HOLOGIC®
AccuProbe®

MYCOBACTERIUM INTRACELLULARE CULTURE IDENTIFICATION TEST

INTENDED USE

The ACCUPROBE MYCOBACTERIUM INTRACELLULARE CULTURE IDENTIFICATION TEST is a rapid DNA probe test which utilizes the technique of nucleic acid hybridization for the identification of *Mycobacterium intracellulare* isolated from culture.

SUMMARY AND EXPLANATION OF THE TEST

Mycobacterium intracellulare (M. intracellulare) is a member of the *Mycobacterium avium* complex (*M. avium* complex) which consists of a number of organisms whose taxonomic relationships are both unclear and controversial, but whose pathogenicity in man is unquestioned (16). *M. intracellulare* has been shown to cause significant disease in immunocompromised patients (12). Treatment of this infection is difficult and the severity of the infection requires rapid diagnosis. Additionally in some laboratories the incidence of *M. avium* complex is equal to or greater than the incidence of *M. tuberculosis.*

Classical methods for identification of mycobacteria rely on staining specimens for acid fast bacilli followed by culture and subsequent biochemical testing. It can take as long as two months to speciate a *Mycobacterium* isolate using these standard culture methods (8).

M. avium complex is generally thought to consist of two species: *M. avium* and *M. intracellulare*. Phenotypically *M. avium* and *M. intracellulare* are virtually indistinguishable and biochemical tests are unable to differentiate between them.

High Performance Liquid Chromatography (HPLC) has been useful in the identification of *M. avium* and *M. intracellulare* (3), however, it is time consuming and not readily available to most clinical laboratories.

Serology has also been used for the differentiation of *M. intracellulare* and *M. avium* strains, using α-antigen sera and may be useful in epidemiological studies. However, serotyping is not generally available and is of limited use in patient management. Currently there are 28 serovars generally accepted within the *M. avium* complex and at various times different serotypes have been assigned to the individual species *M. avium* and *M. intracellulare*. Historically serovars 1 through 3 were considered *M. avium,* while serovars 4 through 28 were considered *M. intracellulare* (15).

In several studies, Baess utilized DNA:DNA hybridization to clarify the taxonomic relationships between these two species. Based upon her analyses, she concluded that serovars 4, 5, 6, and 8, which at that time had been classified as *M. intracellulare*, actually belonged to the species *M. avium*. The status of serovar 9 was unclear (1, 2).

Saito, *et al*, also utilized DNA probe technology and has proposed reassigning the 28 serovars as follows: serovars 1 through 6, 8 through 11, and 21 to *M. avium*; serovars 7, 12 through 20, and 25 to *M. intracellulare,* serovars 22 through 28 (except for serovar 25) were considered to be heterogeneous and could not be assigned to either species (13). In addition, some strains could not be serotyped, and a few agglutinated in more than one antisera.

Other reports utilizing DNA probes for speciating within the *Mycobacterium avium* complex have also been published, including the use of DNA probes for epidemiological studies and the geographical distribution of *M. avium* and *M. intracellulare* (5-7, 9, 11, 14).

The ACCUPROBE MYCOBACTERIUM INTRACELLULARE CULTURE IDENTIFICATION TEST identifies *M. intracellulare* isolated from culture in less than one hour of sample preparation. Identification is based upon the detection of specific ribosomal RNA sequences that are unique to *M. intracellulare*. The ACCUPROBE MYCOBACTERIUM INTRACELLULARE CULTURE IDENTIFICATION TEST offers a rapid, non-subjective and accurate means of identifying *M. intracellulare* isolated from culture.

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 (10). 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 INTRACELLULARE CULTURE IDENTIFICATION TEST are provided in three separate reagent kits:

ACCUPROBE MYCOBACTERIUM INTRACELLULARE PROBE KIT

Probe Reagent. *Mycobacterium intracellulare*.	4 x 5 tubes
Lysing Reagent. Glass beads and buffer.	1 x 20 tubes

ACCUPROBE CULTURE IDENTIFICATION REAGENT KIT

Reagent 1 (Lysis Reagent). Buffered solution containing 0.04% sodium azide.	1 x 10 mL
Reagent 2 (Hybridization Buffer). Buffered solution.	1 x 10 mL
Reagent 3 (Selection Reagent). Buffered solution.	1 x 60 mL

HOLOGIC DETECTION REAGENT KIT

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

WARNINGS AND PRECAUTIONS

A. For *in vitro* diagnostic use.

B. Use universal precautions when performing this assay (4).

C. Use only for the identification of *M. intracellulare* isolated from culture.

D. Use only supplied or specified 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 INTRACELLULARE 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 MYCOBACTERIUM INTRACELLULARE CULTURE IDENTIFICATION TEST is designed to determine the identity of *Mycobacterium intracellulare* 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. intracellulare* 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 INTRACELLULARE CULTURE IDENTIFICATION TEST. Pipette a 100 μL sample from the well mixed broth suspension into the Lysing Tube as described below.

