



## MYCOBACTERIUM TUBERCULOSIS COMPLEX CULTURE IDENTIFICATION TEST

### INTENDED USE

The ACCUPROBE MYCOBACTERIUM TUBERCULOSIS COMPLEX CULTURE IDENTIFICATION TEST is a rapid DNA probe test which utilizes the technique of nucleic acid hybridization for the identification of *Mycobacterium tuberculosis* (TB Complex) isolated from culture. The TB Complex consists of the following species: *M. tuberculosis*, *M. bovis*, *M. bovis BCG*, *M. africanum*, *M. microti* (8), and *M. canetti* (11).

### SUMMARY AND EXPLANATION OF THE TEST

Organisms of the TB Complex are responsible for significant morbidity and mortality in humans. *M. tuberculosis* is the most common TB Complex pathogen isolated in humans. 21,244 new cases of tuberculosis were reported in 1988 (2). *M. bovis BCG* may be transmitted from infected animals to humans (6). *M. africanum* causes pulmonary tuberculosis in tropical Africa (9) and *M. microti* primarily infects animals.

Tuberculosis is highly contagious, therefore rapid diagnosis of the disease is important. For most clinical laboratories assignment of an isolate to the TB Complex is sufficient because the probability that an isolate is a species other than *M. tuberculosis* is extremely small (5, 6, 10). A number of biochemical tests are recommended to speciate members of the TB Complex if further differentiation is required.

Classical methods for identification of mycobacteria rely on staining specimens for acid fast bacilli followed by culture and biochemical testing. It could take as long as two months to speciate an isolate using these standard methods (3).

The ACCUPROBE MYCOBACTERIUM TUBERCULOSIS COMPLEX CULTURE IDENTIFICATION TEST identifies TB Complex isolated from culture in less than an hour.

### 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 (4). 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 MYCOBACTERIUM TUBERCULOSIS COMPLEX CULTURE IDENTIFICATION TEST are provided in three separate reagent kits:

### ACCUPROBE MYCOBACTERIUM TUBERCULOSIS COMPLEX PROBE KIT

Probe Reagent. (4 x 5 tubes)  
*Mycobacterium tuberculosis* complex

Lysing Reagent. (1 x 20 tubes)  
Glass beads and buffer

### 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 TB Complex isolated from culture.
- D. Use only supplied or specified disposable laboratory ware.
- E. Culture handling and all procedural steps through the heat inactivation step should be performed in a Class II Biological Safety Cabinet.
- 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. 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 MYCOBACTERIUM TUBERCULOSIS COMPLEX 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 MYCOBACTERIUM TUBERCULOSIS COMPLEX CULTURE IDENTIFICATION TEST is designed to determine the identity of TB Complex isolated from culture.

- A. **Solid Media Method.** Growth from appropriate solid media, such as Lowenstein-Jensen slants or Middlebrook 7H10 or 7H11 plates, suggestive of TB Complex may be tested. Samples may be tested as soon as growth is visible and during the subsequent 60 days of incubation.
  1. Growth can be removed with a 1 µL disposable plastic loop, a wire loop, or a disposable plastic needle. Swabs should not be used due to the small volume of liquid in which the cells are subsequently resuspended.

2. Avoid taking any of the solid media with the cells.
3. 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 Middlebrook 7H9 broth with turbidity equivalent to or greater than a McFarland 1 Nephelometer Standard may be tested with the ACCUPROBE MYCOBACTERIUM TUBERCULOSIS COMPLEX CULTURE IDENTIFICATION TEST. Pipette a 100 µL sample from the well mixed broth suspension into the Lysing Reagent Tube as described below.

