DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

IQUUM INC
LINGJUN CHEN
COO
700 NICKERSON ROAD
MARLBOROUGH MA 01762-4663

November 4, 2014

Re: K141338
Trade/Device Name: Liat Strep A Assay
Regulation Number: 21 CFR 866.2680
Regulation Name: Streptococcus spp. nucleic acid based assay
Regulatory Class: II
Product Code: PGX
Dated: October 14, 2014
Received: October 15, 2014

Dear Dr. Chen:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Uwe Scherf -S for

Sally Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K141338

Device Name
Liat™ Strep A Assay

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(K) SUMMARY

Summary Date: October 27, 2014

510(k) Number: K141338

Purpose for Submission:

The purpose of this submission is the evaluation of the Liat™ Strep A Assay performed on the Liat™ Analyzer for the detection of *Streptococcus pyogenes*.

Measurand:

The Liat™ Strep A Assay is a rapid, automated *in vitro* diagnostic test for qualitative detection of *S. pyogenes* (Group A *Streptococcus*) from throat swab specimens.

Type of Test:

Nucleic acid assay for qualitative detection of Strep A from throat swab specimens including nucleic acid isolation and real-time PCR amplification using the Liat™ Analyzer.

Applicant:

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700 Nickerson Road
Marlborough, MA 01752
Tel: 508-970-0099
Fax: 508-970-0119

Contact: Lingjun Chen
Title: Vice President, POC Operational Development
Tel: 508-970-0099 ext. 116
Email: lingjun@iquum.com

Proprietary and Established Names:

Liat™ Strep A Assay

Regulatory Information:

Regulation section:

21 CFR 866.2690, *Streptococcus* spp. nucleic acid based assay

Classification:

Class II

Product code:

PGX

Panel:

Microbiology (83)

Intended Use:

Intended use(s):

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*) 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.

Indication(s) for use:

Same as Intended Use

Special conditions for use statement(s):

For prescription use only

Special instrument requirements:

Requires the Liat™ Analyzer

Device Description:

The Liat™ Strep A Assay, performed on the Liat™ Analyzer, is a rapid, automated *in vitro* diagnostic test for the qualitative detection of *Streptococcus pyogenes* (Group A β-hemolytic *Streptococcus,* Strep A) DNA in throat swab specimens in Amies medium.

The Liat™ Strep A Assay targets a well-conserved region of Strep A genome. An Internal Process Control (IPC) is also included. The IPC is present to control for adequate processing of the target bacteria through all steps of the assay process and to monitor the presence of inhibitors in the sample preparation and PCR. The sample-to-result time is ~15 minutes.

The assay utilizes a single-use disposable Liat™ Tube that holds the sample purification and PCR reagents, and hosts the sample preparation and PCR processes. The Liat™ Tube contains all required unit dose reagents pre-packed in tube segments, separated by peelable seals, in the order of reagent use.

The Liat™ Analyzer automates and integrates sample purification, nucleic acid amplification, and detection of the target sequence in biological samples. The Liat™ Analyzer performs all assay steps from clinical sample and reports assay result automatically. During the testing process, multiple sample processing actuators of the analyzer compress the Liat™ Tube to selectively release reagents from tube segments, move the sample from one segment to another, and control reaction volume, temperature, and time to conduct sample preparation, nucleic acid extraction, target enrichment, inhibitor removal, nucleic acid elution and real-time PCR. An

embedded microprocessor controls and coordinates the actions of these sample processors to perform all required assay processes within the closed Liat™ Tube.

Positive and negative controls are provided in the Liat™ Strep A Assay Quality Control Kit. The positive control comprises inactivated Strep A bacteria in a dried format. The negative control comprises Amies medium.

To perform the Liat™ Strep A Assay, an operator first collects a throat swab and places the swab into Amies transport medium. The operator transfers the sample into the Liat™ Strep A Assay tube using a transfer pipette, and scans the tube barcode to identify the test and the sample barcode to code the sample ID with the assay run on the Liat™ Analyzer. The Liat™ Tube is then inserted into the Liat™ Analyzer. The analyzer performs all the test steps and outputs interpreted results (e.g. Strep A Detected, Strep A Not Detected) in ~15 minutes. A report of the interpreted results can be viewed on the Liat™ Analyzer's LCD screen, and printed directly through a USB or network connected printer. No reagent preparation or additional steps are required other than adding the sample to the Liat™ Tube. Because all the reagents are contained within the Liat™ assay tube and no sample or reagent needs to be removed from the tube, cross-contamination between samples is minimized.

