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

I Background Information:

A 510(k) Number

K260333

B Applicant

Diasorin Molecular, LLC

C Proprietary and Established Names

LIAISON NES Group A Strep; LIAISON NES Group A Strep Control Swab Kit

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PGX	Class II	21 CFR 866.2680 – Streptococcus spp. nucleic acid-based assay	MI - Microbiology
OOI	Class II	21 CFR 862.2570 – Instrumentation for clinical multiplex test systems	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

A Dual Submission to obtain 510(k) clearance and CLIA Waiver for the LIAISON NES Group A Strep and LIAISON NES Group A Strep Control Swab Kit.

B Measurand:

Group A *Streptococcus* (GAS; *Streptococcus pyogenes*) nucleic acid

C Type of Test:

Qualitative real-time polymerase chain reaction (PCR)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

LIAISON NES Group A Strep:
The LIAISON NES Group A Strep is a real-time PCR assay intended for use on the LIAISON NES instrument for the *in vitro* qualitative detection of *Streptococcus pyogenes*, Group A *Streptococcus* (GAS) bacterial nucleic acid in throat swab specimens freshly collected from human patients with signs and symptoms of pharyngitis. This test is intended for use as an aid in the rapid diagnosis of Group A *Streptococcus* bacterial infections.

LIAISON NES Group A Strep Control Swab Kit:
The LIAISON NES Group A Strep Control Swab Kit is intended to be used as positive and negative controls with LIAISON NES Group A Strep assay for use on the LIAISON NES instrument. These controls are not intended for use with other assays or systems.

C Special Conditions for Use Statement(s):

Rx – For Prescription Use Only

D Special Instrument Requirements:

To be used only with LIAISON NES Instrument

IV Device/System Characteristics:

A Device Description:

The LIAISON NES Group A Strep is a real-time polymerase chain reaction (PCR) based test intended for the qualitative detection of nucleic acid from *Streptococcus pyogenes* (Group A Strep) in direct, freshly collected throat swabs. The system consists of the LIAISON NES instrument, a sample collection swab, a sample vial, and a test cartridge. The swab is used for sample collection and inserted into the sample vial. The cartridge is preloaded with primers, fluorescent probe, and internal controls. The user prepares the specimen and loads the cartridge into the instrument. The instrument, a PCR thermocycler, is used for the automated amplification and identification of nucleic acid from biological specimens. After the user starts the assay via the touchscreen graphical user interface (GUI), the assay automatically starts the run to generate a test result.

B Principle of Operation:

The LIAISON NES Group A Strep assay consists of the LIAISON NES instrument, LIAISON NES Group A Strep Cartridge containing all the required PCR reagents, a sample vial containing working solution, and a swab for throat sample collection. Following collection by a healthcare professional, the throat swab is directly placed into the sample vial. The sample is released into the working solution, and the entire contents of the vial are transferred into the LIAISON NES

Group A Strep Cartridge. The cartridge is then loaded on the LIAISON NES instrument for subsequent analysis and result generation.

The LIAISON NES Group A Strep assay utilizes dye-labeled fluorescent probes and primers to amplify and detect Group A *Streptococcus* bacterial DNA and internal control (IC) DNA. Conserved regions of Group A *Streptococcus* genome are targeted to identify the bacteria in the specimen, while the IC DNA is used to detect any PCR failures and/or inhibition.

The LIAISON NES Instrument is capable of analysis of a single cartridge containing a single specimen. A set of parameters specific to the assay is included in the instrument software to name target molecules, assign dyes to probes, specify cycling conditions, and analyze data from runs. Fluorescence intensity is monitored at each PCR cycle by detection modules within the instrument. The instrument software controls the thermocycling and, upon completion of the run, automatically interprets and displays results for the specimen.

The instrument incorporates algorithms for processing data and displays results on the graphical user interface (GUI) after completion of the test. The system produces a "Positive", "Negative" or "Invalid" result readout for each target in the assay that is displayed on the GUI.

The LIAISON NES software is a GUI application that is the end-user interface to the LIAISON NES Instrument. The software is installed in an embedded computer. The LIAISON NES software is responsible for providing the environment in which a user runs assays and obtains results.

The LIAISON NES Group A Strep (GAS) Control Swab Kit is provided separately for external controls and is intended to be used as positive and negative controls with LIAISON NES Group A Strep for use on the LIAISON NES instrument. The Control Swab Kit includes: a negative control swab intended to monitor for possible contamination and a positive control swab used for a routine check on the system. The positive control consists of a dry swab coated with inactive lyophilized bacterial cells of *S. pyogenes* (GAS) at a low positive concentration. Quality control result readouts are "Pass" and "Fail".

C Instrument Description Information:

1. Instrument Name:
   LIAISON NES Instrument

2. Specimen Identification:
   The instrument incorporates a barcode reader to scan in specimen identification. Specimen identification may also be entered manually using an on-screen keyboard.

3. Sampling and Handling:
   The NES Swab is utilized by a healthcare provider to collect a throat swab from the patient. After collecting, the fresh throat swab is inserted into the sample vial provided with the kit and mixed by rotating the swab within the sample vial while squeezing the swab head to release the sample. The swab shaft is snapped off at a pre-defined mark on the swab shaft. The sample vial is re-capped and the cap at the end of the sample vial is removed. The sample vial is inserted into the sample port of the cartridge, and the complete contents are

squeezed into the cartridge. The user closes the cartridge lid and inserts the loaded cartridge into the instrument.

