Rapid Diagnosis of Invasive Aspergillosis

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Outline

– Rapid detection of IA
  • Epidemiology
  • Presenting features
  • Conventional diagnostic methods

– Platelia™
  • Non-invasive serum galactomannan antigen EIA test
  • Variability on test performance and clinical utility
    – Sensitivity and specificity
      » FDA Approval data
      » Patient population: BMT vs Solid Organ Transplant
      » Receipt of prior antibiotics
      » Receipt of prior antifungals
      » In vitro vs. in vivo properties of galactomannan
  • Clinical utility- proposed use

– Conclusion
Epidemiology

- *Aspergillus* are ubiquitous soil saprophytes found worldwide.

- *Aspergillus* is acquired by inhalation of airborne conidia by susceptible host.
  - Invasive Aspergillosis
    - Incidence is 5 out of 100,000 people
    - Recent study in *J Hosp Infect*. 2004 Apr;56(4):269-76.
    - Invasive aspergillosis in ICU patients carries a significant attributable mortality of 18.9%.
OI Following BMT

Phases of Opportunistic Infections Among Allogeneic HSCT Recipients

- **Phase I, preengraftment, <50 days**
  - Host immune system defect
  - Neutropenia, mucositis, and acute graft-versus-host disease

- **Phase II, postengraftment, 50–100 days**
  - Impaired cellular immunity and acute and chronic graft-versus-host disease

- **Phase III, late phase, >100 days**
  - Impaired cellular and humoral immunity and chronic graft-versus-host disease

- Device risk
  - Central line

- Allogeneic patients
  - Respiratory and enteric viruses
  - Herpes simplex virus
  - Cytomegalovirus
  - Varicella-zoster virus
  - Epstein-Barr virus lymphoproliferative disease

- Facultative Gram-negative bacilli
  - Staphylococcus epidermidis

- Gastrointestinal tract streptococcal species

- All candida species

- Aspergillus species

- Pneumocystis carinii

- Toxoplasma gondii

- Strongyloides stercoralis

Days after transplant

- 0
- 30
- 100
- 360

- Without standard prophylaxis
- Primarily among persons who are seropositive before transplant

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OI following Solid Organ Transplant

The Timeline of Post-Transplant Infections

- **Nosocomial technical**
  - <4 weeks
  - MRSA, candida, VRE, aspergillus, aspiration, line infection, C. difficile
  - Nosocomial pathogens, donor-derived recipient colonizers

- **Opportunistic, relapsed, residual activation of latent infection**
  - 1-6 months
  - HSV, CMV, HBY, HCY, EBV, listeria, TB, PCP, BK virus, nocardia, toxoplasma, strongyloides, leishmania
  - Period of most intensive immune suppression

- **Community acquired**
  - >6-12 months
  - Community acquired pneumonia, aspergillus, dermatophytes, CMV colitis, UTI
  - Common to rare (depends on exposures and net state of immune suppression)

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Clinical Presentations

- Invasive Aspergillosis-
  - Pulmonary Aspergillosis (most common)
    - CNS aspergillosis
    - Sinonasal aspergillosis
    - Osteomyelitis
    - Endophthalmitis
    - Endocarditis
    - Renal abscesses
    - Cutaneous
Diagnosis

• Definitive diagnosis
  – Requires the demonstration of tissue invasion seen on biopsy specimen.
  – Positive culture obtained from tissue obtained by invasive procedure.
  – These patients are typically very sick/debilitated thereby precluding them from invasive diagnostic procedures.
Diagnosis

• Less or non-invasive tests in the setting of the appropriate clinical setting may suggest the diagnosis.
  – Blood cultures are typically negative.
  – In high risk patient, isolation of isolation of *Aspergillus* from sputum or BAL is strongly suggestive of IA.
  – Serologic *Aspergillus* precipitin assays are rarely elevated in IA and thus are of little clinical value.
Radiographic Presentations

Invasive aspergillosis in a 6-year-old girl with neutropenia and acute lymphocytic leukemia.