The results are interpreted by the Liat™ Analyzer software from measured fluorescent signals and real time curve recognition algorithm. All possible final test results are described below.

Interpretation of Results from the Liat™ Analyzer

	Report Results	Strep A		IPC		Interpretation
		PCR Result	Curve Pattern	PCR Result	Curve Pattern	
1	Strep A Not Detected	–		+	OK	Negative test for Strep A (no Strep A DNA detected)
2	Strep A Detected	+	OK	±		Positive test for Strep A (Strep A DNA present)
3	Strep A Indeterminate. Repeat Assay.	+	Abn	±		Presence or absence of Strep A cannot be determined. Repeat assay with same sample or, if possible, new sample.
4	Assay Invalid. Repeat Assay	–		+	Abn	
		–		–		
5	Assay Aborted	[N/A]	[N/A]	[N/A]	[N/A]	Presence or absence of Strep A cannot be determined. Repeat assay with same sample or, if possible, new sample.

Note: Abn = Abnormal

If the test result is "Indeterminate" or "Invalid", repeat the assay with the same patient specimen, or if possible, collect a new specimen from the patient and repeat the assay using the new

specimen. Specimens that have repeat "Indeterminate" or "Invalid" results should be sent to a laboratory for confirmatory testing.

If an assay is aborted due to run error, or if an assay is aborted by user, repeat the test with the same sample or, if possible, a new sample. Contact IQuum Technical Support if repeat "Errors" are reported.

Substantial Equivalence Information:

Predicate device name(s):

Quidel Lyra™ Direct Strep Test

Predicate 510(k) number(s):

K133883

Comparison with predicate:

Similarities		
Item Name	**Liat™ Strep A**	**Lyra™ Direct Strep**
Intended Use	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*) 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.	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, 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 866.2690	(same)
Product Code	PGX	(same)
Assay Target	*Streptococcus* A	*Streptococcus* A, C/G

Similarities		
Item Name	**Liat™ Strep A**	**Lyra™ Direct Strep**
Sample Type	Throat swab	(same)
Internal Control	Yes	Yes
Strep A Target	Conserved sequence within the genome of *S. pyogenes*	Conserved regions within the genomes of group A streptococci and group C/G streptococci.
Assay Method	PCR for detecting the presence / absence of bacterial DNA in clinical specimens	(same)
Detection Technique	Different reporter dyes for target and Internal Control	(same)
Assay Result	Qualitative	(same)

Differences		
Item Name	**Liat™ Strep A**	**Lyra™ Direct Strep**
Extraction Method	Automated silica-magnetic bead-based nucleic acid extraction and purification	Manual heat lysis
Equipment Required	Liat™ Analyzer	• ABI 7500 Fast Thermocycler • Plate centrifuge for 96 well plate • Heat block • Thermometer • Timer • Micropipette
Automation	Yes: integrated computer controlled sample processing and PCR amplification/detection	No: manual sample processing and PCR set-up
Reagents / Kit Components	• Unitized Liat™ Strep A Assay Tube • Transfer pipette	• Unitized Process Buffer for heat lysis • Bulk PCR Master Mix • Bulk Rehydration Solution for Master Mix
Reagent Format	• Unitized ready for use • Manual reagent manipulation NOT required	• Bulk reagents • Manual pipetting required
Result Interpretation	Automated	Manual
Time-to-result	~15 minutes	~70 minutes

Test Principle:

The Liat™ Strep A Assay uses an established nucleic acid test chemistry and assay protocol for bacterial DNA detection. The sample preparation methodology is based on chaotropic agent-based lysis and silica magnetic bead-based nucleic acid purification. First, the throat swab sample in Amies medium is mixed with an internal process control (IPC) comprising a chemically-inactivated bacterium. Chaotropic and proteolytic reagent then disrupts the three dimensional structure in macromolecules such as proteins and nucleic acids in the sample, and denatures them. Second, nucleic acids are isolated from the lysate through binding to the surface of silica magnetic beads in the presence of a chaotropic salt, which removes water from hydrated molecules in solution. Third, the beads are separated from the lysate using a magnetic field, and the lysate is removed. Fourth, the beads with captured nucleic acids are washed to remove possible inhibitors in the sample. Finally, the captured nucleic acids are then eluted under low-salt conditions into a small volume of elution buffer.

