Cross Reactivity in the BioRad Platelia™ Aspergillus EIA Caused by Histoplasmosis

Running title: Cross reactivity in Platelia Aspergillus EIA

L. Joseph Wheat1, Emily Hackett1, Michelle Durkin1, Patricia Connolly1, Ruta Petraitiene2,3, Thomas J. Walsh2, Kenneth Knox4, Chadi Hage4.

1 MiraVista Diagnostics, Indianapolis, IN, USA

Address correspondence to:
L. Joseph Wheat
MiraVista Diagnostics
4444 Decatur Blvd, Suite 300
Indianapolis, IN 46241
317-856-2681 [phone]
317-856-3685 [fax]
jwheat@miravistalabs.com

2 National Cancer Institute
Pediatric Oncology Branch,
Immunocompromised Host Section,
CRC, Rm 1W-5750,
10 center Drive
Bethesda, MD 20892-1100
Laboratory Animal Sciences Program,

SAIC-Frederick, Inc.,

Frederick, MD 21702

Indiana University School of Medicine

Department of Pulmonary Medicine

Roudebush Veterans’ Administration Hospital

1481 West Tenth Street

Indianapolis, IN 46202
Abstract. We observed false-positive results in the Platelia™ Aspergillus EIA in specimens from patients with histoplasmosis and mice with experimental infection. Platelia™ Aspergillus EIA positive specimens were negative in the second-generation Histoplasma antigen enzyme immunoassay. Care must be taken to exclude histoplasmosis in patients with positive Platelia™ Aspergillus EIA results. The Platelia™ Aspergillus EIA detects a galactomannan antigen produced by several molds (3). However, studies to date did not include Histoplasma capsulatum. We recently observed false-positive results in the Platelia™ Aspergillus EIA in specimens from six patients with culture-proven histoplasmosis, as have others (S. Ranque, F. Dromer, S. Genot, A. Michel-Ngyen, F. Faraut, A. Stein, H. Tissot-Dupont, and H. Dumon, Abstr. 16th Congress of the International Society for Human and Animal Mycology (ISHAM), Abstr. P-0299, 2006). 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. Residual serum and BAL 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 enzyme immunoassay (EIA) (MiraVista Diagnostics, Indianapolis, IN) uses polyclonal antibodies to H. capsulatum and has been described elsewhere (4). Results of ≥ 1 unit were regarded as positive. The Platelia™ Aspergillus EIA (BioRad Laboratories,
Redmond WA) uses monoclonal antibodies produced against *A. fumigatus*. Specimens were pre-treated with EDTA and boiled in accordance with manufacturer’s specifications. Results with a galactomannan index (GMI) of 0.5 or greater are reported as positive. Of note 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 (Figure 1). Positive results were more frequent in specimens that were 40 units or higher in the *Histoplasma* antigen EIA (12/17, 70.6%) than in those with levels below 40 units (11/31, 84.6%), chi square, p=0.043. 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 specimens that were positive *Histoplasma* antigen EIA were positive in the Platelia™ Aspergillus EIA. Results in the *Histoplasma* antigen EIA ranged from 2.2 to 61.7 units in the BAL specimens that were positive in the Platelia™ Aspergillus EIA, compared to 4.2 to 21.2 units in those that were negative. Ten control BAL 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 0.54-9.08, median 1.8) were negative in the *Histoplasma* antigen EIA. Eighteen BAL specimens that were positive in the Platelia™ Aspergillus EIA (GMI range0.84-9.29, median 6.1) were negative in the *Histoplasma* antigen EIA.
Non-immunosuppressed mice were infected intranasally with $10^6$ *H. capsulatum* yeast, and spleens obtained ten 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 to institutional guidelines. Spleens from three 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 in 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, $1.25 \times 10^8$ *A. fumigatus* conidia (NIH isolate 4215, ATCC No. MYA-1163) were administered intratracheally in profoundly neutropenic New Zealand white rabbits (N=9) (Hazelton Research Products, Inc., Denver, PA) (2). Plasma (N=32) and BAL (N=7), which were used in another project and had been stored at -70°C for about two years, all were positive in the Platelia™ *Aspergillus* EIA: plasma (range 0.5 to 5.9 GMI, median 1.1) and BAL (range 1.5 to 6.8 GMI, median 6.4). All were negative in *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 tissue from mice with experimental histoplasmosis. Others have reported a false-positive Platelia™ *Aspergillus* EIA result in a patient with blastomycosis (J. Cummings, G. Jamison, J. Boudreaux, M. Howles, T. Walsh, and R. Hayden, Abstr. 16th Congress of the International Society for Human and Animal Mycology (ISHAM), abstr. P-0376, 2006), and we have observed a false-positive Platelia™ *Aspergillus* EIA in a patient with coccidioidomycosis (Wheat, unpublished data).

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

Recognition of false-positive results in the Platelia™ *Aspergillus* EIA in 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 considering 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. Absence of
severe neutropenia, congenital neutrophil functional impairment, or allogeneic stem cell transplantation make aspergillosis less likely. Immune deficiency states favoring histoplasmosis include AIDS, solid organ transplants, and corticosteroid or tumor necrosis factor inhibitor therapy. Disseminated histoplasmosis also may occur in individuals without known causes for immunosuppression, which is very rare in invasive aspergillosis. In patients with recent or past exposure to endemic areas for histoplasmosis, histoplasmosis should be excluded by antigen testing, serology and culture of relevant tissue or fluid samples. Although rare, dual infection with Aspergillus spp. and H. capsulatum is possible, and should be considered in patients with risk factors and clinical findings compatible with both infections.

This work was conducted in part through the intramural program of the National Cancer Institute (T.J.W and R.P.) and was presented at 46th Interscience Conference on Antimicrobial Agents and Chemotherapy, 2006. We disclose that the following authors are employees of MiraVista Diagnostics, the laboratory that developed the second-generation Histoplasma antigen EIA and performs the Platelia™ Aspergillus EIA (LJW, EH, MD, PC).
Table 1. Clinical specimens identified to have positive results in the second generation *Histoplasma* EIA and the Platelia™ *Aspergillus* EIA

<table>
<thead>
<tr>
<th>Case</th>
<th>2nd generation Histoplasma EIA</th>
<th>Platelia™ Aspergillus EIA GMI²</th>
<th>Type of histoplasmosis</th>
<th>Evidence for aspergillosis</th>
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<tr>
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¹S=serum, U=urine, QNS=quantity not sufficient to test, ND=not done

²GMI=galactomannan index
Figure 1. Comparison of antigen levels in serum (left section) and BAL (middle section) from patients, and spleen tissue from mice (right section) with histoplasmosis tested in the *Histoplasma* EIA (columns labeled Histo) and Platelia™ *Aspergillus* EIA (columns labeled Asper). The vertical axis depicts antigen units. The cut-off for positivity is 1.0 unit for the second generation *Histoplasma* EIA and 0.5 GMI for the Platelia™ *Aspergillus* EIA, shown by the broken horizontal lines. Results for the same specimen tested in both assays are connected by the solid lines.

Reference List

amphotericin B for treatment of histoplasmosis in immunocompetent mice.


