EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Lyra Direct Strep Assay

DECISION SUMMARY

A. 510(k) Number:

K133883

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the Lyra Direct Strep Assay.

C. Measurand:

Group A β-hemolytic *Streptococcus* (*Streptococcus pyogenes*) and pyogenic Group C and G β-hemolytic *Streptococcus* nucleic acids.

D. Type of Test:

The Lyra Direct Strep Assay is a Real-Time PCR in vitro diagnostic test for the rapid and 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.

E. Applicant:

Quidel Corporation

F. Proprietary and Established Names:

Lyra Direct Strep Assay

G. Regulatory Information:

1. Regulation section:

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

2. Classification:

   Class II

3. Product code:

PGX - Group 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):

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.

2. Indication(s) for use:

Same as intended use.

3. Special conditions for use statement(s):

For prescription use only in accordance with 21 CFR 801.109.

The device is not intended for point-of-care use.

4. Special instrument requirements:

ABI 7500 Fast DX Thermocycler

Plate centrifuge for ABI 96 well plate

Dry heating block, capable of heating 1.5 mL tubes at 95^{o}C for 10 minutes

I. Device Description:

The Lyra Direct Strep Assay detects nucleic acids isolated from throat swab specimens obtained from patients with signs and symptoms of pharyngitis. A multiplex Real-time PCR reaction is carried out under optimized conditions in a single tube generating amplicons for Group A β-hemolytic *Streptococcus* (*Streptococcus pyogenes*) and pyogenic Group C and G β-hemolytic *Streptococcus*, and the Process Control (PRC). Identification of Group A, pyogenic Group C/G, and the PRC occurs by the use of target-specific primers and fluorescent-labeled probes that hybridize to conserved regions in the genomes of Group A, pyogenic Group C/G, and the PRC. The assay does not differentiate between Group C and Group G streptococci.

A specimen from a patient's throat swab is transferred to the Process Buffer then heated to lyse the bacteria and expose the DNA. The lysed specimen is then added to a rehydrated Master Mix of targeted oligonucleotide primers, fluorophore and quencher-labeled probes is added to each plate. The plate is placed into the Applied Biosystems® 7500 Fast Dx instrument and the Quidel Molecular Direct Streptococci Assay protocol is initiated.

Quidel Molecular Direct Probe Labels	
Target	**Dye**
Group A streptococci	FAM
Pyogenic group C and G streptococci	CAL Fluor Red®
Process Control (PRC)	Quaser® 670

This assay is based on Taqman® chemistry, and uses an enzyme with DNA polymerase, and 5'-3' exonuclease activities. During DNA amplification, this enzyme cleaves the probe bound to the conserved complementary DNA sequence, separating the quencher dye from the reporter dye. This step generates an increase in fluorescent signal upon excitation by a light source of the appropriate wavelength. With each cycle, additional dye molecules are separated from their quenchers resulting in an increase in the fluorescent signal. If sufficient fluorescence is achieved, the sample is reported as positive for the detected nucleic acid.

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

Not applicable.

K. Test Principle:

The assay detects nucleic acids isolated from throat swab specimens obtained from patients with signs and symptoms of pharyngitis. A multiplex Real-Time PCR reaction is carried out under optimized conditions in a single tube generating amplicons for Group A β-hemolytic Streptococcus (*Streptococcus pyogenes*) and pyogenic Group C and G β-hemolytic Streptococcus, and the Process Control (PRC). Identification of Group A, pyogenic Group C/G streptococci, and the PRC occurs by the use of target-specific primers and fluorescent-labeled probes that hybridize to conserved regions in the genomes of Group A, pyogenic Group C/G, and the PRC. The assay does not differentiate between Group C and Group G streptococci.

During DNA amplification, the Taq polymerase with 5'-3' exonuclease activity cleaves the probe bound to the complementary DNA sequence, separating the quencher dye from the

reporter dye. This step generates an increase in fluorescent signal upon excitation by a light source of the appropriate wavelength. With each cycle, additional dye molecules are separated from their quenchers resulting in an increase in the fluorescent signal. If sufficient fluorescence is achieved, the sample is reported as positive for the detected nucleic acid, otherwise the sample is determined to be negative or invalid, based on the internal PRC control.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision/within laboratory repeatability was demonstrated with a contrived four (4) member panel consisting of moderate positive (3 x LoD), low positive (2 x LoD), high negative (0.3 x LoD) Group A *Streptococcus* and pyogenic Group C *Streptococcus*, and negative samples (negative matrix) tested by two (2) operators, twice a day (2X) for twelve (12) days. The precision study results are acceptable. The results are shown in Table I.

