Chapter

Laboratory Diagnosis of Histoplasmosis: An Update

María J. Buitrago and Clara Valero

Abstract

Early diagnosis of histoplasmosis is essential to establish a suitable antifungal therapy and reduce morbidity and mortality rates. However, laboratory diagnosis remains challenging due to the low availability of proper methods and the lack of clinical suspicion. Conventional diagnosis is still largely used even though limitations are well known. Isolating the fungus is time consuming and requires manipulation in BSL3 facilities, while direct visualization and histopathology techniques show low sensitivity and need skilled personnel. New approaches based on the detection of antibodies and antigens have been developed and commercialized last years. Although sensitivity and specificity of these methods is variable, antigen detection has been recently listed as an essential diagnostic test for AIDS patients due to its excellent performance. DNA detection methods are recognized as promising tools but there is still a lack of consensus among laboratories and there are not commercial tests available. Not all methods are widely available, thus most laboratories combine classical and other tests in order to overcome aforementioned limitations. In this chapter, we review the diagnostic pipeline currently available for the diagnosis of histoplasmosis in microbiological laboratories, from conventional to new developed tests. Most recent approaches are introduced and future perspectives are discussed.

Keywords: histoplasmosis, diagnosis, culture, histopathology, PCR, antigen, antibody

1. Introduction

Histoplasmosis is caused by the thermally dimorphic fungi *Histoplasma capsulatum* and encompasses a broad variety of clinical presentations ranging from asymptomatic infections to subacute, acute and chronic pulmonary infections [1]. In immunosuppressed patients, especially in HIV positive population, it causes a progressive disseminated disease [2]. Mortality rates in AIDS population have been reported as 30–50% when treated, and 100% in absence of treatment [3]. Although this fungus has a cosmopolitan distribution, there are some areas of high endemicity as Ohio and Mississippi basins in North America, several countries from Central and South America and different regions in the African continent [4–6]. Areas of medium-high endemicity have been reported in China and some sporadic cases have been described in other regions of Asia as North of India, Thailand and Philippines [7–10]. The rest of the world is considered as non-endemic regions and reported cases are described as imported by travelers or

immigrants [11]. It has been estimated that about a half million of people acquire the infection per year and around 100,000 develop a disseminated disease [3]. Certain regions of Brazil and Venezuela have been considered hotspots of the disseminated form of the disease [12]. However, in other regions as the African continent the incidence is probably underestimated due to lack of studies [6]. Diagnosis of histoplasmosis is challenging in both endemic and non-endemic regions due to its similarity to other diseases as tuberculosis, usually the first clinical suspicion, and the limitations and low availability of diagnostic tools. Consequently, many efforts have been recently made with the aim of improving diagnosis and combat histoplasmosis [3]. In this chapter, we describe the conventional methods used to diagnose histoplasmosis coupled with an update about the new tests available. The advantages and limitations of each method are detailed as well as their usefulness in the diagnosis regarding the diverse clinical presentations of the disease and their availability in the different regions. The need of continuous work on the development of new methods to achieve an early diagnosis and reduce histoplasmosis morbidity and mortality is also underlined.

2. Conventional diagnostic methods

Conventional diagnostic methods are still widely used for the diagnosis of histoplasmosis. The definite diagnosis is based on the isolation of the fungus in culture or the visualization of intracellular yeasts in tissues or other clinical samples. Thus, despite its known limitations, these methods are very useful and continue to be used in many laboratories, especially in low-resources settings.

2.1 Culture

The isolation of *H. capsulatum* in culture is the gold standard for the diagnosis of histoplasmosis. The recovery of the fungus by culture from clinical samples (blood culture, bronchoalveolar lavage fluid, etc.) is recognized as the criteria for the definition of proven histoplasmosis according to the consensus definitions of the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC-MSGERC) [13].

