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Comparison of Three Commercial Cryptococcal Latex Kits for Detection of Cryptococcal Antigen

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Three commercial latex kits, IBL, MYCO-Immune, and IMMY, for the detection of cryptococcal antigen were compared in regard to sensitivity, specificity, and height of antigen titers. A total of 218 cerebrospinal fluid and 79 serum specimens from 239 patients were included. Twenty-two patients had culture-proven disseminated cryptococcosis. Both the IBL and MYCO-Immune kits had sensitivities of 100%, and the IMMY kit had sensitivities of 82.6 and 45.4% in CSF and serum specimens, respectively. There was one false-positive reaction in serum with the MYCO-Immune kit and one false-negative reaction on screen only with all three kits. Rheumatoid factor-containing sera were used to check the agglutination titers between matching anti-cryptococcal globulin reagents and normal globulin reagents. The finding that agglutination titer with anti-cryptococcal globulin reagents was fourfold higher than with normal globulin reagents in the MYCO-Immune kit is considered to be a cause for a false-positive reaction in serum.

Detection of the polysaccharide antigen of *Cryptococcus neoformans* in body fluids by antibody-coated latex particle agglutination, first described by Bloomfield et al. in 1963 (2), has become an important adjunct to the diagnosis of cryptococcal infection. The test is simple, sensitive, and specific. Despite the increasing use of the latex agglutination test, there has been no report which compares the detection of cryptococcal antigen by various commercial kits. In this paper, we report the sensitivity of three commercial kits in the detection of cryptococcal antigen, the comparability of the height of cryptococcal antigen titers, and the occurrence of false-positive and false-negative results.

MATERIALS AND METHODS

A total of 218 cerebrospinal fluid (CSF) and 79 serum samples from 239 patients were used in the present study. Sixteen cases of culture-proven cryptococcal infection were included in retrospective studies, and the remaining specimens were collected prospectively between November 1981 and November 1982 from patients suspected of systemic mycotic infection at the Clinical Center, National Institutes of Health, Bethesda, Md. In the prospective series, six cases of cryptococcal infections were diagnosed based on the isolation of *C. neoformans* from various body sites including CSF, blood, and urine. Specimens from retrospective series had been stored at −20°C without preservatives for a period between 2 months and 10 years. Before being tested, specimens with a cloudy appearance were cleared by centrifugation at 12,000 × *g* for 5 min.

Three commercial latex cryptococcal antigen test (LCAT) kits were chosen for the study: IBL (International Biological Laboratories, Cranbury, N.J.), MYCO-Immune (American Scientific Products, McGraw Park, Ill. [formerly manufactured by Meridian Diagnostics, Inc., Cincinnati, Ohio]), and IMMY (Immuno-mycologics, Norman, Okla.). Each specimen was divided into three portions and tested according to the manufacturers' instructions. The testing procedures differed somewhat among the three kits; pertinent differences are summarized in Table 1.

Purified polysaccharide preparation from *C. neoformans* serotype A, kindly supplied by J. E. Bennett, National Institute of Allergy and Infectious Diseases, was dissolved at a concentration of 126 μg/ml in 0.1 M glycine-buffered saline (pH 8.4) containing 0.1% bovine serum albumin. Calbiochem-Behring RAPI/TEX-RF kit (Calbiochem-Behring, La Jolla, Calif.) was used to test the presence of rheumatoid factor (RF). In this report, dilution titers are used interchangeably with the reciprocals of the titers.

RESULTS

Agglutination with anti-cryptococcal globulin reagents (ACGR). When known concentrations of purified cryptococcal polysaccharide solution were used, the IBL, MYCO-Immune, and IMMY kits could detect at the minimal concentrations of 0.019, 0.019, and 0.157 μg/ml, respectively. Throughout the study period, a total of five kits (from four lots), four kits (from three

TABLE 1. Procedural differences among three commercial LCAT kits

LCAT kit	Specimen pretreatment		Screen dilution	RF serum control	Antiglobulin control	ACGR: specimen volume ratio	Slide
	Serum	CSF					
IBL	56°C, 30 min	56°C, 30 min	Undiluted	No	No	1:1	Glass
MYCO-Immune	56°C, 30 min	100°C, 3 min	Undiluted	No	Yes	1:1	Glass
IMMY	56°C, 30 min	56°C, 30 min	Undiluted and 1:10	No	Yes	1:2	Disposable card

lots), and three kits (from two lots) of the IBL, MYCO-Immune, and IMMY, respectively, were used. Minimal concentrations of antigen detected by ACGR in each kit showed no variation from kit to kit or from lot to lot with respect to all three products.

