Immunodiagnosis of Histoplasmosis in a Compromised Host

G. A. LAND,* J. H. FOXWORTH, AND K. E. SMITH

Department of Microbiology, Wadley Institutes of Molecular Medicine, Dallas, Texas 75235

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Three serological tests for the diagnosis of histoplasmosis were compared for sensitivity and specificity in serum from blood bank donors, patients with histoplasmosis, and infected or noninfected immunosuppressed patients. The histoplasmin latex agglutination test was positive in 9% of the normal patients, 33% of the histoplasmosis patients, and 61% of the noninfected immunosuppressed patients. Since the test is prone to many false-positive results in patients with inflammatory diseases or non-*Histoplasma* infections, it has limited potential as a screening test among compromised patients. Immunodiffusion and counterimmunoelectrophoresis using a mycelial antigen were found to be more sensitive than either test using a combined yeast and mycelial antigen or a pure yeast phase antigen. Counterimmunoelectrophoresis at pH 7.2 proved to be the test of choice for serodiagnosis of histoplasmosis, resolving 85% of the immunocompetent infected patients and 100% of the infected immunosuppressed patients. Results indicated that counterimmunoelectrophoresis in conjunction with immunodiffusion could be used as a screening protocol to determine infection in incoming patients in a cancer hospital.

The increase in immunoincompetent and otherwise compromised patients prone to secondary fungal infection has been well documented in recent years (3, 4, 11, 15). Although these patients are more susceptible to common airborne fungi, of a more ominous note is their exceptional potential for disseminated disease via the deep-seated mycoses (5, 10, 11, 19). For example, histoplasmosis in a patient with leukemia could be easily disseminated as a result of the increased leukocyte mass of the host, and the predilection of the organism for the reticuloendothelial system. If the patient were also neutropenic, one could predict that the course of the infection would be acute and stormy. In the endemic region, the attack rate of disseminated histoplasmosis among leukemia patients has been reported to occur some 400 times more frequently than in the normal population (i.e., 7.5 versus 0.017%) (5). In fact, it is only when the patient is in remission and accompanying neutropenia has been resolved that the fungal infection can be controlled (5, 19).

Therefore, it becomes necessary for any hospital dealing with compromised patients, particularly those within or near the endemic zones for systemic mycoses, to be acutely aware of possible infection by these fungi. Since our hospital is situated within a endemic area for *Histoplasma capsulatum* and at the fringe of the *Coccidioides immitis* zone, we have been interested in developing a battery of pertinent tests

to screen incoming patients for these systemic agents as well as several of the opportunistic fungi. The histoplasmin latex agglutination (HLA) test was an obvious choice for the screen for three reasons: (i) it is a rapid and easy test to read; (ii) it is designed with the acute disease in mind, picking up the antibody increase associated with early infection; and (iii) the test may be run on anti-complementary serum, a frequent finding with patients on anti-neoplastic chemotherapy (2, 6, 8, 16).

However, there is some controversy as to the value of the HLA test, since false positives have been reported in patients with infections other than histoplasmosis or with inflammatory diseases such as rheumatoid arthritis (6, 13). Thus, the purpose of this study is to determine the diagnostic value of commercial HLA kits with compromised patients, as well as to compare them with the following: (i) a commercial immunodiffusion (ID) kit for *Histoplasma*, (ii) ID using several experimental antigens, and (iii) counterimmunoelectrophoresis (CIEP) using both commercial and experimental antigens.

MATERIALS AND METHODS

Strains. The following ATCC strains of *H. capsulatum* were used to prepare an ID antigen: ATCC 11407, ATCC 12700, ATCC 26028, and ATCC 26032. Additionally, a strain isolated in blood culture at Wadley Institutes from a patient with disseminated disease was also used.

