	0.0	0.0	1.3	3.7	2.3	6.6
Low Positive	GAS [1]	142 [2]	37.8	0.2	0.6	0.0	0.0	0.1	0.4	0.1	0.2	1.0	2.7	1.1	2.8
Moderate Positive	GAS [1]	144	36.5	0.0	0.0	0.3	0.8	0.0	0.0	0.1	0.3	0.9	2.3	0.9	2.5

GAS: Group A Streptococcus (*S. pyogenes*); SPC: Sample Processing Control; SD: Standard Deviation; %CV: Percent Coefficient of Variation

[1] *S. pyogenes* strain ATCC BAA-946

[2] Two (2) samples gave negative results for Group A *Streptococcus*

b. *Linearity/assay reportable range:*

Not applicable.

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

Internal Controls
Each Xpert Xpress Strep A Assay includes two types of Internal Control to monitor the performance of the system:

Sample Processing Control (SPC): Designed to monitor bacterial lysis and sample inhibition. Test results are reported as *INVALID* if the SPC fails.

Probe Check Control (PCC): Fluorescence measurement to monitor probe rehydration and integrity, as well as filling of the reaction tube. Test results are reported as *ERROR* if the PCC fails.

The performance of the Internal Controls was verified by testing 29 potential failure modes due to errors in the manufacture of the Xpert Xpress Strep A Assay cartridges or the assay procedure. Under the conditions tested, no false positive results were obtained and all *S. pyogenes* negative samples were reported as either Negative, Error or Invalid. Two false negative results were obtained with *S. pyogenes* positive samples, the remainder of which were reported as Positive, Error or Invalid. The device manufacturer implemented appropriate measures by which to mitigate the risks associated with the failure modes that gave rise to false negative results, including use of a fixed volume pipette for sample transfer and 100% manual inspection during cartridge assembly.

External Controls
The device labeling includes information regarding the availability of commercially prepared External Positive and Negative Controls. These External Controls were run each day During the Clinical Study for the Xpert Xpress Strep A Assay (**Section M(3)(a)**) prior to testing of any clinical specimens. A summary of the results obtained is shown in **Table 3**.

Table 3. Summary of External Control performance in the prospective Clinical Study for the Xpert Xpress Strep A Assay

	External Control	
	Positive	**Negative**
Tested [1]	258	258
Indeterminate (%)	8/258 (3.1) [2]	6/258 (2.3) [3]
Expected Result (%) [4]	246/250 (98.4)	252/252 (100)

[1] Not including repeat tests
[2] ERROR: 3; NO RESULT: 3; INVALID: 1
[3] ERROR: 2; NO RESULT: 4
[4] On initial testing

An additional evaluation of the recommended External Controls was conducted by testing 3 lots of each control material with 3 lots of Xpert Xpress Strep A Assay reagents. All 180 pairs (100%) of the External Positive Negative Controls tested produced the expected results.

Specimen Stability
The stability of throat swab specimens for use with the Xpert Xpress Strep A Assay was determined analytically by testing ESwabs seeded with pooled throat swab matrix in the presence and absence of *S. pyogenes* strain ATCC BAA-946 at approximately 3X LoD (26 CFU/mL). Specimens were placed at different temperatures and tested at pre-specified intervals. Results were analyzed qualitatively and by ANOVA to assess differences in Ct values for the *S. pyogenes* and SPC targets over time. No false negative or false positive results were observed during the course of the study, although a statistically significant increase in Ct values for the SPC was observed with *S. pyogenes* negative specimens after storage for 8 days at 8°C. As a result, the claimed stability of throat swab specimens at 2-8°C was restricted to ≤6 days. The study also demonstrated the stability of specimens at 15-30°C for ≤2 days.

Reagent Stability
The shelf-life of the Xpert Xpress Strep A Assay cassettes was evaluated in a real-time stability study performed on three lots of reagents that were stored under different conditions. The results from the study to-date support assignment of an expiration date 6 months from the day of manufacture for the assay cassettes when stored under the recommended conditions.

