Diagnosis for systemic fungal infections – non-culture based methods

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Novel diagnostic techniques

- Rapid, sensitive, specific low turn-around time

- Non-culture based
  - Antibody/Antigen detection
  - β-D-glucan detection
  - Metabolite detection
  - PCR
Antibody detection

- Helps in endemic mycoses (histo, blasto, cocci, paracocci, sporo) in immunocompetent hosts
- But, not useful for opportunistic fungal infections in immunocompromised hosts
- Can be used in *Candida* endocarditis
Antigen detection

• Cryptococcosis (LA, ELISA) – excellent
• Histoplasmosis (RIA, ELISA) – very good
• Candidiasis
  • Mannan (LA, ELISA) – promising
  • Enolase (48kD, ELISA) – promising
  • 47kD (broken down product of HSP90) – variable result
  • Secretory aspartyl proteinase – variable result
  • Cand-Tec, Cand-Tec MT – sensitivity 33-71%
• Aspergillosis
  • Galactomannan (LA, ELISA) – promising
Ag detection in Cryptococcosis

Evaluation of a Newly Developed Lateral Flow Immunoassay for the Diagnosis of Cryptococcosis

Mark D. Lindsley, Nanthawan Mekha, Henry C. Baggett, Yupha Surinthong, Rinrapas Aunthateinchan, Pongpun Sawatwong, Julie R. Harris, Benjamin J. Park, Tom Chiller, S. Arumugam Palajee, and Natteewan Poonwan

Clinical Infectious Diseases 2011;53(4):321-325
Ag detection in histoplasmosis

IC – mannan & anti-mannan detection

- **Combined mannan/anti-mannan** (Platelia, Bio-Rad) – meta-analysis of 14 studies – sensitivity (83%), specificity (86%) [Mikulska et al. Crit Care 2010; 14: R222]

- Sensitivity best for *C. albicans* (80-100%), intermediate for *C. tropicalis* & *C. glabrata*, and lowest for *C. parapsilosis* & *C. krusei* (40–50%) [Mikulska et al. Crit Care 2010; 14: R222]
Galactomannan for invasive aspergillosis

- Cell wall component of *Aspergillus* spp., though present in other fungi
- Microbiological criterion for probable IFI in EORTC/MSG
- Cut-off value of GMI - \(?1.5\) or 0.5
- **May be utilized to exclude IA, rather than confirming it**
- **May be detected 5-8d before clinical/radiological findings**
Galactomannan detection in BAL

- In hematological malignancy patients. Marteans et al. (2009) & Niguyen et al. (2010) showed sensitivity – 91% (cut-off – 0.85) & 70% (cut-off-1.0), specificity - >90%

- GM detection in serum little value in non-neutropenic, as neutrophil clears GM by mannose-binding receptor (Mannink-Kersten et al. Lancet Infect Dis 2004; 4: 349-57)

- Meersseman et al., 2008 evaluated 72 non-neutropenic ICU patients, BAL sample with GM cut off 0.5 – sensitivity 88%, specificity 87%

- Standardization of BAL protocol is an issue

- GM recommended in serial samples, problem in BAL
GMI predicts outcome

Patients whose serum aspergillus galactomannan normalized.

Patients whose serum aspergillus galactomannan remained positive.

P<0.001
Galactomannan test

**False-negative**
- Previous antifungal exposure
- Current antifungal therapy
- Inappropriate diagnostic criteria for IA
- Low frequency of testing
- Cut-off value too high
- Disease of low severity
- Low volume of sampling
- Long storage of samples
- Non-neutropenic patients

**False-positive**
- Use of antibiotics
- Pediatrics & neonates
- Infection by certain fungi
- Dialysis
- Autoantibodies
- Bacteraemia
- Plasmolyte (sodium gluconate)
- Contamination with cotton swab
- Multiple myeloma
- 42y-F – HLA matched HSCT from unrelated donor for myeloproliferative disorder
- Serum GM accessed twice weekly from Day 0 of Tx
- GM index increased to 2.22 & 3.01 on D32 & D34
- At that time she had GVHD
- But, she was afebrile with no pulmonary/sinus symptoms
- CT scan of brain, sinus, abdomen – normal
- Voriconazole started on D35
- 42y-F – HLA matched HSCT from unrelated donor for myeloproliferative disorder
- Serum GM accessed twice weekly from Day 0 of Tx
- GM index increased to 2.22 & 3.01 on D32 & D34
- At that time she had GVHD
- But, she was afebrile with no pulmonary/sinus symptoms
- CT scan of brain, sinus, abdomen – normal
- Voriconazole started on D35
False Positive Galactomannan Test after Ice-Pop Ingestion

