Commercial Cryptococcal Latex Kit: Clinical Evaluation in a Medical Center Hospital

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A commercial latex kit for the detection of cryptococcal antigen (LCAT) was used in a medical center hospital to test cerebrospinal fluid (CSF) and serum specimens from patients suspected of having cryptococcal infections. The LCAT was also performed on 8 CSF and 2 serum specimens from other mycotic infections and on 50 serum specimens from healthy controls. Of a total of 561 specimens (489 CSF and 72 sera) from 426 patients, 78 (13.9%) specimens were LCAT positive; these came from 12 patients with culturally proven cryptococcosis. Eleven of these 12 patients were diagnosed as having disseminated cryptococcosis (9 with meningitis). Fourteen other patients, all with positive cultures for *Cryptococcus neoformans* but negative LCATs on sera or CSF, were found not to have disseminated infections. All CSF and sera from other mycotic infections and all 50 control sera were negative. No false positive or false negative tests were encountered. It was concluded that the commercial kit is useful for diagnosis and prognosis of disseminated cryptococcosis, but not as useful for infections such as localized pulmonary or cutaneous cryptococcosis.

Since the work of Bloomfield et al. (2) in 1963 on the detection of *Cryptococcus neoformans* antigen in body fluids, most workers have found the latex test for cryptococcal antigen (LCAT) to provide a reliable means of determining the presence of *C. neoformans* antigen in cerebrospinal fluid (CSF) or sera of patients with disseminated cryptococcosis (1, 4, 6, 7). However, Dolan (3) has reported the LCAT to give false positive reactions and cross-reactions to other mycotic or actinomycotic infections, and Lauter (9) has reported problems with the interpretation of the commercial test.

The first kit to become commercially available was manufactured by Canalco, Inc. in 1971. This kit was later evaluated by workers at the Center for Disease Control, who found that 14 of 24 kits were unsatisfactory for clinical use "due to failure of the latex suspension sensitized with anti-*C. neoformans* globulin (LA) to agglutinate the polysaccharide control necessary to validate the test" (8). The Canalco kit was removed from the market in 1973 but was remarketed by International Biological Laboratories in 1975. Our laboratory has been using the International Biological Laboratories commercial kit for over 3 years. A review of our experience with this kit was compiled to determine (i) its efficacy in detection of antigen in patients culturally proven to have cryptococcosis, (ii) occurrence of any falsely positive or negative tests, (iii) possible detection of

antigen in other mycotic infections and in a normal population, and (iv) prognostic value in culturally proven cryptococcosis.

MATERIALS AND METHODS

Test reagents and controls. Each Crypto-LA Kit (International Biological Laboratories, Rockville, Md.) consists of a Boerner slide and six reagents: latex particles sensitized with rabbit anticryptococcal antibody (LA), latex particles sensitized with normal rabbit globulin (LN), cryptococcal polysaccharide antigen of a stated concentration, a negative control serum, a serum containing rheumatoid factor, and glycine-buffered saline. The test is based upon the principle that latex particles coated with *C. neoformans* antibody will agglutinate in the presence of cryptococcal polysaccharide antigen (2).

Each time the kit is used to test patient specimens, the sensitivity of the reagents should be determined. The cryptococcal polysaccharide antigen is diluted twofold, resulting in known antigen content in each of five tubes: 0.125 through 0.007 $\mu\text{g/ml}$. These dilutions are tested with the reagents and must detect at least 0.06 μg of the antigen per ml with a positive agglutination before the kit can be used. Serum or CSF (heat inactivated) is then tested with both the LA reagent (anticryptococcal latex) and the LN reagent (normal globulin latex). These reagents are also tested with the negative serum and with the rheumatoid factor serum. The LN reagent is used to detect rheumatoid factor, which is known to cause false positive agglutinations in the LCAT (1). The slide is rotated and read for a distinct positive agglutination. Weak agglutination is read as negative. (The criterion of a positive aggluti-

nation in the present study was an intensity of reaction equal to that elicited by the rheumatoid factor control that was provided. Anything less was considered as weak and, therefore, negative.)

