The AccuProbe Blastomyces Dermatitidis Culture Identification Test is a rapid DNA probe test which utilizes the technique of nucleic acid hybridization for the identification of Blastomyces dermatitidis isolated from culture.

Summary and Explanation of the Test

Blastomyces dermatitidis (B. dermatitidis) is the causative agent of the Blastomycosis (North American blastomycosis or Gilchrist's disease), a systemic fungal disease usually occurring as a chronic granulomatous infection of the skin or internal organs. Infection is thought to occur through inhalation of spore-laden dust. From these ports of entry, the organism can spread to the bones, kidneys, lymph nodes, skin, and central nervous system (2, 4, 10, 12). Blastomycosis is not transferrable from person to person or animal to person. It occurs sporadically; individuals engaged in outdoor and agricultural professions are more likely to contract blastomycosis. Most cases occur in the central and southeastern United States and Canada. Indigenous cases have also been reported in South America, Central America, the Middle East, and Africa (12). B. dermatitidis is a thermally dimorphic fungus which, under different environmental conditions, may exist in either the mold or yeast phase. Growth may occur as a filamentous mold at room temperature on media such as Sabouraud Dextrose Agar or in the large broad-based budding yeast form on media such as Brain Heart Infusion Agar at 35° to 37°C (6, 7, 10).

Laboratory identification methods used to identify B. dermatitidis include culturing clinical specimens on nutrient media, colony morphology, and characteristic microscopic sporulation patterns, filamentous to yeast phase conversion and exoantigen tests. Conventional laboratory identification methods begin with culture of the clinical specimen on specialized fungal media. The time required for growth of B. dermatitidis to a visible colony varies from a few days to over two months. The colony morphology of this organism is also quite variable, ranging from flat, glabrous colonies to fluffy-white or brownish colonies (6, 10). Additional growth is needed before the characteristic microscopic sporulation pattern of short conidiophores bearing pyriform conidia may be seen. Many species of fungi other than B. dermatitidis may produce similar colony and sporulation characteristics. These species include Histoplasma capsulatum and Chrysosporium spp. (8). Therefore additional testing is needed to definitively identify this organism. One method is to convert the room temperature mycelial phase growth to the yeast phase by sub-culturing at 35° to 37°C (6, 10).

Preparation of the specimen

Additional growth is needed before the characteristic microscopic sporulation pattern of short conidiophores bearing pyriform conidia may be seen. Many species of fungi other than B. dermatitidis may produce similar colony and sporulation characteristics. These species include Histoplasma capsulatum and Chrysosporium spp. (8). Therefore additional testing is needed to definitively identify this organism. One method is to convert the room temperature mycelial phase growth to the yeast phase by sub-culturing at 35° to 37°C (6, 10). Within a few days or weeks, the characteristic large, fluffy-white or brownish colonies should be no more than one week old from the time growth is first observed. Filamentous colonies DNA:RNA hybrids are measured in a Leader 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 Blastomyces Dermatitidis Culture Identification Test are provided in three separate reagent kits:

- **AccuProbe Blastomyces Dermatitidis Probe Kit**
  - **Probe Reagent** (10 x 2 tubes)
    - Blastomyces dermatitidis
  - **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 identification of B. dermatitidis 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 Blastomyces Dermatitidis 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 Blastomyces Dermatitidis Culture Identification Test kit is designed to determine the identity of Blastomyces dermatitidis isolated from culture. Yeast colonies should be no more than one week old from the time growth is first observed. Filamentous colonies...
Materials Provided

The AccuProbe Blastomyces Dermatitidis Culture Identification Test:

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>102890</th>
<th>20 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probe Tubes</td>
<td>10 x 2 tubes</td>
<td></td>
</tr>
<tr>
<td>Lysing Reagent</td>
<td>1 x 20 tubes</td>
<td></td>
</tr>
</tbody>
</table>

