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Evaluation of a Monoclonal Antibody-Based Latex Agglutination Test for Diagnosis of Cryptococcosis: Comparison with Two Tests Using Polyclonal Antibodies

ALAIN TEMSTET,[1] PATRICIA ROUX,[1] JEAN-LOUIS POIROT,[2] OLIVIER RONIN,[3] AND FRANÇOISE DROMER[3,4]*

Laboratoire de Parasitologie et Mycologie, Hôpital Tenon,[1] Laboratoire de Parasitologie et Mycologie, Hôpital Saint Antoine,[2] Unité de Mycologie, Institut Pasteur,[3] and Institut National de la Santé et de la Recherche Médicale, Unité 283, Hôpital Cochin,[4] Paris, France

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Cryptococcal antigen detection has become a routine biological test performed for patients with AIDS. The poor prognosis of cryptococcosis explains the need for reliable tests. We evaluated the performances of a newly commercialized agglutination test that uses a monoclonal antibody specific for cryptococcal capsular polysaccharide (Pastorex Cryptococcus; Sanofi-Diagnostics Pasteur, Marnes-la-Coquette, France) and compared them with those of tests that use polyclonal immune sera (Cryptococcal Antigen Latex Agglutination System, Meridian Diagnostics, Inc., Cincinnati, Ohio; and Crypto-LA, International Biological Labs Inc., Cranbury, N.J.). The sensitivities and specificities of the tests were compared by using purified polysaccharides and yeast suspensions. Clinical specimens (131 serum samples, 41 cerebrospinal fluid samples, 34 urine samples, and 19 bronchoalveolar lavage samples) from 87 human immunodeficiency virus-positive subjects with (40 patients) and without (47 patients) culture-proven cryptococcosis were retrospectively tested during a blinded study. The effect of pronase treatment of samples was assessed for Pastorex Cryptococcus and the Cryptococcal Antigen Latex Agglutination System, and the antigen titers were compared. Our results show that (i) during the screening, concordance among the three tests was 97%; (ii) the use of pronase enhanced both the sensitivities and specificities of the Pastorex Cryptococcus test; (iii) titers agreed for 67% of the cerebrospinal fluid samples and 60% of the serum samples; and (iv) cryptococcosis was detected equally well with Pastorex Cryptococcus and with the other tests, whatever the infecting serotype (A, B, or D). The meaning of in vitro sensitivity and the relationship between titers and sensitivity are discussed. The results show that Pastorex Cryptococcus is a rapid and reliable test for the detection of cryptococcal antigen in body fluids and suggest that kits cannot be used interchangeably to monitor antigen titers in patients.

The need for sensitive tests for the diagnosis of cryptococcosis has been particularly acute since the beginning of the AIDS epidemic. Cryptococcosis is the most common life-threatening fungal infection in patients with AIDS (4, 6). For several years now, physicians have included the detection of cryptococcal antigen, at least in serum, as a routine screening test whenever a human immunodeficiency virus (HIV)-positive patient has meningitis, unexplained pneumonia, or fever (20). Titration of antigen is used for the diagnosis of cryptococcosis as well as for monitoring antifungal therapy (6). Physicians and biologists are aware of the necessity for a reliable and rapid test. In all but one of the commercialized tests (8), the detection of capsular antigen from *Cryptococcus neoformans* is based on the agglutination of latex beads covered with polyclonal antibodies usually raised in rabbits. A newly commercialized test uses a murine monoclonal antibody specific for cryptococcal polysaccharide (7) to sensitize the beads.

The purpose of the study described here was to report on the performances of this new test, Pastorex Cryptococcus (Sanofi-Diagnostics Pasteur, Marnes-La-Coquette, France), and to compare them with those of two other tests that are currently used in several laboratories and for which some comparative studies have already been published (8, 11, 14, 24). The two other tests are the Cryptococcal Antigen Latex Agglutination System (CALAS; Meridian Diagnostics, Inc., Cincinnati, Ohio) and Crypto-LA (International Biological Labs Inc., Cranbury, N.J.). We compared the sensitivities and specificities of these tests for the detection of cryptococcal antigen in various clinical specimens from patients with AIDS and with or without culture-proven cryptococcosis. For Pastorex Cryptococcus, we also checked the usefulness of pronase treatment of the samples, since this procedure has been reported to eliminate the false agglutination reactions and to improve the sensitivity of the test (9, 11). We tested the influence of the serotype of the infecting *C. neoformans* strain on the ability of Pastorex Cryptococcus to diagnose the infection. We assessed the efficacies of the tests for the detection of cryptococcal antigen in specimens other than serum and cerebrospinal fluid (CSF) specimens by testing bronchoalveolar lavage (BAL) and urine samples. The data show that Pastorex Cryptococcus is a rapid, sensitive, specific, and reliable assay for the detection of cryptococcal antigen in clinical specimens.

