Chapter

Histoplasma capsulatum and *Histoplasmosis:* Current Concept for the Diagnosis

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Abstract

Histoplasmosis is a global deep mycosis caused by *Histoplasma capsulatum (Hc)*, a dimorphic fungus. It exists on two main varieties *Hc capsulatum* and *Hc duboisii* that could be distinguished by their epidemiology, their clinical presentation, and the morphological aspect of the fungus at direct examination of the sample. Laboratory diagnosis of *Hc* remains a real challenge as it required experience and equipment. Through a general review of literature, the different diagnosis tools for *Histoplasma* sp. are analyzed, and strengths and weaknesses are pointed according to the context-based value. Isolation of *Hc* on culture is the gold standard for diagnosis of histoplasmosis. However, it remains less sensitive (sensitivity: up to 77%) and implies long time to result, which can be inappropriate or in adapted for an emergency diagnosis. So, nonculture methods as antigen testing, serology, and molecular biology become available and allow a rapid diagnosis. However, the optimal diagnostic method depends on many parameters as the very wide range of symptomatology, the immune status. Indeed, Ag detection is the best diagnosis tool for PHD (sensitivity: 92–95%) and SCN histoplasmosis (sensitivity: 66%) and serology for the subacute/chronic form (sensitivity: 85–93%). Thus, the clinico-biological dialog is essential, and histoplasmosis management includes an integrated medical approach.

Keywords: *Histoplasma capsulatum*, diagnosis, non-culture methods, culture, performance

1. Introduction

Histoplasmosis caused by *Histoplasma capsulatum* leads to a wide spectrum of symptomatology [1–3] varying from subclinical or acute to chronic form. Histoplasmosis can be localized or it causes a disseminated, life-threatening infection involving various tissues and organs of the body, notably in immunocompromised hosts [1, 4].

This is a thermally dimorphic fungus presenting as a yeast at body temperature and as a hyaline mold in the natural environment. Human histoplasmosis is due to the two varieties of the pathogen: *H. capsulatum* var. capsulatum *(Hcc)* and *H. capsulatum* var. duboisii *(Hcd),* which could be distinguished by their epidemiology, their clinical presentation, and the morphological aspect of the fungus at direct examination of the sample [3].

Hcc, responsible for "small cell" histoplasmosis or American histoplasmosis, is well-documented in the United States mainly in the valleys of Ohio and Mississippi, South America, and Asia. Hcd responsible for "large-form" histoplasmosis or African histoplasmosis is endemic in Central and West Africa and Madagascar [3, 5–6]. We point out a third variety, *H. capsulatum* var. farcimimosum, involved in equine pathology that will not be detailed in this chapter.

Histoplasmosis can be challenging to diagnose because it is laborious and requires special characteristics for its revealing. According to the criteria recommended by 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) and those of the Council of State and Territorial Epidemiologists (CSTE) [7, 8], an integrated approach including clinical, radiographic, and laboratory evidence is required. For laboratory diagnosis, several techniques are available as microbiology, histopathology, and immune-serological assays, depending on the clinical context and laboratory capabilities. Since 1978, the introduction of the *Histoplasma* antigen assay significantly improved the diagnosis by allowing a rapid, noninvasive, and sensitive method. Then, if culture remains the reference approach in most laboratories in endemic and non-endemic areas, non-culture methods are developing and much more improving [2, 9–11].

This review describes the current diagnostics for the laboratory identification of *H. capsulatum*, with focus on their performance and context-based value.

2. Clinical presentations and type of histoplasmosis

For Histoplasma capsulatum var. capsulatum, the inhalation of conidia from the environment (bat or bird guano) leads to Histoplasmosis syndrome or asymptomatic carriage in immunocompetent individuals. It can be self-limited especially in the lung or a progressive disseminated disease. Upon immunosuppression, the fungus can reactivate and be responsible for a localized or disseminated disease. Disseminated histoplasmosis has been classified as an AIDS-defining infection. There is a significant overlap of the pathophysiology and the clinical presentation as tuberculosis or cryptococcosis, sarcoidosis, and malignancy [1–4, 6, 12].

The histoplasmosis syndromes can be evoked according the clinical presentation, the epidemiology, and the disabled. It can be expressed as [1–2, 6]:

- A pulmonary histoplasmosis presenting as acute, subacute, chronic, or pulmonary nodules.
- A progressive disseminated histoplasmosis (PDH) that mainly concerns some particular populations (extreme age groups, immunosuppressed people including AIDS/HIV, iatrogenic origin). However, immunocompetent people defined as without obviously immunodeficiency can develop such syndrome when important inoculum.
- A cerebral nervous system (CNS) histoplasmosis.
- Other clinical findings as mediastinal histoplasmosis (adenitis, granuloma, mediastinitis).

For *Histoplasma capsulatum* var. duboisii, many features remain undiscovered as the reservoir niche, pathogenesis, and epidemiology because of the relatively low

number of case reports. It expresses much more in skin and subcutaneous tissues than in lungs. Association as well with HIV is rare [5].

3. Types of specimens and context base value

All types of clinical specimens can be processed according to symptomatology [1–2, 4, 6–8]:

- Bronchoalveolar lavage (BAL), sputum, and lung biopsy should be performed for patients with pulmonary symptoms
- Bone marrow aspiration
- Punctures of lymph node, pus, or exudates
- Organ biopsies (lymph node, digestive tract, liver, skin, oral mucous)
- Peripheral blood (EDTA or fungal blood culture vacutainers)
- Cerebrospinal fluid (CSF)

Different microbiological approaches can be performed as on one hand mycological examination (direct, culture) and molecular assays (qPCR) and, on the other hand, histopathology. The performances depend on the specimens tested and are much more details on paragraph 5.4 (**Table 1**).

