Laboratory diagnosis and susceptibility testing in fungal infections

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Innsbruck Medical University
### Comparison of true and opportunistic fungal infections

<table>
<thead>
<tr>
<th>Characteristic of Fungus/Disease</th>
<th>True Pathogenic Infections</th>
<th>Opportunistic Infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree of Virulence</td>
<td>Well-developed</td>
<td>Limited</td>
</tr>
<tr>
<td>Condition of Host</td>
<td>Resistance high or low</td>
<td>Resistance low</td>
</tr>
<tr>
<td>Primary Portal of Entry</td>
<td>Respiratory</td>
<td>Respiratory mucocutaneous</td>
</tr>
<tr>
<td>Nature of Infection</td>
<td>Usually primary pulmonary and systemic; often asymptomatic</td>
<td>Varies from superficial skin to pulmonary and systemic; usually symptomatic</td>
</tr>
<tr>
<td>Nature of Immunity</td>
<td>Well-developed, specific</td>
<td>Weak, short-lived</td>
</tr>
<tr>
<td>Infecting Form</td>
<td>Primarily conidial</td>
<td>Conidial or mycelial</td>
</tr>
<tr>
<td>Thermal Dimorphism</td>
<td>Highly characteristic</td>
<td>Absent</td>
</tr>
<tr>
<td>Habitat of Fungus</td>
<td>Soil</td>
<td>Varies from soil to flora of humans and animals</td>
</tr>
<tr>
<td>Geographic Location</td>
<td>Restricted to endemic regions</td>
<td>Distributed worldwide</td>
</tr>
</tbody>
</table>

- Opportunistic fungal pathogen has little or no virulence; host defenses must be impaired.
- Vary from superficial and colonization to potentially fatal systemic disease.
- An emerging medical concern; account for 10% of all nosocomial infections.
Diagnosis of mycotic infections

**Sputum**
- Digest to remove debris
- Negative stain for capsule
- KOH mount (wet mount)
- Special stains, brighteners
- KOH mount
- Direct stains of specimen
- Isolation
- 25°C or 30°C; Selective media
- Stain colonies
- Blood agar
- 37°C; BH/Blood agar
- Stain colonies
- Macroscopic morphology
- • Differential media
  • Biochemical tests
  • Antigen tests
  • Germ tube test
  • Genetic probes
  • Serological tests
- • Test for dimorphism
- • Inspect microscopic morphology after staining
- • Implant specimen on selective media; incubate for 4 weeks
- • Implant onto media selective and differential for fungi
- • Use of wood’s light on hairs
- • Highlight with brighteners; observe microscopically
- • Stain with periodic acid–Schiff (PAS)
- • Observe macroscopically for pigment texture
- • Observe macroscopically for conidial and hyphal morphology
- • Perform hair infection test by inoculating sterile hair with culture isolates
- • Perform hair infection test by inoculating sterile hair with culture isolates

**Blood, Cerebrospinal Fluid**
- Negative stain for capsule
- KOH mount
- KOH wet mount
- Direct stains of specimen
- Isolation
- 25°C or 30°C; Selective media
- Stain colonies
- Blood agar
- 37°C; BH/Blood agar
- Stain colonies
- Macroscopic morphology
- • Differential media
  • Biochemical tests
  • Antigen tests
  • Germ tube test
  • Genetic probes
  • Serological tests
- • Test for dimorphism
- • Inspect microscopic morphology after staining
- • Implant specimen on selective media; incubate for 4 weeks
- • Implant onto media selective and differential for fungi
- • Use of wood’s light on hairs
- • Highlight with brighteners; observe microscopically
- • Stain with periodic acid–Schiff (PAS)
- • Observe macroscopically for pigment texture
- • Observe macroscopically for conidial and hyphal morphology
- • Perform hair infection test by inoculating sterile hair with culture isolates
- • Perform hair infection test by inoculating sterile hair with culture isolates