Materials Required but not Provided

One (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

Materials Available from Hologic

- Leader Luminometer
- Hologic Ultrasonic Water Bath (Cat. No. 901104 or equivalent)
- AccuProbe Culture Identification Reagent Kit (Cat. No. 102800)
- Hologic Detection Reagent Kit (Cat. No. 201791)
- Hologic Heating Block (Cat. No. Single Block 302902; Triple Block 302903)
- Hologic Ultrasonic Water Bath Rack (Cat. No. 901393)

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 ultrasonic water bath to within 1/2 inch from the top of the tank.
   b. Run the ultrasonic water bath 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 Leader 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 *B. dermatitidis* (e.g., American Type Culture Collection, ATCC # 60916) may be used as the positive control while a culture of *Histoplasma capsulatum* (e.g., ATCC # 38904) 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 the 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 labeled Lysing Reagent Tubes as described in the Sample Collection and Preparation section. Pipette a 100 µL sample from the well-mixed broth suspension into the Lysing Reagent Tube as described below.

D. Sample Lysis

1. Push the Lysing Reagent Tubes through the ultrasonic water bath rack so that the reaction mixture in the bottom of the tube is submerged but the caps are above the water. Place the ultrasonic water bath rack on the ultrasonic water bath.
   **Do not allow the tubes to touch the bottom or sides of the ultrasonic water bath.**
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 the fold 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. 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.
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.
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 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 Lysis 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 the addition of Reagent 3.

F. Troubleshooting:

1. Elevated negative control values (H. capsulatum ATCC #38904) 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.

2. Low positive control values (Blastomyces dermatitidis ATCC #60916) 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.

B. Quality Control and Acceptability of Results

Negative control (e.g., H. capsulatum ATCC #38904) and positive control (e.g., B. dermatitidis ATCC #60916) should satisfy the following values:

<table>
<thead>
<tr>
<th>Test</th>
<th>Cut-off Value</th>
<th>Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AccuLDR (formerly PAL)</td>
<td>≥ 1,200 PLU</td>
<td>≥ 44,000 RLU</td>
</tr>
<tr>
<td>Leader</td>
<td>≥ 50,000 PLU</td>
<td>≥ 20,000 RLU</td>
</tr>
</tbody>
</table>

Limitations

The AccuProbe Blastomyces Dermatitidis Culture Identification Test has been shown to produce a positive result with all Paracoccidioides brasiliensis (P. brasiliensis) strains that have been tested. The taxonomic classification of P. brasiliensis remains controversial. It is considered by some to be in the genus Blastomyces (11). P. brasiliensis is the causative agent of paracoccidioidomycosis or South American blastomycosis. This fungal disease follows a progressive, granulomatous pattern similar to North American blastomycosis (11). South American blastomycosis is found almost exclusively in South and Central America and is indigenous to South America (9). The occurrence of paracoccidioidomycosis in the Northern Hemisphere is extremely rare. Approximately 5 cases of paracoccidioidomycosis per year are reported in the United States, compared to approximately 1,000 to 2,000 cases of North American blastomycosis per year. (Pers. comm. Dr. L. Ajello, Centers for Disease Control, Atlanta, GA.)

P. brasiliensis may be distinguished from B. dermatitidis by microscopic examination in the yeast phase. P. brasiliensis is differentiated by the growth of multiple buds from a single cell with thinner cell walls, where B. dermatitidis is typically a single-budding yeast with thicker cell walls. Although it should be noted that occasionally P. brasiliensis may produce single-budding yeast (6, 11).

The AccuProbe Blastomyces Dermatitidis Culture Identification Test has been shown to produce a positive result with the mold, Gymnascella hyalinospora and with Emmonsia parva. These organisms are rarely isolated (3). Emmonsia parva rarely occurs in humans.

The AccuProbe Blastomyces Dermatitidis Culture Identification Test has been shown to produce a positive result with the mold, Chrysosporium charniaggi. The clinical implications of this are not known.

This method has been tested using both fresh growth from agar plates and broth cultures as listed in the Sample Collection and Preparation section. The efficacy of this test has not been demonstrated on direct clinical specimens.

