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Comparison of Commercial Kits for Detection of Cryptococcal Antigen

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Although kits to detect cryptococcal antigen are used widely to diagnose cryptococcal infection, the comparative performance of commercially available assays has not been evaluated in the past decade. Therefore, we compared the sensitivities and specificities of five commercially available kits for detecting cryptococcal antigen (four latex agglutination test kits—Calas [Meridian Diagnostics]), Crypto-LA [International Biological Labs], Myco-Immune [MicroScan], and Immy [Immunomycologics]—and an enzyme immunoassay kit, Premier [Meridian Diagnostics]) with culture for the diagnosis of cryptococcal meningitis and fungemia. Of 182 cerebrospinal fluid (CSF) and 90 serum samples submitted for cryptococcal antigen and fungal culture, 49 (19 and 30 samples, respectively) from 20 patients had a culture positive for *Cryptococcus neoformans*. For CSF specimens, the sensitivities and specificities of all kits were comparable (sensitivity, 93 to 100%; specificity, 93 to 98%). There was a significant difference in sensitivities of the kits when serum samples were tested with the International Biological Labs and MicroScan kits, which do not pretreat serum with pronase. These kits were less sensitive (sensitivity, 83%) than the Immy and Meridian latex kits (sensitivity, 97%), which do pretreat with pronase. The sensitivity of the Meridian enzyme immunoassay kit was comparable to that of the pronase-containing latex kits. These kits were of equivalent specificities (93 to 100%) when testing serum. Some of the currently available kits have limitations that need to be recognized for proper interpretation of results. Specifically, the use of pronase on serum samples reduces the number of false-positive results, and a titer of ≤1:4 can be a false-positive result when CSF samples are being tested.

Cryptococcal meningitis has become increasingly prevalent during the AIDS epidemic, affecting approximately 10% of patients with this syndrome (15). In view of the increasing number of infections, rapid, sensitive, and specific diagnosis of cryptococcal disease has become more important than ever. The detection of cryptococcal capsular antigen in serum and cerebrospinal fluid (CSF) by latex agglutination is rapid and has been documented to be both sensitive and specific (8, 19). There are currently four commercially available latex cryptococcal antigen tests (LCATs) as well as a new enzyme-linked immunoassay (EIA) test to detect cryptococcal capsular antigen. Although detection of cryptococcal antigen in serum and CSF has come to be relied upon in the diagnosis and management of cryptococcosis, there have been no direct comparisons of all commercially available kits. Therefore, we compared the five kits versus cultures to determine their sensitivities and specificities in diagnosing cryptococcal disease. In addition, we compared titers obtained with each of the cryptococcal antigen kits (7, 16, 17), since titration of antigen in serum and CSF has been used to determine prognosis and to evaluate response to therapy, despite use of different kits at different clinical sites in some of the multicenter therapeutic trials.

MATERIALS AND METHODS

Kits. We evaluated the Crypto-LA (International Biological Labs, Inc., Cranbury, N.J.) (IBL), Myco-Immune (American MicroScan, Mahwah, N.J.), Immy Latex-Crypto Antigen (Immuno-Mycologics, Inc., Norman, Okla.), and Calas (Meridian Diagnostics, Inc., Cincinnati, Ohio) LCATs, as well as the Meridian Premier EIA for detection of cryptococcal antigen in serum and CSF. All kits were used according to manufacturers' instructions. The latex kits all required heat inactivation to reduce nonspecific interference and inactivate complement. Three kits provided a normal globulin control to detect nonspecific interference; the Immy kit did not. Two kits, the Immy kit on serum and CSF and the Meridian LCAT on serum only, also used pronase to further limit nonspecific interference. The EIA kit required no specimen preparation beyond centrifugation.

Guidelines for interpretation of LCAT results varied by manufacturer, with all relying on a subjective interpretation by the observer. The EIA titers were calculated with A_{450} values (absorbance × dilution factor × control factor). Some of the pertinent differences in specimen preparation and result interpretation are listed by manufacturer in Table 1.