Invasive aspergillosis in a 58-year-old woman with acute myelocytic leukemia. Transverse CT image depicts the CT halo sign.
Radiographic Presentations

Multiple aspergillomas in the brain of an HIV positive patient with AIDS
Rapid Diagnosis For Aspergillus

• Is there an ideal, sensitive, specific and non-invasive test to assist clinicians with the diagnosis of invasive Aspergillosis?
Platelia™ Aspergillus EIA

- Approved by the FDA in 2003
  - Detects soluble antigen (galactomannan) in serum.
  - Galactomannan is a component of the fungal cell wall and an exoantigen of *Aspergillus*.
- The test can detect as little as 0.5-1 ng/ml of galactomannan in the tested sample
  - Europe: 1.0 for indeterminate and 1.5 for positive.
  - USA: 0.5 positive
- In the dataset evaluated by FDA, the overall sensitivity and specificity of the method were 80.7% and 89.2%, respectively
Platelia™ Aspergillus EIA

False positivity:
Antigenic cross reactivity with other fungi such as *Penicillium chrysogenum*, *Penicillium digitatum*, and *Paecilomyces variotii*

- In some patients galactomannan antigenemia was detected *prior* to the onset of clinical symptoms
  - Favorable for screening pre-symptomatic patients
  - Galactomannan antigen positivity can be detected 5-8 days (average) before clinical signs develop

- This test is not intended to replace any of the other currently used diagnostic tests such as CT scan, bronchoscopy or tissue biopsy with culture.
Platelia™ *Aspergillus* EIA

- Manufacturer’s suggested use:
  - Used in conjunction with other diagnostic procedures.
  - Should be used on high risk patients with consistent clinical features
  - In order to maximize sensitivity, the test should be ordered prior to the initiation of antifungal therapy.
  - To maximize specificity, multiple consecutive specimens should be drawn on separate occasions to confirm a ‘true positive.’
# Platelia™ *Aspergillus* EIA

## Summary of FDA approval data

<table>
<thead>
<tr>
<th>Platelia Test</th>
<th>Probable or Proven IA (31)</th>
<th>No IA (148)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
<td>132</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>25/31 = 81%</td>
<td>132/148 = 89%</td>
</tr>
</tbody>
</table>

However, post-marketing data has shown a significant variability in performance of Platelia™ Aspergillus EIA.
Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation

- Prospective trial in patients at high risk for IA
- Serial screening for circulating galactomannan (GM)
  - Twice weekly, starting at admission, until death or discharge from hospital.
  - Galactomannan positivity was limited to a single positive sample, which exceeded an OD value of 1.5 only once
- All patients:
  - prolonged neutropenia or graft-versus-host disease (GVHD), and/or prolonged use of steroids.

Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation

- **Proven IA**
  - Histopathological evidence of tissue invasion

- **Probable IA**
  - positive culture or cytology for *Aspergillus* species from sputum or BAL fluid together with:
    - **one major** (halo sign, air-crescent sign, or cavity within an area of consolidation on CT imaging)
  - **OR** 2 of 3 **minor** clinical criteria.
    - (1) symptoms of lower respiratory tract infection such as cough, pleuritic chest pain, dyspnea, or hemoptysis; (2) pleural rub; or (3) any new infiltrate not fulfilling the major radiological criteria without an alternative diagnosis.

- **Proven IFI**
  - Positive histopathology without species identification was classified as proven invasive fungal infection (IFI).

- **Probable IFI**
  - Identification of a non-*Aspergillus* mold in identical settings was considered as probable IFI.

