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Antigen Detection in the Diagnosis of Histoplasmosis: A Meta-analysis of Diagnostic Performance

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Abstract We performed a meta-analysis of diagnostic data to evaluate the performance of *Histoplasma* antigen detection tests for diagnosing histoplasmosis. We included all studies involving human subjects that assessed the performance of any antigen detection test for histoplasmosis in urine or serum by carrying out an exhaustive and reproducible search of the literature between 1980 and 2014 from four databases. Quality of the articles was assessed, and meta-analysis was performed under the random effects model, calculating sensitivity, specificity, likelihood and odds ratios, and ROC curve using *Meta-DiSc*(*es*). Nine out of a total of 23 studies met strict quality criteria and were therefore included. The overall sensitivity for antigen detection in serum and urine was 81 % (95 % CI 78–83 %), while specificity was 99 % (95 % CI 98–99 %). Sensitivity for antigenuria and antigenemia was 79 % (95 % CI 76–82 %) and 82 % (95 % CI 79–85 %), respectively; specificity values were 99 % (95 % CI 98–100 %) in urine and 97 % (95 % CI 96–98 %) in serum. The positive and negative likelihood ratios were 49.5 (95 % CI 20.7–118.7) and 0.19 (95 % CI 0.14–0.26), respectively, while the diagnostic OR was 362 (95 % CI 121.2–1080.3) and area under the curve was 0.99. In conclusion, the performance of *Histoplasma* antigen detection assay of urine was not significantly different from that of blood, indicating that antigenuria and antigenemia have equal diagnostic value in histoplasmosis.

Keywords Histoplasmosis · Antigen · Diagnosis · Sensitivity · Specificity

Introduction

A marked increase in invasive fungal infections has been observed during the last decades, including histoplasmosis, a systemic mycosis caused by the dimorphic agent *Histoplasma capsulatum* [1, 2]. This fungus is widely distributed around the world, and exposure to this fungus is common in those people living in endemic areas [1–7]. Lungs are the target organ, from where the fungus can disseminate through the blood to other organs and systems. In 95 % of the cases, the infection is resolved spontaneously. Infected patients who develop symptomatic disease are generally individuals who have inhaled a heavy fungal

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burden or present predisposing factors including advanced age and structural or immunological alterations of the lungs [8].

The clinical presentations of the disease include acute pulmonary, chronic disseminated, acute disseminated and granulomatous mediastinitis or fibrosis, among others, the acute pulmonary histoplasmosis and disseminated forms being the most frequent (50 % of cases) [6]. Moreover, it is important to note that the mycosis behaves as an opportunistic infection in HIV-infected individuals with T CD4+ counts of less that 150 cells/μL [2, 3, 8, 9]. In most cases, this infection defines AIDS [9]. Prevalence of this mycosis ranges from 2.1 to 20 % in Latin America [4].

Diagnosis of histoplasmosis involves a battery of laboratory tests, including: (a) *Direct microscopic examination,* this has a variable sensitivity of 9–43 % [3, 4]; (b) *Culture*, which may take 4–6 weeks to obtain adequate fungal growth and has a sensitivity of 15–85 % [4]; (c) *Immunodiagnostic tests* that may involve both antibody and antigen detection, and (d) *Molecular assays* which allow detection of nucleic acids and have high values for sensitivity and specificity [3].

Antibodies are detected in 71–77 % of cases of disseminated histoplasmosis, rising to 90 % in patients with the chronic form; this variability is due to two factors: (a) the majority of patients with disseminated histoplasmosis present immunodeficiency, and (b) sensitivity and specificity are affected by cross-reactions with other fungal infections [5].