4. Direct examination for mycology

Examination of liquid smears or fine-needle aspiration and/or tissue apposition on slides from all types of samples is carried out by staining with May-Grünwald-Giemsa (MGG). Using 10% KOH with or without calcofluoric acid, a chitin-binding fluorescent stain for the fungal cell wall, the yeasts can be easily visualized between slide and slip cover. For some specimens as the peripherical blood, cerebrospinal fluid, bronchoalveolar fluid, and cytocentrifugation may be required [3, 13, 14].

Diagnosis is possible according to the morphology of the yeasts.

4.1 *H. capsulatum* var. capsulatum

Yeasts are spherical, small (2–5 μm in diameter), intensely violet colored, and surrounded by a clear halo. These yeasts are narrow-based budding yeast cells, usually intracellular (macrophage, histiocyte, etc.), and do not produce any filaments (**Figure 1**). The differential diagnosis includes *Leishmania* species, which is similar in size but has one kinetoplast [3, 6, 13, 14], *Candida* mainly *glabrata* and *Cryptococcus* yeast cell which is predominantly extracellular and strongly MGG stained.

4.2 *H. capsulatum* var. duboisii

Yeasts cells are oval and large (8–15 μm by 4–6 μm), with a "hourglass" or "figure eight" budding form, a thick wall and a narrow budding. They may have inside fat

		PHD	Acute pulmonary syndrom	Subacute pulmonary syndrom	Chronic pulmonary syndrom	Pulmonary nodules	Mediastinal histoplasmosis	CNS histoplasmosis	Other manifestations (pericarditis, rheumatological),
Culture	Pulmonary tissue or secretions (LBA, sputum)	x	x	x	x		(x): differential diagnosis		
	Bone marrow	x						x (if associated PHD)	
	Lymph nodes	x		(x) mediastinal					
	Exudates	x				(x) Non viable yeasts	(x) Non viable yeasts		
	Organ biopsy			(x)		x Non viable yeasts			
	Peripheral blood	x						x (if associated PHD)	
	Cerebrospinal fluid							x	
	histopathology	x	x Cytopathologic examination of BAL (50%)			x Non viable yeasts	(x)		
	Serology	(x)		x	x	X (low sensitivity)	x	X	x
	Antigen	x serum, urine +/-LBA to repeat	x (83%): serum, urine +/-LBA or if associated PDH	(x) up to 40% (urine)	(x) serum, urine +/-LBA			x	

( ): not preferred but can be contributive, PDH: Progressive histoplasmosis disseminated, CNS: Central nervous system, BAL: Broncho-alveolar fluid.

Table 1.
Preferred specimens for diagnosis according the histoplasmosis syndromes [1, 2].

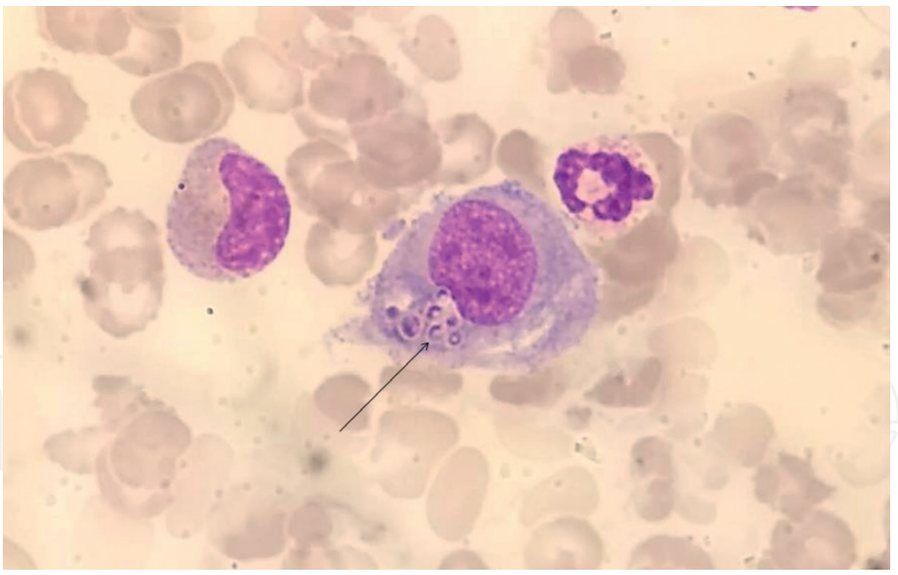

Figure 1.
Histoplasma capsulatum *var. capsulatum on bone marrow smear, intramacrophagic yeast form, microscopic appearance, magnification ×1000 (photo by Emilie GUEMAS).*

droplets. These yeasts are intra- or extracellular, sometimes arranged in short chains of 2 or 3 [3, 6, 13, 14].

Specimens as pus from abscess, draining sinuses and bone marrow lesions, colic biopsy, or BAL smears are relatively contributive to the diagnosis [4, 6, 15] in disseminated histoplasmosis.