Antigen preparation. Strains of *H. capsulatum* were grown in the chemically defined medium of McVeigh and Morton (12), a glucose—salts broth containing $(\text{NH}_4)_2\text{SO}_4$, asparagine, cysteine, vitamins, and trace elements. A 1-liter flask, containing 500 ml of defined medium, was inoculated with either the mycelial or yeast form of each isolate. Thus, each strain was represented by 2 flasks, and there were 10 flasks in all. Mycelial growth was maintained at 30°C for 30 days, whereas yeast growth was at 37°C for 14 days. During the incubation period, both culture sets were shaken at 160 rpm. Before harvesting, each flask was made to 0.5% in Formalin and shaken at 30°C for an additional 3 days, whereupon cells were removed by centrifugation at $3,500 \times g$ at 4°C for 60 min. Each supernatant was collected, pooled, and frozen at -20°C until needed. Pooled cell pellets were suspended to their combined original volume in sterile distilled water and were autolyzed by shaking for 6 days at 37°C. After this reincubation period, cells were removed by centrifugation, and the clarified autolysate was pooled with thawed culture filtrates. The antigen pool was concentrated 10× against a hollow-fiber system (Amicon DC-2) which had a molecular-weight cutoff of 8,000. After concentration, the hollow-fiber unit was switched to the dialysis mode, and the ultrafiltrate (W-Histo) was dialyzed until Formalin was no longer detected. The final retentate was made 1:5,000 with respect to Thimerosal, put up in 3-ml portions, and frozen at -70°C. The resultant histoplasmin, made up of equal parts (according to protein concentration) of yeast phase and mycelial-phase material, had a final protein concentration of 12.0 mg/ml and a hexose concentration of 8.0 mg/ml. Titration of W-Histo against pooled human histoplasmosis sera showed an ID titer of 1:8 to be the optimal dilution for both H and M precipitin bands.

Latex agglutination. Two commercial HLA kits were used in this study. The Inolex kit (Glenwood Park, Ill.) required dilution of the serum to be tested, whereas the Hyland kit (Hyland Laboratories, Division of Travenall Laboratories, Costa Mesa, Calif.) used undiluted serum. Tests were performed as instructed by the manufacturers' package inserts.

Double ID. ID kits using mycelial-phase antigens for *H. capsulatum* and for *C. immitis* (Diagnostic Products, Inc. [DPI], Cincinnati, Ohio; since the compilation of these findings, DPI has become Meridian Diagnostics, Inc.), as well as a kit for *C. immitis* only (Hyland), were set up as per the directions of the manufacturers. Other ID tests used commercially prepared plates, "homemade" antigens, and a commercial antigen. The diffusion media used were as follows: a macroplate made up of 1% agarose in pH 8.0 phosphate buffer, with a well volume of 20 μl (Meloy Laboratories, Springfield, Va.); and a pH 7.2 microplate (5 μl wells) containing 1% agarose in 1 M glycine and 0.15 M NaCl (Diagnostic Products). The antigens tested were the half yeast-half mycelial antigen prepared as described above; a pure yeast phase histoplasmin (a gift from M. Sutcliffe, Veterans Administration Hospital, Nashville, Tenn.); and a mycelial-phase histoplasmin (DPI). The following *C. immitis* antigens were used: Spherulin, a tissue phase antigen (Berkeley Biologicals, Berkeley, Calif.); and two mycelial antigens (Hyland and DPI). Positive control sera for both *His-*

toplasma and *Coccidioides* were also obtained commercially (DPI). The ID tests were set up with the specific antigen in the center well and patient or control sera in the peripheral wells. Incubation was carried out in a humidified chamber at room temperature for 3 days. Daily visual readings were taken by indirect light against a dark background. Lines of identity with positive controls, as well as the presence of H and M bands, were determined.

CIEP. A commercial plate prepared with 1% agarose in pH 8.6 barbitol buffer having two parallel rows of 20 μl volume (Meloy Laboratories) served as the electrophoretic medium for the test. In some instances, these plates were dialyzed overnight against several large volumes of pH 7.2 barbitol buffer to lower the pH of the plate. The pH levels of the dialyzed plates were checked with an electrode made for testing prepared plates (no. 39507; Beckman Instruments, Inc., Palo Alto, Calif.). Running buffer consisted of sodium barbitol (10.8 g), sodium acetate (5.2 g), and 1.0 N HCl (65 ml), and the solution was brought to 2,000 ml. Buffer pH was adjusted to either 7.2 or 8.4 to complement the electrophoretic medium used. The same antigens used in the ID test also served for CIEP. Each antigen was placed in a cathodal well and run against anodally placed patient or positive control sera. Plates were subjected to electrophoresis in a Meloy EP-1A CIEP unit for 90 min at 25 mA/plate, soaked in 4% sodium citrate (wt/vol in water) for 1 h, and blotted dry. Readings were taken immediately and then again after overnight incubation at room temperature. The number of precipitin bands observed with the serum of each patient as well as the identity of those bands with controls were recorded.

