AsTeC
Aspergillus Testing Consortium
Clinical Laboratory Diagnostics for Invasive Aspergillosis
Goal of Study

- Link clinical specimens from pts with IA to approved & experimental laboratory tests for diagnostic proof of principle & test comparisons

IA = Invasive Aspergillosis
How the goal will be accomplished

• Establish & maintain repository of clinical samples from pts at high risk for &/or infected with IA

• Assess new IA diagnostic assays by performance of:
  – replication studies
  – comparison evaluations of experimental IA assays with approved tests
  – studies to determine what conditions may interfere with test performance
Why is this important?
Trends in US Mortality Due to Mycotic Infections

United States, 1980-1997

Why is this important? Making the diagnosis is difficult

- 391 pts recruited to IA therapeutic clinical trial
- Cases reviewed by blinded data review committee
- Modified EORTC-MSG criteria used
  - 102 (26%) excluded due to lack of confirmation of diagnosis

Why is this important?
Other pathogens produce similar clinical syndromes

- Zygomycetes
- *Fusarium*
- *Scedosporium*
- Nocardia (NOT a fungus)
Why is this important?
The galactomannan assay is helpful, but…

- Sensitivity = 0.71
- Specificity = 0.89
- Considerable variability by patient population
- Need for repetitive testing
- Interference by certain antibiotics, TPN additives
- Cross-reactivity with other fungi

Meta-analysis, Pfeiffer CD, Clin Infect Dis 2006; 42: 1417
Why is this important? Starting treatment early matters!

- Nodular Lesions With Halo Sign (n=143)
  - All treated: 52.4%
  - VCZ: 62.3%
  - AmB: 40.9%

- Nodular Lesions Without Halo Sign (n=79)
  - All treated: 29.1%
  - VCZ: 41.5%
  - AmB: 15.8%
Why is this important?
Evaluating treatment responses is hard!

<table>
<thead>
<tr>
<th>Outcome at 12 weeks</th>
<th>Vori</th>
<th>Ampho</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival</td>
<td>71%</td>
<td>58%</td>
</tr>
<tr>
<td>“Success”</td>
<td>53%</td>
<td>32%</td>
</tr>
</tbody>
</table>

Who we are?

Dr. John Wingard  
Overall PI

Clinical Sample Repository  
Dr. Wingard

University of Florida  
Dr. Wingard

Duke University  
Dr. Alexander

BWH/DFCI  
Dr. Baden

PCR & Molecular assays  
Dr. Caliendo  
Emory Univ.  
Dr. Denning  
Univ. Manchester

Antigen Assays  
Dr. Alexander  
Duke University  
Dr. Wheat  
Miravista

Antibody Assays  
Dr. Nguyen & Dr. Clancy  
Univ. of Florida  
Dr. Alexander  
Duke Univ.

Galactomannan Assays  
Dr. Wheat  
Miravista  
Dr. Alexander  
Duke Univ.

Beta Glucan  
Dr. Alexander  
Duke University

Novel Testing*  
Shared responsibility of Drs. Alexander, Caliendo, Wheat, Denning, & Nguyen
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### Patients at our collection sites

<table>
<thead>
<tr>
<th>Types of Patients</th>
<th>University of Florida</th>
<th>Duke</th>
<th>Brigham/Dana Farber</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HSCT patients</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- allogeneic**</td>
<td>150</td>
<td></td>
<td>268</td>
<td>315</td>
</tr>
<tr>
<td>Adult</td>
<td>80</td>
<td></td>
<td>158</td>
<td>175</td>
</tr>
<tr>
<td>Peds</td>
<td></td>
<td>70</td>
<td>110</td>
<td>140</td>
</tr>
<tr>
<td>Cord blood</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-autologous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Other at risk patients</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Acute leukemia</td>
<td>478</td>
<td></td>
<td>1,895</td>
<td>1,510</td>
</tr>
<tr>
<td>- Liver transplant</td>
<td>75</td>
<td></td>
<td>175</td>
<td>130</td>
</tr>
<tr>
<td>- Lung/heart-lung transplant</td>
<td>98</td>
<td></td>
<td>43</td>
<td>0</td>
</tr>
<tr>
<td>- Other solid organ transplant</td>
<td>82</td>
<td></td>
<td>86</td>
<td>50</td>
</tr>
<tr>
<td>- HIV/AIDS</td>
<td>160</td>
<td></td>
<td>191</td>
<td>103</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>63***</td>
<td>1,400</td>
<td>1,227</td>
<td>2,690</td>
</tr>
</tbody>
</table>

