Current Fungal Infection Reports (2020) 14:310–316
https://doi.org/10.1007/s12281-020-00393-4

CLINICAL MYCOLOGY LAB ISSUES (S CORDOBA, SECTION EDITOR)

Innovative Approaches for *Histoplasma* Detection

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Published online: 6 June 2020
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Abstract

Purpose of Review This review highlights innovative approaches for histoplasmosis detection using laboratory technologies published in the past 6 years.

Recent Findings Microscopy and culture are the gold standards for confirming the diagnosis of histoplasmosis, but these can be slow, and their sensitivity and specificity can vary. It is well known that rapid diagnosis is necessary to save lives. Newer technologies based on detection of antibodies, antigens, and DNA can enable rapid and accurate clinical diagnosis.

Summary Based on a systematic review of the literature and evaluation of methodologies in five areas, assay accuracy, rapid testing, point of care testing, commercial availability, and use in animal diagnosis and environmental detection, we describe innovative approaches for detection of *Histoplasma*/histoplasmosis.

Keywords Histoplasmosis · Laboratory diagnosis · *Histoplasma capsulatum* · Antigen · Antibody · DNA · Molecular testing

Introduction

In routine laboratory practice, histoplasmosis is detected using conventional diagnostic methods, such as microscopy and culture, which remain the gold standards. While these methods are well established, they have disadvantages including potentially slow turnaround time, variable analytical performance, and the requirement of specialized laboratory training and infrastructure for the biological containment of the etiologic agent, *Histoplasma capsulatum* [1, 2]. *H. capsulatum* is found in specific environments and restricted geographic locales and can cause disease in non-human mammals, including domestics animals like dogs and cats. For detection in animals or from the environment, the performance of conventional diagnostic methods is poor; *Histoplasma* is rarely grown from environmental specimens, and veterinarians rarely have the laboratory infrastructure necessary to culture specimens from their patients. Because of these limits, the availability of tools to detect animal cases or environmental contamination could lead to a better understanding of histoplasmosis epidemiology [3–5].

Accurate and rapid diagnosis of histoplasmosis has a direct impact on the quality of patient care. It is well know that the use of rapid diagnostic assays has an impact on several aspects of care, treatment, and epidemiology, such as increasing the number of histoplasmosis cases detected (up to 3 times compared with conventional assays), reduction in patient mortality (50% reduction of mortality compared with conventional assays), and identification of new "endemic" regions for histoplasmosis [6–9]. Given the need for improved diagnostics, many efforts are now focused on developing faster and more accurate diagnostic technologies. "Innovative approaches" to *Histoplasma* detection include assays based principally on non-culture technologies such as assays for the detection of antibodies (Ab), antigens (Ag), and deoxyribonucleic acid (DNA). Here, we conducted a systematic review of the literature to identify, evaluate, and describe several laboratory

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This article is part of the Topical Collection on *Clinical Mycology Lab Issues*

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12281-020-00393-4) contains supplementary material, which is available to authorized users.

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methods with high accuracy and short turnaround time that can fill this important diagnostic gap.

Materials and Methods

Systematic Review of Literature

We searched the following databases: Medline, Embase, CAB Abstracts, Global Health, Scopus, the Cochrane Library, PubMed Central, and LILACS. In order to increase the search accuracy, we added a diagnostic methodology search filter that was adapted from the recommendations of the McMaster Health Information Research Unit [10]. This search identified literature published between January 1, 2014, and November 5, 2019, and included the terms "histoplasmosis, histoplasma, sensitivity, diagnostic, and predictive value." In addition, we included articles published in English, Spanish, and Portuguese (Supplemental material 1).

We searched for studies that described the development, standardization, or validation of clinical laboratory assays for detection and/or identification of *Histoplasma* spp. We excluded review articles, editorials, theses, case reports, epidemiological and clinical studies, basic science research, and abstracts or summaries presented at conferences. Studies selected were subjected to a second filter, with the aim of excluding studies related to the development, standardization, or validation of conventional diagnostic methods (like microscopy and culture), and reagents for immunodiffusion and complement fixation. We did not include conventional PCR assays (including Nested PCR) because these assays are not new, but are complex, and often require gel electrophoresis for interpretation. We also excluded studies that did not present a clear methodology. This second filter was designed to focus on the evaluation of innovative laboratory technologies. For evaluation of analytical performance, we analyzed assay accuracy, because this value represents the percentage of cases correctly classified and is a good balance between sensitivity and specify. The studies were evaluated independently by two reviewers using a standard evaluation instrument to show how well they fit the classification criteria (Supplementary material 2).

