5.0 510(K) SUMMARY

510(K) SUMMARY

A. GENERAL INFORMATION

Submission Date: February 21st, 2019

Submitter Information:

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B. PURPOSE FOR SUBMISSION

To obtain a substantial equivalence determination for the GenePOC™ Strep A

C. MEASURAND

Conserved regions of the *Streptococcus pyogenes* bacterial genome

D. TYPE OF TEST

Real-time polymerase chain reaction (PCR)

E. APPLICANT

GenePOC Inc.

F. PROPRIETARY AND ESTABLISHED NAMES

GenePOC™ Strep A

G. REGULATORY INFORMATION

Trade Name:	GenePOC™ Strep A
Classification:	Class II
Regulation:	21 CFR 866.2680
Regulation Name:	*Streptococcus spp*. nucleic acid-based assay
Product Code:	PGX and OOI
Panel:	83 - Microbiology

H. INTENDED USE

1. Intended Use and Indications for Use:
   The GenePOC™ Strep A assay, performed on the revogene™ instrument, is an automated, qualitative *in vitro* diagnostic test that utilizes real-time polymerase chain reaction (PCR) for the direct detection of *Streptococcus pyogenes* (Group A β-hemolytic *Streptococcus*) nucleic acids from throat swab specimens obtained from patients with signs and symptoms of pharyngitis. The GenePOC™ Strep A assay is intended for use as an aid in the diagnosis of Group A *Streptococcus* infection.

2. Special conditions for use statement(s):
   Prescription Use Only

3. Special instrument requirements:
   revogene™

I. INDICATIONS FOR USE

Same as Intended Use.

J. DEVICE DESCRIPTION

The GenePOC™ Strep A assay is a single-use test for qualitative detection of *Streptococcus pyogenes* (group A *Streptococcus* - GAS) nucleic acids from throat swab specimens obtained from patients with signs and symptoms of pharyngitis. The GenePOC™ Strep A assay kit is comprised of the disposable Strep A microfluidic cartridge (PIE), Sample Buffer Tube (SBT), and Disposable Transfer Tool (DTT). These components are used to suspend the sample, extract, amplify, and detect *Streptococcus pyogenes (S. pyogenes)* nucleic acid.

A Process Control (PrC) is also incorporated into each PIE to verify sample processing and amplification steps. The PrC allows for the verification of potential inhibitor substances as well as microfluidic, instrument or reagent failure. The GenePOC™ Strep A assay is designed to be used on the revogene™. The revogene™ is an instrument that automates sample homogenization, sample dilution, cell lysis, DNA amplification and detection of the amplified PCR products.

Each GenePOC™ Strep A assay kit provides components for twenty-four (24) tests. User intervention is required for sample preparation, transferring throat swab specimen into the SBT, using the DTT to transfer the sample into the PIE, and loading/unloading the PIE into the revogene™ carousel. Each PIE is a completely integrated closed device in which a sample is dispensed and processed through different microfluidic chambers and channels that allow for the sample processing and subsequent real-time PCR steps.

During the run and at run completion, the results are computed by the revogene™ from measured fluorescent signals and embedded calculation algorithms. The output results include positive, negative, indeterminate, and unresolved. An Early Positive Result Outcome (E-PRO)

feature provides positive result if the signal from the target DNA reaches a predetermined threshold before the full PCR cycles have been completed. Upon completion of a run, the user removes the used cartridges and disposes of them in normal biological waste. Results may be viewed, printed, transferred, and/or stored by the user.

K. SUBSTANTIAL EQUIVALENCE INFORMATION

1. Predicate Device Name:
   Xpert Xpress Strep A

2. Predicate 510(k) Number:
   K172126

3. Comparison with Predicate:

Item	GenePOC™ Strep A	Xpert Xpress Strep A (Predicate Device)
K Number	K183366	K172126
SIMILARITIES		
Regulation	21 CFR 866.2680	Same
Classification	Class II	Class II
Intended Use	The GenePOC™ Strep A assay, performed on the revogene™ instrument, is an automated, qualitative *in vitro* diagnostic test that utilizes real-time polymerase chain reaction (PCR) for the direct detection of *Streptococcus pyogenes* (Group A β-hemolytic *Streptococcus*) nucleic acids from throat swab specimens obtained from patients with signs and symptoms of pharyngitis. The GenePOC™ Strep A assay is intended for use as an aid in the diagnosis of Group A *Streptococcus* infection.	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.
DNA Target	Group A *Streptococcus*	Same
DNA Extraction	Automated by instrument	Same
Assay Format	Amplification: Real-time PCR Detection: Fluorogenic target-specific hybridization	Same
Detection Probes	TaqMan® Probe	Same
Automatic Assay	Yes-result interpretation	Same
Internal Process Control	To verify presence of potential inhibitory substances as well as any microfluidic, instrument or reagent failures.	Same
External Control	Materials available commercially but not required to run the test	Same
Result Format	Qualitative	Same
Sample Preparation	Automated by revogene™	Automated

