Group A *Streptococcal* Direct Test

INTENDED USE

The Hologic Group A *Streptococcus* Direct Test is a DNA probe assay which uses nucleic acid hybridization for the qualitative detection of Group A Streptococcal RNA as an aid in the diagnosis of Group A Streptococcal pharyngitis from throat swabs.

SUMMARY AND EXPLANATION OF THE TEST

Streptococcus pyogenes (group A β-hemolytic *Streptococcus*) is the etiologic agent of a number of infections in humans including acute pharyngitis, sinusitis, lymphadenitis, pyoderma, endocarditis, meningitis, septicemia, tonsillitis, impetigo, and upper respiratory tract infections (4,5). *Streptococcus pyogenes* infections are of particular concern because serious complications such as glomerulonephritis, rheumatic fever and scarlet fever may result if left untreated (1, 4, 5). Group A β-hemolytic streptococci are universally susceptible to penicillin G, a fact that makes antimicrobial susceptibility testing for this organism unnecessary unless the patient is allergic to penicillin.

Over ninety percent of all streptococcal infections are caused by *Streptococcus pyogenes* (10). Asymptomatic carriers colonized in the nasopharynx, skin, vagina, or rectum are thought to transmit this organism through close person-to-person contact (4, 8). Contaminated food may also be a source of transmission and infections in humans (4).

Presumptive identification of *Streptococcus pyogenes* is traditionally based upon physiological and biochemical traits. These include colony morphology, beta-hemolytic activity on sheep blood agar, gram stain, susceptibility to bacitracin, and the ability to hydrolyze L-pyrrolidonyl-β-naphthylamide (PVR) (8). Commercial antibody tests such as latex agglutination target the *Streptococcus* group A antigen. Occasionally, these tests have been shown to react positively with some strains of *Streptococcus anginosus* containing the group A antigen. In addition, these tests may require repeat testing due to equivocal results. Serological grouping is the method of choice for definitive identification of *Streptococcus pyogenes*. Lancefield serological grouping is determined from group-specific carbohydrate antigen extracted from cell walls and group-specific antisera (8). This method can be time-consuming and costly; therefore, most laboratories rely on the traditional physiological and biochemical methods.

The Hologic Group A *Streptococcus* Direct Test offers a non-subjective, accurate and rapid identification method for definitively identifying *Streptococcus pyogenes* from throat swabs. Identification is based upon the detection of specific ribosomal RNA sequences that are unique to *Streptococcus pyogenes*. The Hologic Group A *Streptococcus* Direct Test identifies *Streptococcus pyogenes* organisms from throat swabs within 60 minutes of sample preparation.

PRINCIPLES OF THE PROCEDURE

Nucleic acid hybridization tests are based on the ability of complementary nucleic acid strands to specifically align and associate to form stable double-stranded complexes (7). The Hologic DNA Probe assay uses a single-stranded DNA probe with a chemiluminescent label which is complementary to the ribosomal RNA of the target organism.

After the ribosomal RNA is released, the labeled DNA probe combines with the target organism's ribosomal RNA to form a stable DNA:RNA hybrid. The Selection Reagent differentiates non-hybridized from hybridized probe. The labeled DNA:RNA hybrids are measured in a Hologic luminometer (Leader® I, 50, 400, 450, or 450i). A positive result is a luminometer reading greater than or equal to the cut-off. A value below this cut-off is a negative result.

REAGENTS

Note: *For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologic.com/sds.*

Hologic Group A *Streptococcus* Direct Test consists of the following:

LR — Lysis Reagent 1 x 30 mL
HEPES buffered solution containing < 2% detergent and 0.04% sodium azide as a preservative.

P — Probe Reagent 2 x 3 mL when reconstituted
Lyophilized non-infectious DNA with a chemiluminescent label (< 30 ng/vial).

HB — Hybridization Buffer 1 x 6 mL
Succinate buffered solution containing < 4% detergent.

S — Selection Reagent 1 x 30 mL
Borate buffered solution containing < 2% surfactant.

PC — Positive Control 1 x 2 mL
Non-infectious *S. pyogenes* nucleic acid in a HEPES buffered solution containing < 2% detergent and 0.04% sodium azide as a preservative.

NC — Negative Control 1 x 2 mL
HEPES buffered solution containing < 2% detergent and 0.04% sodium azide as a preservative.

