



STAPHYLOCOCCUS AUREUS CULTURE IDENTIFICATION TEST

INTENDED USE

The ACCUPROBE STAPHYLOCOCCUS AUREUS CULTURE IDENTIFICATION TEST is a rapid DNA probe test which utilizes the technique of nucleic acid hybridization for the identification of *Staphylococcus aureus* (*S. aureus*) isolated from culture.

SUMMARY AND EXPLANATION OF THE TEST

Staphylococcus aureus (*S. aureus*) is the causative agent of a wide variety of infections in humans including diseases of the skin and soft tissues. Skin pustules, impetigo as well as more serious infections such as bacteremia, osteomyelitis, renal abscess, pneumonia, endocarditis, meningitis, gastroenteritis and toxic shock syndrome are among these diseases (3). *S. aureus* continues to be a prominent agent of nosocomial infections. Methicillin-resistant strains (MRSA) have emerged as a major epidemiological problem in hospitals throughout the United States.

Within the genus *Staphylococcus*, *S. aureus* is the most clinically significant species due to the incidence and severity of the infections it can cause (6).

Current methods used to identify *S. aureus* include Gram stain morphology, cell morphology, production of catalase, coagulase production, pigment production, susceptibility to lysostaphin and lysozyme, and anaerobic production of acid from glucose (4). In addition, there are several commercially available systems that allow strains to be biochemically characterized.

Among *Staphylococcus* species associated with human infections, *S. aureus* is unique in its ability to clot plasma (coagulase). It should be noted that some *Staphylococcus* species found in animals, such as *S. intermedius* and *S. hyicus* may also share this property. Two different coagulase tests are commonly used to identify *S. aureus*. One is a tube test for free coagulase and the other is a slide test for bound coagulase. The tube coagulase test is thought to be the more definitive of the two, however, it can take several hours to overnight to produce a result. The slide coagulase test may yield a negative result for up to 10 to 15 percent of *S. aureus* strains (2).

Because of the existence of problem clinical isolates that may not be identified using current methods and because of the coagulase-positive strains associated with animals, an alternative rapid and accurate identification method is needed. In clinical studies ACCUPROBE STAPHYLOCOCCUS AUREUS CULTURE IDENTIFICATION TEST detected all of the known methicillin-resistant *S. aureus* strains that were tested.

The ACCUPROBE STAPHYLOCOCCUS AUREUS CULTURE IDENTIFICATION TEST offers a rapid, objective method for the definitive identification of *S. aureus* based on the detection of specific ribosomal RNA sequences that are unique to *S. aureus*.

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 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 STAPHYLOCOCCUS AUREUS CULTURE IDENTIFICATION TEST are provided in three separate reagent kits:

ACCUPROBE STAPHYLOCOCCUS AUREUS PROBE KIT

Probe Reagent (4 x 5 tubes).
Staphylococcus aureus.

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 (1).
- C. Use only for the identification of *S. aureus* 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.

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 STAPHYLOCOCCUS AUREUS 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 STAPHYLOCOCCUS AUREUS CULTURE IDENTIFICATION TEST is designed to determine the identity of *S. aureus* isolated from culture.

A. **Solid Media Method.** Growth from appropriate solid media, with morphology suggestive of staphylococci may be tested. The culture should be less than 48 hours old. It can be tested as soon as the growth is visible.

1. Growth 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.** 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 72 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® STAPHYLOCOCCUS AUREUS CULTURE IDENTIFICATION TEST

Cat. No. 2875
Probe Reagent

20 tests
4 x 5 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

Incubator or water bath (35° to 37°C)

Water bath or heating block (60° ± 1°C)

Micropipettes (50 µL, 300 µL)

Re-pipettor (50 µL, 300 µL)

Vortex mixer

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 *S. aureus* (e.g., American Type Culture Collection, ATCC #12600) may be used as the positive control while a culture of *Staphylococcus epidermidis* (e.g., ATCC #14990) may be used as the negative control.

