Laboratory Diagnosis of Invasive Fungal Infection in Transplant Recipients: Theory & Practice

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Clinical classification of fungal infections

Superficial

Oral and genital mucosae

Yeasts

Skin and skin appendages

Moulds (dermatophytes)

Subcutaneous (post-traumatic)

Mycetoma or chromoblastomycosis

Dematiaceous and hyaline moulds

Invasive, deep, or disseminated

Yeasts, moulds, dimorphic fungi

Revised Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group

Table 1. Criteria for proven invasive fungal disease except for endemic mycoses. **(Identification at genus or species level if culture is a/v)**

Analysis and specimen	Molds[a]	Yeasts[a]
Microscopic analysis: sterile material	Histopathologic, cytopathologic, or direct microscopic examination[b] of a specimen obtained by needle aspiration or biopsy in which hyphae or melanized yeast-like forms are seen accompanied by evidence of associated tissue damage	Histopathologic, cytopathologic, or direct microscopic examination[b] of a specimen obtained by needle aspiration or biopsy from a normally sterile site (other than mucous membranes) showing yeast cells—for example, *Cryptococcus* species indicated by encapsulated budding yeasts or *Candida* species showing pseudohyphae or true hyphae[c]
Culture		
Sterile material	Recovery of a mold or "black yeast" by culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding bronchoalveolar lavage fluid, a cranial sinus cavity specimen, and urine	Recovery of a yeast by culture of a sample obtained by a sterile procedure (including a freshly placed [<24 h ago] drain) from a normally sterile site showing a clinical or radiological abnormality consistent with an infectious disease process
Blood **(NOT *Aspergillus* sp.)**	Blood culture that yields a mold[d] (e.g., *Fusarium* species) in the context of a compatible infectious disease process	Blood culture that yields yeast (e.g., *Cryptococcus* or *Candida* species) or yeast-like fungi (e.g., *Trichosporon* species)
Serological analysis: CSF	Not applicable	Cryptococcal antigen in CSF indicates disseminated cryptococcosis

Most hematology / transplant patients

1. Often not a/v
2. Often absent

(*Clin Infect Dis.* 2010;51:1273-80)

Probable Invasive Aspergillosis without Prespecified Radiologic Findings: Proposal for Inclusion of a New Category of Aspergillosis and Implications for Studying Novel Therapies

Possible IFI:
Host factor + clinical criterion but NOT mycological criteria

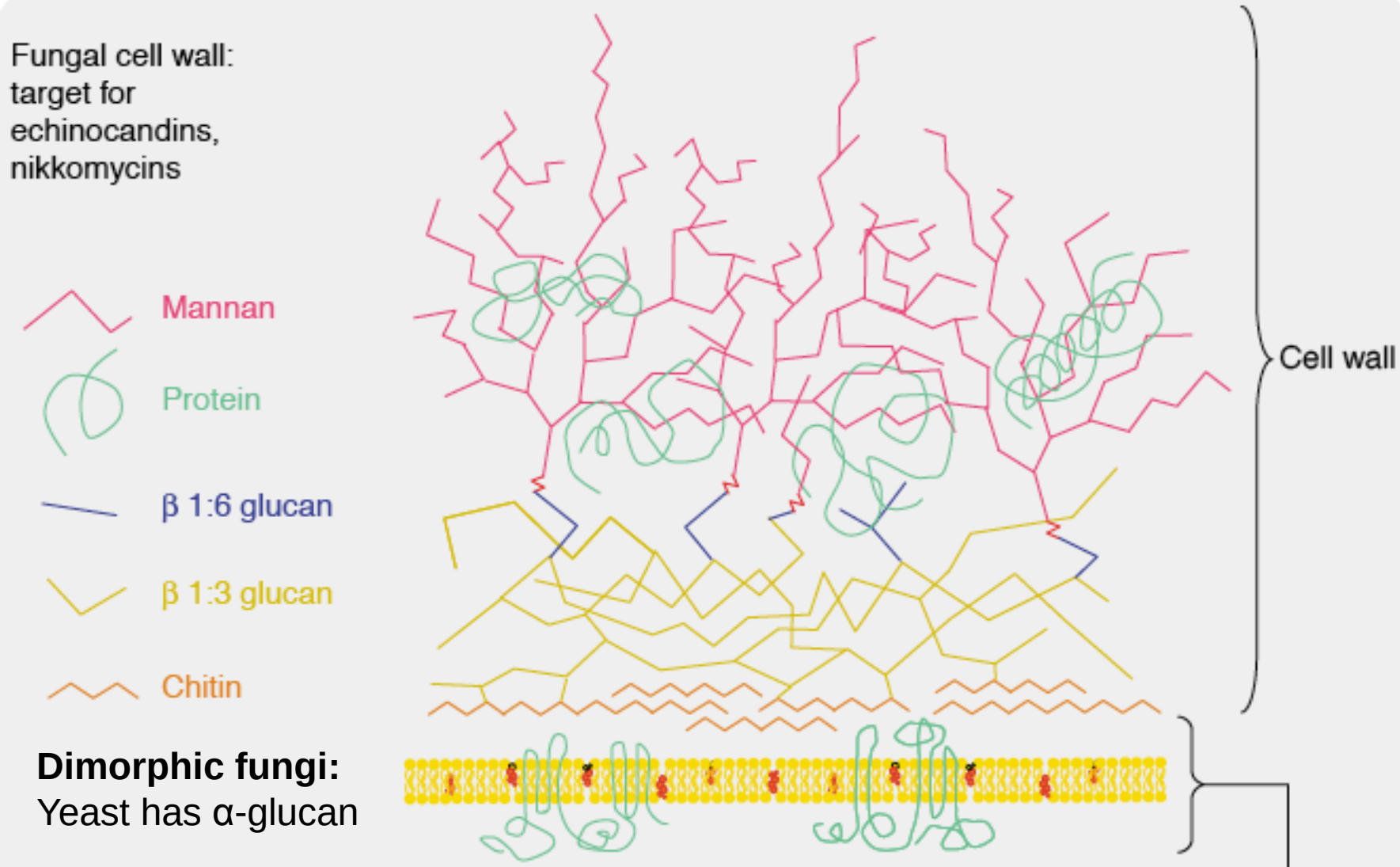
Table 2. Criteria for probable invasive fungal disease except for endemic mycoses.

Host factors[a]
- Recent history of neutropenia (<0.5 × 10^9 neutrophils/L [<500 neutrophils/mm^3] for >10 days) temporally related to the onset of fungal disease
- Receipt of an allogeneic stem cell transplant
- Prolonged use of corticosteroids (excluding among patients with allergic bronchopulmonary aspergillosis) at a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for >3 weeks
- Treatment with other recognized T cell immunosuppressants, such as cyclosporine, TNF-α blockers, specific monoclonal antibodies (such as alemtuzumab), or nucleoside analogues during the past 90 days
- Inherited severe immunodeficiency (such as chronic granulomatous disease or severe combined immunodeficiency)

Clinical criteria[b]
- Lower respiratory tract fungal disease[c]
  - The presence of 1 of the following 3 signs on CT:
    - Dense, well-circumscribed lesions(s) with or without a halo sign
    - Air-crescent sign
    - Cavity
- Tracheobronchitis
  - Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis
- Sinonasal infection
  - Imaging showing sinusitis plus at least 1 of the following 3 signs:
    - Acute localized pain (including pain radiating to the eye)
    - Nasal ulcer with black eschar
    - Extension from the paranasal sinus across bony barriers, including into the orbit
- CNS infection
  - 1 of the following 2 signs:
    - Focal lesions on imaging
    - Meningeal enhancement on MRI or CT
- Disseminated candidiasis[d]
  - At least 1 of the following 2 entities after an episode of candidemia within the previous 2 weeks:
    - Small, target-like abscesses (bull's-eye lesions) in liver or spleen
    - Progressive retinal exudates on ophthalmologic examination

Mycological criteria
- Direct test (cytology, direct microscopy, or culture)
  - Mold in sputum, bronchoalveolar lavage fluid, bronchial brush, or sinus aspirate samples, indicated by 1 of the following:
    - Presence of fungal elements indicating a mold
    - Recovery by culture of a mold (e.g., *Aspergillus, Fusarium,* Zygomycetes, or *Scedosporium* species)
- Indirect tests (detection of antigen or cell-wall constituents)[e]
  - Aspergillosis
    - Galactomannan antigen detected in plasma, serum, bronchoalveolar lavage fluid, or CSF
  - Invasive fungal disease other than cryptococcosis and zygomycoses
    - β-D-glucan detected in serum

Fungal cell wall:
target for
echinocandins,
nikkomycins

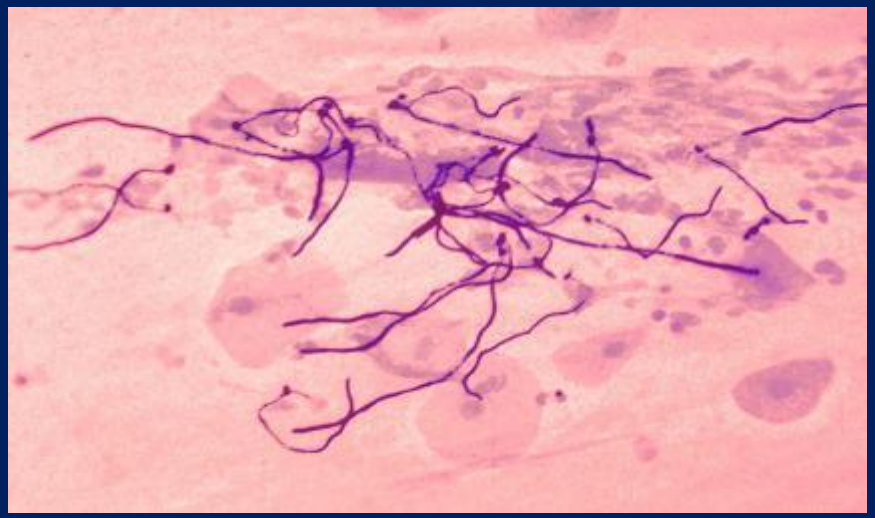


Dimorphic fungi:
Yeast has α-glucan

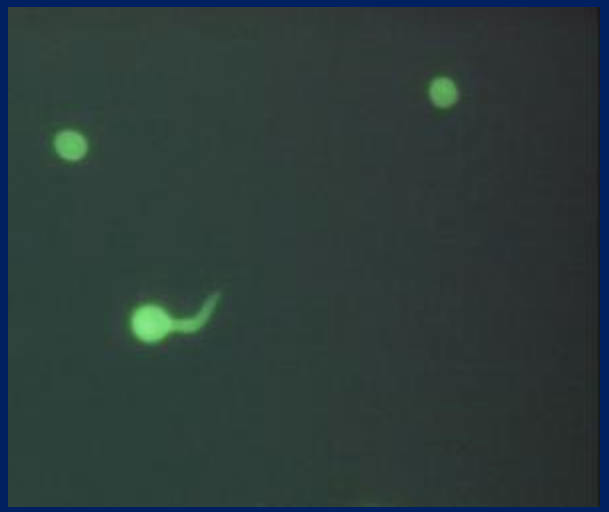
Trends Microbiol. 2003;11:272–279.

