Laboratory Diagnostics for Histoplasmosis

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ABSTRACT The diagnosis of histoplasmosis is based on a multifaceted approach that includes clinical, radiographic, and laboratory evidence of disease. The gold standards for laboratory diagnosis include demonstration of yeast on pathological examination of tissue and isolation of the mold in the culture of clinical specimens; however, antigen detection has provided a rapid, noninvasive, and highly sensitive method for diagnosis and is a useful marker of treatment response. Molecular methods with improved sensitivity on clinical specimens are being developed but are not yet ready for widespread clinical use. This review synthesizes currently available laboratory diagnostics for histoplasmosis, with an emphasis on complexities of testing and performance in various clinical contexts.

KEYWORDS diagnosis, histoplasmosis

Histoplasmosis is the most common endemic fungal infection in North America and causes a wide spectrum of disease, ranging from pulmonary to disseminated and acute to chronic. The etiologic agent, *Histoplasma capsulatum*, is thermally dimorphic, existing as a hyaline mold in the natural environment and as a yeast at body temperature. Demonstration of the yeast on pathological stains and isolation of the mold in culture of clinical specimens constitute the gold standard tests for the diagnosis of histoplasmosis. In 1986, the first *Histoplasma* antigen assay was developed, introducing a novel, highly sensitive, and noninvasive diagnostic modality. Further iterations of this assay have allowed for both greater specificity and quantitative capacity and have revolutionized the diagnosis of histoplasmosis by allowing physicians to make rapid diagnoses in the absence of culture or pathological confirmation. Serologic testing for histoplasmosis is another widely employed method for diagnosis and is particularly useful for chronic disease manifestations in which the sensitivity of antigen detection is suboptimal. Although not yet ready for widespread use, molecular methods have the potential to revolutionize the diagnosis of histoplasmosis.

The European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) have defined criteria for the diagnosis of invasive fungal infections, including those caused by dimorphic fungi (1). A proven diagnosis is contingent on confirmation by either histopathology or culture, while a probable diagnosis is based on the presence of an appropriate clinical presentation, a predisposing condition, and mycological evidence, such as the presence of antigenuria. The Council of State and Territorial Epidemiologists (CSTE) has recently developed a consensus case definition for histoplasmosis to address the variability in designations across different states and as a tool for improved epidemiologic surveillance. Definite and probable criteria for a new case of histoplasmosis include clinical, laboratory, and epidemiologic evidence of disease, as well as a period of 24 months since the last reported onset of histoplasmosis in the same individual (2). As reflected by these criteria, the diagnosis of histoplasmosis often requires a multifaceted approach, includ-

TABLE 1 Summary of diagnostic test for histoplasmosis[a]

| Test | % histoplasmosis result by type | | | |
	Acute pulmonary	Subacute pulmonary	Chronic pulmonary	Progressive disseminated
Culture	0–20	53.8	66.7	74.2
Pathology	0–42	42.1	75.0	76.3
Antigen	82.8–83.3	30.4	87.5	91.8
Serology	64.3–66.7	95.1	83.3	75

[a]See references 14 and 16.

ing convincing laboratory evidence. This review synthesizes current diagnostics for the laboratory identification of *H. capsulatum*, with an emphasis on testing complexities and context-based value.

CULTURE AND MICROBIOLOGY STAINS

Isolation of *H. capsulatum* from clinical specimens remains the gold standard for the diagnosis of histoplasmosis. In the microbiology laboratory, *H. capsulatum* can be identified in culture after specimen is inoculated onto appropriate medium and incubated sufficiently to allow for fungal growth or by staining and direct microscopy on body fluid and tissue specimens. Unlike *Candida* and *Cryptococcus* yeast cells, which are predominantly extracellular, *H. capsulatum* stains poorly with Gram stain and is only rarely detected by this modality. Calcofluor white, a fluorescent stain that binds chitin in the cell wall of all fungi, is useful to identify *H. capsulatum* in clinical specimens sent for microbiological testing. When incubated on appropriate medium at 25 to 30°C, growth of the mycelial phase occurs most commonly within 2 to 3 weeks but may take up to 8 weeks. Once a colony is identified on solid medium, a lactophenol cotton blue test (tease mount) can be performed to determine mold morphology, and depending on the maturity of the mycelia, will first show septated hyphae, followed by the presence of smooth (or, less commonly, spiny) microconidia (2 to 5 μm in size) and finally, characteristic tuberculate macroconidia (7 to 15 μm in size). If plates are originally incubated at 37°C, colonies appear yeast-like, and microscopy will reveal small round narrow-budding yeast. Incubation of the mold form at 37°C will lead to transformation from the mycelial to the yeast phase. Although previously used as a method to confirm the dimorphic nature of *H. capsulatum*, the rate of conversion is low and therefore impractical as a diagnostic tool. Tuberculate macroconidia are highly suggestive of *H. capsulatum*, but other fungi, including *Sepedonium* species, can also produce such structures; therefore, a more specific test is needed prior to making a definitive diagnosis of histoplasmosis. Commercially available molecular probes can be applied to the isolate and yield rapid identification (see Molecular Methods). These have replaced tests for a specific exoantigen, which are more labor-intensive and less practical. *H. capsulatum* poses an infectious risk and must be manipulated in a laboratory with biosafety level 3 safety equipment and facilities.

