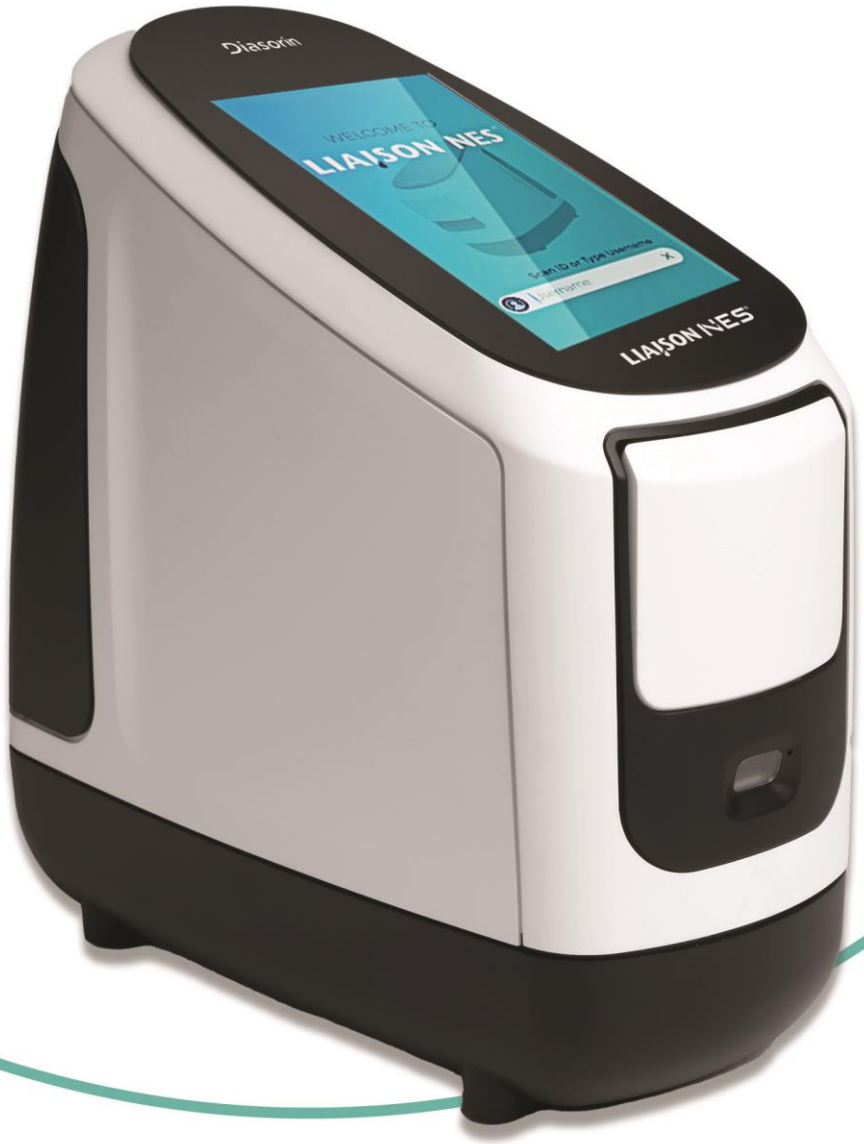
LIAISON NES®

Diasorin

LIAISON NES®
Group A Strep
Instructions for Use



REF NES2850

IVD Rx Only

For In Vitro Diagnostic Use.

CLIA Complexity: WAIVED*

* This statement only applies to customers within the United States:

For use with throat swab specimens.

A Certificate of Waiver is required to perform this test in a CLIA Waived setting. To obtain CLIA Waiver information and a Certificate of Waiver, please contact your state health department. Additional CLIA Waiver information is available at the Centers for Medicare and Medicaid website at www.cms.hhs.gov/CLIA.

Failure to follow the instructions or modification to the test system instructions will result in the test no longer meeting the requirements for waived classification.

INTENDED USE

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* 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.

SUMMARY AND EXPLANATION

The LIAISON NES® Group A Strep assay is a real-time PCR test used on the patented LIAISON NES® instrument to detect *Streptococcus pyogenes* bacterial nucleic acid in throat swab specimens. The LIAISON NES® system is capable of processing biological samples and performing nucleic acid amplification, with fluorometric detection of the resulting amplified products.

Streptococcus pyogenes, also known as Group A *Streptococcus*, is a beta-hemolytic bacterium that belongs to Lancefield serogroup A.[1] Group A *Streptococcus* is the major cause of bacterial acute pharyngitis accounting for 15-30% of the acute pharyngitis cases in children and 5-20% of the cases in adults.[2, 3] Acute pharyngitis can also be caused by viruses such as adenovirus, influenza virus, parainfluenza virus, rhinovirus, and respiratory syncytial virus.[4] The symptoms of bacterial and viral acute pharyngitis overlap broadly and can often be very difficult to differentiate. Identification of Group A *Streptococcus* infection allows the disease to be treated effectively with antibiotics. If left undiagnosed and untreated, Group A *Streptococcus* can cause a number of severe complications including acute rheumatic fever, scarlet fever, glomerulonephritis, and streptococcus toxic shock syndrome.[5, 6, 7] The gold standard for the diagnosis of streptococcal pharyngitis is throat swab culture.[8] However, culture requires 1-2 days to obtain a conclusive result. Rapid antigen detection tests (RADT) that target the presence of Group A Streptococcal carbohydrate on the cell surface can provide test results from a throat swab in 10-20 minutes. RADTs have been well characterized as having good specificity (95.0-98.7%) with lower sensitivity (90.6-97.6%) in comparison to culture, but the requirement for throat swab culture confirmation of negative results has been variable depending on performance.[9,10] Real-time PCR assays have been applied successfully for detecting Group A *Streptococcus* from throat swabs and some of these assays provide sensitivity and specificity levels comparable to that of culture.[11]

