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## Overview

### Useful For

Rapid detection of *Histoplasma capsulatum* and *Blastomyces dermatitidis* DNA

An aid in the rapid diagnosis of histoplasmosis and blastomycosis

### Testing Algorithm

See [Meningitis/Encephalitis Panel Algorithm](#) in Special Instructions.

### Special Instructions

- [Meningitis/Encephalitis Panel Algorithm](#)

### Method Name

Real-Time Polymerase Chain Reaction (PCR)

### NY State Available

Yes

## Specimen

### Specimen Type

Varies

### Ordering Guidance

Urine is not an acceptable source for this assay. Studies indicate that *Histoplasma* DNA is not routinely found in the urine of patients with disseminated histoplasmosis. Therefore, the UHIST / *Histoplasma* Antigen, Urine is the recommended test for this specimen source.

### Additional Testing Requirements

This test should always be performed in conjunction with fungal culture, order FGEN / Fungal Culture, Routine.

### Shipping Instructions

**Specimen must arrive within 7 days of collection; specimens received after 7 days will be rejected.**

**NALC/NaOH-digested specimen must arrive within 7 days of digestion.**

**Necessary Information**

Specimen source is required.

**Specimen Required**

The high sensitivity of amplification by PCR requires the specimen to be processed in an environment in which contamination of the specimen by *Histoplasma* or *Blastomyces* species DNA is not likely.

Submit only 1 of the following specimens:

**Specimen Type:** Body fluid

**Sources:** Body, CSF, bone marrow

**Container/Tube:** Sterile container

**Specimen Volume:** 1 mL

**Specimen Type:** Respiratory

**Sources:** BAL, bronchial washing, sputum

**Container/Tube:** Sterile container

**Specimen Volume:** 1 mL

**Specimen Type:** Tissue or bone

**Container/Tube:** Sterile container

**Specimen Volume:** 5-10 mm

**Collection Instructions:** Collect a fresh tissue or bone specimen.

**Acceptable:**

**Specimen Type:** NALC/NaOH-digested respiratory specimens

**Sources:** Lavage fluid, bronchial washing, gastric washing, respiratory fluid, sputum, or tracheal secretion

**Container/Tube:** Sterile container

**Specimen Volume:** 2 mL

**Collection Instructions:**

1. Submit digested specimen treated with NALC/NaOH.
2. Clearly indicate on container and order form that specimen is a digested specimen.

**Forms**

If not ordering electronically, complete, print, and send a [Microbiology Test Request](#) (T244) with the specimen.

**Reject Due To**

- O Specimen in anaerobe vial or viral transport medium (including but not limited to M4, M5, BD viral transport media, thioglycolate broth) Feces Swab Tissue in formalin fluid Urine Specimen >7 days older

**Specimen Minimum Volume**

Body Fluid or Respiratory Specimen: 0.5 mL

**Specimen Stability Information**

Specimen Type	Temperature	Time	Special Container
Varies	Refrigerated (preferred)	7 days	
	Ambient	7 days	
	Frozen	7 days	

**Clinical & Interpretive**

**Clinical Information**

Infections with *Blastomyces dermatitidis* and *Histoplasma capsulatum* cause a variety of clinical manifestations ranging from self-limited, mild pulmonary illness to potentially life-threatening, disseminated disease. Patients at risk for disseminated disease include neonates and immunosuppressed individuals, particularly those with AIDS, hematologic malignancies, or a recent transplant. Primary infections are acquired through inhalation of microconidia that are present in the environment. In the United States, most cases of blastomycosis and histoplasmosis occur along the Ohio and Mississippi River valleys.