Serum specimens. The specificity and sensitivity of the fungal antigens were determined with serum from the following seven groups of people: (I) 250 normal volunteer blood bank donors; (II) 11 healthy histoplasmin skin test-positive people; (III) 13 coccidioidin skin test-positive people; (IV) 56 patients with culturally proven histoplasmosis; (V) 48 patients with positive complement fixation tests for *H. capsulatum* and/or clinical evidence of histoplasmosis; (VI) 17 patients with active pulmonary coccidioidomycosis; (VII) 5 patients with inactive coccidioidomycosis; and (VIII) 85 serum samples from 54 debilitated patients, 31 of whom had a clinical picture suggestive of histoplasmosis. Sera from groups III and V had been stored frozen at -70°C for a year or more, whereas the remaining sera had been stored for less than 1 year. Additionally, freshly drawn sera from culture-positive and from clinically suspicious patients (36 per group) were tested and compared with the older sera.

RESULTS

Microbiological data from the compromised patient group was as follows: one patient had coccidioidomycosis, three had tuberculosis, three had *E. coli* septicemia, two were heavily colonized with *Candida* as shown by sputum cultures, and six culture-negative patients had evidence of disseminated intravascular coagulation. The primary disease states of this group consisted of 10 patients with hematoproliferative

malignancies, 10 with other neoplasms, 4 with anemia, 3 with myelofibrosis, and 1 each with myasthenia gravis, hepatitis, Waldenstrom's disease, and amyloidosis. All patients at the time of testing had a fever of undetermined origin, with most showing some kind of respiratory involvement.

Both kits for the detection of *H. capsulatum* agglutinating antibody (HLA test) yielded positive results in 23 of 31 immunosuppressed patients with a clinical picture suggestive of acute histoplasmosis. Although 52 of 85 serum samples from these patients were agglutinin positive via the Hyland kit, only 2 of 4 patients with positive cultures for *Histoplasma* had positive agglutinins. The Inolex kit, requiring the titration of patient sera, showed 24 and 28 sera from the compromised group positive at dilutions of 1:4 to 1:8 and $\geq 1:16$, respectively. Of these, only one of the patients with a positive culture had an agglutination titer $\geq 1:16$ (Table 1). In a retrospective study of serum from immunocompetent populations suspected of having histoplasmosis, 31% of the sera tested with the Hyland kit had positive agglutinins, whereas 23% of the Inolex tested sera had titers $\geq 1:4$, but only 3.9% had the diagnostic titer of $\geq 1:16$. Approximately 20% of normal blood bank donors as well as 27% of patients with coccidioidomycosis had positive agglutinins $\geq 1:4$ with the Inolex HLA test; with the Hyland kit, 10 and 28%, respectively, had positive agglutinins.

In general, ID proved to be more specific and sensitive than latex agglutination for determining *H. capsulatum* antibodies in stored sera (Table 2). One serum specimen from a donor having a positive *Coccidioides* skin test was the only positive ID response within the normal group. The commercial ID test using the mycelial antigen was most efficacious with DPI plates and antigen. Precipitin bands appeared in sera from 41% of the total disease group and 49% of the culture-positive patients. The sensitivity of the remaining ID systems, W-Histo (37%) and S-Histo (30%), was similar to that of the HLA test. ID also proved to be highly specific with all three antigens. In the heterologous response, i.e., *Coccidioides* precipitin-positive serum reacting with histoplasmin and vice versa, reactions obtained were of nonidentity (Table 2). Also in this retrospective series, H and M bands occurred together in 20% of the sera tested with the commercial reagents.

The detection of specific precipitins in a small sample of patients with active or inactive coccidioidomycosis showed slightly better results, with 55% of the serum specimens proving positive by ID. There was very little difference between the performance of either of the prepared *Cocci-*

TABLE 1. Retrospective study of the serum responses of patient groups and blood bank donors to histoplasmin-sensitized latex

Group (no. of people)	No. of respondents to: ^a			
	Hyland kit (undiluted)	Inolex HLA test		
		Undiluted to 1:2	1:4 to 1:8	$\geq 1:16$
Normal (274)	9.5	2.2	17.5	2.9
I	23	6	47	7
II	0	0	0	0
III	3	0	1	1
Histoplasmosis (104)	31	2.9	19.2	2.9
IV	22	1	11	5
V	10	2	9	3
Coccidioidomycosis (22)	27	0	13.6	13.6
VI	5	0	3	2
VII	1	0	0	1
Immunosuppressed (85)	61; 50 ^b	0	28; 75 ^b	33; 25 ^b
VIII	52	0	24	28

^a Values in italics indicate percentage of total group responding.

