

## Corrected Version†

# Specificity of the Latex Test for Cryptococcal Antigen: a Rapid, Simple Method for Eliminating Interference Factors

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An enzymatic method involving a protease (pronase) for the elimination of interference factors in the latex test for cryptococcal antigen was developed and compared with dithiothreitol treatment. The two were equivalent in their ability to remove interference factors; however, the enzymatic method generally yielded higher titers. The method is simple, requires only 20 min, and makes the latex test for cryptococcal antigen specific.

The latex test for cryptococcal antigen detection is one of the most reliable fungal serological tests available to clinicians. It is most useful for the diagnosis of cryptococcal meningitis, but circulating capsular polysaccharide antigen can sometimes be detected in the serum of patients having other forms of cryptococcosis.

The detection of cryptococcal antigen in serum is, however, sometimes obscured by the presence of rheumatoid factor (RF) or other interfering substances (1). When this occurs, it is difficult to determine whether the serum contains cryptococcal antigen, interference factors, or both.

Methods involving dithiothreitol (DTT) treatment (4) and, more recently, EDTA-heat extraction (3), have been reported to be helpful in removing interference factors. However, both methods require additional time beyond that necessary to perform the latex test, extra manipulative steps, and dilution of the serum sample before testing.

The following report describes a rapid, simplified enzymatic method that eliminates false-positive test results due to protein interference factors.

A total of 204 sera from the same number of patients having clinically proven rheumatoid arthritis were included in the study. Of these, 118 were obtained from the Mayo Clinic Clinical Immunology Laboratory, and 86 were obtained from the Immunology Branch, Host Factors Division, Centers for Disease Control, Atlanta,

Ga. Also included were 30 sera from 30 patients that gave false-positive results when tested for cryptococcal antigen in the Mayo Clinic Fungal Serology Laboratory; none of the patients in the latter group had cryptococcosis. In addition, 111 sera from normal healthy persons were included. Forty-eight sera and 25 cerebrospinal fluid (CSF) samples from 40 and 20 patients, respectively, with culturally proven cryptococcosis were evaluated.

Latex tests for cryptococcal antigen and RF were performed by using the Crypto-LA test reagents (International Biological Laboratories, Rockville, Md.) and the instructions supplied by the manufacturer. Sera and CSFs were inactivated at 56°C for 30 min before testing. Quantitative titers were determined for all samples tested.

After cryptococcal antigen determinations were made, aliquots of all serum and CSF samples were subjected to treatment with DTT and the enzymatic method.

DTT (Calbiochem-Behring Corp., La Jolla, Calif.), 0.003 M, was prepared in fresh filtered 0.2 M Tris-hydrochloride buffer, pH 8.6. Equal amounts of the heat-inactivated sample and DTT were incubated at room temperature for 1 h before testing for the presence of cryptococcal antigen (4) and RF. Freshly prepared DTT was found to be stable for approximately 2 h after preparation.

A 0.3-g amount of pronase-CB protease (Calbiochem-Behring Corp.) was dissolved in 40 ml of glycine-buffered saline, pH 8.2. Aliquots of 200  $\mu$ l were placed in 1-dram (3.7-ml) vials and lyophilized. Serum or CSF (300  $\mu$ l) was added to the vials to give a final concentration of 5 mg/ml of pronase. After rehydration, samples were

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placed in a 56°C water bath for 15 min, followed by immersion in a boiling water bath for an additional 5 min. Latex tests for cryptococcal antigen and RF were performed on the nonviscous samples immediately after enzyme treatment. Lyophilized vials of pronase were found to be stable for 5 months at 25°C.

Of the sera from patients with arthritis, 173 (85%) had RF titers ranging from 1:40 to 1:40,960 as determined by the method of Singer and Plotz (7) or the Rheumatoid Titration Test (Difco Laboratories, Detroit, Mich.). One hundred thirty-four (66%) of the sera reacted with the anticryptococcal globulin and RF control reagents supplied in the Crypto-LA test. RF titers ranged between 0 and 1:40,960, with mean and median titers of 1:3,336 and 1:1,280, respectively. Only 60 (29%) of the sera were titered with the Crypto-LA RF reagents since the cost became excessive. The range of titers for those tested was 1:2 to 1:1,024. All sera were nonreactive after treatment with DTT or pronase.

All 30 of the sera that gave false-positive results when tested for cryptococcal antigen by the Mayo Clinic Fungal Serology Laboratory were nonreactive after treatment with DTT or pronase; pretreatment titers ranged from 1:1 to 1:256. All 111 of the normal sera were nonreactive before and after treatment.

Pretreatment titers of cryptococcal antigen in the 48 sera of patients known to have cryptococcosis ranged from 1:2 to 1:16,384, with mean and median titers of 1:1,854 and 1:256, respectively. Treatment with DTT gave mean and median titers of 1:1,858 and 1:256, respectively. Figure 1 shows the change from initial cryptococcal antigen titer, by dilution, after treatment with DTT or pronase. Thirty-nine of the sera (81.3%) had titers that remained unchanged ( $\pm 1$  dilution);

eight (16.7%) increased by more than one dilution, and one (2.1%) decreased by two dilutions. Titer changes of  $\pm 1$  dilution are considered to be within acceptable limits of error. Treatment with pronase gave mean and median titers of 1:1,926 and 1:512, respectively. The titers of 32 sera (66.7%) remained unchanged ( $\pm 1$  dilution), and 16 (33.3%) increased by more than one dilution. One patient with invasive pulmonary cryptococcosis had concomitant cryptococcal antigen and RF titers of 1:256 and 1:64, respectively. The RF test was nonreactive after treatment of the serum with DTT or pronase, and the antigen titer remained unchanged.