Item	GenePOC™ Strep A	Xpert Xpress Strep A (Predicate Device)
Interpretation of Test Results	Automated (Diagnostic software of the revogene™)	Automated
Specimen Type	Throat swab (rayon or polyester) in Liquid Stuart or Liquid Amies Transport Medium	Throat swab in Liquid Amies Transport Medium
DIFFERENCES		
Instrument System	revogene™	GeneXpert Dx, GeneXpert Infinity-48s or GeneXpert Infinity-80 instrument systems

L. STANDARDS/GUIDANCE DOCUMENTS REFERENCED

- CLSI Guideline EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline.
- CLSI Guideline EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline.
- CLSI Guideline EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline.

M. TEST PRINICIPLE

The revogene™ automates and integrates nucleic acid extraction and amplification, and detection of the target sequence in complex samples using real-time PCR. A throat swab specimen is collected using a rayon tipped swab, the specimen is transferred into the SBT containing 1.5 mL of sample buffer, and the swab stem is broken. After a 15-second vortex step at maximal speed, the inoculated sample buffer is transferred into the GenePOC™ Strep A PIE using the DTT. The loaded GenePOC™ Strep A PIE is placed into the revogene™ for further sample processing. No operator intervention is necessary once the PIE is loaded onto the revogene™.

Each GenePOC™ Strep A PIE is a completely integrated and self-contained device. Each sample is sequentially transferred by centrifugation from one microfluidic chamber to the next and all reagents specific for the PCR reaction are incorporated and dried within the PCR wells. The stepwise process includes sample homogenization, lysis of cells, sample dilution, and DNA extraction followed by the subsequent real-time PCR steps within one PCR well in the cartridge. An internal PrC is contained in the homogenization chamber and is therefore present in every test to verify critical steps of the analytical process (including sample lysis, dilution and nucleic acid amplification and detection) and for the presence of potential inhibitory substances as well as system or reagent failures. The amplified products are detected in real-time using target-specific TaqMan® chemistry-based probes. The results are computed by the system from measured fluorescent signals and embedded calculation algorithms. An Early Positive Result Outcome (E-PRO) feature provides positive result if the signal from the target DNA reaches a predetermined threshold before the full PCR cycles have been completed. Results may be viewed, printed, transferred, and/or stored by the user.

N. PERFORMANCE CHARACTERISTICS

1. Analytical Performance

a. **Precision/Reproducibility**
Within-Laboratory Precision:

In accordance with the CLSI Guidelines EP05-A3, the Within-Laboratory Precision of the assay was performed at one (1) site and tested specimens with one (1) GenePOC™ Strep A reagent lot over twenty (20) days (consecutives or not) by two (2) operators, at the rate of one (1) run per operator per day using one (1) revogene™ instrument. Two (2) replicates of each sample type were included in a single run and tested daily by each operator for a total of eighty (80) replicates after twenty (20) days.

Description of the Precision/Reproducibility Panels

Sample Composition	Strain	Load Tested (LoD)	Load Tested (CFU/mL of SB)
Low Positive	*S. pyogenes* ATCC 12344	1.95x LoD	650
Moderate Positive	*S. pyogenes* ATCC 19615	3x LoD	3000
Negative	N/A	N/A	N/A

Overall, two-hundred and forty (240) samples (80 low positive, 80 moderate positive and 80 negative samples) were tested in presence of *S. pyogenes*-negative throat matrix during this within-laboratory precision study with the GenePOC™ Strep A assay. The overall agreement of assay results is 97.5% (95% CI: [91.3%-99.7%]) for negative samples, 100% (95% CI: [96.3%-100%]) for low positive samples and 100% (95% CI: [96.3%-100%]) for moderate positive samples.