## **MATERIALS PROVIDED**

The ACCUPROBE® MYCOBACTERIUM TUBERCULOSIS COMPLEX CULTURE IDENTIFICATION TEST

<b>Cat. No. 2860</b>	<b>20 tests</b>
Probe Reagent	4 x 5 tubes
Lysing Reagent	1 x 20 tubes

## **MATERIALS REQUIRED BUT NOT PROVIDED**

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

Control culture strains

Water bath or heating block (59.5° to 61°C)

Water bath or heating block (95° ± 5°C)

Micropipettes (100 µL, 300 µL)

Re-pipettor (100 µL, 300 µL)

Vortex mixer

### **AVAILABLE FROM GEN-PROBE:**

GEN-PROBE® LEADER® Luminometer

GEN-PROBE® Sonicator or equivalent

ACCUPROBE® CULTURE IDENTIFICATION REAGENT KIT (Cat. No. 2800)

GEN-PROBE® DETECTION REAGENT KIT (Cat. No. 1791)

GEN-PROBE® Heating Block (Cat. No. 2775)

GEN-PROBE® Sonicator Rack (Cat. No. 4027)

## **TEST PROCEDURE**

### **A. EQUIPMENT PREPARATION**

1. For optimal transfer of sonic energy, water must be thoroughly degassed according to the following procedure:
  - a. Add enough water to fill the sonicator bath to within 1/2 inch of the top of the tank.
  - b. Run the sonicator for 15 minutes to thoroughly degas the water.
2. Adjust one heating block or water bath to 59.5° to 61°C and another heating block or water bath to 95° ± 5°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 *Mycobacterium tuberculosis* (e.g. American Type Culture Collection, ATCC #25177) may be used as the positive control while a culture of *Mycobacterium avium* (e.g., ATCC #25291) may be used as the negative control.

### **C. SAMPLE PREPARATION**

1. Label a sufficient number of Lysing Reagent Tubes to test the culture isolates and/or controls. Remove and retain the caps.
2. Pipette 100 µL of Reagent 1 (Lysis Reagent) and 100 µL of Reagent 2 (Hybridization Buffer) into all Lysing Reagent Tubes. **If broth cultures are to be tested, do not add Reagent 1 to the Lysing Reagent Tubes.**
3. Transfer the sample from the solid media or 100 µL of a well mixed broth culture into the labeled Lysing Reagent Tubes as described in the SAMPLE COLLECTION AND PREPARATION Section. Twirl the loop or needle in the Reagent 1 and

Reagent 2 diluent mixture to remove the cells if testing growth from solid media.

4. Recap the Lysing Reagent Tubes and briefly VORTEX.

### **D. SAMPLE LYSIS**

1. Push the Lysing Reagent Tubes through the Sonicator Rack so that the reaction mixture in the bottom of the tube is submerged but the caps are above the water. Place Sonicator Rack on water bath sonicator. **DO NOT ALLOW THE TUBES TO TOUCH THE BOTTOM OR SIDES OF THE SONICATOR.**
2. Sonicate for 15 minutes.
3. Place the Lysing Reagent Tubes containing the sonicated organisms in a heating block or water bath for 10 minutes at 95° ± 5°C.
4. Carefully remove the Lysing Reagent Tubes from the heating block or water bath.

### **E. HYBRIDIZATION**

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 100 µL of the lysed specimens from the Lysing Reagent Tubes into the corresponding Probe Reagent Tubes.
4. Recap the Probe Reagent Tubes and incubate for 15 minutes at 59.5° to 61°C in a water bath or heating block.

### **F. 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 10 minutes at 59.5° to 61°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.**

### **G. 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 Hybridization and Selection reactions are temperature dependent. Therefore, it is imperative that the water bath or heat block is maintained within the specified temperature range.
- C. TIME:** 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 10 minutes but no more than 11 minutes.
- D. WATER BATH:** The level of water in the water bath should be maintained to ensure that the Lysing Reagent Tubes are submerged up to, but not above, the level of the sealing ring. It

should also be ensured that the entire liquid reaction volume in the Probe Reaction Tubes is submerged.

- E. VORTEXING: It is critical to have a homogeneous mixture during the SAMPLE PREPARATION and SELECTION Steps, specifically after the addition of cells to Reagents 1 and 2 and after addition of Reagent 3.
- F. TROUBLE-SHOOTING
1. Elevated negative control values (*Mycobacterium avium* ATCC #25291) greater than 10,000 RLU (Relative Light Units) in the LEADER luminometer or 300 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 (*M. tuberculosis* ATCC #25177) less than 30,000 RLU in the LEADER luminometer or 900 PLU in the ACCULDR (formerly PAL) luminometer can be caused by insufficient cell numbers, improper sonication, 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 MYCOBACTERIUM TUBERCULOSIS COMPLEX 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	900 PLU	30,000 RLU
Repeat range	600 - 899 PLU	20,000-29,999 RLU

### B. QUALITY CONTROL AND ACCEPTABILITY OF RESULTS

Negative control (e.g., *M. avium*, ATCC #25291) and positive control (e.g., *M. tuberculosis*, ATCC #25177) should satisfy the following values:

	ACCULDR (formerly PAL)	LEADER
Negative control	<300 PLU	<10,000 RLU
Positive control	>900 PLU	>30,000 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 (e.g., urine, stool, or respiratory specimens).