The LIAISON NES® Group A Strep assay is designed to significantly shorten turnaround time and can be performed outside of laboratories by facilities that obtain CLIA Certificate of Waiver. The LIAISON NES® Group A Strep assay is easy to perform and provides results in about eighteen minutes, allowing for fast and accurate detection of Group A *Streptococcus* without the need for culture confirmation.

PRINCIPLES OF THE PROCEDURE

The LIAISON NES® Group A Strep assay used on the LIAISON NES® instrument is a real-time PCR system that enables the direct amplification and detection of Group A *Streptococcus* bacterial nucleic acid in throat swab specimens.

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, NES Sample Vial containing working solution, and NES Swab for throat sample collection.

Following professional collection, the throat swab is placed into the NES 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.

In the LIAISON NES® Group A Strep assay, dye-labeled fluorescent probes and primers 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 internal control (IC) DNA is used to detect any PCR failures and/or inhibition.

MATERIALS PROVIDED

The LIAISON NES® Group A Strep (NES2850) assay box contains the following:

- (10) Pouched LIAISON NES® Group A Strep Cartridges (NES2851)
- (10) NES Swab for sample collection (NES4001)
- (10) Pouched NES Sample Vials, each containing one foil sealed vial with buffer and one cap (NES1502)
- (1) Printed copy of the Patient Test Quick Reference Guide

  **Use only with professionally collected throat swab specimens*

MATERIALS REQUIRED BUT NOT PROVIDED

- LIAISON NES® Group A Strep Control Swab Kit (NES2860) containing:
  - (10) LIAISON NES® Group A Strep Positive Control Swab (NES2861)
  - (10) LIAISON NES® Group A Strep Negative Control Swab (NES2862)
- Disposable, powder-free nitrile gloves
- Tongue depressor
- Biohazard waste container
- LIAISON NES® instrument (NES1001)

ASSAY DESCRIPTION

The LIAISON NES® Group A Strep assay contains 10 sets of components. Each set consists of 3 components and can be used to perform a single test. Upon receipt, store all components at the temperature range indicated on the label.

Component Name	REF	Number/Kit	Number Needed to Perform One Test
LIAISON NES® Group A Strep Cartridge	NES2851	10	1
NES Sample Vial	NES1502	10	1
NES Swab	NES4001	10	1

COMPONENT DESCRIPTION

Assay Component	Content
LIAISON NES® Group A Strep Cartridge	DNA polymerase, buffers, dNTPs, DNA fragment (internal control) template, dye-labeled fluorescent probes and primers specific for Group A *Streptococcus* and DNA internal control detection.
NES Sample Vial	Working solution containing rinsing buffer
NES Swab	Sterile swab

REAGENT HANDLING AND STORAGE

1. Store LIAISON NES® Group A Strep assay box containing test cartridges, NES swab, and sample vials at the temperature range indicated on the label.
2. Store the assay box away from sunlight.
3. Do not refrigerate or freeze the test cartridges, swabs, and/or sample vials.
4. Do not use assay or test cartridges beyond their expiration dates.
5. After opening the test cartridge from the pouch, use it within 2 hours (120 minutes). Perform the test immediately after preparing the cartridge.

WARNINGS AND PRECAUTIONS

1. Do not open the cartridge pouch until ready to use.
2. Consult the LIAISON NES® User Manual for additional warnings, precautions, and procedures related to the operation of the LIAISON NES® instrument.
3. Wear personal protective equipment, such as (but not limited to) gloves and lab coats when handling kit reagents and equipment. Wash hands thoroughly when finished performing the test.

4. Treat all human specimens and cartridges as potentially capable of transmitting infectious agents.
5. Do not smoke, drink, eat, handle contact lenses, or apply make-up in areas where kit reagents and/or human specimens are handled.
6. Dispose of used test cartridges, used sample vials, used swabs, and other collected specimens according to local, state, and federal regulations.
7. Contamination of patient samples or reagents can produce erroneous results. Handle samples and reagents according to standard laboratory practices.
8. The test should be performed at room temperature (Store all components at the temperature range indicated on the label).
9. Avoid touching, scratching, or tampering with the underside foil on the cartridge. Avoid scratching or tampering with the cartridge wells and reagents located on the bottom of the cartridge. Only open the cap to input the specimen. Do not attempt to break open the cartridge.
10. Before inserting a prepared cartridge (with sample loaded) into the instrument, fully close the cartridge cap.
11. Upon inhalation or skin contact with the reagents, first aid measures should be taken.
12. Refer to the Safety Data Sheet (SDS) of the product for additional information regarding emergency response and exposure measures.
13. Use only the intended sample vial provided in the kit to dispense specimen into the cartridge sample port.
14. Do not use kit or contents if kit packaging or contents appear broken or damaged. Contact our technical support team at (800) 838-4548.

INSTRUCTIONS FOR USE

To run a patient sample, please follow Instructions A and B as detailed below.