Within-Laboratory Precision Qualitative Analysis and Overall Agreement

Sample Type	Strain	Positive Results/Total	Agreement (%) [95% CI]
Low Positive	*S. pyogenes* ATCC 12344	80/80	100 [96.3%-100%]
Moderate Positive	*S. pyogenes* ATCC 19615	80/80	100 [96.3%-100%]
Sample Type	**Strain**	**Negative Results/Total**	**Agreement (%) [95% CI]**
Negative	N/A	78/80	97.5 [91.3%-99.7%]

Results for the Ct Values Analysis of Within-Laboratory Precision Study

S. pyogenes											
Sample Type	**Strain**	**Mean Ct (*S.pyo*)**	**N**	**Between-Days**		**Between-Runs**		**Repeatability**		**Overall**	
				SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Low Positive	*S. pyogenes* ATCC 12344	37.65	80	0.00	0.00	0.00	0.00	1.67	4.44	1.67	4.44
Moderate Positive	*S. pyogenes ATCC 19615*	36.52	80	0.40	1.09	0.43	1.19	1.66	4.54	1.76	4.82
PrC											
Sample Type	**Strain**	**Mean Ct (PrC)**	**N**	**Between-Days**		**Between-Runs**		**Repeatability**		**Overall**	
				SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Negative	N/A	28.56	78	0.08	0.27	0.00	0.00	0.84	2.95	0.85	2.96

The overall %CV of PrC for all 78 negative samples obtained for the Within-Laboratory Precision is 2.96%.

Between-Laboratory Reproducibility:

The Between-Laboratory Reproducibility study was performed with one (1) GenePOC™ Strep A assay reagent lot (Kit Lot #1) on five (5) days (consecutive or not) by two (2) operators, at three (3) sites. Two (2) runs were performed by each operator on each day. Overall, 300 samples (90 low positive, 90 moderate positive and 120 negative samples) were tested in presence of *S. pyogenes*-negative throat matrix during this Between-Laboratory Reproducibility study with the GenePOC™ Strep A assay.

The overall agreement of assay results for the Between-Laboratory Reproducibility study was 100% (95% CI: [96.7%-100%]) for Low Positive (LP) samples, 100% (95%

CI: [96.7%-100%]) for Moderate Positive (MP) samples and 100% (95% CI: [97.5-100%]) for True Negative (TN) samples.

Between-Laboratory Reproducibility Qualitative Analysis and Overall Agreement

Panel Member	Strain	Site 1 Positive Results/Total (% Agreement)	Site 2 Positive Results/Total (% Agreement)	Site 3 Positive Results/Total (% Agreement)	Positive Results/ Total	Overall Agreement (%)
		POS/Total				[95% CI]
Low Positive (1.95x LoD)	*S. pyogenes* ATCC 12344	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90	100.0% [96.7-100.0]
Moderate Positive (3x LoD)	*S. pyogenes* ATCC 19615	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90	100.0% [96.7-100.0]

Between-Laboratory Reproducibility Qualitative Analysis and Overall Agreement for Negative Panel Members

Panel Member	Strain	Site 1 Negative /Total (% Agreement)	Site 2 Negative /Total (% Agreement)	Site 3 Negative/Total (% Agreement)	Negative Results/Total	Overall Agreement (%)
		NEG/Total				[95% CI]
Negative	N/A	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120	100.0% [97.5-100.0]

Results for the Ct Values Analysis of Between-Laboratory Reproducibility Study

S. pyogenes													
Sample Type	Strain	Mean Ct (*S.pyo*)	N	Between-Laboratory		Between-Operators		Between-Days		Repeatability (Residual Error)		Overall	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Positive	*S. pyogenes* ATCC 12344	36.97	90	0.27	0.72	0.72	1.96	0.61	1.65	1.61	4.35	1.88	5.10
Moderate Positive	*S. pyogenes* ATCC 19615	34.98	90	0.26	0.73	0.00	0.00	0.00	0.00	0.71	2.02	0.75	2.15
PrC													
Sample Type	Strain	Mean Ct (PrC)	N	Between-Laboratory		Between-Operators		Between-Days		Repeatability (Residual Error)		Overall	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative	N/A	28.52	120	0.21	0.73	0.00	0.00	0.00	0.00	0.66	2.31	0.69	2.42

Between-Lot Reproducibility:

The Between-Lot Reproducibility study was assessed only at one (1) site where the same experiment was repeated with two (2) additional reagent lots (Kit Lot #2 and Kit Lot #3) for a total of fifteen (15) days of testing (five days per reagent lot). Overall,

300 samples (90 low positive, 90 moderate positive and 120 negative samples) were tested in presence of *S. pyogenes*-negative throat matrix during this Between-Lot Reproducibility study with the GenePOC™ Strep A assay.

