



GROUP B STREPTOCOCCUS CULTURE IDENTIFICATION TEST

INTENDED USE

The ACCUPROBE GROUP B STREPTOCOCCUS CULTURE IDENTIFICATION TEST is a rapid DNA probe test which utilizes the technique of nucleic acid hybridization for the identification of Group B Streptococcus from culture.

SUMMARY AND EXPLANATION OF THE TEST

Group B Streptococcus (*Streptococcus agalactiae*) is one of the leading causes of septicemia and meningitis in newborns. The incidence of Group B disease is estimated to be 2-5 infants per 1000 live births (6). The early onset form of this disease occurs during the first week of life and has a mortality rate greater than 50%. When the onset occurs after day seven, the mortality rate drops to 14-23% (1, 8). Newborns are at increased risk for Group B disease if they are born to women who are colonized with Group B Streptococcus in the vaginal or anorectal areas and who experience prolonged or difficult labor and delivery. As a strategy for prevention of Group B disease, CDC has recommended that all pregnant women be screened for anogenital Group B colonization at 35 to 37 weeks gestation, so that intrapartum antimicrobial prophylaxis can be offered to all women identified as carriers. Screening is accomplished by obtaining vaginal and/or anorectal swabs and culturing them in recommended media such as Lim broth, incubated for 18 to 24 hours (4). Group B Streptococcus can also cause serious illness in adults but it is far less common than in newborns (2).

Presumptive identification is made by traditional physiological and biochemical methods. These include gram stain, catalase reaction, hemolytic activity on sheep blood agar plates, hippurate or PYR hydrolysis, CAMP and bile esculin tests (5). Some Group B Streptococcus colonies are non-hemolytic on sheep blood agar. Therefore, confirmative identification of Group B Streptococcus requires a combination of biochemical tests and/or serological tests. The ACCUPROBE GROUP B STREPTOCOCCUS CULTURE IDENTIFICATION TEST offers a rapid, non-subjective method for the definitive identification of Group B Streptococcus based on the detection of specific ribosomal RNA sequences that are unique to *Streptococcus agalactiae*.

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 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 GEN-PROBE 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

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

ACCUPROBE GROUP B STREPTOCOCCUS PROBE KIT

Probe Reagent (2 x 10 tubes).
Group B Streptococcus

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.

GEN-PROBE 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 (3).
- C. Use only for the identification of Group B Streptococcus 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. To ensure optimal performance we recommend that prior to testing you inspect the tubes for dislodged material. If dislodged material is present, tap tube on the counter top in order to settle contents to the bottom of the tube.

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 GROUP B STREPTOCOCCUS CULTURE IDENTIFICATION TEST may be stored between 2° to 25°C and are stable until the expiration date indicated.

DO NOT FREEZE THE REAGENTS.

SAMPLE COLLECTION AND PREPARATION

The ACCUPROBE GROUP B STREPTOCOCCUS CULTURE IDENTIFICATION TEST is designed to determine the identity of Group B Streptococcus isolated from culture.

- A. **Solid Media Method.** Growth from solid media suggestive of Group B Streptococcus may be tested. Samples may be tested as soon as growth is visible but should be less than 72 hours old.
 1. Colonies can be removed with a 1 µL disposable plastic loop, a wire loop, a disposable plastic needle or an applicator stick. Swabs should not be used due to the small volume of liquid in which the cells are subsequently resuspended.
 2. If a single colony is to be tested, it should be at least 1 mm in diameter. A 1 µL loopful of cells or several (3- 4) smaller colonies can be tested.

3. Avoid taking any of the solid media with the cells.
4. The operator may elect to inoculate another culture plate at this time to confirm the purity of the isolate.

B. **Broth Culture Method.** Growth in broths, such as Todd Hewitt, Lim Broth, Thioglycollate or Trypticase Soy Broth, with turbidity equivalent to or greater than a McFarland 1 Nephelometer standard may be tested with this probe. Vortex broth tube. Pipette a 50 μ L sample from the well mixed broth suspension into the Probe Reagent Tubes as described under Test Procedure below.

C. **Method for Vaginal and/or Anorectal Swab Specimens.** Transport vaginal and/or anorectal swab specimens to the laboratory within 48 hours of collection at ambient temperature. Specimens may be stored in the laboratory an additional 24 hours at 2 to 8°C prior to testing. Immerse the swab into a tube of Lim broth (Todd Hewitt broth with nalidixic acid and colistin) for at least one minute and then express it against the side of the tube. Vortex the broth tube and incubate at 36° ± 1°C for 18 to 24 hours prior to performing the test procedure. After incubation, vortex broth tube and pipette a 50 μ L sample from the well-mixed broth suspension into the Probe Reagent Tubes as described under Test Procedure, Broth Media Method.

