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Histoplasmosis-Associated Cross-Reactivity in the BioRad Platelia *Aspergillus* Enzyme Immunoassay▽

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We observed false-positive results in the Platelia *Aspergillus* enzyme-linked immunoassay (EIA) for specimens from patients with histoplasmosis and mice with experimental infection. Platelia *Aspergillus* EIA-positive specimens were negative in the second-generation *Histoplasma* antigen EIA. Care must be taken to exclude histoplasmosis for patients with positive Platelia *Aspergillus* EIA results.

The Platelia *Aspergillus* enzyme-linked immunoassay (EIA) detects a galactomannan antigen produced by several molds (5). However, studies to date have not included *Histoplasma capsulatum*. We recently observed false-positive results in the Platelia *Aspergillus* EIA for specimens from six patients with culture-proven histoplasmosis, as have others (4). Based upon these observations (Table 1), we conducted a laboratory-based study of specimens submitted for Platelia *Aspergillus* EIA or *Histoplasma* antigen testing and evaluated cross-reactivity in experimental models of histoplasmosis and aspergillosis.

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Residual serum and bronchoalveolar lavage (BAL) fluid specimens that were submitted to MiraVista Diagnostics for *Histoplasma* antigen testing or Platelia *Aspergillus* EIA and were positive were retested the following day in the other EIA. The second-generation *Histoplasma* antigen EIA (MiraVista Diagnostics, Indianapolis, IN) uses polyclonal antibodies to *H. capsulatum* and has been described elsewhere (6). Results of ≥1 unit were regarded as positive. The Platelia *Aspergillus* EIA (Bio-Rad Laboratories, Redmond, WA) uses monoclonal antibodies produced against *Aspergillus fumigatus*. Specimens were pretreated with EDTA for the Platelia *Aspergillus* EIA and boiled in accordance with the manufacturer's specifications. Results with a galactomannan index (GMI) of 0.5 or greater were reported as positive. Note that the Platelia *Aspergillus* EIA is not FDA cleared for specimens other than serum.

Twenty-three of 48 serum specimens positive for antigen in the second-generation *Histoplasma* antigen EIA were positive in the Platelia *Aspergillus* EIA (Fig. 1). Positive results were more frequent for specimens giving levels of 40 units or higher in the *Histoplasma* antigen EIA (12/17 [70.6%]) than for those giving levels below 40 units (11/31 [35.5%]) ($P = 0.043$ by chi-square test). As controls, 12 serum specimens that were negative in the *Histoplasma* antigen EIA were tested in the Platelia *Aspergillus* EIA, and all were negative. Seven of 11 (63.6%) BAL fluid specimens that were positive in the *Histoplasma* antigen EIA were positive in the Platelia *Aspergillus* EIA. Results for the *Histoplasma* antigen EIA ranged from 2.2 to 61.7 units for the BAL fluid specimens that were positive in the Platelia *Aspergillus* EIA, compared to 4.2 to 21.2 units for those that were negative. Ten control BAL fluid specimens that were negative in the *Histoplasma* antigen EIA were negative in the Platelia *Aspergillus* EIA.

Twenty serum specimens that were positive in the Platelia *Aspergillus* EIA (GMI range, 0.54 to 9.08; median, 1.8) were negative in the *Histoplasma* antigen EIA. Eighteen BAL fluid specimens that were positive in the Platelia *Aspergillus* EIA (GMI range, 0.84 to 9.29; median, 6.1) were negative in the *Histoplasma* antigen EIA.

Nonimmunosuppressed mice were infected intranasally with 10^6 *H. capsulatum* yeast cells, and spleens obtained 10 days later were homogenized in 2.0 ml of sterile RPMI (1). The spleen homogenates were tested at a 1:10 dilution in the *Histoplasma* antigen EIA and a 1:1 dilution in the Platelia *Aspergillus* EIA to reduce the chance of overlooking low-level cross-reactivity. All animal experiments were done according

TABLE 1. Clinical specimens identified to have positive results in second-generation *Histoplasma* EIA and Platelia *Aspergillus* EIA

Case no.	Second-generation *Histoplasma* EIA result (U)[a]		Platelia *Aspergillus* EIA result for serum (GMI)	Type of histoplasmosis[b]
	Serum	Urine		
1	42.6	31.4	6.2	Pulmonary
2	QNS	20.4	5.5	Disseminated
3	4.2	12.5	5.5	Cavitary
4	33.9	32.0	1.5	Disseminated
5	24.2	ND	4.7	Disseminated
6	98.7	66.8	7.8	Disseminated

[a] QNS, quantity not sufficient to test; ND, not done.
[b] None of these patients showed evidence of aspergillosis.