The sensitivity of cultures for detection of *H. capsulatum* depends on the clinical manifestation (pulmonary versus disseminated), the net state of immunity of the host, and the burden of disease (Table 1). Patients with disseminated histoplasmosis have a higher rate of positive cultures (74%) than patients with acute pulmonary histoplasmosis (42%) (3). In patients with HIV/AIDS, respiratory cultures may be positive in up to 90%, while blood cultures may be positive in up to 50% (4). Although the routine use of lysis centrifugation tubes for recovery of fastidious bacteria and fungi from blood cultures has fallen out of favor in many laboratories, the sensitivity of this method has been shown to be superior to those of conventional and Bactec MYCO/F Lytic blood cultures for the recovery of *H. capsulatum* (5–7).

HISTOPATHOLOGY

Demonstrating the presence of yeast cells consistent with *H. capsulatum* in tissue supports the diagnosis of histoplasmosis (although not necessarily active infection). *H.*

capsulatum var. *capsulatum* yeast cells are ovoid in shape, measure 2 to 4 μm in size, have thin nonrefractile cell walls, and manifest characteristic narrow base budding. Yeast are predominantly found phagocytosed within macrophages and histiocytes, often in clusters of many organisms but may sometimes be seen in extracellular spaces. *H. capsulatum* var. *duboisii*, the agent of African histoplasmosis, is larger (6 to 12 μm) and easily distinguishable from the more common variety. Organisms to consider when making the histopathologic diagnosis of *H. capsulatum* include *Cryptococcus* spp., *Blastomyces dermatitis*, *Candida glabrata*, *Pneumocystis jirovecii*, *Coccidioides* spp., *Talaromyces* (formerly *Penicillium*) *marneffei*, *Leishmania* spp., *Toxoplasma gondii*, and *Trypanosoma cruzi*. The use of specific histochemical stains facilitates the differentiation of these pathogens, with the Gomori methenamine silver (GMS) and periodic acid-Schiff (PAS) stains being the most useful to visualize *H. capsulatum* in tissue preparations by highlighting the yeast cell wall. Hematoxylin and eosin (H&E) staining is often too insensitive to detect the presence of *H. capsulatum*, except when the burden of organisms is very large. Mucicarmine allows differentiation from *Cryptococcus*, another narrow-budding and slightly larger yeast (3 to 8 μm), by staining its capsule and producing the appearance of characteristic halos. In unencapsulated strains of *Cryptococcus*, Fontana-Masson stain can be used to stain cryptococcal melanin. The majority of *Blastomyces dermatitis* yeast cells are significantly larger (up to 15 μm) than those of *H. capsulatum*, but their broad-based budding and thicker walls can distinguish smaller forms. Because of its small size and lack of pseudohyphal production, the appearance of *C. glabrata* demonstrates the most overlap with *H. capsulatum*. Characteristics that help distinguish these yeasts include predominant cellular location (intracellular for *H. capsulatum*, extracellular for *C. glabrata*), shape and size variation (uniform versus heterogenous), and histopathologic response (granulomatous versus suppurative) (8). *Pneumocystis jirovecii* cysts, like *H. capsulatum*, stain with PAS and GMS but are not encapsulated and do not take up mucicarmine. However, these cysts are larger (5 to 8 μm) than *H. capsulatum* yeast, do not exhibit budding, and are predominantly extracellular. Endospores of *Coccidioides* spp. will approximate the size and shape of *H. capsulatum* and must prompt a search for intact or rupture spherules. *T. marneffei* exhibits a transverse septum that is absent in other yeast and does not bud. *Leishmania* spp., *Toxoplasma gondii*, and *Trypanosoma cruzi* are protozoa that do not stain with GMS or PAS stains but are often evident with H&E. When applied to peripheral blood smears, the Wright-Giemsa stain can identify intracellular clusters of budding yeast, especially in patients with disseminated disease.

The presence of *H. capsulatum* yeast in certain tissues or sterile body fluid (such as skin lesions) and in the appropriate clinical context (such as acute pneumonia) is indicative of active infection. However, nonviable organisms may be found in in mediastinal or lung granuloma tissues for many years after initial infection. Pathology usually shows incomplete granulomas and/or fibrosis rather than a well-formed pyogranulomatous reaction. In these cases, negative cultures, lack of symptoms, and antigenemia can help distinguish between resolved disease, old disease, and active infection.

CYTOPATHOLOGY

Examination of tissue aspirates and fluids for individual cells rather than tissue with preserved architecture can provide presumptive evidence for histoplasmosis. As with histopathology, when stained with GMS or PAS, the cytological preparation will often show narrow-based budding yeast cells mainly within macrophages. Sensitivity varies according to the clinical manifestation (Table 1). Cytopathologic evaluation of bronchoalveolar lavage (BAL) fluid is relatively noninvasive and has a sensitivity of around 50% for acute pulmonary histoplasmosis. When combined with BAL fluid *Histoplasma* antigen testing, the sensitivity rises to 97% (9). Fine-needle aspiration is another safe diagnostic method that can yield a cytodiagnosis of histoplasmosis when applied to a variety of tissues, including lymph nodes and adrenal glands (10).