MATERIALS PROVIDED

ACCUPROBE® GROUP B STREPTOCOCCUS CULTURE IDENTIFICATION TEST

Cat. No. 2820	20 Tests
Probe Reagent	2 x 10 tubes

Cat. No. 2820B	200 Tests
Probe Reagent	20 x 10 tubes

MATERIALS REQUIRED BUT NOT PROVIDED

1 μ L plastic sterile inoculating loops, wire loops, plastic needles, or applicator sticks for selecting colonies.
 Control culture strains (e.g., *S. agalactiae*, ATCC#13813; *S. bovis*, ATCC# 33317)
 Incubator or water bath (35° to 37°C)
 Water bath or heating block (60°±1°C)
 Micropipettes (50 μ L, 300 μ L)
 Re-pipettor (50 μ L, 300 μ L)
 Vortex mixer
 Culture swabs with or without transport medium (such as Stuart's, modified Stuart's, or Amies)
 Broth media (such as Todd Hewitt, Lim Broth, Thioglycollate, or Trypticase Soy Broth)

AVAILABLE FROM GEN-PROBE:

GEN-PROBE® LEADER® Luminometer
 ACCUPROBE® CULTURE IDENTIFICATION REAGENT KIT
 (Cat. No. 2800)
 GEN-PROBE® DETECTION REAGENT KIT (Cat. No. 1791)
 GEN-PROBE® Heating Block (Cat. No. 2775)

TEST PROCEDURE

A. EQUIPMENT PREPARATION

1. Adjust the incubator or water bath to 35° to 37°C.
2. Adjust the water bath or heating block to 60° ± 1°C.
3. Prepare the GEN-PROBE luminometer for operation. Make sure there is sufficient volume of Detection Reagents I and II to complete the tests.

B. CONTROLS

Positive and negative control strains should be tested routinely in each laboratory according to local regulations. A culture of Group B Streptococcus (e.g., *Streptococcus agalactiae*, American Type Culture Collection, ATCC #13813) may be used as the positive control while a culture of *Streptococcus bovis* (e.g., ATCC #33317) may be used as the negative control.

If testing samples from solid media, select a 1 mm sized colony of each control strain from growth on solid media. If testing samples from broth, inoculate one colony from each of the control strains into broth and incubate according to sample test method selected under SAMPLE COLLECTION AND PREPARATION step.

C. SAMPLE PREPARATION

1. 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.**
2. Label a sufficient number of Probe Reagent Tubes to test the culture isolates and/or controls. Remove and retain the caps.
3. Solid Media Method:
 - a) Pipette 50 μ L of Reagent 1 (Lysis Reagent) into all Probe Reagent Tubes.
 - b) Transfer the samples from the solid media 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 and mix thoroughly.
4. Broth Media Method:
 - a) **If broth cultures are to be tested, do not add Reagent 1 to the Probe Reagent Tubes.**
 - b) Transfer 50 μ L of a well-mixed broth culture into the Probe Reagent Tubes as described in the SAMPLE COLLECTION AND PREPARATION section.
5. Recap the Probe Reagent Tubes and incubate at 35° to 37°C for 5 minutes in a water bath or 10 minutes at 35° to 37°C in an incubator.

D. HYBRIDIZATION

1. Remove the Probe Reagent Tubes from the water bath or incubator. Remove and retain the caps. Pipette 50 μ L of Reagent 2 (Hybridization Buffer) into all Probe Reagent Tubes.
2. Recap the Probe Reagent Tubes and incubate for 15 minutes at 60° ± 1°C in a water bath or heating block.

E. SELECTION

1. Remove the Probe Reagent Tubes from the water bath or heating block. Remove and retain the caps. Pipette 300 μ L of Reagent 3 (Selection Reagent) into each tube. Recap the tubes and VORTEX them to mix completely.
2. Incubate the Probe Reagent Tubes for 5 minutes at 60° ± 1°C in a water bath or heating block.
3. 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.

F. DETECTION

1. Select the appropriate protocol from the menu of the luminometer software.
2. 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.
3. When the analysis is complete, remove the tube(s) from the luminometer.

PROCEDURAL NOTES

- A. REAGENT: Reagent 2 (Hybridization Buffer) may precipitate. Warming and mixing the solution at 35° to 60°C will dissolve the precipitate.
- B. TEMPERATURE: The Sample Preparation, Hybridization and Selection reactions are temperature dependent. Therefore, it is imperative that the incubator, water bath or heating block is maintained within the specified temperature range.
- C. TIME:
1. The Hybridization Reaction should be started within 1 hour of adding the cells and Reagent 1 to the Probe Reagent Tubes.
 2. 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.
- D. 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.
- E. VORTEXING: It is critical to have a homogeneous mixture during the SELECTION Step, specifically after the addition of Reagent 3.
- F. TROUBLESHOOTING:

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 luminometer (formerly PAL) 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 agalactiae*, ATCC #13813) less than 50,000 RLU in the LEADER luminometer or 1,500 PLU in the ACCULDR luminometer (formerly PAL) can be caused by insufficient cell numbers or by testing mixed or aged 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.

