NOTES

Cross-Reactivity of Paracoccidioides brasiliensis, Histoplasma capsulatum, and Cryptococcus Species in the Commercial Platelia Aspergillus Enzyme Immunoassay

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Cross-reactivity in the Platelia Aspergillus enzyme immunoassay was evaluated using 120 sera from patients with paracoccidioidomycosis, histoplasmosis, and cryptococcosis. At a cutoff value of 0.5, positivity rates were 50%, 67%, and 50%, respectively. The implications for these findings are discussed.

The sandwich Platelia Aspergillus enzyme immunoassay (EIA) (Bio-Rad, France) is a commercial test that has been used extensively for the diagnosis of invasive aspergillosis (IA). The assay detects galactofuranose-containing side chains of galactomannan, an antigen released from Aspergillus hyphae during growth in the host (1). In addition to varied sensitivity, the test is limited by false-positive results, mostly due to the use of antibiotics. Like Aspergillus species, many other fungi have been shown to produce galactomannan (1, 2, 4–6, 9). Here we evaluate the cross-reactivity of four important pulmonary fungal pathogens—Paracoccidioides brasiliensis, Histoplasma capsulatum, Cryptococcus neoformans, and Cryptococcus gattii—in the commercial galactomannan test.

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A total of 120 serum samples from 102 patients were evaluated. These included 30 samples from each of the following: paracoccidioidomycosis (30 patients), histoplasmosis (n = 26), cryptococcosis due to C. neoformans (n = 28), and C. gattii (n = 18). Sera from patients not suspected to have fungal disease have been tested in parallel as negative controls (n = 12). Platelia Aspergillus EIA testing was performed according to the manufacturer’s specifications, using a cutoff value of 0.5. Samples were obtained from the serum bank at the Santa Casa-Complexo Hospitalar mycology laboratory in Brazil. Most samples had been stored at 20°C for >5 years. A few samples were in the freezer for more than 10 years, including a 17-year-old serum sample.

All patients with paracoccidioidomycosis had positive serology (immunodiffusion) results for P. brasiliensis. The infection was diagnosed by microscopy in 27 cases and by culture in Sabouraud agar for five patients. Histoplasmosis was confirmed by immunodiffusion, microscopy, and culture in 30, 11, and 6 cases, respectively. Cryptococcosis was diagnosed by the Latex-Crypto antigen detection system (Immuno Mycologics, Inc.). C. neoformans and C. gattii were differentiated after culture in canavanine-glycine-bromothymol blue agar.

The results of this study are summarized in Table 1. All sera from patients not suspected to have fungal disease tested negative for the galactomannan test, with optical density indexes ranging between 0.16 and 0.41. Positive results for the galactomannan test occurred for 50%, 67%, 63%, and 37% of the samples from patients with P. brasiliensis, H. capsulatum, C. neoformans, and C. gattii infection, respectively. Lower galactomannan indexes were observed for C. gattii than for C. neoformans (P = 0.017). No correlation was observed between the galactomannan optical density index and the Latex titer for Cryptococcus species (R² = 0.003; P = 0.689).

We looked for other variables that could result in false-positive results in the galactomannan test. Only two patients were on hemodialysis, and none had received piperacillin-tazobactam or amoxicillin-clavulanate. False-positive results usually do not occur with other antimicrobial drugs, even when serum concentrations are high (8). Although the influence of freezing and thawing on the galactomannan antigen assay is not clear, it is usually believed that long-term storage may actually decrease galactomannan levels (1). The impact of serum storage at different freezing temperatures, from −20°C to −80°C, is also unknown.

When more than one sample was obtained from the same patient, concordant results were usually observed. Discordant results occurred for one individual with histoplasmosis and for another one with C. neoformans infection. In both cases, a positive sample was followed by a negative test after antifungal therapy was started, a very well-known phenomenon (1). Sera from seven patients with C. gattii infection were tested more...
than once. For six of these patients, the same serum sample was run in duplicate, and one patient was tested six times. Again, the most discordant results were seen after antifungal therapy was started. Due to the retrospective character of our study, we were not able to obtain additional clinical information for our patients. Thus, any assumption about reduced galactomannan indexes in patients started on antifungal therapy was speculative at most. Moreover, the impact of the mycosis on the clinical presentation (e.g., fungemia versus nondisseminated pulmonary nodules) was run in duplicate, and one patient was tested six times. For six of these patients, the same serum sample was run in duplicate, and one patient was tested six times. Due to the retrospective character of our study, we were not able to obtain additional clinical information for our patients. Thus, any assumption about reduced galactomannan indexes in patients started on antifungal therapy was speculative at most. Moreover, the impact of the mycosis on the clinical presentation (e.g., fungemia versus nondisseminated pulmonary nodules) was not evaluated.

