NOTES

New Cause for False-Positive Results with the Pastorex
Aspergillus Antigen Latex Agglutination Test

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The Pastorex Aspergillus antigen test for detection of Aspergillus galactomanann antigen in the sera of patients with invasive aspergillosis is used in many clinical laboratories. A serum sample contaminated with Penicillium chrysogenum gave a strongly positive reaction (1:128) which was heat stable, was not eliminated by pronase treatment, and was not detected by a normal rabbit globulin control. This observation was shown to be due to cross-reactions of the monoclonal antibody EB-A2 used by the kit with several airborne fungi likely to contaminate serum samples, including Penicillium chrysogenum, Cladosporium herbarum, Acremonium species, Alternaria alternata, Fusarium oxysporum, Wangiella dermatitidis, and Rhodotorula rubra.

The detection of Aspergillus galactomanann antigen in the sera of patients with invasive aspergillosis by radioimmunoassay and enzyme immunoassay was described several years ago (1, 3, 4, 8, 9). It is reported to be nearly 100% specific, whereas its sensitivity is low for patients undergoing bone marrow transplantation (13) and neutropenic patients on chemotherapy for hematological malignancy (7). This may be partly due to the rapid clearance of the antigen by the mannose receptors of macrophages and other cells in the liver and spleen (2, 4), comparable to the clearance of Candida mannan antigen (6). Recently, a latex agglutination (LA) test with the rat monoclonal antibody EB-A2 (11), Pastorex Aspergillus (Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France), became commercially available and since then has been used in many hospitals (12). This test yielded sensitivities of 93.3% for a group of patients with proven invasive aspergillosis and 94.4% for a group of patients suspected of having the disease, while no false-positive results were observed (5). According to the manufacturer, the test exhibits cross-reactivities with antigens from A. fumigatus, A. terreus, A. versicolor, A. niger, and A. flavus. The rat monoclonal antibodies EB-A1 and EB-A2 are reported to cross-react with several fungal exoantigens, including those of Penicillium digitatum, Trichophyton rubrum, Trichophyton mentagrophytes var. interdigitale, Botrytis tulipae, Wallemia sebi, and Cladosporium cladosporioides but not those of Fusarium solani, Trichoderma viride, Candida albicans, Saccharomyces cerevisiae, or Cryptococcus neoformans (11).

In December 1992, a serum sample from a 57-year-old woman on chemotherapy for plasmocytoma was sent to our laboratory to be tested for invasive aspergillosis. The serum was drawn on 22 December and, because of the Christmas holidays, arrived 7 days later, on 29 December. It was routinely examined for anti-Aspergillus antibodies by a hemagglutination test and for Aspergillus antigen by the Pastorex Aspergillus LA test. The hemagglutination test was negative, whereas the LA test gave a titer of 1:128, and the reaction was heat stable, was not eliminated by pronase treatment, and was not detected by a normal rabbit globulin control. Pronase and control latex were not included in the Pastorex Aspergillus LA test but were obtained from Boehringer, Mannheim, Germany, and from the Slidex Crypto Kit (BioMérieux, Marcy-l’Etoile, France), respectively. The serum sample was not completely clear, and was therefore suspected of being contaminated. Cultures in Sabouraud dextrose broth and on blood agar yielded growth of Penicillium chrysogenum and Corynebacterium species. The identity of P. chrysogenum was confirmed by the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. Because the cross-reactivities of the rat monoclonal antibody EB-A2 with P. digitatum and Penicillium marneffei were already reported (11), the reason for this false-positive reaction was obvious.

After this observation, we examined other microorganisms likely to contaminate serum samples, including several airborne fungi and some normal residents of the human skin, for their cross-reactivities with the Pastorex Aspergillus LA test (Table 1). Microorganisms without strain designations were isolated from patients of the University Hospitals of Heidelberg for the year 1992. They were differentiated by standard methods. The exoantigens were prepared as follows. One loop of biomass from the microorganisms grown on standard media for several days or weeks was suspended in 100 μl of treatment solution (which was included in the Pastorex Aspergillus LA test kit; lot no. 2KI34), vigorously homogenized, heated to 100°C for 3 min, and centrifuged at 10,000 × g for 10 min. A serial dilution of the supernatant in diluent buffer (which was included in the kit) was prepared, and 40 μl of each dilution was mixed with 10 μl of Aspergillus latex and rotated on the agglutination card for 5 min at 160 rpm at room temperature before being read for agglutination.

Cross-reactions within the genus Aspergillus were confirmed. In addition to known cross-reactions within the genera Penicillium and Cladosporium, the test also picked up P. chrysogenum and Cladosporium herbarum. Exoantigens from Acremonium species and Alternaria alternata exhibited positive reactions. In contrast to Fusarium solani,
which was reported to lack cross-reactivity (11), we found *Fusarium oxysporum* to cross-react (the identity of *F. oxysporum* was confirmed by the Centraalbureau voor Schimmelcultures). Members of the genera *Geotrichum* and *Rhizopus* were nonreactive.

With *Candida albicans* and *Cryptococcus neoformans*, we confirmed the negative results of Stynen et al. (11). However, red and black yeasts (*Rhodotorula rubra* and *Wangiella dermatitidis*) cross-reacted. The four bacteria tested lacked cross-reactivity.

Stynen et al. (10) mentioned that serum samples should be of good quality, e.g., separated from the clot within 24 h, and transport should be avoided. However, in practice, these conditions are hard to meet. Because the Pastorex *Aspergillus* LA test exhibits significant cross-reactions with widespread airborne fungi, contamination of a serum sample must be ruled out before a positive reaction is accepted.

We recommend that positive results should be reported only if the serum samples are clear and free of any contamination as determined by microscopic examination. Titers greater than 1:32 have rarely been reported for human cases of invasive aspergillosis (4, 5, 7–9, 12, 13). Thus, positive results with a titer of ≥1:32 for serum samples drawn more than 24 h before examination of the titer should indeed be verified by culturing the serum sample prior to reporting the final results.

REFERENCES