Direct examination is inexpensive, and according to the relatively typical appearance of the levuriform elements, it allows to quickly and easily provide presumptive evidence for histoplasmosis. However, doubts may remain, and then it requires trained personnel and further investigations. Indeed confusion can concern *Hcc* versus *Cryptococcus sp., Candida glabrata*, and *Leishmania sp*. and/or if the clinical picture is not very classical [3, 6, 13, 14] or *Hcd* versus *Blastomyces dermatitidis*, which looks like another yeast cell and differs with its broad base budding.

5. Histopathology

Histopathology consists in the research for elements evocative of the yeast form of *H. capsulatum* inside tissues. Different stains can be carried out such as Gomori Methenamine Silver (GMS), Giemsa, or periodic acid-Schiff (PAS) stains that are the most relevant for the diagnosis. Hematoxylin and eosin (H&E) staining is insensitive to detect the presence of *H. capsulatum* [3, 13, 14, 16].

This concept of juggling between different stains and the description of morphologies of the microorganism (shape, size variation, cell disposition) and the tissue (cell response) permit to distinguish other pathogens as *Cryptococcus* spp., *Blastomyces dermatitis*, *Candida glabrata*, *Pneumocystis jirovecii*, *Coccidioides* spp., *Talaromyces* (formerly *Penicillium*) *marneffei*, *Leishmania* spp., *Toxoplasma gondii*, and *Trypanosoma cruzi*. These differences are represented in **Table 2**.

5.1 *H. capsulatum* var. capsulatum *(Hcc)*

It usually shows a granulomatous reaction with "giant cells," containing large rounded or oval-shaped elements (2–4 μm in diameter). *Hcc* is predominantly found phagocytosed within macrophages, histiocytes, and giant cells, often in clusters of many organisms, but sometimes they are in extracellular spaces.

	Size (µm)	Cell disposition	Characteristic	Tissue response	Specific coloration
Hcc	2 - 5	IC	Spherical with halo Narrow-based budding Grouped in clusters into macrophage	Granulamatous tissue response, necrosis	GMS, PAS
Hcd	6 - 12	IC	Oval with halo	Granulamatous tissue response	GMS, PAS
Cryptococcus	3 - 8	Facultative IC	Spherical with characteristic halo (thick capsule) Narrow-based budding	Predominantly granulomatous inflammation, necrosis, +/- fibrosis	Mucicarmin (capsulated yeast) Fontana-Masson (uncapsulated yeast)
Blastomyces dermatitis	> 15	EC	Round, Thick retractile wall Broad-based budding	Mixed suppurative and granulomatous inflammation	GMS, PAS, H&E
Candida glabrata	1 - 4	EC	Oval to round No pseudohyphal production	Suppurative tissue response	GMS, PAS, H&E
Coccidioides	2 - 5	IC/ EC	spherical Confusion when endospores are outside spherules or young spherules without endospores	Mixed suppurative and granulomatous inflammation, (Splendore-Hoëppli phenomenon likely)	GMS, H&E, +/-PAS
Pneumocystis cysts	5-8	EC	Cysts	Minimal reaction	GMS, PAS, H&E
Talaromyces marneffeï	2-5	EC	Small oval-shaped yeast Transverse septum No bud	Mixed suppurative and granulomatous inflammation	
Leishmania	2 - 5	IC	Oval to round kinetoplast		MGG

IC: intracellular, EC: extra cellular, GMS: Gromori Methenamine Silver, PAS: Periodique Acid Schiff:, GE, MGG: May-Grunwald Giemsa.

Table 2.
Diagnosis differential of H. capsulatum [2, 16, 13].

The yeast phase of *Hcc* is very similar to other pathogens and not distinctive in tissues from several other endemic fungi. The misidentification occurs principally with *Candida glabrata, Penicillium marneffei, Pneumocystis jiroveci, Toxoplasma gondii, Leishmania donovani*, and *Cryptococcus neoformans* [9]. However, in the appropriate clinical context, the presence of *H. capsulatum* like yeast is able to

confidently make a diagnosis of histoplasmosis and is indicative of active infection. Moreover, the immunohistochemistry reaction with *H. capsulatum* antibodies can be used to confirm diagnosis of histoplasmosis.

5.2 *H. capsulatum* var. duboisii *(Hcc)*

Hcc is large (6–12 μm) and the wall is thick and highly refractive, with a pseudo-capsulated appearance. The yeast is easily distinguishable from the other endemic fungi especially *Blastomyces dermatitis* [8].

6. Culture

Isolation of *H. capsulatum* remains the gold standard for the laboratory diagnosis of histoplasmosis. However, it requires both BioSafety Level 3 (BSL3) facilities to be manipulated [17] and usually invasive methods to obtain the specimens from which culture can be performed.

6.1 Inoculation and culture of specimens

All specimen types can be used from superficial to deep ones.

Culture is performed on Sabouraud dextrose agar with antibiotics (chloramphenicol +/− gentamicin) +/− actidione that inhibits the contaminants. Other mediums can be used: brain heart infusion (BHI), yeast extract peptone agar (YEP agar) and potato dextrose agar (PDA) [3]. Mediums are incubated at 25–30°C for 6–8 weeks.

As the rate of growth is slow, the growth of the mycelial forms usually takes 2–3 weeks but may take up to 8 weeks. Colony is initially white and smooth and then becomes brown, with a granular or cottony texture. The reverse is white, yellow, or orange (**Figure 2**).