ANTIGEN DETECTION

By virtue of its noninvasive nature, wide accessibility to clinicians, and good performance characteristics, antigen testing has become a leading modality to diagnose histoplasmosis. Although a definitive diagnosis of histoplasmosis necessitates culture or histopathologic confirmation, a probable diagnosis can still be made when a host factor (immunocompromising condition), compatible clinical picture, and mycological evidence (such as antigen positivity) are present (1).

First developed in 1986 as a sandwich radioimmunoassay, the *Histoplasma* antigen was reformulated into an enzyme immunoassay (EIA) in 1989. A second-generation EIA was developed in 2004, which allowed for semiquantitative results, and a third-generation test (MiraVista *H. capsulatum* Galactomannan EIA) with greater specificity and quantitative results became available in 2007. In contrast to the MiraVista assay, which requires processing in a central laboratory, an *in vitro* diagnostic EIA (IMMY ALPHA *Histoplasma* EIA) was approved by the Food and Drug Administration (FDA) on urine specimens in 2007 for use at local facilities. The sensitivity and specificity of this assay were found to be lower than those of the MiraVista assay (11). A subsequently developed analyte-specific reagent (ASR) *H. capsulatum* antigen EIA (IMMY) has shown improved performance characteristics (12), as well as high agreement with the MiraVista EIA (13). However, head-to-head comparisons between IMMY ASR EIA and MiraVista EIAs have shown increased sensitivity and an overall trend toward higher numerical values with the MiraVista EIAs (12, 13).

In a large multicenter study, the sensitivity of the MiraVista EIA *Histoplasma* antigen test was found to be highest in patients with disseminated histoplasmosis in whom the burden of infection is substantial, followed by those with chronic pulmonary histoplasmosis and acute pulmonary histoplasmosis (91.8%, 87.5%, and 83%, respectively), and lowest in patients with subacute histoplasmosis (30%) (14) (Table 1). The sensitivity of the assay is particularly high in patients with HIV/AIDS with disseminated disease, in which antigenuria can be detected in 95% of cases (15). Mediastinal manifestations of histoplasmosis, including mediastinal granuloma and fibrosing mediastinitis, usually do not lead to positive antigen testing.

Detecting antigen in urine has generally proven to be slightly more sensitive than in serum across all manifestations of histoplasmosis. Combining both urine and serum testing increases the likelihood of antigen detection (16). Antigen testing has also been applied to other body fluids, including BAL fluid and cerebrospinal fluid (CSF). In patients with pulmonary histoplasmosis, BAL fluid *Histoplasma* antigen may serve as a useful adjunct to urine and serum testing. An earlier study among HIV/AIDS patients showed a BAL fluid *Histoplasma* antigen sensitivity of 70% compared to 93% in urine and 88.5% in serum (17). However, in a more recent study, the sensitivity of BAL fluid antigen testing was superior (93%) to those of both urine (79%) and serum (65%) and identified cases that were missed by the urine and serum methods (9). In patients with *Histoplasma* meningitis, antigen may be detected in the CSF, with a sensitivity ranging from 40% to 65% (18, 19). More recent data suggest that the CSF *Histoplasma* antigen may be up to 85% sensitive when drawn within 14 days of antifungal initiation in cases of proven CNS histoplasmosis (unpublished data). It is important to note that antigen testing on non-FDA-approved specimens is often based on less robust data, may be hindered by interfering factors, and requires validation studies to establish performance characteristics.

In addition to its utility in diagnosis, the third-generation *Histoplasma* antigen EIA's quantitative nature allows for sequential monitoring of antigen clearance. Antigen levels, particularly in serum, have been shown to decline on effective treatment and to increase with treatment failure, providing a useful marker of treatment response. Data for monitoring of antigenemia and antigenuria have been most rigorous among HIV/AIDS patients; in this population, antigen levels in urine and serum of <2 ng/ml have been proposed as one of the requirements for cure and antifungal discontinuation (20).

TABLE 2 Cross-reactivity of *Histoplasma* antigen with other fungi

Fungus	Cross-reactivity (%) (reference)
Blastomyces dermatitidis	64 (40), 90 (14), 70 (41), 80 (9)
Histoplasma capsulatum var. *duboisii*	100 (40)[a]
Coccidioides immitis and *C. posadasii*	0 (40), 60 (41), 67 (42)
Paracoccidioides brasiliensis	90 (40), 80 (41)
Sporothrix schenckii	100 (43)[a]
Penicillium marneffei	94 (40), 80 (41)
Aspergillus spp.	0 (41)

[a]Data based on single patient.