Table I: Precision

Panel ID	High Negative	Low Positive	Moderate Positive	Negative
Group A Strep (% Detection)	100%	100%	45.8%	0%
Pyogenic Group C Strep (% Detection)	100%	100%	75%	0%

The reproducibility of the Lyra Direct Strep Assay was evaluated at three (3) laboratory sites (two external, one in-house). Reproducibility was assessed using a panel of four (4) simulated samples that include moderate positive and low positive, high negative and negative (as defined in the precision study above) Group A *Streptococcus* (*Streptococcus pyogenes*) and pyogenic Group C *Streptococcus* (*Streptococcus equi* subsp. *zooepidemicus*) samples. The panels and controls were processed and tested on the Applied Biosystems® 7500 Fast Dx. Panels and controls were tested at each site by 2 operators for 5 non-consecutive days (2 operators x 3 replicates x 5 days x 3 sites = 90 results per level for each panel member). The LoD values were based on the values obtained in the LoD study. The reproducibility study results are acceptable. The results are shown in Table II.

Table II: Reproducibility									
Panel ID		Site 1		Site 2		Site 3		Combined	
		Detected Pos/Total	% Pos	Detected Pos/Total	% Pos	Detected Pos/Total	% Pos	Detected Pos/Total	% Pos
Group A Strep	High Neg	7/30	23%	16/30	53%	7/30	23%	30/90	33%
	Low Pos	29/30	97%	30/30	100%	30/30	100%	89/89	99%
	Mod Pos	30/30	100%	30/30	100%	30/30	100%	90/90	100%
	Neg	0/30	0%	0/30	0%	0/30	0%	0/90	0%
Pyo Group C Strep	High Neg	2/30	7%	24/30	80%	2/30	7%	28/90	31%
	Low Pos	30/30	100%	30/30	100%	29/29*	100%	89/90	100%
	Mod Pos	30/30	100%	30/30	100%	30/30	100%	90/90	100%
	Neg	0/30	0%	0/30	0%	0/30	0%	0/90	0%

* 1 replicate removed due to invalid control

b. Linearity/assay reportable range:

Not applicable.

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

Reagents, transport media, specimen storage and hold time studies were performed to characterize the Lyra Direct Strep Assay. Validation studies were performed in house on swabs dipped in contrived negative matrix spiked with Group A *Streptococcus* and pyogenic Groups C and G *Streptococcus* at 3x LoD.
Studies using 4 different swabs (Liquid Amies Single Plastic Applicator, Liquid Stuart Single Plastic Applicator, Puritan Liquid Amies Transport System, and Sterile Puritan Rayon Throat Swabs) demonstrated that samples stored at 2-8 °C and 20-25 ^{o}C produced expected results for up to 13-days.

Study results demonstrated that the rehydrated master mix was stable for 2h at 20-25 ^{o}C, 8 days at 2-8 ^{o}C, and 8 days at -20 ^{o}C.

Study results demonstrated that the Lyra Direct Strep Assay processed samples are stable up to 13 days at 2-8 °C, ambient room temperature, -20 °C, or -70 °C.

The Lyra Direct Strep Assay incorporates a process control which is used to monitor sample processing and evaluate the presence of inhibitory substances. The process control confirms the integrity of assay reagents and detection. Additional controls are performed in accordance with end user laboratory guidelines and requirements.

d. Detection Limit:

The limit of detection (LoD) of the Lyra Direct Strep Assay was determined using 20 quantified (CFU/mL) contrived stocks for each of three strains of Group A streptococci, two strains of pyogenic Group C streptococci and two strains of pyogenic Group G streptococci diluted in a negative matrix (see table below). The

LoD is defined as the lowest concentration at which 95% of all replicates tested positive. The LoD study results are shown in Table III.