Antigen detection has been found to be useful not only for diagnosing the mycosis but also for monitoring the effectiveness of treatment. The first methods to detect antigens using enzymatic immunoassays were described in 1986 [10, 11]. This technique was subsequently adapted to use urine and sera in 1997 [11]. The advantage of this assay is that antigens can be detected much more rapidly than using fungal growth in culture and even before specific antibodies appear in the patients [11, 12]. However, few commercial assays are available to detect antigens of *Histoplasma*, and both commercial and homemade tests show wide variability in sensitivity and specificity values. This variability could be due to the type of technique, clinical sample or antibodies used and the number of individuals or samples analyzed, among other reasons. Given these factors, a through review of the different studies needs to be conducted, since most research only evaluates diagnostic validity in terms of sensitivity and specificity of the method, without exploring other parameters that could help in making clinical decisions. It is important to note that other criteria such as performance, efficiency and safety are important in the evaluation of diagnostic tests; these can be analyzed by means of predictive values, the proportions of correctly and incorrectly diagnosed patients, as well as likelihood and diagnostic odds ratios and ROC curves [13–15]. Thus, in order to determine the value of the antigen detection assay for diagnosing histoplasmosis and to evaluate different parameters using a methodology with clear criteria for the selection and collection of information, reducing selection and extraction bias and increasing the accuracy, power and external validity from the existent literature, we aimed to perform a meta-analysis to evaluate the diagnostic performance of the *Histoplasma* antigen detection tests.

Materials and Methods

Study Type

Systematic review of the literature with meta-analysis.

Identification, Screening, Selection and Inclusion of Studies

In order to ensure completeness of the review, a search for sensitivity of original research articles published in *PubMed, Scopus, Lilacs* and *Scielo* databases was performed. A search for sensitivity without limiting this to Health Sciences Descriptors (DecS) or headers of the Medical Subject Headings (MeSH) allowed a large number of studies to be obtained that were not selected when specificity was the criterion used.

The following terms were used: *histoplasmosis antigen detection, antigenuria* and *antigenemia*.

The following inclusion criteria were applied:

1. articles with the search terms in title and abstracts
2. description of sensitivity and specificity values for the assays
3. urine and serum as the sample type analyzed
4. original articles
5. human studies and
6. full-text articles.

Articles that met the following criteria were excluded:

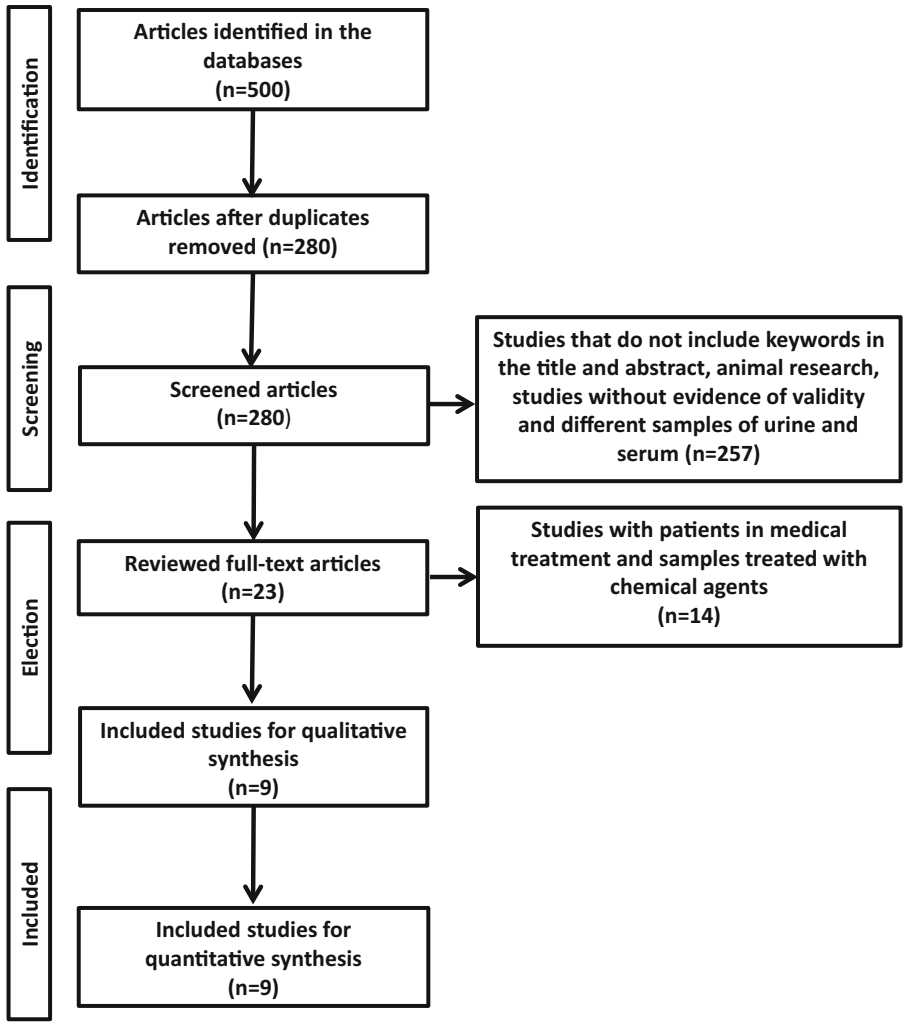

Fig. 1 Algorithm selection of articles

1. studies in patients undergoing medical treatment,
2. clinical samples treated with chemical agents
3. publications with problems of internal validity due to the statistical analysis employed and poor control of selection bias for the data collected.

Criteria set out in the *Quality Assessment of Diagnostic Accuracy Studies* (QUADAS) guide were used to determine the quality of the eligible studies. This involved selecting those that adhered to at least 10 of the 14 items of the guide [16].

Collecting Information

Information was collected independently by two researchers to ensure reproducibility of the search and selection of articles. Discrepancies were resolved by consensus and referral to a third reviewer.

Data Analysis

Sensitivity, specificity, positive and negative likelihoods (PLR and NLR, respectively) and odds ratios (OR) were estimated. The following factors were considered for analysis of these parameters:

1. excellent diagnosis: NLR < 0.1 and PLR > 10
2. good diagnostic aid: NLR 0.1–0.2 and PLR 5–10
3. low clinical utility: NLR 0.21–0.5 and PRL 2–4.9 and
4. assay not useful: NLR 0.51–1 and PRL $<$ to 2.

ROC curves were performed to calculate the area under the curve (AUC) using the *Moses* constant and weighted least squares models. Values greater than 0.89 were considered to be satisfactory and those above 0.96 to be excellent. The diagnostic OR was considered to be of poor diagnostic usefulness when averages were close to 1.0 and excellent to discriminate healthy from infected individuals when values exceeded 100.

All the diagnostic parameters reported in each individual study and also as part of combined or global studies are presented in forest plot, together with their respective confidence intervals.

The information extracted from each study was stored and analyzed using the *Meta-analysis of studies of evaluations of Diagnostic and Screening tests Meta-DiSc*(*es*) software, with a significance level of 0.05. This software generates the combined measures by applying the *Q* (Chi-squared) statistical test from DerSimonian–Laird (REM) under the random effects model.

Results

In all 500 studies were identified, of which nine that met the criteria were analyzed (Fig. 1). Seven of the nine studies performed antigen detection in urine [18–20, 22, 23, 27, 28] and six in serum [17–20, 24, 28], and four evaluated both type of samples [18–20, 28]. The individuals included in such studies were patients with pulmonary and disseminated histoplasmosis ($n = 1029$). It is noteworthy that the patients with the disseminated form of the mycosis all had HIV/AIDS, whereas the controls included healthy individuals ($n = 647$), patients with other mycoses ($n = 1309$) and those with other infections ($n = 239$) (Tables 1, 2).

The overall sensitivity was 81.4 % (95 % CI 79.1–83.5 %), ranging from 56 [17] to 100 % [19]; the specificity was 98.3 % (95 % CI 97.7–98.7 %) and varied between 91 % [24] and 100 % [20, 23, 28] (Fig. 2). When the analysis was performed according to the type of sample, no significant differences were found in the validity, since sensitivity was 79.5 % (95 % CI 76.3–82.4 %) for the antigenuria and 83.9 % (95 % CI 80.5–87.0 %) for the antigenemia, whereas the specificity values were 98.7 % (95 % CI 98.0–99.1 %) in urine and 97.5 % (95 % CI 96.3–98.4 %) in serum (Fig. 2). The PLR was 43.2 (95 % CI 19.5–95.9 %), ranging from 8.2 [24] to 507.6 [28]. In these studies, the percentage weight per study ranged from 4.5 to 9.3 % confirming the robustness of