Cartridge Hold Time
After loading onto the GeneXpert Infinity System, assay cartridges may have to wait for a GeneXpert module to become available before being processed. A study was therefore conducted to determine the maximum permissible interval between addition of a sample to an Xpert Xpress Strep A Assay cartridge and testing on the GeneXpert Infinity System. The study was performed with *S. pyogenes* positive (3X LoD; 26 CFU/mL) and negative samples prepared with simulated throat swab matrix. The samples were added to Xpert Xpress Strep A Assay cartridges that were held for up to 5 hours under different environmental conditions prior to testing (i.e., ambient temperature/humidity, 25°C/75% relative humidity or 35°C/ambient humidity). The expected results were obtained at each time point and there were no important differences in Ct values under any of the conditions tested. The results of the study support the recommended maximum interval of 4.5 hours from sample addition to the Xpert Xpress Strep A Assay cartridges to processing on the GeneXpert Infinity System, with a maximum of 4 hours on the deck of the instrument.

d. Detection limit:

Determination of the Limit of Detection
The analytical sensitivity of the Xpert Xpress Strep A Assay was evaluated by testing dilutions of two strains of *S. pyogenes* in pooled throat swab matrix. The Limit of

Detection (LoD) was determined for each strain using logistic regression according to the guidance in CLSI Document EP17-A2. Testing was performed using two lots of reagents and six different concentrations of each strain ranging from <2 to 35 CFU/mL of ESwab Transport Medium, in addition to *S. pyogenes* negative samples. The LoD point estimates were confirmed by testing an additional 20 replicates of each strain at the claimed LoD target level. At least 19/20 replicates had to produce positive results in order for the LoD to be considered confirmed. The results of the LoD Study are shown in **Table 4**.

Table 4. Analytical sensitivity of the Xpert Xpress Strep A Assay

ATCC Number	Strain	*emm* Sequence Type [1]	LoD Point Estimate (CFU/mL of ESwab Transport Medium)
BAA-946	GAS 10394	6	9
19615	Bruno (CIP 104226)	80	18

ATCC: American Type Culture Collection
[1] https://www.cdc.gov/streplab/m-proteingene-typing.html

Analytical Reactivity/Inclusivity
A study was conducted to demonstrate the ability of the Xpert Xpress Strep A Assay to detect strains of *S. pyogenes* representing different *emm* sequence types, including representatives of those types most commonly isolated in cases of pharyngitis in the U.S. (**Table 5**). A total of 24 strains were tested at a concentration of 26 CFU/mL in simulated throat swab matrix (**Section M(2)(b)**), corresponding to ~3X LoD, with four replicates per strain. All 26 strains were successfully detected. These results are acceptable.

Table 5. Strains of *S. pyogenes* evaluated in the Analytical Reactivity Study

ATCC Number	Strain	*emm* Sequence Type [1]	ATCC Number	Strain	*emm* Sequence Type [1]
12202	NCTC 8370	1	12204	A25	25
12344	T1	1	8135	T27	27
700294	SF370; M1 GAS	1	12365	C107	38
12383	D58X	3	12370	C94	38
12384	C203	3	700497	CDC-SS-1147	75
12385	J17A4	4	700499	CDC-SS-1149	77
12203	NCTC 8709	6	700949	CDC-SS-1397	89
12352	T11	11	BAA-355	N/A	94
BAA-1065	MGAS 2096	12	BAA-356	N/A	95
BAA-1315	MGAS 9429	12	14289	C203 S	*emm* deficient
12357	J17C	18	49399	QC A62	N/A
10403	T22	22	51339	1805	N/A

ATCC: American Type Culture Collection; N/A: not available
[1] https://www.cdc.gov/streplab/m-proteingene-typing.html

Bioinformatic Analysis
The inclusivity of the Xpert Xpress Strep A Assay primers and probe for the targeted region of the *S. pyogenes* genome was analyzed *in silico* using the Basic Local

Alignment Search Tool (BLAST). The assay region was shown to be well conserved, with limited evidence of polymorphisms that could affect primer or probe hybridization. The potential for false-negative Xpert Xpress Strep A Assay results due to unforeseen sequence variation is noted as a Limitation in the device labeling.

e. *Analytical specificity:*

The analytical specificity of the Xpert Xpress Strep A Assay was evaluated by testing a panel of 63 potentially cross-reactive strains of bacteria, including 15 non-Group A species of *Streptococcus,* one strain of yeast and six viruses in simulated throat swab matrix (**Table 6**).

Each strain was tested in triplicate at a concentration of $\geq 10^6$ CFU/mL for bacteria and yeast, and $\geq 10^5$ $TCID_{50}$ for viruses. In all cases, the expected results were obtained and there was no evidence of the potential for false-positive results due to cross-reaction with non-target organisms. These results are acceptable.

