

HOLOGIC® AccuProbe®

COCCIDIOIDES IMMITIS CULTURE IDENTIFICATION TEST

INTENDED USE

The ACCUPROBE COCCIDIOIDES IMMITIS CULTURE IDENTIFICATION TEST is a rapid DNA probe test which utilizes the technique of nucleic acid hybridization for the identification of *Coccidioides immitis* isolated from culture.

SUMMARY AND EXPLANATION OF THE TEST

Coccidioides immitis is the etiologic agent of the fungal disease coccidioidomycosis (San Joaquin Valley Fever). Infection in man and other animals usually occurs following inhalation of arthroconidia (arthrospores) into the lungs. Disease may be evident after an incubation period of from one to four weeks. Approximately 60 percent of those infected are asymptomatic or experience self-limiting upper respiratory infections. The remaining 40 percent of infections proceed to the lower respiratory tract, resulting in mild or severe pneumonia which may resolve spontaneously or progress to form pulmonary nodules or cavities, occasionally resembling tuberculosis or carcinoma. More rarely, the infection may disseminate to almost any organ of the body, including the skin, bone, and central nervous system (1, 8).

Coccidioidomycosis is endemic in semiarid regions of North, Central, and South America. The fungus is found most commonly in areas with alkaline, sandy soils such as central California and Arizona. The incidence of infection with *Coccidioides immitis* is highest in those occupations which work with soil such as archaeology and farming (9).

Coccidioides immitis is a thermally dimorphic fungus which under different environmental conditions may exist in either the filamentous form or as large, round, thick-walled spherules. The fungus grows as a filamentous mold in the soil or at room temperature on fungal media such as Sabouraud Dextrose Agar, producing thin mycelia containing characteristic alternating barrel-shaped arthroconidia. Large spherules containing endospores are formed when the fungus invades mammalian tissues and body fluids, or when grown on highly specialized synthetic media at 40°C and under increased CO₂ tension (6).

Conventional laboratory identification methods used to identify *Coccidioides immitis* include culture on fungal media, growth rate, colony morphology, microscopic morphology, animal inoculation, and biochemical tests. Identification begins with culture of the clinical specimen on fungal media. The time required for growth to a visible, cobweb-like colony varies from 3 to 21 days. The mature colony morphology ranges from flat, glabrous colonies to fluffy-white, gray, or (rarely) brownish-black colonies. Additional growth is needed before the characteristic microscopic sporulation pattern of alternating arthroconidia may be seen. Many species of fungi other than *Coccidioides immitis* may produce similar colony and sporulation patterns, including such naturally occurring soil fungi as *Malbranchea* and *Uncinocarpus* spp (6). Some yeast-like organisms such as *Geotrichum* and *Trichosporon* spp. may also resemble *Coccidioides immitis* (5). Therefore, additional testing is needed to definitively identify this organism. Species-specific spherules characteristic of *Coccidioides immitis* may be produced in tissues after animal inoculation, or by *in vitro* mycelial to spherule conversion, however, these methods are not routinely performed due to procedural difficulties. Other confirmatory tests based on exoantigen extraction have been described (5, 7). These tests may take from 3 to 5 days or longer to perform. The ACCUPROBE COCCIDIOIDES IMMITIS CULTURE IDENTIFICATION TEST identifies *Coccidioides immitis* organisms isolated from culture in less than one hour. Identification is based upon the detection of specific ribosomal RNA sequences that are unique to *Coccidioides immitis*. Colonies may be identified as soon as growth is visible. Sporulation is not necessary. The ACCUPROBE COCCIDIOIDES IMMITIS CULTURE IDENTIFICATION

TEST offers a rapid, non-subjective and accurate identification method for the detection of *Coccidioides immitis* 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 (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 COCCIDIOIDES IMMITIS CULTURE IDENTIFICATION TEST are provided in three separate reagent kits:

ACCUPROBE COCCIDIOIDES IMMITIS PROBE KIT

Probe Reagent (10 x 2 tubes).
Coccidioides immitis.

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). *Coccidioides immitis* arthroconidia represent a major biohazard to laboratory personnel. Biosafety level 3 is recommended for all activities with mold cultures and adherence to appropriate precautions should be rigorously followed (3). Culture samples (e.g., pipetted, vortexed, etc.) and all procedural steps through the heat inactivation step should be performed in a Class II or III Biological Safety Cabinet or a Bacteriological Glove Box.
 1. Arthrospore aerosols may be prevented by wetting growth in the mycelium phase with one or two drops of sterile distilled water.
 2. Appropriate autoclaving procedures for all contaminated materials should be strictly followed.
- C. Use only for the identification of *Coccidioides immitis* isolated from culture.
- D. Use only supplied or specified disposable laboratory ware.
- E. Use routine laboratory precautions. Wash hands thoroughly after handling specimens and kit reagents.