Specimens. We tested 182 CSF and 90 serum samples submitted to the clinical microbiology laboratories for LCAT and fungal culture at Duke University Medical Center (DUMC), Durham, N.C., and Robert Wood Johnson University Hospital (RWJUH), New Brunswick, N.J. A review of patient medical records for clinical evidence of cryptococcal infection was performed for all samples with a positive cryptococcal antigen or culture.

Microbiological methods. Cryptococci were isolated from blood by using the BacT/Alert blood culture system (Organon Teknika, Research Triangle Park, N.C.) at both DUMC and RWJUH. Positive culture bottles were subcultured to chocolate agar at DUMC and chocolate agar and tryptic soy agar

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TABLE 1. Specimen preparation and interpretation by kit

Kit	Heat inactivation				Pronase		Positivity standard
	Serum		CSF		Serum	CSF	
	Min	Temp (°C)	Min	Temp (°C)			
IBL LCAT	30	56	5	100	No	No	Distinct clumps
MicroScan LCAT	30	56	3	100	No	No	1–4
Immy LCAT	30	56	30	56	Yes	Yes	1–4, clumping
Meridian LCAT	15	56	5	100	Yes	No	2–4, clumping
	5	100					
Meridian EIA	None		None		No	No	OD_{450}[a] ≥ 0.150

[a] OD_{450}, optical density at 450 nm.

with 5% sheep blood at RWJUH. Isolates suspected to be cryptococci were identified by the India ink stain, urease testing, and the API 20C test strip (Analytab Products, Plainview, N.Y.). CSF specimens were plated on chocolate agar and tryptic soy agar with 5% sheep blood at both DUMC and RWJUH. CSF specimens for which fungal culture was specifically requested at DUMC were plated on inhibitory mold agar with gentamicin and chloramphenicol and on brain heart infusion agar; at RWJUH these specimens were plated on inhibitory mold agar and on brain heart infusion with gentamicin, chloramphenicol, and 5% sheep blood. Isolates suspected to be cryptococci were identified by the India ink stain and urease testing at DUMC and by these two tests plus an API 20C test strip at RWJUH. Serotyping of cryptococci was not done in either laboratory.

RESULTS

Forty-nine (19 CSF and 30 serum) samples from 20 patients had a culture positive for *Cryptococcus neoformans*. Forty-eight of the 49 cryptococcal culture-positive samples were from human immunodeficiency virus-seropositive patients. The remaining patient had lymphoma. There were no culture-negative patients with clinical evidence of cryptococcal infection.

Sensitivity and specificity for CSF samples. The sensitivities and specificities of the kits were excellent, as indicated in Table 2. Of the 19 true-positive CSF samples, 100% were detected by the IBL, MicroScan, and Meridian LCAT and EIA. The Immy LCAT failed to detect one culture-positive sample that was positive at a low titer (1:2, 1:2, and 1:8 with the IBL, MicroScan, and Meridian LCATs, respectively). The false-positive rate was low for all kits tested: 3, 3, 7, and 4% for the IBL, MicroScan, Immy, and Meridian LCATs, respectively, and 2% for the Meridian EIA. The majority of the false-positive results had titers of less than or equal to 1:4. The number of false-positive tests was highest with the Immy kit. Of these, half were at a titer of 1:1, and all true positives detected with this kit had a titer of greater than or equal to 1:8.

Only 5 of a total of 28 false-positive tests with the four LCAT kits had titers of 1:8 or greater. Of these five false positives, four represented one specimen that was positive by all four LCAT kits with titers of 1:8 to 1:16 but negative by the EIA kit and culture. This patient's serum cryptococcal antigen was negative with all five kits, and a follow-up at 18 months has revealed no evidence of cryptococcal disease. One other patient's CSF was positive by all four LCATs at a titer of 1:4 but also was negative by EIA and culture. Serum cryptococcal antigen was negative, and a follow-up 1 year later has revealed no evidence of cryptococcal infection.

