

STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST

INTENDED USE

The ACCUPROBE STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST is a rapid DNA probe test which utilizes the technique of nucleic acid hybridization for the identification of *Streptococcus pneumoniae* (*Pneumococcus*) isolated from culture.

SUMMARY AND EXPLANATION OF THE TEST

Streptococcus pneumoniae (*S. pneumoniae*) is the leading cause of community acquired bacterial pneumonia in the United States, occurring in approximately one half million cases per year, with a complication and death rate of about 5% (2, 4). It is also the leading cause of otitis media and bacteremia in infants and children (2). *S. pneumoniae* is a common cause of meningitis and has been isolated from patients with conjunctivitis, sinusitis, mastoiditis, pericarditis, occult bacteremia, arthritis, and endocarditis (2). It is the third most common blood culture isolate found (6). *S. pneumoniae* may be considered as part of the normal flora of the upper respiratory tract, however, infants, the elderly, and debilitated patients with other compromising medical conditions of the respiratory tract, or decreased immunological function are at greatest risk for acquiring pneumococcal disease (2, 3, 4).

Presumptive identification is made by traditional physiological and biochemical methods. These include colony morphology, Gram stain, catalase reaction, alpha hemolytic activity on 5% sheep blood agar, optochin susceptibility, and bile solubility (2). More than 80 serotypes have been identified, based on capsular polysaccharides. The Quellung test, based on apparent capsular swelling in response to treatment with antisera to these polysaccharides, is another means of identification. All tests mentioned above are subjective, and no one by itself provides unequivocal identification of *S. pneumoniae*.

The ACCUPROBE STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST offers a rapid, nonsubjective method for the definitive identification of *S. pneumoniae* based on the detection of specific ribosomal RNA sequences that are unique to *S. pneumoniae*.

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 (5). The AccuProbe system uses a single-stranded DNA probe with a chemiluminescent label that is complementary to the ribosomal RNA of the target organism. After the ribosomal RNA is released from the organism, the labeled DNA probe combines with the target organism's ribosomal RNA to form a stable DNA:RNA hybrid. The Selection Reagent allows for the differentiation of non-hybridized and hybridized probe. The labeled DNA:RNA hybrids are measured in a Hologic luminometer. A positive result is a luminometer reading equal to or greater than 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.

Reagents for the ACCUPROBE STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST are provided in three separate reagent kits:

ACCUPROBE STREPTOCOCCUS PNEUMONIAE PROBE KIT

Probe Reagent (10 x 2 tubes).
Streptococcus pneumoniae.

ACCUPROBE CULTURE IDENTIFICATION REAGENT KIT

Reagent 1 (Lysis Reagent). 1 x 10 mL
Buffered solution containing 0.04% sodium azide.

Reagent 2 (Hybridization Buffer). 1 x 10 mL
Buffered solution.

Reagent 3 (Selection Reagent). 1 x 60 mL
Buffered solution.

HOLOGIC DETECTION REAGENT KIT

Detection Reagent I. 1 x 240 mL
0.1% hydrogen peroxide in 0.001 N nitric acid.

Detection Reagent II. 1 x 240 mL
1 N sodium hydroxide.

WARNINGS AND PRECAUTIONS

- A. For *in vitro* diagnostic use.
- B. Use universal precautions when performing this assay (1).
- C. Use only for the identification of *Streptococcus pneumoniae* isolated from culture.
- D. Use only supplied or specified disposable laboratory ware.
- E. 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.
- F. Avoid contact of Detection Reagents I and II with skin and mucous membranes. Wash with water if these reagents come into contact with skin. If spills of these reagents occur, dilute with water before wiping dry.
- G. Do not interchange, mix or combine reagents from kits with different lot numbers.

STORAGE AND HANDLING REQUIREMENTS

Probe Reagent Tubes must be stored in the foil pouches at 2° to 8°C. The Probe Reagent Tubes are stable in the unopened pouches until the expiration date indicated. Once opened, the pouch should be resealed and the tubes should be used within two months and prior to the expiration date.

Other reagents used in the ACCUPROBE STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST may 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 ACCUPROBE STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST is designed to determine the identity of *S. pneumoniae* isolated from culture.

- A. **Solid Media Method.** Growth from appropriate solid media, such as 5% Sheep Blood Agar with morphology suggestive of *S. pneumoniae* may be tested. Samples may be tested as soon as the growth is visible, but should be less than 48 hours old.
 1. Discrete colonies can be taken off the solid media with a 1 µL disposable plastic loop, a wire loop, a disposable plastic needle, or an applicable stick. Swabs should not be used due to the small volume of liquid in which the cells are subsequently resuspended.

- If a single colony is to be tested, it should be at least 1 mm in diameter. Alternatively, several (3 to 4) smaller colonies can be tested. DO NOT USE CONFLUENT GROWTH.
- Avoid taking any of the solid media with the cells.
- The operator may elect to inoculate another culture plate at this time to confirm the purity of the isolate.