H. capsulatum is a so-called fastidious fungus since it can take up to 4 weeks to grow and requires Biosafety level 3 (BSL-3) containment measures [2]. At 25–30°C, it grows as a white and cottony mycelium that evolves to brown, representing the invasive form of the disease. This mycelial form is the most dangerous, as spores could be inhaled causing infection. Some laboratory-acquired infections have been reported by culture manipulation [14]. Microscopically the mycelial form produces the typical tuberculate macroconidia, which are easily identified (**Figure 1A**). However, other species as *Sepedonium* spp., an environmental organism that is usually considered as a laboratory contaminant, produce similar structures. At 37°C (human body temperature), there is a conversion from the mycelial phase to the yeast form, which is the pathogenic form of the disease. These yeasts are easily recognized by their thick wall and ovoid form with a narrow base at the smaller end (**Figure 1B**).

The sensitivity of cultures depends on the clinical status of the patient and the origin of the sample. Cultures are negative in most cases of asymptomatic and mild disease, however they are useful in disseminated and chronic pulmonary histoplasmosis, although their sensitivity varies from 50 to 85% [2, 15].

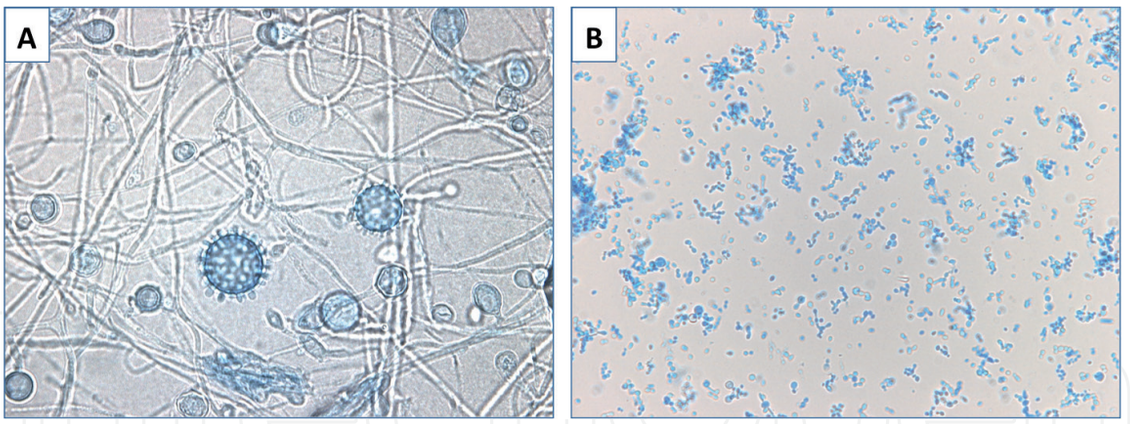

Figure 1.
Photomicrograph of lactophenol cotton blue stains of both filamentous and yeast forms of H. capsulatum. *(A) Culture at 30°C on potato dextrose agar (PDA) showing tuberculate macroconidia (63× magnification). (B) Culture at 37°C on ML-GEMA medium showing yeasts (20× magnification). Both images belong to the image library of the mycology reference laboratory (National Centre of Microbiology, Instituto de Salud Carlos III, Madrid, Spain).*

The main limitation of cultures is the long turnaround time needed to reach a definite diagnosis. Moreover, the microscopical observation of the grown fungus requires further confirmation by other methods. Molecular identification by sequencing the internal transcribed spacer (ITS) region of ribosomal DNA is a powerful tool to confirm the presence of *H. capsulatum*, but extends the time response for the diagnosis even more. Alternatively, single-stranded DNA probes could also be used to detect the fungus in cultures (AccuProbe, Hologic, CA, USA), however the procedure is technically complex and time consuming [16]. In last years, matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-ToF MS) has started to be developed for the identification of *H. capsulatum* strains [17–19]. Although reports are still scarce, results in this area are promising since the system is able for recognizing different varieties (var. *capsulatum*, var. *duboisii*) and forms of the pathogen (yeast and mold), thus reducing response time and decreasing the risk of culture manipulation [19].