To run Quality Controls, please follow Instruction C.

In case of the first use of this device, Quality Controls (**positive and negative controls**) must be run as a first step, before proceeding with the test of a patient sample.

A. Sample Collection, Storage, and Transportation

Throat swab must be collected using the NES Swab provided with the kit by a healthcare provider. Follow the Merck Manual for collection of a throat swab specimen or follow the instructions provided below.

⚠ *Read the entire LIAISON NES® Group A Strep instructions for use (this document) before starting patient sample collection.*

Sample Collection:

1. Disinfect the surface where the procedure will be performed. Remove and lay out the contents of the LIAISON NES® Group A Strep Kit.
2. Wash hands with soap and water. If soap and water are unavailable, use hand sanitizer to disinfect hands.
3. When collecting the sample from a patient, wear powder-free nitrile gloves.
4. Remove the NES Swab from the package. Ensure swab head does not come into contact with gloves or work surfaces.
5. Press the tongue down using a tongue depressor.
6. Gently rub the swab against both tonsils and the posterior pharynx.
7. Place swab back inside the swab tube.

Sample Storage:

The sample, once collected, can be stored inside the swab tube at room temperature. Samples must be processed within 2 hours from collection.

Sample Transportation:

The sample can be transported inside the swab tube at room temperature.

B. Test Procedure *(refer to the quick reference guide provided with the kit for a pictorial depiction of the test procedure).*

1. Take a LIAISON NES® cartridge from a single test box and open the sample port.
2. Take a NES pre-filled sample vial from a single test box and remove the foil seal to open it.

   ⚠ *If any liquid spills or if a vial is leaking – DO NOT USE. Use a new sample vial.*

3. Insert the NES Swab used to collect the patient's sample into the sample vial and rotate the swab **ten (10) times while squeezing** the swab head.
4. After rotation, break the swab shaft at the red mark by snapping along the rim of the sample vial. *(Refer to the quick reference provided with the kit for a pictorial depiction.)*
5. Twist the blue cap onto the sample vial with the swab head inside.

   ⚠ *Dispose of the swab handle into biohazardous waste.*

6. Unscrew the clear nozzle cap of the sample vial.

   ⚠ *Place the cartridge on a hard, flat surface and keep level*.

7. Insert tip of the sample vial into the LIAISON NES® Cartridge sample port.
8. Firmly squeeze the sample vial to dispense ALL the liquid.

   ⚠ *Failure to transfer ALL the liquid into the cartridge can cause invalid results.*

9. Carefully remove the sample vial and close the cartridge securely.

⚠ *Dispose of the sample vial and clear nozzle cap into biohazard waste*.

⚠ *Keep cartridge upright - do not tilt or shake. Perform the test immediately.*

10. Log into the LIAISON NES® instrument.
11. Scan the patient ID barcode using the 'Barcode Scanning Window' on the LIAISON NES® instrument, or, alternatively, enter the patient's information manually. The door will open automatically.
12. Ensure the cartridge is labeled with the sample ID.
13. Insert the LIAISON NES® test cartridge fully into the LIAISON NES® instrument with specimen port facing outwards. *(Refer to the quick reference guide provided with this kit for a pictorial depiction.)*
14. Fully close the LIAISON NES® instrument door.
15. Run the test following on-screen instructions.
16. The door will automatically open when the test run is completed. Remove and dispose of the cartridge into the biohazard waste.
17. Close the door to view the test results.

C. Run Positive and Negative Quality Controls

LIAISON NES® Group A Strep Control Swab Kit (Part Number: NES2860) is needed to perform a quality control test.

1. The LIAISON NES® Group A Strep Control Swab Kit contains positive and negative control swabs. Follow the test procedure (instructions B described above) by using the positive or negative control swab instead of the NES Swab to successfully complete a quality control test.
2. Refer to the instructions for use documentation provided with the LIAISON NES® Group A Strep Control Swab Kit.

RESULT INTERPRETATION

Upon completion of a test run, results will display automatically.

Results	Interpretation
Positive	Result indicates the presence of Group A *Streptococcus* in the patient sample.
Negative	Result indicates the absence of Group A *Streptococcus* in the patient sample.
Invalid	Result indicates an inability to conclusively determine the presence or absence of Group A *Streptococcus* in the patient sample. Collect a fresh sample and re-test with a new test cartridge from the same kit or a new kit. If the problem persists, contact our Technical Support Team at our toll-free number (800) 838-4548.

The results are automatically uploaded to the Diasorin cloud (if connected), LIS (if connected), or can be

saved as a PDF on the USB key. Refer to the LIAISON NES® User Manual for additional details.

Results can be wirelessly printed to any printer connected within the network. Consult the LIAISON NES® User Manual to set up a printer.

INSTRUMENT ERRORS

In case of instrument errors, please refer to the LIAISON NES® instrument User Manual.