Voriconazole therapy

Ice-pop consumption

Galactomannan Antigen Index

Positive threshold

Days after Stem-Cell Transplantation

Gallactomannan Antigen Index

Mr. Freeze
Disney
Yetigel

nicois.guigue@sls.aphp.fr
Galactomannan Testing for Early Diagnosis of *Exserohilum rostratum* Infection

Maya Korem, Itzhack Polacheck, Ayelet Michael-Gayego, Jacob Strahilevitz


<table>
<thead>
<tr>
<th>Sample</th>
<th>Galactomannan ODI(^a) for sample with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No dilution</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
</tr>
<tr>
<td>Upon diagnosis</td>
<td>2.25</td>
</tr>
<tr>
<td>After 2 wk of treatment</td>
<td>1.5</td>
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<tr>
<td>After 3 wk of treatment</td>
<td>0.95</td>
</tr>
<tr>
<td>4 wk following neutrophil recovery</td>
<td>0.52</td>
</tr>
<tr>
<td>Fungal extract</td>
<td></td>
</tr>
<tr>
<td><em>Exserohilum rostratum</em> (our case)</td>
<td>5.08</td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em> (ATCC 64026)</td>
<td>5.02</td>
</tr>
</tbody>
</table>

\(^a\) ODI, optical density index.
Some attempt in mucormycosis

85-100% fraction of different species against sera

Different ammonium sulfate precipitated fraction against sera

Sera raised in rabbit against 85-100% fraction

Antibody detection using purified Ag
1,3-β-D-glucan detection

Fungitec G-test (Seikagaku Corp., Japan)

Glucatell test (Associates of Cape Cod, USA)

Endotoxin (LPS)

Factor C → Activated Factor C

Factor B → Activated Factor B

Preclotting Enzyme

(1→3)-β-D-glucan

Activated Factor G → Factor G

Clotting Enzyme

Bcc-Leu-Gly-Arg-pNA

(Bcc-Leu-Gly-Arg-pNA (Artificial Substrate))
## Comparison of β-D-glucan assay kits

<table>
<thead>
<tr>
<th>Kit</th>
<th>Fungitec G test-MK</th>
<th>BG STA β-glucan test</th>
<th>β-glucan test WAKO</th>
<th>Endosafe-PTS-gulcan</th>
<th>Fungitell</th>
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</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Seikagaku corp. (Japan)</td>
<td>Maruha (Japan)</td>
<td>WAKO pure chemicals (Japan)</td>
<td>Charles River Lab. (USA)</td>
<td>Associates of Cape Cod Inc. (USA)</td>
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<tr>
<td>Lysate</td>
<td><em>Tachypleus tridentatus</em></td>
<td><em>Tachypleus tridentatus</em></td>
<td><em>Limulus polyphemus</em></td>
<td><em>Limulus polyphemus</em></td>
<td><em>Limulus polyphemus</em></td>
</tr>
<tr>
<td>Method</td>
<td>Kinetic chromogenic</td>
<td>Kinetic chromogenic</td>
<td>Kinetic turbidometric</td>
<td>Kinetic chormogenic</td>
<td>Kinetic chromogenic</td>
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<tr>
<td>Detection range (pg/mL)</td>
<td>3.9-500</td>
<td>10-1000</td>
<td>6-600</td>
<td></td>
<td>31.25-500</td>
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<tr>
<td>Cutoff value (pg)</td>
<td>20</td>
<td>20</td>
<td>11</td>
<td>11</td>
<td>60-80</td>
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<td>FDA approval</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
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</table>
1,3-β-D-glucan detection

**Advantages**

- Non-invasiveness of test
- Possibility of early diagnosis
- High sensitivity & specificity
- High –ve predictive value
  (eliminate IFIs)
1,3-β-D-glucan detection

