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CONCISE ARTICLE

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False-Positive Results Caused by Cotton Swabs in Commercial *Aspergillus* Antigen Latex Agglutination Test

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Abstract While testing for *Aspergillus* spp. using a commercially available latex agglutination test on a brain biopsy wrapped in a cotton swab, false-positive results were obtained. Subsequent application of the test on lavage samples obtained using cotton and synthetic swabs resulted positive with the cotton swabs only. This suggests that epitopes cross-reactive with *Aspergillus* galactomannan may be present in cotton.

Introduction

Invasive aspergillosis (IA) is recognized as an increasingly important cause of mortality in immunocompromised patients, especially those with acute leukemia. The mortality rate ranges between 50 and 90%. Improved prognosis needs early diagnosis, but the clinical features of IA are nonspecific. Therefore, diagnosis is suspected/established on the basis of a positive CT scan, the presence of filamentous fungi on microscopic examination of samples, a positive culture, and/or the detection of *Aspergillus* galactomannan antigen in serum [1, 2].

The galactomannan antigen can be detected using either the LAT Pastorex *Aspergillus* latex agglutination test (Bio-Rad, France) or an enzyme-linked immunosorbent assay (Platelia *Aspergillus*, Bio-Rad) [2, 3]. Both procedures have been developed and evaluated to detect the *Aspergillus* polysaccharide in serum samples only. The specificity of the LAT is about 85% [3], and processing a sample with this method takes about 30 min. However, the test is too insensitive to be useful in the early stages of IA [2, 3]. Conversely, in neutropenic patients with signs of aspergillosis on CT scan, utilization of the LAT on bronchoalveolar lavage supernatant has proved an easy and rapid method of confirming the diagnosis of IA [4]. In order to determine the usefulness of applying the LAT on clinical samples other than serum, we decided to use the test on supernatants of ground tissues.

Hyphal elements may be seen on microscopic examination of surgical samples obtained from neutropenic patients who have undergone surgery for extensive lesions and who have clinical, radiological and/or microbiological features of pulmonary aspergillosis [1, 5]. Since these patients may have received antifungal chemotherapy prior to surgery, subsequent culture and identification of the fungal agent is often precluded. However, the reliable performance of LAT on tissue samples could provide a means of overcoming this difficulty. In the present report, we present data suggesting that epitopes cross-reactive with *Aspergillus* galactomannan may be present on cotton swabs used to handle tissues, potentially causing the test to provide false-positive results.

Materials and Methods

In December 1998, we received a brain biopsy wrapped in a cotton swab, which we tested for the presence of *Aspergillus* antigen. After being ground in a potter in sterile 0.9% NaCl, the material was centrifuged at 3,000×g for 10 min at room temperature in a sterile tube, and the supernatant was recovered. The LAT Pastorex *Aspergillus* latex agglutination test (Bio-Rad) was then applied to the ground-biopsy supernatant according to the procedure for serum samples described by the manufacturer, and the result was positive. However, no fungal agents were observed under direct microscopic examination or after Giemsa and Toluidine blue stains of the pellet, and a 2-week culture at 30°C on Sabouraud dextrose agar was negative. Moreover, histopathological examination showed that the lesion was in fact a brain tumor.

This was the first time we had applied the LAT to a biopsy received in a cotton swab. We therefore hypothesized that the posi-

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tive test result may have resulted from cross-reactivity with antigenic components of the swab. This prompted us to test a variety of swabs for false-positive LAT results.

In a first series of experiments, all of the swab types available at our hospital's pharmacy were tested. These included four cotton types and one synthetic type (Table 1). To ensure the reproducibility of the experiments, the following sampling strategy was used. For each type of swab, five packs belonging to each distinct manufacturing set were selected. First, all of the swabs from one pack were tested to establish the absence of variability within a given pack. Then, one swab was chosen randomly from each of the other four packs to test for variability between sets. Altogether, nine swabs were treated for 15 min with 0.9% sterile NaCl. Aliquots from each lavage were recovered for LAT and processed according to the manufacturer's protocol, which included heating of the samples for 3 min at 100°C to eliminate nonspecific reactions. Negative controls consisted of 0.9% sterile NaCl. Positive controls consisted of galactomannan antigen supplied with the test.

A second series of experiments was subsequently performed to determine the level of cross-reactivity of the cotton swabs. To achieve this, twofold dilutions of lavages from one synthetic and three cotton swabs were tested with the LAT (Table 2).