Target amplification and detection uses TaqMan probe-based real-time PCR. The Strep A primer and probe set is designed for the detection of a conserved sequence within the genomes of Strep A bacteria. An IPC primer and probe set is also included to amplify the target region of the IPC bacterium.

Eluted bacterial DNA undergoes PCR where the reaction mixture is repeatedly heated to denature the nucleic acid and cooled to allow annealing of primers and extension of annealed primers by DNA polymerase to logarithmically amplify a specific region of the DNA. Dual-labeled fluorogenic hydrolysis (TaqMan) probes anneal to specific target sequences located between the binding regions of forward and reverse primers. During the extension phase of the PCR cycle, the 5′ nuclease activity of polymerase degrades the probes, causing the reporter dyes to separate from the quenchers, thus generating fluorescent signals. Fluorescence intensities are monitored at each PCR cycle.

The Liat™ Analyzer automatically interprets the results from measured fluorescent signals. Embedded calculation algorithms determine the PCR cycle threshold (Ct) and evaluate the Ct and fluorescence endpoint against the valid range to generate a positive or negative PCR result. Additionally, pattern recognition algorithms inspect the PCR curves to determine if the curve pattern is within specification or abnormal.

All these sample preparation, real-time PCR amplification and detection, and result interpretation processes are conducted in a closed Liat™ Tube in ~15 minutes.

Performance Characteristics:

Analytical performance:

Precision/Reproducibility:

A Reproducibility Study was performed to assess the total variability of the Liat™ Strep A Assay across operators, study sites, testing days, Liat™ Analyzers, and Liat™ assay tube lots. The Liat™ assay was evaluated at 3 sites. Two operators at each of the 3 sites tested a 4 member reproducibility panel in triplicate on 5 different days, for a total of 360 runs (4 panel members × 3 replicates × 2 operators × 5 days × 3 sites). Nine (9) Liat™ Analyzers and 3 Liat™ Strep A Assay tube lots were used. The reproducibility panel comprised a negative, a high negative (C5: 0.03X LOD), a low positive (C95: 1X LOD) and a medium positive (C100: 3X LOD) Strep A sample. For the negative and high negative samples, the expected result was negative; for the low positive and medium positive samples the expected result was positive.

The tables below show the reproducibility results for Strep A and the Internal Process Control (IPC). Total percent agreement was 99.7% for Strep A and 100% for IPC.

Strep A Reproducibility Results

Site	1					2					3					Total					
Sample	Agreement	Ct		Amp		Agreement	Ct		Amp		Agreement	Ct		Amp		Agreement	Ct		Amp		95% CI
		Avg	%CV	Avg	%CV		Avg	%CV	Avg	%CV		Avg	%CV	Avg	%CV		Avg	%CV	Avg	%CV	
Neg.	30/30	-	-	-	-	30/30	-	-	-	-	30/30	-	-	-	-	90/90 (100%)	-	-	-	-	95.9% - 100.0%
C5	30/30	-	-	-	-	30/30	-	-	-	-	30/30	-	-	-	-	90/90 (100%)	-	-	-	-	95.9% - 100.0%
C95	29/30	29.4	2%	1.8	29%	30/30	29.8	4%	1.5	44%	30/30	29.2	3%	1.8	32%	89/90 (99%)	29.5	3%	1.7	35%	94.0% - 99.8%
C100	30/30	27.2	2%	3.2	10%	30/30	27.9	2%	2.8	14%	30/30	26.8	2%	3.2	8%	90/90 (100%)	27.3	3%	3.0	12%	95.9% - 100.0%
Total Agree-ment	119 / 120 (99.2%)					120 / 120 (100%)					120 / 120 (100%)					359 / 360 (99.7%)					98.4%-100.0%

Amp = Endpoint fluorescence value

IPC Reproducibility Results

Site	1					2					3					Total					
Sample	Agreement	Ct		Amp		Agreement	Ct		Amp		Agreement	Ct		Amp		Agreement	Ct		Amp		95% CI
		Avg	%CV	Avg	%CV		Avg	%CV	Avg	%CV		Avg	%CV	Avg	%CV		Avg	%CV	Avg	%CV	
Neg.	30/30	29.0	2%	2.9	13%	30/30	29.0	2%	2.9	12%	30/30	29.1	2%	2.8	12%	90/90 (100%)	29.0	2%	2.9	13%	95.9% - 100.0%
C5	30/30	28.8	2%	3.0	13%	30/30	29.1	2%	2.9	15%	30/30	29.1	2%	2.7	16%	90/90 (100%)	29.0	2%	2.9	15%	95.9% - 100.0%
C95	30/30	28.9	2%	3.0	11%	30/30	28.8	2%	2.9	10%	30/30	29.1	1%	2.6	10%	90/90 (100%)	28.9	2%	2.8	12%	95.9% - 100.0%
C100	30/30	28.5	2%	2.7	12%	30/30	28.8	2%	2.7	11%	30/30	28.7	2%	2.2	16%	90/90 (100%)	28.7	2%	2.5	15%	95.9% - 100.0%
Total Agree-ment	120 / 120 (100%)					120 / 120 (100%)					120 / 120 (100%)					360 / 360 (100%)					98.9-100.0%

