HOLOGIC®

AccuProbe®

MYCOBACTERIUM KANSASII CULTURE IDENTIFICATION TEST

INTENDED USE

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

SUMMARY AND EXPLANATION OF THE TEST

Mycobacterium kansasii (*M. kansasii*) is a slow-growing photochromogenic bacterium that causes chronic human pulmonary disease resembling tuberculosis (1). Disseminated infections caused by non-tuberculous mycobacteria such as *M. kansasii* are becoming an increasing public health concern with the expansion of the AIDS epidemic across the United States (2). *M. kansasii* accounted for 3.5% of the pathogenic isolates reported to the Centers for Disease Control in 1980 (3).

The endemic source of *M. kansasii* is unknown. Extensive soil sampling has failed to isolate *M. kansasii* while some strains have been isolated from water (1).

Classical methods for identification of *Mycobacterium* species rely on staining specimens for acid-fast bacilli followed by culture, colony and cell morphology, growth rate and subsequent biochemical testing. *M. kansasii* cells are moderately long to long acid fast rods. Colonies range from flat to raised with irregular edges and smooth to rough morphology. *M. kansasii* colonies are typically non-pigmented when grown in the dark and turn lemon yellow after exposure to light (photochromogenic). Prolonged light exposure may induce production of dark red ß-carotene crystals on the surface and inside of the colonies. Biochemical reactions include positive results for nitrate reduction, tween hydrolysis, urea hydrolysis, and catalase activity. It could take as long as two months to speciate a *Mycobacterium* isolate using these standard methods (1,4).

The ACCUPROBE MYCOBACTERIUM KANSASII CULTURE IDENTIFICATION TEST offers a rapid, non-subjective and accurate method of identification of *M. kansasii* isolated from culture. Colonies may be identified as soon as growth is visible. The ACCUPROBE MYCOBACTERIUM KANSASII CULTURE IDENTIFICATION TEST identifies *M. kansasii* organisms 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 (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 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 KANSASII CULTURE IDENTIFICATION TEST are provided in three separate reagent kits:

ACCUPROBE MYCOBACTERIUM KANSASII PROBE KIT

Probe Reagent (4 x 5 tubes).
Mycobacterium kansasii

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 laboratory precautions when performing this assay (6).

C. Use only for the determination of *M. kansasii* 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 KANSASII 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 KANSASII CULTURE IDENTIFICATION TEST is designed to determine the identity of *M. kansasii* 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. kansasii* 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 KANSASII 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 KANSASII CULTURE IDENTIFICATION TEST

Cat. No.102855	**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

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 kansasii* (e.g., American Type Culture Collection, ATCC #12478) 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 completely mix.
   2. Incubate the Probe Reagent Tubes for 8 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 of completion of the test**.

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 heating 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 8 minutes but no more than 9 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. 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. kansasii*, ATCC #12478) 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 KANSASII 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. tuberculosis*, ATCC #25177) and positive control (e.g., *M. kansasii*, ATCC # 12478) 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 KANSASII CULTURE IDENTIFICATION TEST should be interpreted in conjunction with other laboratory and clinical data available to the clinician.

EXPECTED VALUES

The ACCUPROBE MYCOBACTERIUM KANSASII CULTURE IDENTIFICATION TEST was compared to standard culture identification methods at two sites using a total of 390 isolates. These isolates were comprised of 121 *M. kansasii* isolates and 269 isolates of 24 other *Mycobacterium* species. Standard culture identification included growth rate, colony and cell morphology (photochromogenic characteristics), microscopic examination and biochemical determinations. The isolates were categorized as either positive (>30,000 RLU) or negative (<30,000 RLU). The range of observations for negative cultures was 663 to 11,021 RLU and 41,966 to 532,925 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	11	0	1	91	91.7%/100%	99.0%
Site 2	95	1	7	106	93.1%/99.1%	96.2%
Site 3	80	1	20	100	80.0%/99.0%	89.6%
Total	**186**	**2**	**28**	**297**	**86.9%/99.3%**	**94.2%**

One AccuProbe negative, culture positive isolate at Site 1 was retested with the ACCUPROBE MYCOBACTERIUM KANSASII CULTURE IDENTIFICATION TEST and continues to yield the same results. Biochemical methods and HPLC identify the isolate as *M. kansasii*.

One AccuProbe positive, culture negative isolate at Site 2 was retested with the ACCUPROBE MYCOBACTERIUM KANSASII CULTURE IDENTIFICATION TEST and produced a negative result. Three AccuProbe negative, culture positive isolates were retested with the ACCUPROBE MYCOBACTERIUM KANSASII CULTURE IDENTIFICATION TEST and produced positive results. One AccuProbe negative, culture positive isolate was determined to be a mixed culture and discarded from the study. The sensitivity and specificity for Site 2 are 97.0% and 100%, respectively.

One AccuProbe positive, culture negative isolate at Site 3 was retested with the ACCUPROBE MYCOBACTERIUM KANSASII CULTURE IDENTIFICATION TEST and produced a negative result. Four AccuProbe negative, culture positive isolates were retested with the ACCUPROBE MYCOBACTERIUM KANSASII CULTURE IDENTIFICATION TEST and produced positive results. Two AccuProbe negative, culture positive isolates were deleted from the study because they had been mislabeled. Two AccuProbe negative, culture positive isolates were re-identified as *M. szulgai* and one was re-identified as *M. smegmatis*. The final sensitivity and specificity for Site 3 are 88.4% and 100%, respectively.

The overall sensitivity, specificity and percent agreement upon repeat testing are 92.8%, 100% and 97.1%, respectively.

PERFORMANCE CHARACTERISTICS

A. WITHIN-RUN PRECISION

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

Sample	**A**	**B**
Number of Replicates	10	10
Mean Response	51,640	93,115
Standard Deviation	1,389	2,883
Coefficient of Variation	2.7%	3.1%

B. BETWEEN-RUN PRECISION

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

Sample	**A**	**B**
Number of Replicates	12	12
Mean Response	51,252	95,276
Standard Deviation	2,734	3,705
Coefficient of Variation	5.3%	3.9%

C. SPECIFICITY

A total of 148 ATCC reference isolates were evaluated using the ACCUPROBE MYCOBACTERIUM KANSASII CULTURE IDENTIFICATION TEST. These isolates represented a total of 122 species representing 44 genera. Eleven isolates of *M. kansasii*, 69 isolates of 54 other *Mycobacterium* species and 68 isolates of 67 species from 43 other genera representing a wide phylogenetic cross-section of organisms were evaluated using the ACCUPROBE MYCOBACTERIUM KANSASII CULTURE IDENTIFICATION TEST. All *Mycobacterium* kansasii isolates tested produced positive reactions using the ACCUPROBE MYCOBACTERIUM KANSASII CULTURE IDENTIFICATION TEST. All other *Mycobacterium* species and non-target genera and species, representing a phylogenetic cross-section, produced negative results using the ACCUPROBE MYCOBACTERIUM KANSASII CULTURE IDENTIFICATION TEST.

D. RECOVERY

M. kansasii ribosomal RNA at concentrations ranging from 1×10^{-4} µg and 5×10^{-1} µg per test was assayed in the presence of 300 million cells of the following non-target species: *Mycobacterium avium*, *Mycobacterium tuberculosis* or *Nocardia asteroides*. The presence of these non-target species did not interfere with the positive signal of the *M. kansasii* rRNA dilutions, nor did they generate a positive reaction alone with the ACCUPROBE MYCOBACTERIUM KANSASII CULTURE IDENTIFICATION TEST.

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