MATERIALS AND METHODS

Study design. Sera collected from 87 HIV-positive patients in four different clinical microbiology laboratories from university hospitals in Paris, France, were used for the retrospective blinded study. These sera were initially sent for a routine screening for cryptococcal antigen by Crypto-LA and were kept frozen for subsequent assays. The

* Corresponding author.

results of this first antigen detection in the sera allowed us to select specimens from 40 patients with culture-proven cryptococcosis (84 serum samples, 30 CSF samples, 12 BAL samples, and 10 urine samples) and 47 patients without a known cryptococcal infection (47 serum samples, 1 CSF sample, and 1 BAL sample). After selection, the samples were divided into aliquots and were randomly assigned a number, their origin being kept from the investigators who did the tests. All samples were stored at −20°C for 3 months to 2 years before testing, without intermediary thawing and freezing cycles. Since there were no control samples other than sera, 24 urine samples collected from HIV-negative subjects and 10 CSF samples (labeled CSF samples 1 to 10) and 6 BAL samples from HIV-positive patients, all of whom were hospitalized for diseases other than cryptococcosis, were subsequently added. All the patients were assigned a number. Those that begin with the letter M indicate those with cryptococcosis; those that begin with the letter T indicate patients in the control group.

Test procedures. (i) Screening. All sera were heat inactivated at 56°C for 30 min. The samples were tested by the three tests by using the procedures recommended by the manufacturers. For the new test (Pastorex Cryptococcus), the influence of enzymatic treatment of the specimens prior to antigen testing on both the sensitivity and the specificity of the test was evaluated. We therefore used in parallel five methods for the screening of undiluted samples, as follows: direct testing with the three different reagents and testing after enzymatic treatment recommended by Sanofi-Diagnostics Pasteur and Meridian. When the available volumes were small (especially for CSF and BAL samples), only direct testing was performed. Specimens were always tested by the three tests on the same day. The procedures were as follows.

For Crypto-LA, 50 μl of the sample was mixed with the same volume of sensitized beads. The kits were from lot number 3864.

For CALAS, 100 μl of the sample was incubated for 15 min at 56°C with the same volume of pronase solution. The reaction was stopped by heating at 100°C for 5 min. Of this mixture, 25 μl was removed and mixed with 1 drop of the sensitized latex beads. Direct testing was performed in the same way with 25 μl of the untreated sample. Lot numbers 404A089, 404A097, and 404A100 were used.

For Pastorex Cryptococcus, the enzymatic treatment was slightly different. The sample (100 μl) was mixed with 35 μl of pronase and heated for 15 min at 56°C. The enzyme was then instantaneously inactivated by the addition of proteinase inhibitor (35 μl). Ten microliters of the sensitized beads was then added to 40 μl of the mixture or untreated sample. Different kits were used, corresponding to lot numbers CN26, CN30, CN36, and 1K104.

(ii) Titrations. Depending on the quantities left after screening, serum and CSF samples that gave a positive agglutination reaction were then serially diluted (twofold dilutions) in the buffer provided with the kits to determine the end point of agglutination. Only 48 serum samples and 9 CSF samples were still available for titration by Pastorex Cryptococcus and CALAS. Titer determination on pronase-treated samples was done on the aliquots that were treated and frozen after the screening. For 14 different samples, we checked that the procedure (pronase reaction) and subsequent storage did not alter antigen detection by Pastorex Cryptococcus and CALAS. Titers were not changed by more than 1 dilution (data not shown). In all reports on antigen titration, changes of ±1 dilution are always considered to be insignificant and within the limit of experimental error. We also assessed, as has been done by others (11), that the procedure of freezing and thawing did not change the antigen titers (data not shown). No titration was done on BAL or urine samples. Volumes of samples treated with pronase according to the protocol of Sanofi-Diagnostics Pasteur were adjusted (addition of 30 μl of buffer) to give a 1:2 starting dilution.