Expected Values

The AccuProbe Blastomyces Dermatitidis Culture Identification Test was compared to standard culture, morphological and biochemical identification methods at two sites using a total of 396 isolates. Of these, 108 were B. dermatitidis isolates (11 isolates were tested in both the yeast and the mycelial phases), 1 P. brasiliensis isolate, and 287 isolates of 68 species from 76 genera, representing a phylogenetic cross-section of organisms. Included among these were 103 isolates of the following dimorphic fungi: 7 Coccidioides immitis; 94 Histoplasma capsulatum; 2 Sporothrix schenckii. Standard culture identification is dependent on microscopic and macroscopic colony morphology, sporulation characteristics, filamentous to yeast phase conversions, 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 936 RLU to 32,072 RLU and 66,052 RLU to 64,940 RLU for positive isolates. A comparison of the AccuProbe Blastomyces Dermatitidis Culture Identification Test and standard culture identification methods is shown below.
The one positive result at Site 1 was a *P. brasiliensis* isolate. As discussed in the Limitations section, the AccuProbe Blastomyces Dermatitidis Culture Identification Test does not differentiate between *B. dermatitidis* and *P. brasiliensis* isolates since both have been shown to produce positive results. The sensitivity, specificity, and percent agreement for Site 1, are therefore 100%. The two isolates initially producing a negative result at Site 2 produced positive results upon retesting with the AccuProbe Blastomyces Dermatitidis Culture Identification Test. The overall sensitivity, specificity and percent agreement, upon retesting, are therefore 100%.

**Performance Characteristics**

A. **Within-Run Precision**

The within-run precision of the AccuProbe Blastomyces Dermatitidis Culture Identification Test was calculated by assaying two concentrations of ribosomal RNA isolated from *B. dermatitidis* using 10 replicates in a single assay.

<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>57,662</td>
<td>135,061</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>5,929</td>
<td>3,694</td>
</tr>
<tr>
<td>Coefficient of Variation</td>
<td>10.3%</td>
<td>2.7%</td>
</tr>
</tbody>
</table>

B. **Between-Run Precision**

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

<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>59,160</td>
<td>180,121</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>6,656</td>
<td>8,537</td>
</tr>
<tr>
<td>Coefficient of Variation</td>
<td>11.3%</td>
<td>4.7%</td>
</tr>
</tbody>
</table>

C. **Specificity**

A total of 102 ATCC reference isolates were evaluated using the AccuProbe Blastomyces Dermatitidis Culture Identification Test. These isolates represented a total of 71 species from 46 genera. Four isolates of *B. dermatitidis*, 4 isolates of *P. brasiliensis*, 6 isolates of *Histoplasma capsulatum*, and 1 isolate of *Sporothrix schenckii* were tested in the filamentous phase. Eighty-seven isolates of 42 other genera representing a phylogenetic cross-section of organisms were evaluated using the AccuProbe Blastomyces Dermatitidis Culture Identification Test. All non-target isolates representing a phylogenetic cross-section of organisms produced negative results using this kit. The AccuProbe Blastomyces Dermatitidis Culture Identification Test does not differentiate between *P. brasiliensis* and *B. dermatitidis* isolates. All *P. brasiliensis* isolates tested in both the filamentous and yeast phases produced positive results. All *B. dermatitidis* isolates tested in both the filamentous and yeast phases produced positive results using the AccuProbe Blastomyces Dermatitidis Culture Identification Test.

D. **Recovery**

*B. dermatitidis* ribosomal RNA at concentrations ranging from 0.04 µg to 1.6 µg per test was assayed alone and in the presence of 0.0045 µg (equivalent to 10^{5} cells) and 0.45 µg (equivalent to 10^{7} cells) ribosomal RNA of either *Histoplasma capsulatum* or *Candida albicans*. There was no interference with the *B. dermatitidis* signal observed and the other organisms present did not react using the AccuProbe Blastomyces Dermatitidis Culture Identification Test.

### Bibliography


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