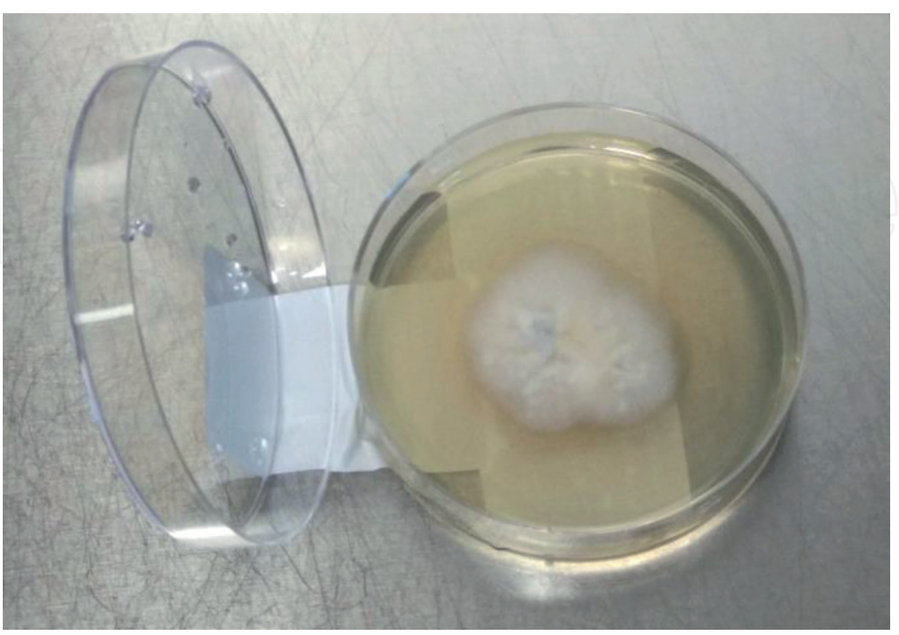

Figure 2.
Culture of H. capsulatum *on Sabouraud dextrose agar. Colony is white with a cottony texture (photo by Emilie GUEMAS).*

6.2 Identification of the fungi

Confirmation of the cultured organism as *H. capsulatum* requires complementary tests:

6.2.1 The lactophenol cotton blue test (LPCB test)

It allows determining microscopy morphology with identification characters [18]:

- **Hyphae**: hyaline septated (2–3 μm in diameter),
- **Characteristic macroconidia**: large, thick-walled, round, typically tuberculate, or knobby (7–15 μm in diameter).
- **Microconidia**: smooth-walled spherical, pyriform (2–5 μm in diameter) on short branches or directly on the sides of the hyphae

The differential diagnosis includes *Sepedonium sp*. (**Figure 3**).

6.2.2 Identification with the MALDI-TOF

The identification of the colony can be carried out by matrix-assisted laser desorption/ionization time off light (MALDI-TOF). Mass spectrometry by MALDI-TOF allows to confirm species accurately and quickly.

This identification technique is based upon the detection of highly abundant proteins in a mass range of 2–20 kDa by calculating their mass (m) to charge (z), m/z values. The spectrum thus obtained is compared with the reference spectra [19, 20].

6.2.3 The conversion from the mold to the yeast form

This can be achieved using enriched media such as BHI or blood agar plates incubated at 35–37°C in a CO_2-enriched atmosphere [3]. However, *H. capsulatum* does not convert easily depending on many parameters [13] as nutriments and temperature conditions, which can explain that this experiment is increasingly abandoned.

6.2.4 "Historic" tests

The urease test allows the distinction between Hcc and Hcd as it reveals the expression of urease strongly for Hcc and weakly for Hcd at 48 hours [3, 5].

Others tests performed from the cultured fungi such as DNA hybridization using a highly specific commercially kit (AccProbe; Gen-Probe, Inc., San Diego, CA®) or the detection of specific precipitin by the exoantigen test was used in some laboratories [10, 13]. They were gradually replaced by less time-consuming method as MALDI-TOF. They will not be more detailed in this chapter.

6.3 Blood culture

The lysis centrifugation method as Isolator® system followed by the inoculation of the collected buffy coat [11] into an appropriate media culture has been proved to be the most efficient for detecting *Histoplasma* in the blood in comparison with other methods such as conventional or automated methods such as Bactec MYCO/F

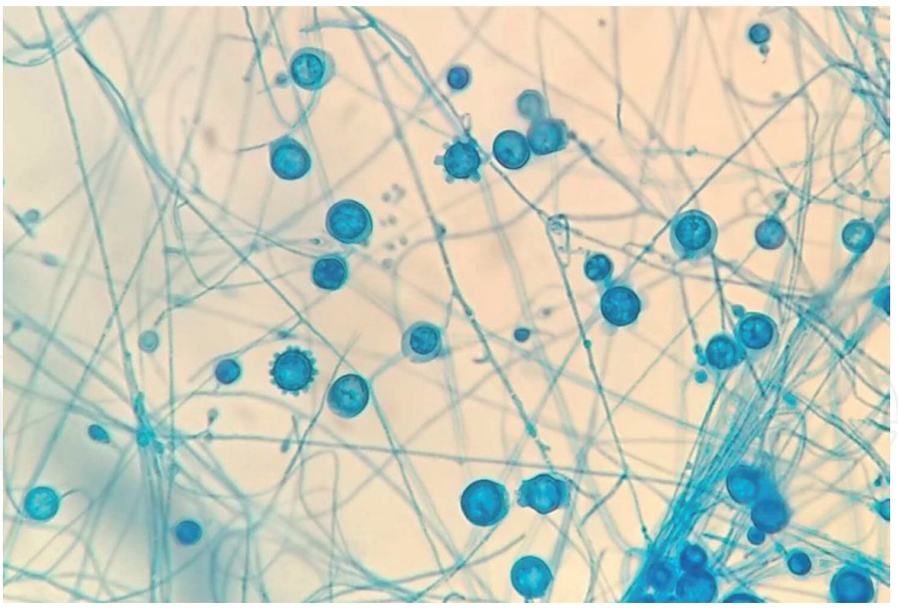

Figure 3.
Histoplasma capsulatum*, microscopic appearance with LPCB test, magnification ×400 (photo by Emilie GUEMAS).*