B. **Broth Culture Method.** Appropriate broth cultures, such as Trypticase Soy or Brain Heart Infusion with turbidity equivalent to or greater than a McFarland 1 Nephelometer Standard may be tested. Broth cultures, incubated for up to 24 hours at 37°C, may be used. Pipette a 50 µL sample from the well mixed broth suspension into the Probe Reagent Tubes, as described below.

MATERIALS PROVIDED

The ACCUPROBE STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST

Cat. No. 102865	20 tests
Probe Reagent	10 x 2 tubes

MATERIALS REQUIRED BUT NOT PROVIDED

1 µL plastic sterile inoculating loops, wire loops, plastic needles, or applicator sticks for selecting colonies

Control culture strains

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

Micropipettes (50 µL, 300 µL)

Re-pipettor (50 µL, 300 µL)

Vortex mixer

*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

ACCUPROBE CULTURE IDENTIFICATION REAGENT KIT

(Cat. No. 102800)

HOLOGIC DETECTION REAGENT KIT (Cat. No. 201791)

Hologic Dry Heat Bath (Cat. No. 102775 or 104006)

TEST PROCEDURE

A. EQUIPMENT PREPARATION

- Adjust the water bath or dry heat bath to 60° ± 1°C.
- Prepare the Hologic luminometer for operation. Make sure there is 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 Operator's Manual.

B. CONTROLS

Positive and negative control strains should be tested routinely in each laboratory according to local regulations. A culture of *S. pneumoniae* (e.g., American Type Culture Collection, ATCC #33400) may be used as the positive control while a culture of *Streptococcus bovis* (e.g., ATCC #33317) may be used as the negative control.

C. SAMPLE PREPARATION

- Open the foil pouch by cutting evenly across the top of the pouch. Remove enough Probe Reagent Tubes to test the culture isolates and/or controls. Reseal the pouch by folding the opened edge over several times and securing with adhesive tape or a clip. **Leave the desiccant pillow in the pouch.**
- Label a sufficient number of Probe Reagent Tubes to test the culture isolates and/or controls. Remove and retain the caps.

- Pipette 50 µL of Reagent 1 (Lysis Reagent) into all Probe Reagent Tubes. **If broth cultures are to be tested, do not add Reagent 1 to the Probe Reagent Tubes.**
- Transfer a 1 mm colony or several smaller colonies from the solid media or 50 µL of a well mixed broth culture into the Probe Reagent Tubes as described in the SAMPLE COLLECTION AND PREPARATION Section. Twirl the loop, needle or stick in Reagent 1 (Lysis Reagent) to remove the cells if testing growth from solid media and mix thoroughly.

D. HYBRIDIZATION

- Pipette 50 µL of Reagent 2 (Hybridization Buffer) into all Probe Reagent Tubes. Recap the Probe Reagent Tubes and mix by shaking or vortexing.
- Incubate for 15 minutes at 60° ± 1°C in a water bath or dry heat bath.

E. SELECTION

- Remove the Probe Reagent Tubes from the water bath or heating block. Remove and retain the caps. Pipette 300 µL of Reagent 3 (Selection Reagent) into each tube. Recap the tubes and VORTEX them to mix completely.
- Incubate the Probe Reagent Tubes for 5 minutes at 60° ± 1°C in a water bath or dry heat bath.
- Remove the Probe Reagent Tubes from the water bath or heating block and leave them at room temperature for at least 5 minutes. Remove and discard the caps. **Read the results in the luminometer within 1 hour after removing from the water bath or dry heat bath.**

F. DETECTION

- Select the appropriate protocol from the menu of the luminometer software.
- Using a damp tissue or paper towel, wipe each tube to ensure that no residue is present on the outside of the tube and insert the tube into the luminometer according to the instrument directions.
- When the analysis is complete, remove the tube(s) from the luminometer.

PROCEDURAL NOTES

- REAGENTS:** Reagent 2 (Hybridization Buffer) may precipitate at 2° to 8°C. Warming and mixing the solution at 35° to 60°C will dissolve the precipitate.
- SAMPLE COLLECTION AND PREPARATION:** It is important to sample growth from an area of the solid media where there are isolated colonies.
- TEMPERATURE:** The Sample Preparation, Hybridization and Selection reactions are temperature dependent. Therefore, it is imperative that the incubator, water bath or dry heat bath is maintained within the specified temperature range.
- TIME:**
 - The Hybridization Reaction should be started within 30 minutes of adding the cells and Reagent 1 to the Probe Reagent Tubes.
 - The Hybridization and Selection reactions are time dependent. Hybridize at least 15 minutes but no more than 20 minutes. Incubate the Probe Reagent Tubes during the SELECTION Step for at least 5 minutes but no more than 6 minutes.
- WATER BATH:** The level of water in the water bath should be maintained to ensure that the entire liquid reaction volume in the Probe Reagent Tubes is submerged, but should not be so high that the water might enter the tubes.
- VORTEXING:** It is critical to have a homogeneous mixture during the SELECTION Step, specifically after the addition of Reagent 3.