Pretreatment titers of the 25 CSF samples ranged from positive in the undiluted specimen to 1:65,536, with mean and median titers of 1:3,528 and 1:16, respectively. Treatment with DTT produced no appreciable change in titers; however, one sample which was positive in the undiluted specimen became negative after treatment. Of the CSF samples, 74% showed no change in titer after treatment with pronase. Six (12%) of the samples exhibited titer increases of two dilutions, and the remaining 12% exhibited increases of three dilutions and a shift in the median to 1:32.

The specificity of the cryptococcal latex test for antigen is obscured only by the presence of rheumatoid interference factors in serum or CSF (1, 2, 5, 6). Of the 9,001 CSF samples and 18,510 sera tested for cryptococcal antigen in the Mayo Clinic Fungal Serology Laboratory during August 1975 to December 1981, 93 (1%) and 446 (2.4%), respectively, yielded false-positive tests due to interference factors. Although the overall false-positivity rate (1.96%) was low, interpretation of results in the presence of interference factors is equivocal and confusing to clinicians.

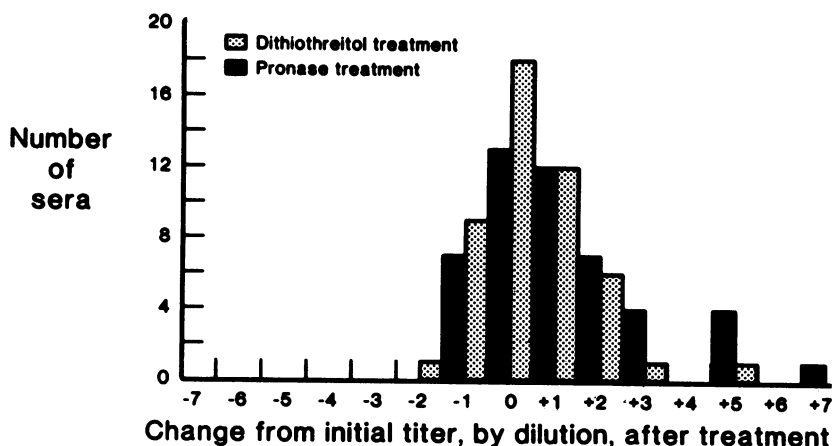


FIG. 1. Changes in cryptococcal antigen titers of 48 sera after treatment with pronase or dithiothreitol.

Comparison of the enzymatic and DTT methods revealed that both were equivalent in their ability to remove interference factors from CSF or serum before testing for cryptococcal antigen. However, the former appeared to increase the sensitivity of the test by frequently yielding somewhat higher titers. We speculate that dissolution of antigen-antibody complexes occurs during the enzyme-heat treatment without destruction of the carbohydrate antigen moiety. If, during the course of infection, the serum or CSF of a patient exhibits interference factor, all previous and subsequent samples should be subjected to treatment with the enzymatic method.

The DTT (4) and EDTA-heat extraction (3) methods have been reported to be effective for the elimination of interference reactions. However, both require that the specimen be diluted with reagents before testing. A review of our serology records showed that 35.5% and 15.5% of the false-positive reactions in CSF and serum, respectively, were observed in undiluted specimens. Elimination of interference factors or cryptococcal antigen or both would have occurred as a result of dilution by these reagents before testing.

The enzymatic method requires no dilution of the specimen, less time for treatment before testing (20 min) than the DTT method, and no centrifugation step, and it yields clearly positive or negative results with the cryptococcal latex test for antigen. In addition, reagents are stable for a longer period of time.

We feel the method is reliable, simple, inexpensive, and rapid, and can be used to clarify interference reactions. We recommend that the method be incorporated into routine use in clinical microbiology laboratories in situations where false-positive reactions occur. Its use should make the cryptococcal latex test for antigen specific and easy to read.

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#### LITERATURE CITED

1. **Bennett, J. E., and J. W. Bailey.** 1971. Control for rheumatoid factor in the latex test for cryptococcosis. *Am. J. Clin. Pathol.* **56**:360-365.
2. **Dolan, C. T.** 1972. Specificity of the latex-cryptococcal antigen test. *Am. J. Clin. Pathol.* **58**:358-364.
3. **Eng, R. H. K., and A. Person.** 1981. Serum cryptococcal antigen determination in the presence of rheumatoid factor. *J. Clin. Microbiol.* **14**:700-702.
4. **Gordon, M. A., and E. W. Lapa.** 1974. Elimination of rheumatoid factor in the latex test for cryptococcosis. *Am. J. Clin. Pathol.* **61**:488-494.
5. **Hay, R. J., and D. W. R. Mackenzie.** 1982. False positive latex tests for cryptococcal antigen in cerebrospinal fluid. *J. Clin. Pathol.* **35**:244-245.
6. **Prevost, E., and R. Newell.** 1978. Commercial cryptococcal latex kit: clinical evaluation in a medical center hospital. *J. Clin. Microbiol.* **8**:529-533.
7. **Singer, J. M., and C. M. Plotz.** 1956. The latex fixation test. I. Application to the serologic diagnosis of rheumatoid arthritis. *Am. J. Med.* **21**:888-892.