**Advantages**

- Non-invasiveness of test
- Possibility of early diagnosis
- High sensitivity & specificity
- High –ve predictive value (eliminate IFIs)

**Disadvantages**

- Non-specificity, cannot identify pathogen
- Proneness to false-+ve results (contamination with cellulose-based dialysates, certain antibiotics, drug containing glucan, gauze, serious bacterial infections, immunoglobulins or albumin, environmental fungi)
- User unfriendliness –send out to reference lab
- Many clinicians are not convinced with the results
Comparison of BDG, CI, CS in ICU

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
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<tbody>
<tr>
<td>BG cut off value, 80pg/ml</td>
<td>92.9</td>
<td>93.7</td>
<td>72.2</td>
<td>98.7</td>
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<tr>
<td>Candida score ≥3</td>
<td>85.7</td>
<td>88.6</td>
<td>57.1</td>
<td>97.2</td>
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<tr>
<td>Colonization index ≥0.5</td>
<td>64.3</td>
<td>69.6</td>
<td>27.3</td>
<td>91.7</td>
</tr>
</tbody>
</table>

BDG detected IC 1-3d before blood culture positive

Posteraro et al. Critical Care 2011; 15: R 249
BDG test in ICU

• Single test with >80pg/mL – sensitivity-97%, specificity-20% (Tissot et al. ICCAC 2010, Boston, 2010)

• Low specificity due high prevalence of bacterial sepsis, albumin infusion, dialysis, contact with gauze

• Two tests with >150pg/mL – sensitivity-73%, specificity-78%

• BDG rise at least 2 days before clinical suspicion

Pre-emptive arm
• Less antifungal use
• 6% proven/probable IC

Empiric arm
• More antifungal use
• 18% proven/probable IC

Metabolite detection

- Diagnostic & prognostic significance
- D-arabinitol in invasive candidiasis, D-mannitol in cryptococcosis & invasive aspergillosis

Methods
- Gas liquid chromatography (GC)
- GC-mass spectrometry (GC-MS)
- Enzymatic - fluorometric
- Enzymatic – colorimetric
- Spectrofluorometrically in COGAS FARA II auto-analyzer (Roche)
Nucleic acid based detection tools

• RNA based detection assay
  o NASBA – Nucleic Acid Sequence Based Amplification (LOD – 1cfu)

• Isothermal reaction
  o RCA – Rolling Circle Amplification
    ▪ Padlock probe & two primer pairs, used on isolates only
    ▪ Differentiate closely related species, can detect SNPs
    ▪ Can be moved into microarray assay functioning at constant temperature

  o LAMP – Loop mediated isothermal amplification
    ▪ Used in detection of *P. brasiliensis*, *O. gallopava*, *P. marneffei* in tissue
Nucleic acid based detection tools

- PCR based detection assay
  - Real time PCR or qPCR or RTQ-PCR
  - High resolution melting curve experiment with new fluorescent dye (EvaGreen, less toxic on polymerase)
    - Melting point & melting behaviour detected
    - Detect SNPs

- Several issues – low fungal DNA, contamination, validation
- Serious attempt only in IA by European Aspergillus Initiative
- No serious attempt yet for IC and mucormycosis
Sensitivity of PCR & β-D-glucan detection

DNA detection – technical issues

- Set up a PCR – need to know DNA sequence to be amplified
- **Implementation for routine use** – much more difficult
- Two major issues – fungal DNA load low, contamination
- To avoid contamination *(fungal spore & DNA in environment & reagents)*
  - Uracyl-N-glycosylase can cut previously amplified product
  - Use real time quantitative PCR
  - **Manipulation under laminar flow** – avoid spore but not DNA
  - Lot of commercial enzymes are produced by fungi
    - Limit use of unnecessary reagents
    - **Commercial tubes containing anticoagulants may have fungal DNA (18%)**
    - Negative extraction control
DNA detection – technical issues

• **Control of amplification yield (avoid PCR inhibitors)** – same result in every test
  - Commercial DNA extraction kits remove residual PCR inhibitors
  - 10-20% tubes used for blood collection may have PCR inhibitors
  - Amplification performance monitored by internal control