Sealing Cards 1 package containing 12 sealing cards.

Polypropylene Tubes (12 x 75) 250 tubes/box.

WARNINGS AND PRECAUTIONS

A. For *in vitro* diagnostic use.

B. Use universal laboratory precautions (1).

C. Use only swabs and/or transport systems that have been qualified for use with this test. Other swab transport systems have been shown to cause high luminometer background readings and potential false positive results.

D. Use only for determining the identity of *Streptococcus pyogenes* from throat swabs.

E. Use only supplied or specified disposable laboratory ware.

F. Reagents in this kit contain sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. Upon disposal of these reagents, always dilute the material with a large volume of water to prevent azide buildup in the plumbing.

G. **Warning: Irritants, Corrosives.** Avoid contact of Detection Reagents I and II with skin, eyes, and mucous membranes. Wash the effected area with water if these reagents come into contact with skin or eyes. If spills of these reagents occur, dilute the spill with water before wiping it dry.

H. Do not interchange, mix, or combine reagents from kits with different lot numbers.

STORAGE AND HANDLING REQUIREMENTS

Probe Reagent must be stored at 2° to 8°C. The Probe Reagent is stable for fourteen days after reconstitution when stored at 2° to 8°C. Positive control must be stored at 2° to 8°C.

Other reagents contained in the Hologic Group A *Streptococcus* Direct Test kit are to be stored between 2° and 25°C and are stable until the expiration date indicated.

DO NOT FREEZE THE REAGENTS.

SAMPLE COLLECTION AND PREPARATION

The Hologic Group A *Streptococcus* Direct Test kit is designed to detect and identify *Streptococcus pyogenes* from throat swabs.

Throat swabs may be transported to the laboratory at ambient temperature for up to 48 hours after specimen collection.

Throat swabs should be stored at 2° to 8°C and tested within 72 hours of receipt at the laboratory.

The following brands of swab transport systems have been qualified for use in this test:

	Catalog Number		
Swab Transport	**Copan**	**Becton Dickinson**	**Remel**
Remel BACTI-SWAB™ Collection and transport System	N/A	N/A	12-100
Liquid Amies Medium Single Swab, Dacron Tip	140CQ	220146	N/A
Liquid Amies Medium Dual Swab, Dacron Tip	138CQ	220147	N/A
Liquid Stuart Medium Single Swab, Dacron Tip	141CQ	220149	N/A
Liquid Stuart Medium Dual Swab, Dacron Tip	139CQ	220148	N/A
Double Dacron Dry Swab - in sterile labeled transport tubes	167CQ	220135	N/A

Other swabs and/or transport systems have not been qualified for use in this test.

MATERIALS PROVIDED

Hologic Group A *Streptococcus* Direct Test

Cat. No. 103890/3890	**100 Tests**
Lysis-Reagent	1 x 30 mL
Probe Reagent	2 x 3 mL when reconstituted
Hybridization Buffer	1 x 6 mL
Selection Reagent	1 x 30 mL
Positive Control	1 x 2 mL
Negative Control	1 x 2 mL
Sealing Cards	1 package
Polypropylene Tubes (12 x 75 mm)	250 tubes/box

MATERIALS REQUIRED BUT NOT PROVIDED

Water Bath or Dry Heat Bath * (60° ± 1°C)

Dry heat bath * (95° ± 3°C)

Micropipettes (50 µL)

Repeat Pipettor (50 µL, 300 µL)

Vortex Mixer

Specimen Collection and Transport System

	Catalog Number		
Swab Transport	**Copan**	**Becton Dickinson**	**Remel**
Remel BACTI-SWAB™ Collection and transport System	N/A	N/A	12-100
Liquid Amies Medium Single Swab, Dacron Tip	140CQ	220146	N/A
Liquid Amies Medium Dual Swab, Dacron Tip	138CQ	220147	N/A
Liquid Stuart Medium Single Swab, Dacron Tip	141CQ	220149	N/A
Liquid Stuart Medium Dual Swab, Dacron Tip	139CQ	220148	N/A
Double Dacron Dry Swab - in sterile labeled transport tubes	167CQ	220135	N/A

*Heating blocks in the dry heat bath should have wells that are correctly sized for 12 x 75 mm tubes. The use of Hologic dry heat baths is recommended.

