Diagnosis of Progressive Disseminated Histoplasmosis (PDH)

Background. Culture is the gold standard for diagnosis [1] and the sensitivity ranges from about 60 to 80% [2-5] (Table 1). However, growth of Histoplasma is slow, taking up to six weeks and culture rarely provides the initial basis for diagnosis. Demonstration of yeast morphologically consistent with *Histoplasma* supports, but does not prove the diagnosis [1] and the sensitivity is comparable to culture [2]. However, false positive results caused by other fungi or staining artifacts complicate diagnosis by pathology. Also, nonviable organisms persist in the tissues for years, and may not indicate active disease. Furthermore, obtaining specimens for culture and pathology may not be feasible in some patients and usually requires invasive procedures. For example, cultures were performed in 37% and pathology in 62% of patients [2]. Antigen detection was the most sensitive method for diagnosis, about 70% in non-immunocompromised and 95% in immunocompromised patients [2].

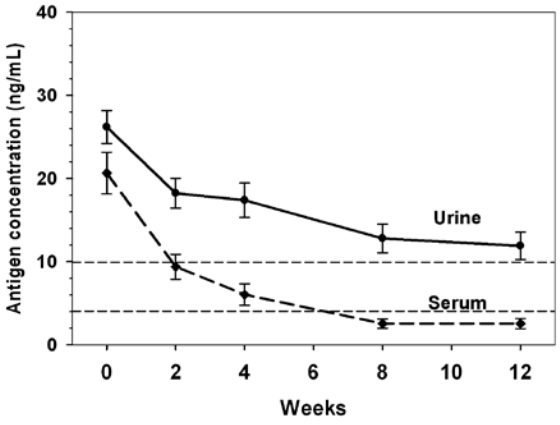
Table 1. Method	Sensitivity	TAT
Culture	56-82%	2-4 weeks
Cyto or histopathology	62-85%	3-5 days
Antigen detection	91-100%	1 day
Antibody detection	38-100%	1-3 days
Antigen & antibody*	98%	1-3 days

*CSF in Histoplasma meningitis: Ag 78%, Ab 82% [9]

Antigen was detected in the urine and serum of over 95% of immunocompromised patients [2-5] (Table 2). Uncertainty exists about the need to test both urine and serum, and some recommended only testing urine [6]. In acute pulmonary histoplasmosis, urine alone was positive in 3%, serum alone in 38%, both in 41% and neither in 17% [7]. Furthermore, we have observed negative results in urine but positive results in serum in patients with PDH (Table 3). Also, monitoring serum antigen levels provides the earliest evidence for response to treatment [8] (Figure 1)

Table 2. Sensitivity of Antigen Detection		
Condition	**Urine**	**Serum**
AIDS (n= 30)	97%	97%
Transplant (n=29)	100%	97%
TNF inhibitor (n= 11)	91%	100%

Figure 1. Antigen clearance during treatment [8]



Recently antibody response in the CSF has been evaluated in patients with *Histoplasma* meningitis [9]. IgG or IgM antibody was detected in 82% compared to 51% for ID or CF. While immunocompromise reduced the sensitivity of antibody detection, sensitivity was higher by EIA (68%) than ID or CF (38%). Combined antigen and IgG and IgM antibody detection provided the most sensitive method for diagnosis: antigen alone, 78%; IgG or IgM antibody alone, 82%; IgG or IgM antibody and antigen, 98% [9].

Table 3. Detection of antigen in the serum in patients with negative results in urine		
Risk Factor	**Serum Antigen ng/mL**	**Pathology or Culture Positive**
TNF blocker	1.7	None
TNF blocker	1.8	Ilium
TNF blocker	3.0	Lymph node
TNF blocker	0.3	None
TNF blocker	0.5	Lung
TNF blocker	0.7	Meninges
TNF blocker	0.3	Bone
AIDS	0.3	None
AIDS	0.4	Scan
Age > 54 years	1.0	Tongue
Age > 54 years	1.7	Lymph node
None	0.3	None
None	4.2	Scan

Until now, results of IgG and IgM antibody detection in serum has not been reported in PDH. Here we present results in immunocompromised patients with PDH [3-5]. The number of cases in non-immunocompromised patients were insufficient to assess antibody detection.