Comparison of sensitivities. The sensitivities of the three kits were assessed by using cryptococcal antigen (positive controls) supplied in the Meridian and the International Biological Labs kits. The limit of detection for the four serotypes was determined by using capsular polysaccharides (CPSs) purified from *C. neoformans* serotypes A, B, C, and D (kindly provided by J. E. Bennett, National Institute of Allergy and Infectious Diseases, Bethesda, Md.). The polysaccharides were serially diluted in buffer and were allowed to react with the three different sensitized latex beads until the end point of agglutination was reached. The minimal amount of antigen detected by each kit was recorded.

The in vitro sensitivities of Pastorex Cryptococcus and Crypto-LA were further assessed on suspensions of live yeasts. *C. neoformans* serotypes A (CDC B551, CDC B236, NIH 68, NIH 271), B (NIH 112, NIH 444, CDC B237), C (NIH 18, NIH 191, CDC B238), and D (NIH 52, NIH 3501, NIH 3502, CBS 132) and *Trichosporon beigelii* (CBS 2936) and *Candida albicans* (NIHB 311) were used. Yeasts were grown in Sabouraud broth in an orbital shaker (for 15 h at 28°C). Cells were then enumerated and diluted in phosphate-buffered saline (PBS; 10 mM; pH 7.4). Agglutination was checked with 10-fold dilutions of the suspensions; the negative control was PBS alone.

Statistical analysis. All calculations were done with a Macintosh SE/30 computer and Statview II software (Abacus Concepts, Inc., Berkeley, Calif.).

RESULTS

Comparison of in vitro sensitivities. The in vitro sensitivities of the tests used in this study are summarized in Table 1. The sensitivity of Pastorex Cryptococcus was similar to those of Crypto-LA and CALAS for reactivity with CPS serotype A and the control CALAS antigen and lower for purified CPS serotypes D, B, and C and the control Crypto-LA antigen. Results were different with yeast suspensions. We first noted a prozone phenomenon when the suspensions were greater than 10^7/ml. The threshold of detection was then determined with 10-fold dilutions starting at 10^7/ml (Table 1). It varied according to the serotype of the strains and the reference strain used (serotype D for Pastorex Cryptococcus and serotypes B, C, and D for Crypto-LA). Overall, the sensitivities of the tests were similar. Cross-reactivities with *T. beigelii* and *C. albicans* were lower with Pastorex Cryptococcus than they were with Crypto-LA.

Screening of the sera by the three tests. After completion of the screening, the results of screening of sera by the three tests were interpreted as positive, negative, or false positive and false negative according to the known infection status of each patient. Results for 84 serum samples from 40 patients with culture-proven cryptococcosis are shown in Fig. 1A. Nine of these serum samples were obtained months before the onset of infection; eight were negative by the three tests, and one serum sample (from patient M24) gave a false-positive reaction by the Pastorex Cryptococcus test, which was resolved by enzymatic treatment. Differences appeared

TABLE 1. Threshold of detection by the three cryptococcal agglutination kits

Antigen	Minimal amount (ng/ml) of antigen detected by:		
	Pastorex Cryptococcus	CALAS	Crypto-LA
Soluble antigens			
CPS serotype A	5	5	5
CPS serotype B	500	10	5
CPS serotype C	1,000	10	10
CPS serotype D	100	10	2.5
CALAS control	25	25	20
Crypto-LA control	250	50	50
Yeast suspensions			
C. neoformans			
Serotype A	10^2	ND[a]	10^3
Serotype B	10^3	ND	10^2–10^3[b]
Serotype C	10^6	ND	10^5–10^6
Serotype D	10^3–10^4	ND	10^3–10^5
T. beigelii	10^7	ND	10^6
C. albicans	None[c]	ND	10^7

[a] ND, not done.
[b] The limit of sensitivity varied depending on the strain used for the test.
[c] No agglutination was seen at 10^7/ml.