Table 6. Strains of bacteria, yeast and viruses evaluated in the Analytical Specificity Study

Gram Negative	Strain	Gram Positive	Strain
Acinetobacter baumannii	ATCC 19606	*Bacillus cereus*	ATCC 13472
Acinetobacter haemolyticum	ATCC BAA-1784	*Corynebacterium diphtheriae*	CCUG 33629b
Bordetella bronchiseptica	ATCC 4617	*Corynebacterium pseudodiphtheriticum*	ATCC 10700
Bordetella pertussis	ATCC 9797	*Enterococcus faecalis*	ATCC 8750
Burkholderia cepacia	ATCC 25416	*Enterococcus faecium*	ATCC19434
Campylobacter rectus	ATCC 33238	*Lactobacillus acidophilus*	ATCC 314
Escherichia coli	ATCC 8739	*Lactococcus lactis* subsp. *lactis*	ATCC 9596
Fusobacterium necrophorum	ATCC 25288	*Listeria monocytogenes*	ATCC 15313
Haemophilus parahaemolyticus	ATCC 10014	*Peptostreptococcus micros*	ATCC 33270
Klebsiella pneumoniae	ATCC 13883	*Staphylococcus aureus*	ATCC 43300
Legionella jordanis	ATCC 700762	*Staphylococcus epidermidis*	NRS8c
Legionella micdadei	ATCC 33204	*Staphylococcus haemolyticus*	NRS50c
Legionella pneumophila	ATCC 33152	*Streptococcus agalactiae*	ATCC 13813
Moraxella catarrhalis	ATCC 43628	*Streptococcus anginosus*	ATCC 33397
Moraxella catarrhalis	ATCC 23246	*Streptococcus bovis*	ATCC 33317
Moraxella lacunata	ATCC 17967	*Streptococcus canis*	ATCC 43496
Neisseria gonorrhoeae	ATCC 700825	*Streptococcus constellatus*	ATCC 27823
Neisseria lactamica	ATCC 23972	*Streptococcus dysgalactiae*	F578943c
Neisseria meningitidis	ATCC 13102	*Streptococcus equi*	ATCC 9525
Neisseria mucosa	ATCC 25998	*Streptococcus gallolyticus*	ATCC 9809
Neisseria sicca	ATCC 29259	*Streptococcus intermedius*	ATCC 27335
Neisseria subflava	ATCC 49275	*Streptococcus mitis*	ATTC 49456
Prevotella (Bacteroides) oralis	ATCC 33269	*Streptococcus mutans*	ATCC 25175
Proteus mirabilis [1]	ATCC 25933	*Streptococcus oralis*	ATCC 9811

Gram Negative	Strain	Gram Positive	Strain
Proteus vulgaris	ATCC 29905	*Streptococcus pneumoniae*	ATCC 49619
Pseudomonas aeruginosa [2]	ATCC 27853	*Streptococcus salivarius*	ATCC 7073
Pseudomonas fluorescens	ATCC 13525	*Streptococcus sanguinus*	ATCC 10556
Serratia marcescens	ATCC 13880	**Gram Indeterminate**	**Strain**
Stenotrophomonas maltophilia	C5669c	*Bordetella parapertussis*	ATCC 15311
Treponema denticola	ATCC 35405	*Haemophilus influenzae* type A	ATCC 9007
Veillonella parvula	ATCC 10790	*Haemophilus parainfluenzae*	ATCC 9796
Yersinia enterocolitica	ATCC 9610	*Mycoplasma pneumoniae*	ATCC 15293
Viruses	**Strain**	**Yeast**	**Strain**
Adenovirus Type 1	3292010a	*Candida albicans*	ATCC 14053
Adenovirus Type 7	07142010LWa		
Cytomegalovirus AD-169	810008a		
Epstein Barr Virus 4	309068a		
Hepatitis B Virus	NATHBV-0003b		
Herpes Simplex Virus [3]	58167228a		

[1] 2 INVALID results obtained on initial testing; both reported as Negative on repeat
[2] 1 INVALID result obtained on initial testing; reported as Negative on repeat
[3] 1 ERROR on initial testing; reported as Negative on repeat

Bioinformatic Analysis
The specificity of the Xpert Xpress Strep A primers and probes for the targeted sequence was also evaluated through extensive *in silico* BLAST analysis which showed that positive results with species other than *S. pyogenes* are unlikely to occur.