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Findings

Antigen detection. Antigen was detected with comparable sensitivity in urine and serum in immunocompromised patients with PDH [3-5] (Table 2). Some patients had negative results in urine but positive results in serum (Table 3). Testing serum is essential in anuric patients. Even if both urine and serum are positive, concentration in serum may be higher than urine, providing the best marker for monitoring treatment.

Antibody detection. IgG antibodies were detected in all patients treated with TNF inhibitors but was less sensitive in patients with AIDS and transplant patients (Table 4). A similar pattern was observed by ID. CF was performed in nine patients, precluding valid assessment.

Table 4. Sensitivity of Antibody Detection

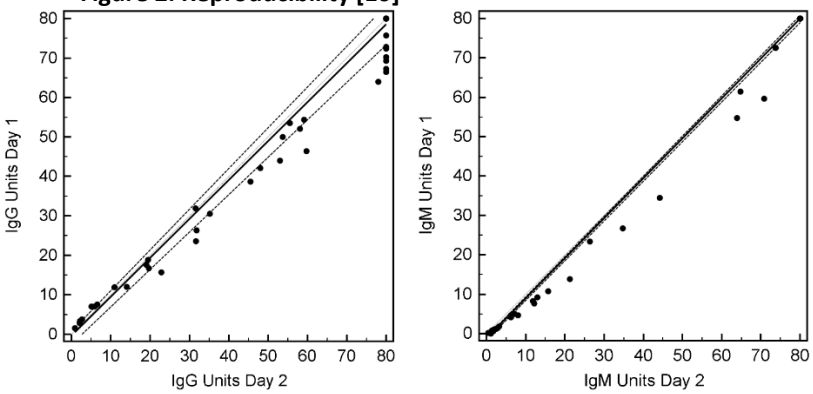
Condition	IgG	IgM	ID
AIDS (n= 30)	77%	26%	74%
Transplant (n=29)	38%	23%	35%
TNF inhibitor (n= 11)	100%	55%	82%

The sensitivity of IgG detection by EIA and ID was similar, and combined IgG and ID testing provided the highest sensitivity (75%), including 15% positive for IgG only and 10% by ID only. The IgG and IgM assays are highly reproducible and permit accurate quantification (Figure 2) [10].

Turnaround time (TAT) is important (Table 1). The antigen test provides results within 1 day. Antibody detection takes 2 to 3 days by CF or ID and 1 to 2 days by EIA. Pathology takes 3 to 5 days and culture requires 2-4 weeks in most patients and up to six weeks in some.

Polymerase chain reaction (PCR). More than 100 studies report useful molecular methods for diagnosis of histoplasmosis, some which were reviewed recently [11]. Most were small (20 patients or less) and the highest sensitivity was observed in tissues [12, 13]. PCR is offered as a laboratory developed test at several reference laboratories, but no FDA cleared test is available in the US. The recent small survey of infectious diseases or pulmonary physicians experienced in the diagnosis of histoplasmosis recommend molecular testing found that less than 10% recommended PCR (unpublished).

Figure 2. Reproducibility [10]



Summary

Testing for both urine and serum antigen provides the most sensitive method for diagnosis by antigen detection and serum is essential in anuric patients. Serum is also useful for monitoring treatment if the serum concentration exceeds that in urine. The IgG and IgM EIA combined with ID offers the most sensitive method for antibody detection, and EIA provides rapid results and accurate quantification.

Answers: Tests for diagnosis of histoplasmosis include culture, cytopathology, histopathology, polymerase chain reaction (PCR), antigen detection and antibody detection.

1. Which is the most sensitive single test? **Antigen**
2. Which provides a proven diagnosis? **Culture**
3. Which two tests provide the most rapid diagnosis? **Antigen & IgG IgM Antibody EIA**
4. Which is least sensitive in immunocompromised patients? **Antibody**
5. Which is the most sensitive combination of two tests? **Antigen & IgG IgM Antibody EIA**

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