LIMITATIONS

1. For in vitro diagnostic use only.
2. For prescription use only.
3. This product can be used only with the LIAISON NES® System.
4. The detection of Group A *Streptococcus* nucleic acid is dependent upon proper sample collection, transport, handling, storage, and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results.
5. False-negative results may occur if: Group A *Streptococcus* is present at a level that is below the analytical sensitivity of the assay; genomic mutations, insertions, deletions, or rearrangements occur in the target regions; or if the test is performed very early in the course of illness.
6. Additional follow-up testing by culture is required if the LIAISON NES® Group A Strep Assay is negative and clinical symptoms persist, or in the event of an outbreak of acute rheumatic fever (ARF).
7. This test is a qualitative test and does not provide the quantitative value for the amount of Group A *Streptococcus* present in the specimen.
8. This test has been designed to detect Group A *Streptococcus*. This test does not detect other viral, fungal, or bacterial pathogens.
9. The performance of this test has not been established for immunocompromised individuals.
10. The performance of this test has not been established for patients without symptoms of pharyngitis infection.
11. The performance of this test has not been established for monitoring the treatment of Group A *Streptococcus* infection.
12. The performance of this test has not been established for use in donor screening tests.
13. This test is designed for use with freshly collected throat swab samples.
14. The performance of this test has not been established for screening blood or blood products for the presence of Group A *Streptococcus* or for use with samples other than NES swab specimens.
15. Positive and negative predictive values are dependent upon prevalence. The performance was established for the 2025 season. Performance may vary depending on the prevalence and the population tested.
16. This test cannot rule out diseases caused by other bacterial or viral agents.
17. Analyte targets (bacterial nucleic acid) may persist in vivo, independent of pathogen viability. Detection of analyte targets does not imply that the corresponding pathogen is infectious or is the causative agent for clinical symptoms.

PERFORMANCE CHARACTERISTICS

EXPECTED VALUES

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.

CLINICAL AGREEMENT

The clinical performance of the LIAISON NES® Group A Strep assay was evaluated using clinical specimens prospectively collected between April 2025 and December 2025 from four geographically diverse clinical sites within the United States. The clinical study utilized prospective specimens collected from pediatric and adult patients exhibiting clinical signs and symptoms of pharyngitis. Throat swabs were professionally collected by a Healthcare Provider. All collection and testing was performed at CLIA-waived facilities, representative of the intended use, point-of-care environment.

A total of 1423 unique prospectively collected specimens, collected from four geographically diverse US sites that met the pre-determined inclusion criteria, were enrolled in the study. Clinical sample testing using the LIAISON NES® Group A Strep assay was performed using the LIAISON NES® instrument by untrained operators at all four collection sites.

Out of the 1423 specimens enrolled in the prospective arm of the study, 21 (1.5%) specimens were disqualified and removed from further analysis. After a single test of each specimen, 1380 specimens generated valid LIAISON NES® Group A Strep assay results for a final success rate of 98.4% (1380/1402).

All clinical specimens were compared to culture followed by latex agglutination and positive confirmation by MALDI-TOF using an FDA-cleared IVD library. Comparator testing was performed at a single external reference lab. The diagnostic performance of the LIAISON NES® Group A Strep assay was determined using Sensitivity, Specificity, and Negative Predictive Value (NPV), along with the associated 95% confidence intervals. The overall (all sites combined) performance of the LIAISON NES® Group A Strep assay when compared to the reference culture method is summarized in **Table 1A**. Performance of the LIAISON NES® Group A Strep assay when compared to the reference culture method was also evaluated by sites, as shown in **Table 1B.**

Table 1 A. 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 1 B. Performance of the LIAISON NES Group A Strep Stratified by Sites

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

REPRODUCIBILITY

Site-to-site reproducibility of the LIAISON NES® Group A Strep assay was evaluated by testing LIAISON NES® Group A Strep assay cartridges with a reproducibility panel, blinded to operators, consisting of eight panel members including negative samples (simulated negative matrix (NTC)), positive control (PC) and six positive panel members. The six positive panel members consisted of contrived samples containing two strains of *Streptococcus pyogenes* individually. Each strain was spiked onto dry swabs at two different concentrations [Low Positive (LP) ~ 1x Limit of Detection (LoD), Moderate Positive (MP) ~ 3x LoD].

Each sample panel member was tested by two different operators at each testing site. Each operator tested each panel member in three replicates each day for a total of five testing days. A total of ninety replicates [three (3) replicates X two (2) operators X three (3) sites X five (5) days] were evaluated for each panel member. A total of fourteen LIAISON NES® instruments [at least two (2) per site] were used to evaluate the reproducibility study.

Table 1. Reproducibility Panel Member Details

Panel Member		Strain	Approximate Concentration	Replicates per day per operator	Total replicates per operator	Total replicates in study
1	*S. pyogenes* M1 LP	*S. pyogenes* M1 (ATCC 700294)	1X LoD	3	15	90
2	*S. pyogenes* M1 MP	*S. pyogenes* M1 (ATCC 700294)	3X LoD	3	15	90
3	*S. pyogenes* M3 LP	*S. pyogenes* M3 (ATCC BAA-595)	1X LoD	3	15	90
4	*S. pyogenes* M3 MP	*S. pyogenes* M3 (ATCC BAA-595)	3X LoD	3	15	90
5	Simulated Negative Matrix (NTC)	N/A	N/A	3	15	90
6	LIAISON NES Group A Strep (GAS) Positive Control (PC)	N/A	N/A	3	15	90
Total				**18**	**90**	**540**

LP = Low Positive, MP = Medium Positive, LoD = Limit of Detection, NTC = No Template Control, PC = Positive Control

