MYCOBACTERIUM AVIUM COMPLEX CULTURE IDENTIFICATION TEST

INTENDED USE
The ACCUPROBE MYCOBACTERIUM AVIUM COMPLEX CULTURE IDENTIFICATION TEST is a rapid DNA probe test which utilizes the technique of nucleic acid hybridization for the identification of Mycobacterium avium complex (M. avium complex) isolated from culture.

SUMMARY AND EXPLANATION OF THE TEST
Infections caused by members of the M. avium complex are the most common mycobacterial infections associated with AIDS and other immunocompromised patients (7,15). The incidence of M. avium complex as a clinically significant pathogen in cases of chronic pulmonary disease is also increasing (8,17). Recently, several laboratories have reported that the frequency of isolating M. avium complex is equivalent to or greater than the frequency of isolating M. tuberculosis (17). The treatment of these infections is difficult and the severity of the infection requires rapid diagnosis.

The M. avium complex consists of those slowly growing mycobacteria that produce little or no pigment, do not hydrolyze Tween 80 or urea, do not reduce nitrate, produce less than 45 mm foam in the semiquantitative catalase test, and produce positive reactions for nicotinamidase and pyrazinamidase. The complex is generally divided into two species, M. avium and M. intracellulare (19). Phenotypically these organisms are virtually indistinguishable and biochemical tests are unable to differentiate between them.

Methods that have commonly been used for identifying an isolate as a member of the M. avium complex have included cultural and biochemical procedures, serotyping, gas liquid chromatography, high performance liquid chromatography (HPLC), and isotopically labeled DNA probes that react with ribosomal RNA (Hologic Rapid Diagnostic System for the MYCOBACTERIUM AVIUM COMPLEX) (1, 3, 4, 5, 6, 9, 10, 12, 13, 16, 18). Furthermore, most strains of the M. avium complex may be identified as either M. avium or M. intracellulare by serotyping with adsorbed sera containing specific antibodies to cell surface antigens. However, recent studies on T-catalase, restriction fragment length polymorphisms, and DNA-DNA hybridization have demonstrated that some serovars formerly thought to be M. intracellulare actually belong to the species M. avium (1, 14, 21).

However, there are a small number of biochemically determined M. avium complex isolates that cannot be reliably speciated by any of the above methods as either M. avium or M. intracellulare. The exact taxonomic status of such strains is currently uncertain. The ACCUPROBE MYCOBACTERIUM AVIUM COMPLEX CULTURE IDENTIFICATION TEST has been designed to detect, M. avium, M. intracellulare, and other isolates more recently identified as belonging to the M. avium complex. It does not differentiate between the species within the complex (20, 22, 23). Rare isolates of the M. avium complex may not produce a positive reaction in this test.

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 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 MYCOBACTERIUM AVIUM COMPLEX CULTURE IDENTIFICATION TEST are provided in three separate reagent kits:

ACCUPROBE MYCOBACTERIUM AVIUM COMPLEX PROBE KIT
Probe Reagent. (4 x 5 tubes)
Mycobacterium avium 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

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 (2).
C. Use only for the identification of M. avium 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 AVIUM 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 AVIUM COMPLEX CULTURE IDENTIFICATION TEST is designed to determine the identity of M. avium 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 M. avium complex may be tested. Samples may be tested as soon as growth is visible and during the subsequent sixty 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 AVIUM 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 AVIUM COMPLEX CULTURE IDENTIFICATION TEST
Cat. No. 102845 20 tests
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 (60° ± 1°C)
Water bath or heating block (95° ± 5°C)
Micropipettes (100 µL, 300 µL)
Re-pipettor (100 µL, 300 µL)
Vortex mixer
McFarland 1 Nephelometer standard

AVAILABLE FROM HOLOGIC:
Hologic Leader® Luminometer
Hologic Sonicator or equivalent
ACCUPROBE CULTURE IDENTIFICATION REAGENT KIT
(Cat. No. 102800)
HOLOGIC DETECTION REAGENT KIT (Cat. No. 201791)
Hologic Heating Block (Cat. No. 102775)
Hologic Sonicator Rack (Cat. No. 104027)

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 hot 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 60° ± 1°C and another heating block or water bath to 95° ± 5°C.

3. Prepare the Hologic 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 avium (e.g. American Type Culture Collection, ATCC #25291) or Mycobacterium intracellulare (e.g., ATCC #13950) may be used as the positive control while a culture of Mycobacterium tuberculosis (e.g., ATCC #25177) 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 60° ± 1°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 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.

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 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 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 Reagent 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 (M. tuberculosis ATCC #25177) 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. avium ATCC #25291 or Mycobacterium intracellulare ATCC #13950) 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 AVIUM 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. If repeat testing yields equivocal results, then the isolate should be subcultured to verify its purity.