4. Calibration:
   The instrument is factory calibrated. No user calibration is performed.

5. Quality Control:
   Refer to Section VII.A.6.A for Quality Control information.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Simplexa Group A Strep Direct and Simplexa Group A Strep Positive Control Pack

B Predicate 510(k) Number(s):

K143651

C Comparison with Predicate(s):

Device & Predicate Device(s):	Device: K260333	Predicate: K143651
Device Trade Name	LIAISON NES Group A Strep and LIAISON NES Group A Strep Control Swab Kit	Simplexa Group A Strep Direct and Simplexa Group A Strep Positive Control Pack
General Device Characteristic Similarities		
Intended Use/Indications For Use	The LIAISON NES Group A Strep is a real-time PCR assay intended for use on the LIAISON NES instrument for the *in vitro* qualitative detection of *Streptococcus pyogenes*, Group A *Streptococcus* (GAS) bacterial nucleic acid in throat swab specimens freshly collected from human patients with signs and symptoms of bacterial pharyngitis. This test is intended for use as an aid in the rapid diagnosis of Group A *Streptococcus* bacterial infections. The LIAISON NES Group A Strep Control Swab Kit is intended to be used as positive and negative controls with the	The Focus Diagnostics Simplexa Group A Strep Direct assay is intended for use on the 3M Integrated Cycler for the *in vitro* qualitative detection of Group A *Streptococcus* (GAS) from throat swabs collected from human patients with signs and symptoms of pharyngitis, such as sore throat. This test is intended for use as an aid in the diagnosis of GAS infection. The assay is intended for use in hospital, reference, or state laboratory settings. The device is not intended for point-of-care use.

Device & Predicate Device(s):	Device: K260333	Predicate: K143651
	LIAISON NES Group A Strep assay for use on the LIAISON NES instrument. These controls are not intended for use with other assays or systems.	Focus Diagnostics' Simplexa Group A Strep Positive Control Pack is intended to be used as a control with Simplexa Group A Strep Direct. This control is not intended for use with other assays or systems.
Measurand	*Streptococcus pyogenes (*Group A *Streptococcus)* nucleic acid	Same
Intended Use Population	Patients with signs and symptoms of pharyngitis	Same
Assay Technology	Real-time polymerase chain reaction	Same
Assay Result	Qualitative	Same
Result Interpretation	Automated	Same
General Device Characteristic Differences		
Sample Type	Freshly collected throat swab (direct testing)	Throat swab in Amies Medium
Time to Result	~ 18 minutes	~ 60 minutes
Instrumentation	LIAISON NES	3M Integrated Cycler
Technological Principle	Dye-labeled fluorescent probes and primers amplify and detect Group A Strep bacterial DNA and internal control (IC) DNA. Utilizes two separate genetic region targets in GAS chromosome.	Bi-functional fluorescent probe-primers used together with corresponding reverse primers amplify Group A Strep bacterial DNA and Internal Control (DNA IC). Utilizes one genetic region target in GAS chromosome.

VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3, *Evaluation of Precision of Quantitative Measurement Procedures*, Third Edition. Wayne, PA: Clinical Laboratory Standards Institute; 2019.
- CLSI EP07, *Interference Testing in Clinical Chemistry*, Third Edition. Wayne, PA: Clinical Laboratory Standards Institute; 2018.
- CLSI EP12, *Evaluation of Qualitative, Binary Output Examination Performance,* Third Edition. Wayne, PA: Clinical Laboratory Standards Institute; 2023.
- CLSI EP15-A3, *User Verification of Precision and Estimation of Bias*, Third Edition. Wayne, PA: Clinical Laboratory Standards Institute; 2019.
- CLSI EP17-A2, *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures*, Second Edition. Wayne, PA: Clinical Laboratory Standards Institute; 2012.

- CLSI EP25-A, *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*. Wayne, PA: Clinical Laboratory Standards Institute; 2009.
- CLSI EP35, *Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures,* 1st Ed. Wayne, PA: Clinical Laboratory Standards Institute; 2019.
- CLSI MM13, *Collection Transport Preparation and Storage of Specimens for Molecular Methods. 2nd Edition*, Wayne, PA: Clinical Laboratory Standards Institute; 2020.
- CLSI MM17, *Verification and Validation of Multiplex Nucleic Acid Assays. 2nd Edition,* Wayne, PA: Clinical Laboratory Standards Institute; 2018.
- IEC 62366-1 Edition 1.1 2020-06 *Consolidated Version; Medical devices – Part 1: Application of usability engineering to medical devices*
- ISO 14971:2019 *Medical Devices – Application of risk management to medical devices*
- ISO 15223-1: 2021-07 *– Medical Devices- Symbols to be used with information to be supplied by the manufacturer – Part 1: General requirements*

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Reproducibility

The reproducibility of the LIAISON NES Group A Strep device was assessed using a panel of contrived positive and negative samples tested at three CLIA-waived sites by two non-laboratory operators at each site. Positive samples were contrived using two strains of *Streptococcus pyogenes* (*S. pyogenes* M1 [ATCC 700294] and *S. pyogenes* M3 ([ATCC BAA-595]) spiked into simulated throat sample negative matrix. Each strain was spiked onto dry swabs at two different concentrations relative to the limit of detection (LoD) as established for each strain: low positive (~1 × LoD) and moderate positive (~3 × LoD). Negative samples were prepared using simulated throat sample negative matrix only. LIAISON NES Group A Strep Positive Control Swabs were also included in testing (**Table 1**). Each operator tested each blinded panel member in three replicates each day for a total of five testing days using one lot of LIAISON NES Group A Strep Cartridges and one lot of external controls. A total of ninety (90) replicates (3 replicates × 2 operators × 3 sites × 5 days) were evaluated for each panel member. A total of fourteen LIAISON NES instruments, with at least two per site, were used to evaluate reproducibility. Reproducibility results for LIAISON NES Group A Strep assay are reported as percent detection (# positive/ # tested × 100%) as summarized in **Table 2**. Agreement between observed and expected results was acceptable (>95%).