Handling of specimens. Patient CSF supernatant or serum was screened with both the LA and LN reagents according to the manufacturer's instructions. Each specimen that gave a positive agglutination reaction with the LA reagent alone was serially diluted to 1:1,024 and retested with the LA reagent to determine the cryptococcal antigen titer. Each specimen that gave positive agglutination reactions with both the LA and LN reagents was serially diluted to 1:1,024 and retested with both reagents. Specimens that had an LA titer at least fourfold higher than the LN titer were interpreted as positive for cryptococcal antigen even though showing interference by nonspecific agglutination; those having a titer less than fourfold higher than the LN titer were interpreted as equivocal due to interference by nonspecific agglutination.

Patients. Of 26 patients culturally proven to have some form of cryptococcosis, 11 were diagnosed as having disseminated cryptococcosis (9 with meningitis) and 15 as having localized primary pulmonary or primary cutaneous cryptococcosis. Patients strongly suspected of having disseminated cryptococcosis, but not culturally proven, were followed by means of a telephone inquiry to the patient's physician at periodic intervals to determine the outcome. Normal sera were obtained from healthy controls.

RESULTS

Over a period of 3 years 561 specimens (72 serum and 489 CSF) from 426 patients suspected of having a cryptococcal infection, and 50 additional serum specimens from healthy controls, were tested with the LCAT. Of the specimens from the patients, 78 (13.9%) (49 CSF and 29 sera from 12 patients culturally proven to have cryptococcosis) were positive. No false positive tests were noted; eight CSF and two sera from other mycotic or actinomycotic infections and all 50 control sera were negative (Table 1). Fourteen CSF specimens (but no serum specimens) exhibited interference due to nonspecific substances; they were positive with the LN as well as with the LA reagent (Table 2). When these specimens were serially diluted and retested with both reagents, only 5 of the 14 specimens met the stated criterion of having at least a fourfold-higher titer with LA than with LN. All five of these specimens yielded *C. neoformans* by culture.

The temporal profile of the LCAT titers on culturally proven cases of disseminated cryptococcosis is shown in Table 3. LCAT titers on multiple specimens were obtained from eight patients with meningitis and two patients with nonmeningeal cryptococcosis. All 10 patients who had more than one LCAT during treatment showed a significant decrease in titer concomitant with their clinical improvement.

TABLE 1. Results of LCAT on CSF and sera from patients and controls during a 3-year period

Source	Specimen	No. tested	No. (%) positive ^a	False positive reactions
Suspected cryptococcosis	CSF	489	49 (10)	0
	Serum	72	29 (40.3)	0
Other known mycotic or actinomycotic infections ^b	CSF	8	0	0
	Serum	2	0	0
Normal controls	Serum	50	0	0

^a All positive tests were from 12 culturally proven cases of cryptococcosis, which included 11 patients with disseminated cryptococcosis (9 with meningitis) and 1 with pulmonary cryptococcosis.

^b Pulmonary nocardiosis (1 CSF), rhino-cerebral phycosporiosis (1 CSF), candidosis (2 CSF, 1 serum), pulmonary aspergillosis (1 CSF, 1 serum), disseminated blastomycosis (1 CSF), disseminated histoplasmosis (1 CSF), actinomycotic brain abscess (1 CSF).

TABLE 2. Interpretation of results obtained on 14 CSF specimens (from 12 patients) which agglutinated both LA and LN reagents in the LCAT

Patient	LA titer	LN titer	Interpretation of LCAT ^a	Culture
1	1:1	1:1	Equivocal	Negative
2	1:8	1:16	Equivocal	Negative
3	1:256	1:16	Positive	<i>C. neoformans</i>
4	1:256	1:8	Positive	<i>C. neoformans</i>
5	1:256	1:16	Positive	<i>C. neoformans</i>
6	1:2	1:2	Equivocal	Negative
7	1:16	1:16	Equivocal	Negative
8	1:64	1:2	Positive	<i>C. neoformans</i>
9	1:4	1:4	Equivocal	Negative
10	1:1	1:1	Equivocal	Negative
11	1:2	1:2	Equivocal	Negative
12 ^b				
(1)	1:8	1:4	Equivocal	Negative
(2)	1:4	1:4	Equivocal	Negative
(3)	1:32	1:4	Positive	<i>C. neoformans</i>

^a Equivocal, Equivocal test for cryptococcal antigen due to interference by nonspecific agglutination. Positive, Positive test for cryptococcal antigen with interference by nonspecific agglutination.