Table 1 Description of studies involving *Histoplasma* antigenemia assays

Author	Country	Healthy individuals or patients with other infections as a control (*n*)	Patients with different clinical forms of histoplasmosis (*n*)	Assay
Hage et al. [18]	USA	Healthy (69)	Disseminated (30)	ELISA
		Other fungal infections (130)	Acute (6)	
			Subacute (41)	
			Pulmonary chronic (6)	
Gómez et al. [24]	UK	Healthy (44)	Disseminated (35)	ELISA
		Other fungal infections (39)		
		Tuberculosis (9)		
Guimarães et al. [17]	Brazil	Healthy (87)	Disseminated (100)	ELISA
		Other fungal infections (35)		
Connolly et al. [19]	USA	Healthy (25)	Disseminated (58)	ELISA
		Other fungal infections (75)		
Wheat et al. [28]	USA	Healthy (295)	Disseminated (89)	ELISA
			Acute (32)	
			Subacute (65)	
			Pulmonary chronic (31)	
Wheat et al. [20]	USA	AIDS (30)	Disseminated (47)	RIA

ELISA enzyme-linked immunosorbent assay, *RIA* radio-immune assay, *AIDS* acquired immunodeficiency syndrome

Table 2 Description of studies for *Histoplasma* antigenuria assays

Author	Country	Healthy individuals or patients with other infections as a control (*n*)	Patients with different clinical forms of histoplasmosis (*n*)	Assay
Hage et al. [18]	USA	Healthy (69)	Acute (6)	ELISA
		Other fungal infections (130)	Pulmonary chronic (8)	
			Disseminated (158)	
			Subacute (46)	
Scheel et al. [22]	USA	Healthy (83)	Disseminated (48)	ELISA
		Other infections (114)		
Connolly et al. [19]	USA	Healthy (25)	Disseminated (72)	ELISA
		Other infections (75)		
Theel et al. [23]	USA	Other fungal infections (941)	Disseminated (62)	ELISA
Wheat et al. [28]	USA	Healthy (295)	Disseminated n (89)	ELISA
			Acute (32)	
			Subacute (65)	
			Pulmonary chronic (31)	
Wheat et al. [20]	USA	AIDS (30)	Disseminated (61)	ELISA
Cáceres et al. [27]	Colombia	Healthy (44)	Disseminated (28)	ELISA
		Other fungal infections (89)		
		Other infections (41)		

ELISA enzyme-linked immunosorbent assay, *RIA* radio-immune assay, *AIDS* acquired immunodeficiency syndrome

the sensitivity analysis to the extent that any study had greater influence on the overall results found. Moreover, the NLR was 0.18 (95 % CI 0.13–0.25) with the lower value reported by Connolly et al., [19] with a 0.01 value and the higher reported by Guimarães et al. [17] with a value of 0.47; the combination of both coefficients was indicative of an excellent capacity to discriminate between healthy and ill individuals for the diagnostic test employed. The above results are supported by the diagnostic OR and AUC (ROC) values of 321 (95 % CI 118–875) and 0.98, respectively (Fig. 3). It is important to note that the previous results showed differences in the absolute measures found when urine and serum were tested, although these were not statistically significant; the PLR for antigenuria was 58.7 (95 % CI 17.4–198.1), NLR 0.20 (95 % CI 0.14–0.30), OR 415.8 (95 % CI 126.0–1372.9) and AUC 0.99, while antigenemia showed a PLR value of 33.7 (95 % CI 10.2–110.9), NLR of 0.16 (95 % CI 0.09–0.31), OR 239.5 (95 % CI 46.7–1226.7) and AUC of 0.94.

A similar pattern was found for the analysis of the different subgroups according to the type of patients studied, in which no statistical difference for the diagnostic evaluation criteria was found. In addition, when estimates were performed of the combined measures obtained by eliminating each of the studies in successive stages, no statistically significant changes were recorded in the conclusion or performance of the overall measure. These results demonstrated the relevance of showing a combined measure for each of the parameters evaluated without differentiating the type of sample or patient (Table 3).