Carry-over Contamination Study
To determine the potential for sample-to-sample contamination using the Xpert Xpress Strep A Assay, testing was performed with alternating "high positive" (>1 x 10^6 CFU/mL of ESwab Transport Medium) and negative samples that were prepared using simulated throat swab matrix. The study was conducted on two GeneXpert Systems (one GX-IV and one GX-XVI) with 20 high positive and 21 negative samples per instrument. No false-positive of false-negative results were obtained on either system and the results were therefore determined to be acceptable.

f. Assay cut-off:

Thresholds and cut-offs for the Xpert Xpress Strep A Assay are embedded within the Assay Definition File that also encodes the instrument settings required to perform the test. The valid Ct ranges for the *S. pyogenes* target and SPC are shown in **Table 7**.

Table 7. Valid Ct ranges for Xpert Xpress Strep A Assay

Target Sequence	Valid Ct Range	
	Minimum	Maximum
S. pyogenes	10.0	43.0
SPC	26.0	43.0

SPC: Sample Processing Control

In addition to fixed algorithm parameters within the software Assay Definition File for the Xpert Xpress Strep A Assay, Lot Specific Parameters (LSP) are also generated for every lot of assay reagents to account for potential variations in production. The LSP are embedded within the barcode of each assay cartridge and are transferred to the GeneXpert instrument system when the barcode is scanned.

g. *Assay interference:*

The potential for interference with the Xpert Xpress Strep A Assay was evaluated with endogenous and exogenous substances that may be present in throat swab specimens, as well as a representative panel of commensal or potentially pathogenic microorganisms. Testing was performed using simulated throat swab matrix (**Section M(2)(b)**) in the presence of *S. pyogenes* ATCC BAA-946 at approximately 3X LoD (26 CFU/mL). *S. pyogenes* negative samples were also included to assess the potential for interference with the SPC. In addition to analysis of qualitative test results, ANOVA was used to assess differences in Ct values for the *S. pyogenes* and SPC targets in relation to appropriate control conditions without any interfering substances or organisms present.

Interfering Substances Study
All *S. pyogenes* positive and negative samples (n = 8 per condition) were correctly identified in the presence of each of the potentially inhibitory substances shown in **Table 8** and there were no statistically significant differences in Ct values between the test and control conditions for either the SPC or *S. pyogenes* targets. Nevertheless, a significant reduction in fluorescence intensity was observed for the *S. pyogenes* target in the presence of antiseptic mouthwash at 6.5% w/v. These results are noted in the device labeling.

Table 8. Potentially interfering substances testing with the Xpert Xpress Strep A Assay

Substance Group	Description/Active Ingredient	Concentration Tested
Saliva	Human Saliva, 100%	6.5% (v/v)
Mucin	Porcine mucin Type III Bound Sialic Acid, 0.5-1.5%	2.5% (w/v)
Blood	Whole Human Blood EDTA anticoagulant	5.0% (v/v)
Antiseptic	Rite Aid Oral Care Tartar Control Plus Antiseptic Mouth Rinse, Ice Mint: Eucalyptol, 0.92% Menthol, 0.042% Methyl Salicylate, 0.060%	6.5% (v/v) [1]

Substance Group	Description/Active Ingredient	Concentration Tested
	Thymol, 0.64%	
Cough Medicine	Wal-Tussin DM Max Cough and Chest Congestion Liquid Berry: Dextromethorphan HBr USP, 10mg Guaifenesin USP, 200mg	5mg/mL [2]
Sugar-containing Cold & Flu Remedies	Vicks NyQuil Severe Cold & Flu Nighttime Relief Berry: Acetaminophen, 650mg Dextromethorphan HBr, 20mg Doxylamine Succinate, 12.5mg Phenylephrine HCl 10mg	6.5% (v/v)
Salt-modifying Remedies	Saline Nasal Spray: Sodium Chloride, 0.65%	6.5% (v/v)
Foods/drinks That Increase Salivary Viscosity	Organic Grade A Whole Milk with Vitamin D3	6.5% (v/v)
pH Modifying Remedies	Orange Juice, 100%	6.5% (v/v)
Antacids	Equate Liquid Maximum Strength Original Classic Antacid/Anti Gas: Aluminum Hydroxide, 400mg Magnesium Hydroxide, 400mg Simethicone, 40mg	6.5% (v/v)