**Pus, Vaginal Secretions**
- Negative stain for capsule
- KOH mount (wet mount)
- Special stains, brighteners
- KOH mount
- Direct stains of specimen
- Isolation
- 25°C or 30°C; Selective media
- Stain colonies
- Blood agar
- 37°C; BH/Blood agar
- Stain colonies
- Macroscopic morphology
- • Differential media
  • Biochemical tests
  • Antigen tests
  • Germ tube test
  • Genetic probes
  • Serological tests
- • Test for dimorphism
- • Inspect microscopic morphology after staining
- • Implant specimen on selective media; incubate for 4 weeks
- • Implant onto media selective and differential for fungi
- • Use of wood’s light on hairs
- • Highlight with brighteners; observe microscopically
- • Stain with periodic acid–Schiff (PAS)
- • Observe macroscopically for pigment texture
- • Observe macroscopically for conidial and hyphal morphology
- • Perform hair infection test by inoculating sterile hair with culture isolates
- • Perform hair infection test by inoculating sterile hair with culture isolates

**Hair, Skin, Nails**
- KOH mount
- Direct stains of specimen
- Isolation
- 25°C or 30°C; Selective media
- Stain colonies
- Blood agar
- 37°C; BH/Blood agar
- Stain colonies
- Macroscopic morphology
- • Differential media
  • Biochemical tests
  • Antigen tests
  • Germ tube test
  • Genetic probes
  • Serological tests
- • Test for dimorphism
- • Inspect microscopic morphology after staining
- • Implant specimen on selective media; incubate for 4 weeks
- • Implant onto media selective and differential for fungi
- • Use of wood’s light on hairs
- • Highlight with brighteners; observe microscopically
- • Stain with periodic acid–Schiff (PAS)
- • Observe macroscopically for pigment texture
- • Observe macroscopically for conidial and hyphal morphology
- • Perform hair infection test by inoculating sterile hair with culture isolates
- • Perform hair infection test by inoculating sterile hair with culture isolates

**Tissue Biopsies, Punches**
- KOH mount
- Direct stains of specimen
- Isolation
- 25°C or 30°C; Selective media
- Stain colonies
- Blood agar
- 37°C; BH/Blood agar
- Stain colonies
- Macroscopic morphology
- • Differential media
  • Biochemical tests
  • Antigen tests
  • Germ tube test
  • Genetic probes
  • Serological tests
- • Test for dimorphism
- • Inspect microscopic morphology after staining
- • Implant specimen on selective media; incubate for 4 weeks
- • Implant onto media selective and differential for fungi
- • Use of wood’s light on hairs
- • Highlight with brighteners; observe microscopically
- • Stain with periodic acid–Schiff (PAS)
- • Observe macroscopically for pigment texture
- • Observe macroscopically for conidial and hyphal morphology
- • Perform hair infection test by inoculating sterile hair with culture isolates
- • Perform hair infection test by inoculating sterile hair with culture isolates
What to do when and how - the major principles!

Specimens

Patients

Tests
Diagnosis of mycotic infections

Diagnosis and identification require microscopic viewing of stained specimens, culturing in selective and enriched media and specific biochemical and serological tests.
Important rules

1. Educate your doctors to give you the „best clinical specimens“
2. Choose tests according your „local epidemiology“ and „patients‘ symptoms & history“
3. Don’t forget culture and microscopic examinations
Patient 1, AML
BAL, Calcofluor White Staining
Patient 2, ICU
BAL, Gram Staining
Serological and molecular methods in the diagnosis of invasive fungal infections