Discussion

This study is the first meta-analysis to determine the performance of laboratory tests based on the detection of antigens for the diagnosis of histoplasmosis. Our findings strongly indicate that detection of *Histoplasma* antigen in urine (antigenuria) and in serum (antigenemia) has equal diagnostic value in histoplasmosis. Herein it was shown that when evaluating antigen detection assays employing serum and urine for the diagnosis of histoplasmosis no significant differences were found between the samples used with respect to validity. The likelihood ratios obtained showed the test possessed an excellent capability to discriminate between healthy and ill individuals

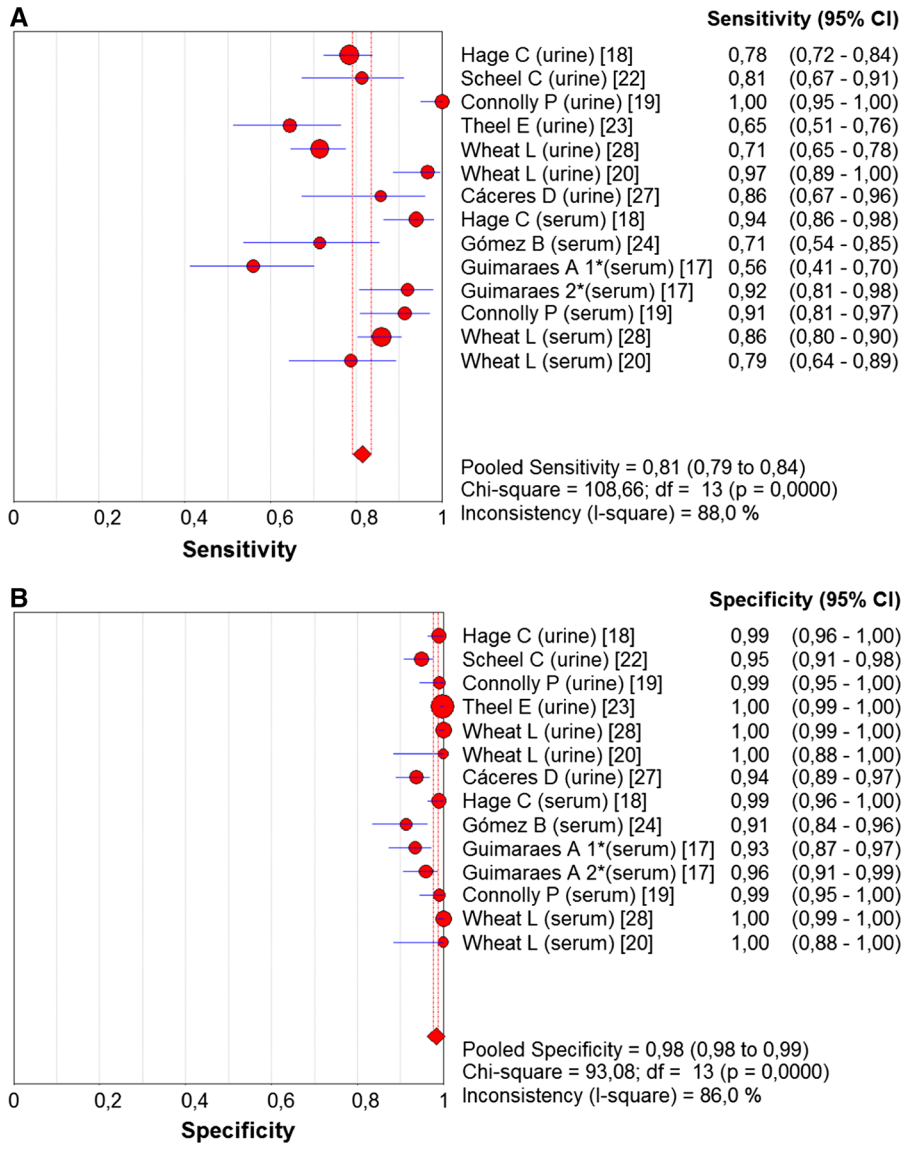

Fig. 2 **a** Sensitivity and **b** specificity of antigenuria and antigenemia tests for the diagnosis of histoplasmosis. *Study with values from two different laboratories (one in Sao Paulo and the other in Rio de Janeiro)

independently of whether analyses were carried out using urine or serum [14].