• **Human DNA yield is much higher than the fungal DNA**
  - Low amplification yield may give positive signal
  - Specific control for each primer set
  - Use heterogeneous DNA (plasmid, virus, mouse DNA) as internal control
    - As quantity of control known – identical result in absence of PCR inhibition
Development of molecular test

Phase I (assay development outcome)
- Research laboratory
- Industry partners

Phase II (clinical validation)
- Diagnostic mycology laboratories (consortium/working group)
- US FDA submission
  - LDT (CLIA/CMS)
- Accuracy and early diagnosis
- Sensitivity and specificity
- Precision and limit of detection
- Work flow and laboratory cost

Phase III (clinical outcome)
- Clinicians
- Improve clinical outcome
- Prognostic value
- Response to treatment
- Healthcare cost saving
Aspergillus PCR: One Step Closer to Standardization

P. Lewis White,1* Stéphane Bretagne,2 Lena Klingspor,3 Willem J. G. Melchers,4 Elaine McCulloch,5 Bettina Schulz,6 Niklas Finnstrom,7 Carlo Mengoli,8 Rosemary A. Barnes,9 J. Peter Donnelly,4 and Juergen Loeffler10 on behalf of the European Aspergillus PCR Initiative

- Frozen or fresh EDTA whole blood is acceptable
- Suitable commercial alternative available from Promega
- Recombinant Protease K preferred
- Ceramic or solid washed glass beads (710-1180μm)

3-4 ml EDTA whole blood

Threshold: 12/18
Step time: Overall Time

15 min

15 min x 2

45 min

PCR performance Panel – 2007
serially diluted A. fumigatus DNA (ATCC strain 3922)

Blinded analysis of results

Blinded analysis of methods then results

Determine optimal protocol by analytical validity

Provide methodological recommendations

Multi-centre QC evaluation of recommendations

Recommended WB DNA extraction protocol

Blinded statistical determination of performance

DNA purification/qqt can be performed using automated or manual processing using the commercial kits highlighted in DNA strategies 3, 6, 8-11, 15 and 16. All reagents should be batch screened for fungal contamination.
Multicenter Comparison of Serum and Whole-Blood Specimens for Detection of *Aspergillus* DNA in High-Risk Hematological Patients

Jan Springer, a C. O. Morton, b* Michael Perry, c Werner J. Heinz, a Melinda Paholcsek, a* Mona Alzheinter, a T. R. Rogers, b Rosemary A. Barnes, d Hermann Einsele, a Juergen Loeffler, a P. Lewis White c


<table>
<thead>
<tr>
<th>Patient characteristic</th>
<th>Value(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>78</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>53:25</td>
</tr>
<tr>
<td>Median age (yr) of males (range)</td>
<td>53 (18–75)</td>
</tr>
<tr>
<td>Median age (yr) of females (range)</td>
<td>51 (20–72)</td>
</tr>
<tr>
<td>Mean no. of specimens per patient (range)</td>
<td>10.4 (3–32)</td>
</tr>
<tr>
<td>No. of AML patients</td>
<td>36</td>
</tr>
<tr>
<td>No. of ALL patients</td>
<td>10</td>
</tr>
<tr>
<td>No. of patients with other underlying diseases b</td>
<td>32</td>
</tr>
</tbody>
</table>