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. 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.**
4. Transfer the sample from the solid media or 50 µL of a well-mixed broth culture into the labeled 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 from solid media and mix thoroughly.
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 µL 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 µL 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 after removing from the water bath or heating block.**

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. **REAGENTS:** 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 heat 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. TROUBLE-SHOOTING
1. Elevated negative control values (*S. epidermidis*, ATCC #14990) 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 (*S. aureus*, ATCC #12600) 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 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 STAPHYLOCOCCUS AUREUS 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., *S. epidermidis*, ATCC #14990) and positive control (e.g., *S. aureus*, ATCC #12600) 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 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 STAPHYLOCOCCUS AUREUS CULTURE IDENTIFICATION TEST should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

EXPECTED VALUES

The ACCUPROBE STAPHYLOCOCCUS AUREUS CULTURE IDENTIFICATION TEST was compared to standard culture biochemical identification methods at 2 sites using a total of 641 clinical isolates. Of these, 309 were *S. aureus* isolates, 40 of the 309 *S. aureus* isolates were known Methicillin-resistant strains, 156 were isolates representing 12 *Staphylococcus* species, 176 were other microbial isolates representing 25 genera.

Five strains of coagulase-positive *Staphylococcus hyicus* and *Staphylococcus intermedius* were evaluated. None of these isolates produced a positive reaction with the ACCUPROBE STAPHYLOCOCCUS AUREUS CULTURE IDENTIFICATION TEST.

Standard identification methods included Gram stain morphology, colony morphology, slide coagulase, tube coagulase test and Staphaurex (Wellcome Diagnostics) at Site 2 only. The isolates were categorized as either positive ($\geq 50,000$ RLU) or negative ($< 50,000$ RLU). The range of observations for negative cultures was 243 to 32,022 RLU and 138,228 to 821,826 for positive cultures. A comparison of the ACCUPROBE STAPHYLOCOCCUS AUREUS 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	221	100%/100%	100%

ACCUPROBE / LATEX AGGLUTINATION IDENTIFICATION

ACCUPROBE LAT. Agg.	Pos POS	Pos NEG	NEG POS	NEG NEG	SENSITIVITY/ SPECIFICITY	PERCENT AGREEMENT
SITE 2	107	0	1*	110	99%/100%	99.5%
SITE 2 After Discrepant Analysis	107	0	0	111	100%/100%	100%

*Site 2 used Staphaurex (Wellcome Diagnostics) for their original identification of *S. aureus*. One apparent false negative isolate was reidentified as "not" *S. aureus* using the tube coagulase test, which gave a negative result.

ACCUPROBE / REFERENCE IDENTIFICATION

ACCUPROBE Ref. I.D.	Pos Pos	Pos Neg	Neg Pos	Neg Neg	Sensitivity/ Specificity	Percent Agreement
Total (of Site 1 & Site 2)	309	0	0	332	100%/100%	100%

This is the total sensitivity, specificity and percent agreement from Site 1 and Site 2 after reidentification of one culture isolate, using the tube coagulase test.

PERFORMANCE CHARACTERISTICS

A. WITHIN-RUN PRECISION

The within-run precision of the ACCUPROBE STAPHYLOCOCCUS AUREUS CULTURE IDENTIFICATION TEST was calculated by assaying two concentrations of *S. aureus* ribosomal RNA using 10 replicates in a single assay.

Sample	A	B
Number of Replicates	10	10
Mean Response	103,813	71,408
Standard Deviation	4,562	2,703
Coefficient of Variation	4.4%	3.8%

B. BETWEEN-RUN PRECISION

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

Sample	A	B
Number of Replicates	12	12
Mean Response	101,064	70,622
Standard Deviation	7,041	4,979
Coefficient of Variation	7.0%	7.1%

C. SPECIFICITY

A total of 88 ATCC culture isolates were evaluated using the ACCU-PROBE STAPHYLOCOCCUS AUREUS CULTURE IDENTIFICATION TEST. These isolates represented a total of 72 species from 50 genera. Thirteen isolates of *S. aureus*, 12 isolates of 8 other *Staphylococcus* species and 63 isolates of 49 other genera representing a phylogenetic cross-section of organisms were evaluated using the ACCU-PROBE STAPHYLOCOCCUS AUREUS CULTURE IDENTIFICATION TEST. All *S. aureus* isolates tested produced positive results using the ACCU-PROBE STAPHYLOCOCCUS AUREUS CULTURE IDENTIFICATION TEST. Other *Staphylococcus* species and the representative phylogenetic cross-section of species did not react using this kit.

D. RECOVERY

Five serial dilutions of *S. aureus* cells ranging from 0 to 10 million cells per assay were tested in the presence of 30 million cells of the following non-target species: *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. The presence of these non-target species did not interfere with the positive signal of the *S. aureus* cell dilutions, nor did they generate a positive reaction with the ACCU-PROBE STAPHYLOCOCCUS AUREUS CULTURE IDENTIFICATION TEST.

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