Table III: LoD for Group A β-hemolytic *Streptococcus* and Pyogenic Group C and G β-hemolytic *Streptococcus*

Strain	Strain ID	CFU/ml
Group A Streptococcal strain 1 (S*treptococcus pyogenes*)	ATCC 19615	6.0E+02
Group A Streptococcal strain 2 (S*treptococcus pyogenes*)	ATCC 700942	2.2E+03
Group A Streptococcal strain 3 (S*treptococcus pyogenes*)	CCUG 33061	1.5E+03
Pyogenic Group C Streptococcal strain 1 (S*treptococcus equi* subsp. *zooepidemicus)*	ATCC 700400	1.7E+04
Pyogenic Group C Streptococcal strain 2 (*Streptococcus dysgalactiae* subsp. *equisimilis)*	CCUG 1483	1.8E+04
Pyogenic Group G Streptococcal strain 1 (*Streptococcus dysgalactiae* subsp. *equisimilis)*	ATCC 12394	1.6E+04
Pyogenic Group G Streptococcal strain 2	CCUG 27477	1.6E+04

These study results are acceptable.

e. Analytical Sensitivity:

Inclusivity studies were conducted with 11 Group A β-hemolytic *Streptococcus* strains, 10 Pyogenic Group C β-hemolytic *Streptococcus* strains and 13 Pyogenic Group G β-hemolytic *Streptococcus* strains against 3 different reagent lots. The strains were cultured, serial diluted in contrived negative matrix and titered to determine the CFU/ml. A rayon swab was twirled in the stock and run with the Lyra Direct Strep Assay. The inclusivity study results and the final organism concentrations tested are shown in Tables IV-VI.

Table IV: Group A β-hemolytic *Streptococcus* Inclusivity

Strain	Strain ID	Conc (x LoD)
Group A Streptococcal strain 1	ATCC 19615	1.1x
Group A Streptococcal strain 2	ATCC 700942	3.8x
Group A Streptococcal strain 3	ATCC 700952	2.6x
Group A Streptococcal strain 4	ATCC 12344	1.2x
Group A Streptococcal strain 5	ATCC 12384	0.7x
Group A Streptococcal strain 6	ATCC 49399	0.4x
Group A Streptococcal strain 7	NCIMB 13285	1.8x

Table IV: Group A β-hemolytic *Streptococcus* Inclusivity		
Strain	**Strain ID**	**Conc (x LoD)**
Group A Streptococcal strain 8	CCUG 33061	1.4x
Group A Streptococcal strain 9	CCUG 33409	0.4x
Group A Streptococcal strain 10	CCUG 39158	0.5x
Group A Streptococcal strain 11	CCUG 53553	0.6x

Table V: Pyogenic Group C β-hemolytic *Streptococcus* Inclusivity		
Strain	**Strain ID**	**Conc (x LoD)**
Streptococcus dysgalactiae subsp. *equisimilis*	ATCC 12388	1.4x
Streptococcus equi subsp. *zooepidemicus*	ATCC 700400	1.3x
Streptococcus dysgalactiae subsp.	CCUG 1483	2.2x
Pyogenic Group C Streptococcal strain (Specific species not identified)	CCUG 27479	2.1x
Pyogenic Group C Streptococcal strain (Specific species not identified)	CCUG 27664	1.2x
Pyogenic Group C Streptococcal strain (Specific species not identified)	CCUG 6713	0.1x
Pyogenic Group C Streptococcal strain (Specific species not identified)	CCUG 21557	0.7x
Pyogenic Group C Streptococcal strain (Specific species not identified)	CCUG 27478	2.6x
Pyogenic Group C Streptococcal strain (Specific species not identified)	CCUG 27480	1.6x
Pyogenic Group C Streptococcal strain (Specific species not identified)	CCUG 28238	1.6x

Table VI: Pyogenic Group G β-hemolytic *Streptococcus* Inclusivity		
Strain	**Strain ID**	**Conc (x LoD)**
Streptococcus dysgalactiae subsp. *equisimilis*	ATCC 12394	0.5x
Streptococcus canis	ATCC 43497	0.6x
Streptococcus dysgalactiae subsp. *equisimilis*	CCUG 502	0.6x
Streptococcus dysgalactiae subsp. *equisimilis*	CCUG 24070	0.4x
Streptococcus dysgalactiae subsp. *equisimilis*	CCUG 27482	0.3x