The inter-run precision of the measurement of cryptococcal antigen titer by the use of each commercial kit was examined by using cryptococcal polysaccharide preparation and antigen-positive serum and CSF specimens. Each specimen was tested in five different runs by the same technologist. Geometric mean titers, geometric standard deviations, and titer ranges were calculated according to Taylor et al. (7). All three kits demonstrated excellent inter-run precision when cryptococcal polysaccharide was tested (Table 2). ACGR from both the IBL and MYCO-Immune kits showed excellent precision in CSF and a geometric standard deviation of less than 1.70 in serum. In contrast, there were considerable inter-run variations in the titers of cryptococcal antigen in both CSF and serum when the IMMY kit was used.

Culture-proven cryptococcal patients. Twenty-two cases of disseminated crytococcosis, including 17 cases of cryptococcal meningitis, were used in the study. Both the IBL and MYCO-Immune kits were able to detect the presence of cryptococcal antigen in all 23 CSFs and 11 sera from these patients (Table 3). Comparing the heights of antigen titers determined by both kits, results of CSF from the MYCO-Immune kit were either twofold higher or lower than those of the IBL. The antigen titers in the serum showed greater variations between these two kits; i.e., antigen titers determined by the MYCO-Immune kit showed an eightfold variation, generally lower, from that of IBL. The antigen titers in CSF determined by the IMMY kit were consistently eightfold lower than that of the IBL. Thus, CSFs that had titers between 1:2 and 1:16 by the IBL kit were negative by the IMMY kit. A similar pattern of lower antigen titers was observed in the serum, and the difference could be as much as 16-fold. Six sera having antigen titers from 1:16 to 1:128 by the IBL kit were determined as negative by the IMMY kit.

Of special interest was one serum with an antigen titer of 1:1,024 by all three kits. On the initial screen, a very weak agglutination was revealed by the IBL and MYCO-Immune kits; with the IMMY kit, there was a completely negative reaction with undiluted specimen but a weak reaction with a specimen diluted 1:10. This specimen was obtained from a fulminant fatal case of disseminated cryptococcosis in a patient with underlying acute lymphoblastic leukemia.

Noncryptococcal patients. A total of 195 CSFs and 68 sera from 217 patients in whom cryptococcal infection could not be documented by culture were studied. Infectious etiologies other than cryptococcosis were established for some patients, including viral meningitis (three cases), systemic mycotic infections such as candidiasis (four cases), histoplasmosis (two cases), blastomycosis (one case), coccidioidomycosis (two cases), actinomycosis (one case), sporotrichosis (one case), and *Exophiala jeanselmei* infection (one case). There were no false-positive results

TABLE 2. Comparison of inter-run precision by manufacturer[a]

Source of sample	IBL			MYCO-Immune			IMMY		
	$\bar{X}_G$	SD_G	Titer range	$\bar{X}_G$	SD_G	Titer range	$\bar{X}_G$	SD_G	Titer range
Cryptococcal polysaccharide (1.2 μg/ml)	64	1	64	64	1	64	8	1	8
CSF	32	1	32	32	1	32	4	2.65	1–8
Serum	256	1	256	42	1.47	32–64	4	2.89	1–16

[a] $\bar{X}_G$, Geometric mean of titers ($\bar{X}_G$ = antilog $\bar{X}_{log}$); SD_G, geometric standard deviation (SD_G = antilog SD_{log}). A distribution with a SD_G of less than 1.70 would have at least 95% of the results within a twofold difference from the mean and would be considered to have acceptable variation. (R. N. Taylor, personal communication).

TABLE 3. Comparison of three commercial LCAT kits in clinical specimens

LCAT kit	No. of samples with the following result in[a]:					
	CSF (n = 218)			Serum (n = 79)		
	True positive	False-positive	False-negative	True positive	False-positive	False-negative
IBL	23	0	0	11	0	0
MYCO-Immune	23	0	0	11	1	0
IMMY	19	0	4	5	0	6

[a] Including multiple specimens from the same patient.

by either the IBL or the IMMY kit. A serum sample from a case of actinomycosis agglutinated with ACGR at a titer of 1:8 and with normal globulin reagents (NGR) at a titer of 1:2 by the MYCO-Immune kit. Since the two titers differed by fourfold, the result was regarded as positive according to the manufacturers' instructions.

Agglutination with NGR. The incidence of interference due to nonspecific agglutination with NGR was zero among 218 CSF specimens tested. There were five sera (6.3%), two from cryptococcal patients and three from noncryptococcal patients, which demonstrated interference with all three commercial kits. The interference titers were generally low, ranging from 1:2 to 1:32. The single false-positive result by the MYCO-Immune kit may be attributed to unequal reactivities between the matching ACGR and NGR in the kit. We explored the possibility by using five sera from noncryptococcal patients known to contain RF titers ranging from 1:80 to 1:2,560 to compare the agglutination reactivities of ACGR and NGR from three commercial kits. The IMMY kit had the lowest cross-reactivity with RF in serum (Table 4). Both the IBL and the IMMY kit demonstrated no more than a twofold difference in the agglutination titers between their respective matching ACGR and NGR. A fourfold difference in the agglutination between ACGR and NGR in the MYCO-Immune kit demonstrated by cases 9 and 10 supported our previous explanation of a false-positive result encountered in clinical specimens.