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The gold standard for diagnosis of blastomycosis and histoplasmosis remains isolation of the organisms in culture. Although sensitive, recovery in culture and subsequent identification may require days to weeks. The organisms can be identified after growth in culture using traditional macro- and microscopic morphologic techniques or through the use of nucleic acid hybridization probes. Hybridization probe-based procedures are rapid and demonstrate good sensitivity and specificity from culture, although some cross-reactivity with relatively uncommon fungal organisms has been reported. Additional diagnostic tests that can be utilized for these organisms include stains, histopathology, serology, and antigen detection with each of these methods offering advantages and limitations depending on the stage of the illness and the status of the patient. Fungal stains (eg, calcofluor white) offer a rapid diagnostic approach, but demonstrate poor sensitivity and specificity. Serologic tests such as complement fixation and immunodiffusion are noninvasive, but are laborious, subjective, and may show low sensitivity, especially in immunocompromised hosts. Antigen detection also offers a noninvasive approach, but has been demonstrated to show cross-reactivity with antigens from closely related fungal species.

Molecular techniques have been established as sensitive and specific methods for the diagnosis of infectious diseases and have the added advantage of a rapid turnaround time for results. Due to the limitations of conventional diagnostic methods for blastomycosis and histoplasmosis, a single tube, real-time PCR assay was developed and verified for the detection and differentiation of *B dermatitidis/gilchristii* and *H capsulatum* directly from clinical specimens.

**Reference Values**

Not applicable

**Interpretation**

A positive result for *Histoplasma capsulatum* indicates presence of *Histoplasma* DNA; a positive result for *Blastomyces dermatitidis/gilchristii* indicates presence of *Blastomyces* DNA.

A negative result indicates absence of detectable *H capsulatum* and *B dermatitidis/gilchristii* DNA. Fungal culture has increased sensitivity over this PCR assay and should always be performed when the PCR is negative.

**Cautions**

This rapid PCR assay detects *Histoplasma capsulatum* and *Blastomyces dermatitidis* nucleic acid and, therefore, does not distinguish between the presence of viable, disease-related organisms and nucleic acid persisting from previous, treated disease. Test results should be correlated with patient symptoms and clinical presentation before a definitive diagnosis is made.

A negative result does not rule out the presence of *H capsulatum* or *B dermatitidis/gilchristii* because the organism may be present at levels below the limit of detection for this assay.

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The sensitivity of the PCR assay from bronchoalveolar lavage (BAL) fluid is low and a negative result does not rule out infection.

### Supportive Data

Analytical Sensitivity and Specificity:

The analytical sensitivity of the assay was determined to be less than or equal to 100 copies/microliter for both *Blastomyces dermatitidis/gilchristii* and *Histoplasma capsulatum*. The *B dermatitidis/gilchristii* melt peak is read at 670 nm and has a melting temperature (T<sub>m</sub>) of 66 degrees C + or - 0.26 degrees C (mean + or - standard deviation), while the *H capsulatum* read at 610 nm has a T<sub>m</sub> of 61 degrees C + or - 0.27 degrees C. A National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) search of the primer, probe, and target sequences for both *B dermatitidis/gilchristii* and *H capsulatum* did not yield any potentially cross-reacting sequences. In addition, testing of nucleic acids from 179 potentially cross-reacting microbes commonly encountered in the clinical laboratory including bacteria, fungi, parasites, and viruses demonstrated no cross-reactivity with other organisms. The list of organisms is available upon request.

Sensitivity and Specificity from Cultured Isolates:

The sensitivity of the assay from isolates grown in culture was 100% (61/61) and 94.5% (51/54) for *B dermatitidis/gilchristii* and *H capsulatum*, respectively. The specificity of the assay was 100% for both organisms as no cross-reactivity was detected in the other non-*B dermatitidis/gilchristii* or non-*H capsulatum* cultures evaluated.