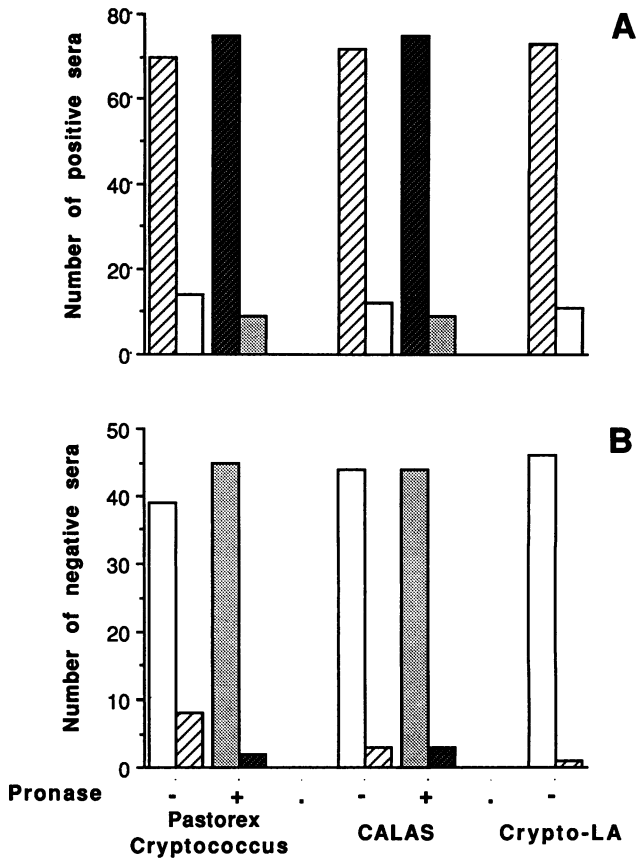

FIG. 1. Screening for cryptococcal antigen in sera from HIV-positive patients. Eighty-four serum samples from 47 patients with culture-proven cryptococcosis (A; 9 serum samples were withdrawn before the onset of the infection) and 47 serum samples from 47 patients without any clinical or laboratory evidence of infection (B) were tested. Three different agglutination tests were used. Bars represent the number of serum samples that were positive (▨) or negative (□) without pronase pretreatment or positive (▨) and negative (▩) after pronase treatment.

when we compared the results for the 75 other serum samples that were withdrawn at the time of diagnosis and during follow-up. False-negative results were seen when the kits were used without pronase pretreatment: five serum samples by Pastorex Cryptococcus, three serum samples by CALAS (these three serum samples were also negative by Pastorex Cryptococcus and were from patients M17, M25, and M35), and two serum samples by Crypto-LA (sera from patients M17 and M35). Positive results were recovered after enzymatic treatment.

Concerning the 47 serum samples from 47 HIV-positive patients without a known cryptococcal infection, a false-positive agglutination was seen for 8 serum samples by Pastorex Cryptococcus, 3 serum samples by CALAS, and 1 serum sample by Crypto-LA (Fig. 1B). After enzymatic treatment, two control serum samples (from patients T47 and T48) remained positive by Pastorex Cryptococcus and CALAS and one (from patient T36) became positive by CALAS. For these patients, there was no other evidence of cryptococcosis.

Screening of other biological samples by the three tests. The presence of cryptococcal antigen was assessed in other clinical specimens. During the blind retrospective study, 30 CSF samples (from 21 patients), 10 urine samples (from 7 patients), and 12 BAL samples (from 8 patients) from among the 40 patients with cryptococcosis were tested by the three tests according to the recommendations of the manufacturers. The use of pronase was therefore tested only by Pastorex Cryptococcus and, when sufficient quantities were available, by CALAS. All 30 CSF samples from infected patients were positive. Some of the CSF samples (i.e., n = 11) were also tested after enzymatic treatment, without modification of the positive results. All the urine samples from infected patients were positive by the three tests. Finally, 2 of the 12 BAL samples (those from patients M11 and M38) required the use of pronase treatment for detection of the antigen by Pastorex Cryptococus. One of these BAL samples (from patient M38) was negative by CALAS and remained negative after enzymatic treatment. All were directly positive by Crypto-LA.