- 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 COCCIDIOIDES IMMITIS 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 COCCIDIOIDES IMMITIS CULTURE IDENTIFICATION TEST kit is designed to determine the identity of *Coccidioides immitis* isolated from culture. Colonies should be no more than one month old from the time growth is first observed. Sporulation is not necessary.

- A. **Solid Media Method.** Growth from appropriate solid media such as Sabouraud Dextrose, Brain Heart Infusion, Mycobiotic (Mycosel), Inhibitory Mold Agar, Cottonseed Agar, Yeast Nitrogen Base Agar or 5% Sheep Blood Agar suggestive of *Coccidioides immitis* may be tested.
- 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.
 - Avoid taking large amounts of the solid media with the cells.
 - A 1 - 2 mm² size sample of growth is recommended for the test.
 - The operator may elect to inoculate another culture plate at this time to confirm the purity of the isolate.
- B. **Broth Culture Method.** Growth from appropriate broth media such as Brain Heart Infusion (BHI) or Trypticase Soy Broth (TSB) with turbidity equivalent to or greater than a McFarland 1 Nephelometer Standard may be tested with the ACCUPROBE COCCIDIOIDES IMMITIS 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 COCCIDIOIDES IMMITIS CULTURE IDENTIFICATION TEST

Cat. No. 102895	20 Tests
Probe Tubes	10 x 2 tubes
Lysing Reagent	1 x 20 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
 Water bath or heating block (60° ± 1°C)
 Water bath or heating block (95° ± 5°C)
 Micropipettes (100 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

- For optimal transfer of sonic energy, water must be thoroughly degassed according to the following procedure:
 - Add enough hot water to fill the sonicator bath to within 1/2" from the top of the tank.
 - Run the sonicator for 15 minutes to thoroughly degas the water.
- Adjust one heating block or water bath to 60° ± 1°C and another heating block or water bath to 95° ± 5°C.
- 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 *Coccidioides immitis* (e.g., American Type Culture Collection, ATCC # 28868) may be used as the positive control while a culture of *Blastomyces dermatitidis* (e.g., ATCC # 60916) may be used as the negative control.

C. SAMPLE PREPARATION

- Label a sufficient number of Lysing Reagent Tubes to test the culture isolates and/or controls. Remove and retain the caps.
- Pipette 100 µL of Reagent 1 (Lysis Reagent) and 100 µL of Reagent 2 (Hybridization Buffer) into the Lysing Reagent Tubes. If broth cultures are to be tested, do not add Reagent 1 to the Lysing Reagent Tubes.
- Transfer the sample from the solid media or 100 µL of a well-mixed broth culture into labeled Lysing Reagent Tubes as described in the SAMPLE COLLECTION AND PREPARATION section. Twirl the loop, needle or stick in the Reagent 1 and Reagent 2 diluent mixture to remove the cells if testing growth from solid medium.
- Recap the Lysing Reagent Tubes and briefly VORTEX.

D. SAMPLE LYSIS

- 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.**
- Sonicate for 15 minutes.
- Place the Lysing Reagent Tubes containing the sonicated organisms in a heating block or water bath for 10 minutes at 95° ± 5°C.
- Carefully remove the Lysing Reagent Tubes from the heating block or water bath.

E. HYBRIDIZATION

- 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.**
- Label a sufficient number of Probe Reagent Tubes to test the culture isolates and/or controls. Remove and retain the caps.
- Pipette 100 µL of the lysed specimens from the Lysing Reagent Tubes into the corresponding Probe Reagent Tubes.

- Recap the Probe Reagent Tubes and incubate for 15 minutes at 60° ± 1°C in a water bath or heating block.

F. SELECTION

- Remove the Probe Reagent Tubes from the water bath. Remove and retain the caps. Pipette 300 µL of Reagent 3 (Selection Reagent) into each tube. If a heating block is used, Reagent 3 may be pipetted directly into the Probe Reagent Tubes. Recap the tubes and VORTEX the tubes to mix completely.
- Incubate the Probe Reagent Tubes for 5 minutes at 60° ± 1°C in a water bath or heating block.
- 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.

G. DETECTION

- Select the appropriate protocol from the menu of the luminometer software.
- 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.
- When the analysis is complete, remove the last tube(s) from the luminometer.

PROCEDURAL NOTES

- REAGENTS:** Reagent 2 (Hybridization Buffer) may precipitate. Warming and mixing the solution at 35° - 60°C will dissolve the precipitate.
- 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.
- 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.
- 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.
- 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 the addition of Reagent 3.
- TROUBLE-SHOOTING**
 - Elevated negative control values (*Blastomyces dermatitidis* ATCC # 60916) 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 an appropriate agar medium and incubated to check for multiple colony types.
 - Low positive control values (*Coccidioides immitis* ATCC #28868) 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, improper sonication, or by testing mixed or aged cultures. Because mixed cultures can occur, a portion of the growth may be streaked onto an appropriate agar medium and incubated to check for multiple colony types.