Table 1. Reproducibility Panel Members

Panel Member	Strain	Concentration
M1 Low Positive	*S. pyogenes* M1 (ATCC 700294)	~1 × LoD
M1 Medium Positive		~3 × LoD
M3 Low Positive	*S. pyogenes* M3 (ATCC BAA-595)	~1 × LoD
M3 Medium Positive		~3 × LoD

Panel Member	Strain	Concentration
Negative Sample	Simulated Negative Throat Matrix only	-
Positive Control	LIAISON NES Group A Strep Positive Control	-

Table 2. Reproducibility of LIAISON NES Group A Strep

Panel Member	% Detection (# Positive / # Tested)				Overall Percent Agreement (95% CI)
	Site 1	Site 2	Site 3	All Sites Combined	
M1 Low Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (95.9% - 100.0%)
M1 Medium Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (95.9% - 100.0%)
M3 Low Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (95.9% - 100.0%)
M3 Medium Positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (95.9% - 100.0%)
Negative Sample	0% (0/30)	0% (0/30)	0% (0/30)	0% (0/30)	100% (95.9% - 100.0%)
Positive Control	100% (30/30)	100% (30/30)	100% (30/30)	100% (30/30)	100% (95.9% - 100.0%)

Within-Laboratory Precision

The within-laboratory, inter-lot precision of the LIAISON NES Group A Strep assay device was assessed using the same sample panel used for reproducibility as shown in **Table 1**. One (1) operator tested three (3) different lots of LIAISON NES Group A Strep Cartridges on three (3) LIAISON NES instruments over the course of eight (8) days with two (2) replicates per day at one (1) internal site. A total of 240 replicates were tested to evaluate combined lot-to-lot reproducibility of the cartridges and instruments. Results are reported as percent detection (positive/ # tested × 100%) as summarized in **Table 3**. Agreement between observed and expected results was acceptable (>95%).

Table 2. Precision of LIAISON NES Group A Strep

Panel Member	% Detection (# Positive / # Tested)	% Agreement
M1 Low Positive	100% (40/40)	100%
M1 Medium Positive	100% (40/40)	100%
M3 Low Positive	100% (40/40)	100%
M3 Medium Positive	100% (40/40)	100%
Negative Sample	0% (0/40)	100%
Positive Control	100% (40/40)	100%

2. Linearity:

Not Applicable.

3. Analytical Sensitivity (Inclusivity):

Wet Testing
The analytical reactivity of the LIAISON NES Group A Strep was evaluated with 15 strains of *S. pyogenes*. Each strain was tested initially in triplicate using 90 CFU/swab (i.e. 3 × LoD level established for *S. pyogenes* strain M3 ATCC BAA-595). Contrived positive samples were prepared by diluting *S. pyogenes* in simulated negative throat matrix and spiking onto a dry swab. The study was designed such that the concentrations for each strain not detected at the initial 90 CFU/swab were increased until 100% detection was obtained. This additional testing was required for 8 of the 15 strains tested. The concentrations tested and results are summarized in **Table 4.**

Table 4. Inclusivity of LIAISON NES Group A Strep

Strep A Strain	Concentration Tested	% Detection (# Positive/# Tested)
M2 (MGAS 10270)	90 CFU/swab	100% (3/3)
M4 (MGAS 10750 [FL01-86])	240 CFU/swab	100% (3/3)
M6 (MGAS 10394)	150 CFU/swab	100% (3/3)
Bruno [CIP 104226]	90 CFU/swab	100% (3/3)
M12 (MGAS 9429)	90 cps/swab	100% (3/3)
M18 (MGAS 8232)	90 cps/swab	100% (3/3)
M58 (CDC-SS-872 [R67/3884])	240 CFU/swab	100% (3/3)
6 (Typing strain S43 [Dochez and Avery S43 (Texas)])	90 CFU/swab	100% (3/3)
23 (Typing strain T23 [F. Griffith strain Barts 102])	150 CFU/swab	100% (3/3)
46 (C105 [20RS14])	90 CFU/swab	100% (3/3)
17 (Typing strain J17E [A. Coburn R9])	240 CFU/swab	100% (3/3)
26 (Typing Strain J17F [A. Coburn R17])	150 CFU/swab	100% (3/3)
40 (Typing strain C143 [C143])	90 CFU/swab	100% (3/3)
M-3 [DLS 88002, Weller]	150 CFU/swab	100% (3/3)
14 [P20080]	240 CFU/swab	100% (3/3)

In silico Analysis
An *in silico* evaluation was performed to assess the predicted reactivity of the LIAISON NES Group A Strep assay with a broad spectrum of *S. pyogenes* isolates. *S. pyogenes* whole genome sequences available in the NCBI GenBank nucleotide database as of August 30, 2025, were analyzed. A total of 930 *Streptococcus pyogenes* sequences with coverage of the assay's primers' sequences were assessed. Based on sequence homology, it was predicted that 100% of the analyzed sequences are detectable by the assay's primers and probes. Based on *in silico* exclusivity analysis, the assay oligos are predicted to have no cross-reactivity to

human genome sequences from the *Homo sapiens* reference genome GRCh38.p14 (Genome Reference Consortium, 2022).