Since aforementioned limitations make culture useless for an early diagnosis of histoplasmosis, a great effort has been made for developing alternative diagnostic methods that could be used alone or in combination with cultures.

2.2 Histopathology and direct visualization

Direct examination of clinical samples or performing histopathological studies on tissues to detect yeast after staining with Gomori methenamine silver (GMS) or periodic acid-Schiff (PAS), are techniques largely used for the diagnosis of histoplasmosis. *H. capsulatum* could appear as a 2- to 4-μm (var. *capsulatum*) or 8- to 15-μm (var. *duboisii*) oval yeast. As yeasts are phagocytosed by macrophages, they could be found forming clusters, which can help the diagnosis. However, several fungi can be misled with *H. capsulatum* such as, among others, the small variant of *Blastomyces dermatitidis*, endospores of *Coccidioides* spp., *Candida glabrata* as well as the causal agents of leishmaniasis, toxoplasmosis, and Chagas' disease [20]. The higher sensitivity could be obtained in respiratory specimens or bone marrow biopsy in patients with disseminated histoplasmosis [21]. However, the low specificity and the need of skilled personnel to achieve a presumptive diagnosis are the main limitations of this technique.

3. Antigen detection

The detection of *H. capsulatum* antigens in clinical samples represented a breakthrough in the early diagnosis of histoplasmosis and has been included in the EORTC/MSG criteria for diagnosis of IFIs for almost 20 years [13, 22, 23]. The *H. capsulatum* polysaccharide antigen can be detected in both serum and urine samples with similar diagnostic value and has been recently listed as an essential diagnostic test for advanced AIDS patients [24, 25]. Two assays based on enzyme linked immunosorbent assay (ELISA) have been commercialized with good diagnostic performances (**Table 1**) although their availability is limited.

MiraVista's test presents great efficiency in serum and urine samples from patients with disseminated infection, but it is reduced in patients with pulmonary forms of the disease [26], in which BALF samples are more suitable for diagnosis [28]. This test is only performed in MVista's facilities then is not accessible out of USA limiting their use. On the other hand, IMMY has recently released an *Histoplasma* GM ELISA test which shows great performance and reproducibility, but has only be tested in urine samples [27]. Despite the high degree of cross-reaction with other fungi, antigen detection assays are widely used to diagnose histoplasmosis and some reports indicate its capacity to monitor treatment response [29, 30]. Nevertheless, their use is restricted mainly to developed endemic areas but it is rarely used in non-endemic regions, probably due to its low cost-effectiveness out of endemic regions [24, 31].

In last years, point-of-care (POC) testing has been emerged as a new diagnostic methodology and immuno-chromatographic assays performed in lateral-flow devices (LFD) are good examples. These "pregnancy tests-like" assays are easy to use, have low turnaround time (less than an hour) and require minimal laboratory equipment which facilitate its implementation in low- and middle-income countries [32, 33]. This technology was first developed for *Aspergillus* GM detection with great results and MVista has recently released a similar test consisting of a dipstick sandwich immunochromatographic assay based on the recognition of *H. capsulatum* GM antigen. Although it has only been tested in serum samples from AIDS patients, its diagnostic performance was very promising (sensitivity of 96%, specificity

Test[a]	Samples	Methodology	Turnaround time	Sensitivity/ specificity	Limitations
MV	Serum, plasma, urine, CSF, BALF, other body fluids	ELISA	Urine, BAL: <24 h Serum, plasma, CSF: 24 h	[b]DH: 92% APH: 83% SAPH: 30% CPH: 88%	• Cross-reactivity with other fungi • Samples are required to be shipped to the company's facilities
IMMY	Urine	ELISA	<2:15H	[c]95-98%/97-98%	• Cross-reactivity with other fungi