Diagnostic utility	Fungal component	Therapeutic utility
PCR (ribosomal, mitochondrial)	Cellular DNA	5-Fluorocytosine
Protein (ribosomal)	MALDI-TOF MS	
Fontana-Masson stain	Melanin	
	Cell membrane	AmpB, nystatin
	Ergosterol	Azoles (flu / itra / vori / posa-conazole) Terbinafine
Caucofluor white stain	Chitin	Nikkomycins
Silver/PAS stain	Glucan	Caspofungin, Micafungin Anidulafungin
Antigen detection	Galactomannan (*Aspergillus*)	
	D-glucan (all fungi)	
Antigen detection (*Cryptococcus*), mucicarmine stain, Indian ink	Capsular material	

Conventional mycology ID methods

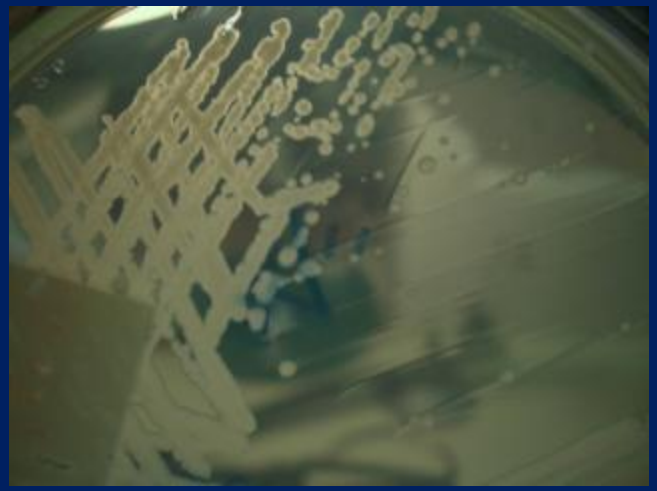


Gram stain: mucosal candidiasis

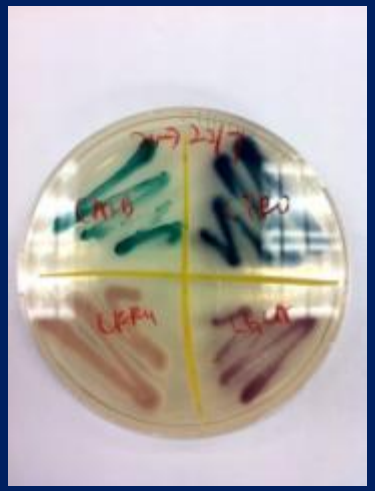


Caucoflour white stain: germ tube formation

Suc Malt Lac Glu
Sugar fermentation tests



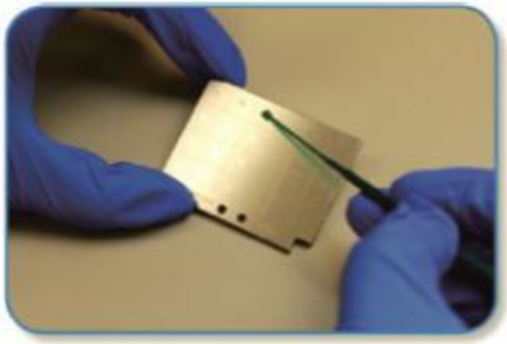
Culture (Sabouraud agar)



Culture (Chromagar)

MALDI-TOF MS workflow

Bacterial / fungal colony



MALDI-TOF MS target plate
(≥1 isolate per run)

Add Formic Acid and Matrix and Dry

Ionization chamber of MS

Comparison against a database of mass spectra

Patel R et al., Clin Infect Dis. 2013, 57:564-72.

Target plate placed into the ionization chamber

Spots shot by an UV N_2 laser desorbing microbial and matrix molecules from the target plate

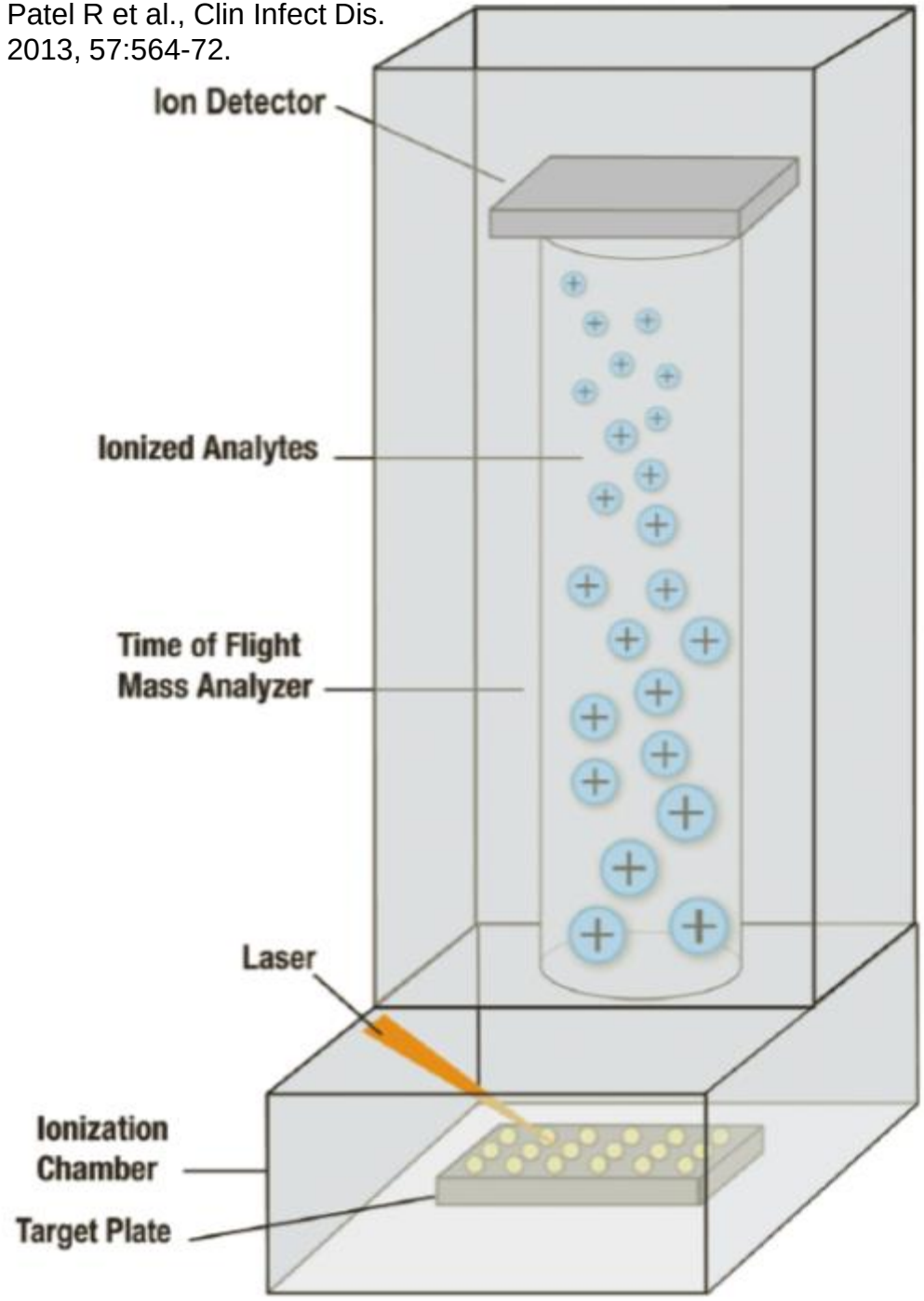
Energy absorbed by the matrix → ionized state

Random collision in the gas phase → charge transferred from matrix to microbial molecules

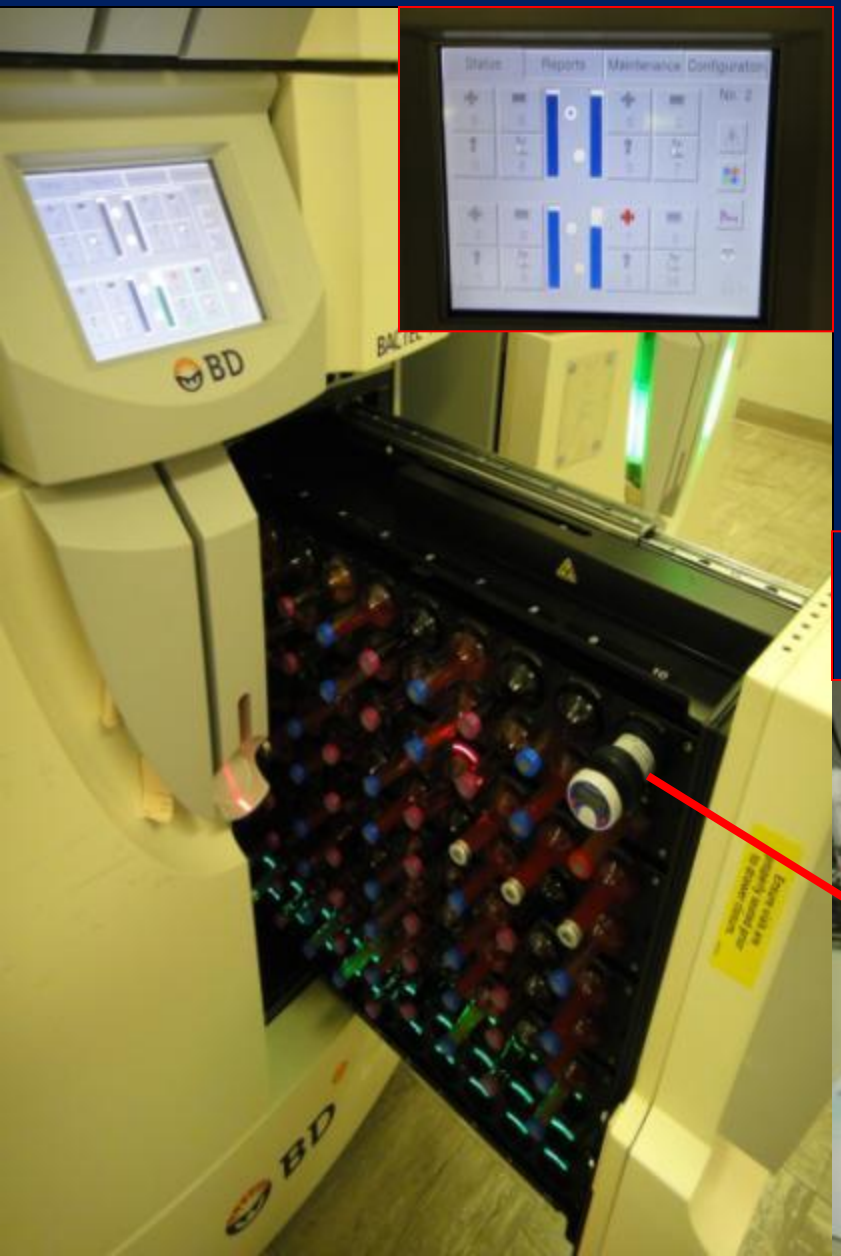
Cloud of ionized molecules funneled through a +ve charged electrostatic field into TOF mass analyzer (tube under vaccum)

Ions collide with an ion detector → mass spectrum (representing # of ions hitting the detector over time)

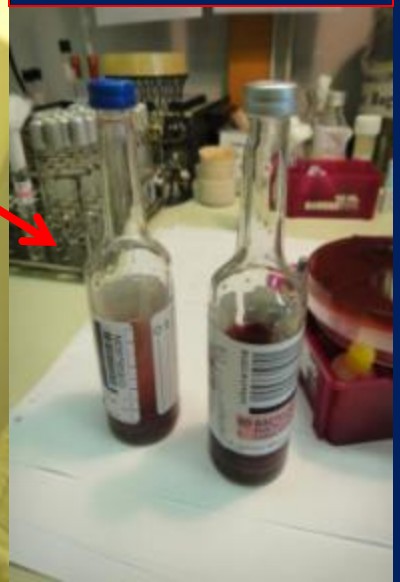
Patel R et al., Clin Infect Dis. 2013, 57:564-72.