Results

Systematic Literature Review

The search strategy identified 1135 references. After performing manual review of article titles and abstracts, 1102 references were excluded for meeting one or more exclusion criteria described above (Fig. 1). Thirty-three manuscripts described the development, standardization, or validation of assays for the detection of *Histoplasma* spp. and therefore met the criteria for inclusion. When the second filter was applied, ten additional manuscripts were excluded, leaving 23 publications for full review. These studies were organized into the following subgroups: [1] immunodiagnostics, [2] molecular diagnostics, [3] veterinary and environmental use, and [4] next generation assays (Fig. 1 and Table 1).

Immunodiagnostic

A total of 12 studies evaluated immunodiagnostic assays. Of those, six reported the use of Ag detection assays, three reported the use of Ab detection assays, two evaluated the combination of Ag and Ab detection assays, and one evaluated an Interferon-Gamma Release Assay (IGRAs). In the studies that detected *Histoplasma* Ag, one study described the validation of a lateral flow assay (LFA), the only study of the 23 publications that evaluated a point of care (POC) assay. This POC assay detected Ag in human serum and showed high analytical performance (greater than 90% accuracy) and rapid delivery of results (~30 min) [13••]. The other five papers that also described Ag detection assays all included using urine as a specimen and the ability to provide results in less than 1 day (enzyme immunoassay (EIA)). Of the EIAs evaluated, two manuscripts described a polyclonal Ab (PoAb) sandwich EIA developed as a Clinical Laboratory Improvement Amendment-approved (CLIA) test by a reference laboratory in the USA and compared it with two commercially available kits. The others described a monoclonal Ab (MoAb) sandwich EIA, and a PoAb sandwich EIA. Better analytical performance was reported using the CLIA-approved PoAb EIA and the commercial MoAb EIA (both reported more that 90% sensitivity and specificity) [14–17, 20].

For Ab detection, Almeida et al. published two articles that discussed the validation of a western blot (WB) assay for the detection of specific antibodies against *H. capsulatum* [21•, 22]. Both studies reported high accuracy, greater than 90%, for the diagnosis of multiple clinical forms of histoplasmosis, including the clinical form associated with HIV. The principal limitations of these two studies were the lack of commercial availability and the need for complex laboratory infrastructure for the performance of the assays. Deppen et al., described a CLIA-approved EIA for the detection of Ab against *H. capsulatum* in human sera. The authors used this Ab detection EIA for the evaluation of suspicious lung nodules, concluding that a positive EIA result for IgM and IgG strongly suggested histoplasmosis. This assay is not commercially available as a kit and is offered only in a reference laboratory in the USA, a condition which significantly affects the access to this test [27].

Richer et al. and Bloch et al. evaluated how the combination of Ag and Ab detection impacts diagnostic analytical performance in detecting pulmonary and central nervous system (CNS) histoplasmosis [18, 19]. These studies evaluated two

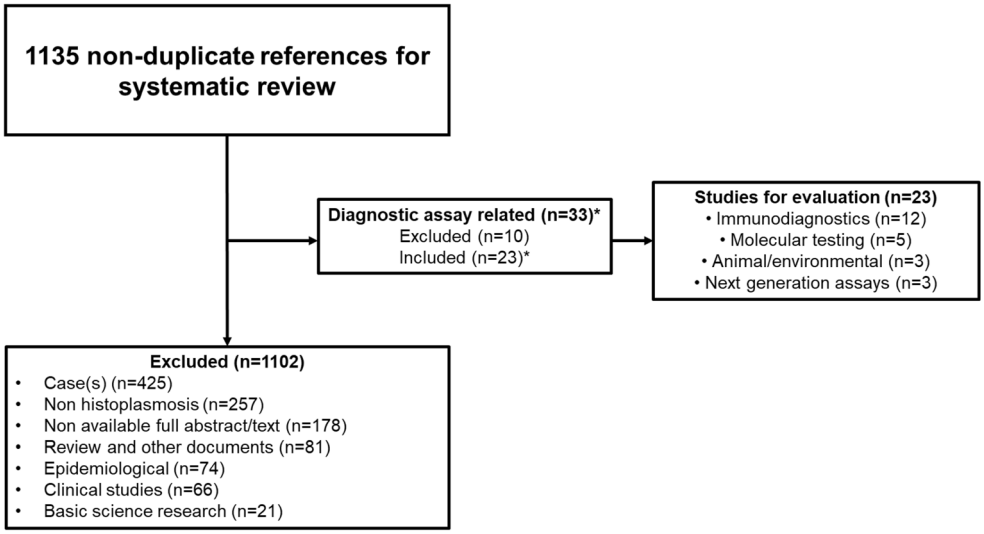

Fig. 1 Flow chart of the literature search and studies selection

CLIA-approved EIA assays for Ag and Ab detection offered in a reference laboratory in the USA. These two studies concluded that the combination of Ag and Ab detection increased the capacity to diagnose pulmonary and CNS histoplasmosis to nearly 100% sensitivity and specificity. Because these assays are EIAs, the results could be obtained in less than 1 day.