There were three false-positive results by EIA. Two were negative by all four LCAT kits and culture of CSF. A third patient with a positive EIA titer of 1:1 did have a positive urine culture for *C. neoformans* with multiple negative CSF cultures. This patient was treated with fluconazole but developed positive serum cryptococcal antigen by all five kits at high titers 1 month later. There was no other evidence of cryptococcal meningitis. There were two samples from different patients that gave indeterminate readings by EIA. One sample was repeatedly indeterminate; testing of the other was not repeated because of inadequate sample volume.

Sensitivity and specificity for serum samples. The sensitivities and specificities of the kits were good to excellent when serum samples were tested (Table 2). Of the 30 true-positive serum samples, only 83% were detected by the IBL and MicroScan kits compared with 97% for the Immy and Meridian LCAT kits. The Meridian EIA had a sensitivity of 93%. Specificity was high for all kits, although the IBL and MicroScan kits were slightly better than the Meridian and Immy kits. The false-positive rate was low for all the kits tested: 1, 0, 5, and 3% for the IBL, MicroScan, Immy, and Meridian LCATs, respectively, and 2% for the Meridian EIA. As with the CSF samples, the number of false positives was highest with the Immy kit. Three of four false-positive results were at a titer of 1:1, whereas all of the true-positive results with this kit were at a titer of greater than or equal to 1:32.

All except one of the false-positive tests with the four LCAT

TABLE 2. False-positive and -negative results, sensitivity, and specificity by kit[a]

Kit (*n*)	% Sensitivity	% Specificity	No. of FP (%)	No. of FN (%)
IBL LCAT				
Serum (88)	83	98	1 (1)	5 (6)
CSF (179)	100	98	4 (3)	0
MicroScan LCAT				
Serum (90)	83	100	0	5 (6)
CSF (181)	100	97	5 (3)	0
Immy LCAT				
Serum (88)	97	93	4 (5)	1 (1)
CSF (177)	93	93	12 (7)	1 (5)
Meridian LCAT				
Serum (90)	97	95	3 (3)	1 (1)
CSF (182)	100	96	7 (4)	0
Meridian EIA				
Serum (85)	93	96	2 (2)	2 (2)
CSF (173)	100	98	3 (2)	0

[a] FP, false positives; FN, false negatives.

TABLE 3. Elimination of serum false negatives by treatment with pronase

Patient	Titer					
	IBL		MicroScan		Immy (with pronase)	Meridian (with pronase)
	Without pronase	With pronase	Without pronase	With pronase		
1	—[a]	1:256	—	1:128	1:32	1:32
2	—	1:64	—	1:1,024	1:32	1:32

[a] —, negative result.

kits had titers of 1:8 or less. One patient's serum was positive by the Meridian and Immy LCATs and the Meridian EIA at titers of 1:32, 1:8, and 1:8, respectively. This patient had biopsy-proven cerebral toxoplasmosis. A follow-up at 18 months has revealed no evidence of cryptococcal infection.

The number of false-negative tests was notably higher for serum samples than for CSF specimens. The two LCAT kits that did not include pronase in specimen preparation had the highest false-negative rates, 6% for both the IBL and the MicroScan kits compared with 1% for the Immy and Meridian LCAT kits and 2% for the Meridian EIA kit. Four of the five false-negative tests with the IBL and MicroScan LCAT kits were from the same four patients. The tests were repeated on two of the patients' samples with the addition of pronase treatment during specimen preparation. The use of pronase eliminated the false-negative results in both cases and, in fact, resulted in positive results of high titers, as shown in Table 3.

The two false-negative tests with the Meridian EIA also were negative by the IBL and MicroScan LCAT kits. Both patients were rheumatoid factor negative. Unfortunately, the specimen volume was insufficient to repeat the EIA after pronase treatment.

Titers. There were substantial variations in titers among LCAT kits for samples whose end point titers were determined. The MicroScan assay consistently had the highest titers, and the Meridian LCAT had the lowest on both CSF and serum. The geometric means for serum and CSF titers are shown by kit in Table 4.