Direct identification of bacteria / yeasts from positive blood culture



Positive blood culture



Conventional

Day 1	Day 2	Day 3
Gram stain Direct ID (Direct ST)	Preliminary ID Preliminary ST Colony ID & ST	Final report (ID & ST)

MALDI-TOF MS

Day 1

(3 hr)

Report ID (+ST?)
(91.3-100% for *Candida* sp.)

Failure:
1. Polymicrobial
2. $<10^4$ CFU/ml ($<5.9 \times 10^5$ CFU/ml threshold)

Buchan BW et al., J Clin Microbiol. 2013;51:1359-66.

HKU/QMH data (colony identification – yeasts)

TABLE 1 Identification results of yeast isolates by Biotyper and Vitek MS IVD (n = 98)

Yeast/fungal species	No. of isolates	MALDI-TOF MS results (no. [%]) using[a]: Biotyper plus fungus RUO database: Unreliable ID[b] (score, <1.6)	Genus-level ID only (score, 1.6–1.99)	Species-level ID (≥2.0)	Vitek MS IVD: Unreliable ID[b] (<90.0%)	Correct ID (90.0–97.9%)	Correct ID (≥98.0%)
Candida spp.							
C. albicans	24	0 (0)	*2 (8.3)	22 (91.7)	1 (4.2)	0 (0)	23 (95.8)
C. boidinii	1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
C. dubliniensis	3	0 (0)	*1 (33.3)	2 (66.7)	3 (100)	0 (0)	0 (0)
C. glabrata	5	0 (0)	*0 (0)	5 (100)	0 (0)	1 (20.0)	4 (80.0)
C. guilliermondii	2	0 (0)	*1 (50.0)	1 (50.0)	0 (0)	0 (0)	2 (100)
C. kefyr (*Kluyveromyces marxianus*)	3	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)
C. krusei (*Issatchenkia orientalis*)	3	0 (0)	*0 (0)	3 (100)	0 (0)	0 (0)	3 (100)
C. lipolytica	2	0 (0)	1 (50.0)	1 (50.0)	0 (0)	0 (0)	2 (100)
C. magnoliae	1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
C. norvegensis	4	0 (0)	0 (0)	4 (100)	0 (0)	0 (0)	4 (100)
C. parapsilosis	18	0 (0)	*5 (27.8)	13 (72.2)	4 (22.2)	1 (5.6)	13 (72.2)
C. pararugosa	1	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)
C. rugosa	2	1 (50)	1 (50)	0 (0)	1 (50)	0 (0)	1 (50)
C. tropicalis	7	0 (0)	*0 (0)	7 (100)	0 (0)	0 (0)	7 (100)
C. (*Clavispora*) *lusitaniae*	2	0 (0)	1 (50)	1 (50)	0 (0)	0 (0)	2 (100)
Subtotal	78	3 (3.8)	12 (15.4)	63 (80.8)	10 (12.8)	2 (2.6)	66 (84.6)
Other yeast and yeast-like fungi							
Blastoschizomyces capitatus	1	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)
Cryptococcus humicola (*Trichosporon mucoides*)	2	0 (0)	1 (50)	1 (50)	2 (100)	0 (0)	0 (0)
Cryptococcus neoformans	7	1 (14.3)	1 (14.3)	5 (71.4)	6 (85.7)	0 (0)	1 (14.3)
Galactomyces candidum	1	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)
Pichia anomala (*Candida pelliculosa*)	1	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)
Pichia ohmeri	2	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	0 (0)
Pseudozyma parantarctica	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)
Saccharomyces cerevisiae	1	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)
Trichosporon asahii	2	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)
Trichosporon dermatis	2	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	0 (0)
Subtotal	20	2 (10.0)	8 (40.0)	10 (50.0)	13 (65.0)	0 (0)	7 (35.0)
Total	98	5 (5.1)	20 (20.4)	73 (74.5)	23 (23.5)	2 (2.0)	73 (74.5)

(94.9%)

(76.5%)

Chen JH et al., J Clin Microbiol. 51:2981-7.

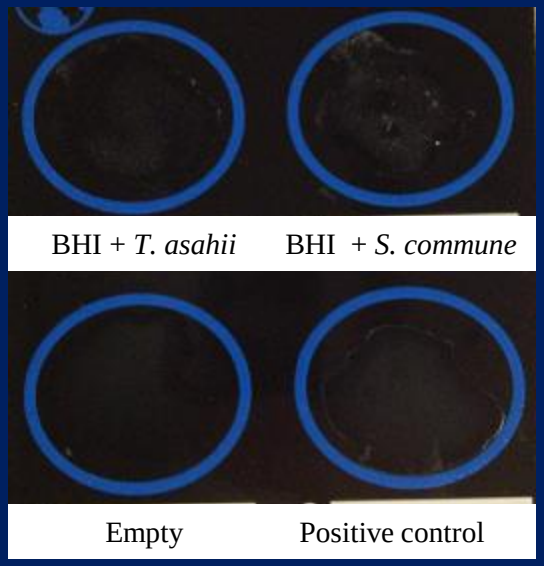
MALDI-TOF MS: identification of medically important fungi

- Yeasts (85-100%):
  - *Candida* sp.
  - *Cryptococcus neoformans & C. gattii*
  - *Saccharomyces* sp.
  - *Trichosporon* sp.
  - *Geotrichum* sp.
  - *Pichia* sp.
- Molds (95-100%):
  - *Aspergillus* sp.
  - *Fusarium* sp.
  - *Rhizopus* sp. & Mucorales
  - *Penicillium* sp.
- Dermatophytes (~100%):
  - *Trichophyton rubrum*
  - *T. interdigitale*
  - *T. tonsurans*
  - *A. benhamiae*
- Dimorphic fungi?
- Antifungal susceptibility tests:
  - Fluconazole (*C. albicans*)
  - Echinocandins (*Candida* sp. & *Aspergillus* sp.)

Marianch C et al., Proteomics. 2009;9:4627-31.
De Carolis E et al., Clin Microbiol Infect. 2012;18:475-84.
Alanio A et al., Clin Microbiol Infect. 2011;17:750-5.
Firacative C et al., PLoS One. 2012;7:e37566.
Vella A et al., J Clin Microbiol. 2013;51:2964-9.

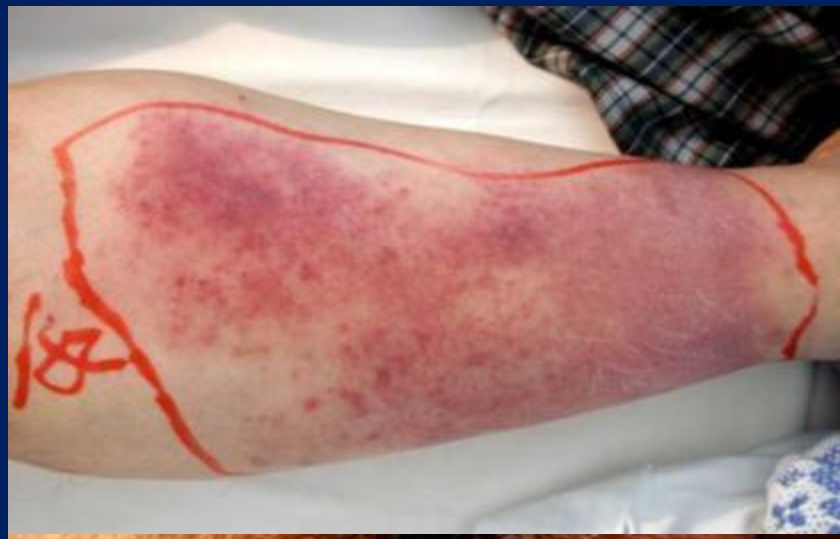
Cryptococcal antigen

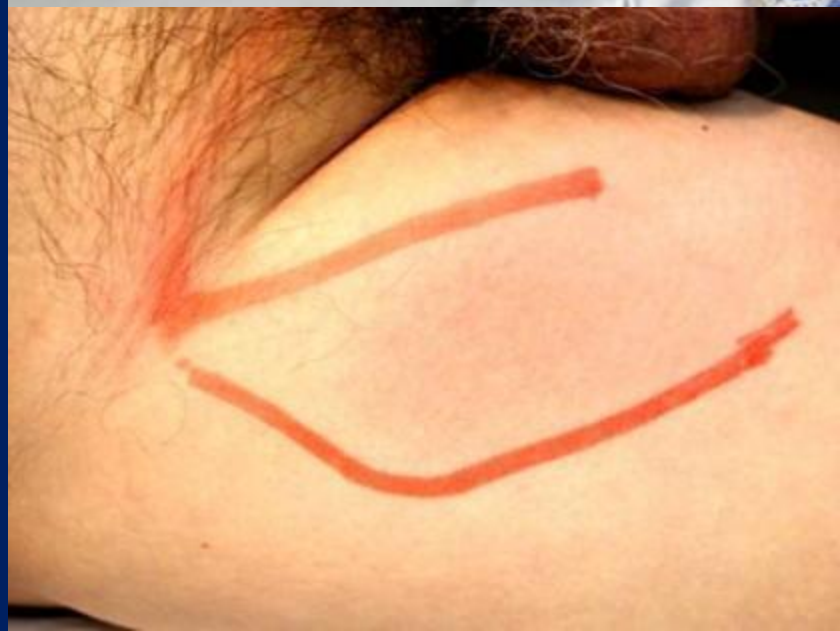
- Latex agglutination or EIA (>90% concordance) to detect capsular polysaccharide of *C. neoformans*
- Sensitivity:
  - 87% (vs blood culture; 42%);
  - higher in AIDS (disseminated) than non-AIDS / SOT (?BMT) patients (95% vs 77%)
  - Meningitis: similar to culture (97%)
  - Pulmonary: 62%
- Specificity:
  - Serum & CSF: 93-100%
  - False +ve:
    - Low titers (1:1-1:8)
    - Hemic malignancy with CNS involvement: may be false +ve
    - *Trichosporon asahii (begielii)*
    - *Schizophyllum commune*
- Qualitative + semi-quantitative
- Prospective testing in HIV