<table>
<thead>
<tr>
<th>Method</th>
<th>Indication</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactomannan</td>
<td>Early detection of invasive aspergillosis (IA)</td>
<td>A screening test to accompany conventional diagnostic methods in patients at high risk of IA.</td>
<td>In non-neutropenic patients: not the same diagnostic and prognostic value</td>
</tr>
<tr>
<td>(GM)</td>
<td>2 serum samples/week, positive cut-off index &gt; 0.5.</td>
<td>In neutropenic adults</td>
<td>Mold-active antifungal drug therapy is one of the factors that may have an impact on sensitivity</td>
</tr>
<tr>
<td></td>
<td>1 single sample, positive cut-off index &gt; 0.7.</td>
<td>In neutropenic children</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum value &gt; 1: sign of therapeutic failure in adults and children</td>
<td>Quantification in BAL (cut-off &gt;1) and CSF (cut-off &gt; 0.5) (useful in neutropenic and non-neutropenic patients)</td>
<td>Persistent GM antigenemia during therapy is a poor prognostic sign and should prompt a reassessment</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>Method</th>
<th>Indication</th>
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<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-D-glucan (BG)</td>
<td>Diagnosis of IFI</td>
<td>Pan-fungal marker in critically ill patients and in cases of P. jiroveci pneumonia</td>
<td>False - (+) results (bacteraemia)</td>
</tr>
<tr>
<td></td>
<td>2 samples/week</td>
<td>Does not cover Mucormycetes and Cryptococcus neoformans</td>
<td>Limited experience (less widely used than GM)</td>
</tr>
<tr>
<td></td>
<td>(minimum)</td>
<td>A frequency of 2 tests per week seems an appropriate screening strategy</td>
<td>The threshold for positive results depends on the test that is used:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37% false positive result: 1x 80 pg/mL</td>
<td>Fungitell &gt; 80 pg/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23% false positive results: 2x 80 pg/mL</td>
<td>Wako &gt; 70 pg/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increases the specificity but decreases the sensitivity</td>
<td>Declines slowly in most IA, IC and PCP patients with appropriate antifungal therapy;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Site of infection may be important: patients with tissue infections failed to show a significant drop in BG levels despite successful outcomes</td>
<td>May persists above the usual threshold for positivity long after clinical resolution of the original infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Less accurate in hematological patients</td>
</tr>
</tbody>
</table>
### Serological and molecular methods in the diagnosis of invasive fungal infections

<table>
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<tr>
<th>Method</th>
<th>Indication</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannan plus Anti-mannan</td>
<td>Candidemia</td>
<td>Good sensitivity and specificity when combined in ICU patients</td>
<td>Limited experience</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Early diagnosis prior to blood culture results</td>
<td>Non-mycological criterion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ESCMID Diagnostic &amp; Management Guideline for Candida Diseases 2012 recommend this test-combination, high negative PV</td>
<td>The sensitivity and specificity were 87.5% and 85.5% for (1→3)-β-D-glucan and 89.3% and 63.0% for mannan antigen plus anti-mannan antibody</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C. parapsilosis and C. guilliermondii fungemias were not detected by the Platelia Candida Ag Plus assay</td>
</tr>
</tbody>
</table>

Mikulska et al, Crit Care Med 2010 14: R222  
Held J et al, C Clin Microbiol 2013, 51(4):1158-64
Serological and molecular methods in the diagnosis of invasive fungal infections