AVAILABLE FROM HOLOGIC:

Hologic Leader Luminometer

Hologic Detection Reagent Kit (Cat. No. 101791/1791)

Hologic Dry Heat Bath (Cat. No. 104006/4006)

TEST PROCEDURE

A. EQUIPMENT PREPARATION

1. Adjust the dry heat bath and/or water bath to the appropriate temperature (95° or 60°C).
2. Prepare the Leader luminometer for operation. Make sure there is a sufficient volume of Detection Reagents I and II to complete the tests. If sufficient reagents are not contained in the reservoirs, fill the reservoirs according to the instructions in the instrument operator's manual.

B. REAGENT PREPARATION

1. Allow all kit components to reach room temperature prior to use.
2. Hybridization Buffer

   The Hybridization Buffer will precipitate at lower temperatures. Warming and mixing the solution at 35° to 60°C will dissolve the precipitates. Vortex to ensure homogeneity.
3. Probe Reagent

   Allow lyophilized Probe Reagent and Hybridization Buffer to reach room temperature. To reconstitute, pipette 3.0 mL of Hybridization Buffer into the lyophilized Probe Reagent vial. Warm the reconstituted Probe Reagent by swirling the vial for 10 to 20 seconds in a 60°C water bath or place the vial in a 37°C incubator for 5 minutes. Vortex to ensure homogeneity of the newly prepared Probe Reagent. Following reconstitution, the Probe Reagent will precipitate at lower storage

temperatures. Briefly warm the solution at 35° to 60°C and mix thoroughly to dissolve the precipitate before each set of tests is run.

C. CONTROLS

1. Kit Controls

   One positive and one negative control should be included with each assay run. Pipette 50 µL of the Positive and Negative Controls each into a clean polypropylene test tube. Set the tubes aside for use at the Hybridization step (E) below.

D. SAMPLE PREPARATION

1. Place each throat swab in 300 µL (use 400 µL Lysis Reagent if using dry swab) of Lysis Reagent in a polypropylene tube. Heat the tubes containing the swabs in a dry heat bath for 10 minutes at 95° ± 3°C. Remove the tubes from the dry heat bath.
2. While the tubes cool for 5 minutes, thoroughly express each swab against the side of its tube to remove as much liquid as possible. Discard the swab. Remove 50 µL of the liquid and transfer it to a clean, labeled polypropylene tube. Any remaining swab lysate liquid may be stored frozen or refrigerated for up to 7 days for repeat hybridization testing, if desired.

E. HYBRIDIZATION

1. Pipette 50 µL of the well-mixed reconstituted Probe Reagent into all specimen and control tubes (positive and negative kit controls).
2. Cover the tubes with sealing cards, mix by shaking gently (do not vortex) and incubate all tubes at 60° ± 1°C for 30 minutes in a water bath or dry heat bath.

F. SELECTION

1. Remove the tubes from the water bath or dry heat bath. Remove the sealing cards and pipette 300 µL of Selection Reagent into every tube. Cover the tubes with sealing cards. ***Vortex all tubes thoroughly.***
2. Incubate the tubes for 7 minutes at 60° ± 1°C in a water bath or dry heat bath.
3. Remove the tubes from the water bath or dry heat bath. Remove the sealing cards and allow the tubes to cool at room temperature for 5 minutes. Read the results in the luminometer within 1 hour after completion of the test.

G. DETECTION

1. Select the appropriate protocol from the menu of the luminometer software.
2. Using a damp tissue or lint-free paper towel, wipe each tube to ensure that no residue is present on the outside of the tube and static charge is eliminated. Insert the tube into the luminometer according to the instrument directions.
3. When the reading is complete, remove the tube(s) from the luminometer.

PROCEDURAL NOTES

A. REAGENTS: Hybridization Buffer may precipitate at 2° to 8°C. Warming and mixing the solution at 35° to 60°C will dissolve the precipitates.

B. TEMPERATURE: The sample preparation, hybridization and selection reactions are temperature dependent. Therefore, it is imperative that the water bath or dry heat bath is maintained within the specified temperature range.

C. TIME:

1. After the sample preparation step (Test Procedure, step D.1) the reaction tubes may remain at room temperature for 1 hour before proceeding with the hybridization step.
2. The selection step incubation time is important. Do not exceed 8 minutes at 60° ± 1°C.
3. Tubes may remain at room temperature for up to 60 minutes after the selection step before reading in the luminometer without a significant loss of chemiluminescent signal.