Table 2. LIAISON NES® Group A Strep Reproducibility

	Site 1	Site 2	Site 3	All Sites
Panel Member	**Agreement with expected results**	**Agreement with expected results**	**Agreement with expected results**	**Agreement with expected results**
S. pyogenes M1 LP	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
S. pyogenes M1 MP	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
S. pyogenes M3 LP	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
S. pyogenes M3 MP	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
NTC	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
PC	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)

LP = Low Positive, MP = Medium Positive, PC = Positive Control, NTC = No Template Control

Table 3. LIAISON NES® Group A Strep Internal Control Reproducibility

	Site 1	Site 2	Site 3	All Sites
Panel Member	**Agreement with expected results**	**Agreement with expected results**	**Agreement with expected results**	**Agreement with expected results**
S. pyogenes M1 LP	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
S. pyogenes M1 MP	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
S. pyogenes M3 LP	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
S. pyogenes M3 MP	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
NTC	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)
PC	30/30 (100%)	30/30 (100%)	30/30 (100%)	90/90 (100%)

LP = Low Positive, MP = Medium Positive, NTC = No Template Control, PC = Positive Control

ANALYTICAL SENSITIVITY/LIMIT OF DETECTION

The LoD of the LIAISON NES® Group A Strep assay was determined to be the lowest detectable concentration of verified (re-grown and re-titered) bacterial stock (CFU/swab) at which ≥ 95% of all replicates were detected. Two (2) serotypes of *S. pyogenes* were serially diluted in human negative matrix and spiked onto NES swab to determine the LoD. The LoD results are shown in Table 7.

Table 4. LIAISON NES® Group A Strep Assay Limit of Detection

Bacterial Serotype	LoD (CFU/swab)
S. pyogenes serotype M1 ATCC 700294	90.7
S. pyogenes serotype M3 ATCC BAA-595	29.7

ANALYTICAL REACTIVITY/CROSS REACTIVITY

Analytical Reactivity

The analytical reactivity of the LIAISON NES® Group A Strep assay was evaluated. A total of fifteen (15) strains were tested. Verified (re-grown and re-titered) bacterial material was diluted in simulated negative matrix and spiked onto NES swab at the concentrations listed in Table 8 below (corresponding to 3X LoD or higher) and tested in triplicate. Each strain was tested initially in triplicate using 90

CFU/swab (i.e. 3 X LoD) level established for *S. pyogenes* strain M3 ATCC BAA-595). 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. Overall, seven (7) strains were detected at 100% at 3x LoD, four (4) strains were detected at 100% at 5x LoD and four (4) strains were detected at 100% at 8x LoD. The concentrations tested and results are shown in Table 8.

Table 5. LIAISON NES® Group A Strep Assay Analytical Reactivity

Target	Tested Concentration	Agreement with Expected Results: %Detection (#Detected/#Total)
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 Analytical Reactivity/Inclusivity

An *in silico* inclusivity analysis of the assay oligo sequences in the LIAISON NES® Group A Strep assay was performed. All primer and probe sets were aligned against sequences available in the GenBank database (as of August 30, 2025). Specifically, a total of 930 *Streptococcus pyogenes* sequences with coverage of the assay's oligo sets were assessed in this analysis. Based on the *in silico* analysis, LIAISON NES® Group A Strep assay exhibits 100% inclusivity of the analyzed sequences available in the aforementioned database.

Cross-Reactivity (Analytical Specificity)

Cross-reactivity of the LIAISON NES® Group A Strep assay was evaluated by testing forty-seven (47) whole microorganisms including related bacteria, high-prevalence pathogenic microorganisms or normal flora that are reasonably likely to be present in the clinical sample.

Specimens were prepared by diluting cultured or inactivated organisms, in simulated negative matrix and spiked onto NES swab. Cross-reactivity was determined based on three replicates. Results from cross-reactivity testing are summarized in Table 9. No cross-reactivity was observed with any of the microorganisms tested.

Table 6: LIAISON NES® Group A Strep Assay Cross-Reactivity (Analytical Specificity)

Organism	Tested Concentration	Agreement with Expected Results: % Detection (#Detected/#Total)
Adenovirus Type 1	10^5 $TCID_{50}$/mL	0% (0/3)