- **Possible IFI** (1) a positive microbiological criterion  OR (2) one major (or 2 minor) criteria

Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation

<table>
<thead>
<tr>
<th></th>
<th>Proven IA</th>
<th>Probable IA</th>
<th>Probable IFI</th>
<th>Possible IFI</th>
<th>NO IA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>7</td>
<td>29</td>
<td>2</td>
<td>83</td>
<td>132</td>
<td>191</td>
</tr>
<tr>
<td>Number of samples</td>
<td>256</td>
<td>529</td>
<td>12</td>
<td>1119</td>
<td>2384</td>
<td>4300</td>
</tr>
<tr>
<td>Number of patients with at least one positive test</td>
<td>7 (100%)</td>
<td>20 (66.6%)</td>
<td>0</td>
<td>15 (15.5%)</td>
<td>2 (0.9%)</td>
<td>44 (12.1%)</td>
</tr>
<tr>
<td>Antigenemia present in all patients with proven IA</td>
<td>GM antigenemia was expressed in less than 2% of study episodes that carried a high risk but demonstrated no evidence of IA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Positive samples</td>
<td>116 (45.1%)</td>
<td>218 (41.2%)</td>
<td>0</td>
<td>157 (14%)</td>
<td>11 (0.5%)</td>
<td>502 (11.7%)</td>
</tr>
<tr>
<td>Multiple positive samples were reported</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients with Single positive sample</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>7</td>
<td>12 (3.3)</td>
</tr>
</tbody>
</table>

So what is the sensitivity and specificity?
First…desired qualities of a good screening/diagnostic test

• Sensitivity
  – Positive test given disease
• Specificity
  – Negative test given absence of disease
• Positive predictive value
  – Disease given a positive test
• Negative predictive value
  – Absence of disease given a negative test
<table>
<thead>
<tr>
<th>Test</th>
<th>Disease Present</th>
<th>Disease Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive</strong></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td><strong>Negative</strong></td>
<td>C</td>
<td>D</td>
</tr>
</tbody>
</table>

- **Sensitivity**: \( \frac{A}{A+C} \)
- **Specificity**: \( \frac{D}{B+D} \)
Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation

Different estimates were used to calculate sensitivity, specificity, and predictive values from 2 × 2 tables.

Method A: calculated the statistical values based on episodes that are known to be truly positive and truly negative. Cases without any clue referring to IA and those with proven non-Aspergillus fungal infections were considered true negative for all estimates.

Method B: assumed that all episodes of proven and probable IA were true-positive episodes and that the uninfected cases were true-negative ones.

Method C: assumed that all cases of possible IFI were true-positive

Method D: assumed that all cases of possible IFI were true-negative

<table>
<thead>
<tr>
<th>Estimate, %</th>
<th>Analysis based on premortem stratification</th>
<th>Final analysis after incorporating autopsy findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100</td>
<td>72.9</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.1</td>
<td>98.1</td>
</tr>
<tr>
<td>PPV</td>
<td>77.7</td>
<td>93.1</td>
</tr>
<tr>
<td>NPV</td>
<td>100</td>
<td>95.7</td>
</tr>
<tr>
<td>Efficacy</td>
<td>99.1</td>
<td>95.4</td>
</tr>
</tbody>
</table>

HDG indicates analysis of histologically documented group; PPV, positive predictive value; and NPV, negative predictive value.

Estimates can dramatically change sensitivity (range: 31.3% to 100%) and predictive values.

Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation

The presence of 2 (or more) consecutive positive sera samples increased the specificity and positive predictive value of this assay in all subgroups, with a minimal decrease in sensitivity from 92.3% to 89.7%.

The absolute value of a single sample appears less informative than the demonstration of a rising or decreasing titer in antigenemia.

Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation

Time course of antigenemia in 8 selected patients.

Six patients, including 4 survivors, cleared GM.

Patients nos. 4 and 8 represent a larger group of patients with rising antigen titers; they all died of or with IA.

In cases of a favorable clinical response, the titer of the antigen tends to either decline or does not change significantly compared to the baseline titer.

In contrary, it increases significantly in case of treatment failure
Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation

• The Authors’ conclusions:
  – After incorporating postmortem findings to allow a more accurate final analysis, this approach proved to have a sensitivity of 89.7% and a specificity of 98.1%.
  – The positive and negative predictive values equaled 87.5% and 98.4%, respectively.
  – False-positive reactions occurred at a rate of 14%.
  – Serial sampling appeared to be necessary to maximize detection.
  – ‘Serial screening for GM, complemented by appropriate imaging techniques, is a sensitive and noninvasive tool for the early diagnosis of IA in high-risk adult hematology patients. The excellent sensitivity and negative predictive value makes this approach suitable for clinical decision making.’