A limitation of *Histoplasma* antigen testing is the significant cross-reactivity with other fungal antigens, including *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *T. marneffei*, and less commonly, *Coccidioides immitis* and *Coccidioides posadasii* (see Table 2). False-positive reactions have also been shown to occur in 15% of transplant patients receiving anti-thymocyte globulin as part of anti-rejection treatment (21). Although low-positive results are more likely to be false positives, they are often of clinical significance and cannot be ignored. In one study of 25 patients with low-positive results and no history of histoplasmosis, 13 patients were proven to have active histoplasmosis by other diagnostic methods (histopathology, culture, serology, or PCR), and 5 patients were determined to have other fungal infections (blastomycosis or coccidioidomycosis) endemic to the area (22).

SEROLOGY

Antibodies require 4 to 8 weeks to become detectable in peripheral blood and are therefore unsuitable for the diagnosis of early acute infection. Antibody testing is most useful for subacute and chronic forms of histoplasmosis (including mediastinal histoplasmosis), in which circulating antibodies are present and the sensitivity of antigen detection is suboptimal (Table 1). As with other serologic testing, a positive antibody test for *H. capsulatum* indicates that the patient was exposed to the fungus at some point in the past. However, in some scenarios, serologic testing may provide evidence of acute infection (Table 3). The 3 most common serologic assays for histoplasmosis include the immunodiffusion (ID) test, complement fixation (CF) test, and enzyme immunoassay (EIA). The CF method detects the presence of antibodies in a patient's serum, based on the extent of complement fixation to complexes of patient antibodies with yeast-phase and mycelium-phase (histoplasmin) antigens. With this assay, acute infection is defined as a ≥4-fold rise in antibody titers between acute and convalescent-phase sera. A titer of 1:8 is positive, indicating previous exposure to *H. capsulatum*. A titer of ≥1:32 or a 4-fold rise in antibody titer from acute- to convalescent-phase serum is strongly suggestive of active infection (23). Titers usually decrease with disease resolution, but the decline is slow and often incomplete, making antibody clearance impractical as a tool to assess treatment response. The ID test detects the presence of serum antibodies that precipitate on agar gel after binding with H and M *H. capsulatum*

TABLE 3 Serologic evidence of acute infection per CSTE criteria[a]

Criterion[b]	Specimen source[c]
≥4-fold rise in *H. capsulatum* CF titers taken at least 2 wk apart	Serum
Detection of H band by *H. capsulatum* ID test	Serum
Detection of M band by *H. capsulatum* ID test after documented lack of M band on previous test	Serum
Detection of *H. capsulatum* antibodies by single CF titer of ≥1:32	Serum or CSF
Detection of M band by *H. capsulatum* ID test without previous negative test	Serum or CSF

[a]Fulfillment of a single criterion is sufficient for a diagnosis of acute histoplasmosis.
[b]CF, complement fixation; ID, immunodiffusion.
[c]CSF, cerebrospinal fluid.

antigens. The M band is detectable in most patients with acute histoplasmosis (80%) but persists for long periods of time; therefore, a single positive M band cannot distinguish active from latent or resolved disease. H precipitins are rarely seen (20%) but, when present, confirm acute infection. The CF method is more sensitive than ID (90% versus 80%, respectively) (23). Cross-reactivity in the setting of other fungal infections or other conditions (particularly granulomatous disease, such as tuberculosis and sarcoidosis) can occur with both assays but is more common with CF (24). An EIA method that is more sensitive than CF and ID but with decreased specificity has been described (25). Serology can be useful even in areas that are highly endemic for the disease, where surprisingly, less than 5% of individuals have positive serology on CF or ID (23). The presence of antibodies in the CSF by CF or ID is sufficient to make the diagnosis of *Histoplasma* meningitis and is more sensitive than the isolation of *H. capsulatum* on CSF cultures (26). Serologic parameters that provide confirmation of acute infection have been delineated by the CSTE, and the presence of a single criterion is sufficient for diagnosis of acute infection (Table 3). Immunosuppressed patients, particularly those with impaired humoral immunity, may not mount an antibody response. Data suggest that the majority of patients on tumor necrosis factor inhibitors will have positive serology, whereas only 25 to 30% of recipients of solid organ transplant patients develop an antibody response (27, 28). Combining antibody and antigen testing may lead to significantly improved sensitivity for diagnosing acute pulmonary histoplasmosis (29).

MOLECULAR METHODS

Molecular methods offer the advantage of high analytical specificity, combined with turnaround times shorter than those of other diagnostics. However, there are no currently FDA-approved molecular assays for *H. capsulatum* that are directly applicable to clinical specimens. Laboratory-developed PCR assays using a variety of molecular targets have been developed (Table 4). Compared to culture and a criterion-based or clinical diagnosis of histoplasmosis, the sensitivity of molecular assays in published studies has ranged between 67 and 100% (30–35) and 33 and 87% (36, 37), respectively. A fluorescence *in situ* hybridization (FISH) technique that successfully detects *H. capsulatum* rRNA in blood cultures may circumvent the need for colony growth to obtain a definitive and timely diagnosis (35). Although culture has typically been considered the gold standard for diagnosis, molecular methods may in fact be more sensitive. Indeed, in a study comparing real-time PCR to culture for the detection of *H. capsulatum*, 10 of 11 patients with culture-negative PCR-positive samples were confirmed to have histoplasmosis based on positive cultures from other specimens or positive histopathology (33). Fewer studies have examined molecular methods in comparison to antigen and antibody detection. A PCR-enzyme immunoassay-based method was only 18.5% sensitive in comparison to high-level antigenuria (>20 U) (34), while a nested PCR detected 86% of cases with elevated *H. capsulatum*-specific antibodies (1:320 to 1:2,560) (38). The generalizability of these results is limited by the heterogeneity of molecular assays, targets used, small numbers of patients included, variation in the clinical specimens studied, and the comparator diagnostic method. Nonetheless, molecular methods clearly have the potential to revolutionize the diagnosis of histoplasmosis, and assays with improved performance characteristics will likely play a larger role in years to come.