The control samples were tested in two different ways. Very few samples were included within the first round of screening (one CSF sample and one BAL sample); the other samples were added after the first round of screening. The results of all rounds of screening are presented together. All 11 control CSF samples were negative by Pastorex Cryptococcus (with and without pronase treatment); 2 CSF samples each were positive by CALAS (CSF samples 2 and 3) and Crypto-LA (sample from patient T44 and CSF sample 3). Agglutination of the control latex beads provided with the kits and the use of pronase in the case of CALAS allowed us to classify CSF samples 2 and 3 as negative samples, but it left the sample from patient T44 slightly positive. All 24 control urine samples were negative by CALAS and Crypto-LA (with 3 of them agglutinating both the control and the sensitized beads) and Pastorex Cryptococcus after a 1:2 dilution. It should be noted that in the case of cloudy specimens, the reactions were performed on the supernatants obtained after sedimentation. None of the seven control BAL samples was positive by Pastorex Cryptococcus; three of the BAL samples gave a positive reaction with the

TABLE 2. Discrepancies among the three tests during screening

Sample	Patient no.	Result by the following test:			Clinical diagnosis
		Pastorex	CALAS	Crypto-LA	
Serum	M17	+	+	−	+
Serum	M35	+	+	−	+
BAL	M38	+	−	+	+
Serum	T47	+	+	−	−
Serum	T36	−	+	−	−
CSF	T44	−	−	+	−

antibody-sensitized and control beads provided with the CALAS and Crypto-LA kits.

Discrepancies among the three kits at the end of screening. Overall, of the 131 serum samples, 31 CSF samples, and 13 BAL samples tested during the blinded study, only 6 (3%) discrepancies were noted by the three tests, if we considered the results of both Pastorex Cryptococcus and CALAS after enzymatic treatment. Discordant results are summarized in Table 2. They concerned three samples from patients with cryptococcosis and three samples from control subjects. For patients M17 and M35, the negative reaction seen with Crypto-LA can be attributed to the lack of enzymatic treatment, since both were negative by Pastorex Cryptococcus and CALAS before the pronase reaction. The BAL sample from patient M38 was negative by CALAS, despite a positive antigen determination in CSF and serum samples. Concerning the control samples, no explanation is available for the faint positivity seen in the serum sample from patient T36 by CALAS (titer, 1:2 after pronase treatment) and in the serum sample from patient T47 by Pastorex Cryptococcus and CALAS (titer, 1:8 whatever procedure was used). These patients had no clinical or laboratory evidence of cryptococcosis (a serum sample withdrawn from patient T47 almost 10 months after the first serum sample was obtained was negative by Pastorex Cryptococcus even after pronase treatment). The weak positivity seen by Crypto-LA for the CSF sample from patient T44 has not been confirmed as specific by follow-up of the patient (one serum sample withdrawn on the same day as the CSF sample was obtained as well as several additional serum samples were negative by the three tests). One possible explanation is a cross-reactivity (18) with *T. beigelii* (cultured from the BAL sample), but there was no dissemination (negative blood cultures).

The sensitivity of Pastorex Cryptococcus was thus 100%, whereas it was 99% for CALAS and 98% for Crypto-LA when all of the results are summarized. The specificities of the tests were 97, 96, and 93%, respectively.

Comparison of antigen titers determined by Pastorex Cryptococcus and CALAS. We titrated 48 of the 75 positive serum samples (from 25 different patients) by Pastorex Cryptococcus and CALAS. Correlations between titers before and after pronase treatment were similar for Pastorex Cryptococcus and CALAS (r^2 = 0.856 and 0.821, respectively) (Fig. 2). Pronase treatment did not modify the antigen titers (±1 dilution) in 80 and 59% of the serum samples tested by Pastorex Cryptococcus and CALAS, respectively. The modifications were seen only for sera with an initial titer of less than 1:256; the geometric means before and after pronase treatment were 1:28 and 1:46, respectively, by Pastorex Cryptococcus (P = 0.02 by the Wilcoxon signed-rank test) and 1:17 and 1:52, respectively, by CALAS (P = 0.001). The small numbers of serum samples with increased titers after pronase treatment determined by Pastorex Cryptococcus