Lytic bottle [21]. However, many laboratories preferred those alternative systems than the manual isolator system because they permit less pre-analytic processing and continuous automated monitoring of bottles for growth when automated [9].

6.4 Performances of the culture

The sensitivity of culture depends on the clinical manifestation, the clinical specimen, the state of immunity of the host, and the burden of disease [2, 9, 14]. Sensitivity is lower for patients with acute pulmonary histoplasmosis (40%) than for patients with disseminated histoplasmosis (75%) [2, 9, 10].

The diagnosis of disseminated histoplasmosis blood culture processed by lysis methodology and bone marrow shows higher sensitivity (60–90%). Conversely, respiratory samples presented poor sensitivity (0–60%) [2, 9, 10].

The limitations of the culture are the time frame for diagnosis, the mycological expertise required, and safety. Several weeks for growth of the fungus to establish the diagnosis are not consistent with the severity of the disease in immunosuppressed patients. Moreover, *H. capsulatum* poses an infectious risk and must be manipulated in a laboratory with BSL3.

Conversely, detection of *H. capsulatum* by automated methods such as the Bactec system has not shown optimal results with sensitivity of less than 50% [2, 9–11, 13, 14] in acute and disseminated histoplasmosis that need to be rapidly diagnosed for the prompt initiation of therapy.

7. Antibody detection

Several protocols do exist based on *Histoplasma*-specific antibody detection with three routinely used assays: immunodiffusion test (ID), fixation complement test (FC), and indirect immunological assays (EIA) [9–11, 13, 14, 22–26]. Serology aims to confirm a previously contact with the pathogen, and it usually does not mean that the patient is making an acute infection. For histoplasmosis, in certain circumstances, serology can detect the acute phase of histoplasmosis. It is reported

that the antibodies detection period is between 4 and 8 weeks but can be negative in immunodeficient patients [10, 14]. The serologic targets are the specific *Histoplasma* proteins M (a catalase) and H (a β-glucosidase) that are present in the entire yeast or an antigenic extract (histoplasmin or HMIN) used as deglycosylate or not, from mycelial culture. Another protein can be targeted, the protein C (a carbohydrate, galactomannan), that is less specific and can be responsible of cross-reactions with other fungi [9]. Their characteristics are summarized in the **Table 3**.

7.1 Immunodiffusion (ID)

Immunodiffusion assay qualitatively detects the precipitating antibodies to antigens M and H on agar gel. The H band appears after the M band, and the presence of both bands is highly significant for histoplasmosis diagnosis. Thus, M band is detectable in most patients with acute infection and persists for long periods of time up to 3 years after disease resolution and is often present in chronic forms [9, 10, 14]. It does not distinct between active from latent or resolved infection. H band is less frequent, confirms acute infection only in 7% according [13], remains present 1–2 years after disease resolution, and indicates a more severe form of the disease. This method is simple, reliable, and inexpensive.

7.2 Complement fixation (FC)

Complement fixation method quantitatively measures the presence of complex antigen antibodies in a patient's serum against the entire yeast form or mycelial antigen (HMIN) from 3 to 6 weeks following infection [13]. Interlaboratory results vary as it is entirely strain-dependent for the antigen preparation. It is quite more sensitive bus less specific than the ID. Indeed, it presents numerous cross-reactions with other fungi and interference with rheumatoid factor and cold agglutinins [13]. A threshold defined as up to 1:32 or titer a 4-fold rise indicates an active infection.

7.3 Enzyme-linked immunosorbent assay (ELISA)

This method does not allow differentiation between an active infection and an old infection. Indeed, antibodies require 4–8 weeks to become detectable in peripheral blood. Serology is unreliable in patients with a reduced ability to produce antibodies, and then it is often negative in immunocompromised patients [13, 14]. Antibody testing is most useful for subacute and chronic forms of histoplasmosis [2].

7.4 Western blot

Western blot immunoassay uses Ag proteins of different kDa that will complex with the specific *Histoplasma* antibodies on a nitrocellulose band. It showed good results but does not exist as a commercial kit, so it can be fastidious to set up. The sensibility is improved by the treatment of the Ag (Histoplasmin) [22].

7.5 Other tests

Interferon gamma release assay (IGRA): this test is based on the quantification of lymphocyts-released interferon (IFN) -γ after restimulation in vitro of the cell-mediated immunity with the same specific antigens. Recently, Rubio-Carrasquilla et al. [23] considered it as a promising screening method to detect individuals with latent Hc infection, even decades after the primary infection.