<table>
<thead>
<tr>
<th>Cut-off value</th>
<th>Repeat range</th>
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<tbody>
<tr>
<td>900 PLU</td>
<td>600 - 899 PLU</td>
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<tr>
<td>30,000 RLU</td>
<td>20,000 - 29,999 RLU</td>
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</table>

B. QUALITY CONTROL AND ACCEPTABILITY OF RESULTS

Negative control (e.g., M. tuberculosis, ATCC #25177) and positive control (e.g., M. avium, ATCC #25291), whether from broth culture or solid media, should satisfy the following values:

<table>
<thead>
<tr>
<th>Negative control</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 300 PLU</td>
<td>&gt; 900 PLU</td>
</tr>
<tr>
<td>&lt; 10,000 RLU</td>
<td>&gt; 30,000 RLU</td>
</tr>
</tbody>
</table>

If the positive control or negative control values are not in the required range, the test results must not be reported.

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 (e.g., urine, stool, or respiratory specimens).

The ACCUPROBE MYCOBACTERIUM AVIUM COMPLEX CULTURE IDENTIFICATION TEST does not differentiate between members of the M. avium complex. Isolates of either species will be identified as M. avium complex.

There are a small number of biochemically determined M. avium complex isolates that cannot be differentiated by serology or HPLC methods as either M. avium or M. intracellulare. Some of these strains also may not be detected by the ACCUPROBE MYCOBACTERIUM AVIUM COMPLEX CULTURE IDENTIFICATION TEST. The exact taxonomic status of such strains is at present uncertain. The ACCUPROBE MYCOBACTERIUM AVIUM COMPLEX CULTURE IDENTIFICATION TEST identifies M. avium complex strains that belong to this complex based on traditional biochemical methods HPLC or GLC procedures. ACCUPROBE MYCOBACTERIUM AVIUM COMPLEX CULTURE IDENTIFICATION TEST may detect unusual M. avium complex strains, the clinical significance of which is not well established.

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

EXPECTED VALUES

The ACCUPROBE MYCOBACTERIUM AVIUM COMPLEX CULTURE IDENTIFICATION TEST was compared to standard biochemical culture identification methods at three sites. Site 1 was Hologic Incorporated; Sites 2 and 3 were Reference Laboratories. Seven hundred seventeen isolates of M. avium complex (51 M. avium, 42 M. intracellulare, and 624 M. avium complex) and 235 strains of other Mycobacterium strains representing 22 species were tested. The results were categorized as either positive (< 30,000 RLU) or negative (< 30,000 RLU). The range of observations for negative cultures was 1,353 to 14,675 RLU and 30,829 to 2,742,691 RLU for positive cultures. A comparison of these results to standard culture identification methods is shown below.

<table>
<thead>
<tr>
<th>AccuProbe</th>
<th>Pos</th>
<th>Neg</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>44</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Site 2</td>
<td>146</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Site 3</td>
<td>526</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>716</td>
<td>0</td>
<td>100%</td>
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</table>

Upon Discrepant Resolution

<table>
<thead>
<tr>
<th>ACCUPROBE/STANDARD BIOCHEMICAL PROCEDURES</th>
</tr>
</thead>
<tbody>
<tr>
<td>AccuProbe Culture</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Site 1</td>
</tr>
<tr>
<td>Site 2</td>
</tr>
<tr>
<td>Site 3</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
The one discrepant sample (2416) was analyzed by the CDC, Atlanta, Georgia, and was identified as an *M. avium* complex by HPLC analysis. The drug susceptibility pattern of this organism was unusual for a member of the *M. avium* complex, and biochemical results were also atypical.

Site 3 had 17 original discrepant. Two of these discrepant (7755, 5113) were misidentified and have been re-identified by HPLC and GLC as *M. nonchromogenicum*. Three cultures were deleted from the study because two were mixed (4750, 8168), and one culture was no longer viable (0601). Two other cultures were deleted from the study because they could not be definitively identified as belonging to the MAC: 2344 and 5124 are identified as "most closely resembles MAC." HPLC results on seven of the ten remaining discrepant were received from the CDC, Atlanta, Georgia. Strains PE09 and 6458 were identified as *M. xenopi* 2, while 9714 and 8310 were identified as *M. terrae* complex. Discrepants 1264 and 3634 were identified as *M. scrofulaceum* by the CDC based on their HPLC patterns. Strain 1264 exhibited pattern SC007 while strain 3634 showed pattern EM002.

Strain 0214 was identified as *M. simiae*, while 8153 has been confirmed as a MAIS HPLC pattern EM005. Isolates 2888 and 2971 were identified as "unidentified scotochromogen." Members of the *M. avium* complex are not scotochromogenic.