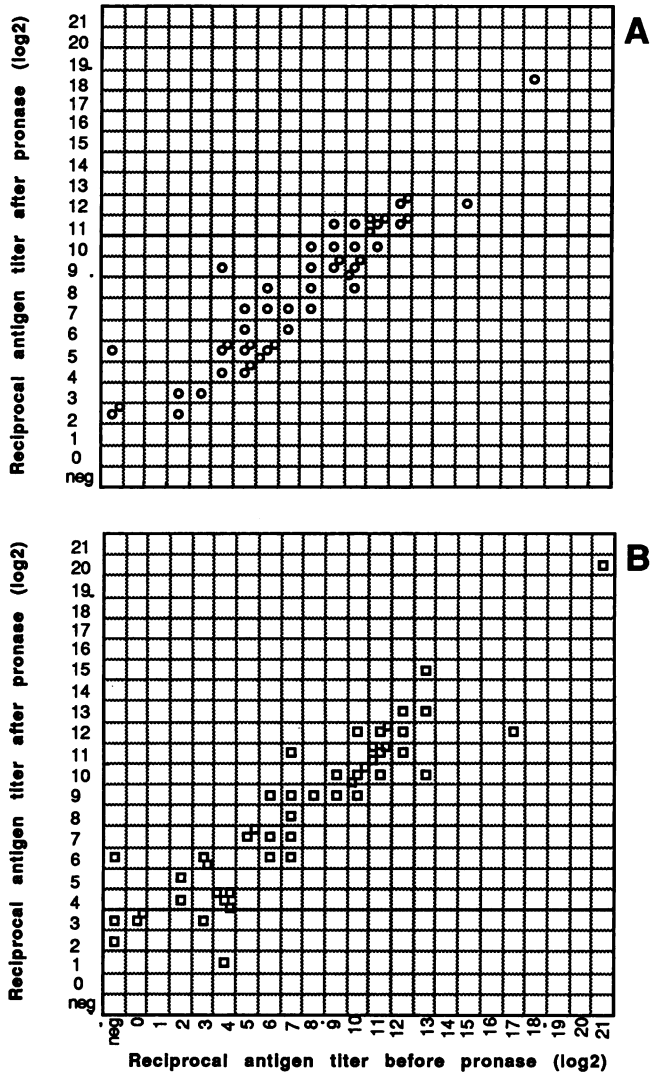

FIG. 2. Antigen titers (reciprocal titers in $\log_2$) in sera from HIV-positive patients with culture-proven cryptococcosis. Sera were tested by Pastorex Cryptococcus (A) or CALAS (B) with and without pronase pretreatment.

compared with those determined by CALAS were not related to the activity of the enzyme provided by the manufacturers. An increase in the pronase concentration (two to three times) did not enhance further the titer for 12 different serum samples (data not shown).

Titers determined with both kits agreed (within 1 dilution) for 61 and 57% of serum specimens, respectively, before and after pronase treatment. For falsely negative specimens, titers after pronase treatment were within 1 dilution of each other when determined by Pastorex Cryptococcus and CALAS and were equal to or less than 1:64. Titers determined for the nine CSF samples (range, 1:2 to 1:16,384) agreed for 67% of the samples after pronase treatment. For three different patients, we had additional samples that allowed us to repeat a comparison of the antigen levels over time. In these three patients, the evolution of titers was similar by both tests (data not shown).

Contribution of pronase treatment to antigen detection. It should be noted that one prozone phenomenon was not

resolved by pronase treatment, a clear-cut positive reaction being seen only after dilution of this serum sample (from patient M21).

As shown in Fig. 1, pronase treatment uncovered all the false-negative and most of the false-positive reactions in sera. As noted above, unexplained positive results remained for two patients in the control group (patients T47 and T48), which was disturbing in view of the other results. Pronase treatment uncovered two false-negative results (for serum samples from patients M17 and M35), showing that these patients were already infected with *C. neoformans* 232 and 153 days, respectively, prior to mycological proof of infection or the positivity of the Crypto-LA.