^b This patient had three CSF specimens taken as an out-patient. It is not known if the titer of the first and second reflect early cryptococcal meningitis or if her neurological disorder caused nonspecific agglutination. Specimen 2 was taken 6 days later. Specimen 3 was taken 30 days after no. 2, and patient was admitted for therapy (see also patient no. 8, Table 3).

There were four cases of meningitis (patients 4, 5, 7, and 8, Table 3) for whom therapy was initiated solely on the basis of the LCAT. India-ink smears on these patients' CSF were repeatedly negative, but the CSF cultures yielded *C.*

TABLE 3. *Laboratory and clinical data of 11 patients with disseminated cryptococcosis, monitored by the LCAT^a*

Patient	Underlying problems	Positive culture sites	LCAT specimen and titers ^b	IFA ^c	Outcome
1	Chronic active hepatitis	CSF, lung	CSF: 1:128 ^d	—	Expired
2	Diabetes; possible lymphoma	CSF	CSF: 1:256 ^d ; (9) 1:256; (10) 1:256; (9) 1:128; (8) 1:256; (27) 1:256; (12) 1:128; (14) 1:128 ^e ; (134) 1:128; (52) 1:64; (41) 1:64; (69) 1:64	—	Discharged
3	Chronic lymphocytic leukemia; pneumonia	CSF, urine	CSF: 1:64 ^d ; (12) 1:32; (24) 1:4; (15) 1:4 (12) ^e ; (19) negative; (10) 1:1 ^{d,f} ; (8) 1:1; (19) negative; (15) negative (5) ^g ; (11) negative	—	Discharged
4	Chronic lymphosarcoma, Stage IV	CSF, urine	CSF: 1:128 ^d ; (8) 1:256; (12) 1:128; (13) 1:128; (7) 1:32 (2) ^e	—	Expired after discharge
5	Arthritis; pneumonia	CSF	CSF: 1:256 ^d ; (8) 1:256; (20) 1:64; (13) 1:64 ^e	—	Discharged
6	Systemic lupus erythematosus; pulmonary nocardiosis	CSF, blood	CSF: 1:256 ^d ; (6) 1:128; (16) 1:64; (34) 1:4 ^e	+	Discharged
7	Chronic active hepatitis	CSF	CSF: 1:256 ^d ; (13) 1:64; (17) 1:8; (18) 1:4 ^e ; (205) negative	+	Discharged
8	Alcoholic; chronic liver disease; neurological disorder	CSF	CSF: equivocal ^h ; (6) equivocal; (30) 1:32 ^d ; (39) negative	—	Currently undergoing therapy
9	Hodgkin's disease	CSF	CSF: 1:512 ^h ; (32) 1:256	—	Expired
10	Hyper-immunoglobulin M syndrome	Urine, cervical lymph node, skin	Serum: 1:128 ^d ; (12) 1:32; (42) 1:4 (14) ^e CSF: Negative	—	Expired after discharge
11	T-cell defect	Blood, cervical lymph node	Serum: 1:512 ^d ; (30) 1:32; (16) 1:16; (22) 1:4 ^e ; (42) negative; (74) 1:8 ^{d,i} ; (19) negative (58) ^e CSF: Negative	—	Discharged

^a Various laboratory and clinical data of 11 patients with disseminated cryptococcosis. Their clinical response to therapy was monitored by means of the LCAT. Therapy, in all cases, was amphotericin B + 5-fluorocytosine.

^b Number (in parentheses) preceding titer denotes interval in days since last LCAT titer.