Finally, Rubio et al. developed IGRAs for histoplasmosis to detect individuals with latent histoplasmosis. Preliminary results were promising, but future validation is necessary to determine the analytical performance of this assay. Currently, IGRAs present several disadvantages and limitations, including the fact that blood samples had to be processed within 8–30 h after collection (viability of white blood cells), and require complex laboratory capacity and personal experience, with an overall high cost for the assay [31].

Molecular Diagnostics

Five papers described molecular detection assays. These reports evaluated three real-time PCRs (RT-PCR), two loop-mediated isothermal amplification (LAMP) assays, and one fluorescent in situ hybridization assay (FISH). For the RT-PCR assays, the study published by Gago et al. stands out [24••]. This study described the development of a multiplex RT-PCR for the diagnosis of pneumonia caused by *H. capsulatum*, *Pneumocystis jirovecii*, and *Cryptococcus neoformans/gattii* in bronchoalveolar lavage (BAL) samples. This multiplex assay could have high applicability in the care of people with advanced HIV, and would also be useful for the care of other immunocompromised patients [24••]. The multiplex nature of this assay might also be very useful for diagnosis of histoplasmosis in areas where *Histoplasma* is not thought to be endemic, especially in patients with travel. Muraosa et al. described the development of two RT-PCR methods and although promising on a limited basis, the accuracy of these two RT-PCR assays was low (< 70%) [26]. The limitations of these methodologies include the fact that they require complex laboratory infrastructure and trained personnel and that they are not available commercially and so would they have to be independently validated [26].

Two papers described the development of assays based on LAMP. It is important to highlight that LAMP assays have many advantages, including the ability to rapidly and economically detect *Histoplasma* DNA without complex laboratory infrastructure. The assay developed by Zatti et al. evaluated for both bone marrow and blood samples, reported an accuracy greater than 80% [25, 29].

Silva et al. described the development of a fluorescently labeled rRNA-targeting FISH for the diagnosis of histoplasmosis in positive blood cultures. Blood culture from HIV positive patients with invasive fungal infection and blood cultures spiked with *H. capsulatum* were tested. The FISH assay was able to identify all blood cultures with *H. capsulatum* ($n = 7$). The principal limitation was the low number of samples tested, and the fact that it can only be used on positive blood culture, which can take up to a few weeks for *Histoplasma* [33]. It would be a major advantage in endemic regions if the same FISH assay could be used on bronchoalveolar lavage fluid.

One of the principal limitations of the molecular diagnostics presented here are that the specimens used for validation were obtained by invasive procedures. Validation of non-invasive specimens like urine or sputum would make them more useful, but even then, the lack of availability of commercial kits will keep them from becoming more widespread.

Veterinary and Environmental Assays

Three studies were identified in this category, two studies evaluated *Histoplasma* Ag detection assays using animal