At present, the main procedure for molecular diagnosis of histoplasmosis is by applying a rapid DNA probe to fungus isolated from culture. One such test is the AccuProbe, which uses a single-stranded DNA probe with a chemiluminescent label that is complementary to a sequence of fungal rRNA. Fluorescence generated by labeled DNA:RNA hybrids are then measured by a luminometer. Signals greater than or equal to predetermined cutoff values are considered positive (39). Certain commercial laboratories offer tissue-based PCR testing and sequencing, including a broad-range PCR of fungal 28S ribosome and internal transcribed spacer (ITS) sequence, as well as

TABLE 4 Laboratory-developed molecular methods for detection of *H. capsulatum* in clinical specimens

Molecular method[a]	*H. capsulatum* molecular target[b]	No./type of patients[c]	No. of clinical samples	Specimen source(s) (n)[d]	Comparator test	Sensitivity (%)	Specificity (%)[e]
LAMP (32)	hcp100 gene locus	6 HIV+ with PDH, 10 controls	16	Urine	Culture	67	100
Nested PCR (31)	hcp100 gene locus	15 HIV+ with PDH*, 12 controls*	40	Bone marrow (11), hepatic biopsy sample (9), bronchial aspirations (6), BAL fluid (4), lymph node (2), gut biopsy (2), blood (2), CSF (2), serum (2)	Culture	100	100
PCR-EIA (34)	*H. capsulatum*-specific gene sequence (99 bp)	51 with positive urine *Histoplasma* antigen, 25 controls	76	Urine	Culture	80	100
					Urine antigen (1–19.9 U)	0	NR
					Urine antigen (>20 U)	18.5	NR
Real-time PCR (30)	192-bp region of *GAPDH* gene	Suspected fungal infection, N NR	797 (15 culture-positive samples)	Bronchial washings (346), BAL fluid (212), pleural fluid (157), tracheal secretions (35), tissue (14), sputum (13), lung washes (6), blood (4), bone marrow (5), peritoneal fluid (3), other body fluids (2)	Culture	73	100
FISH (35)	Ribosomal 18S subunit	3 HIV+ with clinical diagnosis of invasive mycosis, 30 controls	33	Blood culture	Culture	100	100
PCR (35)	rRNA	3 HIV+ with clinical diagnosis of invasive mycosis, 30 controls	33	Blood	Culture	100	100
Real-time PCR (44)	*H. capsulatum*-specific gene sequence (99 bp)	9 with histoplasmosis	9	FFPE	Culture	89	ND
Real-time PCR (33)	Internal transcribed spacer region of rRNA gene complex	Suspicion for clinical mycoses, N NR	348 (71 culture-positive samples)	Bone marrow (108), CSF (55), blood (48), BAL fluid (43), intestinal biopsy (31), liver biopsy (30), lymph nodes (25), skin biopsy (8)	Culture	96	96
PCR (36)	*RYP1* gene	15 HIV+ with histoplasmosis, 6 controls	21	Blood	Diagnosis of histoplasmosis (specific comparator not reported)	87	100
Nested PCR (37)	Conserved regions of NAALADase genes	5 with proven (4) or probable (1) histoplasmosis per EORTC criteria	9	Serum (4), FFPE (4), BAL fluid (1)	Diagnosis of histoplasmosis per EORTC criteria	77	ND
Real-time PCR (37)	Conserved regions of NAALADase genes	5 with proven (4) or probable (1) histoplasmosis per EORTC criteria	9	Serum (4), FFPE (4), BAL fluid (1)	Diagnosis of histoplasmosis per EORTC criteria	33	ND
Nested PCR (38)	hcp100 gene locus	7 with acute pulmonary histoplasmosis	7	Serum	Serology (EIA; titer range, 1:320–1:2,560)	86	ND
Simplex PCR (38)	*H. capsulatum*-specific gene sequence (1281–1283 [220] bp)	7 with acute pulmonary histoplasmosis	7	Serum	Serology (EIA; titer range, 1:320–1:2,560)	86	ND

a LAMP, Loop-mediated isothermal amplification; EIA, enzyme immunoassay; FISH, fluorescence *in situ* hybridization.
b NAALADase, N-acetyl-L-aspartyl-L-glutamate peptidase. "[220]" refers to a 220-bp fragment that was amplified using primers 1281 to 1283.
c PDH, progressive disseminated histoplasmosis; EORTC, European Organization for Research and Treatment of Cancer. *, all patients except 1 were HIV positive. N NR, number not reported.
d BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; FFPE, formalin-fixed paraffin-embedded tissue.
e NR, not reported; ND, not done.

a *Histoplasma*-specific PCR assay. However, the performance and clinical validation of these assays have not been well clarified.