Organism	Tested Concentration	Agreement with Expected Results: % Detection (#Detected/#Total)
Arcanobacterium haemolyticum	10^6 CFU/mL	0% (0/3)
Bacillus cereus	10^6 CFU/mL	0% (0/3)
Bordetella pertussis	10^6 CFU/mL	0% (0/3)
Burkholderia cepacia	10^6 CFU/mL	0% (0/3)
Campylobacter rectus	10^6 CFU/mL	0% (0/3)
Candida albicans	10^6 CFU/mL	0% (0/3)
Corynebacterium diphtheriae	10^6 CFU/mL	0% (0/3)
Enterococcus faecalis	10^6 CFU/mL	0% (0/3)
Escherichia coli	10^6 CFU/mL	0% (0/3)
Fusobacterium necrophorum	10^6 CFU/mL	0% (0/3)
Haemophilus influenzae	10^6 CFU/mL	0% (0/3)
Human Influenza virus A	10^5 $TCID_{50}$/mL	0% (0/3)
Human Influenza virus B	10^5 $TCID_{50}$/mL	0% (0/3)
Human metapneumovirus-9	10^5 $TCID_{50}$/mL	0% (0/3)
Klebsiella pneumoniae	10^6 CFU/mL	0% (0/3)
Lactobacillus acidophilus	10^6 CFU/mL	0% (0/3)
Lactococcus lactis	10^6 CFU/mL	0% (0/3)
Legionella longbeachae	10^6 CFU/mL	0% (0/3)
Moraxella catarrhalis	10^6 CFU/mL	0% (0/3)
Mycoplasma pneumoniae	10^6 cps/mL	0% (0/3)
Neisseria gonorrhoeae	10^6 CFU/mL	0% (0/3)
Parainfluenza Type 3	10^5 $TCID_{50}$/mL	0% (0/3)
Peptostreptococcus micros	10^6 CFU/mL	0% (0/3)
Pseudomonas aeruginosa	10^6 CFU/mL	0% (0/3)
Respiratory syncytial virus Type B-9320	10^5 $TCID_{50}$/mL	0% (0/3)
Rhinovirus 1A	10^5 $TCID_{50}$/mL	0% (0/3)
Saccharomyces cerevisiae	10^6 CFU/mL	0% (0/3)
Staphylococcus epidermidis	10^6 CFU/mL	0% (0/3)
Stenotrophomonas maltophilia	10^6 CFU/mL	0% (0/3)
Streptococcus agalactiae	10^6 CFU/mL	0% (0/3)
Streptococcus anginosus	10^6 CFU/mL	0% (0/3)
Streptococcus bovis	10^6 CFU/mL	0% (0/3)
Streptococcus constellatus subsp. Pharyngis	10^6 CFU/mL	0% (0/3)
Streptococcus dysgalactiae subsp. Equisimilis	10^6 CFU/mL	0% (0/3)
Streptococcus gallolyticus	10^6 CFU/mL	0% (0/3)
Streptococcus intermedius	10^6 CFU/mL	0% (0/3)
Streptococcus mitis	10^6 CFU/mL	0% (0/3)

Organism	Tested Concentration	Agreement with Expected Results: % Detection (#Detected/#Total)
Streptococcus mutans	10^6 CFU/mL	0% (0/3)
Streptococcus oralis	10^6 CFU/mL	0% (0/3)
Streptococcus pneumoniae	10^6 CFU/mL	0% (0/3)
Streptococcus salivarius	10^6 CFU/mL	0% (0/3)
Streptococcus sanguinis	10^6 CFU/mL	0% (0/3)
Treponema denticola	10^6 CFU/mL	0% (0/3)
Veillonella parvula	10^6 CFU/mL	0% (0/3)
Hoylesella oralis	10^6 CFU/mL	0% (0/3)
Streptococcus canis	10^6 CFU/mL	0% (0/3)

CFU/mL= colony forming units/milliliter
copies/mL= copies/milliliter
$TCID_{50}$/mL= tissue culture infectious dose/milliliter

In silico Cross Reactivity/Exclusivity

The analytical specificity of the LIAISON NES® Group A Strep assay was further evaluated with an *in silico* analysis to predict potential cross-reactivity of the assay oligos through a BLAST comparison of all primers and probes in the assay to the human reference genome and the GenBank nt sequence database. Based on the *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. *In silico* assessment of the analyzed potential cross-reactive organisms, with sequences available in the GenBank nt database as of October 18, 2025, the assay oligos are predicted to have no potential cross-reactivity to any of the analyzed organisms.

INTERFERING SUBSTANCES

Potentially interfering substances from respiratory specimens were tested for the ability to generate false positive or false negative results. Samples were prepared by: 1) diluting each potentially interfering substance into a baseline sample consisting of simulated negative matrix and *S. pyogenes* (M1 ATCC 700294 or M3 ATCC BAA-595 serotype) at 3xLoD and 2) diluting each potentially interfering substance into a baseline sample consisting of simulated negative matrix. Prepared test samples were spiked onto NES swab and interference based on three replicates was determined. The results are shown in Table 10 and Table 11.

No substances tested in Table 10 showed any interference with the detection of *S. pyogenes* at the concentrations tested. No interference was observed for negative samples.

Table 7: LIAISON NES® Group A Strep Assay Interference for Positive Sample

Potentially Interfering Substances	Active Ingredient	Tested Concentration	*S. pyogenes* M1 % Detection (#Detected/ #Tested)	*S. pyogenes* M3 % Detection (#Detected/ #Tested)
Blood (Human)	N/A	4% (v/v)	100% (3/3)	100% (3/3)
Chloraseptic® Max Sore Throat Spray	Phenol, Glycerin	8% (v/v)	100% (3/3)	100% (3/3)
Robitussin Maximum Strength Severe Multi-Symptom Cough + Flu	Acetaminophen, Dextromethorphan Guaifenesin	8% (v/v)	100% (3/3)	100% (3/3)
Cool Mint Listerine antiseptic mouthwash	Eucalyptol, Menthol, Methyl Salicylate, Thymol	8% (v/v)	100% (3/3)	100% (3/3)
Crest Pro-Health	Stannous Fluoride	8% (v/v)	100% (3/3)	100% (3/3)

