Overview

Useful For
Aids in the diagnosis of invasive aspergillosis and assessing response to therapy

Method Name
Enzyme Immunoassay (EIA)

NY State Available
Yes

Specimen

Specimen Type
Lavage

Specimen Required
Container/Tube: Sterile, leak-proof container

Specimen Volume: 2 mL

Additional Information: To prevent specimen contamination, avoid opening/transferring specimen.

Forms
If not ordering electronically, complete, print, and send a Microbiology Test Request (T244) with the specimen.

Specimen Minimum Volume
1.5 mL

Reject Due To

<table>
<thead>
<tr>
<th>Bronchial washing</th>
<th>Specimen in a nonleak proof container</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thick/viscous/mucoid specimens</td>
</tr>
</tbody>
</table>

Specimen Stability Information

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lavage</td>
<td>Frozen (preferred)</td>
<td>14 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Refrigerated</td>
<td>5 days</td>
<td></td>
</tr>
</tbody>
</table>

Clinical and Interpretive

Clinical Information
Invasive aspergillosis (IA) is a severe infection that occurs in patients with prolonged neutropenia following transplantation or in conjunction with aggressive immunosuppressive regimens (e.g., prolonged corticosteroid use,
chemotherapy). The incidence of IA is reported to vary from 5% to 20% depending on the patient population. IA has an extremely high mortality rate of 50% to 80%, due in part to the rapid progression of the infection (ie, 1-2 weeks from onset to death). Approximately 30% of cases remain undiagnosed and untreated at death.

Definitive diagnosis of IA requires histopathological evidence of deep-tissue invasion or a positive culture. However, this evidence is often difficult to obtain due to the critically ill nature of the patient and the fact that severe thrombocytopenia often precludes the use of invasive procedures to obtain a quality specimen. The sensitivity of culture in this setting also is low, reportedly ranging from 30% to 60% for bronchoalveolar lavage (BAL) fluid.

Accordingly, the diagnosis is often based on nonspecific clinical symptoms (unexplained fever, cough, chest pain, dyspnea) in conjunction with radiologic evidence (computed tomography scan), and a definitive diagnosis is often not established before fungal proliferation becomes overwhelming and refractory to therapy.

Recently, a serologic assay was approved by the FDA for the detection of galactomannan, a molecule found in the cell wall of Aspergillus species. Serum galactomannan (Aspergillus antigen) can often be detected a mean of 7 to 14 days before other diagnostic clues become apparent, and monitoring of Aspergillus antigen can potentially allow initiation of preemptive antifungal therapy before life-threatening infection occurs.

The clinical utility of Aspergillus antigen testing in BAL specimens as an early prognostic indicator of IA has recently been assessed. These studies demonstrated equivalent or higher sensitivity compared to detection of Aspergillus antigen in serum. (1-4) This assay may be useful in the assessment of therapeutic response as antigen levels typically decline in response to effective antimicrobial therapy.

**Reference Values**

<0.5 Index

**Interpretation**

A positive result in bronchoalveolar lavage (BAL) fluid supports a diagnosis of invasive, pulmonary aspergillosis. Positive results should be considered in conjunction with other diagnostic procedures, such as microbiologic culture, histological examination of biopsy specimens, and radiographic evidence (see Cautions).

A negative result in BAL fluid does not rule out the diagnosis of invasive aspergillosis (IA). Patients at risk of IA should be monitored twice a week for Aspergillus antigen levels in serum until determined to be clinically unnecessary.

**Aspergillus** antigen levels typically decline in response to effective antimicrobial therapy.

**Cautions**

False-positive results are reported to occur at rates of 8% to 14% with this assay when performed on serum. Numerous foods (pasta, rice, etc) contain galactomannan. It is thought that damage to the gut wall by cytotoxic therapy, irradiation, or graft-versus-host disease enables translocation of the galactomannan from the gut lumen into the blood and may be partially responsible for the high false-positive rate of this assay when serum is tested. Whether false-positive results in bronchoalveolar lavage (BAL) fluid are associated with the consumption of certain foods, as is observed in serum samples, remains to be determined.