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<tr>
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<th>Indication</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular methods (polymerase chain reaction; PCR)</td>
<td>DNA detection mainly of Aspergillus less experience for Candida Blood, BAL, .....</td>
<td>Early diagnosis (rapid techniques), high NPV High sensitivity (multicopy genes), capacity for rapid speciation and ability to quantitate fungal burden Low burden of organisms during bloodstream infections: &lt;10 CFU/mL (in 25% &lt;1 CFU/mL) and intermittent nature of candidaemia due to hepatic clearance of fungal cells and/or periodic release of cells from deep organ sites into circulation</td>
<td>Additional techniques Non-mycological criterion (they are still in development) Limited to reference laboratories (low availability) High costs, improve technical equipment Technical difficulties of efficient fungal DNA extraction from complex clinical samples</td>
</tr>
<tr>
<td>Most experience of in-house tests</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name</th>
<th>Affigene Aspergillus Tracer</th>
<th>MycAssay Aspergillus</th>
<th>Aspergillus spp. Q-PCR Alert Kit</th>
<th>MycoReal Aspergillus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company</td>
<td>Cepheid</td>
<td>Myconostica</td>
<td>Nanogen</td>
<td>Inogenetix</td>
</tr>
<tr>
<td>IVD accredited</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Detection</td>
<td>Genus Aspergillus</td>
<td>Genus Aspergillus</td>
<td>Genus Aspergillus</td>
<td>A. fumigatus, A. terreus, A. flavus, A. niger, A. nidulans and others</td>
</tr>
<tr>
<td>Species identification</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Sample materials</td>
<td>full blood, serum, plasma</td>
<td>BAL, sputum</td>
<td>BAL, sputum</td>
<td>blood, liquor, BAL, puncture specimen, tissues, paraffin</td>
</tr>
<tr>
<td>DNA extraction kits</td>
<td>no kit/protocol</td>
<td>MycXtra</td>
<td>EXTRAcell protocol</td>
<td>protocol</td>
</tr>
<tr>
<td>Technology</td>
<td>Scorpions (FAM) amplification graphs</td>
<td>Molecular Beacons (FAM) amplification graphs</td>
<td>TaqMan-MGB (FAM) amplification graphs</td>
<td>HybProbes (LC640, 705) amplification and melting graphs</td>
</tr>
<tr>
<td>Target</td>
<td>18S-rRNA-gene plasmid (ROX)</td>
<td>18S-rRNA-gene plasmid (HEX)</td>
<td>18S-rRNA-gene plasmid (ROX)</td>
<td>ITS-region</td>
</tr>
<tr>
<td>Internal control</td>
<td>beta-globin-gene (VIC)</td>
<td>beta-globin-gene (VIC)</td>
<td>beta-globin-gene (VIC)</td>
<td>beta-globin-gene (VIC)</td>
</tr>
<tr>
<td>PCR-platform</td>
<td>Mx3000P and Mx300P iQ and iQ5 Rotor-Gene 3000</td>
<td>Light Cycler 2.0 ABI 7500, SmartCycler MX3000</td>
<td>ABI 7500</td>
<td>LightCycler 2.0</td>
</tr>
<tr>
<td>Analysis</td>
<td>automatically</td>
<td>automatically</td>
<td>manually</td>
<td>manually</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0,54 genome equivalents/µl</td>
<td>1,3 copies of genome/PCR</td>
<td>10 copies of target/PCR</td>
<td>3 CFU/PCR</td>
</tr>
<tr>
<td>Specificity</td>
<td>cross reaction (Penicillium)</td>
<td>cross reaction (Penicillium)</td>
<td>cross reaction (Penicillium)</td>
<td>specific for Aspergillus</td>
</tr>
</tbody>
</table>
BAL Lateral Flow Device Test for IA

- Point-of-care test for invasive aspergillosis
- Detects an extracellular glycoprotein secreted during active growth of *Aspergillus* via mAB JF 5
- Developed by Christopher Thornton, University of Exeter, UK
- Simple, rapid (15 min), single-use test
  Can be performed in rudimentary facilities using BAL or serum specimens
Predicting IFIs based on colonization or other factors

Some studies identified colonization of non-sterile body sites as a relevant risk factor (1,2), others have not (3). In general, recommending guidelines using fungal colonization as a risk factor for disease are impractical, largely because of quality control issues.

For example,
• how many cultures or body sites should be examined?
• What are the criteria for positive versus negative cultures?
• Can the expense of initial or repeat cultures be justified?

Important rules

1. Educate your doctors to give you the „best clinical specimens“
2. Choose tests according your „local epidemiology“ and „patients‘ symptoms & history“
3. Don‘t forget culture and microscopic examinations
4. Define indirect tests as an „add on“ and have „assay variabilities“ in mind
6. Be aware of the pro & cons
7. No test covers alle fungi!
Why Susceptibility Testing?

- to provide information for therapy
- to provide early warning
- to get epidemiological data
- to get information for species ID
Facts: Susceptibility Testing

We lack clinically derived breakpoint for most drug/bug combinations

- Technical factors influence MICs
- Which in vitro method reflects best outcome
- Host factors are most important for survival
Methods: Susceptibility testing

- Standards: CLSI, EUCAST
- E-test, Agar-diffusion
- Ready to use test assays
- Others
Overall good inter/intralaboratory agreement exists!
## Common Problems with Microdilution Methodology

<table>
<thead>
<tr>
<th>Problem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual determination of azole MIC endpoint (trailing growth) &quot;prominent reduction&quot;</td>
</tr>
<tr>
<td>MEC (Minimal effective drug concentration): Aspergillus and Candins</td>
</tr>
<tr>
<td>Dramatic increase in azole MIC from 24 to 48 hours</td>
</tr>
<tr>
<td>Poor growth of some isolates in tray</td>
</tr>
<tr>
<td>Time consuming</td>
</tr>
<tr>
<td>&quot;Clustering&quot; of AMB MICs</td>
</tr>
<tr>
<td>Candins: Drug solubility/stability</td>
</tr>
<tr>
<td>Batch to batch variations</td>
</tr>
<tr>
<td>Molds: not easy &quot;to read&quot;</td>
</tr>
</tbody>
</table>