Table VI: Pyogenic Group G β-hemolytic *Streptococcus* Inclusivity		
Strain	**Strain ID**	**Conc (x LoD)**
Streptococcus dysgalactiae subsp. *equisimilis*	CCUG 27483	2.8x
Streptococcus dysgalactiae subsp. *equisimilis*	CCUG 33645	1.2x
Streptococcus dysgalactiae subsp. *equisimilis*	CCUG 33802	1.0x
Pyogenic Group G Streptococcal strain (Specific species not identified)	CCUG 1859	N/A*
Pyogenic Group G Streptococcal strain (Specific species not identified)	CCUG 15679	0.3x
Pyogenic Group G Streptococcal strain (Specific species not identified)	CCUG 15680	1.0x
Pyogenic Group G Streptococcal strain (Specific species not identified)	CCUG 26147	0.3x
Pyogenic Group G Streptococcal strain (Specific species not identified)	CCUG 27477	2.3x

* The concentration of the glycerol stock of the CCUG 1859 strain was below detectable levels and therefore could not be titered, therefore it was tested at the neat concentration.

These study results are acceptable.

Co-infection Studies:

Competitive interference studies with the Lyra Direct Strep Assay were conducted to ensure that both Group A *Streptococcus* and pyogenic Group C/G *Streptococcus* could be detected in the same reaction in cases of co-infection. Group A *Streptococcus* and pyogenic Group C *Streptococcus* stocks at 2 to 3 x LoD concentrations in contrived negative matrix were tested in the presence of varying amounts of the other analyte.

No competitive interference was observed with 2 to 3 x LoD concentrations of Group A *Streptococcus* when tested with high concentrations (2E05, 1E06, 1E07 and 1E08 CFU/ml) of pyogenic Group C *Streptococcus* in the same reaction. No competitive interference was observed with 2 to 3 x LoD concentrations of pyogenic Group C *Streptococcus* when tested with high concentrations (2E03, 1E04, 1E05 and 1E06 CFU/ml) of Group A *Streptococcus* in the same reaction. These study results are acceptable.

f. Analytical Specificity:

An *in silico* BLAST analysis of primers used in the Lyra Direct Strep Assay against the NCBI database against sixty (60) potential interfering organisms did not show evidence of cross-reactivity.

A study was performed on the Applied Biosystems® 7500 Fast Dx to evaluate the performance of the Lyra Direct Strep Assay in the presence of forty-four (44) other microorganisms commonly found in throat specimens. Each potentially interfering microorganism was tested in the presence of 2 x LoD Group A *Streptococcus* (2 strains), a pyogenic Group C *Streptococcus* strain and a pyogenic Group G streptococcus strain in the presence of clinically relevant levels of viruses (10^5pfu/ml) and bacteria (10^6cfu/mL) or higher. All strain combinations were spiked into contrived negative matrix. The strains included in the cross-reactivity study are shown in Tables VII.

Table VII: Strains Included in Cross-Reactivity

Strain		
Acinetobacter lwoffii	*Moraxella catarrhalis*	*Streptococcus oralis*
Arcanobacterium haemolyticum	*Neisseria gonorrhoeae*	*Streptococcus pneumoniae*
Bacillus cereus	*Neisseria subflava*	*Streptococcus salivarius*
Bordetella pertussis	*Pseudomonas aeruginosa*	*Streptococcus sanguinis*
Burkholderia cepacia	*Serratia marcescens*	*Streptococcus suis*
Corynebacterium diphtheria	*Staphylococcus aureus*	*Veillonella parvula*
Enterococcus faecalis vanB	*Staphylococcus epidermidis*	*Candida albicans*
Escherichia coli	*Stenotrophomonas maltophilia*	Adenovirus Type 1
Fusobacterium necrophorum	*Streptococcus agalactiae*	Adenovirus Type 11 (Slobitski)
Haemophilus influenza	*Streptococcus anginosus*	Influenza A/ Victoria/ 3/75/ H3N2
Klebsiella pneumonia	*Streptococcus bovis*	Influenza B/Panama/45/90
Lactococcus lactis	*Streptococcus gordonii* (Virdans type)	Influenza C/Taylor/1233/47
Legionella jordanis	*Streptococcus intermedius*	Parainfluenza virus 4a
Legionella micdadei	*Streptococcus mitis*	Rhinovirus Type 15 (1734)
Legionella pneumophila	*Streptococcus mutans*	

None of the forty-four (44) microorganisms tested that might be found in throat specimens cross-react with the assay.