	Complement fixation (FC)	**Immunodiffusion (ID)**	**ELISA**	**Western Blott**
Mechanisms	A supplemented specific Ag induces Ag-Ab complexes	Precipitins Ac-Ag (H/M) on gel agar	Indirect sandwich ELISA	Visualisation of band profiles on nitrocellulose membranes
Type of Ag	Entire yeast or HMIN	HMIN Prot M, Prot H	Yeast cell extract, ribosome, HMIN (glycosylayte/ deglycosylate)	Histoplasma antigens of 115, 91,88, 83, 70 and 38 kDa from HMIN (glycosylayte/deglycosylate)
delay	3 -6 weeks	4-6 weeks IgG anti-M → IgG M+H	2 -4 weeks	2-4 weeks
Duration of positive antibodies after resolution	Months to years Months to years Persistent in recurrences	Prot M: up to 3 years Prot H: 1-2 years	Months to years	Months to years
Sensitivity	72-95%	70-95%	75-100%	45-100%
Specificity	70-80%	100%	91-100%	94-100%
Histoplasmosis meningitidis	When positive (culture usually negative (63%)	When positive (culture usually negative (44%)	Specificity: 93% Sensibility: 82% (26)	No data
Acute infection	++	Band M (80%) Band H (7-20%)	66-100% (9-10, 13)	45-94% (13, 22)
Chronic infection	+	Band M	90% (9-10, 13)	94-100% (13, 22)
Special remarks	Interference with Rheumatoid factor and cold agglutinins	Positive after skin test Histoplasmine (M +++/H+)	Ribosome antigen and ptHMIN induces better response than glycosylate HMIN antigene. Best results with ELISA using ferrous metal	Better results when using ptHMIN Can detect early in the infection
Kits/tests	House-made tests	House-made tests	House-made tests Commercialized tests with IgG, IgM, IgA	House-made tests

Histoplasmine : HMIN, deglycosylated HMIN: tpHMIN.

Table 3.
Summary of serological assays [2, 9, 10, 13, 14, 22, 26].

Latex agglutination tests were developed as a commercial kit, but false positives results were found in patients with tuberculosis. It was compared as more sensitive than CF [13].

A hemagglutination test was available but failed to differentiate *B. dermatitidis* [13].

A recent meta-analysis [9] interested in the global sensitivity of antibody detection for disseminated histoplasmosis and showed a low sensitivity of 58% in contrast with high specificity (100%). But there is a real distinction between the different assays. WB and ELISA methods have the highest analytical performance, with sensitivity of up to 90% when used in ptHMIN. This can explain that it is relevant to associate different methods in order to improve the diagnosis.

Cross-reactivity with granulomatous disease, tuberculosis, and sarcoidosis can occur with immunodiffusion and complement fixation tests. Moreover, serologic cross-reaction can occur with other common fungal pathogens like *Blastomyces dermatitidis*, *Coccidioides immitis*, *Aspergillus fumigatus*, and *Paracoccidioides brasiliensis* [24, 25].

The presence of antibodies in the CSF allows making the diagnosis of Histoplasma meningitis [2, 26].

8. Antigen detection

8.1 Target antigens

*8.1.1 Specific-*Histoplasma *antigen detection*

Circulating specific-*Histoplasma* polysaccharide antigen (HPA) detection is a useful option for diagnosis [9, 13–14, 27–28]. *Histoplasma* galactomannan is the target of the antigenic detection. Since its introduction in 1986 [13–14], as a solid-phase radioimmunoassay, it was developed into a sandwich enzyme immunoassay (EIA) [2, 13, 27–29] with different improved generations and recently into a lateral flow assay (LFA) [30]. Their performance is related to the choice of the antibodies used monoclonal versus polyclonal, and the conjugate (Biotine or horseradish peroxidase) [13].

8.1.2 Galactomannan antigen detection

This polysaccharide mostly found in the cell wall of *Aspergillus* sp. is commonly used for invasive aspergillosis diagnosis in high-risk immunosuppressed patients suffering of solid organ transplantation or hematological malignancies. Cross-reactivity with the histoplasma antigen detection is reported suggesting that this test could be a potentially helpful diagnostic test for histoplasmosis in HIV-infected patient because of the low incidence of invasive aspergillosis in this population. The sensibility of this test for diagnosis of disseminated histoplasmosis in AIDS patients is 77% and specificity is 100% [31].

8.2 Technical methods

8.2.1 Enzyme immunoassay (EIA)

This noninvasive method can be performed in urine, serum, and other body fluids as LBA [2, 9, 10, 13–14] and is the simplest diagnostic method, easily implemented in low- and middle-income countries.

It allows a rapid diagnosis especially for immunosuppressed patients with severe acute or disseminated histoplasmosis. It is less sensitive than serology for the diagnosis of subacute and chronic pulmonary histoplasmosis [2, 9].

Antigen detection in urine is more sensitive than in serum for the diagnosis of disseminated histoplasmosis (95% versus 86% for HIV-infected patient) [9, 10–11]. However, antigen detection in urine is less useful for pulmonary forms or chronic histoplasmosis.

This method has also been applied to other body fluids, including BAL for patient with pulmonary symptomatology and CSF for *Histoplasma* meningitis. Antigen was detected in the CSF of 66% of immunosuppressed-infected patient patients who had *Histoplasma* meningitis [26].

If useful for the diagnosis, it can also monitor the antigen clearance, particularly in serum and thus appears as an useful marker for treatment response [32].

Moreover, it has proven its applicability to infected animals that may act as potential reservoirs for humans [28, 29].