The overall sensitivity of the test was 81.4 %, indicating that most patients with the infection can be diagnosed and favoring the establishment of a model involving early treatment, evolution, follow-up of the disease and epidemiological tracking in vulnerable populations. However, it is important to note that a significant number (18.6 %) of patients could not be diagnosed correctly by this assay, a fact that could be due to inherent factors of histoplasmosis such as fungal burden when performing the test, the clinical form of the disease involved or underlying comorbidities in these patients. Other important variables to be considered include the type of antibody (monoclonal or polyclonal), the technique employed (e.g., conventional, inhibition or sandwich ELISA, RIA), and other factors of the pre-analytical phase including sample collection, storage and processing of clinical samples, among others [22]. Most studies reported and analyzed in the present study employed polyclonal antibodies [17–28], and only one trial utilized monoclonal antibodies to detect antigens [24].

The specificity of the method was 98.3 %, indicating an excellent capacity of the test to differentiate healthy individuals from ill patients [14, 15]. The

remaining 1.7 % were false-positive cases which can be attributed to cross-reactions, particularly with other fungal infections caused by *Paracoccidioides* spp. and *Blastomyces* spp. [5, 6, 15]. However, these findings could contribute toward the improvement of screening tests in immunocompromised patients, helping to rule out other fungal infections as described above [21].

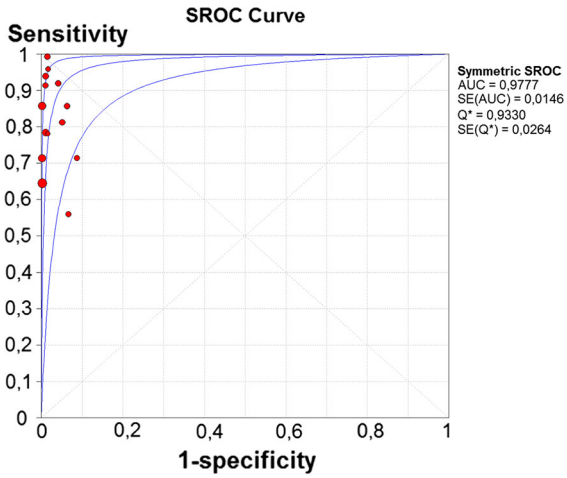

Fig. 3 Area under the curve (AUC) for the antigenuria and antigenemia tests used to diagnose histoplasmosis

One of the central findings of this research was that the global sensitivity and specificity data do not vary with the type of sample. However, individual analyses of the studies revealed that the sensitivity of tests employing serum samples are slightly higher than those with urine, contrasting with the results of previous studies. The results of the present analysis indicate no significant differences for diagnosis between the two types of clinical samples, suggesting that the use of either to detect *Histoplasma* antigens may give the same diagnostic value.

Two platforms were employed in the studies analyzed, i.e., ELISA [17–19, 22–24, 27, 28] and radioimmunoassay (RIA) [20]. Although studies using RIA to detect antigens showed high sensitivity and specificity values, the main disadvantage of this technique is the sophisticated equipment it requires, hampering its routine implementation in laboratories with limited resources. It is important to note that antigen detection assays are useful not only for diagnosis but also for monitoring the response of patients to treatment; thus in AIDS patients treated with amphotericin B, the antigen levels fall during the