The overall agreement of assay results for the Between-Lot Reproducibility study was 97.8% (95% CI: [92.2-99.7%]) for Low Positive (LP) samples, 100% (95% CI: [96.7-100%]) for Moderate Positive (MP) samples and 100% (95% CI: [97.5-100%]) for True Negative (TN) samples.

Between-Lot Reproducibility Qualitative Analysis and Overall Agreement

Panel Member	Strain	Lot #1 Positive Results/Total (% Agreement)	Lot #2 Positive Results/Total (% Agreement)	Lot #3 Positive Results/Total (% Agreement)	Positive Results/ Total	Agreement (%)
		POS/Total[1]				[95% CI]
Low Positive (1.95x LoD)	*S. pyogenes* ATCC 12344	30/30 (100%)	29/30 (96.6%)	29/30 (96.7%)	88/90	97.8% [92.2-99.7]
Moderate Positive (3x LoD)	*S. pyogenes* ATCC 19615	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90	100.0% [96.7-100.0]

Between-Lot Reproducibility Qualitative Analysis and Overall Agreement for Negative Panel Members

Panel Member	Strain	Lot #1 Negative/ Total Agreement)	Lot #2 Negative/ Total Agreement)	Lot #3 Negative/ Total Agreement)	Negative/Total	Agreement (%)
		NEG/Total				[95% CI]
Negative	N/A	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120	100.0% [97.5-100.0]

Results for the Ct Values Analysis of Between-Lot Reproducibility Study

S. pyogenes													
Sample Type	Strain	Mean Ct (Spyo)	N	Between-Lot		Between-Operators		Between-Days		Repeatability (Residual Error)		Overall	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Low Positive	*S. pyogenes* ATCC 12344	37.85	88	0.00	0.00	0.92	2.42	1.17	3.08	1.76	4.65	2.30	6.08
Moderate Positive	*S. pyogenes* ATCC 19615	35.49	90	0.00	0.00	0.43	1.21	0.00	0.00	1.55	4.37	1.61	4.53
PrC													
Sample Type	Strain	Mean Ct (PrC)	N	Between-Lot		Between-Operators		Between-Days		Repeatability (Residual Error)		Overall	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative	N/A	28.55	120	0.34	1.20	0.00	0.00	0.04	0.12	0.66	2.31	0.74	2.61

The maximal overall %CV of PrC Ct values for Negative samples obtained for both Between-Laboratory and Between-Lot Reproducibility studies was 2.61% (%CV values).

b. **Linearity/Assay Reportable Range**
Not applicable.

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

Internal process control (PrC):

Each PIE contains a PrC that controls for amplification inhibition, assay reagents, and sample processing effectiveness

External Controls:

Two (2) External Controls that must be prepared by the end user. These are recommended but not required to allow the user to select the most appropriate for their laboratory quality control program.

GenePOC recommends using a 0.5 ± 0.05 MacFarland (McF) suspension of a *S. pyogenes* commercially available strain (e.g., ATCC® 12344™) diluted 1/100 in saline as a Positive External Control and a 0.5 ± 0.05 McF suspension of a *Streptococcus salivarius* commercially available strain (e.g., ATCC® 13419™) as a Negative External Control.

Sample Stability in the PIE:

The GenePOC™ Strep A assay PIE (microfluidic cartridge) stability study was validated using positive samples comprised of the *S. pyogenes* (GAS) strain ATCC® 12344™ and negative samples in presence of *S. pyogenes*-negative throat matrix. The study was conducted using three (3) GenePOC™ Strep A kit lots, three (3) replicates of *S. pyogenes* and negative samples (one replicate per kit lot), and five (5) revogene™ instruments.

The results of the study support the recommended maximum interval of one (1) hour from the opening of the PIE pouch and sample addition into the GenePOC™ Strep A assay PIE to the processing on the revogene™ instrument.

Nested Specimen Stability:

This study evaluated the stability of the throat swab specimens collected with rayon swabs with Liquid Stuart transport media, and the inoculated SBT used in the GenePOC™ Strep A assay. The time periods tested in this study established specimen stability between i) throat swab specimen collection and SBT inoculation and ii) SBT inoculation and loading of the GenePOC™ Strep A PIE prior to initiation of the revogene™ run.

The nested stability was determined by testing positive samples comprised of the *S. pyogenes* (GAS) strain ATCC® 12344™, and negative samples in presence of *S. pyogenes*-negative throat matrix. The study included three (3) lots GenePOC™ Strep A assay kits. For each sample type (positive or negative), and for each time point, a total of twelve (12) swabs were inoculated (4 swabs for each matrix pool, i.e., 4 replicates per kit lot).