Cross-reactivity of antigen testing occurs in patients who have other fungal infections, including infections by *Blastomyces dermatitidis, Paracoccidioides brasiliensis,* and *Penicillium marneffei* [33]. The most problematic issue is differentiating histoplasmosis and blastomycosis because the geographic areas overlap.

8.2.2 Lateral flow assay (LFA)

Recently, a lateral flow assay (LFA) has been developed and evaluated for rapid diagnosis of histoplasmosis. This point of care antigen detection in serum allows rapid results with high analytical performance. It is based on the using of rabbit polyclonal antibody that recognizes galactomannan antigen of *H. capsulatum*. Indeed, sensitivity is under 95% and specificity is 90%. Cross-reactivity occurs principally with paracoccidioidomycosis [30].

9. Molecular tests

9.1 Polymerase chain reaction (PCR)

Molecular methods can improve and accelerate the histoplasmosis diagnosis with a high analytical sensibility and specificity [9, 10, 34–36]. This combined with turnaround times shorter than those of other diagnostics. It is much more safety and reduces the risk of laboratory acquired histoplasmosis when handle its positive culture. Several applications can be linked to the use of PCR based techniques as (i) human diagnosis from different samples, with an increasingly use for the imported cases diagnosis in non-endemic areas [9–11, 13], (ii) environmental exploration for revealing the histoplasmosis reservoir [35], (iii) and prevention policies and strategies for exposure risk [2].

There are no currently official approved molecular assays for H. capsulatum that are directly applicable to clinical specimens and no commercially kits are available [9, 10, 34]. However, there are numerous reports of the laboratory-developed PCR assays (in house PCR) using the different type of PCR (conventional, nested and quantitative) and a variety of molecular targets. The most relevant are the Internal Transcribed Spacer (ITS) multicopy region of the ribosomal DNA, genes encoding the M antigen or the 100-kDa-like protein [9, 34]. Most studies in the literature evaluated the performance of nested PCR in the diagnosis of PDH, this assay is associated with the increased risk of amplicon contamination, because of the manipulation of amplified products.

A multiplex approach can also be performed. For example, in a reference laboratory in Spain they developed a multiplex qPCR for *H. capsulatum, Pneumocystis jirovecii* and *Cryptococcus neoformans* [11].

As a full tool to Histoplasmosis diagnosis, molecular assays can be performed on different types of specimens including respiratory secretions, biopsies, bone marrow, blood, or sera.

For diagnosis of disseminated histoplasmosis (PDH), sensitivity and specificity are 95% and 99%, respectively [2, 9]. Sample of blood and bone marrow have an excellent sensitivity (100%) in immunocompromised patients with disseminated histoplasmosis, whereas it is weaker in immunocompetent patients [2, 11].

Moreover, sensitivity increases by testing more than one sample per patient in cases with extra-pulmonary histoplasmosis. So, in case of high suspicion, clinicians should repeat and diversify samples [2, 9, 11, 34].

Recent findings in molecular biology permit to evaluate genetic diversity using multilocus sequence typing (MLST) OR RAPD-PCR [36]. This could be Interesting to associate genotypes and clinical presentation.

9.2 Loop-mediated isothermal amplification (LAMP)

9.2.1 Principe

Loop-mediated isothermal amplification (LAMP) is a highly efficient, sensitive, specific and cost-effective isothermal amplification method that uses at least four primers, recognizing six different regions in the target sequence (**Figure 4**) and results in a self-primed DNA [37].

In LAMP, the target sequence is amplified at a constant temperature of 60–65°C using either two or three sets of primers and a polymerase with high strand displacement activity in addition to a replication activity. The amount of DNA produced in LAMP is considerably higher than PCR-based amplification.

To increase the reaction sensitivity, the ITS region was used as target, since it is a multicopy sequence and also because it is considered an important barcoding sequence for fungal identification, being conserved among *H. capsulatum* strains and divergent from other fungi, ensuring high specificity.

Forward inner primer (FIP) and backward inner primer (BIP) have inverted sequences attached at the 5′ end, named F1c and B1c, which are complementary to an internal sequence from the amplified strand, forming a loop at each extremity of a single strand DNA.

The outer primers (F3 and B3) anneal upstream to the FIP and BIP, acting as a binding site for DNA polymerase, which, in the LAMP reaction, also contains the strand displacement activity.

LAMP results can be observed using several strategies with minimal ambiguity with real-time turbidimetry (magnesium pyrophosphate formation), fluorescent compounds (Sybr Green, Eva Green, SYTO, calcein), magnesium colorimetric titration, fluorescent-labeled probes, quencher-labeled primers, dye-labeled primers, and PH-sensitive dyes.

9.2.2 Indications

LAMP can be used on samples of whole blood or bone marrow for patients suspected of progressive disseminated histoplasmosis (PDH).

A new assay, the so-called ITS LAMP, showed no cross-reactivity when assayed with DNA from other pathogenic or environmental fungi. The assay is able to detect isolates from all geographical clades of *H. capsulatum*, including Hcd. In comparison with Hcp100 nPCR, it reached sensitivity of 83% and specificity of 92% [35].