These study results are acceptable.

g. Assay Cut-off:

The Lyra Direct Strep Assay has fixed cut-offs based on fluorescence values attained for each fluorophore associated with each analyte. Ct cut-off values were determined after testing Group A streptococci and pyogenic Group C and G streptococci at the limit of detection (contrived specimens spiked in negative matrix) and a set of clinical specimens in the Lyra Direct Strep Assay on the Applied Biosystems® 7500 Fast Dx instrument. The Ct value cut-offs were selected to fall above the highest true positive Ct value observed for an analyte in both the analytical sensitivity and clinical specimen testing study and to minimize cycle time. A Ct cut-off of 40 cycles was recommended for both analytes and the PRC.

The baseline, threshold, and Ct cut-off settings for the Lyra Direct Strep Assay on the Applied Biosystems® 7500 Fast Dx are described in Table VIII:

Table VIII: Lyra™ Direct Strep Assay ABI 7500 Fast Dx Baseline, Threshold and Ct Cut-off

Analyte	Fluorescence Threshold	Baseline	Ct Cut-off
Group A *Streptococcus*	8.00E+04	Auto Baseline	40 Cycles
Pyogenic Group C/G *Streptococcus*	1.00E+05		
Process Control (PRC)	1.90E+04		

2. Comparison studies:

a. Method comparison with predicate device:

Clinical performance was based on comparison of the Lyra Direct Strep Assay results to those obtained by a composite culture of directly plated patients' throat swabs and culture of the transport fluid material at a central location.

b. Matrix comparison:

A comparison study was conducted between negative clinical matrix and a contrived negative matrix in order to validate the use of the contrived negative matrix in place of a clinical negative matrix for the analytic studies in section M1 above. Contrived negative matrix was constructed to mimic challenging clinical specimens, and consisting of Porcine Gastric Mucin (PGM), Phosphate Buffered Saline (PBS), Bovine Serum Albumin and sodium azide. The matrix comparison study results are shown in Table IX.

Table IX: Matrix Comparison Study

Panel ID		Contrived Negative Matrix		Pooled Negative Clinical Matrix	
		Detected	% Pos	Detected	% Pos
Group A *Streptococcus*	3 x LoD	20/20	100%	20/20	100%
	1 x LoD	19/20	95%	20/20	100%

Table IX: Matrix Comparison Study					
Panel ID		Contrived Negative Matrix		Pooled Negative Clinical Matrix	
		Detected	% Pos	Detected	% Pos
Pyo Group C *Streptococcus*	3 x LoD	20/20	100%	20/20	100%
	1 x LoD	19/20	95%	20/20	100%
Pyo Group G *Streptococcus*	3 x LoD	20/20	100%	20/20	100%
	1 x LoD	20/20	100%	20/20	100%

These studies demonstrate that the contrived negative matrix is equivalent to a clinical matrix.

3. Clinical studies:

a. Clinical Sensitivity:

The clinical performance of the Lyra Direct Strep Assay was demonstrated with one thousand two hundred ninety three (1293) prospectively collected fresh throat specimens at three (3) sites across the United States. The assay was evaluated for the qualitative detection and differentiation of Group A β-hemolytic *Streptococcus* and pyogenic Group C and G β-hemolytic *Streptococcus* using nucleic acids isolated from the patients' throat swabs. A single specimen was collected per patient. Samples were collected using Polyester or Rayon Swab with liquid Amie's or Polyester Swab or Rayon with liquid Stuart's.

All one thousand two hundred ninety three (1293) fresh throat specimens were cultured for Group A β-hemolytic *Streptococcus* (GAS), pyogenic Group C and G β-hemolytic *Streptococcus* (GCS/GGS) and tested with the Lyra Direct Strep Assay. The specimens were cultured at the testing sites and the transport fluid was cultured at a central location. The specimen was considered positive if culture from either the swab or the transport fluid was positive for Group A β-hemolytic *Streptococcus* and/or pyogenic Group C and G β-hemolytic *Streptococcus*, as appropriate. Cultured isolates were typed by latex agglutination. β-hemolytic isolates that were typed as Group C or G were subcultured and the species were determined using MALDI TOF.