^b Second value indicates percentage of culture-positive respondents.

dioides ID kits, with 54 and 40% of the specimens positive when the DPI reagents and the Hyland kit, respectively, were used. Spherulin proved to be the least sensitive antigen for antibody directed against *Coccidioides* and was the most cross-reactive as well. It was also our experience that differences in well size had no effect on increasing the sensitivity of either the *Histoplasma* or *Coccidioides* ID test. We did, however, see a 20% increase in H and M bands when the plate with the larger wells was used for *Histoplasma* precipitin testing.

CIEP gave the greatest degree of clinical correlation of the tests investigated in this study (Table 3). The DPI reagent proved to be the most sensitive antigen in CIEP testing, reacting with sera from 64% of the culture-positive and 85% of the suspected patients. In comparison, the W-Histo and S-Histo performed equally well with culture-positive sera (66% and 59%) but detected only 46% of the clinically suspected sera. The increased sensitivity of CIEP in serodiagnosing histoplasmosis also extended to the detection of antibodies to *C. immitis*, with 82% patients who had active coccidioidomycosis demonstrating precipitins via the DPI *Coccidioides* antigen/CIEP test. The other two *Coccidioides* antigens were less sensitive, with only

TABLE 2. *Retrospective study on the Histoplasma and Coccidioides ID precipitin responses among patient groups and blood bank donors*

Group (no. tested)	No. of respondents to: ^a					
	DPI-Histo	W-Histo	S-Histo	DPI Cocci	Hyl and Cocci	Spherulin
Normal (274)	0	0	0	0.36	0.36	0
I ^b	0	0	0	0	0	0
II	0	0	0	0	0	0
III	0	0	0	1	1	0
Histoplasmosis (104)	41.3	36.5	29.8	24.0 NI	18.2 NI	40.3 NI
IV ^c	27	21	16	16 NI	12 NI	27 NI
V ^c	16	17	15	9 NI	7 NI	15 NI
Coccidioidomycosis (22)	4.5 NI	0	0	54.5	40.9	18.2
VI	1 NI	0	0	11	9	4
VII	0	0	0	1	0	0
Immunosuppressed (85)	0	0	0	1.2; 100 ^d	0	0
VIII	0	1 ^e	1 ^e	1 ^f	0	0

^a Values in italics indicate percentage of total group responding. Cocci, *Coccidioides* antigen; NI, reaction of nonidentity.

^b Serum was tested both at normal and at 10-fold concentrations.

^c Sera were positive for either H and M bands or for M band only.

^d Second value indicates percentage of culture-positive respondents.

^e One-fourth of the patients had culturally proven histoplasmosis; serum was concentrated 10-fold.

^f One patient with culturally proven coccidioidomycosis; serum concentrated 10-fold.

TABLE 3. *Retrospective determination of precipitins to Histoplasma and Coccidioides by CIEP within patient groups and blood bank donors*

Group (no. tested)	No. of respondents to: ^a					
	DPI-Histo	W-Histo	S-Histo	DPI Cocci	Hyl and Cocci	Spherulin
Normal (274) ^b	1.1	0.7	0.36	2.2	0.7	1.1
I	0	0	0	0	0	0
II	1	1	0	2	1	2
III	2 NI	1 NI	1 NI	4	1	1
Histoplasmosis (104)	74	56.7	52.9	35.6 NI	34.6 NI	56.7 NI
IV	36	37	33	16 NI	12 NI	27 NI
V	41	22	22	21 NI	24 NI	32 NI
Coccidioidomycosis (22)	9.1 NI	4.5 NI	0	77.2	45.5	45.5
VI	2 NI	1 NI	0	14	9	10
VII	0	0	0	3	1	0
Immunosuppressed (85) ^b	100 ^c	100 ^c	100 ^c	100	100	0
VIII	4 ^c	4 ^c	4 ^c	2 ^d	1 ^d	0

^a Values in italics indicate percentage of total group responding. Cocci, *Coccidioides* antigen; NI, reaction of nonidentity.