D. WATER BATH

The level of water in the water bath should be maintained to ensure that the liquid reaction volume in the tube is submerged, but the water level should not be so high that the water might enter the tubes.

E. TROUBLESHOOTING

1. Elevated Negative Control values greater than 3,000 Relative Light Units (RLU) may be caused by insufficient mixing after adding the Selection Reagent or by incubation below 59°C during the hybridization or selection steps. In addition, elevated Negative Control values will be seen if the Selection Reagent is not added or if the 60°C incubation does not occur.
2. Low Positive Control values below 15,000 RLU can be caused by elevated incubation temperatures during the hybridization and selection steps, prolonged incubation during the selection step or if the volume of Selection Reagent or Probe Reagent is not correct.

RESULTS

A. INTERPRETATION OF RESULTS

The results of the Hologic Group A *Streptococcus* Direct Test are calculated automatically by the Leader luminometer and are based on the following cut-off values:

Positive

$\geq$ 4,500 RLU

Negative

$<$ 4,500 RLU

The 4,500 RLU cut-off value was established after testing 849 throat swab specimens at locations throughout the United States.

The luminometer prints the specimen RLU response and compares this signal to the assigned reporting threshold of 4,500 RLU. A positive or negative interpretation based on this cut-off is printed. See the operator's manual for detailed protocols.

Example:

Sample 1	437,530 RLU	POS
Sample 2	1,532 RLU	NEG

A negative result should be reported as: “No Group A Streptococcus rRNA detected.”

A positive result should be reported as: “Positive for Group A Streptococcus rRNA.”

Laboratories wishing to further maximize sensitivity should culture or repeat in duplicate (from the remaining lysate) those specimens that give results close to the cutoff (6).

B. QUALITY CONTROL AND ACCEPTABILITY OF RESULTS

The expected value of the Positive Control should be $\geq$ 15,000 and $<$ 45,000 RLU. The expected value of the Negative Control should be $\leq$ 3,000 RLU. These values were established using 144 different runs at 8 locations throughout the U.S.

If the Positive and Negative Controls do not yield the expected results, the test results are invalid and must not be reported.

Each laboratory under their normal operating conditions should establish their own mean and range for the Negative and Positive Controls and maintain records according to standard laboratory Quality Control practices (1, 9).

Additional controls which may be used for the lysis step are:

A positive cell control for the 95°C lysis step may be performed by sampling 1 to 4 colonies of Group A Streptococcus (i.e., *S. pyogenes* ATCC # 12344) from an 18 to 72 hour blood agar plate culture with a Hologic qualified clean, sterile swab. In order to properly duplicate specimen processing, follow the swab manufacturer's instructions as specified. Ensure ampule is activated to moisten the swab if indicated on the instructions.The swab should be run according to the complete assay procedure, beginning with Test Procedure Step D. This should produce a positive result of ≥ 500,000 RLU.

A negative cell control for the 95°C Lysis Step may be performed by sampling 1 to 4 colonies of Group B Streptococcus (i.e., S. agalactiae ATCC # 13813) from an 18 to 72 hour blood agar plate culture with a Hologic qualified clean, sterile swab. In order to properly duplicate specimen processing, follow the swab manufacturer's instructions as specified. Ensure ampule is activated to moisten swab if indicated on instructions. The swab should be run according to the complete assay procedure, beginning with Test Procedure Step D. This should produce a negative result of < 4,500 RLU.

LIMITATIONS

This method has been tested using throat swab specimens only. Performance with other specimens has not been assessed.

Results from the Hologic Group A *Streptococcus* Direct Test should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

A negative test does not exclude the possibility of infection because test results may be affected by improper specimen collection, procedural errors, or clerical errors.

The following substances have been tested and do not interfere with the Hologic Group A *Streptococcus* Direct Test: white blood cells, saliva, mucous, and sheep blood agar. Blood and, potentially, other chemiluminescent substances may interfere with this test. Do not perform this test on throat swabs that contain visible blood.

A negative test does not exclude the possibility that the numbers of Group A Streptococcal cells may be below the level of detection of the assay. Culture may be used to detect those specimens with lower levels of Group A Streptococcus due to infection or colonization. This test is not intended to differentiate carriers of Group A Streptococcus from those with infection due to Group A Streptococcus.