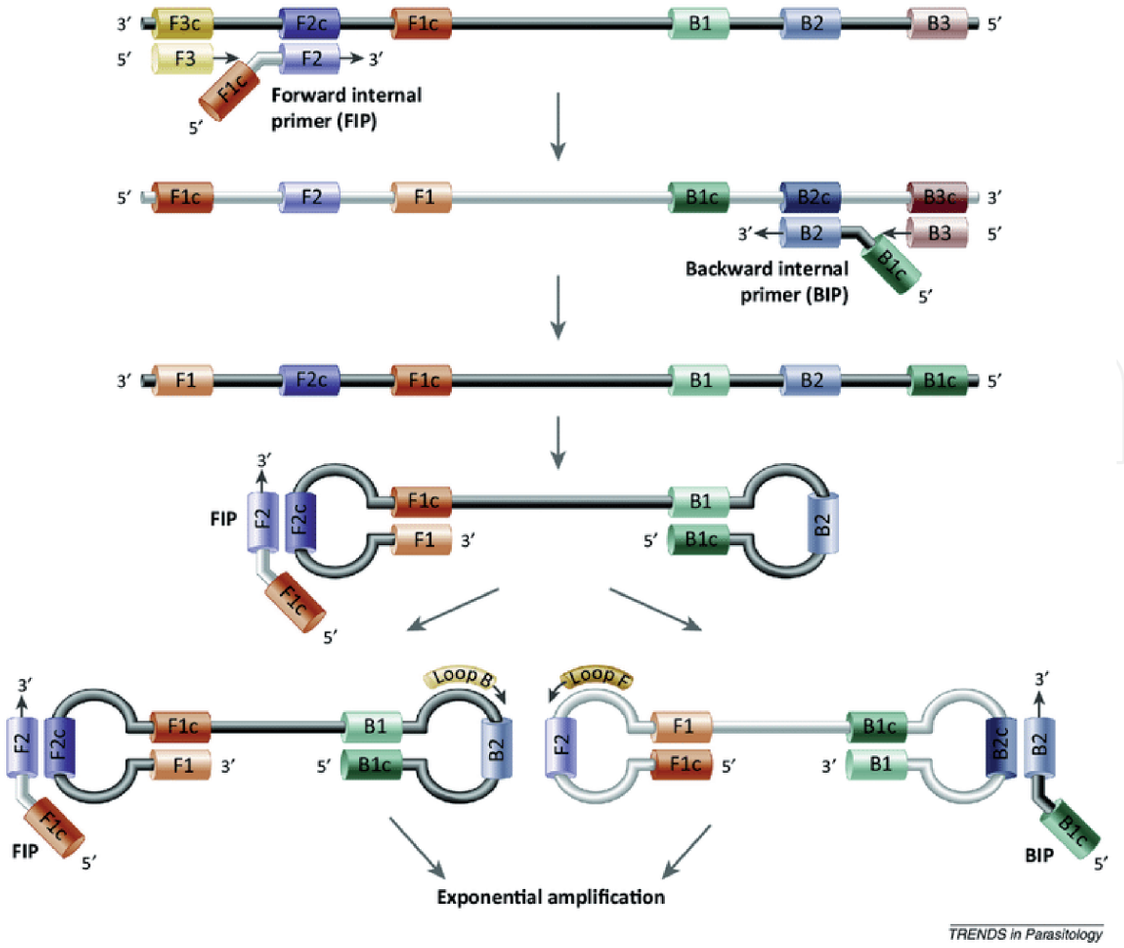

Figure 4.
Principe of LAMP [38].

This method remains cost-effective with or without the extraction DNA step and did not require a thermocycler or an electrophoresis apparatus. It saves time with test performed in less than 200 minutes [35]. However, this little-known and recent method is currently underused and much more reserved for resource-limited laboratories. It should evolve in the next years to come and need further evaluation to be routinely used.

10. Summary of the different categories of tests and their sensitivity (%)

The performance for each test according the clinical context is summarized in **Table 4**. It considers global sensitivity without any distinction between the different protocols (antigen preparation, methods, ...).

It highlights that Ag detection is the best diagnosis tool for PHD and SCN histoplasmosis, serology for the subacute/chronic form, and the culture although this is the reference method remains less sensitive.

11. Conclusion

Histoplasmosis remains a severe and neglected disease for which early diagnosis of invasive fungal infections is critical to allow a prompt patient care. Indeed the mortality rate among HIV/AIDS patients diagnosed with histoplasmosis is high: 42% mortality for disseminated histoplasmosis if treatment is delayed and 100% if antifungal therapy is not prescribed.

	PHD		Acute pulmonary syndrom	Subacute pulmonary syndrom	Chronic pulmonary syndrom	Mediastinal histoplasmosis	CNS histoplasmosis	
	(2)	(9)						
Ag detection	92	95	83	30	88	5	66	
							81	31
Serology	75	58	64	95	83	83	59	
							63	54
Pathology	76	NE	20	42	75	75		
culture	74	77	42	54	67	67	38	
							40	33
Molecular Biology		95						

PDH: Progressive histoplasmosis disseminated, CNS: Central nervous system, NE: Non evaluated.

Table 4.
The different performances (sensibilité %)of the methods are summarized in this table adapted to azar et al. (2), Caceres et.al (9) and Wheat et.al (26).

The gold standard for histoplasma diagnosis remains culture. It's a time-consuming process and has limitations in sensitivity. Moreover, it requires invasive procedure and mycological expertise.

Nonculture methods have been developed to improve and accelerate diagnosis of histoplasmosis, such as histoplasma antigen detection, antibody detection, and molecular biology. It should have conjunction between the different tools of diagnosis to be reliable in the histoplasmosis management regarding the wide range of clinical features.

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