RESULTS

A. INTERPRETATION OF RESULTS

The results of the ACCUPROBE COCCIDIOIDES IMMITIS 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., *Blastomyces dermatitidis* ATCC # 60916) and positive control (e.g., *Coccidioides immitis* ATCC # 28868) 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

The ACCUPROBE COCCIDIOIDES IMMITIS CULTURE IDENTIFICATION TEST has been evaluated using fresh growth from agar plates and from broth. The performance of this test has not been determined on direct clinical specimens.

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

EXPECTED VALUES

The ACCUPROBE COCCIDIOIDES IMMITIS CULTURE IDENTIFICATION TEST was compared to standard culture, morphological and biochemical identification methods, for the identification of *Coccidioides immitis* at 2 sites using a total of 471 isolates. These isolates were comprised of 166 *Coccidioides immitis* isolates and 305 isolates representing 82 species from 77 genera. These non-target isolates represented a wide phylogenetic cross-section of organisms. Standard culture identification included selective growth media, biochemical identification methods, microscopic and macroscopic colony morphology, sporulation characteristics and in some cases exoantigen tests. Isolates were either categorized as positive (≥ 50,000 RLU) or negative (< 50,000 RLU). The range of observations for negative isolates was 346 RLU to 34,301 RLU and 67,641 RLU to 812,451 RLU for positive isolates. A comparison of the ACCUPROBE COCCIDIOIDES IMMITIS 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	84	0	2	130	97.7%/100%	99.1%
Site 2	80	0	0	175	100%/100%	100%
Total	164	0	2	305	98.8%/100%	99.6%

The two AccuProbe negative, culture positive isolates at Site 1 were retested with the ACCUPROBE COCCIDIOIDES IMMITIS CULTURE IDENTIFICATION TEST and again gave negative results. These two isolates were then subjected to *Coccidioides immitis* exoantigen tests and found negative. The Sensitivity, Specificity, and Percent Agreement for Site 1, upon retesting, is therefore 100%.

PERFORMANCE CHARACTERISTICS

A. WITHIN-RUN PRECISION

The within-run precision of the ACCUPROBE COCCIDIOIDES IMMITIS CULTURE IDENTIFICATION TEST was calculated by assaying two concentrations of ribosomal RNA isolated from *Coccidioides immitis* using 10 replicates in a single assay.

Sample	A	B
Number of Replicates	10	10
Mean Response	42,027	136,815
Standard Deviation	2,370	7,004
Coefficient of Variation	5.6%	5.1%

B. BETWEEN-RUN PRECISION

The between-run precision was calculated by assaying the same two concentrations of *Coccidioides immitis* ribosomal RNA using single determinations in 10 consecutive runs.

Sample	A	B
Number of Replicates	10	10
Mean Response	51,104	150,687
Standard Deviation	2,924	12,449
Coefficient of Variation	5.7%	8.3%

C. SPECIFICITY

A total of 106 ATCC reference isolates were evaluated using the ACCUPROBE COCCIDIOIDES IMMITIS CULTURE IDENTIFICATION TEST. These isolates represented a total of 71 species from 45 genera. Six of the total number of isolates tested with *Coccidioides immitis* isolates. Six isolates of *Histoplasma capsulatum*, four isolates of *Blastomyces dermatitidis*, three isolates of *Paracoccidioides brasiliensis*, and one isolate of *Sporothrix schenckii* were tested in the yeast phase. Twelve of these isolates were also tested in the yeast phase. Eighty-six isolates of 39 other genera representing a phylogenetic cross-section of fungal organisms were evaluated using the ACCUPROBE COCCIDIOIDES IMMITIS CULTURE IDENTIFICATION TEST. Included among the non-target genera representing a phylogenetic cross-section were species of *Malbranchea*, *Uncinocarpus*, *Myxotrichum*, *Oidiodendron*, *Arachniotus*, *Gymnoascus*, *Geotrichum*, and *Trichosporon*. All non-target isolates produced negative results using this test in both the filamentous and the yeast phases. All *Coccidioides immitis* isolates tested produced positive results using the ACCUPROBE COCCIDIOIDES IMMITIS CULTURE IDENTIFICATION TEST.

D. RECOVERY

Coccidioides immitis ribosomal RNA (rRNA) at concentrations of 0.09 µg and 0.27 µg per test were assayed alone and in the presence of either *Histoplasma capsulatum* or *Candida albicans* rRNA concentrations ranging from 0.002 µg (equivalent to 5×10^4 cells) to 0.2 µg (equivalent to 5×10^6 cells). The presence of these non-target rRNA concentrations did not interfere with the positive signal of *Coccidioides immitis*, nor did they generate a positive reaction with the ACCUPROBE COCCIDIOIDES IMMITIS CULTURE IDENTIFICATION TEST.

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