

**HISTOPLASMA CAPSULATUM
CULTURE IDENTIFICATION TEST****INTENDED USE**

The ACCUPROBE HISTOPLASMA CAPSULATUM CULTURE IDENTIFICATION TEST is a rapid DNA probe test which utilizes the technique of nucleic acid hybridization for the identification of *Histoplasma capsulatum* isolated from culture.

SUMMARY AND EXPLANATION OF THE TEST

Histoplasma capsulatum (*H. capsulatum*) is the causative agent of the systemic fungal disease histoplasmosis. The organism occurs throughout the world, but is most commonly found in soil from the fertile river valleys of North America. Large numbers of infectious conidia are especially associated with areas containing bird or bat excrement. (6, 10) The organism is also found in two other varieties: *H. capsulatum* var. *duboisii* (African histoplasmosis) and *H. capsulatum* var. *farciminosum* (epizootic lymphaginitis of horses and mules). The teleomorph (mating or sexual) state of *H. capsulatum* is *Ajellomyces capsulatus*. (8)

H. capsulatum is a dimorphic fungus. The organism grows as a filamentous fungus under appropriate soil conditions or on room temperature fungal media. In mammalian cells or on specialized fungal media at 37°C, the organism grows as a small budding yeast. Conventional laboratory identification methods begin with culture of the clinical specimen at room temperature on specialized fungal media, which isolates the slower growing *H. capsulatum* colonies from possible contaminants such as bacteria and faster growing saprobic fungi. Growth of *H. capsulatum* to a visible colony may take from two to four weeks or longer. (4, 8) Some clinical laboratories hold their cultures for as long as twelve weeks before reporting them as negative. (7)

Additional growth is needed before the characteristic colony morphology and microscopic sporulation pattern with tuberculate macroconidia may be demonstrated. About 10% of cultures produce only smooth walled macroconidia. (8) Some cultures fail to sporulate. (4, 5) Unusual glabrous, brown or gray colonies are sometimes encountered. Many species of fungi other than *H. capsulatum* produce similar colony and sporulation characteristics. Some examples are *Blastomyces dermatitidis*, *Chrysosporium* sp., and *Sepedonium* sp. (4, 8) Therefore, additional testing is needed to definitively identify the organism. One method is to convert the fungus colony from the filamentous, mycelial phase to the yeast phase by subculture to highly enriched cysteine-containing media and incubation at 35° to 37°C. Conversion to the yeast form is often difficult and may require several transfers at three-day intervals. (4) Other confirmatory identification methods based on exoantigen extraction have been described, which take from three to five days or longer to perform. (2, 4, 9).

The ACCUPROBE HISTOPLASMA CAPSULATUM CULTURE IDENTIFICATION TEST identifies both phases regardless of sporulation in less than an hour based on identification of specific ribosomal RNA sequences that are unique to *H. capsulatum*.

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 (3). 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 less than 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 HISTOPLASMA CAPSULATUM CULTURE IDENTIFICATION TEST are provided in three separate reagent kits:

ACCUPROBE HISTOPLASMA CAPSULATUM PROBE KIT

Probe Reagent (10 x 2 tubes).
Histoplasma capsulatum.

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 (1).
- C. Use only for the determination of *H. capsulatum* 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 HISTOPLASMA CAPSULATUM 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 HISTOPLASMA CAPSULATUM CULTURE IDENTIFICATION TEST is designed to determine the identity of *H. capsulatum* isolated from culture. Colonies may be tested as soon as growth is visible. Yeast colonies should be no more than one week old from the time growth is first observed. Filamentous 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, Corn Meal Agar, Yeast Nitrogen Base Agar or 5% Sheep Blood Agar suggestive of *H. capsulatum* may be tested.

1. 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.
2. Avoid taking large amounts of the solid media with the cells.
3. A 1-2 mm² size sample of yeast or filamentous growth is recommended for the test.
4. 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 Brain Heart Infusion broth with turbidity equivalent to or greater than a McFarland 1 Nephelometer Standard may be tested with the ACCUPROBE HISTOPLASMA CAPSULATUM 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 HISTOPLASMA CAPSULATUM CULTURE IDENTIFICATION TEST

Cat. No. 102910	20 Tests
Probe Reagent	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, 300 µL)

Repipettor (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 *H. capsulatum* (e.g., American Type Culture Collection, ATCC #38904) 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

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, needle or stick 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 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. 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 tubes. 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.