^b Serum was tested at normal and at 10-fold concentrations.

^c Four of four patients with positive *H. capsulatum* cultures detected (unconcentrated sera).

^d Positive *C. immitis* culture detected only one patient with positive precipitins; one reaction of nonidentity.

60% of the coccidioidomycosis cases showing demonstrable precipitins. Spherulin proved more sensitive with CIEP than it did for ID, but again this antigen was the most cross-reactive of the *C. immitis* antigens tested. Cross-reactions for both fungi determined by the CIEP test were of nonidentity. In most cases, identity of the precipitin bands could be determined between the directly opposed wells of the Meloy plates, but with difficulty.

All tests were further evaluated against two groups of sera (36 sera per group) drawn from patients with recent positive cultures or with

recent clinical and serological evidence of histoplasmosis (Table 4). There appeared to be some contrasting results between antibodies detected in fresh sera and those detected in frozen, stored sera. Both latex agglutination tests had the same overall sensitivity as in the earlier study, picking up approximately 32% of the suspected cases. The tests differed slightly in specificity, in that the Hyland reagents seemed more sensitive for serum from culture patients, whereas the Inolex test had more positive respondents within the suspected group. ID, however, appeared to be a more sensitive test with the more recent speci-

mens. The most noteworthy increase in sensitivity was with the DPI system, with detected serum precipitins in 68% of the combined recent groups (Table 4) as opposed to 45% of the combined groups in the retrospective study. The ID test using W-Histo was also more sensitive with fresh serum than it was for stored specimens, whereas S-Histo ID results were equivalent to those of the retrospective study.

A slight increase in sensitivity was also observed when testing the fresh serum with CIEP. The most striking observation was the 92% sensitivity of the mycelial antigen with fresh serum specimens from clinically suspicious patients (Table 4), imparting an overall sensitivity of 85% to the test. Additionally, CIEP with the mycelial antigen at a plate and buffer pH of 7.2 produced more detectable precipitin bands than did pH 8.2 (Table 5). Moreover, more H and M doublets

were seen at the lower pH. This was especially true in the clinically suspected group, whereby the 7.2 buffer system resolved over twice as many cases as did the higher pH. By comparison, as the amount of yeast material in the antigen increased, the number of cases detected by CIEP decreased, such that S-Histo (yeast antigen) reacted with only half the serum specimens that were positive with the mycelial antigen.

DISCUSSION

The HLA test, designed for the detection of serum precipitins during acute histoplasmosis, performed acceptably with sera from apparently healthy individuals. The 9.5% rate was somewhat higher than the 3.3% predicted by the Hyland package insert, but was still acceptable. The sensitivity of the HLA test for patients with suspected histoplasmosis was inadequate for di-

TABLE 4. Sensitivity of serological tests for *H. capsulatum* antibody in patients recently suspected of having histoplasmosis^a

Test group (no. tested)	Respondents (%) to:							
	Hyland latex	Inolex latex	DPI Histo		W-Histo		S-Histo	
			ID	CIEP	ID	CIEP	ID	CIEP
Culture positive (36)	44	19.4	66.7	77.7	61.1	77.7	41.6	61.1
Serologically or clinically suspected cases (36)	19.4	47.2	69.4	91.6	50	41.6	25.0	44.4
Total (72)	31.9	33.3	68.0	84.7	55.5	59.6	33.3	52.8

^a Specificity was 100%, since all reactions with heterologous antigens were of nonidentity.

TABLE 5. Effect of pH on the precipitin response of culture-positive and suspected histoplasmosis patients as determined by CIEP

Test group (no. tested)	pH	Precipitin response	Sera reacting (% $\pm$ SE) to: ^a			
			DPI Histo		DPI Cocci	
			Total	+ Only ^b	Total	+ Only ^b
Culture positive (36)	8.2	H	53.3	0	16.6 NI	5.5 NI
		H+M	± 8.3 13.8	± 0 0		
	7.2	H	47.2	8.3	2.7 NI	5.5 NI
		H+M	± 6.6 33.3	± 7.2 16.6		
Serologically or clinically suspected (36)	8.2	H	33.3	13.8	25.0 NI	8.3 NI
		H+M	± 8.0 2.8	± 5.7 0		
	7.2	H	62.8	22.2	38.8 NI	8.3 NI
		H+M	± 7.2 28.8	± 8.0 13.8		

^a SE, Standard error of H and H+M groups combined. Standard error = $\pm \sqrt{P_1(1 - P_1)/n}$, where n = number of cases. P , % respondents. NI, reactions of nonidentity; Cocci, *Coccidioides* antigen.