Detection of Circulating *Aspergillus fumigatus* Galactomannan: Value and Limits of the Platelia Test for Diagnosing Invasive Aspergillosis

- Prospective evaluation of galactomannan detection (by Platelia™) in 3,327 sera samples from 807 patients over a 3 year period.
  - Patients from Hematology service and ICUs in a French tertiary care medical center.
  - All patients with suspected or proven IA
  - Detection of galactomannan was carried out exactly by manufacturer’s instruction.

Detection of Circulating *Aspergillus fumigatus* Galactomannan: Value and Limits of the Platelia Test for Diagnosing Invasive Aspergillosis

In the 3 Proven IA; all the Ag tests were negative

Proven and Probable IA:
Sensitivity = 50.0%
Specificity = 99.6%

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<table>
<thead>
<tr>
<th>Patient case category</th>
<th>No. of patients with:</th>
<th>Total no. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive Platelia result&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Negative Platelia result</td>
</tr>
<tr>
<td>Proven IA</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Probable IA</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>Possible IA</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>IA not retained</td>
<td>3</td>
<td>748</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>773</td>
</tr>
</tbody>
</table>

<sup>a</sup> Patients with at least two positive antigenemia results were included.

Efficacy of Galactomannan Antigen in the **Platelia Aspergillus** Enzyme Immunoassay for Diagnosis of Invasive Aspergillosis in Liver Transplant

- 154 patients followed prospectively for one year (9/2002-10/2002).
- IA was diagnosed by the EORTC/MSG criteria
  - (European Organization for Research and Treatment of Cancer/Mycoses Study Group)
  - Investigators were blinded to the test results.
- Blood samples were collected twice weekly during the post transplant and subsequent hospitalizations
- Samples with an index of 0.5 or greater were retested and treated as positive only if repeat test was positive
- Sensitivity and Specificity was tested in reference to the diagnosis of IA

Efficacy of Galactomannan Antigen in the **Platelia Aspergillus** Enzyme Immunoassay for Diagnosis of Invasive Aspergillosis in Liver Transplant

- **Results:**
  - 1594 sera were analyzed
    - IA developed in 1 of 154 (0.6%) of the patient
      - 31 sera for the suspected IA case
        - Positive result on the first initial sample but not on retesting
    - 153 patients without IA
      - 1563 sera
        - 20 patients without IA had 23 false positive tests
        - Sample agreement was 98.5% (1,540 /1,563)

Efficacy of Galactomannan Antigen in the Platelia Aspergillus Enzyme Immunoassay for Diagnosis of Invasive Aspergillosis in Liver Tranplant

- Specificity: 87%
- Sensitivity: could not be meaningfully calculated due to presence of only one case.
- Conclusion:
  - In a prospective cohort of 154 orthotopic liver transplant recipients, there was one case of IA.
    - IA case had 3 positive and 28 negative samples
  - The Platelia test demonstrated a specificity of 87%.
  - Sensitivity could not be calculated
  - 20 patients had false positive results calculated

What effect will anti-infectives have on Platelia™ Aspergillus EIA test performance?
Treatment with Piperacillin-Tazobactam and False-Positive 
*Aspergillus* Galactomannan Antigen Test Results for Patients 
with Hematological Malignancies

**Method:**
- The GM antigen assay available since 1997 at Institut Gustave-
  Roussy, Villejuif, France.
- GM antigenemia is monitored *weekly* in those patients with 
  hematological malignancies who are likely to experience severe 
  neutropenia.
- ELISA testing of serum samples was performed with the Platelia 
  *Aspergillus* kit
- Results were expressed as the ratio (index) between the optical 
  density (OD) obtained from the patient serum sample and that of 
  the control serum containing 1 mg of GM per mL.
  - Results were considered positive for samples with an OD 
    index of >1.5 and were considered indeterminate for those 
    with OD indexes of 1 and 1.5.
A case-control study was performed to analyze the risk factors for false-positive test results.