G. TROUBLE-SHOOTING

1. Elevated negative control values (*Streptococcus bovis*, ATCC #33317) greater than 20,000 RLU (Relative Light Units) in the Leader luminometer or 600 PLU (Photometric Light Units) in the AccuLDR (formerly PAL) luminometer can be caused by insufficient mixing after adding Reagent 3 (Selection Reagent) or by testing mixed cultures. Because mixed cultures can occur, a portion of the growth may be streaked onto the appropriate agar medium and incubated to check for multiple colony types.
2. Low positive control values (*Streptococcus pneumoniae*, ATCC #33400) less than 50,000 RLU in the Leader luminometer or 1,500 PLU in the AccuLDR (formerly PAL) luminometer can be caused by insufficient cell numbers, by testing mixed or aged cultures, or by leaving cells in Reagent 1 more than 30 minutes before adding Reagent 2. Because mixed cultures can occur, a portion of the growth may be streaked onto the appropriate agar medium and incubated to check for multiple colony types.

RESULTS

A. INTERPRETATION OF RESULTS

The results of the ACCUPROBE STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST are based on the following cut-off values. Samples producing signals greater than or equal to these cut-off values are considered positive. Signals less than these cut-off values are considered negative. Results in repeat ranges should be repeated.

	AccuLDR (formerly PAL)	Leader
Cut-off value	1,500 PLU	50,000 RLU
Repeat range	1,200-1,499 PLU	40,000-49,999 RLU

B. QUALITY CONTROL AND ACCEPTABILITY OF RESULTS

Negative control (e.g., *Streptococcus bovis*, ATCC #33317) and positive control (e.g., *Streptococcus pneumoniae*, ATCC #33400) should satisfy the following values:

	AccuLDR (formerly PAL)	Leader
Negative control	< 600 PLU	< 20,000 RLU
Positive control	> 1,500 PLU	> 50,000 RLU

LIMITATIONS

This method has been tested using fresh growth from solid media and from broth cultures listed in the SAMPLE COLLECTION AND PREPARATION Section. The efficacy of this test has not been demonstrated on direct clinical specimens.

Results from the ACCUPROBE STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

EXPECTED VALUES

The ACCUPROBE STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST was compared to standard culture biochemical identification methods at two sites using a total of 662 clinical isolates. Of these, 305 were *S. pneumoniae* isolates, 185 were isolates of other *Streptococcus* species, 172 were other microbial isolates representing 25 genera. Standard identification methods included Gram stain, colony morphology, catalase reaction, hemolytic activity on 5% sheep blood agar, optochin susceptibility, and bile solubility. The isolates were categorized as either positive ($\geq 50,000$ RLU) or negative ($< 50,000$ RLU). The range of observations for negative culture isolates was 344 to 32,911 RLU and 59,223 to

863,193 RLU for positive cultures. A comparison of the ACCUPROBE STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST and standard culture identification methods is shown below.

ACCUPROBE / CULTURE IDENTIFICATION

AccuProbe Culture	Pos Pos	Pos Neg	Neg Pos	Neg Neg	Sensitivity/ Specificity	Percent Agreement
Site 1	202	0	0	212	100%/100%	100%
Site 2	103	0	0	145	100%/100%	100%
Total	305	0	0	357	100%/100%	100%

All *S. pneumoniae* isolates produced a positive result and all other clinical isolates representing a phylogenetic cross-section of organisms produced a negative result with the ACCUPROBE STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST. The percent sensitivity, percent specificity, and percent agreement for the ACCUPROBE STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST compared to standard culture identification methods is 100%.

PERFORMANCE CHARACTERISTICS

A. WITHIN-RUN PRECISION

The within-run precision of the ACCUPROBE STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST was calculated by assaying two concentrations of ribosomal RNA isolated from *S. pneumoniae* using 10 replicates in a single assay.

Sample	A	B
Number of Replicates	10	10
Mean Response	101,292	56,025
Standard Deviation	4,293	1,743
Coefficient of Variation	4.2%	3.1%

B. BETWEEN-RUN PRECISION

The between-run precision was calculated by assaying the same two concentrations of *S. pneumoniae* ribosomal RNA using single determinations in 12 consecutive runs.

Sample	A	B
Number of Replicates	12	12
Mean Response	101,322	53,288
Standard Deviation	3,660	1,776
Coefficient of Variation	3.6%	3.3%

C. SPECIFICITY

A total of 95 ATCC reference isolates were evaluated using the ACCUPROBE STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST. These isolates represented a total of 78 species from 51 genera. Five isolates of *S. pneumoniae*, 24 isolates of 13 other *Streptococcus* species and 66 isolates of 50 other genera representing a phylogenetic cross-section of organisms were evaluated using the ACCUPROBE STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST. All *S. pneumoniae* isolates tested produced positive results using the ACCUPROBE STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST. Other *Streptococcus* species and the representative phylogenetic cross-section of species did not react using this kit.

D. RECOVERY

Seven serial dilutions of *S. pneumoniae* cells ranging from 3 thousand to 30 million cells per assay were tested in the presence of 30 million cells of the following non-target species: *Streptococcus salivarius* and

Streptococcus bovis. The presence of these non-target species did not interfere with the positive signal of the *S. pneumoniae* cell dilutions, nor did they generate a positive reaction with the ACCUPROBE STREPTOCOCCUS PNEUMONIAE CULTURE IDENTIFICATION TEST.

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