Specificity testing with heavy inocula of pure cultures (both ATCC and clinical isolates) showed that some strains of *Streptococcus equi* and rare strains of non-hemolytic streptococci which react with Group G antisera may result in a false positive reaction.

The Hologic Group A *Streptococcus* Direct Test has been shown to produce a positive result with *Streptococcus porcinus*. The incidence of human infection is very infrequent (3).

EXPECTED VALUES

The Hologic Group A *Streptococcus* Direct Test was used to detect and identify *Streptococcus pyogenes* from clinical throat swab specimens. A single swab was used for both the reference culture method and the Hologic Group A *Streptococcus* Direct Test. Rigorous culture procedures were used to compare this test with standard culture methods, including swab inoculation onto either selective or non-selective blood agar media and incubation in an aerobic (5% CO2) or anaerobic atmosphere for up to 72 hours, with a subculture from the primary quadrant of the plate to either selective or non-selective media following the first 18 to 24 hours of incubation. Standard identification methods included colony morphology, beta-hemolytic activity on sheep blood agar, the ability to hydrolyze L-pyrrolidonyl-p-naphthylamide (PYR) and bacitracin susceptibility. Confirmatory antigen latex agglutination methods included Streptex® (Murex Diagnostics Ltd., Dartford, Kent, England) and PathoDx® (Diagnostic Products Corporation, Los Angeles, California).

A two-site clinical study was conducted. Samples were either categorized as positive (Sample RLU ≥ 4,500) or negative (Sample RLU < 4,500). Site A used Remel Selective Strep Agar incubated anaerobically for 72 hours and included a subculture to Remel Selective Strep Agar, with PYRE and Streptex as confirmatory methods. Site B used BBL® ssA™ Agar incubated anaerobically for 48 hours without subculture. Confirmatory testing was by PYR and Streptex serotyping.

Site A was a pediatric hospital that collected both outpatient and inpatient samples. Site B was an HMO medical center that included both pediatric and adult samples, primarily from outpatients.

A comparison of the results with the Hologic Group A *Streptococcus* Direct Test and culture with confirmatory testing is shown below.

Before Discrepant Resolution:

PROBE TEST/CULTURE

	P/P	P/N	N/P	N/N	Total Tested	Sens	Spec	Percent Agreement
Site A	54	1	4	134	193	93.1%	99.3%	97.4%
Site B	233	16	23	833	1105	91.0%	98.1%	96.5%
Both Sites	***287***	***17***	***27***	***967***	***1298***	***91.4%***	***98.3%***	***96.6%***

After Discrepant Resolution

Ten samples that were probe-positive and culture-negative were recultured in Todd-Hewitt broth. The pledget from the Becton-Dickinson Culturette swab system was cultured in Todd-Hewitt broth for 24 to 48 hours at 37°C. A 50 µL sample was plated onto sheep red blood cell agar, incubated aerobically for 24 to 48 hours at 35° to 37°C and examined for beta-hemolytic colonies. All beta-hemolytic colonies were selected and confirmed using the Streptex group serotyping method.

All 10 samples were Todd-Hewitt broth culture positive for Group A Streptococcus. If these samples are reclassified as true positives, then the following performance data are obtained.

PROBE TEST/CULTURE

	P/P	P/N	N/P	N/N	Total Tested	Sens	Spec	Percent Agreement
Site A	55	0	4	34	193	93.2%	100.0%	97.9%
Site B	242	7	23	833	1105	91.3%	99.2%	97.3%
Both Sites	***297***	***7***	***27***	***967***	***1298***	***91.7%***	***99.3%***	***97.4%***

These results show that the Hologic Group A *Streptococcus* Direct Test, when compared with culture, gave a sensitivity of 91.7%, a specificity of 99.3%, and an overall agreement of 97.4%.

In this evaluation of the Hologic Group A *Streptococcus* Direct Test, growth of any number of colonies of *Streptococcus pyogenes* on any of the media used was considered a positive test.

The positive and negative predictive values for various prevalence rates in a given population at a sensitivity of 91.7% and a specificity of 99.3% are shown below.