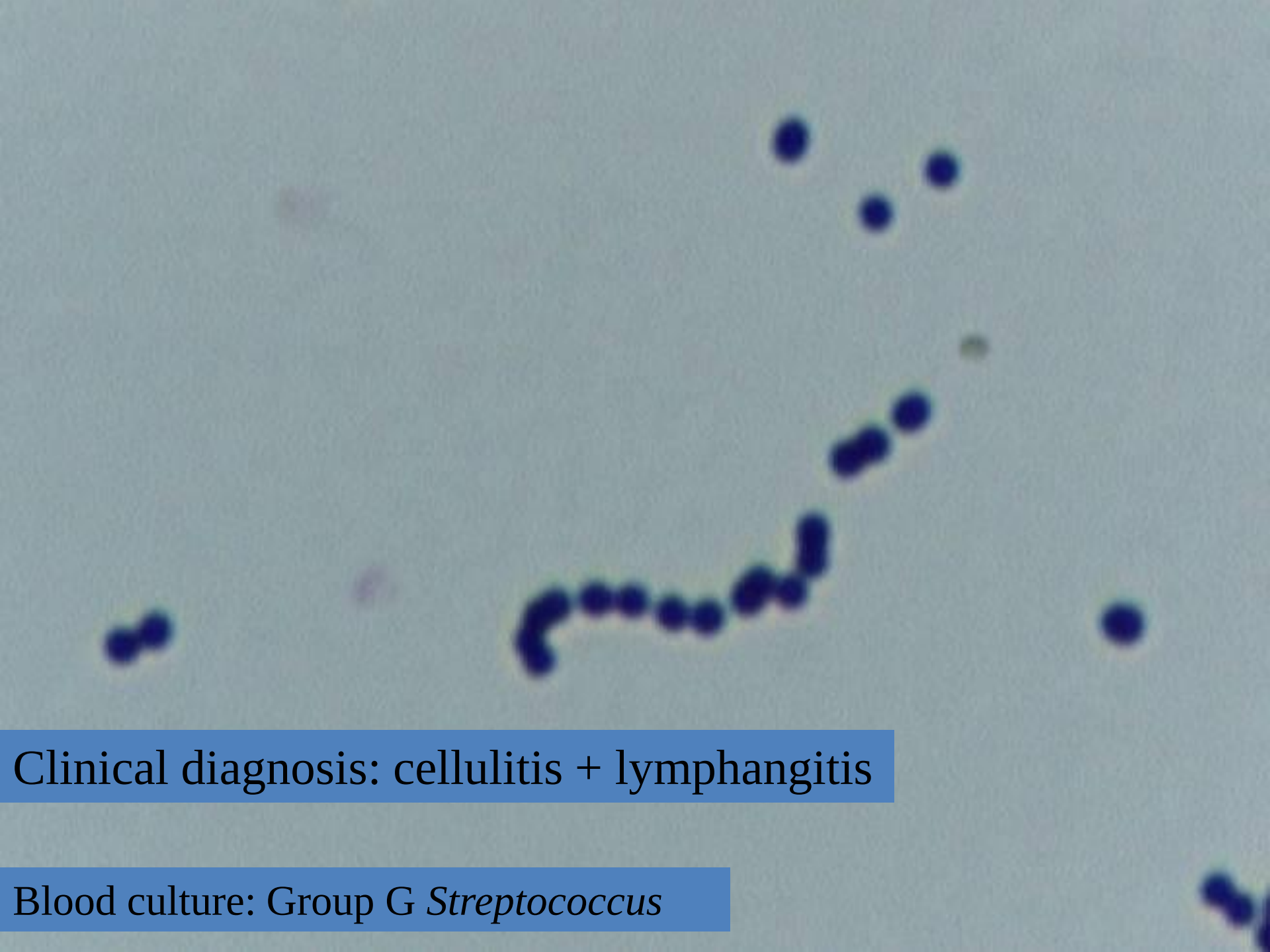


Case

- M/65
- PMH:
  - Follicular lymphoma (1985)
    - Chemotherapy & radiotherapy
    - Relapse: 1989 & 1997
- HPI:
  - Admitted KWH (4/6/2002)
  - Fever, severe right leg pain
  - Dx: cellulitis; Rx: ampicillin + cloxacillin
- Ix:
  - WBC 10.6, ANC 8.9, Lym 1.7
  - Hb 8.9, Plt 58, INR 1.1,
  - L/RFT: N







Clinical diagnosis: cellulitis + lymphangitis

Blood culture: Group G *Streptococcus*

Specimen Type: Marrow Blood

Report :
Hb 8.7 WBC 3.7 Pl 54
The marrow aspirate is aparticulate. Marrow smears show predominantly leukaemic promyelocytes. Blast cells and promyelocytes number 5% and 94% of the nucleated cell count respectively. Occasional Auer rods are seen. Normal haemopoiesis is severely suppressed.

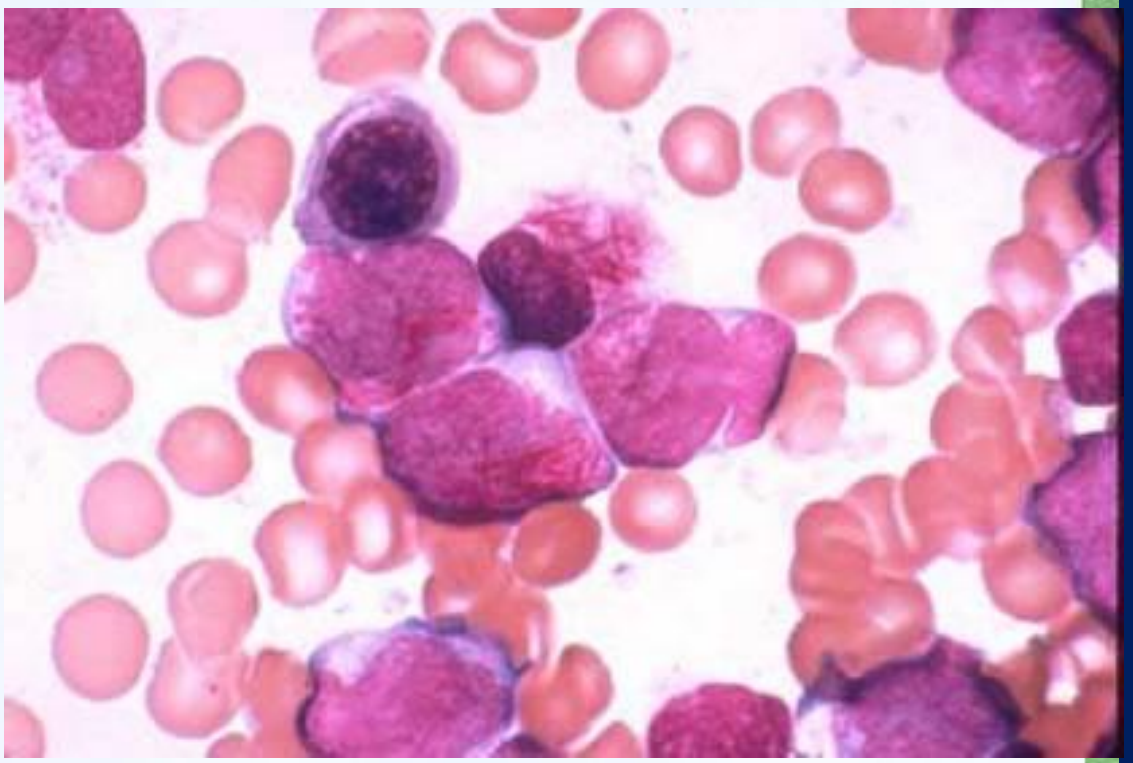
Conclusion :
Findings show acute promyelocytic leukaemia.

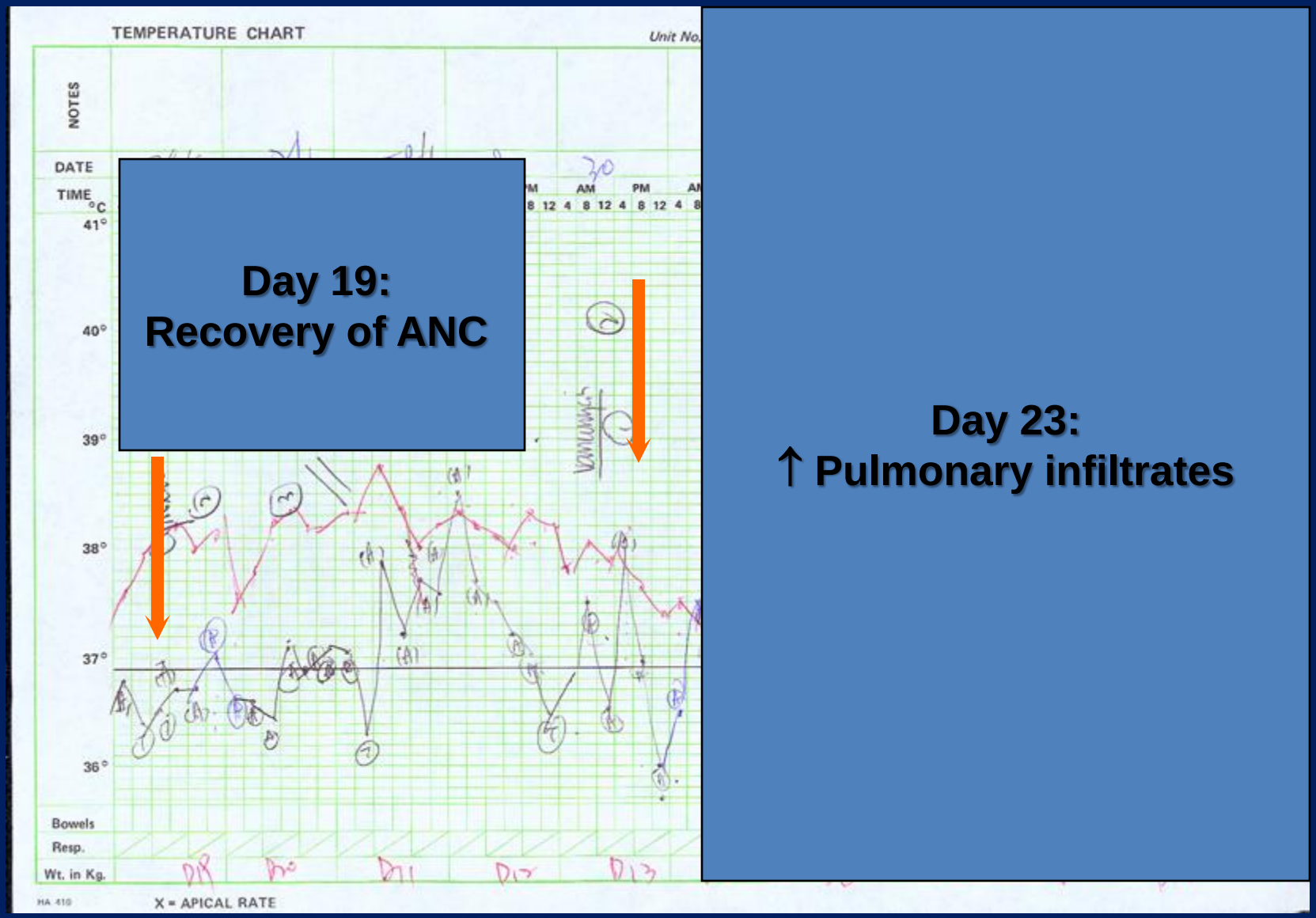
Pathologist: [redacted]

