JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1994, p. 2307–2308
0095-1137/94/$04.00+0

Vol. 32, No. 9

Effect of Potential Interference Factors on Performance of Enzyme Immunoassay and Latex Agglutination Assay for Cryptococcal Antigen

HOWARD D. ENGLER* AND YVONNE R. SHEA

Microbiology Service, Clinical Pathology Department, Warren Grant Magnuson Clinical Center, Bethesda, Maryland 20892

Received 24 March 1994/Returned for modification 6 May 1994/Accepted 9 June 1994

The PREMIER Cryptococcal Antigen Enzyme Immunoassay (Meridian Diagnostics) did not give discrepant results with rheumatoid factor, syneresis fluid, or serum macroglobulins from systemic lupus erythematosis patients. The Cryptococcal Antigen Latex Agglutination System (Meridian Diagnostics) did cross-react with syneresis fluid but not with the other serum factors tested.

Cryptococcus neoformans var. *neoformans* is an encapsulated yeast that ranks as the fourth most common opportunistic infection in patients with AIDS in the United States (9). With the development and availability of latex agglutination (LA) assays utilizing anticryptococcal globulin-coated particles to detect *C. neoformans* capsular polysaccharide antigen (CAg) in serum and cerebrospinal fluid (CSF), the diagnosis of cryptococcosis became rapid, more sensitive and specific, and more reliable. However, nonspecific interference has been demonstrated with these latex assays. False-positive reactions due to the presence of rheumatoid factor (RF) (1, 7) and other macroglobulins in serum from patients with rheumatoid arthritis, systemic lupus erythematosus (SLE), sarcoidosis, and other conditions have been reported. False-negative reactions occur because of prozone effects seen during CAg excess (10) or because of the masking of CAg by unknown nonspecific proteins (6). These nonspecific reactions are eliminated by pretreatment of sera with pronase and/or by dilution of the specimen (5, 6, 11). Contamination of CSF samples with syneresis fluid condensation (SF) from the surface of agar has also been shown to be a cause of false positivity (2). Other false-positive reactions are known to occur because of cross-reactivity of an antigen of *Trichosporon beigelii* with CAg in those patients with disseminated *Trichosporon* infection (8). These reactions are not eliminated by pretreatment of the specimen with pronase.

An enzyme immunoassay (EIA) is now commercially available to detect CAg in serum and CSF. This assay utilizes anticryptococcal polyclonal capture antibodies followed by a monoclonal detection antibody conjugated to horseradish peroxidase. The EIA is comparable to LA in simplicity, cost, shelf life, and technologist time required. Total test time for either assay is 40 to 45 min. Early studies indicate the EIA to be more sensitive than LA for detecting CAg (4). The manufacturer reports that *T. beigelii* was found to cross-react in the EIA. The purpose of this study was to determine if other factors previously described as causing interference in CAg LA assays would cause similar false-positive reactions with the PREMIER Cryptococcal Antigen Enzyme Immunoassay (PREMIER EIA) (Meridian Diagnostics, Inc., Cincinnati, Ohio) and the Cryptococcal Antigen Latex Agglutination System (CALAS) (Meridian Diagnostics).

CSF and serum specimens were obtained from the Immunology Service of the Clinical Pathology Department at the National Institutes of Health. All sera were stored at −70°C prior to testing and were tested without heat or pronase pretreatment. All CSF specimens were obtained from patients with no culture documentation or clinical evidence of cryptococcal disease and were stored at 2 to 8°C prior to testing.

LA assays were performed with the CALAS according to the manufacturer's instructions, with 25-μl samples of serum or CSF. Samples were mixed with an equal amount of anticryptococcal reagent or normal globulin reagent on a slide and placed on a rotating platform at 120 rpm for 5 min, and agglutination reactions were assessed and graded as either negative or 1+ to 4+ positive. The PREMIER EIA was performed according to the manufacturer's instructions, with 50 μl of serum or CSF. Samples were incubated with horseradish peroxidase-conjugated CAg-specific monoclonal antibodies in polyclonal antibody-coated microwells, followed by incubation with the substrate system. Reactions were read spectrophotometrically at 450 nm with a Dynatech MR5000 EIA reader (Dynatech Laboratories, Inc., Chantilly, Va.). Results were interpreted according to the manufacturer's instructions. An absorbance (OD_{450}) reading of ≥0.15 was considered to be positive. Positive and negative controls were included with each test run of both assays.

To evaluate the effect of RF on CALAS and PREMIER EIA results, 20 sera negative for RF and 55 sera with RF titers ranging from 27 to 7,440 IU/ml were tested. All of these specimens were found to give negative results by both CAg assays, with EIA OD_{450} readings ranging from 0.041 to 0.099. A positive control tested at the same time gave 4+ agglutination and an OD_{450} reading of 0.272.