SUMMARY AND CONCLUSIONS

Isolation of *H. capsulatum* on culture or identification of yeast on histopathology are the gold standards for diagnosis. Antigen testing and serology are also available, with antigen testing being both highly sensitive and easily interpretable, making it widely accessible to clinicians. Molecular methods may be the next frontier in *Histoplasma* diagnostics, but current assays have not been FDA approved for routine clinical use. As with most other infectious diseases, the optimal diagnostic method is contingent on the time point in the natural course of the disease, the site of infection, the clinical specimen being sampled, and the net state of immunosuppression. Selecting the appropriate tests requires an understanding of the performance characteristics of various diagnostic methods in each clinical setting. The clinical microbiology laboratory can serve as an important resource for clinicians seeking to select and interpret diagnostic tests for histoplasmosis.

REFERENCES

1. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, Pappas PG, Maertens J, Lortholary O, Kauffman CA, Denning DW, Patterson TF, Maschmeyer G, Bille J, Dismukes WE, Herbrecht R, Hope WW, Kibbler CC, Kullberg BJ, Marr KA, Munoz P, Odds FC, Perfect JR, Restrepo A, Ruhnke M, Segal BH, Sobel JD, Sorrell TC, Viscoli C, Wingard JR, Zaoutis T, Bennett JE, European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group, National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. 2008. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis 46:1813–1821. https://doi.org/10.1086/588660.
2. CSTE. 2016. Standardized surveillance case definition for histoplasmosis. Council of State and Territorial Epidemiologists, Atlanta, GA. http://www.cste.org/resource/resmgr/2016PS/16_ID_02.pdf.
3. Hage CA, Azar MM, Bahr N, Loyd J, Wheat LJ. 2015. Histoplasmosis: up-to-date evidence-based approach to diagnosis and management. Semin Respir Crit Care Med 36:729–745. https://doi.org/10.1055/s-0035-1562899.
4. Kauffman CA. 2007. Histoplasmosis: a clinical and laboratory update. Clin Microbiol Rev 20:115–132. https://doi.org/10.1128/CMR.00027-06.
5. Santiago AR, Hernandez B, Rodriguez M, Romero H. 2004. A comparative study of blood culture conventional method vs. a modified lysis/centrifugation technique for the diagnosis of fungemias. Rev Iberoam Micol 21:198–201. (In Spanish.)
6. Vetter E, Torgerson C, Feuker A, Hughes J, Harmsen S, Schleck C, Horstmeier C, Roberts G, Cockerill F, III. 2001. Comparison of the BACTEC MYCO/F Lytic bottle to the isolator tube, BACTEC Plus Aerobic F/bottle, and BACTEC Anaerobic Lytic/10 bottle and comparison of the BACTEC Plus Aerobic F/bottle to the Isolator tube for recovery of bacteria, mycobacteria, and fungi from blood. J Clin Microbiol 39:4380–4386. https://doi.org/10.1128/JCM.39.12.4380-4386.2001.
7. Ramanan P, Vetter E, Milone AA, Patel R, Wengenack NL. 2016. Comparison of BACTEC MYCO/F Lytic bottle to the Wampole Isolator for recovery of fungal and mycobacterial organisms. Open Forum Infect Dis 3:1557. https://doi.org/10.1093/ofid/ofw172.1258.
8. Sangoi AR, Rogers WM, Longacre TA, Montoya JG, Baron EJ, Banaei N. 2009. Challenges and pitfalls of morphologic identification of fungal infections in histologic and cytologic specimens: a ten-year retrospective review at a single institution. Am J Clin Pathol 131:364–375. https://doi.org/10.1309/AJCP99OOOZSNISCZ.
9. Hage CA, Davis TE, Fuller D, Egan L, Witt JR, III, Wheat LJ, Knox KS. 2010. Diagnosis of histoplasmosis by antigen detection in BAL fluid. Chest 137:623–628. https://doi.org/10.1378/chest.09-1702.
10. Gupta N, Arora SK, Rajwanshi A, Nijhawan R, Srinivasan R. 2010. Histoplasmosis: cytodiagnosis and review of literature with special em-