- 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 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 tubes into the luminometer according to the instrument directions.
- When the analysis is complete, remove the tube(s) from the luminometer.

PROCEDURAL NOTES

- REAGENTS:** Reagent 2 (Hybridization Buffer) may precipitate. Warming and mixing the solution at 35° to 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 homogeneous 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 (*B. 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 the appropriate agar medium and incubated to check for multiple colony types.
 - Low positive control values (*H. capsulatum* ATCC #38904) 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 the appropriate agar medium and incubated to check for multiple colony types.

RESULTS

A. INTERPRETATION OF RESULTS

The results of the ACCUPROBE HISTOPLASMA CAPSULATUM 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
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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., *B. dermatitidis*, ATCC #60916) and positive control (e.g., *H. capsulatum*, ATCC # 38904) should satisfy the following values:

	AccuLDR (formerly PAL)	Leader
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Negative control	<600 PLU	<20,000 RLU
Positive control	>1,500 PLU	>50,000 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., respiratory specimens or CSF).

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

EXPECTED VALUES

The ACCUPROBE HISTOPLASMA CAPSULATUM CULTURE IDENTIFICATION TEST was compared to standard culture identification methods at two sites using a total of 281 isolates: 114 isolates of *H. capsulatum* (12 isolates were tested in both the yeast and the filamentous phase), 26 isolates of 3 other genera of dimorphic fungi (18 *Blastomyces dermatitidis*, 6 *Coccidioides immitis*, 2 *Sporothrix schenckii*) and 141 other isolates representing 73 genera. Standard culture identification is dependent on microscopic and macroscopic-characteristics of the colony, sporulation characteristic and exoantigen test. The isolates were categorized as either positive (>50,000 RLU) or negative (<50,000 RLU). The range of observations for negative cultures was 703 to 23,846 RLU and 180,071 to 720,550 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	60	0	0	40	100%/100%	100%
Site 2	54	0	0	127	100%/100%	100%
Total	114	0	0	167	100%/100%	100%

PERFORMANCE CHARACTERISTICS

A. WITHIN-RUN PRECISION

The within-run precision of the ACCUPROBE HISTOPLASMA CAPSULATUM CULTURE IDENTIFICATION TEST was calculated by assaying two concentrations of ribosomal RNA isolated from *H. capsulatum* using 10 replicates in a single assay.

Sample	A	B
Number of Replicates	10	10
Mean Response	48,941	103,783
Standard Deviation	1,684	2,589
Coefficient of Variation	3.4%	2.5%

B. BETWEEN-RUN PRECISION

The between-run precision was calculated by assaying the same two concentrations of *H. capsulatum* ribosomal RNA using single determinations in 15 consecutive runs.

Sample	A	B
Number of Replicates	15	15
Mean Response	54,965	100,201
Standard Deviation	6,373	5,548
Coefficient of Variation	11.6%	5.5%

C. SPECIFICITY

A total of 96 ATCC culture isolates were evaluated using the ACCUPROBE HISTOPLASMA CAPSULATUM CULTURE IDENTIFICATION TEST. These isolates represented a total of 68 species from 41 genera. Six isolates of *Histoplasma capsulatum* including one each of the varieties *duboisii* and *farciminosum* (filamentous and yeast phase), 6 isolates of 3 other dimorphic fungi (*Blastomyces dermatitidis*, *Coccidioides immitis*, *Sporothrix schenckii*) and 84 isolates of 37 other genera representing a phylogenetic cross-section of organisms were evaluated using the ACCUPROBE HISTOPLASMA CAPSULATUM CULTURE IDENTIFICATION TEST. All *Histoplasma capsulatum* isolates tested and all varieties of *Histoplasma capsulatum* tested produced a positive result using the ACCUPROBE HISTOPLASMA CAPSULATUM CULTURE IDENTIFICATION TEST. The representative phylogenetic cross-section isolates and other dimorphic fungi did not react in this test.

D. RECOVERY

Histoplasma capsulatum ribosomal RNA at concentrations ranging from 0.02 µg to 0.06 µg per test was assayed in the presence of ribosomal RNA of either *Blastomyces dermatitidis* or *Candida albicans* ranging from 0.0045 µg (equivalent to 10⁵ cells) to 0.45 µg (equivalent to 10⁷ cells). There was no interference of *Histoplasma capsulatum* signal observed and the other organisms present did not react using the ACCUPROBE HISTOPLASMA CAPSULATUM CULTURE IDENTIFICATION TEST.

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