Table 1 Innovative approaches to *Histoplasma*/histoplasmosis detection

Category*	Authors (year of publication)	Ref.	Assay description	Paper's evaluation categories (scores)					Assay/ paper final score
				Assay accuracy	Rapid test	POC	Commercially available	Double use¥	
3	Rothenburg, L et al. (2019)	[11•]	MoAb EIA for *Histoplasma* Ag detection in cat's urine	0.8	1	0	1	1	**3.8**
			PoAb EIA for *Histoplasma* Ag detection in cat's urine	1	1	0	0.5	1	**3.5**
3	Cunningham, L et al. (2015)	[12•]	PoAb EIA for *Histoplasma* Ag detection in dog's urine)	1	1	0	0.5	1	**3.5**
1	Caceres, DH et al. (2019)	[13••]	PoAb Ag LFA for *Histoplasma* Ag detection in human serum	1	1	1	0	0	**3**
1	Caceres, DH et al. (2018)	[14]	MoAb Ag EIA for *Histoplasma* Ag detection in human urine	1	1	0	1	0	**3**
1	Hoffmann, ER et al. (2017)	[15]	PoAb Ag EIA for *Histoplasma* Ag detection in human urine	1	1	0	1	0	**3**
1	Torres, P et al. (2018)	[16]	PoAb Ag EIA for *Histoplasma* Ag detection in human urine	0.8	1	0	1	0	**2.8**
1	Zhang, C et al. (2015)	[17]	MoAb Ag EIA for *Histoplasma* Ag detection in human urine	0.8	1	0	1	0	**2.8**
			PoAb Ag EIA for *Histoplasma* Ag detection in human urine	1	1	0	0.5	0	**2.5**
			PoAb Ag EIA for *Histoplasma* Ag detection in human urine	0.2	1	0	1	0	**2.2**
1	Richer, M. L. et al. (2016)	[18]	EIAs for *Histoplasma* Ag and Ab detection in human samples	1	1	0	0.5	0	**2.5**
1	Bloch, T et al. (2018)	[19]	EIAs for *Histoplasma* Ag and Ab detection in human CSF	1	1	0	0.5	0	**2.5**
1	Theel, S et al. (2015)	[20]	Evaluation of a MoAb EIA for detection of *Histoplasma* Ag	0	1	0	1	0	**2**
1	Almeida, MA et al. (2019)	[21•]	WB for *Histoplasma* Ab detection in PLHIV	1	1	0	0	0	**2**
1	Almeida MA et al. (2016)	[22]	WB for *Histoplasma* Ab detection in histoplasmosis	1	1	0	0	0	**2**
3	Gomez, L et al. (2019)	[23••]	PCR for detection of *Histoplasma* DNA in organic fertilizers	0	1	0	0	1	**2**
2	Gago, S et al. (2014)	[24••]	Multiplex PCR	1	1	0	0	0	**2**
2	Zatti, M et al. (2019)	[25]	LAMP	0.8	1	0	0	0	**1.8**
2	Muraosa, Y et al. (2016)	[26]	Nested real-time PCR	0.6	1	0	0	0	**1.6**
			Cycling probe-based real-time PCR	0	1	0	0	0	**1**
1	Deppen S et al. (2019)	[27]	EIA for detection of *Histoplasma* Ab in human samples	0	1	0	0.5	0	**1.5**
4	McTaggart, L et al. (2019)	[28]	NGS (mycobiome sequencing)	1	0	0	0.5	0	**1.5**
2	Scheel, C M et al. (2014)	[29]	LAMP	0.4	1	0	0	0	**1.4**
4	Valero, C et al. (2018)	[30]	MALDI TOF	0.8	0	0	0.5	0	**1.3**
1	Rubio, M et al. (2019)	[31]	IGRAs	0.6	0	0	0	0	**0.6**
4	Frickmann, H et al. (2019)	[32]	NGS	0	0	0	0.5	0	**0.5**
2	da Silva Jr. R et al. (2015)	[33]	In situ hybridization in pre-incubated blood culture	0	0	0	0	0	**0**

(*) Category; *1*, immunodiagnostics; *2*, molecular; *3*, veterinary and environmental use; *4*, new generation assays. Parameters for paper scores are summarized in supplementary material 2

Ref., reference; *POC*, point of care; ¥, animal and environmental detection; *MoAb*, monoclonal antibody; *PoAb*, polyclonal antibody; *Ag*, antigen; *Ab*, antibody; *EIA*, enzyme Immunoassays; *LFA*, lateral flow assay; *BAL*, bronchoalveolar lavage; *CSF*, cerebrospinal fluid; *PLHIV*, people living with HIV; *WB*, western blot; *PCR*, polymerase chain reaction; *DNA*, deoxyribonucleic acid; *LAMP*, loop-mediated isothermal amplification; *NGS*, next generation sequencing; *MALDI TOF*, matrix assisted laser desorption/ionization time of flight; *IGRAs*, interferon-gamma release assays

specimens, and one study described a method for detection of *Histoplasma capsulatum* DNA in fertilizers. Two manuscripts validated Ag detection assays in cats and dogs. One study compared two assays for the diagnosis of feline histoplasmosis, one commercial MoAb sandwich EIA, and one EIA offered by a reference laboratory in the USA. This study tested 35 cats with histoplasmosis and 59 cats without histoplasmosis [11•]. The second study evaluated the same EIA offered by a reference laboratory in the USA described in the study above, using 58 samples from dogs (including 17 canine histoplasmosis cases) [12•]. Both studies concluded that Ag testing presented high accuracy (greater than 85%) and rapid turnaround time for results even though the specimens were from animals.