The end point titers of 20 serum samples were compared among LCAT kits. The Meridian LCAT was chosen as the reference kit because, overall, it was the most sensitive and specific kit (Table 2). There was agreement (±1 twofold dilution) among the Meridian and the IBL, MicroScan, and Immy LCATs 60, 30, and 55% of the time, respectively. The IBL and MicroScan kits ranged from 6 dilutions less to 5 dilutions greater than the Meridian LCAT. The range for the Immy LCAT was from 3 dilutions less to 8 dilutions greater than the Meridian LCAT. Twenty percent of the titers with the IBL LCAT were more than 1 dilution greater than the titers with the Meridian LCAT, and 20% were more than 1 dilution less than the Meridian LCAT titers. Thirty-five percent of the MicroScan titers were more than 1 dilution greater, and 35% were more than 1 dilution less than the Meridian titers. Ten percent of the Immy titers were more than 1 dilution greater, and 35% were more than 1 dilution less than the Meridian titers.

TABLE 4. Geometric means of titers by LCAT kit

Samples	Geometric mean			
	IBL	MicroScan	Immy	Meridian
CSF	912	1,445	407	288
Serum	2,399	3,981	3,648	1,905

The end point titers of seven CSF samples were determined. Comparison of titers among kits revealed 57, 42, and 57% agreement (±1 twofold dilution) among the IBL, MicroScan, and Immy and the reference Meridian LCAT. The IBL and MicroScan LCATs ranged from 1 dilution less to 9 dilutions greater than the Meridian LCAT. The Immy LCAT ranged from 2 dilutions less to 8 dilutions greater than the Meridian LCAT. All of the IBL and MicroScan titers that were not in agreement were more than 1 dilution greater than the Meridian LCAT titers. Fourteen percent of the Immy titers were more than 1 dilution greater, and 29% were more than 1 dilution less than the Meridian LCAT titers.

A positive India ink preparation suggests a high antigen load. Thirteen (68%) of 19 true-positive CSF samples had a positive India ink stain. Ninety-six percent of India ink-positive CSF samples had titers ≥1:256 by all four kits. Of the 19 true-positive samples, 18 had sufficient specimen volume for titration. Fifteen (83%) of these 18 true positives had a higher titer (≥1:256) by the Meridian LCAT. The three other LCAT kits were in agreement that the titer was high 95% of the time. Three of the 18 (17%) true positives were at a low titer (<1:256) by the Meridian LCAT. The three other LCAT kits agreed that the titer was low only 56% of the time.

Twenty-two (73%) of the 30 true-positive serum samples had titers of ≥1:256 by the Meridian LCAT. There was 91% agreement that the titer was high by the three other LCAT kits. Eight of 30 (27%) true-positive samples by the Meridian LCAT had low titers. The other three LCAT kits agreed that the titer was low 48% of the time.

The Meridian EIA kit titers roughly paralleled the LCAT kits (i.e., high titers by LCAT were also high by EIA). However, the values obtained by EIA were always higher than the LCAT values, although the correlation was not linear.

DISCUSSION

This is the first systematic comparison of all commercially available kits for detection of cryptococcal antigen since 1983 (19) and the first during the AIDS epidemic. There were three LCAT kits compared in 1983: IBL, MicroScan (formerly marketed by American Scientific Products), and Immy. In that study (19), the IBL kit had a sensitivity and specificity of 100% for both serum and CSF, the MicroScan had a sensitivity of 100% for both serum and CSF and specificities of 99% for serum and 100% for CSF, and the Immy had sensitivities of 45% for serum and 83% for CSF and a specificity of 100% for both serum and CSF. On the basis of the methodologies used in 1983 (19), there have been substantive changes to the Immy kit since 1983, including the addition of pronase treatment. Our data show that both its sensitivity and its specificity have improved significantly. Moreover, 6 of 12 and 3 of 4 Immy false positives from serum and CSF, respectively, could have been eliminated if the threshold for a positive result had been defined as a titer of 1:2 or greater rather than 1:1. This interpretive revision would have further improved specificity and not reduced sensitivity at all.