Diagnosis:

Bacteraemic cellulitis + lymphangitis (Group G *Streptococcus*) in AML-M3

Rx: Tazocin 4.5 g iv q8h
All-trans-retinoic acid

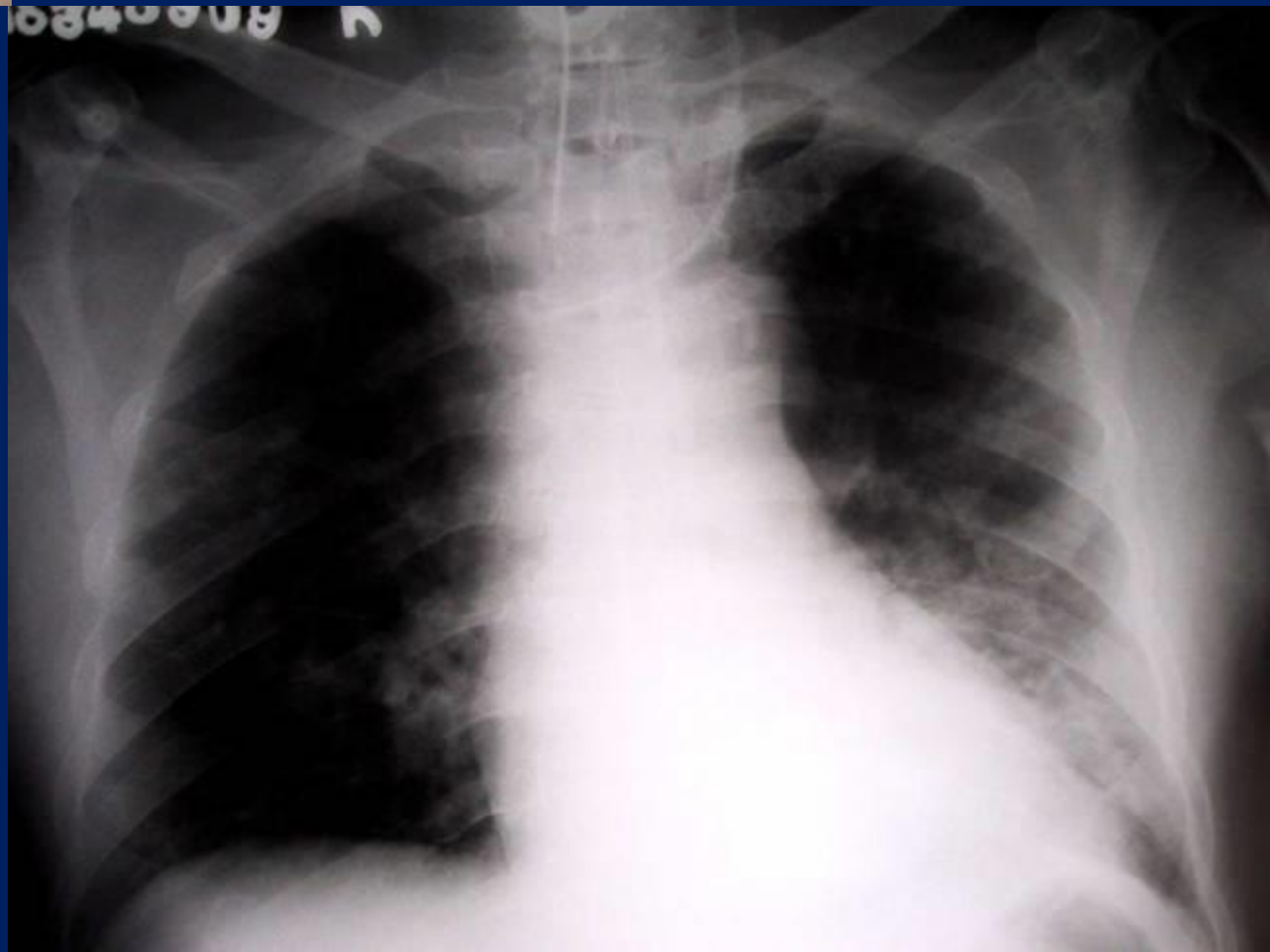


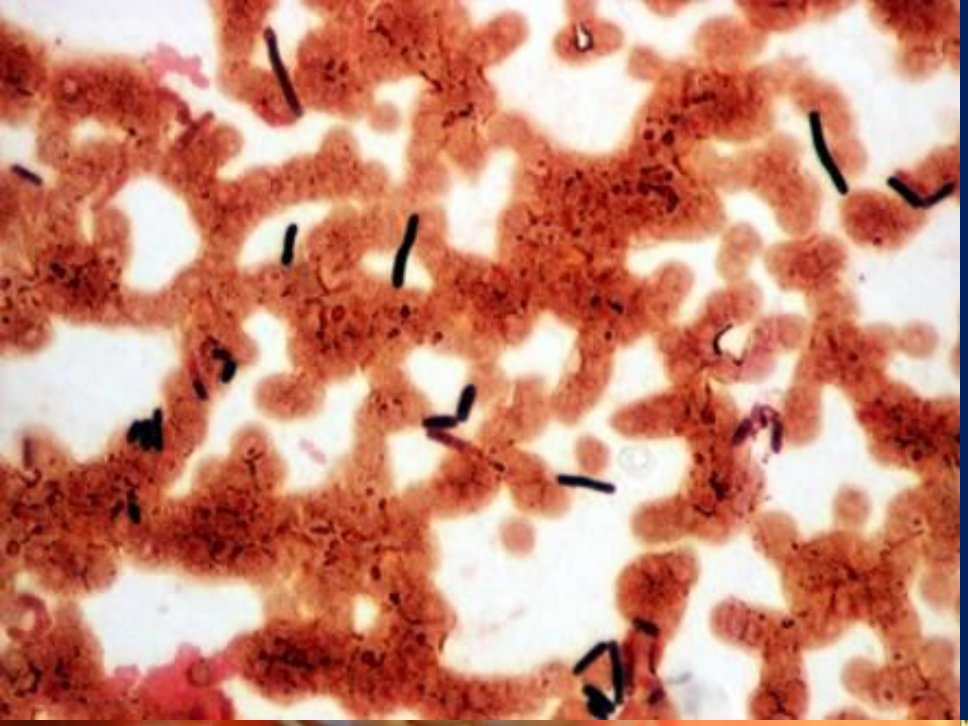

TEMPERATURE CHART
Unit No.
NOTES
DATE
TIME
°C
41°
40°
39°
38°
37°
36°
Bowels
Resp.
Wt. in Kg.
X = APICAL RATE
Day 19:
Recovery of ANC
Day 23:
↑ Pulmonary infiltrates

Day 23: Nosocomial pneumomia

Serum cryptococcal antigen positive (1:8)

Blood and ETA grew a yeast which forms pseudoytype and fragments into arthroconidia





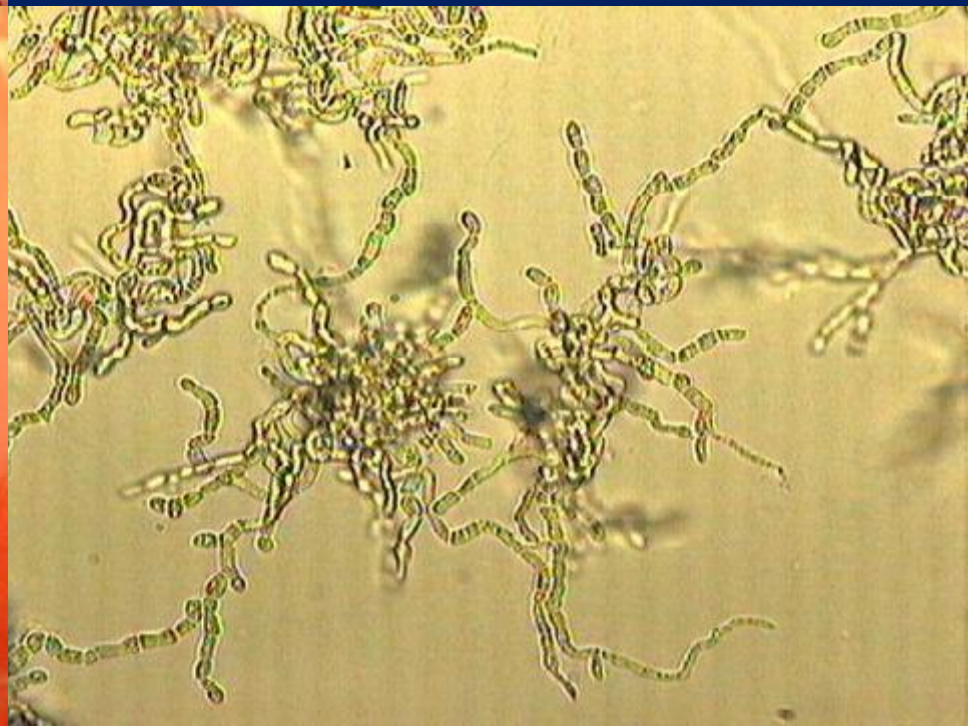
Day 26:
Blood culture: yeast
Disseminated trichosporonosis

Rx: Amphotericin B 1 mg/kg/day; itraconazole 200mg bd for 3 days then 100 mg bd

Volcano like colony morphology

Arthroconidia in agar culture

Capsular polysaccharide
Glucuronoxylomannan (GXM): xylose, arabinose, mannose
Mannose as cross-reactive antigenic epitope leading to false positive cryptococcal antigen test



1-3-β-D-Glucan

- 1-3-β-D-glucan:
  - Cell wall component of most fungal species including *Candida* sp., *Trichosporon* sp., *Saccharomyces cerevisiae*, *Acremonium* sp., *Aspergillus* sp., *Fusarium* sp., *Coccidioides immitis*, *Histoplasma capsulatum*, *Pneumocystis jiroveci* (NOT zygomycetes, *Cryptococcus* sp., *Blastomyces dermatitidis*)
  - 4 commercially available assays (different sources of substrate for the chromogenic reaction → different reactivities / cutoffs for positivity)
  - Monitoring strategy: 2-3 times / week

Marchetti O et al., Bone Marrow Transplant. 2012;47:846-54.

Table 3 Performance of the commercially available β-glucan assays for the diagnosis of IFD in hematological patients

Commercial kit	*% Sensitivity*	*% Specificity*	*% Positive PV*	*% Negative PV*
Case–control studies[a]				
Fungitell[b]	64–78	71–92	72–89	73–77
Fungitec-G[c]	90–95	86–100	59–81	96–97
Wako-Maruha	NA	NA	NA	NA
Cohort studies[d]				
Fungitell[e]	64–100	45–90	37–61	91–100
Fungitec-G[f]	63	76	19	96
Wako-Maruha[g]	50–55	89–98	56–67	87–96

No significant difference among the different assays

Concerns: different study designs & patient groups

Good NPV, poor SEN (ie: not useful for exclusion)

*Good PPV & good SPE (ie: good for ruling-in IFI)

1-3-β-D-Glucan

- Practical considerations:
  - Non-specific: IC & IA similar (different Rx options)
  - May precede clinical s/s by 1-10 days (good for ruling-in IFI)
  - Always requires clinical, radiological & mycological support
  - Trend of BDG: may be useful for monitoring treatment response
  - PJP: pooled SEN 94.8% & SPE 86.3%
  - False –ve:
    - *Cryptococcus* sp.
    - Zygomycetes
    - Antifungal Rx / prophylaxis
  - False +ve:
    - Blood transfusion
    - HD/hemofiltration
    - β-lactams
    - IVIG
    - Cellulose dressings
    - Laboratory contamination
    - Bacterial infections (*S. pneumoniae, P. aeruginosa*, *Alcaligenes faecalis*, etc.)