- Cases patients were patients who had 1 positive test result (OD index, >1.5) and were not suspected of having IA.
- Control patients were chosen from patients only with negative test results (OD index, <1.0).

Case patients and control patients were matched for both the date of hospitalization (±15 days) and the duration of surveillance.

The surveillance period for the case patients began at admission and ended when the first serum sample with a positive test result was obtained.

The surveillance period for the control patients began at admission and ended after a number of days equal to that of the surveillance period of the matched case patient.
## Treatment with Piperacillin-Tazobactam and False-Positive Aspergillus Galactomannan Antigen Test Results for Patients with Hematological Malignancies

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of patients</th>
<th>By GM antigenemia assay results</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>All negative</td>
<td>1 positive</td>
</tr>
<tr>
<td>IA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proven</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Probable</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Possible</td>
<td></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>No IA</td>
<td></td>
<td>173</td>
<td>38</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>176</td>
<td>42</td>
</tr>
</tbody>
</table>

None of the 218 patients had a diagnosis of proven IA.

Positive antigen reported in 1 out of 2 with PROBABLE IA.
Positive antigen reported in 3 out of 5 with POSSIBLE IA.
Positive antigen reported in 38 out of 211 patients with no evidence of IA.

42 (19.3%) of them had 1 positive result.

*Clinical Infectious Diseases* 2004;38:917-920
Treatment with Piperacillin-Tazobactam and False-Positive Aspergillus Galactomannan Antigen Test Results for Patients with Hematological Malignancies

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control patients (n = 24)</th>
<th>Case patients (n = 24)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex</td>
<td>7</td>
<td>8</td>
<td>1.2 (0.31 5.0)</td>
<td>1</td>
</tr>
<tr>
<td>Age &gt;50 years</td>
<td>12</td>
<td>13</td>
<td>1.2 (0.27 6.3)</td>
<td>1</td>
</tr>
<tr>
<td>Stay in room without HEPA filters</td>
<td>7</td>
<td>8</td>
<td>1.3 (0.23 9.1)</td>
<td>1</td>
</tr>
</tbody>
</table>
Treatment with Piperacillin-Tazobactam and False-Positive *Aspergillus* Galactomannan Antigen Test Results for Patients with Hematological Malignancies

<table>
<thead>
<tr>
<th>Therapy received</th>
<th>Case</th>
<th>Control</th>
<th>OR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperacillin-tazobactam</td>
<td>24</td>
<td>14</td>
<td>13.9</td>
<td>.002</td>
</tr>
</tbody>
</table>

Univariate analysis of risk factors for false-positive galactomannan (GM) antigen assay results for 48 matched case patients and control patients.

*Clinical Infectious Diseases* 2004;38:917-920
Treatment with Piperacillin-Tazobactam and False-Positive Aspergillus Galactomannan Antigen Test Results for Patients with Hematological Malignancies

- **Case vs. Controls:**
  - Piperacillin-tazobactam was the only treatment significantly associated with the occurrence of false-positive results.
  - Univariate analysis showed a trend for an increased risk of false-positive results with administration of hematopoietic growth factors ($P = .07$) and a trend for a decreased risk with amikacin administration ($P = .03$).

- After adjusting for exposure to piperacillin-tazobactam, these associations did not persist.

*Clinical Infectious Diseases* 2004;38:917-920
Reactivity of Platelia Aspergillus Galactomannan Antigen with Piperacillin-Tazobactam: Clinical Implications Based on Achievable Concentrations in Serum

• Study Goals:
  – Assess whether anti-infectives would test positive in the Platelia Aspergillus EIA.
  – For anti-infectives testing positive as undiluted samples
    • Retest for Platelia reactivity at serum concentrations consistent with achievable serum concentrations.