Table 3 Analysis of the sensibility values for parameters of diagnostic validity

Omitted study	Sample	Sen (95 % CI)	Spe (95 % CI)	PLR (95 % CI)	NLR (95 % CI)	OR (95 % CI)
Wheat et al. [20]	Urine	81 (78–83)	98 (98–99)	43 (19–97)	0.19 (0,14–0.27)	296 (106–828)
Wheat et al. [28]	Urine	83 (81–86)	98 (97–99)	38 (18–81)	0.16 (0.11–0.24)	291 (105–803)
Connolly et al. [19]	Urine	80 (78–82)	98 (97–99)	42 (18–96)	0.19 (0.14–0.26)	266 (98–717)
Scheel et al. [22]	Urine	82 (80–84)	98 (98–99)	49 (20–122)	0.18 (0.12–0.25)	379 (123–1168)
Hage et al. [18]	Urine	82 (79–84)	98 (97–99)	41 (18–93)	0.17 (0.11–0.25)	323 (110–946)
Theel et al. [23]	Urine	82 (80–84)	97 (96–98)	36 (16–80)	0.16 (0.11–0.24)	296 (103–848)
Caceres et al. [27]	Urine	81 (79–83)	99 (98–99)	50 (21–119)	0.18 (0.13–0.25)	371 (123–1115)
Overall measure	Urine	79 (76–82)	99 (98–99)	59 (17–198)	0.20 (0.14–0.30)	416 (126–1373)
Wheat et al. [20]	Serum	81 (79–84)	98 (98–99)	43 (19–98)	0.17 (0.12–0.25)	331 (116–943)
Gómez et al. [24]	Serum	82 (79–84)	99 (98–99)	51 (22–119)	0.17 (0.12–0.24)	405 (144–1142)
Wheat et al. [28]	Serum	80 (78–83)	98 (97–99)	37 (18–79)	0.18 (0.13–0.26)	274 (101–739)
Guimarães et al. [17][a]	Serum	82 (80–85)	99 (98–99)	51 (22–120)	0.17 (0.12–0.23)	406 (156–1057)
		81 (79–83)	98 (98–99)	47 (19–116)	0.19 (0.13–0.26)	334 (112–995)
Connolly et al. [19]	Serum	81 (78–83)	98 (98—99)	41 (18–93)	0.19 (0.14–0.26)	295 (104–833)
Hage et al. [18]	Serum	80 (78–83)	98 (98–99)	40 (18–92)	0.20 (0.14–0.27)	278 (100–774)
Overall measure	Serum	84 (80–87)	97 (96–98)	34 (10–111)	0.16 (0.09–0.31)	239 (47–1227)
Total measure	Urine and serum	81 (79–84)	98 (98–99)	43 (19–96)	0.18 (0.13–0.25)	321 (118–875)

Sen sensitivity, *Spe* specificity, *PLR* positive likelihood ratio, *NLR* negative likelihood ratio, *OR* odds ratio

[a] Study with values from two different laboratories (one in Sao Paulo and the other in Rio de Janerio)

first weeks of therapy and they are stabilized at low levels during treatment, whereas in immunocompetent patients elimination of the antigen occurs more rapidly and completely [19, 23]. These antigen detection tests complement rather than replace conventional ones (detection of antibodies, direct examination and culture), improving the overall sensitivity and increasing the chances of opportune and timely diagnosis, especially in patients with severe forms of the disease. Besides its high cost, the main disadvantage of this method is that it is only available in referral centers in the USA and has only recently been implemented in a few countries of Latin America [4, 22, 27].

Although antigen detection in both urine and serum have good sensitivity and specificity values, the presence of false positives may be explained by various factors such as presence of nonspecific human IgG antibodies and rheumatoid factor; false-negative results could be due to a low fungal burden when sampling or to the use of antifungal treatment [24].

Because meta-analysis is a relatively new technique, it has certain limitations, one of its main problems being the quality of the different studies and the heterogeneity of the combined clinical trials, representing a challenge to the validity of the method itself.

Early diagnosis of histoplasmosis has become a constant public health challenge because of its high mortality rate, especially in immunocompromised patients, including those with HIV/AIDS. Conventional diagnostic methods have many difficulties, related primarily to the time it takes to obtain results and the high variability in sensitivity and specificity values [4, 5]. It is therefore important to evaluate the effectiveness and validity of immunodiagnostic methods as useful and timely tools to diagnose histoplasmosis. It is important to note that researchers should keep trying to improve and validate existing techniques or to design new technologies in order to increase sensitivity and their diagnostic values.

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