4. Analytical Specificity/Interference:

A. Cross-Reactivity and Microbial Interference

A cross-reactivity (analytical specificity) study was conducted with the LIAISON NES Group A Strep to evaluate potential cross-reactivity with related bacteria, high-prevalence pathogenic microorganisms or normal flora that are reasonably expected to be present in the clinical sample. In this study, forty-seven (47) microorganisms were tested. Each test sample was prepared by spiking dry swabs with microorganisms at concentrations of at least 10^6 CFU/mL for bacteria and fungi and at least 10^5 $TCID_{50}$/mL for viruses. Microorganisms were prepared in simulated negative matrix prior to spiking. All samples were tested in triplicate and evaluated by three operators using two (2) lots of LIAISON NES Group A Strep Cartridge (**Table 5**).

A microbial interference (exclusivity) study was conducted with the LIAISON NES Group A Strep to evaluate the device's ability to exclusively detect Group A *Streptococcus*. This study utilized the same 47 microorganisms tested in the cross-reactivity study. Microorganisms were prepared in simulated negative matrix at the same concentrations as the cross-reactivity study and tested in triplicate in the presence of *S. pyogenes* M1 ATCC 700294 or *S. pyogenes* M3 ATCC BAA-595 at 3 × LoD. Samples were spiked into dry swabs, tested in triplicate, and evaluated by three operators using two (2) lots of LIAISON NES Group A Strep Cartridge (**Table 5**).

External negative and positive controls were run once per day, per instrument, during testing. All control results were acceptable.

No cross-reactivity or microbial interference was observed for the LIAISON NES Group A Strep assay with the microorganisms at the concentrations tested and the results are acceptable.

Table 5. Cross-reactivity & Microbial Interference of LIAISON NES Group A Strep

Organism	% Detection (# Positive/# Tested)		
	Absence of Strep A	**Presence of Strep A M1** (3 × LoD)	**Presence of Strep A M3** (3 × LoD)
Adenovirus Type 1	0% (0/3)	100% (3/3)	100% (3/3)
Arcanobacterium haemolyticum	0% (0/3)	100% (3/3)	100% (3/3)
Bacillus cereus	0% (0/3)	100% (3/3)	100% (3/3)
Bordetella pertussis	0% (0/3)	100% (3/3)	100% (3/3)
Burkholderia cepacia	0% (0/3)	100% (3/3)	100% (3/3)
Campylobacter rectus	0% (0/3)	100% (3/3)	100% (3/3)
Candida albicans	0% (0/3)	100% (3/3)	100% (3/3)
Corynebacterium diphtheriae	0% (0/3)	100% (3/3)	100% (3/3)
Enterococcus faecalis	0% (0/3)	100% (3/3)	100% (3/3)

Organism	% Detection (# Positive/# Tested)		
	Absence of Strep A	**Presence of Strep A M1** (3 × LoD)	**Presence of Strep A M3** (3 × LoD)
Escherichia coli	0% (0/3)	100% (3/3)	100% (3/3)
Fusobacterium necrophorum	0% (0/3)	100% (3/3)	100% (3/3)
Haemophilus influenzae	0% (0/3)	100% (3/3)	100% (3/3)
Hoylesella oralis	0% (0/3)	100% (3/3)	100% (3/3)
Human Influenza virus A	0% (0/3)	100% (3/3)	100% (3/3)
Human Influenza virus B	0% (0/3)	100% (3/3)	100% (3/3)
Human metapneumovirus-9	0% (0/3)	100% (3/3)	100% (3/3)
Klebsiella pneumoniae	0% (0/3)	100% (3/3)	100% (3/3)
Lactobacillus acidophilus	0% (0/3)	100% (3/3)	100% (3/3)
Lactococcus lactis	0% (0/3)	100% (3/3)	100% (3/3)
Legionella longbeachae	0% (0/3)	100% (3/3)	100% (3/3)
Moraxella catarrhalis	0% (0/3)	100% (3/3)	100% (3/3)
Mycoplasma pneumoniae	0% (0/3)	100% (3/3)	100% (3/3)
Neisseria gonorrhoeae	0% (0/3)	100% (3/3)	100% (3/3)
Parainfluenza Type 3	0% (0/3)	100% (3/3)	100% (3/3)
Peptostreptococcus micros	0% (0/3)	100% (3/3)	100% (3/3)
Pseudomonas aeruginosa	0% (0/3)	100% (3/3)	100% (3/3)
Respiratory syncytial virus Type B-9320	0% (0/3)	100% (3/3)	100% (3/3)
Rhinovirus 1A	0% (0/3)	100% (3/3)	100% (3/3)
Saccharomyces cerevisiae	0% (0/3)	100% (3/3)	100% (3/3)
Staphylococcus epidermidis	0% (0/3)	100% (3/3)	100% (3/3)
Stenotrophomonas maltophilia	0% (0/3)	100% (3/3)	100% (3/3)
Streptococcus agalactiae	0% (0/3)	100% (3/3)	100% (3/3)
Streptococcus anginosus	0% (0/3)	100% (3/3)	100% (3/3)
Streptococcus bovis	0% (0/3)	100% (3/3)	100% (3/3)
Streptococcus canis	0% (0/3)	100% (3/3)	100% (3/3)
Streptococcus constellatus subsp. *pharyngis*	0% (0/3)	100% (3/3)	100% (3/3)
Streptococcus dysgalactiae subsp. *equisimilis*	0% (0/3)	100% (3/3)	100% (3/3)
Streptococcus gallolyticus	0% (0/3)	100% (3/3)	100% (3/3)
Streptococcus intermedius	0% (0/3)	100% (3/3)	100% (3/3)
Streptococcus mitis	0% (0/3)	100% (3/3)	100% (3/3)
Streptococcus mutans	0% (0/3)	100% (3/3)	100% (3/3)
Streptococcus oralis	0% (0/3)	100% (3/3)	100% (3/3)
Streptococcus pneumoniae	0% (0/3)	100% (3/3)	100% (3/3)
Streptococcus salivarius	0% (0/3)	100% (3/3)	100% (3/3)
Streptococcus sanguinis	0% (0/3)	100% (3/3)	100% (3/3)
Treponema denticola	0% (0/3)	100% (3/3)	100% (3/3)
Veillonella parvula	0% (0/3)	100% (3/3)	100% (3/3)