Reactivities of commonly used antibiotics of fungal, nonfungal, and nonmicrobial or synthetic sources with the Platelia *Aspergillus* galactomannan assay were assessed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Index*</th>
<th>Interpretation of the test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>0.167</td>
<td>NA*</td>
</tr>
<tr>
<td>Threshold control</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Positive control</td>
<td>3.228</td>
<td>NA</td>
</tr>
<tr>
<td>0.9% saline</td>
<td>0.061</td>
<td>Negative</td>
</tr>
<tr>
<td>5% dextrose</td>
<td>0.067</td>
<td>Negative</td>
</tr>
<tr>
<td>Amoxicillin powder in phosphate buffer</td>
<td>0.125</td>
<td>Negative</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>4.968</td>
<td>Positive</td>
</tr>
<tr>
<td>Piperacillin-tazobactam, lot A</td>
<td>&gt;5.168</td>
<td>Positive</td>
</tr>
<tr>
<td>B1</td>
<td>&gt;5.168</td>
<td>Positive</td>
</tr>
<tr>
<td>B2</td>
<td>&gt;5.168</td>
<td>Positive</td>
</tr>
<tr>
<td>B3</td>
<td>&gt;5.168</td>
<td>Positive</td>
</tr>
<tr>
<td>C1</td>
<td>&gt;5.168</td>
<td>Positive</td>
</tr>
<tr>
<td>C2</td>
<td>&gt;5.168</td>
<td>Positive</td>
</tr>
<tr>
<td>C3</td>
<td>&gt;5.168</td>
<td>Positive</td>
</tr>
<tr>
<td>C4</td>
<td>&gt;5.168</td>
<td>Positive</td>
</tr>
<tr>
<td>Naflonial</td>
<td>0.067</td>
<td>Negative</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>0.127</td>
<td>Negative</td>
</tr>
<tr>
<td>Ceftazolin</td>
<td>0.047</td>
<td>Negative</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0.048</td>
<td>Negative</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.062</td>
<td>Negative</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.062</td>
<td>Negative</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0.069</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>0.078</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* Index values represent the test results for duplicate wells. NA, not applicable. 
* The index for piperacillin-tazobactam for each lot in lots A, B, and C was >3.166 for duplicate wells; the value is therefore presented only once per lot. The OD of the piperacillin-tazobactam wells was not of the absorbance range of the plate reader. The index was calculated with an OD value of >3.080.

Piperacillin-Tazobactam administered as an undiluted sample was associated with a strongly positive galactomannan Ag EIA.

Achievable serum piperacillin-tazobactam concentrations may potentially result in a positive test for galactomannan. The timing of the collection of serum samples from patients may influence the test results.

Sera with typical peak doses of Piperacillin/Tazobactam tested positive for galactomannan antigen by EIA.

Sera with typical trough level doses of Piperacillin/Tazobactam tested negative for galactomannan antigen by EIA.

Could the in-vivo galactomannan secretion affect Platelia™ Aspergillus EIA test performance?
In Vivo vs. In Vitro Galactomannan Variability

• The growth of *Aspergillus* species has been well studied in-vitro. This data has been largely *extrapolated* in-vivo.
  – In vitro growth:
    • **Phase I:** growth on glucose with organic acid production and concomitant drop in pH.
      – Galactomannan is incorporated into the fungal cell wall
    • **Phase II:** Glucose becomes limited, organic acids are used as a secondary carbon source with a concomitant increase in pH.
      – Phase II characterized by a declining growth rate followed by stationary growth
    • **Phase III:** after reaching a maximum the pH stabilizes
      – End of phases II and III are characterized by lysis and breakdown of mycelium.
In Vivo vs. In Vitro Galactomannan Variability

• In the presence of sufficient nutrients in vitro, *Aspergillus* species readily release galactomannan during growth.

• The presence of nutrients may be restricted in certain in vivo environments.

• Growth phase and pH affected by:
  – Infarction and necrosis of tissue
  – Infarction/thrombosis of blood supply.
  – Accumulation of waste products

The *in-vivo mellieu* may result in an erratic or less predictable secretion of Galactomannan. As such, detection may be compromised.
What effect will anti-fungals have on **Platelia™ Aspergillus** EIA test performance?
## Prior Exposure to Antifungal Agents.

