

## Fungal Diagnostics: Old Tools and New Problems

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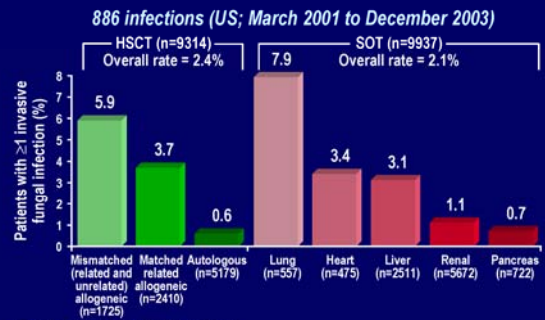
Fungal infections...in the mind of your neighbor



### Invasive Fungal Infections (aka systemic fungal infections)

- Almost exclusively in severely immunocompromised patients
- bone-marrow/stem cell transplant
  - solid organ transplant
  - HIV/AIDS

### TRANSNET Incidence of Systemic Fungal Infection by Type of Transplant

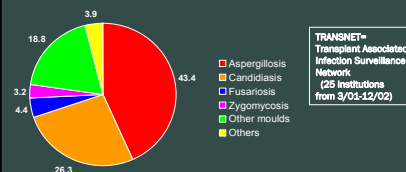


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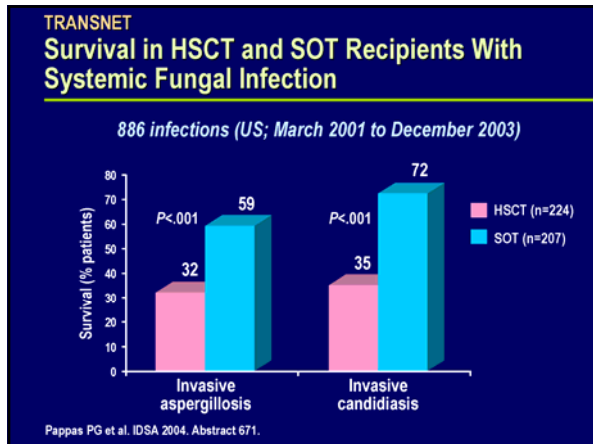
Only a significant clinical problem in the past 20-25 years

### Etiologic agents of IFIs in transplant patients



#### More common organisms:

- *Aspergillus* spp (especially *fumigatus*)
- *Candida* spp (especially *albicans/ glabrata*)
- *Fusarium* spp
- Zygomycetes (Mucormycosis not an accurate term...  
- *Rhizopus*, *Rhizomucor* more common...*Mucor* actually quite rare)
- *Cryptococcus neoformans* (especially in AIDS)
- *Scedosporium/ Pseudoallescheria* spp.
- *Histoplasma capsulatum/ Blastomyces dermatitidis* (especially AIDS)



**Why are IFI Mortality Rates so high?**

- Typically Severely Impaired Host
  - minimal reserve
- Therapeutics Often Fail
  - innate resistance
  - evidence of acquired resistance to azoles
  - toxicity of pharmaceuticals
  - new drug targets needed
- Early Diagnosis is Uncommon
  - clinical presentation variable and non-specific
  - reliability of current diagnostic tools is limited
  - diagnosis often made late or at autopsy
  - only 1/4 of IFIs diagnosed pre-mortem

**HOW CAN WE BETTER IDENTIFY FUNGAL INFECTIONS EARLIER???**

**Diagnosis of an IFI...What Could We Detect?**

<u>Fungal Factors:</u>	<u>Host Factors:</u>
1. Viable fungus -culture	1. Antibodies
2. Fungal elements -direct exam	2. Elements of host response
3. Fungal antigens/secreted molecules -cell wall fragments -secreted proteins	
4. Nucleic Acids -DNA/RNA	

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Radiology:

- Commonly used
- Can help, especially with aspergillosis, but not very sensitive or specific
- May be some future advances here that could be useful...

**Culture—The Conventional Diagnostic Tool**

I. Low Sensitivity for IFIs:

- Candidiasis = 50-60%
  - > organisms cleared from blood through both antibody and non-antibody dependent receptor-ligand interactions
  - > Rand, K.H. et al *Mol cell probes* 1994: ~25% of *Candida* positive blood cultures have <math><1\text{ cfu/mL}</math> and ~50% have <math><10\text{ cfu/mL}</math>
- Aspergillosis  $\leq 10\%$ 
  - > *Aspergillus* is angioinvasive
- Patient on anti-fungal prophylaxis
  - > effect on blood samples...