Clinical Sensitivity and Specificity Directly from Specimens:

A total of 797 clinical specimens were tested concurrently by fungal culture and the real-time PCR assay to assess clinical sensitivity and specificity. The sensitivity and specificity of the PCR assay for *B dermatitidis/gilchristii* was 86% (12/14 positive) and 99.4% (778/783 negative), respectively. The overall sensitivity and specificity of the PCR assay for *H capsulatum* was 73.3% (11/15 positive) and 100% (782/782 negative), respectively. Of note, the recovery of *H capsulatum* from bronchoalveolar lavage (BAL) fluid was low (2 PCR positives of 6 culture positives), which accounted for all of the falsely negative specimens. Therefore, a negative result from BAL fluid does not rule out *H capsulatum* infection due to low sensitivity from this specimen source.

Due to the low number of positive specimens obtained clinically despite testing almost 800 specimens over a period of 1 year, spiking studies using a plasmid control for both organisms were also performed using negative specimens representing various specimen types (30 each of pleural fluid, sputum, BAL fluid, cerebrospinal fluid, tissue, bone, sterile body fluids, urine, and blood). The spiking studies demonstrated a sensitivity of the PCR assay of 100% (30/30 positive) for *B dermatitidis/gilchristii* across all specimen types except blood (97% sensitive; 29/30 positive). The PCR assay had 100% (30/30) sensitivity for *H capsulatum* across all spiked specimen types except sputum and blood which had 98% and 97% sensitivity respectively.

**Inhibition studies:**

The overall extraction and amplification inhibition rate of the assay using both targets was less than 1% (2/330 inhibited). Extraction inhibition occurred in 1 of 30 blood specimens for *B dermatitidis/gilchristii* and *H capsulatum*, and 1 of 60 sputum specimens for *H capsulatum*.

**Clinical Reference**

1. Kauffman CA: Histoplasmosis. Clin Chest Med 2009;30:217-225
2. Wheat LJ, Freifeld AG, Kleiman MB, et al: Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America. Clin Infect Dis 2007;45:807-825
3. Chapman SW, Bradsher RW Jr, Campbell GD Jr, et al: Practice guidelines for the management of patients with blastomycosis. Infectious Diseases Society of America. Clin Infect Dis 2000;30:679-683

**Performance****Method Description**

Following specimen processing, nucleic acids are extracted using the MagNA Pure Compact (Roche Applied Sciences). The extract is then transferred to a cuvette for amplification using the LightCycler real-time PCR platform (Roche Applied Sciences). The LightCycler is an automated instrument that amplifies and monitors the development of target nucleic acid (amplicon) after each cycle of PCR. The detection of amplicon is based on fluorescence resonance energy transfer (FRET), which utilizes hybridization probes. The presence of the specific organism nucleic acid is confirmed by performing a melting curve analysis of the amplicon. Primers and FRET hybridization probes were designed to target a 174-base pair (bp) region of the histidine kinase (*DRK-1*) gene of *Blastomyces dermatitidis/gilchristii* and a 192-bp region of the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene of *Histoplasma capsulatum*, respectively. The acceptor probe for *B dermatitidis/gilchristii* was labeled with a Red-705 dye, while the acceptor probe for *H capsulatum* was labeled with a Red-640 dye. Labeling the acceptor probes with 2 different dyes allows for simultaneous detection and differentiation of *B dermatitidis/gilchristii* and *H capsulatum* within a single PCR assay. (Babady NE, Buckwalter SP, Hall L, et al: Detection of *Blastomyces dermatitidis* and *Histoplasma capsulatum* from Culture Isolates and Clinical Specimens by Use of Real-Time PCR. J Clin Microbiol 2011;49:3204-3208)

**PDF Report**

No

**Specimen Retention Time**

7 days

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**Performing Laboratory Location**

Rochester

**Fees & Codes****Test Classification**

This test was developed, and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the US Food and Drug Administration.

**CPT Code Information**

87798 x 2

**LOINC® Information**

Test ID	Test Order Name	Order LOINC Value
HBRP	Histoplasma/Blastomyces PCR	81653-8

Result ID	Reporting Name	LOINC®
SRC78	Histo/Blasto Source	31208-2
32457	Histo/Blasto Result	81653-8