

## NOTES

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

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Received 8 March 1993/Returned for modification 14 May 1993/Accepted 5 June 1993

The Pastorex *Aspergillus* antigen test for detection of *Aspergillus* galactomannan 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* galactomannan 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*, *Walleria 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'Étoile, 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 marneffeii* 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 isolates 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  $\mu$ l of treatment solution (which was included in the Pastorex *Aspergillus* LA test kit; lot no. 2K134), vigorously homogenized, heated to 100°C for 3 min, and centrifuged at 10,000  $\times$  g for 10 min. A serial dilution of the supernatant in diluent buffer (which was included in the kit) was prepared, and 40  $\mu$ l of each dilution was mixed with 10  $\mu$ l 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 reconfirmed. 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*,

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TABLE 1. Reactivities of Pastorex *Aspergillus* LA test with exoantigen preparations from different microorganisms

Microorganism <sup>a</sup>	Reciprocal titer
<i>Aspergillus fumigatus</i> strain 1.....	64
<i>Aspergillus fumigatus</i> strain 2.....	256
<i>Aspergillus flavus</i> .....	4,000
<i>Aspergillus terreus</i> .....	1,000
<i>Penicillium chrysogenum</i> .....	256
<i>Cladosporium herbarum</i> .....	256
<i>Acremonium</i> species.....	4,000
<i>Fusarium oxysporum</i> .....	16
<i>Alternaria alternata</i> .....	16
<i>Rhizopus stolonifer</i> .....	Negative
<i>Geotrichum candidum</i> .....	Negative
<i>Candida albicans</i> NCPF 3205 serotype A.....	Negative
<i>Cryptococcus neoformans</i> .....	Negative
<i>Rhodotorula rubra</i> .....	2,000
<i>Wangiella dermatitidis</i> ATCC 34100.....	256
<i>Wangiella (Exophiala) dermatitidis</i> .....	128
<i>Corynebacterium striatum</i> .....	Negative
<i>Pseudomonas aeruginosa</i> ATCC 27853.....	Negative
<i>Staphylococcus aureus</i> ATCC 25923.....	Negative
<i>Escherichia coli</i> ATCC 25922.....	Negative

<sup>a</sup> Microorganisms without strain designations are clinical isolates from the University Hospitals of Heidelberg obtained in 1992.

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 Schimmecultures). 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 contam-

ination 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  $\geq 1:32$  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.

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