[1] Although all samples were reported appropriately as positive, reduced fluorescent signal was observed for the *S. pyogenes* target in the presence of antiseptic mouthwash at 6.5% v/v

[2] 1 *S. pyogenes* negative specimen produced an ERROR on initial testing; reported as Negative on repeat

Microbial Interference Study

The list of microorganisms tested is shown in **Table 9**. Each of the species was tested at $\geq 10^6$ CFU/mL of ESwab Transport Medium. The Xpert Xpress Strep A Assay correctly identified all the *S. pyogenes* positive and negative specimens in the study (n = 4 per condition) and there were no statistically significant differences in Ct values between the test samples and controls. However, a reduction in fluorescence intensity was observed for both the *S. pyogenes* target and SPC in the presence of a high concentration of *N. lactamica*. The results of the study are noted in the device labeling.

Table 9. Species of commensal organisms tested with the Xpert Xpress Strep A Assay in the Microbial Interference Study

Species	Strain	Species	Strain
Acinetobacter baumannii	ATCC 19606	*Streptococcus constellatus*	ATCC 27823
Candida albicans	ATCC 14053	*Streptococcus dysgalactiae*	F578943
Enterococcus faecalis	ATCC 8750	*Streptococcus equi*	ATCC 9525
Fusobacterium necrophorum	ATCC 25288	*Streptococcus gallolyticus*	ATCC 9809
Haemophilus influenzae type A	ATCC 9007	*Streptococcus intermedius*	ATCC 27335
Lactobacillus acidophilus	ATCC 314	*Streptococcus mitis*	ATCC 49456
Neisseria lactamica [1]	ATCC 23972	*Streptococcus mutans*	ATCC 25175
Peptostreptococcus micros	ATCC 33270	*Streptococcus oralis*	ATCC 9811
Prevotella (Bacteroides) oralis	ATCC 33269	*Streptococcus pneumoniae*	ATCC 49619
Staphylococcus epidermidis	NRS8	*Streptococcus salivarius*	ATCC 7073

Species	Strain	Species	Strain
Streptococcus agalactiae	ATCC 13813	*Streptococcus sanguinis*	ATCC 10556
Streptococcus anguinosus	ATCC 33397	*Treponema denticola*	ATCC 35405
Streptococcus bovis	ATCC 33317	*Veillonella parvula*	ATCC 10790
Streptococcus canis	ATCC 43496		

[1] Although all samples were reported appropriately as positive or negative, reduced fluorescent signal was observed for both the *S. pyogenes* target and SPC in the presence of a high concentration of *N. lactamica*

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable.

b. Matrix comparison:

Comparison of Fresh and Frozen Cell Stocks
Specimens for analytical evaluation of assay performance were prepared using *S. pyogenes* cell stocks that were grown on Tryptic Soy Agar plates for 18-24 hours, suspended in Typtic Soy Broth containing 15% (v/v) glycerol and frozen at -80°C. The concentration of the stocks was determined by performing viable counts. Testing of samples prepared from fresh cell stocks and cell stocks that were frozen at -80°C for 10 days showed negligible difference in qualitative assay performance or Ct values. Use of frozen cells stocks in preparation of samples for analytical testing was therefore deemed acceptable.

Comparison of Performance with Natural and Simulated Matrices
To provide a sufficient quantity of material for testing, a simulated throat matrix was used for the majority of Analytical Studies. To represent a worst case scenario, for each sample, the ESwab Transport Medium was seeded with 150µL of simulated matrix (equal to the absorptive capacity of the swab head). Studies were performed in accordance with the standard assay procedure by transferring 300µL of the simulated matrix to the Xpert Xpress Strep A Assay cartridge.

To assess the suitability of the simulated matrix for use in analytical testing, a comparison study was conducted to confirm that the Xpert Xpress Strep A Assay performed similarly in the presence of natural and simulated throat swab matrix. Testing was performed with *S. pyogenes* ATCC BAA-946 at levels close to the LoD of the assay. The results are summarized in **Table 10** and show that the pre-defined acceptance criteria were met at each target level and therefore that the assay exhibited similar analytical sensitivity when performed in the presence of either natural or simulated matrix. Ct values for the *S. pyogenes* and SPC targets were also similar in both matrices. The results of this study support the use of simulated matrix in the Analytical Studies to characterize the performance of the Xpert Xpress Strep A Assay.