Other genera of fungi such as Penicillium and Paecilomyces have shown reactivity with the rat EBA-2 monoclonal antibody used in the assay. These species are rarely implicated in invasive fungal disease. Specimens containing Histoplasma antigen may cross-react in the Aspergillus antigen assay. Cross-reactivity with Alternaria species also has been reported.

Semisynthetic antibiotics such as piperacillin, amoxicillin, and Augmentin, which are based on natural compounds derived from the genus Penicillium, have been demonstrated to cross-react with the rat EBA-2 monoclonal antibody used in the assay.
The specificity of the assay for *Aspergillus* species cannot exclude the involvement of other fungal pathogens with similar clinical presentations such as *Fusarium*, *Alternaria*, and *Mucorales*.

The performance of the assay has not been evaluated other specimen types such as urine or cerebrospinal fluid.

The assay may exhibit reduced detection of *Aspergillus* antigen in patients with chronic granulomatous disease and Job syndrome.

The concomitant use of antifungal therapy in some patients with invasive aspergillosis may result in reduced sensitivity of the assay.

False-positive results are possible in patients receiving PLASMA-LYTE for intravenous hydration or if PLASMA-LYTE is used during bronchoscopy for the collection of BAL fluid.

**Supportive Data**

In clinical studies submitted to the FDA, the sensitivity of the test for serum was reported to be 81% for proven or probable invasive aspergillosis (n=31 patients), and the specificity was 89% (n=148 patients). The positive and negative predictive values were reported as 68% and 96% respectively, based on an average prevalence of 14% in the study population. In a low prevalence population (5%), the positive predictive value decreases to 31%; the negative predictive value remains at 96%.(Package insert: Platelia Aspergillus EIA, Bio-Rad, Redmond, WA, 6/2003).

Accuracy:

The performance characteristics of the Platelia *Aspergillus* EIA for the detection of galactomannan in BAL fluid were validated at Mayo Clinic Laboratories by comparison of results obtained from an outside reference laboratory using the same assay. These studies demonstrated 95.6% (240/251) agreement between sites (Table 1).

Table 1: Comparison of Platelia *Aspergillus* Antigen results at Mayo Clinic Laboratories and an Outside Reference Laboratory using BAL fluid (n=251).

<table>
<thead>
<tr>
<th>MCL <em>Aspergillus</em> Antigen Result</th>
<th>Outside Reference Lab Aspergillus Antigen Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>216</td>
</tr>
</tbody>
</table>

Percent Agreement: 95.6% (240/251) (95% confidence interval; 92.2-97.6)

Kappa value: 0.79

For 10 of the 11 discordant results, testing at Mayo Clinic Laboratories correlated with either serum *Aspergillus* antigen levels or fungal culture. Ä

Precision:

Intra- and interassay precision was tested for negative, midrange and high-positive, and spiked bronchoalveolar lavage (BAL) specimens. The mean index values, standard deviation and percent coefficient of variation were all acceptable, indicating excellent precision. (Table 2-3)
Table 2: Intra-assay precision studies

<table>
<thead>
<tr>
<th></th>
<th>Mean Index</th>
<th>Standard Deviation</th>
<th>% Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0.23</td>
<td>0.05</td>
<td>22.1</td>
</tr>
<tr>
<td>Mid-Positive</td>
<td>2.23</td>
<td>0.16</td>
<td>Å  6.9</td>
</tr>
<tr>
<td>High Positive</td>
<td>4.29</td>
<td>0.43</td>
<td>Å  9.9</td>
</tr>
</tbody>
</table>