Amp = Endpoint fluorescence value

Controls:

The Liat Strep A Assay has 3 controls: (1) internal process control, (2) positive control and (3) negative control.

Internal Process Control

The internal process control (IPC) comprises an inactivated bacterium that is pre-packed in each Liat™ tube. When conducting an assay, the IPC is first mixed with sample and then goes through all the test processes to monitor both the sample processing and PCR performance. The IPC DNA is detected in a separate channel by IPC specific primers and probe. If IPC target Ct and fluorescence endpoint are not above a minimum value and Strep A is not detected, the assay run report indicates "Assay Invalid. Repeat test" to avoid false negative results due to excessive sample inhibition or system operation outside the normal range.

Positive Control

The positive control is provided in the Liat™ Strep A Assay QC Kit. The positive control comprises inactivated Strep A bacteria in a dried format. The target level for the positive control is designed to be close to the LOD of the assay.

To use the positive control, an operator transfers the Amies medium contained in the Dilution Amies tube into the positive control tube using a transfer pipette to rehydrate and mix the dried positive control, and then transfers the entire mixture into the Liat™ Tube. The Liat™ Tube is then run on a Liat™ Analyzer according to the Package Insert.

The positive control is required to be run during the "Add Liat™ Tube Lot" process, in which the Liat™ Tube lot and end user site procedures are checked at the end user site. Additional positive control runs may be performed by the end-user to confirm the performance of a Liat™ Analyzer and a Liat™ Tube lot through detection of *S. pyogenes* target DNA, or as required by the end user's quality control standards.

Negative Control

The negative control is provided in the Liat™ Strep A Assay QC Kit. The negative control comprises Amies medium. The solution is provided in unit dose quantity and labeled as Dilution Amies.

To use the negative control, an operator transfers the Amies media directly into the Liat™ Tube using a transfer pipette and runs the assay following the Package Insert.

The negative control is required to be run during the "Add Liat™ Tube Lot" process, in which potential contamination and end user site procedures are checked at the end user site. Additional negative control runs may be performed by the end-user to check if there is contamination resulting in a false positive result, or as required by the end user's quality control standards.

Detection Limit:

The Limit of Detection (LOD) of the Liat™ Strep A Assay was determined by limiting dilution studies using titered bacteria of 4 Strep A strains. The bacteria were spiked into throat swab sample matrix, and then tested using the Liat™ Strep A Assay. The LOD was determined as the lowest bacterial concentration that was detected ≥95% of the time (i.e. at least 19 out of 20 replicates tested positive). The Liat™ assay detected all strains tested, with an LOD in the range of 5 – 20 CFU/mL, or 1 – 4 CFU/test.

Strain	LOD	
	CFU/mL	CFU/test
ATCC BAA-946	5	1
ATCC 12370	10	2
ATCC BAA-1066	10	2
ATCC 700294	20	4

Analytical Specificity (reactivity):

A Reactivity Study was performed to evaluate the ability of the Liat™ Strep A Assay to detect Strep A strains representing temporal and geographical diversity. In addition to those strains tested in LOD study, the Liat™ Strep A Assay was evaluated for reactivity with 5 Strep A strains at 20 – 80 CFU/mL or 4 – 16 CFU/test. The bacteria were spiked into throat swab sample matrix, and then tested using the Liat™ Strep A Assay. The assay detected all strains tested.

Strain	Test Concentration		Strep A Result
	CFU/mL	CFU/test	
ATCC 700497	20	4	+
ATCC 700949	20	4	+
ATCC 700499	40	8	+
ATCC 21548	40	8	+
ATCC 10403	80	16	+

Analytical Specificity (Cross-reactivity):

A Cross-reactivity Study was performed to evaluate the potential of the Liat™ Strep A Assay to cross-react with other microorganisms that may be present in throat swab samples. The Liat™ assay was evaluated against a panel of 72 microorganisms. Bacteria were tested at $\geq 10^6$ CFU/mL. Viruses were tested at $\geq 10^5$ $TCID_{50}$/mL or the highest available concentration. The Liat Strep A Assay showed no cross reactivity with the tested microorganisms.