The third study in this category described the development of a PCR assay for the detection of *H. capsulatum* DNA in organic fertilizer, which is important to consider because fertilizer is often made with bird or bat guano which can be contaminated with *Histoplasma*. This was the only study that showed the standardization of a laboratory assay able to detect *H. capsulatum* in the environment. This type of technology has the potential for widespread application in epidemiological studies and outbreak investigations [23••]. Because this study was a description and not a validation, the accuracy could not be determined.

Next Generation Assays

We identified three studies that employed newer technology, two using Next Generation Sequencing (NGS) and one using MALDI TOF. McTaggart et al. described the development of a next-generation sequencing (NGS) assay for the analysis of the fungal microbiome (mycobioma) in respiratory specimens. In this study, the pipeline used for the sequencing of the Internal transcribed spacer 1 (ITS1) was able to detect 96% of species that could be potential fungal lung pathogens. In this study, a total of six culture-proven histoplasmosis specimens were tested, and five of the six (83%) were positive by this assay. Frickmann et al. evaluated the use of NGS for detection of rare invasive infections in potentially polymicrobially contaminated, formalin-fixed, paraffin-embedded (FFPE) tissues, including five tissue blocks histologically diagnosed with histoplasmosis. In this study, the authors had several conclusions as follows: (1) the major disadvantage of pathogen nonspecific NGS was the low sensitivity compared with specific PCR, (2) the high susceptibility of contamination in FFPE tissue samples, and (3) the need for further standardization of the methodology using a larger set of specimens to increase the power of discrimination of the assay. These new reports (both published in 2019) expand the available knowledge of histoplasmosis and other fungal disease diagnostics, and future efforts will certainly be directed toward optimizing this type of technology [28, 32].

Valero et al. developed a MALDI-TOF reference database for the identification of *H. capsulatum*. The authors tested a total of 63 fungal isolates, including 30 *H. capsulatum* isolates (tested in mycelia and yeast phase). All the isolates were correctly identified, but better scores were obtained using the mycelial rather than the yeast phase of *H. capsulatum*. While identification could be performed rapidly and with great accuracy, this study's limitation was the need for a fungal isolate, similar to conventional diagnostics [30].

Conclusion

We identified and evaluated 23 recently published studies that described culture-independent (with the exception of the FISH and MALDI-TOF) assays for the detection and identification of *Histoplasma*/histoplasmosis. The results of this review show that even though this disease has been present for a long time, there are still ongoing advances in technologies used to detect histoplasmosis. Newer technologies can detect and quantify the pathogen in less time, with greater accuracy and, in the cases of LAMP and LFA, without the need of complex technology. However, the availability and integration of these technologies is influenced by the clinical suspicion of histoplasmosis and access to these diagnostic assays. Additionally, we observed that many of these methodologies utilized multiple types of biological specimens, which is a significant advance, as this could increase the possibility of diagnosing the disease, especially in atypical clinical forms such as central nervous system histoplasmosis.

Antigen detection assays have been extensively studied and improved since their first report back in late 1980s [34]. This type of assay includes several advantages such as a short turnaround time (e.g., a new point of care assay) and commercial availability. Antigen detection assays were included in the World Health Organization's (WHO) most recent Second Model List of Essential In Vitro Diagnostics [35]. These are also the only type of assay evaluated in non-human mammals for histoplasmosis diagnosis.

Beyond immunodiffusion (ID) and complement fixation (CF) assays for Ab detection, additional novel technologies have now also been proven to be able to provide accurate and rapid results for Ab testing, reducing the turnaround time from days to hours. Combining Ag and Ab testing has also been shown to significantly increase the detection of histoplasmosis, especially, in low-frequency clinical forms like CNS histoplasmosis. We also observed that more molecular testing is starting to be conducted in the form of simple, multiplex assays (including for environmental use), and portable technology. This new approach to molecular assays could impact future testing in more laboratories around the world if cost and technological requirements can be decreased. We expect that soon, next generation technologies like NGS, MALDI-

TOF, and IGRAs will be developed and integrated into histoplasmosis diagnostics options.

In summary, an ideal diagnostic assay needs to meet some criteria, including the use of non-invasive biological specimens, high accuracy and reproducibility, rapid turnaround time, and portable and simple assay performance. These types of technology should be accessible, low cost, and with continuous production and distribution in developing countries where histoplasmosis is frequently diagnosed. Some of the assays presented here may eventually meet that challenge.

Acknowledgments We want to thank Mark Mezzullo and Pooja Gandhi for editing the manuscript, Karlyn D. Beer for manuscript review, and Martha Knuth for developing the systematic review of the literature.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Compliance with Ethical Standards

Conflict of Interest Diego Caceres, Norma Fernandez, and Shawn Lockhart declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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