| Assay                | Performance value (% [95% CI])
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
</tr>
<tr>
<td>GM</td>
<td>80.9 (67.5–89.6)</td>
</tr>
<tr>
<td>WB PCR</td>
<td>85.1 (72.3–92.6)</td>
</tr>
<tr>
<td>Serum PCR</td>
<td>78.7 (65.1–88.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Combination testing</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>LR⁺ b</th>
<th>LR⁻</th>
<th>DOR c</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM/GM</td>
<td>48.9 (35.3–62.8)</td>
<td>100 (89.0–100)</td>
<td>100 (85.7–100)</td>
<td>56.4 (43.3–68.7)</td>
<td>&gt;489</td>
<td>0.51</td>
<td>&gt;958.9</td>
</tr>
<tr>
<td>GM/WB</td>
<td>68.1 (53.8–79.6)</td>
<td>100 (89.0–100)</td>
<td>100 (89.3–100)</td>
<td>67.4 (53.0–79.1)</td>
<td>&gt;681</td>
<td>0.32</td>
<td>&gt;2,128.1</td>
</tr>
<tr>
<td>GM/serum</td>
<td>59.6 (45.3–72.4)</td>
<td>100 (89.0–100)</td>
<td>100 (87.9–100)</td>
<td>62.0 (48.2–74.1)</td>
<td>&gt;596</td>
<td>0.40</td>
<td>&gt;1,490</td>
</tr>
<tr>
<td>WB/WB</td>
<td>46.8 (33.3–60.8)</td>
<td>93.5 (79.3–98.2)</td>
<td>91.7 (74.2–97.7)</td>
<td>53.7 (40.6–66.3)</td>
<td>7.2</td>
<td>0.57</td>
<td>12.6</td>
</tr>
<tr>
<td>WB/serum</td>
<td>57.4 (43.3–70.5)</td>
<td>96.8 (83.8–99.4)</td>
<td>96.4 (82.3–99.4)</td>
<td>60.0 (46.2–72.4)</td>
<td>17.9</td>
<td>0.44</td>
<td>40.7</td>
</tr>
<tr>
<td>Serum/serum</td>
<td>53.2 (39.2–66.7)</td>
<td>100 (89.0–100)</td>
<td>100 (86.7–100)</td>
<td>58.5 (45.1–70.7)</td>
<td>&gt;532</td>
<td>0.47</td>
<td>1,131.9</td>
</tr>
</tbody>
</table>
### Commercial molecular assay

<table>
<thead>
<tr>
<th>Assay</th>
<th>Vendor (location)</th>
<th>Method</th>
<th>Target</th>
<th>Specimen type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast Traffic Light®</td>
<td>AdvanDx (MA, USA)</td>
<td>PNA–FISH</td>
<td>26S rRNA; <em>Candida</em> sp.</td>
<td>Blood (from positive blood-culture bottles)</td>
</tr>
<tr>
<td>MycAssay™ Pneumocystis</td>
<td>Trinity Biotech (County Wicklow, Ireland)</td>
<td>Real-time PCR</td>
<td>miSU; <em>Pneumocystis jiroveci</em></td>
<td>BAL; sputum</td>
</tr>
<tr>
<td>MycAssay™ <em>Aspergillus</em></td>
<td>Trinity Biotech</td>
<td>Real-time PCR</td>
<td>18S rRNA; <em>Aspergillus</em> sp.</td>
<td>BAL; serum</td>
</tr>
<tr>
<td>Luminex xTAG® fungal assay</td>
<td>Luminex Molecular Diagnostics (ON, Canada)</td>
<td>Multiplex PCR coupled with bead probe fluid array</td>
<td>Up to 23 fungi</td>
<td>BAL; blood</td>
</tr>
<tr>
<td>IBIS Plex-ID fungal spectrum assay</td>
<td>IBIS Biosciences (CA, USA)</td>
<td>Multiplex PCR coupled with electrospray ionization mass spectrometry</td>
<td>Large-subunit rRNA; up to 75 fungi</td>
<td>BAL; blood</td>
</tr>
<tr>
<td>Seeplex® ACE PCR system</td>
<td>Seegene Diagnostics (Seoul, South Korea)</td>
<td>Multiplex PCR coupled with electrophoresis separation with fluorescence detection</td>
<td><em>Candida</em> sp.</td>
<td>Blood</td>
</tr>
<tr>
<td>RenDx™ Fungiplex panel</td>
<td>Renishaw Diagnostics (Glasgow, UK)</td>
<td>Multiplex PCR coupled with surface-enhanced resonance Raman scattering detection</td>
<td>Up to 50 fungi</td>
<td>Blood</td>
</tr>
<tr>
<td>ICEPlex 16-plex fungal panel</td>
<td>PrimeraDx (MA, USA)</td>
<td>Multiplex PCR coupled with sequential separation of amplicons by capillary electrophoresis and multicolor quantitative detection</td>
<td>Multiple fungi</td>
<td>Blood</td>
</tr>
<tr>
<td>Prove-it™ sepsis assay</td>
<td>Mobidiag (Helsinki, Finland)</td>
<td>Multiplex PCR coupled with microarray</td>
<td>ITS; <em>Candida</em> sp.</td>
<td>Blood</td>
</tr>
<tr>
<td>T2Candida®</td>
<td>T2 Biosystems (MA, USA)</td>
<td>Nanoparticles and T2 magnetic resonance detection platform</td>
<td>ITS2</td>
<td>Whole blood</td>
</tr>
</tbody>
</table>
Summary of molecular tests