The breakdown of performance by analyte is summarized in Tables X and XI:

Table X: Clinical Performance Data for the Lyra Direct Strep Assay vs. Composite Cultures for Group A β-hemolytic *Streptococcus*			
All Sites			
Lyra	**Composite Culture**		
	GAS	**Negative**	**Total**
GAS	109	24	133
Negative	4	1156	1160
Total	113	1180	1293
Sensitivity: 96.5% (109/113) 95% CI (91.3%-98.6%) **Specificity:** 98.0% (1156/1180) 95% CI (97.0%-98.6%)			
Site 1			
Lyra	**Composite Culture**		
	GAS	**Negative**	**Total**
GAS	18	1	19
Negative	0	246	246
Total	18	247	265
Sensitivity: 96.5% (18/18) 95% CI (82.4%-100.0%) **Specificity:** 99.6% (246/247) 95% CI (97.7%-99.9%)			
Site 2			
Lyra	**Composite Culture**		
	GAS	**Negative**	**Total**
GAS	54	17	71
Negative	2	556	558
Total	56	573	629
Sensitivity: 96.4% (54/56) 95% CI (87.9%-99.0%) **Specificity:** 97.0% (556/573) 95% CI (95.3%-98.1%)			
Site 3			
Lyra	**Composite Culture**		
	GAS	**Negative**	**Total**
GAS	37	6	43
Negative	2	354	356
Total	39	360	399
Sensitivity: 94.9% (37/39) 95% CI (83.1%-98.6%) **Specificity:** 98.3% (354/360) 95% CI (96.4%-99.2%)			

These study results are acceptable.

Table XI: Clinical Performance Data for the Lyra Direct Strep Assay vs. Composite Cultures for Pyogenic Group C and G β-hemolytic *Streptococcus*			
All Sites			
Lyra	**Composite Culture**		
	GCS/GGS	**Negative**	**Total**
GCS/GGS	67	21	88
Negative	3	1202	1205
Total	70	1223	1293
Sensitivity: 95.7% (67/70) 95% CI (88.1%-98.5%) **Specificity:** 98.3% (1202/1223) 95% CI (97.4%-98.9%)			
Site 1			
Lyra	**Composite Culture**		
	GCS/GGS	**Negative**	**Total**
GCS/GGS	29	8	37
Negative	0	228	228
Total	29	236	265
Sensitivity: 100.0% (29/29) 95% CI (88.3%-100.0%) **Specificity:** 96.6% (228/236) 95% CI (93.5%-98.3%)			
Site 2			
Lyra	**Composite Culture**		
	GCS/GGS	**Negative**	**Total**
GCS/GGS	22	12	34
Negative	2	593	595
Total	24	605	629
Sensitivity: 91.7% (22/24) 95% CI (74.2%-97.7%) **Specificity:** 98.0% (593/605) 95% CI (96.6%-98.9%)			
Site 3			
Lyra	**Composite Culture**		
	GCS/GGS	**Negative**	**Total**
GCS/GGS	16	1	17
Negative	1	381	382
Total	17	382	399
Sensitivity: 94.1% (16/17) 95% CI (73.0%-99.0%) **Specificity:** 99.7% (381/382) 95% CI (98.5%-100.0%)			

These study results are acceptable.

The external quality control isolates used in these studies were from the Lyra A+G Control Set #M111 consisting of *Streptococcus pyogenes* Z018 (for the GAS primer set) and *Streptococcus dysgalactiae* Z068 (for the GCS/GGS primer set), which serve as processing and extraction controls. The control isolates were tested with acceptable results.

*b. Clinical specificity**:***

See table 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 overall incidence of Group A β-hemolytic *Streptococcus* in patients tested during this study was 8.4% (108/1293). The combined overall incidence of pyogenic Group C and G β-hemolytic *Streptococcus* was 5.2% (67/1293). All clinical specimens collected during this study were collected between August, 2013 and October 2013.

M. Instrument Name:

ABI 7500 Fast DX Thermocycler

N. System Descriptions:

1. Modes of Operation:

Please see the Decision Summary for the Applied Biosystems 7500 Fast Dx – k082562.

2. Software:

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

Yes ____X____ or No _____

The results of the validation and verification testing results were provided for v1.4 of the Applied Biosystems 7500 Fast Dx software. These results are acceptable.

3. Specimen Identification:

Not applicable – specimen identification is manually entered.

4. Specimen Sampling and Handling:

   Specimens are manually transferred to processing tubes prior to amplification.

5. Calibration:

   Please see the manual for the Applied Biosystems 7500 Fast Dx.