Study results support throat swab specimen storage at 25±2°C up to two (2) days or 2-8°C for up to seven (7) days. SBT inoculated with throat swab specimens may be stored up to three (3) days at 25±2°C or 2-8°C for up to seven (7) days prior testing with the GenePOC™ Strep A assay.

Nested Specimen Stability – Various Swabs:

This study confirms the cumulative stability (nested stability) of throat swabs specimens when collected and conserved with Rayon Liquid Amies, Polyester Liquid Stuart, Polyester Liquid Amies and the inoculated SBT used with the GenePOC™ Strep A assay at defined temperature of 25 ± 2°C and 2-8°C.

The nested confirmation study was executed by testing *S. pyogenes* (GAS) strain ATCC® 12344™ at 3x LoD (999 CFU/ml) of Sample Buffer (SB) and negative samples in presence of *S. pyogenes*-negative throat matrix. The study included three (3) to four (4) lots GenePOC™ Strep A assay kits for each type of swab. For each sample type (positive or negative), and for each time point, a total of twelve (12) swabs were inoculated (four (4) swabs for each matrix pool, i.e., four (4) replicates per kit lot).

Study results support throat swab specimen nested stability for GenePOC™ Strep A assay at 25±2°C up to two (2) days or 2-8°C for up to seven (7) days; SBT inoculated with throat swab specimens may be stored up to three (3) days at 25±2°C or 2-8°C for up to seven (7) days prior testing with the GenePOC™ Strep A assay, as confirmed with the rayon swab in Liquid Amies transport media and Polyester swab in Liquid Stuart and Liquid Amies transport media.

Reagent Stability

The shelf life of the GenePOC™ Strep A assay reagents was evaluated in a real time stability study performed on three (3) lots of reagents that were stored under different conditions. GenePOC claims a shelf life of 63 days (2 months) at 2-8°C and 25 ± 3°C with 60 ± 10% RH. GenePOC will claim reagent stability of two (2) months in the product labeling at the time of 510(k) submission.

d. **Detection Limit**

The Limit of Detection (LoD) was established and confirmed in two separate studies. The LoD of the GenePOC™ Strep A assay was established using throat swab specimen matrix collected from rayon swab in Liquid Stuart transport media (previously tested negative for *S. pyogenes*) spiked with various concentrations of *S. pyogenes* bacterial suspensions. Three (3) strains of *S. pyogenes* (ATCC® 12344™, ATCC® 19615™, and

ATCC® 700942™) were tested in twenty-four (24) replicates per concentration with three (3) GenePOC™ Strep A kit lots.

In accordance with the CLSI Guideline EP17-A2, the confirmation of the LoD was conducted during three (3) days using one (1) GenePOC™ Strep A kit lot and one (1) revogene™ instrument per strain. Two (2) different preparations of each *S. pyogenes* strain (ATCC® 19615, ATCC® 12344, and ATCC® 700942) were used in the study. A total of twenty (20) replicates per strain in presence of *S. pyogenes*-negative throat matrix was tested. The LoD of the GenePOC™ Strep A assay ranges from 333 to 1,333 CFU/mL of SB.

The LoD for each *S. pyogenes* strain was also confirmed with the rayon swab in Liquid Amies transport media and polyester swab in Liquid Stuart and Liquid Amies transport media.

Analytical Reactivity/Inclusivity

The analytical reactivity (inclusivity) of the GenePOC™ Strep A assay was determined for twelve (12) strains of *Streptococcus pyogenes* GAS (Lancefield group A) representing six (6) different *emm* sequence types from various geographic origins. Nine (9) strains were tested in presence of *S. pyogenes*-negative throat matrix at a load of 999 CFU/mL of SB (3xLoD). Three (3) lots of GenePOC™ Strep A kits were used for sample testing (n=3 strains/kit lot). Eight (8) *S. pyogenes* strains were detected with 100% positivity (in 9/9 replicates) by the GenePOC™ Strep A assay at a load of 999 CFU/mL of SB. The *S. pyogenes* ATCC® 49399™ strain was detected with 100% positivity at a load of 1667 CFU/mL of SB.