phasis on differential diagnosis on cytomorphology. Cytopathology 21:240–244. https://doi.org/10.1111/j.1365-2303.2009.00693.x.
11. Zhang X, Gibson B, Jr, Daly TM. 2013. Evaluation of commercially available reagents for diagnosis of histoplasmosis infection in immunocompromised patients. J Clin Microbiol 51:4095–4101. https://doi.org/10.1128/JCM.02298-13.
12. Zhang C, Lei GS, Lee CH, Hage CA. 2015. Evaluation of two new enzyme immunoassay reagents for diagnosis of histoplasmosis in a cohort of clinically characterized patients. Med Mycol 53:868–873. https://doi.org/10.1093/mmy/myv062.
13. Theel ES, Jespersen DJ, Harring J, Mandrekar J, Binnicker MJ. 2013. Evaluation of an enzyme immunoassay for detection of Histoplasma capsulatum antigen from urine specimens. J Clin Microbiol 51:3555–3559. https://doi.org/10.1128/JCM.01868-13.
14. Hage CA, Ribes JA, Wengenack NL, Baddour LM, Assi M, McKinsey DS, Hammoud K, Alapat D, Babady NE, Parker M, Fuller D, Noor A, Davis TE, Rodgers M, Connolly PA, El Haddad B, Wheat LJ. 2011. A multicenter evaluation of tests for diagnosis of histoplasmosis. Clin Infect Dis 53:448–454. https://doi.org/10.1093/cid/cir435.
15. Wheat LJ, Kauffman CA. 2003. Histoplasmosis. Infect Dis Clin North Am 17:1–19, vii. https://doi.org/10.1016/S0891-5520(02)00039-9.
16. Swartzentruber S, Rhodes L, Kurkjian K, Zahn M, Brandt ME, Connolly P, Wheat LJ. 2009. Diagnosis of acute pulmonary histoplasmosis by antigen detection. Clin Infect Dis 49:1878–1882. https://doi.org/10.1086/648421.
17. Wheat LJ, Connolly-Stringfield P, Williams B, Connolly K, Blair R, Bartlett M, Durkin M. 1992. Diagnosis of histoplasmosis in patients with the acquired immunodeficiency syndrome by detection of Histoplasma capsulatum polysaccharide antigen in bronchoalveolar lavage fluid. Am Rev Respir Dis 145:1421–1424. https://doi.org/10.1164/ajrccm/145.6.1421.
18. Wheat LJ, Azar MM, Bahr NC, Spec A, Relich RF, Hage C. 2016. Histoplasmosis. Infect Dis Clin North Am 30:207–227. https://doi.org/10.1016/j.idc.2015.10.009.
19. Wheat LJ. 2001. Laboratory diagnosis of histoplasmosis: update 2000. Semin Respir Infect 16:131–140. https://doi.org/10.1053/srin.2001.24243.
20. Myint T, Anderson AM, Sanchez A, Farabi A, Hage C, Baddley JW, Jhaveri M, Greenberg RN, Bamberger DM, Rodgers M, Crawford TN, Wheat LJ. 2014. Histoplasmosis in patients with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS): multicenter study of outcomes and factors associated with relapse. Medicine (Baltimore) 93:11–18. https://doi.org/10.1097/MD.0000000000000016.
21. Wheat LJ, Connolly P, Durkin M, Book BK, Tector AJ, Fridell J, Pescovitz MD. 2004. False-positive Histoplasma antigenemia caused by antithymocyte globulin antibodies. Transpl Infect Dis 6:23–27. https://doi.org/10.1111/j.1399-3062.2004.00045.x.
22. Theel ES, Ramanan P. 2014. Clinical significance of low-positive Histoplasma urine antigen results. J Clin Microbiol 52:3444–3446. https://doi.org/10.1128/JCM.01598-14.
23. Wheat J, French ML, Kohler RB, Zimmerman SE, Smith WR, Norton JA, Eitzen HE, Smith CD, Slama TG. 1982. The diagnostic laboratory tests for

histoplasmosis: analysis of experience in a large urban outbreak. Ann Intern Med 97:680–685. https://doi.org/10.7326/0003-4819-97-5-680.

24. Wheat J, French ML, Kamel S, Tewari RP. 1986. Evaluation of cross-reactions in Histoplasma capsulatum serologic tests. J Clin Microbiol 23:493–499.

25. Wheat LJ, Kohler RB, French ML, Garten M, Kleiman M, Zimmerman SE, Schlech W, Ho J, White A, Brahmi Z. 1983. Immunoglobulin M and G histoplasmal antibody response in histoplasmosis. Am Rev Respir Dis 128:65–70. https://doi.org/10.1164/arrd.1983.128.1.65.

26. Wheat J, French M, Batteiger B, Kohler R. 1985. Cerebrospinal fluid Histoplasma antibodies in central nervous system histoplasmosis. Arch Intern Med 145:1237–1240. https://doi.org/10.1001/archinte.1985.00360070115018.

27. Hage CA, Bowyer S, Tarvin SE, Helper D, Kleiman MB, Wheat LJ. 2010. Recognition, diagnosis, and treatment of histoplasmosis complicating tumor necrosis factor blocker therapy. Clin Infect Dis 50:85–92. https://doi.org/10.1086/648724.

28. Cuellar-Rodriguez J, Avery RK, Lard M, Budev M, Gordon SM, Shrestha NK, van Duin D, Oethinger M, Mawhorter SD. 2009. Histoplasmosis in solid organ transplant recipients: 10 years of experience at a large transplant center in an endemic area. Clin Infect Dis 49:710–716. https://doi.org/10.1086/604712.

29. Richer SM, Smedema ML, Durkin MM, Herman KM, Hage CA, Fuller D, Wheat LJ. 2016. Improved diagnosis of acute pulmonary histoplasmosis by combining antigen and antibody detection. Clin Infect Dis 62:896–902. https://doi.org/10.1093/cid/ciw007.