Table 10. Comparison of Xpert Xpress Strep A Assay performance with natural and simulated throat swab matrix

Matrix	Target Level [1]	*N*	Xpert Xpress Strep A Positive (%)		Ct Value			
					S. pyogenes [2]		SPC [3]	
			Expected	Observed	Mean	SD	Mean	SD
Natural	Negative	10	0 (0)	0 (0)	NA	NA	35.1	0.8
	<1X	10	1-9 (10-90)	6 (60)	39.8	1.3	34.9	0.5
	2X	30	≥27 (≥95)	30 (100)	37.9	0.6	NA	NA
	5X	10	10 (100)	10 (100)	36.4	0.5	NA	NA
	10X	5	5 (100)	5 (100)	35.5	0.3	NA	NA
Simulated	Negative	10	0 (0)	0 (0)	NA	NA	34.4	0.4
	<1X	10	1-9 (10-90)	7 (70)	40.1	0.6	34.3	0.3
	2X	30	≥27 (≥95)	30 (100)	38.2	0.9	NA	NA
	5X	10	10 (100)	10 (100)	36.7	0.6	NA	NA
	10X	5	5 (100)	5 (100)	35.8	0.3	NA	NA

NA: Not Applicable; SD: Standard Deviation; SPC: Sample Processing Control

[1] Multiple of Limit of Detection (LoD = 9 CFU/mL ESwab Transport Medium)
Actual concentrations tested (CFU/150µL matrix): <1X: 3; 2X: 17; 5X: 42; 10X: 84

[2] Values shown are for samples that were *S. pyogenes*-positive by the Xpert Xpress Strep A Assay

[3] Values shown are for samples that were *S. pyogenes*-negative by the Xpert Xpress Strep A Assay

3. Clinical studies:

a. Clinical Sensitivity:

The performance of the Xpert Xpress Strep A Assay was evaluated in two multicenter studies that were conducted at a total of nine (9) clinical sites in the U.S. Specimens were collected from subjects who presented with a sore throat and at least one other symptom that was characteristic of pharyngitis (e.g., tonsillar exudate, tender cervical lymphadenopathy or fever). All the specimens were collected using the Copan Liquid Amies Elution Swab (ESwab) Collection and Transport System and were used for both Xpert Xpress Strep A testing and the reference culture method. One study evaluated performance of the Xpert Xpress Strep A Assay using residual, de-identified specimens that were left over from standard of care testing (1st swab), while the second study evaluated specimens that were collected prospectively under informed consent following the collection of swabs for use in standard of care patient management (2nd swab).

Testing with the Xpert Xpress Strep A Assay was conducted at each of the clinical sites. Residual sample after Xpert testing was shipped at 2-8°C to a central laboratory for reference culture (inoculation of 10µL transport medium onto Trypticase Soy Agar with 5% Sheep Blood and incubation at 35°C in 5% CO_2). All reference cultures were inoculated within 48 hours of specimen collection. Isolated colonies

that exhibited β-hemolysis were typed by latex agglutination (Remel Streptex). Culture plates that did not exhibit β-hemolytic colonies after 48 hours were recorded as negative for Group A *Streptococcus*.

Eight hundred and forty-four (844) specimens were initially enrolled in the two studies. Of these, 261 were excluded from the analysis of performance due to failure to comply with the inclusion criteria (19), reference culture procedural error (184), delay in reference culture inoculation (31), delay in shipment (26) or labeling error (1). A total of 583 specimens was therefore included in the analysis. On initial testing, 18/583 specimens (3.1%) produced indeterminate results (Error [9], Invalid [6], No Result [3]). Sixteen of the 18 were retested, of which 12 produced valid results that were included in the analysis of performance for a final indeterminate rate of 6/583 (0.9%) (**Table 11**).