***S. pyogenes* Strains utilized in the Analytical Inclusivity Study**

Species	Strain Identification	Lancefield Group	*Emm*-type
Streptococcus pyogenes	ATCC 12384	A	3
	ATCC 49399	A	N/A
	CCUG 33409	A	N/A
	CCUG 39158	A	N/A
	CCUG 53553	A	N/A
	ATCC 700952	A	92
	ATCC 700294	A	1
	ATCC 12357	A	18
	CCUG 65322	A	N/A
	ATCC 19615[1]	A	80
	ATCC 12344[1]	A	1
	ATCC 700942[1]	A	82

[1] Strains used for the LoD determination and therefore not retested

Analytical Specificity

A total of fifty (50) various analytes found in clinical throat swab specimens (different from *S. pyogenes*) were selected. These included:

- Commensal and pathogenic microorganisms (bacteria, yeast and viruses) from the throat or mouth;
- Species phylogenetically related to *S. pyogenes*;
- Human gDNA.

Forty-two (42) bacteria and one (1) yeast were tested at a concentration of $\geq 10^6$ CFU/mL of SB in presence of *S. pyogenes*-negative throat matrix. Six (6) viral nucleic acid preparations and human genomic DNA were tested at a concentration of $\geq 10^5$ cp/mL of SB in presence of *S. pyogenes*-negative throat matrix. No cross-reactivity was observed among the fifty (50) non-specific analytes tested.

Cross-Reactants Tested in the Analytical Specificity Study

Name	Identification	Name	Identification
Bacteria and Yeasts			
Acinetobacter lwoffii	ATCC 15309	*Klebsiella pneumoniae*	ATCC 27736
Arcanobacterium haemolyticum	ATCC BAA-1784	*Lactococcus lactis*	ATCC 19435
Bacillus cereus	ATCC 14579	*Legionella jordanis*	ATCC 33623
Bordetella pertussis	ATCC 9797	*Legionella micdadei (Tatlockia micdadei)*	CCUG 31229
Burkholderia cepacia	ATCC 25416	*Legionella pneumophila*	ATCC 33152
Corynebacterium diphtheriae	ATCC 13812	*Moraxella catarrhalis*	ATCC 25238
Enterococcus faecalis	ATCC 19433	*Neisseria gonorrhoeae*	ATCC 43069
Escherichia coli	ATCC 11775	*Neisseria subflava*	ATCC 49275
Fusobacterium necrophorum	ATCC 25286	*Parvimonas micra*	ATCC 33270
Haemophilus influenza	ATCC 9006	*Pseudomonas aeruginosa*	ATCC 35554
Serratia marcescens	ATCC 13880	*Streptococcus dysgalactiae* subsp. *dysgalactiae*	ATCC 9926
Staphylococcus aureus	ATCC 33592	*Streptococcus dysgalactiae* subsp. *dysgalactiae*	ATCC 43078
Staphylococcus epidermidis	ATCC 14990	*Streptococcus gordonii*	ATCC 10558
Stenotrophomonas maltophilia	ATCC 13637	*Streptococcus intermedius*	ATCC 27335
Streptococcus agalactiae	ATCC 12403	*Streptococcus mitis*	NCIMB 13770
Streptococcus anginosus	ATCC 33397	*Streptococcus mutans*	ATCC 25175
Streptococcus anginosus subsp. *whileyi*	CCUG 39159	*Streptococcus oralis*	ATCC 6249
Streptococcus bovis	ATCC 33317	*Streptococcus pneumoniae*	ATCC 49619
Streptococcus canis	ATCC 43496	*Streptococcus salivarius*	ATCC 13419
Streptococcus constellatus subsp. *viborgensis*	CCUG 62387	*Streptococcus sanguinis*	ATCC 10556
Streptococcus suis	CCUG 7984	*Veillonella parvula*	ATCC 10790
		Candida albicans	ATCC 10231
Viruses and Human DNA			
Adenovirus Type 1	ATCC VR-1D	Influenza A/Aichi/2/68/H3N2	ATCC VR-1680D
Influenza B/Taiwan/2/62	ATCC VR-1735D	Parainfluenza virus 4b	ATCC VR-1377D
Rhinovirus Type 17	ATCC VR-1663D	Human DNA	N/A
Adenovirus type 11 (Slobitski)	ATCC VR-12D		

Bioinformatic Analysis

The *in silico* specificity of the GenePOC™ Strep A assay was validated through the analysis of the assay's primers and probe sequences selected for the specific detection of *S. pyogenes* and to demonstrate, *in silico*, the level of sequence conservation within the targeted region.

The *in silico* study suggests that no non-specific amplifications or detection will occur with the primers and probe of the GenePOC™ Strep A assay for the detection of the targeted *S. pyogenes* sequence. The primers and probes have been proven to be specific to their respective target following Basic Local Alignment Search Tool (BLAST) analysis. No other target than *S. pyogenes* was found to have significant level of homology with the *S. pyogenes* primers and probe. Therefore, there is no evidence of potential cross-reactivity for this target.