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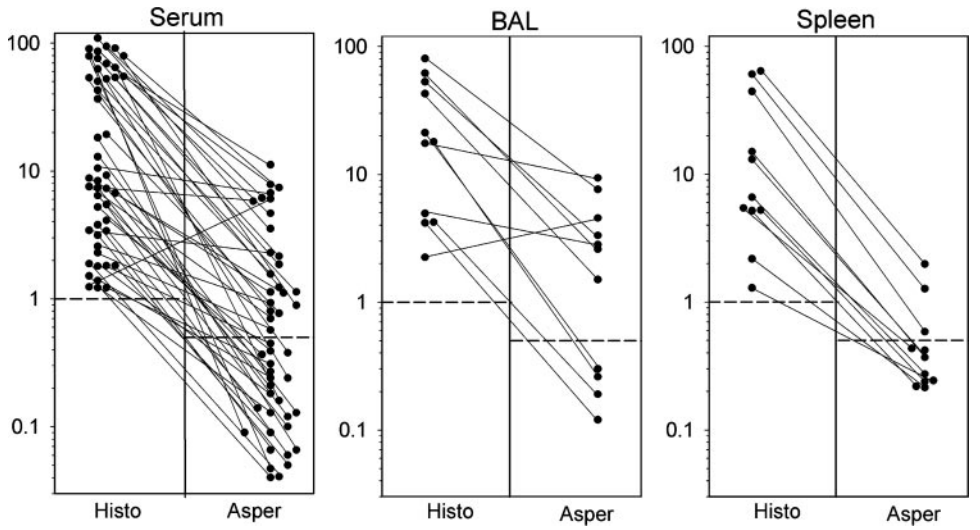

FIG. 1. Comparison of antigen levels in sera (left) and BAL fluids (middle) from patients with histoplasmosis and in spleen tissues from mice with histoplasmosis (right), tested in the *Histoplasma* EIA (Histo) and Platelia *Aspergillus* EIA (Asper). The vertical axis depicts antigen units (Histo) and GMI (Asper). The cutoffs for positivity are 1.0 unit for the second-generation *Histoplasma* EIA and 0.5 GMI for the Platelia *Aspergillus* EIA, as shown by broken horizontal lines. Results for the same specimens tested in both assays are connected by solid lines.

to institutional guidelines. Spleens from 3 of 11 (27.3%) mice were positive in the Platelia *Aspergillus* EIA. These three specimens exhibited the highest results in the *Histoplasma* antigen EIA (44.4 units, 60.5 units, and 64.0 units). Results for the eight spleen homogenates that were negative in the Platelia *Aspergillus* EIA ranged from 1.3 to 15.0 units in the *Histoplasma* antigen EIA.

In an experimental model of invasive pulmonary aspergillosis, 1.25×10^8 *A. fumigatus* conidia (NIH isolate 4215; ATCC MYA-1163) were administered intratracheally to profoundly neutropenic New Zealand White rabbits ($n = 9$) (Hazelton Research Products, Inc., Denver, PA) (3). Plasma ($n = 32$) and BAL fluid ($n = 7$) samples, which were used in another project and had been stored at −70°C for about 2 years, were all positive in the Platelia *Aspergillus* EIA (plasma GMI range, 0.5 to 5.9 [median, 1.1]; BAL fluid GMI range, 1.5 to 6.8 [median, 6.4]). All were negative in the *Histoplasma* antigen EIA.

These findings indicate that the antigen detected in body fluids from patients with histoplasmosis is detected in the Platelia *Aspergillus* EIA. Cross-reactivity correlated with the level of positivity in the *Histoplasma* antigen EIA, occurring twice as often in specimens with *Histoplasma* antigen levels of 40 units or more. Cross-reactivity also was observed in spleen tissues from mice with experimental histoplasmosis. Others have reported a false-positive Platelia *Aspergillus* EIA result for a patient with blastomycosis (2), and we have observed a false-positive Platelia *Aspergillus* EIA result for a patient with coccidioidomycosis (L. J. Wheat, unpublished data).

Surprisingly, specimens that were positive in the Platelia *Aspergillus* EIA, including those from rabbits with aspergillosis and very high *Aspergillus* antigen levels in BAL fluid, were negative in the *Histoplasma* antigen EIA. The reasons for this discrepancy are not fully understood but may relate to differences in the magnitudes of antigenemia in the two mycoses.

Recognition of false-positive results in the Platelia *Aspergillus* EIA for specimens from patients with histoplasmosis is important because treatment for aspergillosis may not be effective for histoplasmosis. For example, the echinocandins are neither active nor recommended for histoplasmosis, and experience using voriconazole is insufficient to recommend it. If only the Platelia *Aspergillus* EIA is ordered and the result is positive, the patient may be treated for aspergillosis without consideration that the result may be due to histoplasmosis.

Care must be taken to exclude histoplasmosis in patients with a positive Platelia *Aspergillus* EIA if histoplasmosis is deemed more likely than aspergillosis. An absence of severe neutropenia, congenital neutrophil functional impairment, or allogeneic stem cell transplantation makes aspergillosis less likely. Immune deficiency states favoring histoplasmosis include AIDS, solid organ transplantation, and corticosteroid or tumor necrosis factor inhibitor therapy. Disseminated histoplasmosis may also occur in individuals without known causes of immunosuppression, which is very rare for invasive aspergillosis. In patients with recent or past exposure to areas where histoplasmosis is endemic, histoplasmosis should be excluded by antigen testing, serology, and culture of relevant tissue or fluid samples. Although it is rare, dual infection with *Aspergillus* spp. and *H. capsulatum* is possible and should be considered for patients with risk factors and clinical findings compatible with both infections.

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We disclose that L.J.W., E.H., M.D., and P.C. are employees of MiraVista Diagnostics, the laboratory that developed the second-generation *Histoplasma* antigen EIA and performs the Platelia *Aspergillus* EIA.

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