CFU = Colony Forming Unit, $TCID_{50}$ = Median Tissue Culture Infectious Dose, CPS = copies

In silico analysis was also performed to evaluate potential cross-reactivity with the LIAISON NES Group A Strep assay. Exclusivity assessment of the oligo sequences incorporated in the assay were queried against off panel organisms (**Table 6**) based on sequences available in the NCBI GenBank nucleotide database as of October 18, 2025. The assay oligos are predicted to have no cross-reactivity to these off-panel organisms.

Table 6. *In Silico* Analysis for Potential Cross-reactive or Interfering Organisms

Bacteria/Fungi		Viruses
Acinetobacter baumannii	*Neisseria subflava*	Alphapapillomavirus
Arcanobacterium haemolyticum	*Parvimonas micra*	Coronavirus 229E
Bacillus cereus	*Peptostreptococcus* spp.	Coronavirus HKU1
Bacteroides ovatus	*Proteus mirabilis*	Coronavirus NL63
Bordetella bronchiseptica	*Proteus vulgaris*	Coronavirus OC43
Bordetella parapertussis	*Pseudomonas* spp.	Cytomegalovirus
Bordetella pertussis	*Saccharomyces cerevisiae*	Enterovirus A
Burkholderia cepacia	*Serratia marcescens*	Enterovirus B
Campylobacter rectus	*Staphylococcus aureus*	Enterovirus C
Candida spp.	*Staphylococcus epidermidis*	Enterovirus D
Chlamydia pneumoniae	*Staphylococcus haemolyticus*	Epstein-Barr virus
Chlamydia trachomatis	*Stenotrophomonas maltophilia*	Hepatitis B Virus
Corynebacterium diphtheriae	*Streptococcus* spp. (excluding *S. pyogenes*)	Human alphaherpesvirus 1 (HSV-1)
Corynebacterium pseudodiphtheriticum	*Treponema denticola*	Human alphaherpesvirus 2 (HSV-2)
Enterococcus spp.	*Veillonella parvula*	Human influenza virus A
Escherichia coli	*Yersinia enterocolitica*	Human influenza virus B
Fusobacterium necrophorum		Human mastadenovirus A
Haemophilus influenzae		Human mastadenovirus B
Haemophilus parahaemolyticus		Human mastadenovirus C
Haemophilus parainfluenzae		Human mastadenovirus D
Hoylesella oralis		Human mastadenovirus E
Klebsiella spp.		Human mastadenovirus F
Lactobacillus acidophilus		Human mastadenovirus G
Lactococcus lactis		Human metapneumovirus
Legionella spp.		Human parainfluenza virus 1
Listeria monocytogenes		Human parainfluenza virus 2
Moraxella catarrhalis		Human parainfluenza virus 3
Moraxella lacunata		Human parainfluenza virus 4
Mycoplasma pneumoniae		Respiratory syncytial virus A
Neisseria gonorrhoeae		Respiratory syncytial virus B
Neisseria lactamica		Rhinovirus A
Neisseria meningitidis		Rhinovirus B
Neisseria mucosa		Rhinovirus C
Neisseria sicca		SARS-CoV-2

B. Interfering Substances

An interfering substances study was performed to evaluate the performance of the LIAISON NES Group A Strep device in the presence of potentially interfering substances which may be present in throat swab specimens. Potential interfering substances were tested in triplicate at clinically relevant concentrations in the absence or presence of *S. pyogenes* M1 ATCC 700294 or M3 ATCC BAA-595 at 3 × LoD. Samples were prepared in the simulated negative throat matrix and spiked onto a dry swab. A total of 19 interfering substances were tested by two operators (**Table 7**).

The interfering substances study demonstrated no inhibition or no interference on the performance of the LIAISON NES Group A Strep with the listed substances at the tested concentrations.

Table 7. Interfering Substances Evaluated with the LIAISON NES Group A Strep

Interfering Substances	Active Ingredient	Concentration Tested
Advil Liqui-Gels	Ibuprofen	8% (v/v)
Amoxicillin	Amoxicillin	0.8% (w/v)
Aspirin	Acetylsalicylic acid	0.62 mg/ml
Blood (Human)	N/A	4% (v/v)
Cepacol Extra Strength Sore Throat Lozenges	Benzocaine, Menthol	0.024% Benzocaine (w/v), 0.0058% Menthol (w/v)
Cephalexin	Cephalexin	0.2% (w/v)
Chloraseptic Max Sore Throat Spray	Phenol, Glycerin	8% (v/v)
Cool Mint Listerine antiseptic mouthwash	Eucalyptol, Menthol, Methyl Salicylate, Thymol	8% (v/v)
Crest Pro-Health	Stannous Fluoride	8% (v/v)
Food Dye Blue 1	N/A	8% (v/v)
Food Dye Yellow 5 (Tartrazina)	N/A	8% (v/v)
Halls	Menthol	8% (v/v)
Miralax	Polyethylene Glycol	2.43% (w/v)
Mucin: porcine stomach Type II	Purified mucin protein	0.8% (w/v)
Orange Juice	N/A	8% (v/v)
Penicillin G	Penicillin G Sodium Salt	0.8% (w/v)
Robitussin Maximum Strength Severe Multi-Symptom Cough + Flu	Acetaminophen, Dextromethorphan Guaifenesin	8% (v/v)
Sucrets Sore Throat & Cough	Dyclonine Hydrochloride, Menthol, Pectin	0.0048% Dyclonine, Hydrochloride (w/v), 0.012% Menthol (w/v), Pectin 0.014% (w/v)
Tums Ultra Strength	Calcium Carbonate	2.4 mg/ml