DISCUSSION

Both the IBL and MYCO-Immune kits achieved 100% sensitivity in detecting cryptococcal antigen in clinical specimens, and the IMMY kit had sensitivities of 82.6 and 45.4% in CSF and serum, respectively. Relatively less potent ACGR provided by the IMMY kit is a major factor for the low sensitivity. If the volume ratio of ACGR to specimen in the final reaction mixture were 1:1 instead of 1:2, the sensitivity of IMMY kit might be improved slightly. The disposable card used in the IMMY kit also presented several disadvantages as compared with the glass slides. The disposable card did not provide a clear-cut background for discerning the intensity of agglutination and occasionally absorbed moisture from the reaction mixture.

The comparability of the titers of the cryptococcal antigen among different commercial kits depended on the nature of the specimens. Antigen titers in sera gave wider variations than those in CSF. With the IMMY kit, the run-to-run variation was also more pronounced in the serum samples.

A false-negative result on initial screen of a serum sample with a titer of 1:1,024 could not be

TABLE 4. Results of ACGR and NGR titers from sera of study cases and patients with rheumatoid arthritis

Case no.	Diagnosis	RF titer	ACGR (NGR) titer		
			IBL	MYCO-Immune	IMMY
1	Cryptococcosis	<1:10	1:16 (1:2)	1:8 (1:2)	1:2 (0)
2	Cryptococcosis	<1:10	1:16 (1:2)	1:8 (1:1)	1:2 (1:2)
3	Actinomycosis	<1:10	1:16 (1:8)	1:8 (1:2)	0 (0)
4	Histoplasmosis	<1:10	1:32 (1:16)	1:16 (1:8)	0 (0)
5	Hodgkin's disease	1:320	1:32 (1:32)	1:32 (1:32)	1:16 (1:16)
6	Rheumatoid arthritis	1:80	0 (0)	0 (0)	0 (0)
7	Rheumatoid arthritis	1:320	1:2 (1:2)	1:1 (0)	0 (0)
8	Rheumatoid arthritis	1:640	1:128 (1:128)	1:2 (1:1)	0 (0)
9	Rheumatoid arthritis	1:1,280	1:512 (1:512)	1:512 (1:128)	1:64 (1:64)
10	Rheumatoid arthritis	1:2,560	1:512 (1:265)	1:256 (1:64)	1:4 (1:2)

attributed entirely to the antigen excess present in the serum, since two other clinical specimens with considerably higher titers (2,048 in serum, 8,192 in CSF) showed no evidence of prozone phenomenon. The occurrence of blocking antibody in some clinical specimens should be considered. Routine dilution by the laboratory on all negative specimens does not seem to be cost-effective. As an adjunct diagnostic test, the LCAT should be repeated on diluted specimens in cases in which there are positive findings in India ink preparation or culture.

The detection of cryptococcal antigen is sometimes obscured by the presence of RF and other interfering substances (1). Our results revealed that the interference is limited in serum. The fact that we did not encounter interference in CSF could be attributed to the small number of samples in our study. Stockman and Roberts reported a 1% interference incidence in their study of 9,000 CSF specimens (6).

According to all three manufacturers, the titer of ACGR must be at least fourfold higher than that of NGR to validate a positive result in the presence of interference. Such recommendation, however, lacks literature support. A fourfold difference may not be wide enough in cases of unequal reactivities of matching ACGR and NGR, such as the MYCO-Immune kit. The problem of unequal reactivities might have been avoided if the latex from NGR had been sensitized with preimmune globulin taken from the same rabbit used to produce the anti-*C. neoformans* globulin. Consequently, both globulins should contain identical allotypes. Information provided by the manufacturers indicated that pooled globulins were used in all three commercial kits. Based on our findings, we recommend that ACGR and NGR from a new kit should be checked quantitatively by a RF-containing serum (titer 1:1,280 or greater) or anti-globulin control reagents as provided in the MYCO-Immune and IMMY kits. If the agglutination titer produced by ACGR is fourfold or greater than by NGR, an additional procedure to eliminate interfering substances in clinical specimens should be used. Methods for the latter purpose include dithiothreitol treatment (5) and EDTA-heat extraction (4). One disadvantage of these two methods is the dilution of specimens with reagents before testing. More recently, Stockman and Roberts described an enzymatic procedure involving pronase treatment of clinical specimens (6). The enzymatic method required no dilution of specimens and seemed simple to perform.

In this study, however, we did not have a sufficient number of cases to allow serial LCAT using the three commercial kits. Although the antigen titers may be used as an indication of the response to treatment (3), considerable variation may be expected due to variability in the sensitivity of a particular commercial kit. Work to establish prognostic criteria by using different commercial LCAT kits is in progress.

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