II. Optimal sample often not obtainable

**Culture—The All Too Common Scenario...**

- > Blood, fluid or tissue comes to the lab for fungal culture
- > Processed (NEVER grind!) and plated onto media to support fungal growth
- > Then incubated...
- > And incubated...
- > And incubated...
- > 'Hey, I see an itty bitty fuzzy!'
  - 'Let's incubate this another day...'
- > 'I did a tape prep and all I see is hyphae'
  - 'OK, let's sub it and set up a slide culture'
- > 'And incubate those for a few days...'

## Culture—The Conventional Diagnostic Tool

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- Patient on anti-fungal prophylaxis
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### II. Optimal sample often not obtainable

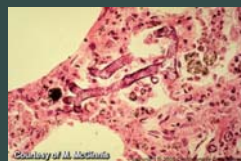
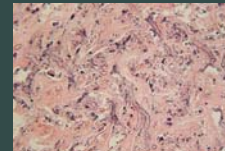
### III. Slow (we'll touch on this again, later...)

## Direct Exam: If you see it...

- ...but often you don't
- samples can be difficult to obtain
- sampling errors

Not possible to ID based on morphology in tissue sample

- maybe zygomycete...



## Secreted/Shed Fungal Molecules—An Overview

### Tests in use that have made a significant difference:

- Cryptococcal Antigen Test (Glucuronoxylomannan)
- Histoplasma Urine Antigen (Galactomannoprotein)

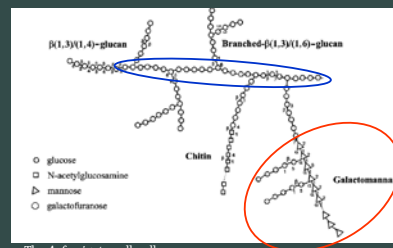
### Tests in use that have made a slight or questionable difference:

- Galactomannan EIA (for diagnosis of *Aspergillus*)
- Beta-Glucan Test
- Mannan Test (*Candida*)

### Tests on the horizon...

- Secreted proteins of *Aspergillus fumigatus* and other fungi

## Cell Wall Galactomannan and $\beta$ -1,3-Glucan



The *A. fumigatus* cell wall

Bernard & Latge. 2001. *Med. Mycol.*

## Galactomannan (GM) Assay (Bio-Rad Platelia®)

Blood sample (serum or plasma)

ELISA-monoclonal antibody against *A. fumigatus* galactomannan

Requires two consecutive positive results

Cut-off controversial

## Galactomannan (GM) Assay (Bio-Rad Platelia®)

Patient group	Sensitivity (%)	Specificity (%)	Reference
Galactomannan: CO 0.5 on consecutive specimens unless otherwise noted			
Allo HSCT, neutropenia, ALL on steroids	96	99	Maertens et al. (6)
Neutropenia, suspected IA, GyHD, steroids	100	93	Kawazu et al. (7)
Allo HSCT	81	89	Mari et al. (8)
Hematologic malignancy, single specimen	59-89 <sup>a</sup>	92	Mari et al. (9)
Neutropenia, CO 1.5	88	90	Pazos et al. (10)
Hematologic malignancy	80	82	Weisser et al. (11)

From: Wheat, LJ *Transpl Inf Dis* 2006

- When serially monitored (2-3 times/week), GM preceded conventional diagnosis by around 1 week

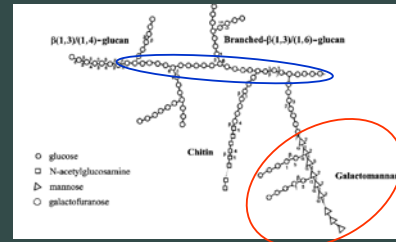
- Sensitivity may be better if BAL fluid used, but that sample type is not always available and not yet FDA approved...

## Galactomannan (GM) Assay (Bio-Rad Platelia®)

### The Issues with GM:

- Sensitivity lower in patients on anti-fungal therapy and in less susceptible patients
- Negative test can't rule-out IA
- False positive tests with some chemotherapy drugs and cGVHD
- False positive tests in patients on piperacillin-tazobactam (Zosyn) and other beta lactam antibiotics
- Other fungal organisms contain an apparent cross reacting molecule  
-Examples: *Penicillium*, *Alternaria*, and *Paeclomyces*
- Unclear whether the assay is useful in monitoring treatment
- The assay is simply a bit flaky...