5. Detection Limit and Assay Reportable Range:

Limit of Detection (LoD)

The limit of detection (LoD) of the LIAISON NES Group A Strep was determined using contrived samples prepared by spiking two serotypes of *Streptococcus pyogenes*, M1 (ATCC 700294) and M3 (ATCC BAA-595), that were diluted into a pooled negative human throat matrix onto dry swabs. The LoD was defined as the lowest Strep A concentration detected ≥95% of the time (at least 19 out of 20 replicates testing positive). This study was performed using a two-step approach entailing a preliminary range-finding LoD study followed by LoD confirmation. Preliminary testing was conducted using a series of linear dilutions with five replicates per concentration for both Strep A strains. Confirmation studies were then performed with concentrations around the preliminary LoD using 20 replicates per concentration across three lots for a total of sixty (60) replicates per concentration for each Strep A strain. Based on the confirmation study results, the limit of detection was established as 90.7 CFU/swab (129.6 CFU/mL) for *S. pyogenes* M1 ATCC 700294 and 29.7 CFU/swab (42.4 CFU/ml) for *S. pyogenes* M3 ATCC BAA-595 (**Table 8**).

Table 8. Limit of Detection (LoD) for LIAISON NES Group A Strep

Strep A Strain	LoD (CFU/swab)[1]	LoD (CFU/ml)[2]
M1 ATCC 700294	90.7	129.6 CFU/ml
M3 ATCC BAA-595	29.7	42.4 CFU/ml

[1]Number of organisms (colony forming units, CFU) applied to swab prior to placing it into the NES Sample Vial.
[2]Final concentration of organisms after 10 µL of bacterial dilution is spiked onto the dry swab and released in 700 µL of working solution contained in NES Sample Vial (assuming 100% recovery of bacteria)

6. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

A. Quality Control

The LIAISON NES Group A Strep assay incorporates one internal control built-in in the LIAISON NES cartridge. Two external controls are provided separately as the LIAISON NES Group A Strep Control Swab Kit.

- *Internal Control:*

The LIAISON NES Group A Strep assay includes an internal control (IC) intended to indicate that the test is functioning as expected and to detect any PCR failures and/or inhibition. The assay cartridge contains dye-labeled fluorescent probes and primers to amplify and detect IC DNA (*Arabidopsis thaliana* template DNA). The dye-labeled fluorescent probe of internal control is detected on a separate channel than the *S. pyogenes* (GAS) target. Detection of the IC DNA is required for valid result generation in the absence of a positive ("Positive") call. Interpretation of results are shown in **Table 9**. An "Invalid" result indicates the inability to conclusively determine the presence or absence of *S. pyogenes* DNA in the sample and the inability to conclusively detect the presence of the IC. A new sample must be acquired and tested.

Table 9. Interpretation of results

Group A Strep DNA	Internal Control	Result Interpretation
Detected	Not Applicable	Positive
Not Detected	IC Detected	Negative
Not Detected	IC Not Detected	Result invalid; Repeat testing

- *External Controls (Positive and Negative)*

  External controls are provided separately in the LIAISON NES Group A Strep Control Swab Kit containing ten (10) LIAISON NES Group A Strep Positive Control Swabs and ten (10) LIAISON NES Group A Strep Negative Control Swabs. The LIAISON NES Group A Strep Positive Control Swab is a single-use swab (the same swab type as used for sample collection) coated with lyophilized, inactive GAS at a low positive concentration. The positive control mimics the intended specimen and confirms that the bacterial lysis and amplification reaction occur as expected during an assay run. The LIAISON NES Group A Strep Negative Control Swab is a single-use swab (the same swab type as used for sample collection) without sample or matrix. The negative control swab is intended to monitor for possible GAS contamination. The device instructions for use recommend that positive and negative external controls are tested when receiving a new lot of reagents or when a new operator uses the test before proceeding with patient sample testing to ensure the test is functioning as expected and the assay procedure is performed correctly. Control swabs are ready for use without any preparation and are tested following the same procedure used for patient samples.

B. Evaluation of Control Performance

The performance of the LIAISON NES Group A Strep Control Swab Kit was evaluated for a lot-to-lot variability. Three (3) different lots of each control swab were tested in four (4) replicates by one (1) operator over three (3) consecutive days generating a total of 72 test runs (3 lots × 2 control swabs × 4 replicates × 3 days = 72 runs). The performance of the controls is summarized in **Table 10**. All results were as expected.