The ACCUPROBE MYCOBACTERIUM TUBERCULOSIS COMPLEX CULTURE IDENTIFICATION TEST does not differentiate between members of the TB Complex, i.e., *M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, and *M. microti*. The Probe Reagent does not react with any mycobacteria other than tubercle (MOTT) bacilli.

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

## EXPECTED VALUES

The ACCUPROBE MYCOBACTERIUM TUBERCULOSIS COMPLEX CULTURE IDENTIFICATION TEST was compared to standard culture biochemical identification methods at two sites using a total of 612 isolates of the TB Complex, 748 isolates of 28 other *Mycobacterium* species, and 7 other microbial isolates representing 1 genus. Standard culture identification is dependent on growth rate, colony morphology, microscopic examination, and a series of biochemical reactions. The isolates were categorized as either positive (> 30,000 RLU) or negative (< 30,000 RLU). The range of observations for negative cultures was 226 to 33,343 RLU and 4,163 to 646,053 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	422	1	1	541	99.8%/99.1%	99.8%
Site 2	185	0	4	213	98.9%/100%	99.0%
<b>Total</b>	<b>607</b>	<b>1</b>	<b>5</b>	<b>754</b>	<b>99.2%/99.9%</b>	<b>99.6%</b>

When the discordant samples were retested, the correct results were obtained with the exception of one isolate from Site 2 which was nonviable.

## PERFORMANCE CHARACTERISTICS

### A. WITHIN-RUN PRECISION

The within-run precision of the ACCUPROBE MYCOBACTERIUM TUBERCULOSIS COMPLEX CULTURE IDENTIFICATION TEST was calculated by assaying two concentrations of ribosomal RNA isolated from *Mycobacterium tuberculosis* using 10 replicates in a single assay.

Sample	A	B
Number of Replicates	10	10
Mean Response	51,939	126,563
Standard Deviation	1,980	5,869
Coefficient of Variation	3.8%	4.6%

### B. BETWEEN-RUN PRECISION

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

Sample	A	B
Number of Replicates	12	12
Mean Response	51,522	126,227
Standard Deviation	1,952	4,575
Coefficient of Variation	3.8%	3.6%

### C. SPECIFICITY

A total of 94 ATCC culture isolates were evaluated using the ACCUPROBE MYCOBACTERIUM TUBERCULOSIS COMPLEX CULTURE IDENTIFICATION TEST. These isolates represented a total of 92 species from 40 genera. Six isolates of TB Complex (*M. africanum*, *M. bovis*, *M. microti* and *M. tuberculosis*), 25 isolates of 25 other *Mycobacterium* species, and 63 isolates of 39 other genera representing a phylogenetic cross-section of organisms were evaluated using the ACCUPROBE MYCOBACTERIUM TUBERCULOSIS COMPLEX CULTURE IDENTIFICATION TEST. All TB Complex isolates tested produced a positive result using the ACCUPROBE MYCOBACTERIUM TUBERCULOSIS COMPLEX CULTURE IDENTIFICATION TEST. Other *Mycobacterium* species and the representative phylogenetic cross-section isolates did not react in this test.

#### D. RECOVERY

*Mycobacterium tuberculosis* ribosomal RNA at concentrations ranging from  $5 \times 10^{-4}$   $\mu\text{g}$  to  $1 \times 10^{-1}$   $\mu\text{g}$  per test was assayed in the presence of 30 million cells of either *M. avium*, *M. kansasii*, or *Nocardia asteroides*. There was no interference with *M. tuberculosis* signal observed and the other organisms present did not react using the ACCUPROBE MYCOBACTERIUM TUBERCULOSIS COMPLEX CULTURE IDENTIFICATION TEST.

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