- Most molecular tests are in-house with variable results
- Even evaluation of these tests are limited & controversial
- Data on cost-effective analysis is further limited
- Standardization of molecular tests is a big issue – only initiative
- EAPCRI for aspergillosis
- Commercial molecular tests much used for fungal identification
- Need of development of more consortia research
- Areas of interest – detection of fungi in blood, in formalin-fixed tissue, & identification of antifungal drug resistance directly in clinical samples
New techniques yet to be standardized in multi-centers
Antibody specific to thioredoxin reductase as a new biomarker for serodiagnosis of invasive aspergillosis in non-neutropenic patients

Li-ning Shi \textsuperscript{a,b}, Fang-qi Li \textsuperscript{b}, Jing-fen Lu \textsuperscript{b}, Xiao-xiang Kong \textsuperscript{b}, Shi-qin Wang \textsuperscript{b}, Mei Huang \textsuperscript{b}, Hai-feng Shao \textsuperscript{b}, Shi-he Shao \textsuperscript{a,*}


- Sensitivity – 96% (poor in neutropenic – 43.5%)
- Sensitivity significantly higher than GM (80.9% vs. 52.3%; \(p<0.01\)) – combining two sensitivity – 88.1%
- Negative in other fungal & bacterial infections, negative even in \textit{A. flavus} & \textit{A. niger} infections
- Antibody appears within 7-9 days of infection
Aspergillus specific extracellular glycoprotein Ag
Secreted during active growth of fungi
Mab (JF5) developed
Lateral-flow device (point of care)
Useful in BAL
Evaluation of Real-Time PCR, Galactomannan Enzyme-Linked Immunosorbent Assay (ELISA), and a Novel Lateral-Flow Device for Diagnosis of Invasive Aspergillosis

P. Lewis White, a Christian Parr, a, b Christopher Thornton, c Rosemary A. Barnes b


<table>
<thead>
<tr>
<th>Demographic characteristic</th>
<th>Proven IA (n = 8)</th>
<th>Probable IA (n = 14)</th>
<th>Possible IA (n = 22)</th>
<th>No IFD (n = 59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. male/no. female</td>
<td>5/3</td>
<td>10/4</td>
<td>15/7</td>
<td>43/16</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>48.3</td>
<td>45.1</td>
<td>53.6</td>
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<tr>
<td>Hematological malignancy</td>
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<td>AML/MDS, 6; lymphoma, 1;</td>
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<td>CLL, 1</td>
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<td>AML/MDS, 6; ALL, 3; CML, 2;</td>
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<td>lymphoma, 1; CLL, 1; SAA, 1</td>
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<td>IPA, 3; IPA/sinusitis, 3;</td>
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<td>cerebral, 1; IPA/Dissem, 1</td>
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<td>Allo, 9; Auto, 1</td>
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<td>IPA, 10; IPA/sinusitis, 1;</td>
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<td>IPA/Dissem, 1;</td>
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<td>IPA/cerebral/sinusitis, 1</td>
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<td>Disease manifestation</td>
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<td>(type, no.)</td>
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<td>Allo, 5</td>
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<td>IPA, 16; sinusitis, 5;</td>
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<td>cerebral/sinusitis, 1;</td>
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<td>None</td>
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</table>

% assay sample positivity" (% difference; 95% CI; P)