^c Indirect fluorescent test (IFA) for cryptococcal antibody was performed at the South Carolina Bureau of Laboratories.

^d Antifungal therapy was started on same day as given titer or within 48 h afterwards.

^e Antifungal therapy discontinued on same day as given titer or on day in parentheses after given titer.

^f Clinically apparent relapse of cryptococcal meningitis; readmitted and retreated.

^g Nonspecific agglutination; see also patient no. 12, Table 2.

^h Patient diagnosed and started on antifungal therapy at another hospital; titer given is on second specimen.

ⁱ Patient developed a fibrotic lesion at C₆–C₇ vertebra, resulting in paraplegia. It was concluded that this lesion represented a cryptococcoma. He was retreated.

neoformans after 8 to 10 days. All four patients were started on antifungal therapy well before the cultures were positive in spite of the negative India-ink smears.

There were 15 isolations of *C. neoformans* in patients with localized cryptococcal infections other than meningitis, viz. 14 pulmonary and 1 cutaneous. Only one of these patients had detectable antigen (Table 4).

DISCUSSION

The purpose of the LCAT is twofold: (i) to rapidly detect cryptococcal antigen in serum or CSF to facilitate early diagnosis, and (ii) to provide a means of prognosis by detecting progressive changes in antigen level. This laboratory has found that both of these criteria are adequately met with the commercial kit. In all

TABLE 4. Laboratory and clinical data on 15 patients with localized pulmonary, cutaneous or possibly transient cryptococcosis whose specimens were tested with LCAT

Patient	Underlying problem	Positive culture sites ^a	No. of <i>C. neoformans</i> colonies	LCAT on CSF and serum ^b	Placed on anti-fungal therapy	IFA ^c	Outcome
A	Chronic bronchitis	Sputum	<50	Negative	No	+	Discharged
B	Liposarcoma; bronchial pneumonia	Sputum	<10	Negative	No	ND	Discharged
C	Pulmonary granulomatous infection; sarcoid	Bronchial washings	>100	Negative	Yes	+	Discharged
D	Diabetes; bacteremia and pneumonia	Sputum	<10	Negative	No	ND	Expired
E	Bilateral pneumonia	Sputum	<10	Negative	No	-	Discharged
F	Chronic bronchitis	Transtacheal aspiration	<50	Negative	Yes	+	Discharged
G	Chronic bronchitis	Sputum	<10	Negative	No	+	Discharged
H	Chronic bronchitis	Sputum	<10	Negative	No	-	Discharged
I	None found	Skin lesion ^d	>100	Negative	Yes	+	Discharged
J	Bilateral lung infiltrates; pneumonia	Sputum ^e	>100	1:32 (serum only)	Yes	+	Discharged
K	Chronic bronchitis	Sputum	<10	Negative	No	ND	Discharged
L	Right cavitory lung lesion	Bronchial washings	<50	Negative	No	+	Discharged
M	Sarcoid; extensive right lung infiltrates	Bronchial washings	>100	Negative	Yes	+	Discharged
N	Active tuberculosis	Sputum	<10	Negative	No	-	Discharged
O	Active tuberculosis	Sputum	<10	Negative	No	ND	Discharged

^a Source listed was the only culturally positive site for *C. neoformans* although multiple sites were cultured (CSF, urine, blood) from all patients except J, N, and O (sputum only).

^b LCAT was performed on both CSF and serum on all patients except J, N, and O (serum only).

^c Indirect fluorescent cryptococcal antibody test (IFA) performed at the South Carolina Bureau of Laboratories, on serum only. ND, Not done.

^d After extensive culturing and study of this patient, it was concluded that the skin lesion was a case of true primary cutaneous cryptococcosis due to a splinter.

^e It is possible that this patient had early disseminated cryptococcosis. He had a history of chronic pneumonia for many months and multiple sputum specimens grew many colonies of *C. neoformans*; urine, blood, and CSF were not submitted for cultures; he was discharged on 5-fluorocytosine and lost to follow-up.