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Becker et al. <em>J. Antimicrobial Chemotherapeutics</em> 2003;52: 428-34</td>
<td>Rat model of invasive Aspergillosis</td>
<td>Exposure to Amphotericin resulted in decrease expression of cell wall components</td>
</tr>
<tr>
<td>Becker et al. <em>British Journal of Haematology</em> 2003; 121:448-57</td>
<td>GM detection in CT based BAL fluid and serum for the diagnosis of invasive pulmonary aspergillosis (IPA) in haematologic patients with neutropenia.</td>
<td>Detection of galactomannan in BAL fluid was greatly diminished by receiving antifungals for &gt; 3 days</td>
</tr>
</tbody>
</table>
In vivo variability of circulating Galactomannan antigen

Probable IA; decrease titer on Rx

Probable IA, Rx, died

Probable IA, Rx, Survived

Proven IA; treated, died

Proven IA, Rx, survived

No Invasive aspergillosis

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Test Variability

Figure 2. Changes in the sensitivity, but not specificity, over the years.

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Test Variability

Factors that influence the performance of antigen detection in invasive aspergillosis.

<table>
<thead>
<tr>
<th>Biological factors</th>
<th>Epidemiological factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site of infection</td>
<td>Patient population</td>
</tr>
<tr>
<td><em>Aspergillus</em> species causing infection</td>
<td>Sampling strategy</td>
</tr>
<tr>
<td>Microenvironment at the site of infection: nutrients, oxygen level, pH</td>
<td>Definition of a positive result</td>
</tr>
<tr>
<td>Exposure to antifungal agents</td>
<td>Definition of an infected patient</td>
</tr>
<tr>
<td>Molecule structure of released galactomannan</td>
<td>Prevalence of infection</td>
</tr>
<tr>
<td>Underlying condition/level of immunosuppression</td>
<td>Cut-off</td>
</tr>
<tr>
<td>Renal clearance, hepatic metabolism</td>
<td>Laboratory experience</td>
</tr>
<tr>
<td>Presence of galactomannan antibodies</td>
<td></td>
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<tr>
<td>Storage of sample</td>
<td></td>
</tr>
<tr>
<td>Pretreatment procedure</td>
<td></td>
</tr>
</tbody>
</table>
So how can the galactomannan EIA test be best employed as a screening/diagnostic test in patients at risk for invasive aspergillosis?
Twice weekly monitoring for patients at high risk

Sensitivity 29-100%
Specificity >85%

Ask yourself:
1. Positive antigen test in the absence of localizing physical or radiographic findings: how does this test affect your management?
2. Negative test in the presence of physical/radiographic findings. How does this test affect management?

*Figure 8. A strategy for managing patients at high risk of developing invasive pulmonary aspergillosis.*

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Conclusion

• *Aspergillus* is a significant opportunistic pathogen in immunosuppressed patients.
  – These patients are typically very sick/debilitated thereby precluding them from invasive diagnostic procedures.
  – A non-invasive test for IA would be beneficial if it were able to detect early disease, confirm suspected disease, or exclude disease in patients at high risk.
Conclusion

• **Platelia™ Aspergillus EIA** - approved by the FDA in 2003
  – Detects soluble antigen (galactomannan) in serum.
  – Studies submitted for FDA approval reported sensitivity of 81 percent and a specificity of 82-93 percent.
  – In some patients galactomannan antigenemia was detected prior to the onset of clinical symptoms
    • Favorable for screening pre-symptomatic patients
Conclusion

- Significant test variability has been reported post marketing.
  - Sensitivity 29-100%
  - Specificity >85%
- False positive reactions may be observed
  - 1-18% of the tested samples

- Test performance is likely affected by:
  - In vivo characteristics of galactomannan secretion of Aspergillus.
  - Patient population
    - BMT vs Solid Organ transplant
  - Receipt of prior antibiotics and antifungals

- The use and interpretation of Platelia™ Aspergillus EIA may be of some, yet limited, value in the diagnosis of IA in high risk patients.