Lass-Flörl et al., Mycoses 2009
MICs defined via E-test

Growth of fungus

MIC = zone of inhibition
Acquired resistance to echinocandins in *Candida albicans*: case report and review

Marie-Thérèse Baixench¹, Naji Aoun², Marie Desnos-Ollivier³, Dea García-Hermoso³, Stéphane Bretagne³, Sandrine Ramires², Christophe Piketty² and Eric Dannaouï¹,³*
## Interpretive guidelines for in vitro susceptibility testing of *Candida* spp.

breakpoints according CLSI and **EUCAST**

<table>
<thead>
<tr>
<th>Antifungal Agent</th>
<th>Susceptible (S)</th>
<th>Susceptible dose-dependent (S-DD)</th>
<th>Resistant (R)</th>
<th>Non-susceptible (NS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anidulafungin</td>
<td>&lt;2 n.v.</td>
<td>- n.v.</td>
<td>-</td>
<td>&gt;2 n.v.</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>&lt;2 n.v.</td>
<td>- n.v.</td>
<td>-</td>
<td>&gt;2 n.v.</td>
</tr>
<tr>
<td>Micafungin</td>
<td>&lt;2 n.v.</td>
<td>- n.v.</td>
<td>-</td>
<td>&gt;2 n.v.</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>≤8 &lt;2 16-32 4  ≥64 &gt;4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>≤0.125 n.v.</td>
<td>0.25-0.5 n.v.</td>
<td>≥1 n.v.</td>
<td>-</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>≤1 E cutoff &lt; 0.125 2 n.v. ≥4 E- cutoff &gt; 0.125</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>n.v. n.v. n.v.</td>
<td>n.v. n.v. n.v.</td>
<td>n.v. n.v.</td>
<td>-</td>
</tr>
</tbody>
</table>
## EUCAST Antifungal Clinical Breakpoint Table v. 4.1, valid from 2012-03-05

**MIC method** (EUCAST standardised broth microdilution method)
- **Medium:** RPMI1640-2% glucose, MOPS buffer
- **Inoculum:** Final 0.5x10⁵ – 2.5x10⁵ cfu/mL
- **Incubation:** 18-24h
- **Reading:** Spectrophotometric, full inhibition for amphotericin B but 50% growth inhibition for other compounds

**Quality control:** C. parapsilosis ATCC 22019 or C. krusei ATCC 6258

### Antifungal agent

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>C. albicans</th>
<th>C. glabrata</th>
<th>C. krusei</th>
<th>C. parapsilosis</th>
<th>C. tropicalis</th>
<th>C. guilliermondii</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>IE</td>
</tr>
<tr>
<td>Anidulafungin</td>
<td>0.03</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>-</td>
<td>0.06</td>
<td>IE²</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>Note³</td>
<td>Note³</td>
<td>Note³</td>
<td>Note³</td>
<td>-</td>
<td>Note³</td>
<td>IE²</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>2</td>
<td>4</td>
<td>IE²</td>
<td>IE²</td>
<td>-</td>
<td>2</td>
<td>IE²</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
</tr>
<tr>
<td>Micafungin</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
<td>IP</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>0.06</td>
<td>0.06</td>
<td>IE²</td>
<td>IE²</td>
<td>0.06</td>
<td>0.06</td>
<td>IE²</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.12⁴</td>
<td>0.12⁴</td>
<td>IE</td>
<td>IE</td>
<td>0.12⁴</td>
<td>0.12⁴</td>
<td>IE²</td>
</tr>
</tbody>
</table>

**Notes**

1. Non-species related breakpoints have been determined mainly on the basis of PK/PD data and are independent of MIC distributions of specific species. They are for use only for organisms that do not have specific breakpoints.