Microorganism	Test Concentration	Strep A Result
Acinetobacter baumannii	1.25×10^6 CFU/mL	–
Arcanobacterium haemolyticum	4.40×10^6 CFU/mL	–
Bacillus cereus	2.90×10^6 CFU/mL	–
Bacteroides oralis	1.55×10^6 CFU/mL	–
Bordetella bronchiseptica	1.25×10^6 CFU/mL	–
Bordetella parapertussis	1.25×10^6 CFU/mL	–
Bordetella pertussis	1.25×10^6 CFU/mL	–
Burkholderia cepacia	1.25×10^6 CFU/mL	–
Campylobacter rectus	1.45×10^6 CFU/mL	–
Candida albicans	1.25×10^6 CFU/mL	–
Chlamydia pneumoniae	1.40×10^5 $TCID_{50}$/mL	–
Chlamydia trachomatis	1.25×10^6 EB/mL	–
Corynebacterium diphtheriae	1.25×10^6 CFU/mL	–
Corynebacterium pseudodiphtheriticum	1.25×10^6 CFU/mL	–
Enterococcus faecalis	1.25×10^6 CFU/mL	–
Enterococcus faecium	1.25×10^6 CFU/mL	–
Escherichia coli	1.25×10^6 CFU/mL	–
Haemophilus influenzae	1.25×10^6 CFU/mL	–
Haemophilus parahaemolyticus	1.25×10^6 CFU/mL	–
Haemophilus parainfluenzae	1.25×10^6 CFU/mL	–
Klebsiella pneumoniae	1.25×10^6 CFU/mL	–
Lactobacillus acidophilus	1.20×10^6 CFU/mL	–
Lactococcus lactis	1.25×10^6 CFU/mL	–
Legionella jordanis	1.25×10^6 CFU/mL	–
Legionella micdadei	1.25×10^6 CFU/mL	–
Legionella pneumophila	1.25×10^6 CFU/mL	–
Listeria monocytogenes	1.25×10^6 CFU/mL	–
Moraxella catarrhalis (2 strains)	1.25×10^6 CFU/mL	–
Moraxella lacunata	1.25×10^6 CFU/mL	–
Mycoplasma pneumoniae	1.25×10^6 copies/mL†	–
Neisseria gonorrhoeae	1.25×10^6 copies/mL†	–
Neisseria lactamica	1.25×10^6 CFU/mL	–
Neisseria meningitidis	1.25×10^6 CFU/mL	–

Microorganism	Test Concentration	Strep A Result
Neisseria mucosa	1.25×10^6 CFU/mL	–
Neisseria sicca	1.25×10^6 CFU/mL	–
Neisseria subflava	1.25×10^6 CFU/mL	–
Proteus mirabilis	1.25×10^6 CFU/mL	–
Proteus vulgaris	1.25×10^6 CFU/mL	–
Pseudomonas aeruginosa	1.25×10^6 CFU/mL	–
Pseudomonas fluorescens	1.25×10^6 CFU/mL	–
Serratia marcescens	1.25×10^6 CFU/mL	–
Staphylococcus aureus	1.25×10^6 CFU/mL	–
Staphylococcus epidermidis	1.25×10^6 CFU/mL	–
Staphylococcus haemolyticus	1.25×10^6 CFU/mL	–
Stenotrophomonas maltophilia	1.25×10^6 CFU/mL	–
Streptococcus agalactiae	1.25×10^6 CFU/mL	–
Streptococcus anginosus	1.25×10^6 CFU/mL	–
Streptococcus bovis	1.25×10^6 CFU/mL	–
Streptococcus canis	1.25×10^6 CFU/mL	–
Streptococcus constellatus	1.25×10^6 CFU/mL	–
Streptococcus dysgalactiae	1.25×10^6 CFU/mL	–
Streptococcus equi	1.25×10^6 CFU/mL	–
Streptococcus gallolyticus	1.60×10^6 CFU/mL	–
Streptococcus intermedius	1.10×10^6 CFU/mL	–
Streptococcus mitis	1.25×10^6 CFU/mL	–
Streptococcus mutans	1.25×10^6 CFU/mL	–
Streptococcus oralis	1.25×10^6 CFU/mL	–
Streptococcus pneumoniae	1.25×10^6 CFU/mL	–
Streptococcus salivarius	1.25×10^6 CFU/mL	–
Streptococcus sanguis	1.25×10^6 CFU/mL	–
Treponema denticola	1.63×10^6 copies/mL[†]	–
Veillonella parvula	2.13×10^6 CFU/mL	–
Yersinia enterocolitica	1.25×10^6 CFU/mL	–
Adenovirus, Type 1	4.45×10^5 $TCID_{50}$/mL	–
Adenovirus, Type 7	4.45×10^4 $TCID_{50}$/mL	–
Cytomegalovirus	1.00×10^5 $TCID_{50}$/mL	–
Epstein-Barr virus	2.15×10^5 copies/mL	–