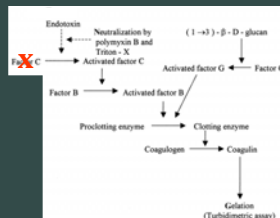
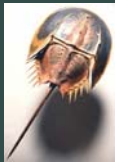
## Cell Wall Galactomannan and $\beta$ -1,3-Glucan



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## $\beta$ -1,3-Glucan Assay



## $\beta$ -1,3-Glucan Assay (Fungitell®)

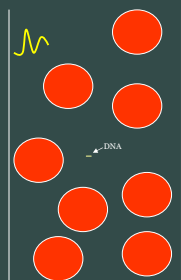
- Detects a number of different fungi, but no idea of which one  
- does not detect *Cryptococcus*, *Zygomycetes*

Patient group	Sensitivity (%)	Specificity (%)	Reference
Glucan: CO 60 pg/mL on consecutive specimens unless otherwise noted			
Neutropenia, suspected IA, GVHD, steroids	55	95	Kawazu et al. (7)
Hematologic disease	88	85	Horiguchi (12)
Neutropenia, CO 120 pg/mL, single	88	90	Pazos et al. (10)

From: Wheat, LJ *Transpl Inf Dis* 2006

- In head-to-head with GM, was less sensitive, less specific and detected later (Kawazu, et al *J Clin Micro*. 2004)
- Big specificity problems, especially in an ICU or surgical setting  
- many medical products contain glucan
- Assay technically difficult (need glucan free tubes, etc)

## PCR Detection of Fungal Infections



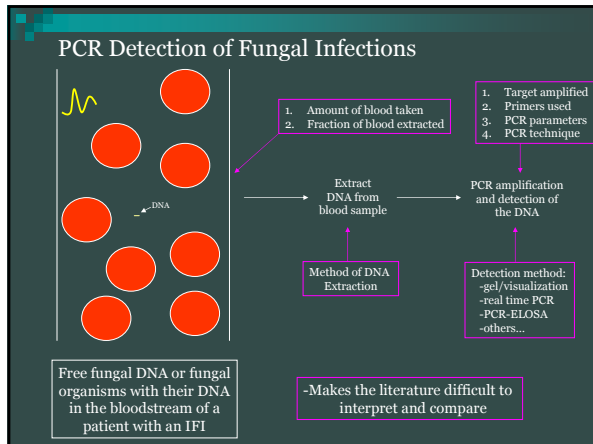
Free fungal DNA or fungal organisms with their DNA in the bloodstream of a patient with an IFI

Extract DNA from blood sample

PCR amplification and detection of the DNA

## PCR Detection of Fungal Infections

- Numerous studies evaluating 'home brew' assays  
-no commercial assays exist
- No standardization among the different assays...



### PCR Detection of Fungal Infections

Patient group	Sensitivity (%)	Specificity (%)	Reference
PCR			
Neutropenia, suspected IA, GHD, steroids	45	93	Kawazu et al. (7)
Neutropenia and HSCT	36	92	Buchheid et al. (13)
Hematologic disease	50	100	Bretagne et al. (14)

From: Wheat, LJ Transpl Inf Dis 2006

➤ Question of amount of free DNA or fungal organism in blood during IFI  
-may be limited by some of the same problems as culture  
-problem not in PCR technique, but in sampling

Bottom-line...may be some promise, but needs standardization

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- And incubated...
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- 'Hey, I see an itty bitty fuzzy!' → Molecular identification  
- 'Let's incubate this another day...'
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### Molecular ID of Medically Important Molds

Extract DNA

PCR amplification  
-pan-fungal or species-specific primers

Targets:  
-ITS  
-D1/D2  
-V9 domain } ribosomal DNA regions

Detection of products:  
-simple gel (species-specific primers)  
-sequencing  
-RFLP  
-Luminex  
-padlock probes  
-melting point analysis

**Fungal identification**

### Summary/Overview

- Invasive fungal infections are bad
- Diagnosis of these infections is difficult
- The conventional diagnostics fail often or are too slow  
-component of high mortality
- New diagnostic methodologies are available  
-galactomannan assay for *Aspergillus*  
-β-1,3 glucan assay to detect most fungi  
-inherent problems limit their effectiveness
- Molecular methods for direct detection of fungi from clinical samples have not proven to be as useful as one would expect
- New technologies are certainly needed for IFI diagnosis