[a]*MV:* Histoplasma *Quantitative EIA test (MiraVista Diagnostics, Indianapolis, IN, USA); IMMY: CLARUS* Histoplasma *GM EIA kit (IMMY, Norman, OK, USA).*
[b]*DH [26].*
[c]*In 95-98% [27].*
ELISA: enzyme-linked immunosorbent assay; CSF: cerebrospinal fluid; BALF: bronchoalveolar lavage fluid; DH: disseminated histoplasmosis; APH: acute pulmonary histoplasmosis; SAPH: sub-acute pulmonary histoplasmosis; CPH: chronic pulmonary histoplamosis; GM: galactomannan.

Table 1.
Main characteristics and diagnostic performance of commercial assays for detecting H. capsulatum *antigens.*

of 90%) [34]. However, false positive and negative results still occurred due to cross-reactivity with other endemic fungi and antibiotic treatment, respectively, and a pre-treatment step was required extending turnaround times. Currently, a new LFD test is being developed and validated by using urine samples [35].

4. Antibody detection

The main advantages of antibody detection tests are the requirement of minimally invasive samples and the achievement of results when culture is still negative reducing the need of handling potentially infectious fungi [36]. Serological techniques such as complement fixation or immunodiffusion are useful when testing samples from travelers coming from endemic regions for the first time. Both techniques are commercially available (Immuno Mycologics, Norman, OK, USA) and the sensitivity of these tests in acute and subacute pulmonary histoplasmosis has been reported as 95% [37]. However, sensitivity is very limited in immunosuppressed patients due to low or absent antibody titers. A recent meta-analysis described sensitivity and specificity values of 58% and 100%, respectively, in samples from HIV patients [38]. Finally, since seropositivity remains long time after disease, interpretation of serological results could be challenging [39, 40]. Despite the limitations previously described, antibody detection tests are still considered as valuable diagnostic tools and have been demonstrated to improve diagnostic yield when combined with other diagnostic methods [41, 42].

5. DNA based detection methods

PCR methods based on the detection of fungal DNA directly from clinical samples are currently implemented in the routine of several laboratories for the diagnosis of main fungal infections, but there are considerably fewer PCR tests for the diagnosis of histoplasmosis. Their advantages rely on their simplicity, high specificity and short turnaround time with the bonus that real-time PCR (qPCR) formats allow for determining the fungal burden in patients by using non-specific DNA-binding dyes or fluorescently labeled probes [36, 43]. However, this technique also has some limitations as the moderate amount of DNA in low invasive samples, the lack of standardization and the low availability of widely validated commercial systems [44, 45]. Recently, PCR based methods have been included in the EORTC/MSG criteria for the diagnosis of some fungal infections such as invasive aspergillosis or candidiasis but not for endemic mycoses [13].

The majority of PCR tests for the diagnosis of histoplasmosis have been developed in house and none of them has been commercialized. They have been recently reviewed in several reports with different purposes [31, 38, 46]. Most developed methods targeted specific multicopy regions of the ribosomal DNA or the single-copy Hcp100 gene and were performed by using conventional, nested or qPCR formats. The sensitivity and specificity of these assays depends on the type of sample analyzed, the clinical characteristics of patients and the PCR format used for DNA detection. These tests showed an excellent analytical performance (overall sensitivity of 95% and specificity of 99%) when testing samples of HIV patients [38], but sensitivity decreased when testing blood and serum samples from immunocompetent patients [31, 46]. Panfungal or broad-range PCRs are used when there is not a clear suspicion of the fungus involved in the infection, since universal primers are used to detect any fungal DNA in the clinical sample. Although studies are scarce, several reports achieved the detection of

H. capsulatum DNA in clinical samples by using this technique, which could be especially useful in non-endemic regions [47–51].