Table 10. Performance of LIAISON NES Group A Strep Controls

Sample Type	Sample Lot	% Detection (# Positive /# Tested)	% Detection (# Positive /#Tested)
Positive Control	Lot 1	100% (12/12)	100% (12/12)
	Lot 2	100% (12/12)	100% (12/12)
	Lot 3	100% (12/12)	100% (12/12)
	All lots	100% (36/36)	100% (36/36)
Negative Control	Lot 1	0% (0/12)	100% (12/12)
	Lot 2	0% (0/12)	100% (12/12)
	Lot 3	0% (0/12)	100% (12/12)
	All lots	0% (0/36)	100% (36/36)

C. Sample Stability

The instructions for use (package insert) for the LIAISON NES Group A Strep state that once collected, throat swab samples can be stored inside the swab tube at room temperature for up to 2 hours from collection before processing. To evaluate sample stability, contrived swabs were prepared by spiking two different strains of *S. pyogenes* (M1 ATCC 700294 and M3 ATCC BAA-595) in pooled human negative throat swab matrix. Testing was performed after storing the prepared swabs at 30°C and 80% relative humidity for six (6) different timepoints: 0, 30, 60, 90, 120, and 150 minutes. Five (5) negative samples (10 µL of human negative throat swab matrix spiked on the dry swab), five (5) samples at 5 × LoD, and twenty (20) samples at 2 × LoD for each serotype of *S. pyogenes* were tested at each timepoint. Sample stability results are summarized in **Table 11**. The LIAISON NES Group A Strep assay demonstrated acceptable performance with swab samples stored inside the swab tube at room temperature for 2 hours from the collection time.

Table 11. Sample Stability test for LIAISON NES Group A Strep assay

Timepoints Tested	% Detection (# Positive /# Tested)				
	Negative Sample	M1 ATCC 700294		M3 ATCC BAA-595	
		2 × LoD	5 × LoD	2 × LoD	5 × LoD
0 minutes	0% (0/10)	100% (20/20)	100% (5/5)	100% (20/20)	100% (5/5)
30 minutes	0% (0/10)	100% (20/20)	100% (5/5)	100% (20/20)	100% (5/5)
60 minutes	0% (0/10)	100% (20/20)	100% (5/5)	100% (20/20)	100% (5/5)
90 minutes	0% (0/10)	100% (20/20)	100% (5/5)	100% (20/20)	100% (5/5)
120 minutes	0% (0/10)	100% (20/20)	100% (5/5)	100% (20/20)	100% (5/5)

7. Assay Cut-Off:

Not Applicable

8. Carryover/Cross-contamination

The carry-over and cross-contamination study for the LIAISON NES Group A Strep assay was assessed by testing alternating high positive samples and negative samples between successive runs on the LIAISON NES instrument. The high positive samples consisted of *S. pyogenes* M3 serotype (ATCC BAA-595) spiked onto NES swabs at 10^6 CFU/swab prepared in simulated negative matrix, while the negative samples used simulated negative matrix only spiked on NES swabs. No evidence of carry-over or cross-contamination was observed, demonstrating that alternating between highly positive and negative samples does not result in cross-contamination.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not Applicable

2. Matrix Comparison:

A matrix equivalency study was performed to evaluate the performance of samples prepared with human negative throat swab matrix and simulated negative matrix on the LIAISON NES Group A Strep. Simulated negative matrix was formulated to be representive of natural throat swab clinical matrix and consisted of IDTE buffer (10 mM Tris pH 8.0, 0.1 mM EDTA buffer) with mucin, NaCl, and Human Genome DNA. Human negative throat swab matrix was prepared by pooling throat swabs collected from healthy donors. Prior to the study, both matrices were screened to ensure they were negative for GAS bacteria. Each matrix was contrived with two *S. pyogenes* serotypes, M1 (ATCC 700294) and M3 (ATCC BAA-595), at concentrations of 2 × LoD, 5 × LoD, and 10 × LoD. In addition, ten (10) replicates of both matrices without adding GAS were tested as negative samples. Testing was conducted by two (2) operators over the course of two (2) non-consecutive days using one lot of LIAISON NES Group A Strep Cartridge, one lot of NES Sample Vial and one lot of NES Swab Tubes. The results are summarized in **Table 13**. The study results demonstrate equivalency between natural and simulated matrix and support the use of simulated matrix in select analytical studies.

Table 13. Matrix Equivalence of LIAISON NES Group A Strep

LoD Level	M1 (ATCC 700294)				M3 (ATCC BAA-595)			
	Simulated Negative Matrix		Human Negative Matrix		Simulated Negative Matrix		Human Negative Matrix	
	Positive /Tested	% Detection	Positive /Tested	% Detection	Positive /Tested	% Detection	Positive /Tested	% Detection
2 × LoD	30/30	100%	30/30	100%	30/30	100%	30/30	100%
5 × LoD	10/10	100%	10/10	100%	10/10	100%	10/10	100%
10 × LoD	5/5	100%	5/5	100%	5/5	100%	5/5	100%
Negative	0/10	0%	0/10	0%	0/10	0%	0/10	0%

C Clinical Studies:

1. Clinical Sensitivity and Specificity:

The clinical performance of the LIAISON NES Group A Strep assay was evaluated using clinical specimens prospectively collected between April 2025 and December 2025 across four geographically diverse clinical sites within the United States. The prospective specimens were collected from pediatric and adult patients exhibiting clinical signs and symptoms of pharyngitis. All collection and testing activities were performed at CLIA-waived facilities. Two throat swab specimens were collected from each participant: one throat swab for testing on the LIAISON NES Group A Strep and another throat swab placed in 1mL Copan liquid Amies for reference testing consisting of blood agar culture growth followed by latex agglutination and positive confirmation of GAS isolates by MALDI-TOF mass spectrometry.