MATERIALS PROVIDED

The ACCUPROBE MYCOBACTERIUM INTRACELLULARE CULTURE IDENTIFICATION TEST

Cat. No. 102840	**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 dry heat bath* (60° ± 1°C)
Water bath or dry heat bath* (95° ± 5°C)
Micropipettes (100 μL, 300 μL)
Re-pipettor (100 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
Hologic Sonicator or equivalent
ACCUPROBE CULTURE IDENTIFICATION REAGENT KIT
(Cat. No. 102800)
HOLOGIC DETECTION REAGENT KIT
(Cat. No. 201791)
Hologic Dry Heat Bath
(Cat. No. 102775)
Hologic Sonicator Rack
(Hologic 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 dry heat bath or water bath to 60° ± 1°C and another dry heat bath 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 *M. intracellulare* (e.g., American Type Culture Collection, ATCC #13950) may be used as the positive control while a culture of *M. 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 dry heat bath or water bath for 10 minutes at 95° ± 5°C.
4. Carefully remove the Lysing Reagent Tubes from the dry heat bath 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 dry heat bath.

F. SELECTION

1. Remove the Probe Reagent Tubes from the water bath or dry heat bath. 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 dry heat bath.
3. Remove the Probe Reagent Tubes from the water bath or dry heat bath 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 precipitates.

B. TEMPERATURE: The Hybridization and Selection reactions are temperature dependent. Therefore, it is imperative that the water bath or dry heat bath 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 homogenous 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. TROUBLESHOOTING:

1. Elevated negative control values (*M. 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. 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 INTRACELLULARE 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. intracellulare,* ATCC #13950) 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).

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

EXPECTED VALUES

The ACCUPROBE MYCOBACTERIUM INTRACELLULARE CULTURE IDENTIFICATION TEST was compared to standard culture biochemical identification methods from two sites, site 1 and 2, using a total of 259 isolates: 109 isolates of the *M. intracellulare* and 147 isolates of 19 other *Mycobacterium* species. Standard culture identification is dependent on growth rate, colony morphology, microscopic examination, and a series of biochemical reactions. In addition, the ACCUPROBE MYCOBACTERIUM INTRACELLULARE CULTURE IDENTIFICATION TEST was compared to High Performance Liquid Chromatography (HPLC) at site 3 using a total of 97 *Mycobacterium* strains. HPLC identified 31 isolates as *M. intracellulare*, 36 isolates as *M. avium*, and 36 as isolates representing 12 other *Mycobacterium* species. Using the ACCUPROBE MYCOBACTERIUM INTRACELLULARE CULTURE IDENTIFICATION TEST all isolates were categorized as either positive (≥ 30,000 RLU) or negative (< 30,000 RLU). The range of observations for negative cultures was 266 to 3,405 RLU and 33,851 to 559,708 RLU for positive cultures. A comparison of these results to standard culture identification methods (Sites 1 and 2) and HPLC methods (Site 3) are shown below.

ACCUPROBE / CULTURE AND HPLC IDENTIFICATION

AccuProbe Culture	**Pos Pos**	**Pos Neg**	**Neg Pos**	**Neg Neg**	**Sensitivity/ Specificity**	**Percent Agreement**
Site 1	28	0	0	50	100% / 100%	100%
Site 2	81	0	0	100	100% / 100%	100%
Site 3	31	0	0	66	100% / 100%	100%
Total	**140**	**0**	**0**	**216**	**100% / 100%**	**100%**

PERFORMANCE CHARACTERISTICS

A. WITHIN-RUN PRECISION

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

Mycobacterium intracellulare

Sample	**A**	**B**
Number of Replicates	10	10
Mean Response	39,179	71,587
Standard Deviation	764	2,123
Coefficient of Variation	2.0%	3.0%

B. BETWEEN-RUN PRECISION

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

Mycobacterium intracellulare

Sample	**A**	**B**
Number of Replicates	12	12
Mean Response	37,541	72,189
Standard Deviation	3,318	4,352
Coefficient of Variation	8.8%	6.0%

C. SPECIFICITY

A total of 106 ATCC culture isolates were evaluated using the ACCUPROBE MYCOBACTERIUM INTRACELLULARE CULTURE IDENTIFICATION TEST. These isolates represented a total of 91 species from 39 genera. Five isolates of *M. intracellulare,* 37 isolates of 25 other *Mycobacterium* species, and 64 isolates of 38 other genera representing a phylogenetic cross-section of organisms were evaluated using the ACCUPROBE MYCOBACTERIUM INTRACELLULARE CULTURE IDENTIFICATION TEST. Only *M. intracellulare* isolates tested produced a positive result using the ACCUPROBE MYCOBACTERIUM INTRACELLULARE CULTURE IDENTIFICATION TEST. Other *Mycobacterium* species and the representative phylogenetic cross-section species did not react using this kit.

D. RECOVERY

M. intracellulare ribosomal RNA at concentrations ranging from 5 x 10^{-4} μg and 1 x 10^{-1} μg per test was assayed in the presence of 15 million cells of either *M. tuberculosis, M. simiae,* or *Nocardia asteroides.* No interference of *M. intracellulare* signal was observed and the other organisms present did not react using the ACCUPROBE MYCOBACTERIUM INTRACELLULARE CULTURE IDENTIFICATION TEST.

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