In addition, it was concluded that the *S. pyogenes* target primers and probe do not interact with the PrC sequence. Thus, there is no risk to produce false positive results. Further, the PrC primers did not demonstrate cross-reactivity with other organisms.

Carry-over and Cross-Contamination Studies

Carry-over (between-run) and cross-contamination (within-run) were evaluated by running two studies using two (2) revogene™ instruments, one (1) for the cross-contamination study and one (1) for the carry-over study.

For the carry-over study, a total of ten (10) runs were performed by two (2) operators with the GenePOC™ Strep A assay on one (1) revogene. Testing was performed with alternating "high positive" (10^6 CFU/mL of Sample Buffer) and negative samples in presence of *S. pyogenes*-negative throat matrix. For the cross-contamination study, a run of eight (8) replicates of high positive samples followed by a run of eight (8) replicates of negative samples were performed by two (2) operators, for a total of ten (10) runs on one revogene.

No false positive results were detected in the within-run cross-contamination study (n=80) or in the between-run carry-over study (n=80).

e. **Assay Cut-off:**
Thresholds and cut-offs for the GenePOC™ Strep A assay are embedded within the Assay Definition File that also encodes the instrument settings required to perform the test. Both the native (throat swab specimens) and contrived samples were used to set the thresholds of positivity and negativity of the GenePOC™ Strep A assay. The assay cutoff was determined by testing n=509 native and contrived samples.

f. **Assay Interference**
The potential for interference with the GenePOC™ 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.

Interference with Exogenous and Endogenous Substances:

Interference on the GenePOC™ Strep A assay was evaluated with nineteen (19) potentially interfering substances, which included three (3) endogenous substances and sixteen (16) exogenous substances, identified because they could be present in a throat specimen.

Two (2) strains of *S. pyogenes* (ATCC® 12344™ and ATCC® 19615™) were tested at a moderate load (3xLoD, 999 CFU/mL and 3000 CFU/mL of SB, respectively) in the presence of potentially interfering substances and *S. pyogenes*-negative throat matrix. Negative samples were also tested in the presence of potentially interfering substances to evaluate the impact on the Process Control (PrC). The potentially interfering substances were tested at the highest concentration that may be encountered in a throat swab specimen.

Results demonstrated that Analgesic / Antipyretic (Tylenol®), Nonsteroidal anti-inflammatory drug (Aspirin®, Advil®), Bronchodilator (Albuterol sulfate), Whole Blood and Mucin showed a potentially inhibitory effect on the detection of PrC or *S. pyogenes* when any of these substances was present in SBT at a concentration of 4.3% (w/v or v/v). These substances showed no reportable interference with the GenePOC Strep A assay when tested at 0.4% (w/v) for Tylenol®, Advil®, Albuterol sulfate and Mucin or at 0.1% (w/v) or 0.1% (v/v) for Aspirin® and Whole Blood, respectively.

Microbial Interference:

The microbial interference study for the GenePOC™ Strep A assay evaluated thirty (30) potentially interfering non-targeted microorganisms genetically close to the assay analytes, flora potentially found in the mouth/throat/respiratory tract and the most frequent throat and upper respiratory tract infection causative agents. Two (2) *S. pyogenes* strains, ATCC® 12344™ and ATCC® 19615™, were tested at 3xLoD (999 CFU/mL and 3000 CFU/mL of SB respectively) in triplicate in presence of potentially interfering non-targeted microorganisms and *S. pyogenes*-negative throat matrix. Negative samples were also tested in the presence of potentially interfering non-targeted microorganisms to evaluate the impact on the PrC. None of the thirty (30) organisms present at 10^6 CFU/mL of SB for bacteria and yeast and 10^5 copies/mL of SB for viruses interfered with detection of PrC or with *S. pyogenes* strain ATCC 19615.

A combinatory effect of *Streptococcus mutans*, *Streptococcus oralis*, *Streptococcus salivarius* and *Moraxella catarrhalis*, at $\geq 10^6$ CFU/mL of SB or the presence of *Streptococcus dysgalactiae* subsp. *dysgalactiae*, at $\geq 10^6$ CFU/mL of SB, may have an inhibitory effect on the detection of *S. pyogenes* ATCC® 12344™. These results are noted in the device labeling.

2. Comparison Studies
   a. **Method Comparison with predicate device**
      Not applicable.

   b. **Matrix Comparison**
      Not applicable.