Clinical sample testing using the LIAISON NES Group A Strep assay was performed by non-laboratory health professionals who are representative of typical intended use operators (e.g., nurses, medical assistants, research assistants, clinical coordinators). No assay-specific training was provided, and testing was performed using the Instructions for Use and Quick Reference Guides (QRI) provided prior to study initiation. Testing was performed within 2

hours of specimen collection. The second throat swab concurrently collected was shipped refrigerated (2-8°C) to the reference laboratory and reference culture method testing was initiated within 72 hours of collection.

A total of 1423 unique specimens were prospectively collected and 43 specimens were excluded (**Table 14**). There were no specimens excluded from data analysis due to protocol deviations. A total of 1380 specimens generated evaluable results for the final determination of performance.

Table 14. Summary of Excluded Specimens

Exclusion Reason	Excluded Specimens
Delay (>72 hrs) to start culture	13
Reference lab mislabeling	4
Indeterminate reference result	1
Inability to verify time window of collection	3
Error/Invalid results with LIAISON	22
Total Excluded Specimens	**43**

To establish clinical performance of the LIAISON NES Group A Strep assay, estimates of sensitivity, specificity, and negative predictive value (NPV) for the prospective data set were calculated based on comparison between the reference method result and the LIAISON NES Group A Strep assay result. Discordant analysis was performed when the LIAISON NES Group A Strep assay results were different from those of the reference assay. Discordant analysis consisted of testing with three FDA-cleared molecular assays intended to detect GAS from direct throat swab samples. The results from discordant analysis are added as footnotes to the performance table. The overall (all sites combined) performance of the LIAISON NES Group A Strep assay when compared to reference culture method is summarized in **Table 15**. Performance of the LIAISON NES Group A Strep assay when compared to the reference culture method was also evaluated by site, as shown in **Table 16**.

Table 15. Clinical Performance of LIAISON NES Group A Strep

All sites combined		Reference Culture Method		
		Positive	Negative	Total
LIAISON NES Group A Strep	Positive	104	53†	157
	Negative	0	1223	1223
	Total	104	1276	1380

Sensitivity	100% (104/104)	95% CI (96.44% - 100%)
Specificity	95.85% (1223/1276)	95% CI (94.61% - 96.81%)
PPV	66.24% (104/157)	95% CI (58.54% - 73.17%)
NPV	100% (1223/1223)	95% CI (99.69% - 100%)

† Discordant result analysis showed that Strep A was detected in 51/53 False Positive specimens by at least one of three FDA-cleared molecular assays.

Table 16. Performance of the LIAISON NES Group A Strep Stratified by Site

Site	Total	TP	FN	TN	FP	Sensitivity [95% CI]	Specificity [95% CI]
Site 1	330	21	0	296	13	100% (21/21) [84.54% - 100%]	95.79% (296/309) [92.94% - 97.53%]
Site 2	434	50	0	365	19	100% (50/50) [92.86% - 100%]	95.05% (365/384) [92.40% - 96.81%]
Site 3	379	20	0	347	12	100% (20/20) [83.89% - 100%]	96.66% (347/359) [94.25% - 98.08%]
Site 4	237	13	0	215	9	100% (13/13) [77.19% - 100%]	95.98% (215/224) [92.54% - 97.87%]
All Sites	1380	104	0	1223	53	100% (104/104) [96.44% - 100%]	95.85% (1223/1276) [94.61% - 96.81%]

TP = true positive, FN = false negative, TN = true negative, FP = false positive, CI = confidence interval

2. Clinical Cut-Off:

   Not Applicable

3. Other Clinical Supportive Data (When 1. Is Not Applicable):

Exclusion of culture confirmation of negative LIAISON NES Group A Strep results:

Support for the exclusion of culture confirmation of assay-negative GAS results from this assay was assessed using parameters and criteria as previously assessed for K141173. Briefly, the following criteria for sensitivity and negative predictive value (NPV) should be demonstrated to exclude the statement requiring follow-up culture for negative results in the Intended Use for GAS assays:

1) Overall sensitivity ≥98%, with the lower bound of the 95% CI of ≥93% from ≥ 100 positive specimens;
2) Overall NPV ≥99%, with the lower bound of the 95% CI of ≥97% extrapolated based on 30% prevalence (recent studies presented to the FDA indicated that it is unlikely that the prevalence for GAS will exceed 30% during the peak season for GAS pharyngitis);
3) Each testing site demonstrates an NPV of ≥98% and an approximately even distribution of samples is observed among the sites.

The overall sensitivity of the LIAISON NES Group A Strep assay is 100% with the lower bound of the 95% CI of 96.4%, the overall NPV is 100% with the lower bound of the 95% CI of 99.7% at a prevalence of 7.5% encountered in this study. When extrapolated to a prevalence of 30%, the overall NPV is 100%. Samples tested are approximately distributed evenly (17% to 31%) across the testing sites. Based on these values and the criteria noted above, there is no need to add a statement requiring follow-up culture for negative results in the Intended Use for this assay.

However, to further mitigate the risks of a false negative result, the following limitation is added to the limitation section of the package insert:

"*Additional follow-up testing using the culture method is required if the result is negative and clinical symptoms persist, or in the event of an acute rheumatic fever (ARF) outbreak*."

D Expected Values/Reference Range:

The clinical study analysis included results from 1380 throat swab specimens collected prospectively from four geographically diverse clinical sites within the United States between April 2025 and December 2025. The overall prevalence of *S. pyogenes* was 7.54% (104/1380) as determined by reference culture and the overall positivity was 11.4% (157/1380) as determined by the LIAISON NES Group A Strep assay.

E Other Supportive Instrument Performance Characteristics Data:

Not Applicable

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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