6. Quality Control:

   The Lyra Direct Strep Assay incorporates internal and external controls to monitor assay performance. The Process Control is used during sample processing and amplification in the assay. External positive and negative controls streptococcal controls can be purchased commercially or a previously characterized specimen may be used. These controls must be treated as a patient specimen and should be performed in accordance with lab standards.

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

Not applicable.

P. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10, 21 CFR 801.109, and the special controls.

Q. Identified Potential Risks and Required Mitigation Measures:

Identified Potential Risks	**Mitigation Measures**
Incorrect identification of a pathogenic microorganism by the device can lead to improper patient management.	Special controls (1), (2), (3), (4), (5) and (6)
Failure to correctly interpret test results	Special control (7)
Failure to correctly operate the instrument	Special controls (8)

R. Benefit/Risk Analysis:

Summary	
Summary of the Benefit(s)	When used for the proposed intended use, the benefits to the clinician and the patient include: 1) the first assay submitted to FDA to detect pyogenic group C and group G streptococcus as part of a multiplexed assay that also detects group A streptococcus 2) establishment of the device performance in a manner that demonstrates consistent accurate test results; and 3) ability to use a well validated device to diagnose group A streptococcus and pyogenic G/C streptococcus from nucleic acids isolated from throat swab specimens obtained from patients with signs and symptoms of pharyngitis, which will allow prompt patient management including that ability to initiate disease specific treatment.
Summary of the Risk(s)	The Lyra™ Direct Strep Assay can yield false positive and false negative results through 1) incorrect identification of a pathogenic microorganism by the device, leading to improper patient management, 2) failure to correctly interpret test results, and 3) failure to correctly operate the instrument. Risks associated with the device are false negative and false positive results; false negative results are well mitigated by culture confirmation of negative results, which is clearly indicated in device labeling. The risk from a false positive result is unnecessary treatment. The risks of unnecessary treatment from penicillin (and alternative antibiotics) are well recognized and include rash, allergic/hypersensitivity reactions, gastrointestinal reactions, candidiasis, and many less common possible adverse effects. Serious adverse reactions are uncommon.
Conclusions Do the probable benefits outweigh the probable risks?	The probable benefits of this device outweigh the probable risks associated with its use. There are no substantial clinical concerns with the classification of this device in Class II given the combination of general and special controls.

S. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.2680 with special controls. FDA believes that special controls, along with the applicable general controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Device Type: *Streptococcus spp.* nucleic acid-based assay

Class: II (special controls)

Regulation: 21 CFR 866.2680

(a) *Identification*. A *Streptococcus spp.* nucleic acid-based assay is a qualitative *in vitro* diagnostic device intended to simultaneously detect and identify various *Streptococcus spp.* nucleic acids extracted directly from clinical specimens. The device detects specific nucleic acid sequences for organism identification. The identification aids in the diagnosis of diseases caused by bacteria belonging to the genus *Streptococcus* and provides epidemiological information on these diseases. Pathogenic streptococci are associated with infections, such as sore throat, impetigo (an infection characterized by small pustules on the skin), urinary tract infections, rheumatic fever, and kidney disease.

(b) *Classification*. Class II (special controls). The special controls for this device are:

1) Premarket notification submissions must include detailed device description documentation, including the device components, ancillary reagents required but not provided, and a detailed explanation of the methodology including primer/probe sequence, design, and rationale for sequence selection.
2) Premarket notification submissions must include detailed documentation from the following analytical and clinical performance studies: Analytical sensitivity (Limit of Detection), reactivity, inclusivity, precision, reproducibility, interference, cross reactivity, carry-over, and cross contamination.
3) Premarket notification submissions must include detailed documentation from a clinical study. The study, performed on a study population consistent with the intended use population, must compare the device performance to results obtained from well-accepted reference methods.
4) Premarket notification submissions must include detailed documentation for device software, including, but not limited to, software applications and hardware-based devices that incorporate software.
5) Premarket notification submissions must include database implementation methodology, construction parameters and quality assurance protocols, as appropriate.
6) The device labeling must include limitations regarding the need for culture confirmation of negative specimens, as appropriate.
7) A detailed explanation of the interpretation of results and acceptance criteria must be included in the device's 21 CFR 809.10(b)(9) compliant labeling.
8) Premarket notification submissions must include details on an end user device training program that will be offered while marketing the device, as appropriate.