Concerning the other biological samples, pronase treatment also seemed to be beneficial for the detection of cryptococcal antigen in both BAL and CSF samples from infected patients. However, use of pronase should be avoided for urine samples, since it constantly gave false-positive results (data not shown). Urine testing required only a 1:2 dilution of clarified supernatant to prevent nonspecific interference with the sensitized beads. This dilution would not have prevented the agglutination on specimens from infected patients, since the titers were $\geq$1:2 for two urine samples and $\geq$1:10 for the other eight urine samples that were titrated.

Overall, the use of pronase enhanced the sensitivities and the specificities of the tests. When looking at the results for the 127 positive samples corresponding to patients with culture-proven cryptococcosis and the 98 negative samples, the sensitivity increased from 94 to 100% for Pastorex Cryptococcus and from 97 to 99% for CALAS. The specificity increased from 86 to 97% for Pastorex Cryptococcus and from 89 to 96% for CALAS.

Influence of the serotype on the ability of Pastorex Cryptococcus to detect soluble antigen in patients. We assessed the performances of Pastorex Cryptococcus with samples withdrawn from patients for whom knew the serotypes of the infecting strains. Strains of *C. neoformans* corresponding to 14 patients in the present study were available for serotyping (12): all were *C. neoformans* var. *neoformans*, 10 were serotype A, 3 were serotype D (patients M9, M16, and M22), and 1 was untypeable (patient M13). There was no discrepancy between Pastorex Cryptococcus and the other tests for samples from these three patients. Four other patients with a serotype D infecting strain were further tested by one of the polyclonal antibody-based tests and Pastorex Cryptococcus without a false-negative result and with concordant antigen titers (data not shown). We also tested three serum samples from one patient infected with a serotype B strain (kindly provided by B. Dupont, Unité de Mycologie, Institut Pasteur, Paris, France). The titers determined by Pastorex Cryptococcus and Crypto-LA were 1:32 and 1:16, 1:1,024 and 1:1,024, and 1:256 and 1:32, respectively.

DISCUSSION

We assessed the performances of a newly commercialized test that uses a monoclonal antibody to detect cryptococcal antigen in clinical specimens and compared them with those of two other kits using polyclonal antibodies. Purified polysaccharides and a total of 131 serum samples, 41 CSF samples, 34 urine samples, and 19 BAL samples were tested by the three tests.

For Pastorex Cryptococcus, we found a sensitivity similar to those of the other tests for polysaccharide serotype A and lower than those for serotypes B, C, and D. When tested with yeast suspensions, the performances of Pastorex Cryptococcus and Crypto-LA with strains of the four serotypes varied with the reference strain used and were very close to each other. The differences seen with antigens that were either chemically purified or present on live cells suggest a variability in antigen expression on yeasts of the same serotype. It might partly explain the variability in antigen titers during the infection. However, the restricted specificity of the monoclonal antibody compared with those of the polyclonal antibodies did not prevent an accurate detection of cryptococcal antigen. First, in our study, Pastorex Cryptococcus was even more sensitive than Crypto-LA for samples from two patients and could have allowed the diagnosis of the infection several months before the Crypto-LA test detected it. Second, the serotype of the infecting strains did not seem to influence the efficiency of the test. At present, the vast majority of patients with cryptococcosis have AIDS (17). According to published reports, probably more than 98% are infected with *C. neoformans* var. *neoformans*, i.e., serotypes A and D, with a higher proportion of serotype A (2, 15, 17, 19, 22). We checked that Pastorex Cryptococcus as well as polyclonal antibody-based tests detected the infection in patients infected with serotype D strains. Among the patients infected with *C. neoformans* var. *gattii*, i.e., serotypes B and C, all the reported cases were of serotype B and, when noted, antigen was not detectable by conventional tests (1, 5, 13, 21, 23). For one patient infected with a serotype B strain, soluble antigen was detected by Pastorex Cryptococcus as well as Crypto-LA. To our knowledge, infections with serotype C strains have rarely been diagnosed except in Southern California before the AIDS epidemic (16). Unfortunately, we did not have the opportunity to test samples from such patients. The results of the study described here showed that the in vitro sensitivity and specificity of Pastorex Cryptococcus should allow the detection of cryptococcal antigen in all infected patients.