To rule out the possibility that a positive LAT might result from contamination of the swabs with fungal spores or hyphae, aliquots from each lavage were cultured on Sabouraud dextrose agar at 30°C for 2 weeks. Moreover, in the second series of experiments the lavage samples were treated by centrifugation at 7,000×g for 5 min. The resulting pellets were suspended in 100 µl of distilled water. After DNA extraction (Qiamp DNA mini kit; Qiagen, Germany), samples were subjected to a panfungal polymerase chain reaction (PCR) that targets the ribosomal RNA repeat [6, 7]. Positive controls consisted of *Aspergillus fumigatus* spores suspended in distilled water (threshold of detection equivalent to DNA from 5 spores per PCR reaction). Samples were amplified in duplicate by adding DNA from *Candida albicans* to ensure that negative results were not due to the presence of PCR inhibitors. In addition, the pellets were examined microscopically for fungal elements.

Results and Discussion

All 39 cotton swabs tested in the two series of experiments resulted positive with the LAT, whereas all of the 10 synthetic swabs resulted negative. None of the samples grew *Aspergillus* on culture, thereby ruling out the possibility of contamination with viable *Aspergillus* spores. Moreover, PCR amplification with an assay based on universal primers complementary to the coding regions of the fungal rRNA was negative (Table 2), and direct microscopic examination of the lavage samples failed to detect fungal elements. This makes it very unlikely that the presence of nonviable fungi caused the positive LAT results.

Although only a limited sample of all commercially available swabs was tested in this study, our data strongly suggest that cotton swabs release components that cause false-positive LAT results. It has previously been reported that disinfectants and soaps used to clean ring slides were found to cause false-positive latex agglutination results in a cryptococcal antigen latex agglutination assay [8]. Since cotton may be treated with detergents and bleaching agents, the positive LAT results observed with lavage samples from cotton swabs could be due to nonspecific interference with remnants of detergents present on the swabs. An alternative hypothesis is that epitopes cross-reactive with *Aspergillus* galactomannan are present on cotton, thus explaining the false-positive LAT result observed with the brain biopsy from our patient.

Galactomannan is a well-characterized polysaccharide antigen of the cell wall of *Aspergillus fumigatus*, and its molecular structure has been established: the mannan core has a linear configuration containing α(1–2)- and α(1–6)-linked residues, and the antigenic side chains branched on α(1–2)-linked mannose residues are composed of β(1–5) galactofuranosyl residues [2]. The LAT uses a monoclonal antibody (Mab) that recognizes the β(1–5) galactofuranosyl side chains of the *Aspergillus* galactomannan [2]. On the other hand, cotton contains cellulose, which is composed of β(1–4) glucopyranose units [9], and glucose is an isomeric form of galactose [9]. Therefore, despite the fact that furanosyl and pyranosyl residues have distinct cycle organizations, we cannot rule out the possibility that the Mab used in the LAT also reacted with the β(1–4) glucose units of cellulose. Interestingly, viscose (which accounts for about 70% of the composition of swabs manufactured by Smith Nephews and Sylamed) is a textile fiber obtained from cellulose, in which cellulose is transformed by a complex procedure including sodium hydroxide and acid treatment; this may account for the destruction of reactive epitopes and the lack of false-positive agglutination with this type of swab.

Table 1 Results of LAT reactivity and culture of lavage supernatants on different types of swabs

Manufacturer	Swab type	LAT	Culture
BastosViegas	cotton (100%)	positive	negative
Tetra medical	cotton (100%)	positive	negative
Rauscher	cotton (100%)	positive	negative
Hartmann	cotton (100%)	positive	negative
Smith Nephews	synthetic (67% viscose, 33% polyester)	negative	negative

Table 2 Results of LAT, titration, culture, microscopy and panfungal PCR of lavage samples on three cotton swabs and one synthetic swab

Manufacturer	Swab type	LAT	Titer	Culture	Microscopy	PCR
Mefra	cotton (100%)	positive	1/64	negative	negative	negative
Sylamed	synthetic (70% viscose, 30% polyester)	negative	0	negative	negative	negative
Hydrex (small)	cotton (100%)	positive	1/16	negative	negative	negative
Hydrex (large)	cotton (100%)	positive	1/64	negative	negative	negative

According to the manufacturer, the LAT exhibits cross-reactivities with antigens from *Aspergillus terreus*, *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus versicolor*, allowing the test to detect IA caused by *Aspergillus* species other than *fumigatus*. The LAT has also been reported to cross-react with fungal exoantigens from non-*Aspergillus* species [10], reducing its specificity for the diagnosis of IA. In addition, nonfungal causes of false-positivity have been described, such as poor quality or bacterial contamination of the serum samples tested, or utilization of wooden toothpicks during preparation of the reaction [11]. The latter observation is particularly interesting in the context of our study, since wood, like cotton, contains cellulose [9]. Finally, unidentified serum components have also been shown to cross-react with an enzyme-linked immunosorbent assay test based on the same monoclonal antibody as the LAT [12], and a false-positive LAT result has been reported from the urine of a rat receiving cyclophosphamide [13]. The current study adds a new cause to the list of substances that may cross-react with the LAT. More generally, it also emphasizes that great care should be exercised when using a test under conditions other than those recommended by the manufacturer and for which it has not been thoroughly evaluated.

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