30. Babady NE, Buckwalter SP, Hall L, Le Febre KM, Binnicker MJ, Wengenack NL. 2011. Detection of Blastomyces dermatitidis and Histoplasma capsulatum from culture isolates and clinical specimens by use of real-time PCR. J Clin Microbiol 49:3204–3208. https://doi.org/10.1128/JCM.00673-11.

31. Maubon D, Simon S, Aznar C. 2007. Histoplasmosis diagnosis using a polymerase chain reaction method. Application on human samples in French Guiana, South America. Diagn Microbiol Infect Dis 58:441–444. https://doi.org/10.1016/j.diagmicrobio.2007.03.008.

32. Scheel CM, Zhou Y, Theodoro RC, Abrams B, Balajee SA, Litvintseva AP. 2014. Development of a loop-mediated isothermal amplification method for detection of Histoplasma capsulatum DNA in clinical samples. J Clin Microbiol 52:483–488. https://doi.org/10.1128/JCM.02739-13.

33. Simon S, Veron V, Boukhari R, Blanchet D, Aznar C. 2010. Detection of Histoplasma capsulatum DNA in human samples by real-time polymerase chain reaction. Diagn Microbiol Infect Dis 66:268–273. https://doi.org/10.1016/j.diagmicrobio.2009.10.010.

34. Tang YW, Li H, Durkin MM, Sefers SE, Meng S, Connolly PA, Stratton CW, Wheat LJ. 2006. Urine polymerase chain reaction is not as sensitive as urine antigen for the diagnosis of disseminated histoplasmosis. Diagn Microbiol Infect Dis 54:283–287. https://doi.org/10.1016/j.diagmicrobio.2005.10.008.

35. da Silva RM, Jr, da Silva Neto JR, Santos CS, Cruz KS, Frickmann H, Poppert S, Koshikene D, de Souza JV. 2015. Fluorescent in situ hybridization of pre-incubated blood culture material for the rapid diagnosis of histoplasmosis. Med Mycol 53:160–164. https://doi.org/10.1093/mmy/myu080.

36. Brilhante RS, Guedes GM, Riello GB, Ribeiro JF, Alencar LP, Bandeira SP, Castelo-Branco DS, Oliveira JS, Freire JM, Mesquita JR, Camargo ZP, Cordeiro RA, Rocha MF, Sidrim JJ. 2016. RYP1 gene as a target for molecular diagnosis of histoplasmosis. J Microbiol Methods 130:112–114. https://doi.org/10.1016/j.mimet.2016.09.006.

37. Muraosa Y, Toyotome T, Yahiro M, Watanabe A, Shikanai-Yasuda MA, Kamei K. 2016. Detection of Histoplasma capsulatum from clinical specimens by cycling probe-based real-time PCR and nested real-time PCR. Med Mycol 54:433–438. https://doi.org/10.1093/mmy/myv106.

38. Frias-De-Leon MG, Ramirez-Barcenas JA, Rodriguez-Arellanes G, Velasco-Castrejon O, Taylor ML, Reyes-Montes MDR. 2017. Usefulness of molecular markers in the diagnosis of occupational and recreational histoplasmosis outbreaks. Folia Microbiol (Praha) 62:111–116. https://doi.org/10.1007/s12223-016-0477-4.

39. GenProbe, Inc. 2011. AccuProbe Histoplasma capsulatum culture identification test. GenProbe Inc., San Diego, CA. http://www.hologic.ca/sites/default/files/package%20inserts/102962RevK.pdf.

40. Wheat J, Wheat H, Connolly P, Kleiman M, Supparatpinyo K, Nelson K, Bradsher R, Restrepo A. 1997. Cross-reactivity in Histoplasma capsulatum variety capsulatum antigen assays of urine samples from patients with endemic mycoses. Clin Infect Dis 24:1169–1171. https://doi.org/10.1086/513647.

41. Connolly PA, Durkin MM, LeMonte AM, Hackett EJ, Wheat LJ. 2007. Detection of Histoplasma antigen by a quantitative enzyme immunoassay. Clin Vaccine Immunol 14:1587–1591. https://doi.org/10.1128/CVI.00071-07.

42. Kuberski T, Myers R, Wheat LJ, Durkin M, Connolly P, Kubak BM, Bruckner D, Pegues D. 2007. Diagnosis of coccidioidomycosis by antigen detection using cross-reaction with a Histoplasma antigen. Clin Infect Dis 44:e50–54. https://doi.org/10.1086/511684.

43. Assi M, Lakkis IE, Wheat LJ. 2011. Cross-reactivity in the Histoplasma antigen enzyme immunoassay caused by sporotrichosis. Clin Vaccine Immunol 18:1781–1782. https://doi.org/10.1128/CVI.05017-11.

44. Koepsell SA, Hinrichs SH, Iwen PC. 2012. Applying a real-time PCR assay for Histoplasma capsulatum to clinically relevant formalin-fixed paraffin-embedded human tissue. J Clin Microbiol 50:3395–3397. https://doi.org/10.1128/JCM.01705-12.