<table>
<thead>
<tr>
<th>Patient population</th>
<th>GM vs PCR</th>
<th>GM vs LFD</th>
<th>PCR vs LFD</th>
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<tbody>
<tr>
<td>Proven IA</td>
<td>14.9 vs 28.2 (13.3; 0.43–26.1; 0.0501)</td>
<td>14.9 vs 17.2 (2.3; −8.8–13.3; 0.8389)</td>
<td>28.2 vs 17.2 (11.0; 24.0–−2.1; 0.1240)</td>
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<tr>
<td>Proven/probable IA</td>
<td>31.0 vs 27.9 (3.1; 12.1; −6.0; 0.5050)</td>
<td>31.0 vs 26.6 (4.4; 13.1; −4.4; 0.3807)</td>
<td>27.9 vs 26.6 (1.3; 10.2; −7.6; 0.8192)</td>
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<tr>
<td>Proven/probable/possible IA</td>
<td><strong>17.8 vs 29.1 (11.3; 4.9–17.5; 0.0007)</strong></td>
<td>17.8 vs 20.1 (2.3; −3.5–8.0; 0.5019)</td>
<td><strong>29.1 vs 20.1 (9.0; 2.5–15.4; 0.0072)</strong></td>
</tr>
<tr>
<td>No IFD</td>
<td>9.7 vs 10.8 (1.1; −5.3–8.1; 0.7236)</td>
<td>9.7 vs 5.7 (4.0; 9.9; −1.7; 0.2289)</td>
<td>10.8 vs 5.7 (5.1; 11.6–−5.1; 0.1128)</td>
</tr>
</tbody>
</table>
T2 Magnetic Resonance Enables Nanoparticle-Mediated Rapid Detection of Candidemia in Whole Blood

A

~2 ml blood sample → Blood cell lysis & *Candida* cell concentration → Remove supernatant → Lyse *Candida* cells → PCR lysate → Aliquot & hybridize with particles → T2 detection

B

Target complementary capture probe A

Target complementary capture probe B

Add sample (i.e., blood containing target DNA)

DNA target hybridizes to capture probes forming interparticle linkages. A change in T2 is measured as agglomeration ensues.
Genetic susceptibility to IFIs

• Invasive aspergillosis
  - Genetic variability in plasminogen pathway (Zaas et al. Plos Genet 2008; 4: e1000101)
  - Toll-like receptor 4 polymorphisms (Bochud et al. NEJM 2008; 359: 1766)

• Invasive candidiasis
  - Variations in Dectin-1/CARD9 recognition pathway (Rosentul et al. J Infect Dis 2011; 204: 1138)
  - Toll-like receptor 1 polymorphisms (Johnson et al. CID 2012; 54:502)
  - CASPASE- 12 alleles (Rosentul et al. Eur J Clin Microbiol Infect Dis 2012; 31: 277)
<table>
<thead>
<tr>
<th>Method</th>
<th>Turnaround time</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>1,3-β-D-glucan detection</td>
<td>&lt;24h</td>
<td>73</td>
<td>78</td>
<td>Tissot, 2010</td>
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<td></td>
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<td>64</td>
<td>84</td>
<td>Koo, 2009</td>
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<td></td>
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<td>56</td>
<td>73</td>
<td>Nguyen, 2012</td>
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<tr>
<td>Mannan &amp; anti- mannan detection</td>
<td>&lt;24h</td>
<td>79</td>
<td>84</td>
<td>Prella, 2005</td>
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<td></td>
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<td>83</td>
<td>86</td>
<td>Mikulska, 2010</td>
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<tr>
<td>Galactomannan</td>
<td>&lt;24h</td>
<td>71%</td>
<td>89%</td>
<td>CID, 2006</td>
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<td>PCR</td>
<td>6 h</td>
<td>91</td>
<td>100</td>
<td>McMullan, 2008</td>
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<td>80</td>
<td>70</td>
<td>Nguyen, 2012</td>
</tr>
</tbody>
</table>
Mortality of opportunistic fungal infections

Variation due to:

- timing of intervention (timely diagnosis)
- patients’ defense system

97%

22%
Screening improved?

FUNGAL BURDEN diagnostics

High resolution CT scan
Galactomannan
β-D-Glucan
PCR

New tools

Traditional diagnosis

22%

FUNGAL BURDEN
Future direction

- Need of *in-vitro* biomarker for point of care
- May think about *in-vivo* biomarkers
- MS for identification of isolate – still a challenge
- Mass spectrometry (MS) of tissue for identification of fungi, but limited due to difficulty to acquire good tissue
- Carbohydrate MS need to be evaluated, as carbohydrate surface of fungi differ from species to species
- Chip technology
Thank you!

- Any solution to a problem changes the problem.
  — R. W. Johnson

- Life would otherwise be boring, no?