Potentially Interfering Substances	Active Ingredient	Tested Concentration	*S. pyogenes* M1 % Detection (#Detected/ #Tested)	*S. pyogenes* M3 % Detection (#Detected/ #Tested)
Halls	Menthol	8% (v/v)	100% (3/3)	100% (3/3)
Advil Liqui-Gels	Ibuprofen	8% (v/v)	100% (3/3)	100% (3/3)
Food Dye Blue 1	N/A	8% (v/v)	100% (3/3)	100% (3/3)
Food Dye Yellow 5 (Tartrazina)	N/A	8% (v/v)	100% (3/3)	100% (3/3)
Orange Juice	N/A	8% (v/v)	100% (3/3)	100% (3/3)
Cepacol® Extra Strength Sore Throat Lozenges	Benzocaine, Menthol	0.024% Benzocaine (w/v), 0.0058% Menthol (w/v)	100% (3/3)	100% (3/3)
Sucrets® Sore Throat & Cough	Dyclonine Hydrochloride, Menthol, Pectin	0.0048% Dyclonine Hydrochloride (w/v), 0.012% Menthol (w/v), Pectin 0.014% (w/v)	100% (3/3)	100% (3/3)
Miralax	Polyethylene Glycol	2.43% (w/v)	100% (3/3)	100% (3/3)
Tums Ultra Strength	Calcium Carbonate	2.4 mg/ml	100% (3/3)	100% (3/3)
Penicillin G	Penicillin G Sodium Salt	0.8% (w/v)	100% (3/3)	100% (3/3)
Cephalexin	Cephalexin	0.2% (w/v)	100% (3/3)	100% (3/3)
Mucin: porcine stomach Type II	Purified mucin protein	0.8% (w/v)	100% (3/3)	100% (3/3)
Amoxicillin	Amoxicillin	0.8% (w/v)	100% (3/3)	100% (3/3)
Aspirin	Acetylsalicylic acid	0.62 mg/ml	100% (3/3)	100% (3/3)

mg/mL = Milligrams/milliliter

v/v = Volume/Volume

w/v = Weight/Volume

Table 8: LIAISON NES® Group A Strep Assay Interference for Negative Sample

Potentially Interfering Substances	Active Ingredient	Tested Concentration	*S. pyogenes* % Detection (#Detected/ #Tested)	IC % Detection (#Detected/ #Tested)
Blood (Human)	N/A	4% (v/v)	0% (0/3)	100% (3/3)
Chloraseptic® Max Sore Throat Spray	Phenol, Glycerin	8% (v/v)	0% (0/3)	100% (3/3)
Robitussin Maximum Strength Severe Multi-Symptom Cough + Flu	Acetaminophen, Dextromethorphan Guaifenesin	8% (v/v)	0% (0/3)	100% (3/3)
Cool Mint Listerine antiseptic mouthwash	Eucalyptol, Menthol, Methyl Salicylate, Thymol	8% (v/v)	0% (0/3)	100% (3/3)
Crest Pro-Health	Stannous Fluoride	8% (v/v)	0% (0/3)	100% (3/3)

Potentially Interfering Substances	Active Ingredient	Tested Concentration	*S. pyogenes*	IC
			% Detection (#Detected/ #Tested)	% Detection (#Detected/ #Tested)
Halls	Menthol	8% (v/v)	0% (0/3)	100% (3/3)
Advil Liqui-Gels	Ibuprofen	8% (v/v)	0% (0/3)	100% (3/3)
Food Dye Blue 1	N/A	8% (v/v)	0% (0/3)	100% (3/3)
Food Dye Yellow 5 (Tartrazina)	N/A	8% (v/v)	0% (0/3)	100% (3/3)
Orange Juice	N/A	8% (v/v)	0% (0/3)	100% (3/3)
Cepacol® Extra Strength Sore Throat Lozenges	Benzocaine, Menthol	0.024% Benzocaine (w/v), 0.0058% Menthol (w/v)	0% (0/3)	100% (3/3)
Sucrets® Sore Throat & Cough	Dyclonine Hydrochloride, Menthol, Pectin	0.0048% Dyclonine Hydrochloride (w/v), 0.012% Menthol (w/v), Pectin 0.014% (w/v)	0% (0/3)	100% (3/3)
Miralax	Polyethylene Glycol	2.43% (w/v)	0% (0/3)	100% (3/3)
Tums Ultra Strength	Calcium Carbonate	2.4 mg/ml	0% (0/3)	100% (3/3)
Penicillin G	Penicillin G Sodium Salt	0.8% (w/v)	0% (0/3)	100% (3/3)
Cephalexin	Cephalexin	0.2% (w/v)	0% (0/3)	100% (3/3)
Mucin: porcine stomach Type II	Purified mucin protein	0.8% (w/v)	0% (0/3)	100% (3/3)
Amoxicillin	Amoxicillin	0.8% (w/v)	0% (0/3)	100% (3/3)
Aspirin	Acetylsalicylic acid	0.62 mg/ml	0% (0/3)	100% (3/3)

mg/mL = Milligrams/milliliter

v/v = Volume/Volume

w/v = Weight/Volume

INHIBITION BY OTHER MICROORGANISMS

The LIAISON NES® Group A Strep assay was evaluated by testing the ability to detect a low concentration of *S. pyogenes* when other potentially inhibitory microorganisms were present. Specimens were prepared by diluting the potentially inhibitory cultured isolates or inactivated organisms into simulated negative matrix in the presence of a low concentration (3X LoD) of either *S. pyogenes* M1 ATCC 700294 or M3 ATCC BAA-595 serotype. Forty-seven (47) potentially inhibitory microorganisms were individually spiked on NES swab and tested in triplicate.

No inhibition by any organisms was observed for *S. pyogenes* at the concentrations indicated in Table 12.