Positive: >0.5
Negative: <0.5

Table 3: Inter-assay precision studies

<table>
<thead>
<tr>
<th></th>
<th>Mean Index</th>
<th>Standard Deviation</th>
<th>% Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0.20</td>
<td>0.05</td>
<td>25.5</td>
</tr>
<tr>
<td>Mid-Positive</td>
<td>2.32</td>
<td>0.39</td>
<td>17.1</td>
</tr>
<tr>
<td>High Positive</td>
<td>4.45</td>
<td>0.84</td>
<td>18.9</td>
</tr>
</tbody>
</table>

Positive: >0.5
Negative: <0.5

Analytical Specificity:

Cross-reactivity studies were performed by testing analyte-negative BAL specimens that had been spiked with varying concentrations of positive control material for the following organisms: *Histoplasma capsulatum, Blastomyces dermatitidis,* or *Cryptococcus neoformans.* These studies demonstrated that high concentrations of *Histoplasma* and *Blastomyces* antigen in BAL may yield positive results by the Platelia *Aspergillus* antigen assay. This has been demonstrated in prior published studies (5), and it is a known limitation of this test that there may be cross-reactivity with dimorphic fungal pathogens.

In addition to the studies above, an analyte-negative BAL specimen was spiked with a pleural fluid that was known to be positive for *Streptococcus pneumoniae* antigen. This spiked-specimen was tested by the Platelia assay and was negative at all dilutions tested.

Clinical Reference


### Performance

#### Method Description

The Platelia *Aspergillus* enzyme immunoassay (EIA) is a 1-stage immunoenzymatic sandwich microplate assay that detects galactomannan in bronchoalveolar lavage (BAL) specimens. The assay uses the rat monoclonal antibody EBA-2, which is directed against *Aspergillus* galactomannan. The monoclonal antibody is used 1) to coat the wells of the microplate and bind the antigen and 2) as the detector antibody in the conjugate reagent (peroxidase-linked monoclonal antibody).

Samples are heat-treated in the presence of EDTA in order to dissociate immune complexes and to precipitate proteins that could possibly interfere with the test. The treated samples and conjugate are added to the wells coated with the monoclonal antibody and incubated. A monoclonal antibody-galactomannan-monoclonal antibody/peroxidase complex is formed in the presence of *Aspergillus* antigen.

The strips are washed to remove any unbound material, and the substrate solution is added, which will react with the complex bound to the well to form a blue color reaction. The enzyme reaction is stopped by the addition of acid, which changes the blue color to yellow. The optical absorbance of specimens and controls is determined with a spectrophotometer set at 450 nm and 620/630 nm wavelengths.

Negative, cutoff (low-positive), and high-positive controls are analyzed each time the assay is performed. The presence or absence of *Aspergillus* (galactomannan) antigen in the test sample is determined by calculation of an index for the specimen. The index is the optical density (OD) value of the specimen divided by the mean OD of wells containing the cutoff control serum (low-positive control).(Package insert: Platelia Aspergillus EIA, Bio-Rad, Redmond, WA, 5/2008).

#### PDF Report

No

#### Day(s) and Time(s) Test Performed

Monday; 4 p.m., Tuesday through Friday; 9 a.m. and 4 p.m., Sunday; 8 a.m.

#### Analytic Time

1 day

#### Maximum Laboratory Time

2 days

#### Specimen Retention Time

14 days

#### Performing Laboratory Location

Rochester
Fees and Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification

This test has been cleared or approved by the U.S. Food and Drug Administration and is used per manufacturer's instructions. Performance characteristics were verified by Mayo Clinic in a manner consistent with CLIA requirements.

CPT Code Information

87305

LOINC® Information

<table>
<thead>
<tr>
<th>Test ID</th>
<th>Test Order Name</th>
<th>Order LOINC Value</th>
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</thead>
<tbody>
<tr>
<td>ASPBA</td>
<td>Aspergillus Ag, BAL</td>
<td>62467-6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
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<th>Test Result Name</th>
<th>Result LOINC Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>61009</td>
<td>Aspergillus Ag, BAL</td>
<td>62467-6</td>
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