PREVALENCE

	10%	15%	20%	25%
Positive Predictive Value	93.6%	95.8%	97.0%	97.7%
Negative Predictive Value	99.1%	98.5%	97.0%	97.3%
Efficiency	98.5%	98.2%	97.8%	97.4%

PERFORMANCE CHARACTERISTICS

A. WITHIN-RUN PRECISION

The within-run precision of the Hologic Group A *Streptococcus* Direct Test was calculated by assaying two concentrations of ribosomal RNA isolated from *Streptococcus pyogenes* and a single concentration of *S. pyogenes* cells using 10 replicates in a single assay.

	High Level RNA	Low Level RNA	Cells
Number of Replicates	10	10	10
Mean Response (RLU)	28,412	12,387	5,851
Standard Deviation	1,201	411	103
Coefficient of Variation	4.2%	3.3%	1.8%

B. BETWEEN-RUN PRECISION

The between-run precision was calculated by assaying one concentration of *Streptococcus pyogenes* ribosomal RNA, and two suspensions of *Streptococcus pyogenes* per assay. Each of three operators ran one replicate in each of four separate runs.

	High Level RNA	Low Level Cells+Swab	High Level Cells+Swab
Number of Replicates	12	12	12
Mean Response (RLU)	22,830	6,937	87,076
Standard Deviation	1,900	517	11,157
Coefficient of Variation	8.3%	7.5%	12.8%

C. CLINICAL REPRODUCIBILITY STUDY

A clinical reproducibility study was conducted at three sites using coded materials for inoculation onto swabs. All sites tested two coded swabs for each of the variables listed below. Two replicate hybridization assays were run on each swab.

Combined data for all three test sights

Swab Inoculum	Intersite Mean RLU (n=12)	S.D.	% CV
Lysis Reagent	1,882	406	21.6
Throat Swab Material Only	1,731	416	24.0
Low Group A Cells Level #1	14,253	2,785	19.5
Medium Group A Cells Level #2	49,457	12,934	26.2
Medium Group A Cells Level #1	97,045	21,236	21.9
High Group A Cells Level #2	182,806	38,006	20.8
High Group A Cells Level #1	608,513	172,252	28.3
Negative Control	1,146	313	27.3
Positive Control	38,581	2,392	6.2

D. SPECIFICITY

A total of 93 ATCC reference isolates were evaluated using the Hologic Group A *Streptococcus* Direct Test. These isolates represented a total of 78 species from 48 genera. Three isolates of *Streptococcus pyogenes*, 25 isolates of 13 other *Streptococcus* species, and 65 isolates of 47 other genera representing a phylogenetic cross-section of organisms were evaluated. All *Streptococcus pyogenes* isolates tested produced a positive result using the Hologic Group A *Streptococcus* Direct Test. All other *Streptococcus* species and a representative phylogenetic cross-section of species gave negative results using the Hologic Group A *Streptococcus* Direct Test.

In addition, 256 clinical isolates representing 41 species from 21 genera were evaluated with this test, including 64 *Streptococcus pyogenes* isolates, 75 strains of beta-hemolytic *Streptococcus* from groups B, C, F, G and non-typeable; 30 strains of Enterococcus species; 31 strains of alpha-hemolytic *Streptococcus*; 14 strains of *Streptococcus pneumoniae*; and 42 strains of other common clinically isolated bacteria. All *Streptococcus pyogenes* isolates tested produced a positive result, and all other organisms tested gave negative results with the Hologic Group A *Streptococcus* Direct Test, with the exception of two strains of *Streptococcus equi* and one strain of a non-beta hemolytic species of *Streptococcus* which gave false positive results.

E. RECOVERY

Eight dilutions of *Streptococcus pyogenes* cells ranging from 630 to 84,000 cells per assay were tested alone and in the presence of 2 million cells of the following non-target species: *Streptococcus agalactiae* and *Streptococcus mitis.* The presence of these non-target species did not interfere with the positive signal of the *Streptococcus pyogenes* cells, nor did they generate a positive result alone with the Hologic Group A *Streptococcus* Direct Test.

F. ANALYTICAL SENSITIVITY

The analytical sensitivity of the Hologic Group A *Streptococcus* Direct Test was determined by measuring the RLU output from three separate serial dilutions of *Streptococcus pyogenes* cells. Using a cut-off of 4,500 RLU places the assay sensitivity at about 1,200 CFU per test or the equivalent of 7,200 CFU per swab, or 24,000 CFU/mL of Lysis Reagent.

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