Experience with the Use of Pronase To Eliminate Interference Factors in the Latex Agglutination Test for Cryptococcal Antigen

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Cryptococcal antigen titers of 70 cerebrospinal fluid and 57 serum specimens from patients suspected of having cryptococcosis were determined both before and after treatment with pronase. Median titers of cerebrospinal fluid specimens before and after treatment were 128 and 128, respectively; mean geometric titers of these specimens before and after treatment were 102 and 204, respectively. Median titers of the serum specimens before and after treatment were 16 and 512, respectively; mean geometric titers of these specimens before and after treatment were 19 and 631, respectively. The modified latex agglutination test did not detect antigen in any of 50 cerebrospinal fluid and 51 serum specimens from patients not suspected of having cryptococcosis. These results suggest that the pronase modification increases the sensitivity of the latex agglutination test and that the modification be routinely incorporated into it.

The latex agglutination test (LAT) for the detection of cryptococcal capsular polysaccharide antigen in serum and cerebrospinal fluid (CSF) is a rapid and reliable serologic method for the diagnosis of cryptococcosis (3, 9, 10). Results of the LAT can be used as the basis for making decisions concerning prognosis and therapy (2, 4, 6, 9).

Several investigators have suggested that false-positive LAT results can occur with specimens from patients with immunological abnormalities (9) or noncryptococcal infections (2, 7, 8, 11). These results were apparently attributed to nonspecific cross-reactions. More commonly, false-positive results obtained with the LAT have been thought to be due to rheumatoid factor (1-4, 6) or other interference factors. Immune complexes involving the binding of cryptococcal antigen have been proposed as a possible cause of artificially low titers obtained occasionally with the use of the LAT (10). Several studies have resulted in the development of modifications of the LAT to eliminate false-positive results or artificially low titers or both and include the following: (i) treatment of specimens with heat (5), pronase (10), or dithiothreitol (4, 6); (ii) EDTA-heat extraction (3); and (iii) use of a control reagent (latex beads coated with normal [nonimmune] rabbit globulin) (1, 2).

The present study presents experience with the pronase treatment method of Stockman and Roberts (10). This method has been used with a commercial LAT kit in the Mayo Clinic Fungal Serology Laboratory for the past 5 years and has been found to eliminate interference factors and increase the sensitivity of the LAT. The method is simple, reliable, and reproducible. This report presents the experience of using the pronase treatment method to examine CSF and serum specimens received in our laboratory over a 13-year period.

A commercially available LAT was used to test 70 CSF and 57 serum specimens which were submitted to the Mayo Clinic Fungal Serology Laboratory from 1974 to mid-1987 for testing for cryptococcal antigen. All 127 specimens were from patients suspected of having cryptococcosis. In addition, 51 CSF and 50 serum specimens from the same number of patients who did not have clinical or laboratory evidence of cryptococcosis also were examined by the modified LAT and were used as controls. All specimens were heat inactivated for 30 min at 56°C before being tested.

Cryptococcal antigen pretreatment titers of all specimens were determined by using Crypto-LA test reagents (Wampole Laboratories, Cranbury, N.J.) and the instructions supplied by the manufacturer. Subsequently, each specimen was also treated with pronase according to the method of Stockman and Roberts (10), and cryptococcal antigen posttreatment titers were determined immediately after enzyme treatment. The pronase treatment method (Fig. 1) was performed as follows. Pronase-CB protease (Calbiochem-Behring Corp., La Jolla, Calif.) was dissolved in glycine-buffered saline (pH 8.2) to 7.5 mg/ml. 100-μl aliquots of the pronase solution were dispensed into 3.7-ml screw-cap vials, and the aliquots were lyophilized. Samples (150 μl) of each CSF or serum specimen were added to individual vials to give a final pronase concentration of 5 mg/ml. Treated samples were heat inactivated at 56°C for 15 min. Enzymatic activity was stopped by immersing the vials into boiling water for 5 min.

The geometric means of pre- and posttreatment titers were obtained after transforming all variables, i.e., after adding a value of 0.1 to all titers.

Cryptococcal antigen was not detected either before or after treatment with pronase in any of the 101 CSF or serum specimens used as controls. These results suggest that the pronase treatment procedure did not elicit false-positive titers for CSF or serum specimens from patients who were not suspected of having cryptococcosis.

Pretreatment titers of the 70 CSF specimens from patients suspected of having cryptococcosis ranged from 0 to 65,536; the median and geometric mean titers were 128 and 102, respectively. After pronase treatment, titers of the CSF specimens ranged from 1 (undiluted) to 262,144; the median titer remained 128, and the geometric mean titer increased only 1 dilution to 204. Titer changes of ±1 dilution are considered to be insignificant and within acceptable limits of experimental error. Fifty-six (80.6%) of the 70 CSF specimens had titers which remained unchanged (±1 dilution), 12 (17.1%) increased in titer by 2 dilutions, and two (2.9%) increased in titer by 3 dilutions (Fig. 2).

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Pretreatment titers of the 57 serum specimens from patients suspected of having cryptococcosis ranged from 0 to 65,536; the median and geometric mean titers were 16 and 19, respectively. After pronase treatment, titers of the serum specimens ranged from 8 to 262,144; both the median and geometric mean titers increased approximately 5 dilutions to 512 and 631, respectively. Only 11 (19.3%) of the 57 serum specimens had titers which remained unchanged (±1 dilution). All of the remaining 46 specimens increased in titer (e.g., six [10.5%), eight [14.0%], seven [12.3%], and two [3.5%]) specimens showed increases in titers of three, five, seven, and nine dilutions, respectively (Fig. 2). Pronase did not elicit a reduction in the titer of any serum specimen.

The 127 specimens examined in this study were submitted by Mayo Clinic physicians and by laboratories which use the Mayo Clinic reference laboratory (Mayo Medical Laboratories). Examination of patient records and telephone inquiries revealed the following information about the specimens: 91 specimens were from patients who had positive cultures for Cryptococcus neoformans, 7 specimens were from patients who had negative cultures, 6 specimens were from patients who did not have fungal cultures performed, and 23 specimens were submitted by laboratories whose personnel were not able to determine (in retrospect) whether specimens from patients were cultured for fungi. Median and geometric mean titers of CSF and serum specimens before and after treatment with pronase were calculated and analyzed, including and excluding the data obtained from the 36 specimens from patients not documented to have cultures positive for C. neoformans but who were suspected of having cryptococcosis. There were no significant differences between the pre- and posttreatment titers of the 91 specimens from patients who had positive cultures and the titers of the entire group of 127 specimens.

Pronase treatment modification of the LAT (i) produced positive results only in specimens from patients who were suspected of having cryptococcosis and (ii) increased the sensitivity of the LAT by increasing the cryptococcal antigen titer of some CSF and most serum specimens. Therefore, the data suggest that protease treatment of CSF and serum specimens is a useful and simple modification of the LAT. We recommend the use of pronase with routine testing by LAT.

LITERATURE CITED