2. The ECOFFs for these species are in general higher than for C. albicans.

3. Due to significant inter-laboratory variation in MIC ranges for caspofungin, EUCAST breakpoints have not yet been established.

4. Strains with MIC values above the S/I breakpoint are rare or not yet reported. The identification and antimicrobial susceptibility tests on any such isolate must be repeated and if the result is confirmed the isolate sent to a reference laboratory. Until there is evidence regarding clinical response for confirmed isolates with MIC above the current resistant breakpoint (in italics) they should be reported resistant.
Aspergillus spp.

EUCAST Antifungal Clinical Breakpoint Table v. 4.1, valid from 2012-03-05

**MIC method (EUCAST standardised broth microdilution method)**

**Medium:** RPMI1640-2% glucose, MOPS as buffer

**Inoculum:** Final 1x10(5) – 2.5x10(5) cfu/mL

**Incubation:** 48h

**Reading:** Visual

**Quality control:** A. fumigatus ATCC 204305, A. flavus ATCC 204304, A. fumigatus F 6919, A. flavus CM 1813, C. parapsilosis ATCC 22019 (read after 18-24 h) or C. krusei ATCC 6258 (read after 18-24 h)

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>A. flavus</th>
<th>A. fumigatus</th>
<th>A. nidulans</th>
<th>A. niger</th>
<th>A. terreus</th>
<th>Non-species related breakpoints¹</th>
<th>Notes</th>
</tr>
</thead>
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<tr>
<td><strong>Amphotericin B</strong></td>
<td>IE²</td>
<td>IE²</td>
<td>1</td>
<td>2</td>
<td>Note³</td>
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<td><strong>Anidulafungin</strong></td>
<td>IE²</td>
<td>IE²</td>
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<tr>
<td><strong>Caspofungin</strong></td>
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<td><strong>Fluconazole</strong></td>
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<td><strong>Itraconazole⁴</strong></td>
<td>0.12⁶</td>
<td>0.25⁶</td>
<td>0.12⁶</td>
<td>0.25⁶</td>
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<td><strong>Micafungin</strong></td>
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<tr>
<td><strong>Posaconazole</strong></td>
<td>IE²</td>
<td>IE²</td>
<td>0.12⁶</td>
<td>0.25⁶</td>
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<tr>
<td><strong>Voriconazole</strong></td>
<td>IP</td>
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¹ Non-species related breakpoints have been determined mainly on the basis of PK/PD data and are independent of MIC distributions of specific species. They are for use only for organisms that do not have specific breakpoints.

² The ECOFFs for these species are in general one step higher than for A. fumigatus.

³ There are too few MIC data to establish ECOFFs and hence to suggest any breakpoints.

⁴ Monitoring of itraconazole trough concentrations in patients treated for fungal infection is recommended.

⁵ The MIC values for isolates of A. niger and A. versicolor are in general higher than those for A. fumigatus. Whether this translates into a poorer clinical response is unknown.

⁶ Provided adequate drug exposure has been confirmed using therapeutic drug monitoring (TDM). There remains some uncertainty regarding cut-off values for posaconazole concentrations that separate patients with a high probability of clinical success from those with a low probability of clinical success. In some circumstances (e.g. patients with persistent and profound neutropenia, large lesions, or those with other features associated with a poor clinical outcome) a relatively high trough concentration should be sought. Preclinical and clinical data suggest this value should be >1 mg/L at steady state. For other patient groups a lower trough concentration may be acceptable. For prophylaxis a target concentration of >0.7 mg/L has been suggested.
Conclusions

*in vitro* susceptibility testing, local epidemiology and patients’ immune status

- **Yeast**
  - sterile body site
  - plus non-*C. albicans*
  -azole (?)
  - non-responder
  - rare species

- **Molds**
  -non *A. fumigatus*
  - all: non responder
  - long treatment & azole
  - rare species
Take Home Message

1. LABORATORY-Standard
   - Direct microscopic examination in freshly obtained (adequate) samples
   - histopathological examination of tissue sections
   - cultivation of the causative fungus and species identification, susceptibility testing if indicated

2. Variable contribution of diagnostic tools according to the underlying disease

3. Screening tests are helpful, but may interfere with antifungal therapy (BAL, serum)
Thank you very much for your attention!