Non-PCR based methods are also able to amplify and detect *H. capsulatum* DNA from clinical samples. Loop-mediated isothermal amplification (LAMP) rests on the use of a DNA polymerase with high displacement strand activity and a set of specifically designed primers to amplify targeted DNA [52]. Some reports described the implementation of this technique for the molecular diagnosis of histoplasmosis showing great results [53, 54]. The high efficiency, sensitivity, specificity and cost-effectiveness of these assays make them good candidates to be implemented in the diagnostic routine of resource-limited laboratories.

6. Conclusions and future perspectives

Early diagnosis of histoplasmosis is essential to establish a suitable antifungal therapy, which results in the reduction of mortality rates [55, 56]. This becomes especially important in certain hyper-endemic regions since they usually are disfavored areas where patients develop the disease in its disseminated form. While culture and histopathological examination are considered the gold standards methods for histoplasmosis diagnosis, these techniques show moderate sensitivity. In addition, culture is time consuming, requiring handling fungi in BSL-3 facilities. New approaches as MALDI-ToF MS technology allow for a rapid identification, but studies are still scarce. Antibody and antigen detection are useful tools for an early detection of the pathogen in low invasive clinical samples such as serum and urine. Despite limitations concerning sensitivity in certain populations and specificity have been widely reported, *H. capsulatum* antigen detection has been recently included in the second edition of the list of essential in vitro diagnostics (https://www.who.int/medical_devices/diagnostics/selection_in-vitro/selection_in-vitro-meetings/sage-ivd2nd-meeting/190318-2ndEditionofEDL-open-session.pdf?ua=1). Regarding molecular methods, in the last decade a great effort has been made on the development of PCR tests for the detection of *H. capsulatum* DNA in a broad spectrum of clinical samples showing excellent diagnostic performance. However, a consensus among laboratories about the best samples, targets and procedures is mandatory. Consequently, no commercial tests are available to date limiting the use of this technique. To date, only an inter-laboratory study focused on molecular techniques for the diagnosis of histoplasmosis has been published [57]. In this work, authors concluded that qPCR targeting a multicopy genomic region was the best option for a suitable sensitivity. A European initiative is being launched to carry out multicenter studies in this regard.

All these problems have gained attention thanks to different initiatives coming from researchers from hyper-endemic regions [58] or international foundations as the Global Action Fund for Fungal Infections (GAFFI). As a result, proposals such as the Manaus declaration have been launched, specifically to get access to rapid testing for histoplasmosis in the Americas and Caribe until 2025 (https://www.gaffi.org/the-manaus-declaration-on-histoplasmosis-in-the-americas-and-caribbean-100-by-2025/). However, in addition to these excellent initiatives further work is required to improve diagnosis. In this sense, novel techniques as next generation sequencing (NGS) have been found to be useful in the diagnosis of several infections. Sequences obtained from clinical samples through NGS can be compared against reference databases enabling their identification to the genus and species level [59]. This technique has been used recently to identify *H. capsulatum* as the causal agent of a case of chronic meningitis [60] and for the differential diagnosis among histoplasmosis, leishmaniosis and talaromycosis [61]. Another approach