Perhaps most notable among the LCAT kits were the differences in sensitivities of these kits in detecting cryptococcal antigen in serum. In our study the sensitivity of those kits that included pronase treatment of serum was remarkably higher than the sensitivity of those that did not (97 versus 83%). Among those kits that did not use pronase, we found a 6% false-negative rate and, in the two samples we were able to

test, demonstrated that these could be eliminated by pronase treatment. This false-negative rate is lower than the 19 and 27% rates reported by Gray and Roberts (11) and Hamilton et al. (12), respectively. The value of pronase in eliminating false negatives for serum samples has been demonstrated previously (11, 12). It is apparent, therefore, that if serum cryptococcal antigen titers are to be used as a screening test for cryptococcosis in AIDS patients, pronase treatment should be used to avoid a significant loss of sensitivity.

In contrast with serum specimens, it does not appear that pronase treatment is necessary for CSF samples. There were no positives detected with the Immy kit, which uses pronase on CSF, that were not detected by the kits that do not use pronase treatment of CSF, confirming the findings of Hamilton et al. (12).

Our data indicate that LCAT kits from different manufacturers should not be used interchangeably for determining titers. Hamilton and colleagues demonstrated that the IBL and Meridian LCAT kits were concordant for only 54% of serum specimens and 86% of CSF samples when used according to manufacturers' instructions (12). Our data are remarkably similar: 60% agreement between the IBL and Meridian LCAT kits for serum and 57% for CSF. Concordance between the Immy and Meridian kits was 55% for serum and 57% for CSF. The MicroScan and Meridian kits were in agreement for only 30 and 42% of the serum and CSF titers, respectively.

Although it is clear that titers from different kits cannot be used interchangeably in individual patients, it is equally important to recognize that many clinical studies of cryptococcosis have not controlled for kits (9, 14, 17). Some have provided no data as to which kits were used and, nevertheless, drawn conclusions pertaining to clinical outcomes based on titers (1–4, 13, 18, 20). Others have used titers obtained by different test methods (e.g., complement fixation and latex agglutination) interchangeably (6). In light of our data, any LCAT titer for which clinical prognostic significance is ascribed should be suspect, unless the investigators have controlled for the specific test kit used. Saag et al. (17) used different kits at different centers in their multicenter study of fluconazole versus amphotericin B as therapy for cryptococcal meningitis (16a). Given this information, the prognostic significance of a titer of ≥1,024 in their study is uninterpretable. Further, when studies have controlled for particular kits, reported titers apply only to the specific kits used (5, 7, 15). We believe that too much emphasis has been placed on antigen titer in the clinical management of cryptococcal disease, especially in association with AIDS. Indeed, there are as yet no published studies, controlled for test kit, that have associated specific titers with clinical course or outcome in a systematic fashion. Powderly et al. (16) have looked retrospectively at the clinical utility of serum and CSF antigen titers using data from trials that did not control for kits and have found them to be of limited use. At this time, culture and clinical response would seem more reliable than antigen titers as measures of response to anticryptococcal therapy. In this study, all LCAT kits showed 90% concordance when titers were high (≥1:256). Agreement among kits for low-titer samples was not as good, but the small number of samples did not permit any definite conclusions. Thus, a breakpoint such as ≥1:256 may be as useful clinically as a titer of ≥1,024, but this needs to be demonstrated in a controlled prospective trial.

In general, the LCAT kits should be viewed as equivalent in diagnosing cryptococcal disease in CSF. However, the Meridian and Immy are superior to the IBL and MicroScan for serum because they include pronase. The Meridian LCAT appears to be the most sensitive and specific kit overall. All kits were easy to use, although end point determination was easiest with the Meridian LCAT kit.

The Meridian EIA kit was as sensitive and specific as the best LCAT kit. The most significant problem we encountered concerned two kits that included controls that were repeatedly out of range. This problem is being remedied by the manufacturer (10a). As with most EIA kits, this kit is best used with fairly large numbers of samples that can be batched. We found that a washer was useful for infection control purposes, allowing us to deal with human immunodeficiency virus-positive body fluids without risk of splashing. As shown by Gade et al. (10), titers with the EIA are calculated and bear no numerical relation to LCAT titers (10). However, in general, high titers by EIA were also high by LCAT.

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