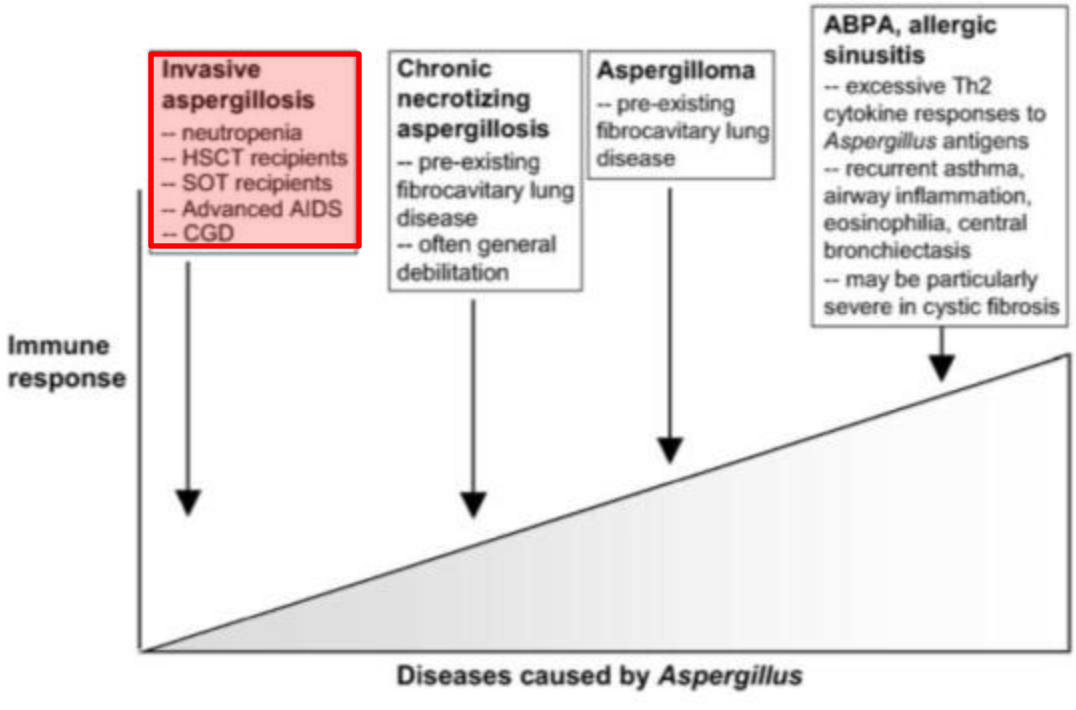
- Unknowns:
  - Optimal cutoff values?
  - # of +ve samples needed? (2 consecutive better than 1)
  - Frequency of tests?
  - Utility in follow-up of IFI?

Marchetti O et al., Bone Marrow Transplant. 2012;47:846-54.
Lamoth F et al., Clin Infect Dis. 2012;54:633-43.

Invasive aspergillosis and the *Aspergillus* Galactomannan antigen

TABLE 1. PATIENT POPULATIONS AT RISK FOR INVASIVE ASPERGILLOSIS

Patient Populations*	Comments
Neutropenia	• Severe (absolute neutrophil count < 100/ul) and prolonged (> 10 d) neutropenia pose high risk for IA • Patients at highest risk include: receiving cytotoxic chemotherapy for acute leukemia; aplastic anemia
Allogeneic HSCT recipients	• First month neutropenia from conditioning regimen is the major risk factor for IA • 1–6 mo: defective cellular immunity contributes to risk of IA; high-dose corticosteroids cause global immunosuppression and disable phagocyte and cellular immunity • > 6 mo: partial reconstitution of cellular immunity expected in absence of significant immunosuppression for GVHD†
Autologous HSCT recipients	• Invasive aspergillosis much less common in autologous compared to allogeneic HSCT • CD34-enriched autografts have higher risk of IA • Patients receiving more than one HSCT and those with prior treatment with potent immunosuppressive regimens for refractory malignancy are at higher risk for IA
Solid organ transplantation	• Lung transplant recipients at highest risk for IA • Major risk factor is intensity of immunosuppression to treat allograft rejection
Other patients receiving potent immunosuppressive therapy (e.g., for autoimmune diseases)	• High-dose systemic corticosteroids (e.g., prednisone equivalent > 20 mg/d for > 3 wk), calcineurin inhibitors, anti-lymphocyte immunoglobulin preparations; anti-TNF-α agents (e.g., infliximab) • Combination of high-dose corticosteroids and other immunosuppressive or cytotoxic agents (such as used for Wegener's granulomatosis) carries highest risk of IA among patients with autoimmune disease
AIDS	• Occurs in patients with advanced AIDS (CD4 < 100/ul) • Incidence of IA in AIDS is significantly reduced in era of highly active antiretroviral therapy
CGD	• Inherited disorder of phagocyte NADP reduced oxidase • IA is a leading cause of mortality in CGD • Diagnosis of IA in absence of known risk factors should prompt evaluation for CGD • Serum galactomannan lacks sensitivity in IA in CGD
Other primary immune disorders	• Job's syndrome (*Aspergillus* colonizing pneumatoceles may lead to invasive disease) • MELAS syndrome, Pearson's syndrome, and severe combined immunodeficiency are rarely associated with IA



Segal BH et al., Am J Respir Crit Care Med. 2006;173:707-17.

Platelia™ *Aspergillus* Ag Kit

Early detection is key for the diagnosis and treatment of Invasive Aspergillosis

The Galactomannan Test

- Detection of the *Aspergillus* galactomannan antigen, six days before onset of clinical symptoms and 10 days before diagnosis[1]
- Results in three hours
- EIA microplate format with ready-to-use reagents and controls

(Double-sandwich ELISA)

[1] *Marr KA, et al. J Infec Dis 2004 Aug 1; 190(3):641–649.*

FDA-cleared for serum and BAL specimens from adults and pediatric patients

BIO-RAD

https://www.bio-rad.com/webroot/web/pdf/cdg/literature/J115_Microbiology.pdf

Testing and Result Interpretation

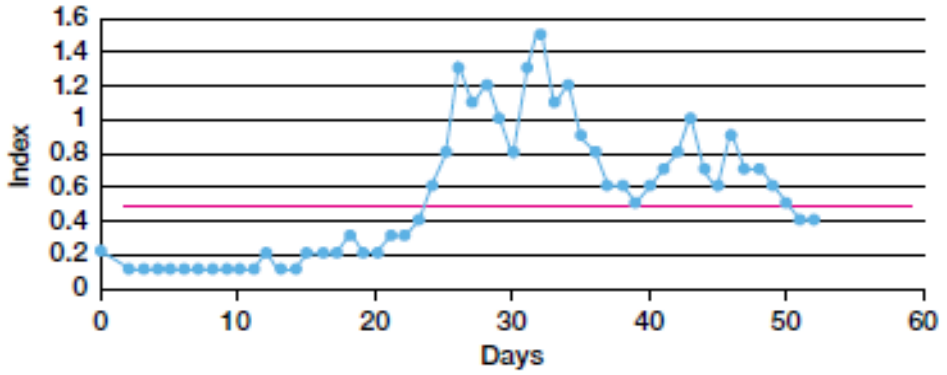
Positive Result = Index ≥ 0.5

- For all positive patient results, re-testing the same Serum/BAL sample (new aliquot) is recommended
- Regular screening (twice weekly) of serum samples of high risk patients is recommended
- Positive results should be considered in conjunction with other diagnostic procedures

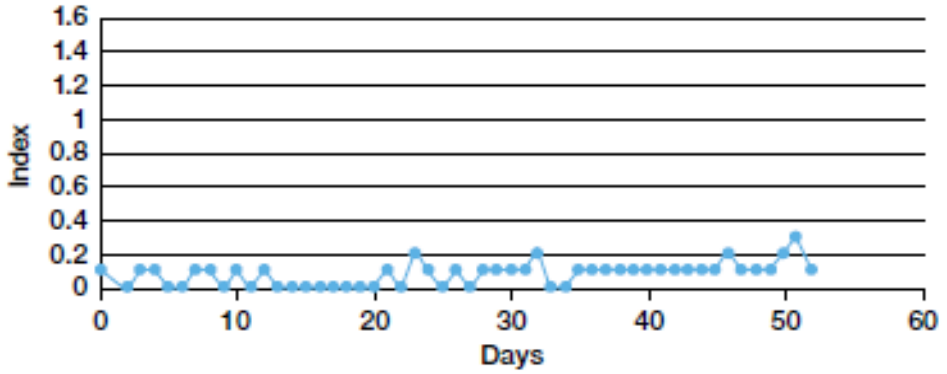
Negative Result = Index < 0.5

- Repeat testing is recommended if result is negative but disease is suspected

Positive Patient: Proven Invasive Aspergillosis Patient



Negative Patient: Control Patient



https://www.bio-rad.com/webroot/web/pdf/cdg/literature/J115_Microbiology.pdf

Convenient

- 96 well microplate with breakable strips
- Ready-to-use colored conjugate

Objective

- Results reported as index

Efficient

- Excellent sensitivity and specificity
- Faster initiation of appropriate treatment

Aspergillus Galactomannan antigen

- Meta-analysis (serum):
  - Sensitivity: 61-71%
  - Specificity: 89-93%
  - PPV: 26-53%
  - NPV: 95-98%
  - (ie: good at ruling out the Dx of IA, but less good at confirming the Dx) – only used when high pretest probability (ie: >5-10%; high-risk groups)
- Concerns:
  - Heterogeneous patient groups (hemic better than SOT)
  - Cut-off value (signal in test sample : signal of reference sample) - ≥1.5 (manufacturer), ≥1.0? ≥0.5?
  - Differences in clinical practice and lack of study on clinicians' response to the result
- Impact:
  - Shorten the time to diagnosis (days before clinical/radiological findings) and pre-emptive antifungal therapy
  - Predicts outcome:
    - High baseline serum GM Ag at Dx of IA associated with poor outcome
    - Serum GMI trend: week 1 GMI (responder < non-responder; 0.62+/-0.12 vs 1.15+/-0.22; $p<0.05$); rising absolute GMI <0.5 → >0.5 at week 2 despite Rx – poor clinical outcome)
  - Other specimens: BAL, CSF (urine, pleural fluid, sputum?)

Pfeiffer CD et al., Clin Infect Dis. 2006;42:1417-27.
Rex JH et al., Clin Infect Dis. 2006;42:1428-30.
Bergeron A et al., J Clin Microbiol. 2012;50:823-30.
Chai LY et al., J Clin Microbiol. 2012;50:2330-6.