Table 9: LIAISON NES® Group A Strep Assay Microbial Inhibition

Organism	Tested Concentration	Agreement with Expected Results: %Detection (#Detected/#Total)	
		***S. pyogenes* M1**	***S. pyogenes* M1**
Adenovirus Type 1	10^5 TCID50/mL	100% (3/3)	100% (3/3)
Arcanobacterium haemolyticum	10^6 CFU/mL	100% (3/3)	100% (3/3)
Bacillus cereus	10^6 CFU/mL	100% (3/3)	100% (3/3)
Bordetella pertussis	10^6 CFU/mL	100% (3/3)	100% (3/3)
Burkholderia cepacia	10^6 CFU/mL	100% (3/3)	100% (3/3)
Campylobacter rectus	10^6 CFU/mL	100% (3/3)	100% (3/3)
Candida albicans	10^6 CFU/mL	100% (3/3)	100% (3/3)
Corynebacterium diphtheriae	10^6 CFU/mL	100% (3/3)	100% (3/3)
Enterococcus faecalis	10^6 CFU/mL	100% (3/3)	100% (3/3)
Escherichia coli	10^6 CFU/mL	100% (3/3)	100% (3/3)
Fusobacterium necrophorum	10^6 CFU/mL	100% (3/3)	100% (3/3)
Haemophilus influenzae	10^6CFU/mL	100% (3/3)	100% (3/3)
Hoylesella oralis	10^6 CFU/mL	100% (3/3)	100% (3/3)
Human Influenza virus A	10^5 TCID50/mL	100% (3/3)	100% (3/3)
Human Influenza virus B	10^5 TCID50/mL	100% (3/3)	100% (3/3)
Human metapneumovirus-9	10^5 TCID50/mL	100% (3/3)	100% (3/3)
Klebsiella pneumoniae	10^6 CFU/mL	100% (3/3)	100% (3/3)
Lactobacillus acidophilus	10^6 CFU/mL	100% (3/3)	100% (3/3)
Lactococcus lactis	10^6 CFU/mL	100% (3/3)	100% (3/3)
Legionella longbeachae	10^6 CFU/mL	100% (3/3)	100% (3/3)
Moraxella catarrhalis	10^6 CFU/mL	100% (3/3)	100% (3/3)
Mycoplasma pneumoniae	10^6 cps/mL	100% (3/3)	100% (3/3)
Neisseria gonorrhoeae	10^6 CFU/mL	100% (3/3)	100% (3/3)
Parainfluenza Type 3	10^5 TCID50/mL	100% (3/3)	100% (3/3)
Peptostreptococcus micros	10^6 CFU/mL	100% (3/3)	100% (3/3)
Pseudomonas aeruginosa	10^6 CFU/mL	100% (3/3)	100% (3/3)
Respiratory syncytial virus Type B-9320	10^5 TCID50/mL	100% (3/3)	100% (3/3)
Rhinovirus 1A	10^5 TCID50/mL	100% (3/3)	100% (3/3)
Saccharomyces cerevisiae	10^6 CFU/mL	100% (3/3)	100% (3/3)

Organism	Tested Concentration	Agreement with Expected Results: %Detection (#Detected/#Total)	
		***S. pyogenes* M1**	***S. pyogenes* M1**
Staphylococcus epidermidis	10^6 CFU/mL	100% (3/3)	100% (3/3)
Stenotrophomonas maltophilia	10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus agalactiae	10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus anginosus	10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus bovis	10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus canis	10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus constellatus subsp. pharyngis	10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus dysgalactiae subsp. equisimilis	10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus gallolyticus	10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus intermedius	10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus mitis	10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus mutans	10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus oralis	10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus pneumoniae	10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus salivarius	10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus sanguinis	10^6 CFU/mL	100% (3/3)	100% (3/3)
Treponema denticola	10^6 CFU/mL	100% (3/3)	100% (3/3)
Veillonella parvula	10^6 CFU/mL	100% (3/3)	100% (3/3)

CFU/mL= colony forming units/milliliter
Cps/mL= copies/milliliter
$TCID_{50}$/mL= tissue culture infectious dose/milliliter

CARRY-OVER AND CROSS-CONTAMINATION

Amplification carry-over and cross-contamination for the LIAISON NES® Group A Strep assay has been assessed. The study was designed by processing the samples alternating between highly positive and negative samples. High Positive (HP) samples were formulated by spiking *S. pyogenes* M3 serotype **(**ATCC BAA-595) into simulated negative matrix and then spiked on the NES swab at a final concentration of 10^6 CFU/swab. Simulated negative matrix onto NES swab was used as negative sample. The same alternating order was maintained when loading the corresponding cartridges into the instruments. No evidence of carry-over or cross-contamination was observed.

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GLOSSARY*

	Caution		Telephone
	Consult instructions for use	IVD	In vitro diagnostic medical device
	Biological risks	REF	Catalog number
	Contains sufficient for <n> tests	MD	Medical Device
	Temperature limit	LOT	Batch code
	Manufacturer	CONT	Kit contents
	Use by	Rx Only	Prescription only
	Do not reuse		Keep Dry
	Keep away from sunlight	STERILE EO	Sterilized using ethylene oxide
	Do not use if package is damaged		Do not resterilize
	Single sterile barrier system		Date of manufacture
SN	Serial number		

*ISO 15223-1

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