3. Clinical Studies
   GenePOC conducted a prospective multicenter trial at eight (8) geographically diverse clinical trial sites (seven (7) collecting sites and one (1) reference center), two (2) in Canada and six (6) in United States. Throat swab specimens were collected from patients with signs and symptoms of pharyngitis. Samples were tested with both the Reference Method (culture) and the GenePOC™ Strep A. Out of seven hundred and sixty-seven (767) enrolled specimens, one hundred and sixty-three (163) specimens were excluded: three (3) failed to comply with the revogene testing procedures, thirteen (13) exceeded the delay in shipment, twenty-three (23) did not meet inclusion criteria, twenty-seven (27) did not have complete testing results, and ninety-seven (97) failed to comply with the reference culture protocol. Six hundred and four (604) fully compliant samples were tested with both the Reference Method and the GenePOC™ Strep A assay for the performance estimation study. A total of three (3) lots of the GenePOC™ Strep A assay kits were used for testing. Because the primary objective of the trial was to establish the performance of the system**,** the results were not used in the patient management for *S. pyogenes* infection. This was done according to hospital standard operating practices in place at each institution.

   The GenePOC™ Strep A assay sensitivity and specificity on the six hundred and four (604) prospective specimens were 98.1% (151/154) and 94.7% (426/450) respectively.

   Overall performance (all sites combined) with the GenePOC™ Strep A assay in comparison with the Reference Method

All sites		**Reference Method**		**Total**
		Positive	**Negative**	
GenePOC™ Strep A test	**Positive**	151	24[a]	175
	Negative	3[b]	426	429
	Total	154	450	604
Sensitivity	98.1% (151/154)		[94.4% - 99.3%]	
Specificity	94.7% (426/450)		[92.2% - 96.4%]	
PPV	86.3% (151/175)		[80.4% - 90.6%]	
NPV	99.3% (426/429)		[98.0% - 99.8%]	
Prevalence	25.5% (154/604)		[22.2% - 29.1%]	

Numbers between brackets indicate Wilson Score 95% CI

[a] Results from alternative PCR with bi-directional sequencing: 17 of 24 were Strep A Positive; 6 of 24 were Strep A Negative and 1 was inconclusive.

[b] Results from alternative PCR with bi-directional sequencing: 1 of 3 was Strep A Positive and 2 were inconclusive.

4. Clinical Cut-off
   Not applicable.

5. Expected Values/Reference Range
   GenePOC conducted a multi-site prospective investigational study at eight (8) geographically diverse clinical sites (seven (7) collection centers and one (1) reference center) at which testing for *S. pyogenes* was performed with culture and the GenePOC™ Strep A assay. Per the CLSI Guideline EP28-A3c, *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory*, GenePOC calculated the prevalence of *S. pyogenes* using the culture positive results from the Clinical Performance Study. The overall *S. pyogenes* prevalence rate as determined by reference method was 25.5% (154/604).

O. INSTRUMENT NAME

revogene™

P. SYSTEM DESCRIPTIONS

1. Modes of Operation:
   Real-time polymerase chain reaction with fluorogenic detection of amplified DNA.

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:
   Barcodes are used to identify patient specimens. The GenePOC™ Strep A assay's SBT and PIE are both pre-labeled with a unique barcode to identify both specimen and assay. The instrument has two barcode readers to identify reagents and patient specimens. It provides traceability of the sample ID to the PIE ID, SBT ID, and assay ID.

4. Specimen Sampling and Handling:
   User intervention is required for discharging the patient sample into the SBT, transferring the sample into the microfluidic cartridge using the DTT and for loading the microfluidic cartridge into the revogene™. All further specimen handling is automated.

5. Calibration:
   The system is factory calibrated by the manufacturer. The calibration is verified annually. Upon the verification, maintenance is performed if required.

6. Quality Control:
   A PrC is provided in each microfluidic cartridge of the GenePOC™™ Strep A assay. The PrC is lysed, amplified, and detected along with each specimen tested and verifies the

efficacy of the DNA extraction and PCR amplification processes. Commercially available strains (e.g., ATCC® 12344™ and ATCC® 13419™) can be used as a Positive and Negative External Controls, respectively.

Q. OTHER SUPPORTIVE INSTRUMENT PERFORMANCE CHARACTERISTICS DATA NOT COVERED IN THE "PERFORMANCE CHARACTERISTICS" SECTION

Not applicable.

R. PROPOSED LABELING

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

S. CONCLUSION

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