^b + Only, Sera positive for this test, this pH, and this antigen combination only.

agnostic purposes. In sera from culture-positive patients, both recent and retrospective, between 40 and 44% of the patients had a positive response. These results correlated well with the Hyland kit results of 45% positives among suspected patients and with the results of Hill and Campbell from 35 patients with chronic pulmonary disease (8). On the other hand, the test was positive in only 19% of the clinically suspected group. The Inalex test seemed to be more specific, because it reacted with only 2.9% of normal serum specimens at a $\geq 1:16$ dilution; but appeared to be less sensitive because it was positive in only 3.9% of the older histoplasmosis sera and 16.6% of the recent group. If the positive cutoff point were lowered to $\geq 1:8$, then 23% of the stored sera and 33% of the fresh would be agglutinin positive.

The appearance of *Histoplasma* agglutinins has been reported in patients with inflammatory processes or infections other than histoplasmosis (6, 13). Our initial concern was that cancer patients, many having constant fever and inflammation due to their primary disease, might also display this nonspecific agglutination. The fact that 52 of 85 serum samples from immunosuppressed patients reacted with both HLA tests further points to the inadequacy of the test in the compromised host. In the Hyland test, the positive reaction in these specimens was usually 3+ to 4+, whereas in the Inalex test, 28 specimens had $\geq 1:16$ reaction, and 24 were positive at 1:8. At the latter titer, 63% of these patients (including four of four culture-positive patients) would be positive, but the test specificity would only be 39%.

Since the experience of earlier investigators with HLA suggested a 3+ to 4+ response at serum dilutions of $\geq 1:16$ or $\geq 1:32$ as indicative of acute disease, low agglutination titers are not alarming when observed within normal patient populations. However, in immunosuppressed patients having limited capacities to produce antibodies in response to infection, low agglutination titers might become meaningful. For example, if this test were to be adopted for the routine screening of incoming cancer patients for histoplasmosis, several points should be considered: (i) a positive reaction is meaningless without an anti-inflammatory or rheumatoid factor control (17), (ii) patients incapable of forming antibody may not achieve diagnostic titers of $\geq 1:16$, (iii) repeat specimens should be taken every 21 days if the admission test results are equivocal, in order that titer movement might be observed, and (iv) other serological tests must be used for confirmation of positive HLA results.

In infected and noninfected immunocompetent patients and similar groups of immunoin-

competent patients, ID and CIEP proved to be more specific and sensitive than the HLA test (Tables 4 and 6). The DPI-ID kit using a mycelial antigen proved to be the best ID system tested, in that serum from 68% of the histoplasmosis patients demonstrated positive precipitins. These results were substantially lower than those reported by Bauman and Smith (1) who, using a similar ID system, found that sera from 63 of 70 (90%) proven histoplasmosis patients were precipitin positive. Several explanations for our lower detection rate are possible. (i) Precipitin antibody titers are extremely low in early infection. Exposure of the diffused antibody to an antigen excess, as is possible in ID, would lead to soluble antigen-antibody complexes rather than a precipitin band. (ii) Our sample group consisted of patients that were suspected of having, but not proven to have, the disease. It is, therefore, possible that some patients within this group did not have histoplasmosis, but the strong response of these same sera to CIEP discounts this possibility. (iii) Decreased positive responses with stored serum specimens may be due to antibody decay during prolonged freezing. The fact that all of the serological tests had more positive responses when fresh serum was used supports this conclusion. A similar hypothesis has been offered as an explanation for the conflicting results seen between retrospective and immediate studies of *Candida* precipitins, whereby some positive sera which had been frozen and stored for an extended period seemed less apt to form precipitins (18).

Using standard error of difference to statistically evaluate the data, the W-Histo (yeast and mycelial antigen) appears equivalent to DPI-Histo in terms of sensitivity and specificity in the ID test. However, results obtained with the yeast phase antigen ID system do not correlate well with suspected infection (Table 6). When these same antigens were used with the CIEP system, the DPI mycelial antigen proved to be more sensitive than either of the two antigens containing yeast material. This lack of sensitivity may reflect a depressed humoral response to yeast phase antigen during early infection.