RESULTS

A. INTERPRETATION OF RESULTS

The results of the ACCUPROBE GROUP B STREPTOCOCCUS 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

Results of repeated samples are based on the positive cut-off value ($\geq 1,500$ PLU; $\geq 50,000$ RLU) and negative cut-off value ($< 1,500$ PLU; $< 50,000$ RLU).

B. QUALITY CONTROL AND ACCEPTABILITY OF RESULTS

Negative control (e.g., *S. bovis*, ATCC #33317) and positive control (e.g., *S. agalactiae*, ATCC # 13813) should satisfy the following values:

	ACCULDR (formerly PAL)	LEADER
Negative control	<600 PLU	$<20,000$ RLU
Positive control	$>1,500$ PLU	$>50,000$ RLU

If the negative control or positive control values do not meet the control criteria, the test results are invalid and must not be reported.

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, on cultured swabs taken from neonates, or on cultured swabs taken from clinical sources other than the vagina or anorectum.

Cultures from prenatal screening samples have only been tested for use in the ACCUPROBE GROUP B STREPTOCOCCUS CULTURE IDENTIFICATION TEST from Lim broth (Todd Hewitt broth with nalidixic acid and colistin); other selective broth media have not been tested.

The ACCUPROBE GROUP B STREPTOCOCCUS CULTURE IDENTIFICATION TEST will react positively with some isolates of *Streptococcus equi*.

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

EXPECTED VALUES

The ACCUPROBE GROUP B STREPTOCOCCUS CULTURE IDENTIFICATION TEST was compared to standard culture biochemical identification methods using 299 isolates of the Group B Streptococcus species and 419 other bacterial isolates from 16 genera. Standard identification methods include Gram stain, catalase reaction, PYR and CAMP tests. The isolates were categorized as either positive ($\geq 50,000$ RLU) or negative ($< 50,000$ RLU). The range of observations for negative cultures was 221 to 8,800 RLU and 56,300 to 1,360,000 RLU for positive cultures. A comparison of these results to 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	99	0	0	152	100%/100%	100%
Site 2	100	0	0	100	100%/100%	100%
Site 3	100	0	0	167	100%/100%	100%
Total	299	0	0	419	100%/100%	100%

In addition, the ACCUPROBE GROUP B STREPTOCOCCUS CULTURE IDENTIFICATION TEST was compared to standard culture and serological or biochemical identification using 1,110 vaginal and/or anorectal swab specimens collected from pregnant women at 35 to 37 weeks gestation, and cultured in Lim broth (Todd Hewitt broth with nalidixic acid and colistin) for 18 to 24 hours. The Lim broth was also subcultured to blood agar, incubated for 48 hours, and examined for Group B Streptococcus colonies. A positive culture result from either culture method was considered a positive result.

Lim broth subcultures were tested according to the ACCUPROBE package insert protocol, Method for Vaginal and/or Anorectal Swab Specimens. Test results were read on a GEN-PROBE LEADER luminometer and categorized as positive ($\geq 50,000$ RLU, negative ($< 40,000$ RLU) or in the repeat range (40,000 to 49,999 RLU). The range of observations upon initial testing were 217 positive results at

68,127 to > 2,000,000 RLU, 880 negative results at 1,034 to 36,777 RLU, and 2 results in the repeat range at 47,779 and 49,254 RLU. Repeat results were determined based on a 50,000 RLU cutoff, yielding true negative results upon retest. A comparison of culture and ACCUPROBE results are shown below. Results are presented by site and include sensitivity, specificity, and corresponding 95% confidence intervals (95% CI).

ACCUPROBE /CULTURE IDENTIFICATION
(CULTURED VAGINAL AND/OR ANORECTAL
SWAB SPECIMENS)

Results of Initial Determination:

ACCUPROBE Culture	Pos Pos	Neg Neg	Neg Pos	Neg Neg	Sensitivity (95% CI)	Specificity (95% CI)
Site 1	26	0	1	151	96.3% (81.0-99.9%)	100% (97.6-100%)
Site 2	80	5	2	306	97.6% (91.5-99.7%)	98.4% (96.3-99.5%)
Site 3	111	3	2	423	98.2% (93.8-99.8%)	99.3% (98.0-99.9%)
Total	217	8	5	880	97.7% (94.8-99.3%)	99.1% (98.2-99.6%)

Range of Control Values Observed in the 3 site Study:

	RLU Values	
	Range	Mean
Positive Controls	52,700 to 1,499,003 ¹	510,505
Negative Controls	253 to 27,560 ²	6,060

¹65/66 runs had positive control values greater than 100,000 RLU.
²64/65 runs had negative control values less than 15,000 RLU (1 run was invalid due to a negative control value of 27,560 RLU).