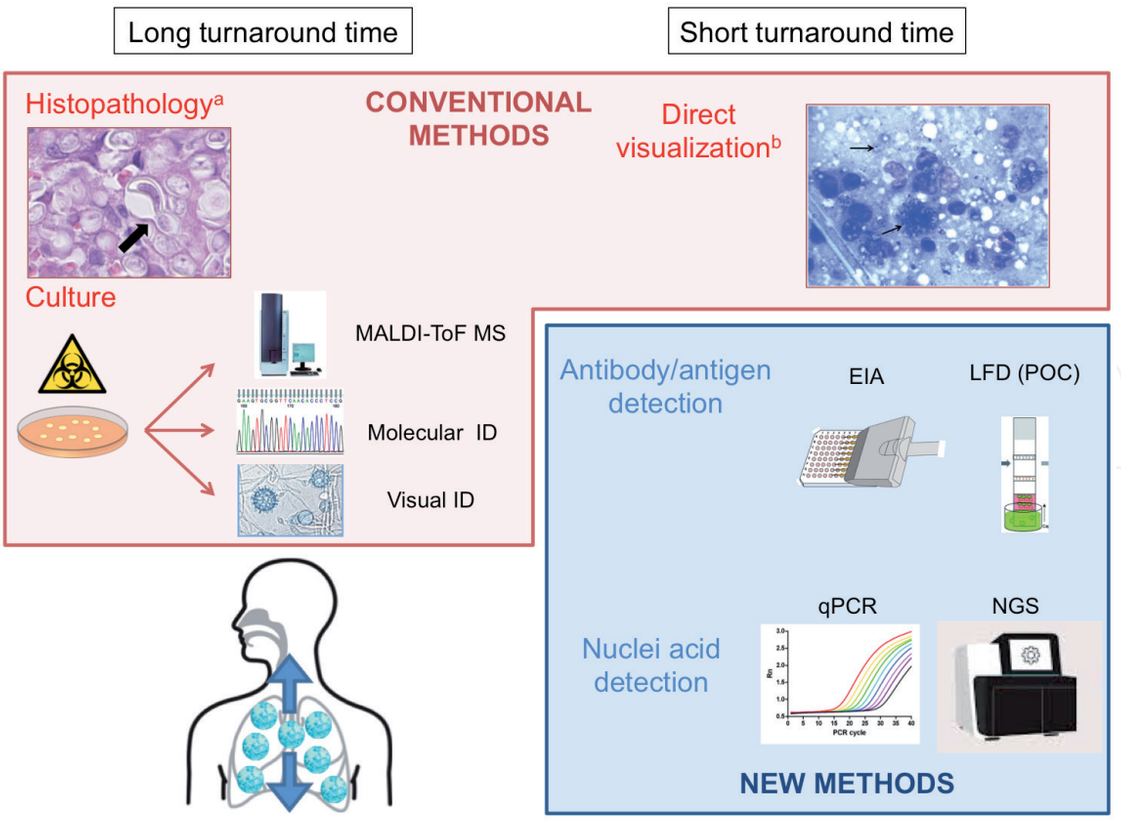

Figure 2.
Diagnostic pipeline currently available for the microbiological diagnosis of histoplasmosis. [a]Histopathological preparation showing a H. capsulatum *yeast budding [62]. [b]Direct visualization of fine needle aspiration cytology showing intra- and extracellular yeasts of* H. capsulatum *[63]. MALDI-ToF MS: matrix-assisted laser desorption/ionization-fime of flight mass spectrometry; ID: identification; EIA: enzyme immunoassay; LFD: lateral flow device; POC: point of care; qPCR: quantitative PCR; NGS: next generation sequencing.*

in constant development is based on determining host and fungal biomarkers that could indicate the presence of *H. capsulatum* in human body fluids. Recent advances in antibody detection are related to the refinement of antibody detection platforms (*Histoplasma* antibody IgG IgM EIA, MiraVista Diagnostics), while most efforts in antigen detection are focused on improving diagnostic yields of POC methods [35].

In summary, the aim of this chapter was to summarize the diagnostic pipeline currently available for the diagnosis of histoplasmosis in microbiological laboratories (**Figure 2**). Although so much progress has been made in the area, much certainly remains to be done to improve the early diagnosis of histoplasmosis, allowing the establishment of a prompt antifungal therapy and consequently reducing morbidity and mortality rates of this infection.

Conflict of interest

The authors declare no conflict of interest.

Author details

María J. Buitrago[1*] and Clara Valero[1,2]

1 Reference Laboratory in Mycology, National Center for Microbiology, Instituto de Salud Carlos III, Madrid, Spain

2 School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

*Address all correspondence to: buitrago@isciii.es

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