Microorganism	Test Concentration	Strep A Result
Hepatitis B virus	5.00×10^5 copies/mL	−
Herpes simplex virus 1	2.80×10^5 $TCID_{50}$/mL	−
Human papilloma virus, Type 11	2.50×10^5 copies/mL	−
Human papilloma virus, Type 6	2.50×10^5 copies/mL	−

† Testing was performed with genomic DNA due to difficulties in propagation of these bacteria.

Interfering Microorganisms:

An Interfering Microorganism Study was conducted to evaluate whether other microorganisms that may be present in throat swab samples can interfere with the detection of Strep A by the Liat™ assay. The 72 microorganisms were tested for potential interference with Strep A detection. Bacteria were tested at $\geq10^6$ CFU/mL, and viruses were tested at $\geq10^5$ $TCID_{50}$/mL, or the highest available concentration, in the presence of a Strep A at concentration of 3x LOD in throat swab matrix. Results show that the presence of the tested microorganisms did not interfere with the detection of Strep A.

Microorganism	Test Concentration	Strep A Result
Acinetobacter baumannii	1.25×10^6 CFU/mL	+
Arcanobacterium haemolyticum	4.40×10^6 CFU/mL	+
Bacillus cereus	2.90×10^6 CFU/mL	+
Bacteroides oralis	1.55×10^6 CFU/mL	+
Bordetella bronchiseptica	1.25×10^6 CFU/mL	+
Bordetella parapertussis	1.25×10^6 CFU/mL	+
Bordetella pertussis	1.25×10^6 CFU/mL	+
Burkholderia cepacia	1.25×10^6 CFU/mL	+
Campylobacter rectus	1.45×10^6 CFU/mL	+
Candida albicans	1.25×10^6 CFU/mL	+
Chlamydia pneumoniae	1.40×10^5 $TCID_{50}$/mL	+
Chlamydia trachomatis	1.25×10^6 EB/mL	+
Corynebacterium diphtheriae	1.25×10^6 CFU/mL	+
Corynebacterium pseudodiphtheriticum	1.25×10^6 CFU/mL	+
Enterococcus faecalis	1.25×10^6 CFU/mL	+
Enterococcus faecium	1.25×10^6 CFU/mL	+
Escherichia coli	1.25×10^6 CFU/mL	+

Microorganism	Test Concentration	Strep A Result
Haemophilus influenzae	1.25×10^6 CFU/mL	+
Haemophilus parahaemolyticus	1.25×10^6 CFU/mL	+
Haemophilus parainfluenzae	1.25×10^6 CFU/mL	+
Klebsiella pneumoniae	1.25×10^6 CFU/mL	+
Lactobacillus acidophilus	1.20×10^6 CFU/mL	+
Lactococcus lactis	1.25×10^6 CFU/mL	+
Legionella jordanis	1.25×10^6 CFU/mL	+
Legionella micdadei	1.25×10^6 CFU/mL	+
Legionella pneumophila	1.25×10^6 CFU/mL	+
Listeria monocytogenes	1.25×10^6 CFU/mL	+
Moraxella catarrhalis (2 strains)	1.25×10^6 CFU/mL	+
Moraxella lacunata	1.25×10^6 CFU/mL	+
Mycoplasma pneumoniae	1.25×10^6 copies/mL†	+
Neisseria gonorrhoeae	1.25×10^6 copies/mL†	+
Neisseria lactamica	1.25×10^6 CFU/mL	+
Neisseria meningitidis	1.25×10^6 CFU/mL	+
Neisseria mucosa	1.25×10^6 CFU/mL	+
Neisseria sicca	1.25×10^6 CFU/mL	+
Neisseria subflava	1.25×10^6 CFU/mL	+
Proteus mirabilis	1.25×10^6 CFU/mL	+
Proteus vulgaris	1.25×10^6 CFU/mL	+
Pseudomonas aeruginosa	1.25×10^6 CFU/mL	+
Pseudomonas fluorescens	1.25×10^6 CFU/mL	+
Serratia marcescens	1.25×10^6 CFU/mL	+
Staphylococcus aureus	1.25×10^6 CFU/mL	+
Staphylococcus epidermidis	1.25×10^6 CFU/mL	+
Staphylococcus haemolyticus	1.25×10^6 CFU/mL	+
Stenotrophomonas maltophilia	1.25×10^6 CFU/mL	+
Streptococcus agalactiae	1.25×10^6 CFU/mL	+
Streptococcus anginosus	1.25×10^6 CFU/mL	+
Streptococcus bovis	1.25×10^6 CFU/mL	+
Streptococcus canis	1.25×10^6 CFU/mL	+
Streptococcus constellatus	1.25×10^6 CFU/mL	+