*Children represent 28% of HSCT pts and 10% of all pts
**66% of pediatric HSCT are cord blood grafts
***Hospitalized only
How many IA cases we estimate we can collect

<table>
<thead>
<tr>
<th>Types of Patients</th>
<th>Nos. of pts at risk</th>
<th>Estimated rate of IA (%)</th>
<th>Total no. of IA cases</th>
<th>Nos. of Proven IA Cases* (% of pts at risk)</th>
<th>Nos. of Probable IA Cases*</th>
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<td></td>
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</tr>
<tr>
<td>Adult</td>
<td>733</td>
<td>15%</td>
<td>75</td>
<td>29 (4%)</td>
<td>46</td>
</tr>
<tr>
<td>Peds</td>
<td>413</td>
<td></td>
<td>62</td>
<td>24 (5%)</td>
<td>38</td>
</tr>
<tr>
<td>Cord blood</td>
<td>297</td>
<td></td>
<td>13</td>
<td>5 (2%)</td>
<td>8</td>
</tr>
<tr>
<td>-autologous</td>
<td>116</td>
<td>4%</td>
<td>5 (2%)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>4%</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>320</td>
<td>4%</td>
<td>13</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>- Acute leukemia</td>
<td>3,883</td>
<td>9%</td>
<td>84</td>
<td>31 (2%)</td>
<td>53</td>
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<td>- Liver transplants</td>
<td>380</td>
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<td>34</td>
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<td>21</td>
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<td>6</td>
<td>2 (1%)</td>
<td>4</td>
</tr>
<tr>
<td>- Other transplants</td>
<td>218</td>
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<td>13</td>
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<td>8</td>
</tr>
<tr>
<td>- HIV/AIDS</td>
<td>454</td>
<td>1%</td>
<td>4</td>
<td>1 (&lt;1%)</td>
<td>3</td>
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<td></td>
<td>2,690</td>
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<td>27</td>
<td>10 (&lt;1%)</td>
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<tr>
<td>Totals</td>
<td>4,616</td>
<td></td>
<td>159</td>
<td>60</td>
<td>99</td>
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*We estimate that 39% of total IA cases are proven, the remainder probable

**Includes heart-lung transplants

***hospitalized only
Sample Plan

• Two strategies
  – Longitudinal collection of samples from those at highest risk (Group 1)
    • Allogeneic BMT
    • Acute leukemia
    • Lung transplant
      – **Pros:** baseline samples before onset IA
      – **Cons:** Very inefficient

  – Collection of samples from others who become infected (etiology suspected to be IA) (Group 2)
    • Any pt group
      – **Pros:** More efficient, data in pt groups not in the highest risk
      – **Cons:** no baseline uninfected samples
Our patients and how many IA cases we estimate we can collect

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*We estimate that 39% of total IA cases are proven, the remainder probable

**Includes heart-lung transplants

***Hospitalized only
Collection timeline for adult Allogeneic BMT (Group 1)

Total blood collected 142 – 177.5 ml for adults (in toto)

T1 (baseline)

T2
Days 15-20
(Just before engraftment)

T3
Onset on GVHD

T4
One week after onset of GVHD

T5
3 wks after onset of GVHD if Prednisone > 1 mg/kg/d or salvage therapy

Candida

Aspergillus
Collection timeline for AML patients (Group 1)

T1 (baseline)

Aspergillus

T2
Reassess; 2nd induction

T3
Day T2+7d

T4
Day T2+14d

T5
Day T2+21d
Collection timeline for Lung Transplant (Group 1)

T1 (baseline)

- T2: 2 wks after transplant
- T3: Onset of Rejection
- T4: One week after Rejection
- T5: If 2nd line rejection therapy given
- T6: After 2nd rejection treatment

Aspergillus
What we expect to get from longitudinal sampling (Group 1)

- Longitudinal samples from 42 IA-infected patients/yr
- Longitudinal samples from 67 pts with probable IA/yr
- Baseline samples from 902 pts not infected/yr; follow-up sampling is driven by the occurrence of clinical factors that put pt at risk for IA (about 30-40% of pts enrolled at baseline)
Collection timeline...
An IA infection occurs (Group 2, Group 1 --> 2)

- T1 (baseline)
- T2 Days 15-20 (Just before engraftment)
- T3 Onset of GVHD
- T4 1 week after onset GVHD

Candida
Aspergillus
IA
Collection timeline…

When an IA infection occurs (Group 2, Group 1 --> 2)