Aspergillus Galactomannan antigen

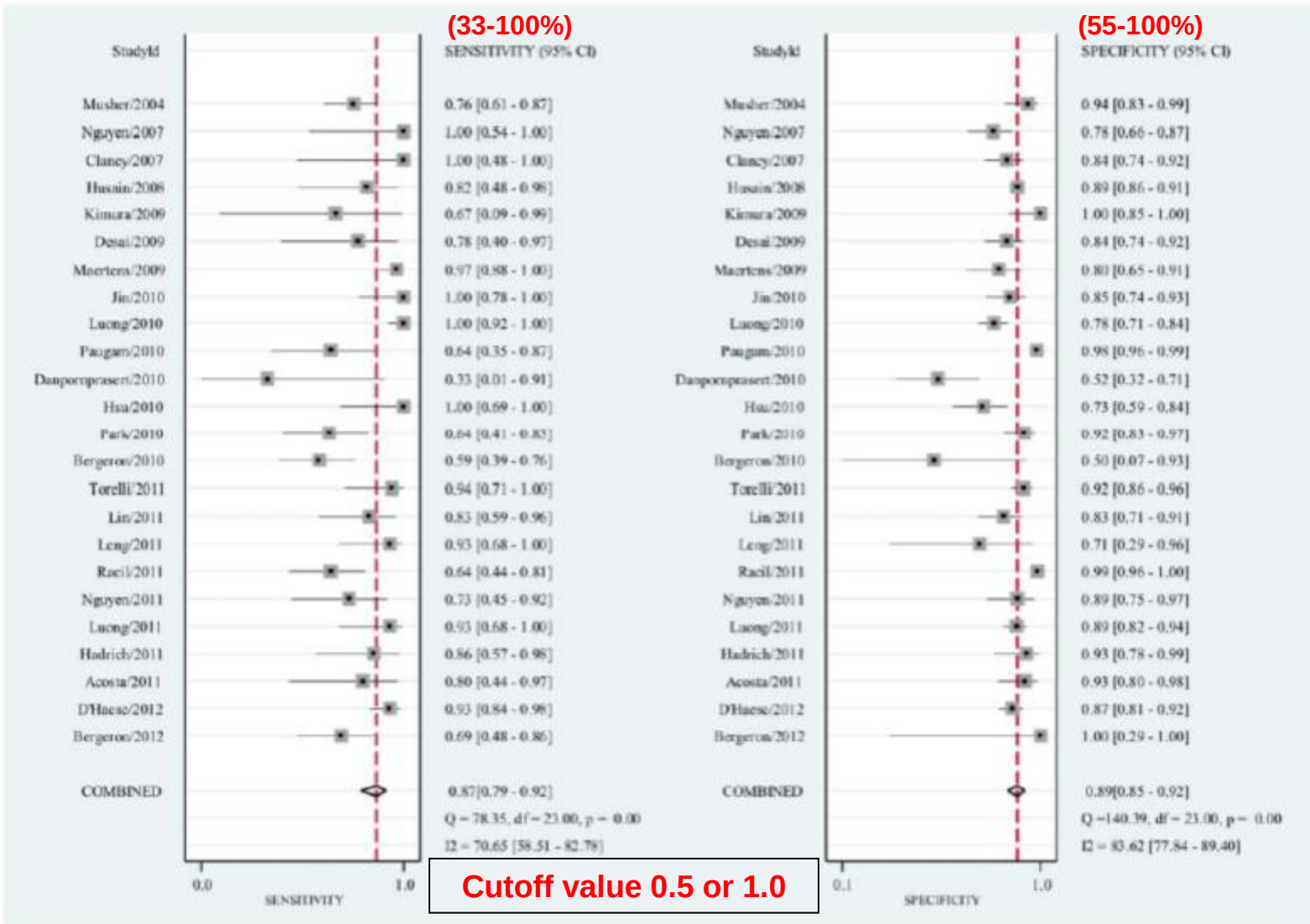
- Limitations:
  - False +ve:
    - Procedure-related
    - GI tract mucosal breach:
      - *Bifidobacterium* sp.
      - GVHD / severe mucositis
    - Other fungal infections:
      - *Cryptococcus neoformans* – AIDS
      - *Penicillium marneffei* – AIDS
      - *Histoplasma* sp.
      - *Fusarium* sp.
      - *Alternaria* sp.
      - *Paecilomyces* sp.
      - *Geotrichum* sp.
      - *Trichosporon dermatis*
      - *Prototheca* sp. (algae)
    - Drugs / infusate:
      - β-lactams: piperacillin-tazobactam, amoxicillin-clavulanate
      - Blood products: pooled platelet concentrates, FFP, packed RBCs, anticoagulant (Fresenius Kabi)
      - Gluconate-containing IVF
  - False -ve:
    - Procedure-related
    - Antifungal Rx
    - Patients with CGD / Job's syndrome
  - Neonatal / pediatric serum samples: lack of data

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

Biological factors	Epidemiological factors
Site of infection	Patient population
Aspergillus species causing infection	Sampling strategy
Microenvironment at the site of infection: nutrients, oxygen level, pH	Definition of a positive result
Exposure to antifungal agents	Definition of an infected patient
Molecule structure of released galactomannan	Prevalence of infection
Underlying condition/level of immunosuppression	Cut-off
Renal clearance, hepatic metabolism	Laboratory experience
Presence of galactomannan antibodies	
Storage of sample	
Pretreatment procedure	

Mennink-Kersten MA et al., Lancet Infect Dis. 2004;4:349-57.

Aspergillus Galactomannan antigen (BAL)



Forest plot of sensitivities and specificies from test accuracy studies of BAL-GM in the Dx of IA

Zou M et al., PLoS One. 2012;7:e43347.

Aspergillus Galactomannan antigen (BAL)

- Meta-analysis:
  - Serum GM vs BAL-GM:
    - Pooled SEN: 65% vs 85% (larger fungal burden in bronchial tree; hyphae secrete more antigenic GM than conidia)
    - Pooled SPE: 95% vs 86% (different stages of disease – BAL: airway cellular invasion of *Aspergillus* sp; serum: later penetration of hyphae via endothelial cells)
  - PCR vs BAL-GM:
    - Pooled SEN: 82% vs 78-94%
    - Pooled SPE: 98% vs 91-97%

Avni T et al., J Clin Microbiol. 2012;50:3652-8.
Zou M et al., PLoS One. 2012;7:e43347.

TABLE 2 Direct comparisons of PCR and GM **(BAL)**

Comparison (no. of studies)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	DOR (95% CI)	*P* value
PCR vs GM with an ODI of >0.5 (7)				0.088
PCR	86.4 (68.2–95)	96.2 (90.2–98.6)	163 (47–560)	
GM	82 (52.7–94.9)	96.6 (92.2–98.6)	129 (32–516)	
PCR vs GM with an ODI of >1.0 (7)				0.01
PCR	92.6 (69.9–98.5)	97.7 (92.6–99.3)	516 (82–3,248)	
GM	85.1 (62.5–95.1)	99.7 (97.3–100)	1,731 (202–14,802)	
Any positive result vs GM (10)[a]				0.001
GM or PCR	97 (83–99.5)	97.5 (92.9–99.1)	1,258 (155–10,215)	
GM	89 (63–97.5)	98.5 (94.5–99.6)	516 (74–3,611)	

[a] Comparison between a positive result defined by either PCR or GM positivity when both tests are performed and GM alone. Paired analysis was conducted with all studies reporting on GM in BAL fluid preferably using the results obtained with an ODI cutoff of >0.5.

HKU/QMH data (07/2003-06/2013)

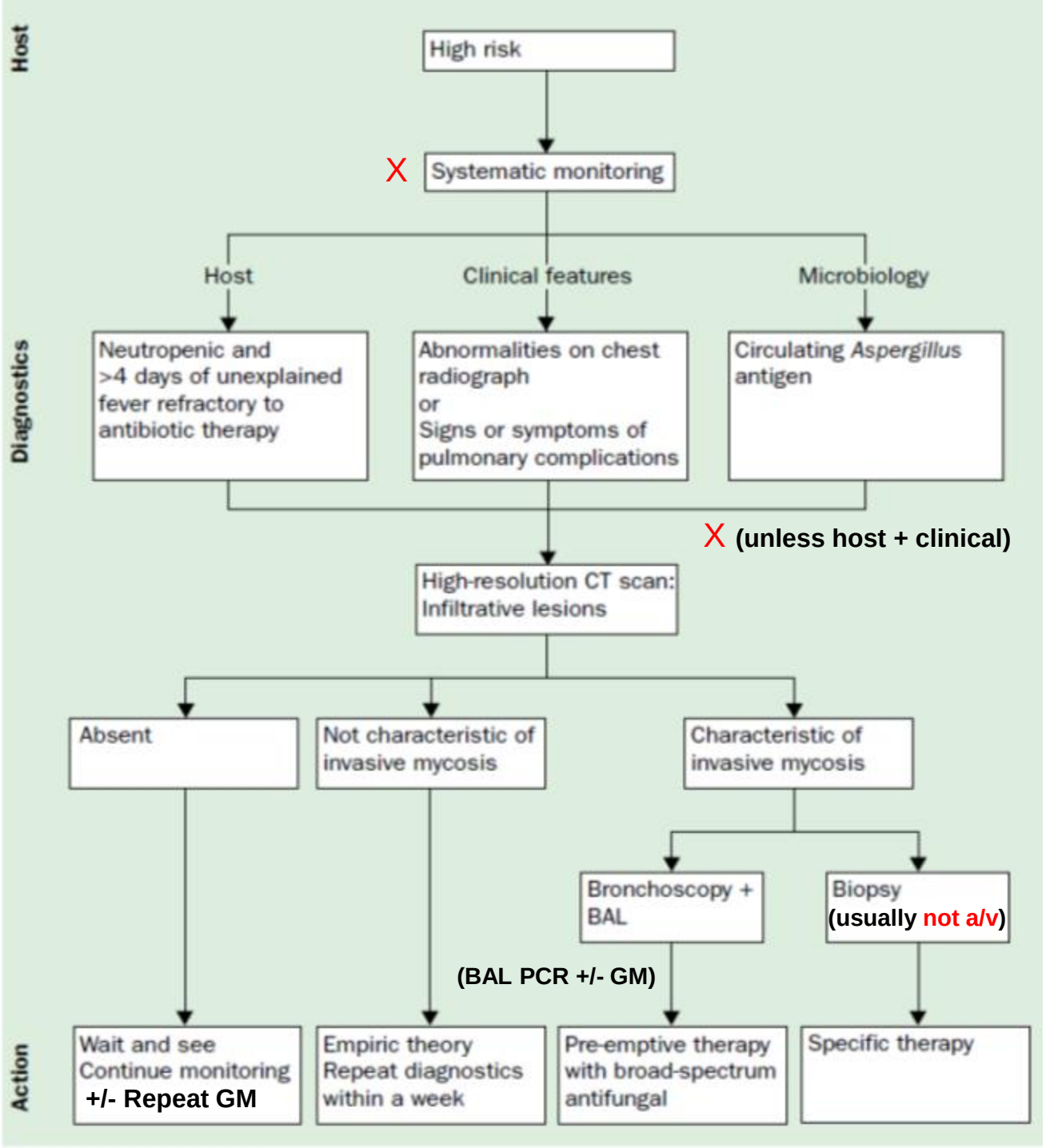
Parameters	Values
Age (median, range)	53.0 / 24 days to 92 years
Sex	M:F = 66:41
Working diagnosis:	
Neutropenic fever with pneumonia	14 (13.1%)
Neutropenic fever without pneumonia	7 (6.5%)
*Non-neutropenic fever with pneumonia	57 (53.3%)
Non-neutropenic fever without pneumonia	18 (16.8%)
Radiological abnormality without symptoms	8 (7.5%)
Others	3 (2.8%)
Underlying conditions:	
*Neutropenia for >10 days before onset of symptoms	21 (19.6%)
Previous pulmonary tuberculosis	21 (19.6%)
Active pulmonary tuberculosis	5 (4.7%)
Chronic pulmonary disease	29 (27.1%)
Chronic cardiac disease	24 (22.4%)
Chronic renal disease	8 (7.5%)
Chronic hepatic disease	16 (15.0%)
Central nervous system disease	9 (8.4%)
Diabetes mellitus	13 (12.1%)
Autoimmune disease	11 (10.3%)
Human immunodeficiency virus infection	11 (10.3%)
Congenital immunodeficiency	0 (0%)
*Corticosteroids / immunosuppressive drugs	31 (29.0%)
Chemotherapy in preceding 30 days	25 (23.4%)
Solid organ malignancy	9 (8.4%)
*Hematological malignancy	34 (31.8%)
*Solid organ transplantation	7 (6.5%)
*Hematopoietic stem cell transplantation	21 (19.6%)
Presenting symptoms:	
Fever	64 (59.8%)
Cough	46 (43.0%)
Sputum	32 (29.9%)
Hemoptysis	20 (18.7%)
Dyspnea	50 (46.7%)
*Asymptomatic (radiological abnormality only)	8 (7.5%)