Microorganism	Test Concentration	Strep A Result
Streptococcus dysgalactiae	1.25×10^6 CFU/mL	+
Streptococcus equi	1.25×10^6 CFU/mL	+
Streptococcus gallolyticus	1.60×10^6 CFU/mL	+
Streptococcus intermedius	1.10×10^6 CFU/mL	+
Streptococcus mitis	1.25×10^6 CFU/mL	+
Streptococcus mutans	1.25×10^6 CFU/mL	+
Streptococcus oralis	1.25×10^6 CFU/mL	+
Streptococcus pneumoniae	1.25×10^6 CFU/mL	+
Streptococcus salivarius	1.25×10^6 CFU/mL	+
Streptococcus sanguis	1.25×10^6 CFU/mL	+
Treponema denticola	1.63×10^6 copies/mL†	+
Veillonella parvula	2.13×10^6 CFU/mL	+
Yersinia enterocolitica	1.25×10^6 CFU/mL	+
Adenovirus, Type 1	4.45×10^5 $TCID_{50}$/mL	+
Adenovirus, Type 7	4.45×10^4 $TCID_{50}$/mL	+
Cytomegalovirus	1.00×10^5 $TCID_{50}$/mL	+
Epstein-Barr virus	2.15×10^5 copies/mL	+
Hepatitis B virus	5.00×10^5 copies/mL	+
Herpes simplex virus 1	2.80×10^5 $TCID_{50}$/mL	+
Human papilloma virus, Type 11	2.50×10^5 copies/mL	+
Human papilloma virus, Type 6	2.50×10^5 copies/mL	+

† Testing was performed with genomic DNA due to difficulties in propagation of these bacteria.

Interfering Substances

The Liat™ Strep A Assay was evaluated with 28 substances that may be encountered in throat swab specimens. Medically and/or physiologically relevant concentrations of potential interferents were tested in throat swab matrix in the presence and absence of Strep A at 3x LOD. Results showed that none of the substances tested interfered with the Liat™ Strep A Assay.

Potential Interferent	Concentration	Strep A Result	
		Strep A 3x LOD	Neg. Matrix
Acetaminophen (Tylenol)	100 µg/mL	+	−
Adult Robitussin Peak Cold, Maximum Strength, Cough + Chest	5% v/v	+	−
Adult Robitussin Peak Cold, Nighttime, Multi-symptom cold	5% v/v	+	−
Amoxicillin	25 µg/mL	+	−
Blood (human)	5% v/v	+	−
Brompheniramine Maleate	60 ng/mL	+	−
Cepacol Sore Throat	5 mg/mL	+	−
Cepacol Ultra Sore Throat Spray	5% v/v	+	−
Children's Dimetapp Cold & Cough	5% v/v	+	−
Children's Robitussin Cough & Cold	5% v/v	+	−
Children's Dimetapp Nighttime Cold & Congestion	5% v/v	+	−
Chloraseptic Max	5% v/v	+	−
Chlorpheniramine Maleate	25 ng/mL	+	−
Cool Mint Listerine, antiseptic	5% v/v	+	−
Crest Pro-Health	5% v/v	+	−
Dextromethorphan HBr	20 ng/mL	+	−
Diphenhydramine HCl	350 ng/mL	+	−
Doxylamine Succinate	300 ng/mL	+	−
Erythromycin	15 µg/mL	+	−
Guaifenesin (Guaiacol glyceryl)	5 mg/mL	+	−
Halls Mentho-lyptus Cherry	5 mg/mL	+	−
Halls Mentho-lyptus Sugar Free	5 mg/mL	+	−
Ibuprofen (Advil)	25 µg/mL	+	−
Mucin: bovine submaxillary gland, type I-S	25 mg/mL†	+	−
Penicillin G	1.2 mg/mL	+	−
Sucrets Complete	5 mg/mL	+	−
Tussin Adult Chest Congestion	5% v/v	+	−
Tylenol Cold Sore Throat	5% v/v	+	−