Table 11. Expert Xpress Strep A Assay Clinical Performance vs Reference Culture (1st and 2nd swab data combined)

		Reference Culture		
		Positive	**Negative**	**Total**
Xpert Xpress Strep A	**Positive**	138	26 [1, 2]	**164**
	Negative	0	413	**413**
	Total	**138**	**439**	**577** [3]
Sensitivity		138/138 = 100% (95% CI: 97.3-100%)		
Specificity		413/439 = 94.1% (95% CI: 91.5-95.9%)		
Positive Predictive Value		138/164 = 84.1% (95% CI: 77.8-88.9%)		
Negative Predictive Value		413/413 = 100% (95% CI: 99.1-100%)		

95% CI: Two-sided 95% score confidence interval

[1] 25/26 specimens were tested by an alternative PCR assay with bi-directional sequencing of the amplified products; 21/25 were positive for *S. pyogenes* by this method

[2] 10/26 subjects were positive by a rapid antigen test method used as standard of care

[3] On initial testing, 18/583 specimens (3.1%) produced indeterminate results (Error (9), Invalid (6), No Result (3)); 16/18 were retested, of which 12 produced valid results that were included in the analysis of performance for a final indeterminate rate of 6/583 (0.9%)

The results of the two Clinical Studies were also analyzed separately to determine the performance of the Xpert Xpress Strep A Assay with residual clinical specimens from standard of care testing (1st swab) and specimens that were collected prospectively (2nd swab) (**Table 12**). Although both residual and prospectively collected specimen types yielded 100% sensitivity, higher specificity was observed with the residual specimens (96.4% vs 90.9%).

Table 12. Expert Xpress Strep A Assay Clinical Performance vs Reference Culture

1st swab: Residual Specimens				
		Reference Culture		
		Positive	**Negative**	**Total**
Xpert Xpress Strep A	**Positive**	65	9 [1, 2]	**74**
	Negative	0	244	**244**
	Total	**65**	**253**	**318** [3]
Sensitivity		65/65 = 100% (95% CI: 94.4-100%)		
Specificity		244/253 = 96.4% (95% CI: 93.4-98.1%)		
Positive Predictive Value		65/74 = 87.8% (95% CI: 78.5-93.5%)		
Negative Predictive Value		244/244 = 100% (95% CI: 98.5-100%)		
2nd swab: Prospective Specimens				
		Reference Culture		
		Positive	**Negative**	**Total**
Xpert Xpress Strep A	**Positive**	73	17 [4, 5]	**90**
	Negative	0	169	**169**
	Total	**73**	**186**	**259** [6]
Sensitivity		73/73 = 100% (95% CI: 95.0-100%)		
Specificity		169/186 = 90.9% (95% CI: 85.9-94.2%)		
Positive Predictive Value		73/90 = 81.1% (95% CI: 71.8-87.9%)		
Negative Predictive Value		169/169 = 100% (95% CI: 97.8-100%)		

95% CI: Two-sided 95% score confidence interval

[1] 8/9 specimens were tested by an alternative PCR assay with bi-directional sequencing of the amplified products; 7/8 were positive for *S. pyogenes* by this method

[2] All 9 subjects who had Xpert Xpress Strep A false positive results were also evaluated with a rapid antigen test method used as standard of care (SOC); 3/9 subjects were positive by the SOC test method

[3] On initial testing, 9/321 specimens (2.8%) produced indeterminate results (Error (4), Invalid (4), No Result (1)); 7/9 were retested, of which 6 produced valid results that were included in the analysis of performance for a final indeterminate rate of 3/321 (0.9%)

[4] All 17 specimens were tested by an alternative PCR assay with bi-directional sequencing of the amplified products; 14/17 were positive for *S. pyogenes* by this method

[5] All 17 subjects who had Xpert Xpress Strep A false positive results were also evaluated with an SOC rapid antigen test method; 7/17 subjects produced a positive result by the SOC test method

[6] On initial testing, 9/262 specimens (3.4%) produced indeterminate results (Error (5), Invalid (2), No Result (2)); all 9 were retested, of which 6 produced valid results that were included in the analysis of performance for a final indeterminate rate of 3/262 (1.1%)

The performance of the Xpert Xpress Strep A Assay stratified by clinical site and 1st or 2nd swab (residual or prospectively collected) is shown in **Table 13**. The Xpert Xpress Strep A Assay exhibited low specificity at Sites 5 and 6, although 7/16 subjects at these sites with false positive Xpress Strep A Assay results were positive for *S. pyogenes* by a

standard of care rapid antigen test and 13/16 were positive by PCR/bidirectional sequencing. These results are acceptable.