T1 (baseline)  T2  Day 2-4  T3  Day 5-7  T4  Day 9-10  T5  Week 8

IA

7 days  14 days  Week 8
What we expect to get from sampling patients suspected to have IA (Group 2)

- Pts with suspected IA/yr
- 30-40% will have documented IA
- Of IA cases 30-40% will have proven IA (the rest probable)
- To collect a target of 30-50 pts with proven IA, we will collect samples from 188-556 pts with suspected IA
## Sample Types for Testing

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Antigen</th>
<th>Antibody</th>
<th>Metabolite</th>
<th>Molecular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood* **</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Urine**</td>
<td>√</td>
<td>X</td>
<td>√</td>
<td>X</td>
</tr>
<tr>
<td>BAL**</td>
<td>√</td>
<td>X</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Tissue**</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
</tbody>
</table>

* Group 1   ** Group 2
Application of manufacturer for testing of new assay

- Application process
  - Completion of application
  - Review of application by AsTeC Review Committee
    - Scientific merit
    - Practicality
    - Performance characteristics
## Template to evaluate candidate assay

<table>
<thead>
<tr>
<th>Parameter [scoring guideline]</th>
<th>Finding</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of detection [&lt;1 ng/ml=1, &gt;100 ng/ml=5]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity, clinical [&gt;90%=1, &lt;50%=5]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity, clinical [% negative] &gt;90%=1, &lt;50%=5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precision positive control [CV &lt;5%=1, &gt;50%=5]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interference [none=1, &gt;3 common conditions=5]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross-reactivity [none=1, &gt;3 common causes=5]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample stability [room temperature=1, frozen within 1 hour=5]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample preparation [none=1, multiple steps=5]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equipment requirement [none=1, non-standard expensive item=5]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assay complexity [dipstick=1, &gt;5 steps=5]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time requirement [&lt;30 min=1, overnight=5]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Training requirement [&lt;2 hr=1, &gt;1 wk=5]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost for kit [&lt;$10/test=1, &gt;$100/test=5]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Total score

<table>
<thead>
<tr>
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<th>Finding</th>
<th>Score</th>
</tr>
</thead>
</table>

### Comment

1. Some factors include multiple steps at different temperatures; reagents that have to be combined before use.
Plan for Test Evaluation

• SOPs for test evaluation will be designed using Clinical Laboratory Standard Institute (CLSI) Reference Procedures and FDA Guidance Documents

• Pre-IDE packet including draft SOPs will be submitted for FDA review and comment
Planned Analyses

• Replication (Reproducibility) Studies
  – Initial Repeatability Study
  – Precision
  – Limit of Detection
  – Linearity
  – Accuracy

• Comparison Studies

• Interfering Medical Conditions

• Inter-laboratory Reproducibility Studies

Using Consortium Specimens

Using NON-Consortium Specimens
Testing Milestones

Milestone I
Replicate Manufacturers Specification

Review with Project Officer

Sample Selection
EMMES
Data Evaluation

Milestone II
Compare New Test to Predicate Device

Review with Project Officer

Milestone III
Interference Characterization

Review with Project Officer

Milestone IV
Recommendation for Further Development

Consideration for FDA clearance
Technical Issues and plans to address them...

Specimen Stabilization and Integrity
- Particular issue for RNA and DNA samples
- Minimum manipulation at collection site preferred
  - reduces risk of contamination
  - less technical expertise and equipment at site
Transporting & Storing Fungal DNA

- May lose sensitivity by storing whole blood
- Will assess RNALater® and NucliSENS Lysis Buffer® for stabilization
  - Work with Animal Models group to assess acute stability
  - Perform series of studies to compare DNA stability over time using an existing repository of samples (some stored up to 6 years in Lysis Buffer)
  - Work to improve storage strategies over time...*dynamic process*
Detecting Fungal DNA

Additional problems

Breaking open fungal cell walls for DNA release

- Requires complex, time-consuming enzymatic or mechanical manipulation
- Einsele method considered standard in literature, but VERY labor-intensive, open to contamination

Fungal DNA contamination of reagents and supplies is a MAJOR, well-documented problem

No PCR Assay standardized or commercialized for use as a reference method
Timelines of study

- IRB approved protocol: early to mid November
- Collection of samples to begin January 08
- Estimated collection of 3 proven IA cases per month
- Start of repeatability studies spring 08
- Start of comparison studies summer 08
Proposed Interactions with IAAM

• Divided responsibilities for testing diagnostics
  – Early work with manufacturers: IAAM
  – Preparatory for licensure: AsTeC
• IAAM will provide standards for repeatability and reproducibility testing
• Coordinated meetings