CIEP, when used with the DPI antigen at pH 7.2, was the most sensitive serodiagnostic test used in our study (Tables 5 and 6). Precipitins were determined in 85% the immunocompetent histoplasmosis patients and four of four culture-positive immunosuppressed patients. These results correlated closely with those of Kleger and Kaufman (9), who reported better results with a pH 7.2 buffer system and an overall sensitivity for the test of 81%. Furthermore, CIEP at pH 7.2 has been shown to be 100% sensitive in suspected patients within the endemic zone, sug-

TABLE 6. Comparison of the probable error of difference of antigens and serological tests used for detection of *H. capsulatum* antibody in patients with suspected histoplasmosis

Comparison of:	Antigen	(%) Total positive response	Standard error (%)	Standard error of difference ^a
ID antigens	DPI Histo	68.0	±5.9	
	W-Histo	55.5	±5.9	12.5 ± 8.3; <i>P</i> < 3×
	S-Histo	33.3	±5.3	34.7 ± 7.9; <i>P</i> > 3×
CIEP antigens	DPI Histo	84.7	±4.3	
	W-Histo	59.6	±5.4	25.1 ± 6.9; <i>P</i> > 3×
	S-Histo	45.8	±5.9	38.9 ± 7.3; <i>P</i> > 3×
CIEP tests using best antigen	DPI Histo (pH 7.2)	84.7	±4.3	
	DPI Histo (pH 8.2)	41.6	±5.8	43.1 ± 7.6; <i>P</i> > 3×
Agglutination and ID with pH 7.2 CIEP	DPI Histo (pH 7.2)	84.7	±4.3	
	ID DPI-Histo	68.0	±5.9	16.7 ± 7.3; <i>P</i> < 3×
	Inolex latex agglutinin	33.0	±5.5	51.7 ± 7.0; <i>P</i> > 3×

^a Standard error of difference = $\pm \sqrt{\frac{P_1(1-P_1)}{n_1} + \frac{P_2(1-P_2)}{n_2}}$, where *n* = number of cases and *P* is % respondents. To be significant, error of difference should be at least three times (3×) greater than its error.

gesting to those authors that CIEP for *Histoplasma* antibody may serve as a rapid means of screening suspected patients (14). A final advantage to consider is that CIEP is less prone to false-negative results due to antigen excess. Since the antigen and antibody are "focused" or brought toward each other by means of electrophoresis, a concentration gradient is established, enabling the antibody to find an equivalent zone of antigen concentration for maximum precipitation to occur.

A disadvantage of the CIEP test is that it is prone to the same interpretation problems as the ID test, i.e., a positive M precipitin band may reflect active infection, a recent skin test, or past infection (14, 17). In the latter case, positive M bands have been reported as much as 5 years after the acute disease. It appears that the H band has diagnostic potential in that it is present with M bands during active histoplasmosis (14, 17). However, the production of both H and M bands occurs in only 25 to 35% of proven or suspected cases. One further disadvantage is the lack of commercially available reagents and plates for the test. Without standardization of the test and quality control of reagents as well as commercial development of plates having proper well configuration and pH, test results will vary from laboratory to laboratory. To minimize the mechanical disadvantages of CIEP and to provide the test with some modicum of standardization and reproducibility, we have set forth a simple procedure using dialysis to lower the pH of commercially prepared plates. These plates, in combination with commercially available ID antigens and antisera, would enable a laboratory of any size to use CIEP as a rapid screen for histoplasmosis.

In conclusion, our results indicate to us that

the HLA test has limited potential with immunosuppressed patients suspected of having histoplasmosis. Using the test in its present state, with uncorrected false-positives, physicians may be misled by results. We suggest that an early screen for histoplasmosis consist of both ID and CIEP at pH 7.2 using a mycelial antigen. More precipitins would be detected via the dual tests than with either test alone. A final suggestion is that, in clinically suspicious patients, repeat specimens be taken every 21 days to (i) note the appearance of H bands, and (ii) determine complement fixation titers and their subsequent movement. A protocol such as this for a cancer hospital within the endemic zone would establish an adequate serological base line for the noninfected patient and at the same time will permit an early diagnosis of histoplasmosis, which is of paramount importance in the compromised host.

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