We compared the performances of the tests on various clinical specimens. Discrepancies were found for only six specimens (3%). The performances of Pastorex Cryptococcus were excellent and very similar (97% specificity, 100% sensitivity) to those found for CALAS (96% specificity, 99% sensitivity) and Crypto-LA (93% specificity, 98% sensitivity) in this and previous (8, 9, 11) studies. For Pastorex Cryptococcus as well as for CALAS, the use of pronase enhanced both the sensitivity and the specificity, as reported previously (9, 11). However, it did not resolve one prozone phenomenon that showed the need for sample dilution in view of clinical symptoms suggestive of cryptococcosis (10). Pronase resolved all the other false-negative reactions (6% for Pastorex Cryptococcus and 4% for CALAS). These values are lower than those presented in a previous study (11), in which 19% of the sera gave false-negative reactions that were uncovered by pronase treatment. Despite pronase treatment, two false-positive reactions were unresolved, since clinical and laboratory evidence was against the diagnosis of cryptococcosis. However, it is noteworthy that in two patients a positive antigen in serum samples was predictive of a later infection. Pronase was also effective for other biological samples, especially BAL samples, in our hands. Results for none of the positive CSF samples were modified by pronase treatment (a finding already noted by others [11]). Pronase pretreatment should be avoided for urine samples. A 1:2 dilution of clarified supernatants prevented all false-positive reactions. For none of these different specimens was there a false-positive reaction by Pas-

torex Cryptococcus that would have been clarified by the use of control globulin-coated latex beads as provided by Meridian or International Biological Labs. The use of a very specific reagent and the systematic treatment of all specimens except urine with pronase therefore seem sufficient for detecting all positive and negative samples. In our opinion, manipulations with this test are not more fraught with problems than those with a polyclonal antibody-based test, owing to a simplified procedure for enzymatic treatment.

The antigen titers determined by Pastorex Cryptococcus and CALAS agreed (within 1 dilution) for 61 and 57% of serum specimens, respectively, before and after pronase treatment and for 67% of the titrated CSF samples, a value close to that given in a recent report (11). The fact that titers are usually higher after pronase treatment when tests like Crypto-LA (9, 11) or CALAS (11; this study) are used but are not higher by Pastorex Cryptococcus, even at higher pronase concentrations, suggests that the monoclonal antibody may be responsible. One hypothesis is that this antibody detects an antigenic determinant that is less hidden by the interfering substances (whatever they are). Nevertheless, in our opinion, there is a misunderstanding between the antigen titer determined by a test and the sensitivity of the test. A reliable assay should be able to detect the infection early and to monitor antifungal therapy and the evolution of the disease. Both Pastorex Cryptococcus and CALAS proved to be efficient in our hands.

This is the first study of cryptococcal antigen detection in clinical specimens other than CSF and serum specimens. Urine samples are easy to test, and positive antigen detection in samples diluted 1:2 was, in this study, complementary evidence of cryptococcosis. It is often necessary to obtain BAL samples from patients with AIDS. An abnormal chest X-ray and the occurrence of cryptococcal pneumonia have been reported several times in patients with AIDS (3). Testing of the BAL sample for the presence of cryptococcal antigen seems easy and may be helpful (unpublished data): first, in cases in which culture is negative but antigen detection in serum and CSF samples is positive, a positive BAL sample could reinforce the presumption and, second, during pneumonia caused by *C. neoformans* and another organism which can prevent *C. neoformans* from growing in culture. We therefore think that testing of BAL and urine samples can be recommended when cryptococcosis is suspected. However, other studies are needed to determine whether the hypothesis based on our experience is fully justified.

We conclude from the results of this study that Pastorex Cryptococcus provides a perfectly adequate, rapid, and reliable means of diagnosing and monitoring cryptococcosis in patients. Our results show the need for pronase pretreatment to prevent false-positive and false-negative reactions for all specimens but urine samples. These data suggest the value of a systematic screening for cryptococcal antigen on a regular basis in patients with AIDS. The data also underline the fact, as has been done previously (11), that kits for the detection of cryptococcal antigen cannot be used interchangeably. Finally, when confronted with a negative result, clinicians and biologists should keep in mind the need for repeated cultures and antigen testing whenever the evidence of infection is strong enough.

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