Previous studies have described that cross-reactivity might occur with several fungi in the commercial galactomannan test. These include Geotrichum capitatum (4) and Penicillium marneffei (5), molds that contain galactomannan in the cell wall. The cross-reactivity of H. capsulatum and C. neoformans has also been described (2, 5, 6, 9). The mechanism of H. capsulatum cross-reactivity is not yet elucidated. Although samples from patients with histoplasmosis may give positive results for the Platelia Aspergillus EIA, samples from patients with aspergillosis are negative for the Histoplasma antigen EIA (9). The C. neoformans cell wall contains galactoxylomannan, an antigen similar to galactomannan, which may result in a cross-reaction in the galactomannan test (2). However, this was not confirmed in a recent study (3). The hypothesis that genotypic variations could explain the differences observed among studies with C. neoformans remains to be tested.

To the best of our knowledge, this is the first study in which reactivity with antibody used for the Platelia assay was tested for patients infected with P. brasiliensis and C. gattii. These are important pulmonary pathogens, affecting mostly nonimmunocompromised hosts. Galactomannan is a cell wall component for both the yeast and the mycelial forms of P. brasiliensis. In addition, H. capsulatum and P. brasiliensis are phylogenetically closely related fungi (7). Similarly for C. neoformans, the presence of an epitope causing cross-reactivity may also occur for C. gattii. Actually a previous investigation showed that soluble antigens from one reference strain of C. gattii tested positive in the Platelia Aspergillus EIA (2).

This study reinforces that caution should be taken when considering a positive galactomannan test for a patient with respiratory infection. The diagnostic of IA using galactomannan may be tricky for patients coming from areas where paracoccidioidomycosis and histoplasmosis are endemic. Cryptococcus also affects solid organ transplant recipients, a population at risk for IA, which may present with multiple pulmonary nodules. Moreover, these mycoses may differ in terms of response to antifungal drugs. For instance, echinocandins are not active against Histoplasma species, and experience using voriconazole for histoplasmosis remains limited (9). Both Geotrichum and Cryptococcus species are usually susceptible to amphotericin B and azoles but intrinsically resistant to echinocandins. Conversely, P. brasiliensis infection can be treated with sulfonamides. In order to properly interpret the meaning of a positive galactomannan test, clinical and epidemiological data should be taken into consideration.

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### REFERENCES


### TABLE 1. Results of serum galactomannan testing from patients infected with *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, and *Cryptococcus gattii*

<table>
<thead>
<tr>
<th>Agent</th>
<th>Serum galactomannan optical density index*</th>
<th>No. (%) of samples with optical density index of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range, Mean value, 95% Confidence interval</td>
<td>&gt;2.0, &gt;1.0 to 2.0, ≥0.5 to 1.0, &lt;0.5</td>
</tr>
<tr>
<td><em>Paracoccidioides brasiliensis</em></td>
<td>0.23–5.47, 0.99, 0.53–1.44</td>
<td>3 (10.0), 5 (16.6), 7 (23.3), 15 (50.0)</td>
</tr>
<tr>
<td><em>Histoplasma capsulatum</em></td>
<td>0.24–5.38, 1.43, 0.88–1.98</td>
<td>7 (23.3), 7 (23.3), 6 (20.0), 10 (33.3)</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>0.18–&gt;10, 1.95, 0.95–2.94</td>
<td>9 (30.0), 7 (23.3), 5 (16.6), 11 (36.6)</td>
</tr>
<tr>
<td><em>Cryptococcus gattii</em></td>
<td>0.18–&gt;9, 1.03, 0.38–1.68</td>
<td>4 (13.3), 3 (10.0), 4 (13.3), 19 (63.3)</td>
</tr>
</tbody>
</table>

* Optical density indexes higher than or equal to 0.5 are considered positive. High indexes (i.e., >1.0) increase the test specificity in the diagnosis of invasive aspergillosis (1).

b n = 30 (samples) for each group.