The effect of interference factors in sera from patients with SLE was examined by testing 23 sera obtained from SLE patients with anti-nuclear antigen titers ranging from 1:80 to 1:1,280 with homogeneous, speckled, and atypical-speckled patterns. All of these specimens gave negative results by both CALAS and PREMIER EIA, with EIA OD_{450} readings ranging from 0.051 to 0.097. A positive control tested at the same time gave 4+ agglutination and an OD_{450} reading of 0.485.

SF was collected from chocolate agar (Remel, Lenexa, Kans.) in order to assess the effect of SF contamination of CSF

* Corresponding author. Mailing address: Microbiology Service, Clinical Pathology Department, Bldg. 10, Room 2C-385, National Institutes of Health, Bethesda, MD 20892-0001. Phone: (301) 496-4433. Fax: (301) 402-1886. Electronic mail address: hengler@pop-server.nih.gov.

on the performance of the CALAS and PREMIER EIA. CSF specimens from 10 patients were divided into six aliquots of 1 ml each. Initially, one aliquot was tested directly, one was heated for 3 min at 100°C and then tested, and one was tested after being treated with pronase for 15 min at 56°C and then heated for 5 min in a 100°C sand bath to inactivate the pronase. One hundred microliters of SF was then added to each of the remaining three aliquots, and the aliquots were treated in the same manner as described above. Prior to the addition of SF to the CSF, both the CALAS and the PREMIER EIA gave negative results for all 10 of the CSF samples. However, addition of SF to the CSF resulted in the CALAS becoming falsely positive with all samples, despite pretreatment with heat or pronase, while the PREMIER EIA results remained negative.

Interference factors long have been a problem when testing clinical specimens for the presence of cryptococcal antigen. As others have shown, our results confirm that the CALAS clearly gives false-positive results following SF contamination of CSF samples, and laboratory technologists must be consciously aware of this problem when they process their CSF samples for culture. We have demonstrated, however, that the PREMIER EIA, which does not require pronase treatment of the specimen prior to testing, does not yield false-positive results with patient specimens containing SF, RF, or serum macroglobulins from patients with SLE, a significant advantage over the LA tests. This lack of cross-reactivity seen with the PREMIER EIA is most probably due to the increased specificity one would expect when using a monoclonal detection antibody, as does the EIA, rather than a polyclonal antibody-based system, as is found with the CALAS. Interestingly, unlike previous studies which have recognized RF as a cause of false-positive results with LA tests, the CALAS did not react with sera containing high titers of RF or anti-nuclear antigen, even though no pretreatment of the sera was performed. Eng and Person previously demonstrated false-positive CAg results when they tested RF-spiked sera and sera from patients with rheumatoid arthritis using a LA kit from another manufacturer (3). Whether or not the results we have seen with the CALAS are intrinsic to this particular manufacturer's kit and whether a combination of interference factors and/or cofactors may be needed to produce false-positive results with some kits are not known.

REFERENCES

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. **Boom, W. H., D. J. Piper, K. L. Ruoff, and M. J. Ferraro.** 1985. New cause for false-positive results with the cryptococcal antigen test by latex agglutination. J. Clin. Microbiol. **22:**856–857.
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. **Gade, W., S. W. Hinnefeld, L. S. Babcock, P. Gilligan, W. Kelly, K. Wait, D. Greer, M. Pinilla, and R. L. Kaplan.** 1991. Comparison of the PREMIER cryptococcal antigen enzyme immunoassay and the latex agglutination assay for detection of cryptococcal antigens. J. Clin. Microbiol. **29:**1616–1619.
5. **Gray, L. D., and G. D. Roberts.** 1988. Experience with the use of pronase to eliminate interference factors in the latex agglutination test for cryptococcal antigen. J. Clin. Microbiol. **26:**2450–2451.
6. **Hamilton, J. R., A. Noble, D. W. Denning, and D. A. Stevens.** 1991. Performance of cryptococcus antigen latex agglutination kits on serum and cerebrospinal fluid specimens of AIDS patients before and after pronase treatment. J. Clin. Microbiol. **29:**333–339.
7. **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.
8. **McManus, E. J., and J. M. Jones.** 1985. Detection of a *Trichosporon beigelii* antigen cross-reactive with *Cryptococcus neoformans* capsular polysaccharide in serum from a patient with disseminated *Trichosporon* infection. J. Clin. Microbiol. **21:**681–685.
9. **Perfect, J. R.** 1989. Cryptococcosis. Infect. Dis. Clin. N. Am. **3:**77–102.
10. **Stamm, A. M., and S. S. Polt.** 1980. False-negative cryptococcal antigen test. JAMA **244:**1359.
11. **Stockman, L., and G. D. Roberts.** 1983. Specificity of the latex test for cryptococcal antigen: a rapid, simple method for eliminating interference factors. J. Clin. Microbiol. **17:**945–947.