PERFORMANCE CHARACTERISTICS

A. WITHIN-RUN PRECISION

The within-run precision of the ACCUPROBE GROUP B STREPTOCOCCUS CULTURE IDENTIFICATION TEST was calculated by assaying two concentrations of ribosomal RNA isolated from *Streptococcus agalactiae* (A,B) and a third sample (C) containing a mixture of ribosomal RNA isolated from *Streptococcus agalactiae*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Neisseria gonorrhoeae*, using 10 replicates in a single assay.

Sample	A	B	C
Number of Replicates	10	1010	
Mean Response	70,552	150,691	77,612
Standard Deviation	5,472	11,428	2,767
Coefficient of Variation	7.8%	7.6%	3.6%

B. BETWEEN-RUN PRECISION

The between-run precision was calculated by assaying the same two concentrations of *Streptococcus agalactiae* and the same mixed culture of ribosomal RNA using single determinations in 12 consecutive runs.

Sample	A	B	C
Number of Replicates	12	1212	
Mean Response	74,825	147,682	78,676
Standard Deviation	5,125	8,248	2,966
Coefficient of Variation	6.9%	5.6%	3.8%

C. ANALYTICAL SENSITIVITY

The analytical sensitivity (limits of detection) of the ACCUPROBE GROUP B STREPTOCOCCUS CULTURE IDENTIFICATION TEST has shown to detect 1.05×10^5 CFU of Group B Streptococcus per assay from solid or broth methods. Vaginal/anorectal swab samples tested with the Group B Streptococcus Culture Identification Test have shown to detect as few as 300 CFU of Group B Streptococcus when inoculated into Lim broth and incubated for 18 to 24 hours.

D. ANALYTICAL SPECIFICITY

A total of 93 culture isolates were evaluated using the ACCUPROBE GROUP B STREPTOCOCCUS CULTURE IDENTIFICATION TEST. These isolates represented a total of 93 species from 47 genera. Twenty-five isolates of 14 *Streptococcus* species including all 9 serotypes of Group B Streptococcus, 12 *Enterococcus* species and 54 other species from 45 genera representing a phylogenetic cross-section of organisms were evaluated using the ACCUPROBE GROUP B STREPTOCOCCUS CULTURE IDENTIFICATION TEST. All 9 serotypes of Group B Streptococcus reacted positively with the probe. In addition, some strains of *Streptococcus equi* reacted positively with the probe. All other species tested were negative.

Bloody swab specimens have not been proven to interfere with assay performance.

E. RECOVERY

Streptococcus bovis were added at a concentration of 21 million cells per test alone and to samples containing between 2 thousand and 21 million cells of *S. agalactiae* and did not interfere with the recovery of *S. agalactiae* using the ACCUPROBE GROUP B STREPTOCOCCUS CULTURE IDENTIFICATION TEST.

BIBLIOGRAPHY

1. **Anthony, B. F., and D. M. Okada.** 1977. The emergence of Group B streptococci infections of the newborn infant. *Ann. Rev. Med.* **28**: 355-369.
2. **Bayer, A. S., A. W. Chow, B. F. Anthony, and L. B. Guze.** 1976. Serious infections in adults due to Group B streptococci; clinical and serotypic characterization. *Am. J. Med.* **61**:498-503.
3. **Centers for Disease Control.** 1988. United States Morbid. and Mortal. Weekly Rep. **37**:377-382, 387-388.
4. **Centers for Disease Control.** 1996. Prevention of perinatal group B streptococcal disease: a public health perspective. *United States Morbid. and Mortal. Weekly Rep.* **45**:1-24.
5. **Facklam, R. R., and R. B. Carey.** 1985. Streptococci and aerococci. p. 154-175. *In* E. H. Linnette, et al. (ed.) *Manual of clinical microbiology*, 4th ed. American Society for Microbiology, Washington, D. C.
6. **Fisher, G., R. E. Horton and R. Edelman.** 1973. Summary of the National Institutes Of Health workshop on group B streptococcal infections. *J. Infect. Dis.* **14**:163-166.
7. **Kohne, D. E., A. G. Steigerwalt, and D. J. Brenner.** 1984. Nucleic acid probe specific for members of the genus *Legionella*. p. 107-108. *In* C. Thornsberry, et al. (ed.), *Legionella: proceedings of the 2nd international symposium*. American Society for Microbiology, Washington, D. C.
8. **Patterson, M. J., and A. El Batool Hafez.** 1976. Group B streptococci in human disease. *Bacteriol. Rev.* **40**:774-792.

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