11 cases of disseminated cryptococcosis (9 with meningitis) and in 1 case of pulmonary cryptococcosis, a positive LCAT was obtained. In four of the nine patients with cryptococcal meningitis, the LCAT was the only means by which the diagnosis was made without the delay of waiting for a positive culture.

As shown in Table 3, 10 patients responded to chemotherapy with at least a fourfold drop in the LCAT titer, thus demonstrating the prognostic value of the test.

LCAT titers were negative in 14 of 15 cases of localized primary pulmonary or primary cutaneous cryptococcosis, which is expected in infections with low concentrations of cryptococcal antigen. Bloomfield and associates (2) reported that negative LCAT results may occur in chronic low-grade cryptococcosis, in early infections, and during remission following antifungal therapy. Further laboratory and clinical follow-up is sug-

gested by Randhawa and Pal (10) for all patients whose specimens yield *C. neoformans* even when definitive manifestations of cryptococcosis are absent. We make every attempt to follow these patients with additional cultures, LCAT, and indirect fluorescent tests for cryptococcal antibody regardless of the number of colonies of *C. neoformans* isolated.

Nonspecific agglutination reactions, observed only in CSF, could not be clinically related to rheumatoid arthritis in any of the patients. Many of these patients, however, were known to have other single or multiple immunological problems, such as anti-nuclear antibodies, or to have neoplasms that produce abnormal proteins, such as myeloma proteins. In any case, it appears that screening CSF specimens with normal globulin in the LCAT is desirable to prevent false positive tests, since eight patients (Table 2) would otherwise have been reported as LCAT

positive. Nine specimens from these eight patients were culturally negative for *C. neoformans*. It should be noted, however, that Gordon and Vedder (5) have reported that heating CSF to 100°C for 10 min will eliminate some nonspecific reactions, a procedure of which we were unaware at the time our study was undertaken.

Recently, a letter by Lauter (9) criticized the commercial LCAT for demonstrating titers that were higher than a patient's symptoms reflected, and, in his view, caused unjustifiably prolonged treatment with amphotericin B. We have found that the main pitfall in reading an LCAT titer is in the interpretation of a "positive" agglutination reaction. Interpretation of a weak reaction as positive will invariably cause higher titers and false positive tests. In fact, in several instances we have observed that when the positive agglutination reaction stopped at lower dilutions, a weak reaction was present up to 1:1,024. Kaufman and Blumer (7) addressed themselves to this problem in response to poor results reported by Dolan (3) with the LCAT. They emphasized that laboratories should record as positive only those reactions that show an intensity of agglutination equal to or stronger than the positive control. Unfortunately, the commercial LCAT contains no positive control as such, but the cryptococcal polysaccharide antigen reagent may be used as a positive control. We have found, however, that the antigen content of this reagent rapidly decreases unless it is stored at -20°C. The rheumatoid factor positive control serum provided in the Crypto-LA kit consistently produces a distinct positive agglutination reaction for comparison so that weak reactions are not misinterpreted as positive. This latter technique is not suggested in the kit's instructions, but we feel that such incorporation of a positive agglutination control alleviates confusion and allows consistent interpretation.

In our experience, the sensitivity of the Crypto-LA kit has been reliable. We have used some 27 kits with nine different lot numbers, and each kit consistently detected 0.03 to 0.06 µg of the cryptococcal polysaccharide reagent per ml.

In our retrospective study of the Crypto-LA

kit on CSF and serum specimens we determined that (i) the kit provided a rapid, reliable index of cryptococcal antigen for diagnostic and prognostic use in cases of disseminated cryptococcosis; (ii) it did not detect cryptococcal antigen in serum or CSF from most localized pulmonary or cutaneous cryptococcal infections, in CSF or sera from other mycotic infections, or in healthy control sera; (iii) nonspecific agglutination was found only in CSF (it could not be clinically related to rheumatoid arthritis), but this reaffirms the need to screen CSF as well as sera with normal globulin to prevent false positive tests; and (iv) use of the rheumatoid factor positive control serum as a positive agglutination control delineated a positive agglutination reaction from a weak reaction, thus facilitating interpretation of the test.

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