Table 13. Xpert Xpress Strep A performance stratified by site and 1st or 2nd swab (residual or prospectively collected)

Specimen Source	Site	Culture Positive (%)	Xpert Xpress Strep A (%; 95% Confidence Interval)	
			Sensitivity	Specificity [1, 2]
1st Swab (Residual)	**1**	28/157 (17.8)	28/28 (100; 87.9-100)	125/129 (96.9; 92.3-98.8)
	3	37/161 (23.0)	37/37 (100; 90.6-100)	119/124 (96.0; 90.9-98.3)
	Total	**65/318 (20.4)**	**65/65 (100; 94.4-100)**	**244/253 (96.4 93.4-98.1)**
2nd Swab (Prospective)	**2**	11/37 (29.7)	11/11 (100; 74.1-100)	26/26 (100; 87.1-100)
	4	3/23 (13.0)	3/3 (100; 43.9-100)	19/20 (95.0; 76.4-99.1)
	5	4/17 (23.5)	4/17 (100; 51.0-100)	10/13 (76.9; 49.7-91.8)
	6	38/111 (34.2)	38/38 (100; 90.8-100)	60/73 (82.2; 71.9-89.3)
	7	9/39 (23.1)	9/9 (100; 70.1-100)	30/30 (100; 88.7-100)
	8	6/17 (35.3)	6/17 (100; 61.0-100)	11/11 (100; 74.1-100)
	9	2/15 (13.3)	2/15 (100; 34.2-100)	13/13 (100; 77.2-100)
	Total	**73/259 (28.2)**	**73/73 (100; 95.0-100)**	**169/186 (90.9; 85.9-94.2)**
Overall		**138/577 (23.9)**	**138/138 (100; 97.3-100)**	**413/439 (94.1; 91.5-95.9)**

[1] Standard of care rapid antigen test results on subjects with discordant Xpert Xpress Strep A and reference culture results: **Site 1**: 0/4 positive; **Site 3**: 3/5 positive; **Site 4**: 0/1 positive; **Site 5**: 2/3/ positive; **Site 6**: 5/13 positive

[2] PCR/bidirectional sequencing results on subjects with discordant Xpert Xpress Strep A and reference culture results: **Site 1**: 3/4 positive (1 not tested); **Site 3**: 4/5 positive; **Site 4**: 1/1 positive; **Site 5**: 3/3/ positive; **Site 6**: 10/13 positive

b. Clinical specificity:

Refer to **Section M(3)(a)**, 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 performance of the Xpert Xpress Strep A Assay was evaluated in a Clinical Study conducted at multiple sites in the US (**Section M(3)(a)**). The overall prevalence of *S. pyogenes* (Group A *Streptococcus*) in throat swab specimens was 28.4% as determined by the Xpert assay and 23.9% as determined by culture. The prevalence of Group A *Streptococcus* as determined by the Xpert assay is shown in **Table 14**, stratified by the age and gender of the subjects.

Table 14. Prevalence of *S. pyogenes* positive subjects by age and gender

Age/Gender	Number	Xpert Xpress Strep A Positive	% Prevalence [1]
0 to 1 years	3	0	0
2-5 years	76	28	36.8
6-12 years	189	80	42.3
13-21 years	129	25	19.4
>22-65 years	170	30	17.6
>65 years	10	1	10.0
Male	251	74	29.5
Female	326	90	27.6
Total	**577**	**164**	**28.4**

[1] As determined by the Xpert Xpress Strep A Assay

N. Instrument Name:

GeneXpert Instrument Systems (GeneXpert Dx, GeneXpert Infinity-48s and GeneXpert Infinity-80).

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 ___X___ or No _____

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:

   Barcode scan or manual entry.

4. Specimen Sampling and Handling:

   Throat swab samples are collected using the Copan Liquid Amies Elution Swab (ESwab) and transported to the testing laboratory. The test operator briefly shakes the ESwab tube containing the swab to elute the sample and adds 300µL of the transport medium to an Xpert Xpress Strep A Assay cartridge using a transfer pipette. The operator then loads the cartridge into the GeneXpert System and initiates the run from the user interface.

5. Calibration:

   No calibration by the user is required.

6. Quality Control:

   Quality control is addressed for each separately cleared assay for use on the GeneXpert systems. Refer to **Section M(1)(c)** for information on internal and external controls.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable.

Q. Proposed Labeling:

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

R. Conclusion:

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