† In the presence of bovine mucin at 25 mg/mL, Strep A Ct was delayed and endpoint fluorescence was suppressed, though all Strep A 3x LOD samples were detected as positive.

Assay cut-off:

The result algorithm for the Liat™ Strep A Assay employs cut-offs for both the cycle threshold (Ct) value and endpoint amplitude, in addition to other parameters. The cut-offs were determined through analysis of a combination of negative clinical samples and samples that were spiked with different strains of *S. pyogenes* at the LOD target level. The Liat Analyzer software evaluates the assay Ct and endpoint amplitude of Strep A and IPC against the cut-offs and interprets the results automatically.

Carry-over/Cross-contamination:

A study was conducted to demonstrate that the single-use, self-contained Liat™ assay tube reduces the risk of carry-over contamination when alternating high positive and negative samples are tested in series. High positive samples comprised of Strep A spiked into negative throat swab matrix at 3.13×10^5 CFU/mL, while negative samples comprised negative throat swab matrix. Eighty (80) tests were conducted on 2 Liat™ Analyzers with high positive and negative samples alternating analyzer-to-analyzer and run-to-run. All 40 high positive samples tested were correctly reported as "Strep A Detected". All 40 negative samples tested were correctly reported as "Strep A Not Detected". There was no carry-over or cross contamination observed during this study.

Clinical Performance:

Clinical Sensitivity and Specificity:

The Liat™ Strep A Assay was evaluated in December 2013 to April 2014 by six clinical sites representing geographically distinct regions throughout the United States. Clinical specimens were collected from patients with symptom characteristics of pharyngitis. Performance characteristics of the assay were determined by comparison to culture and latex agglutination for Strep A typing. Discordant results were investigated using PCR and bi-directional sequencing based on published methods.

The tables below summarize the clinical performance of the Liat Strep A Assay. Assay sensitivity was 98.3% (95% CI: 95.0 – 99.4%) and assay specificity was 94.2% (95% CI: 91.5 – 96.1%).

Strep A		Comparative Culture		
		Positive	Negative	Total
Liat	Positive	170	23[a]	193
	Negative	3[b]	374	377
	Total	173	397	570

[a] Of 23 Liat positive, culture negative specimens, all 23 were Strep A positive by PCR/sequencing.

[b] Of 3 Liat negative, culture positive specimens, 3 were Strep A positive by PCR/sequencing. All 3 were also positive when the Liat assay was repeated using residual specimen after culture.

	#	%	95% CI
Sensitivity	170 / 173	98.3%	95.0% - 99.4%
Specificity	374 / 397	94.2%	91.5% - 96.1%
Accuracy	544 / 570	95.4%	93.4% - 96.9%
Prevalence	173 / 570	30.4%	26.7% - 34.2%
PPV	170 / 193	88.1%	82.8% - 91.9%
NPV	374 / 377	99.2%	97.7% - 99.7%
Invalid [c]	7 / 577	1.2%	0.6% - 2.5%

[c] Rate includes all Invalid, Indeterminate and Assay Aborted results. In all cases, re-test of the same specimens gave a valid result.

Expected Values/Reference Range:

In multicenter clinical studies for the Liat™ Strep A Assay, 570 throat swab specimens were analyzed. The number and percentage of positive cases per specified age group and gender, as determined by the Liat™ Strep A Assay, are presented in the table below:

Age	# Samples	% Samples	# Positive	% Positive
≤5 years	141	24.7%	59	41.8%
6-21 years	401	70.4%	130	32.4%
22-59 years	25	4.4%	4	16.0%
≥60 years	3	0.5%	0	0.0%
Total	570	100%	193	33.9%

Sex	# Samples	% Samples	# Positive	% Positive
M	268	47.0%	100	37.3%
F	302	53.0%	93	30.8%
Total	570	100%	193	33.9%