Thus, upon resolution, the overall sensitivity is 99.9%, specificity is 100%, and the percent agreement is 99.9%.

In a separate evaluation of 148 unusual MAC isolates referred to reference laboratories because of difficulties in identification, 120 reacted positively with the *M. avium* reference laboratories because of difficulties in identification, 120 reacted positively with the *M. avium* culture identification test. These isolates represented a total of 93 species from 37 genera. Three isolates of *M. avium*, *M. intracellulare* or non-*M. avium*, non-*M. intracellulare* *M. avium* complex using 12 replicates in a single assay.

**Mycobacterium avium**

<table>
<thead>
<tr>
<th>Sample</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Replicates</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Mean Response</td>
<td>67,574</td>
<td>112,246</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>2,900</td>
<td>3,429</td>
</tr>
<tr>
<td>Coefficient of Variation</td>
<td>4.3%</td>
<td>3.1%</td>
</tr>
</tbody>
</table>

**Mycobacterium intracellulare**

<table>
<thead>
<tr>
<th>Sample</th>
<th>A</th>
<th>B</th>
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</thead>
<tbody>
<tr>
<td>Number of Replicates</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Mean Response</td>
<td>61,758</td>
<td>100,736</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>3,941</td>
<td>3,275</td>
</tr>
<tr>
<td>Coefficient of Variation</td>
<td>6.4%</td>
<td>3.3%</td>
</tr>
</tbody>
</table>

**M. avium complex**

<table>
<thead>
<tr>
<th>Sample</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Replicates</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Mean Response</td>
<td>64,148</td>
<td>113,049</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>3,384</td>
<td>3,249</td>
</tr>
<tr>
<td>Coefficient of Variation</td>
<td>5.3%</td>
<td>2.9%</td>
</tr>
</tbody>
</table>

B. **BETWEEN-RUN PRECISION**

The between-run precision was calculated by assaying the same two concentrations of *M. avium*, *M. intracellulare* non-*M. avium*, non-*M. intracellulare* *M. avium* complex ribosomal RNA using single determinations in 10 consecutive runs.

**Mycobacterium avium**

<table>
<thead>
<tr>
<th>Sample</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Replicates</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mean Response</td>
<td>65,790</td>
<td>125,506</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>4,535</td>
<td>9,115</td>
</tr>
<tr>
<td>Coefficient of Variation</td>
<td>6.9%</td>
<td>7.3%</td>
</tr>
</tbody>
</table>

**Mycobacterium intracellulare**

<table>
<thead>
<tr>
<th>Sample</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Replicates</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mean Response</td>
<td>60,175</td>
<td>104,203</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>6,339</td>
<td>9,239</td>
</tr>
<tr>
<td>Coefficient of Variation</td>
<td>10.5%</td>
<td>8.9%</td>
</tr>
</tbody>
</table>

**M. avium complex**

<table>
<thead>
<tr>
<th>Sample</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Replicates</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mean Response</td>
<td>64,187</td>
<td>111,197</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>4,659</td>
<td>10,011</td>
</tr>
<tr>
<td>Coefficient of Variation</td>
<td>7.3%</td>
<td>9.0%</td>
</tr>
</tbody>
</table>

C. **SPECIFICITY**

A total of 122 ATCC culture isolates were evaluated using the ACCUPROBE MYCOBACTERIUM AVIUM COMPLEX CULTURE IDENTIFICATION TEST. These isolates represented a total of 93 species from 37 genera. Three isolates of *M. avium* complex, 60 isolates of 55 other *Mycobacterium* species, and 59 isolates of 36 other genera representing a phylogenetic cross-section of organisms were evaluated using the ACCUPROBE MYCOBACTERIUM AVIUM COMPLEX CULTURE IDENTIFICATION TEST. Only *Mycobacterium avium* complex isolates tested produced a positive result using the ACCUPROBE MYCOBACTERIUM AVIUM COMPLEX CULTURE IDENTIFICATION TEST. Other *Mycobacterium* species and the representative phylogenetic cross-section isolates did not react in this test.

D. **RECOVERY**

*M. avium*, *M. intracellulare*, and *M. avium* complex ribosomal RNA, each at concentrations ranging from 2.5 x 10^-3 µg to 4.0 x 10^-2 µg per test was assayed in the presence of 15 million cells of either *Mycobacterium terrae*, *M. simiae*, or *Nocardia asteroides*. No interference of *M. avium* complex signal was observed and the other organisms present did not react using the ACCUPROBE MYCOBACTERIUM AVIUM COMPLEX CULTURE IDENTIFICATION TEST.

**BIBLIOGRAPHY**