HKU/QMH data (07/2003-06/2013)

Laboratory parameters (median, range):	
Total white cell count, x 10^9 cells/liter	5.90/0.03-40.80
*Neutrophil count, x 10^9 cells/liter	4.13/0.00-38.10
Lymphocyte count, x 10^9 cells/liter	0.80/0.00-91.50
Hemoglobin, g/dl	10.30/6.40-16.60
Platelet count, x 10^9 cells/liter	163.00/4.00-731.00
Sodium, mmol/liter	138.00/124.00-151.00
Potassium, mmol/liter	3.90/2.00-8.70
Urea, mmol/liter	5.80/1.80-62.20
Creatinine, µmol/liter	76.00/23.00-603.00
Random glucose, mmol/liter	6.35/2.70-22.10
Albumin, g/liter	29.00/11.00-54.00
Globulin, g/liter	34.00/12.00-78.00
Total bilirubin, µmol/liter	12.00/2.00-301.00
Alkaline phosphatase, IU/liter	98.00/11.00-989.00
Alanine transaminase, IU/liter	27.00/7.00-843.00
Aspartate transaminase, IU/liter	28.50/8.00-116.00
ESR, mm/hour	65.00/6.00-138.00
C-reactive protein, mg/dl	5.28/0.35-45.00
Chest radiograph features:	
No abnormality	17 (16.0%)
*Consolidation / collapse	62 (58.5%)
*Cavity **(Halo / air-crescent)**	22 (20.8%)
Nodule / granuloma	7 (6.6%)
Pleural effusion	20 (18.9%)
Fibrosis	21 (19.8%)
Site of chest radiograph abnormality:	
RUL	8 (7.5%)
RML	7 (6.6%)
RLL	8 (7.5%)
LUL	5 (4.7%)
LLL	9 (8.5%)
*Multilobar	53 (50.0%)

HKU/QMH data (07/2003-06/2013)

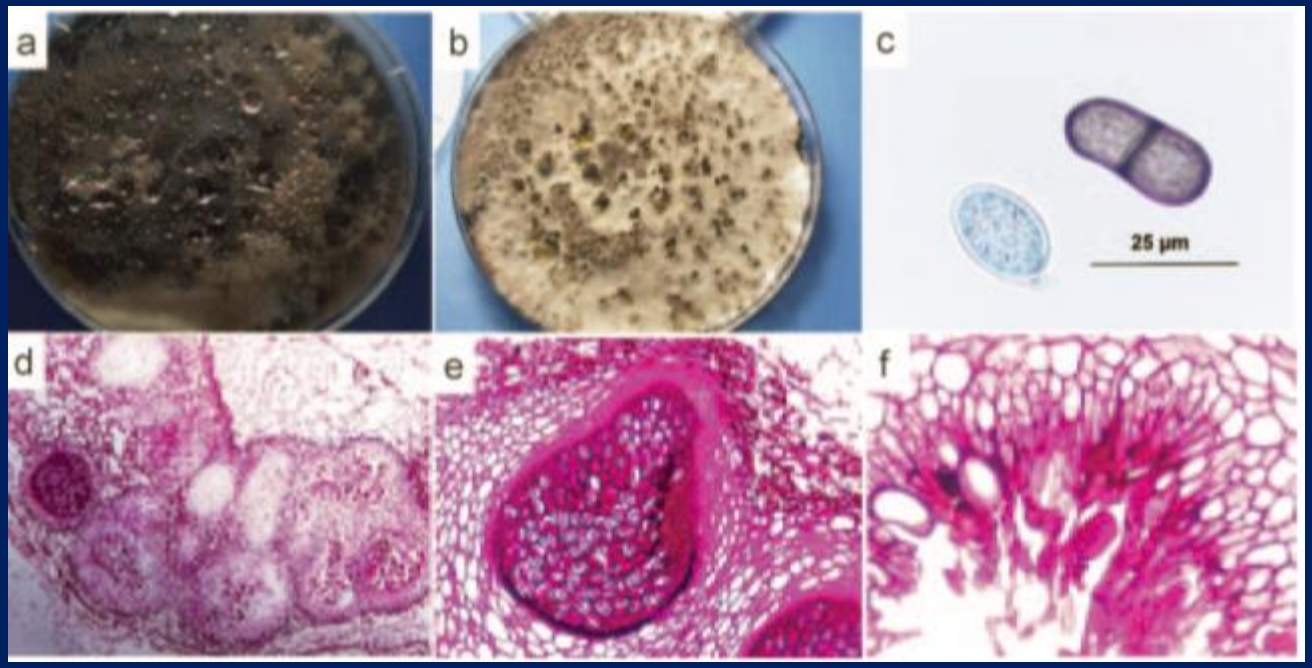
Alternative reasons for positive Ag in "no IA" group (n = 55):	
*Use of amoxicillin-clavulanate / piperacillin-tazobactam	21/55 (38.2%)
*Fungemic penicilliosis	4/55 (7.3%)
False-positive with alternative diagnosis	5/55 (9.1%)
No alternative reasons for positive Ag:	
Non-invasive pulmonary aspergillosis	10/55 (18.2%)
Community-acquired pneumonia (unknown etiology)	9/55 (16.4%)
HIV with fever	5/55 (9.1%)
Leukemia with fever	1/55 (1.8%)



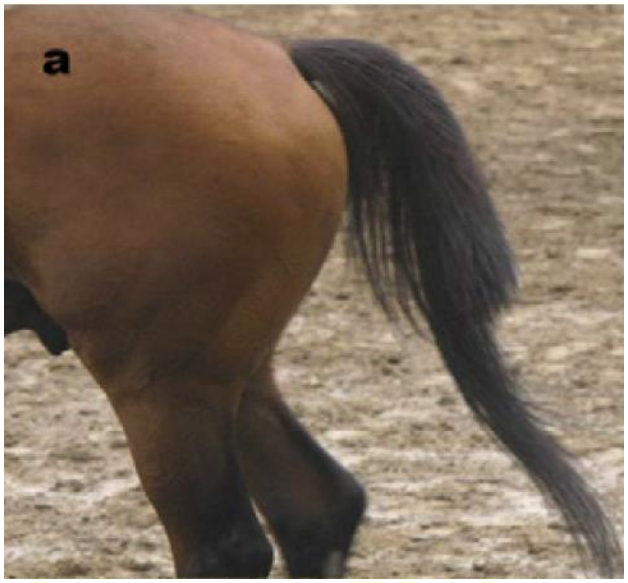
Mennink-Kersten MA et al., Lancet Infect Dis. 2004;4:349-57.

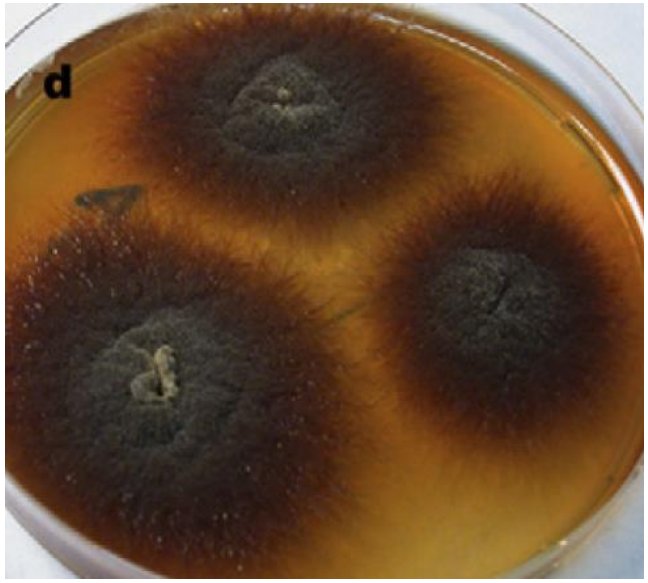
Polymerase chain reaction

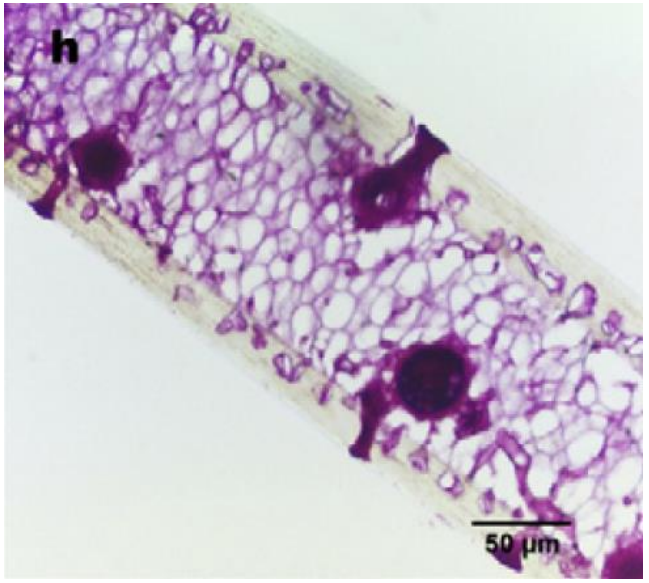
- Not included in EORTC/MSG definitions of IFD:
  - Not standardized
  - Not thoroughly evaluated
  - Mainly investigated in IA
  - Gene targets: ITS, 18S rRNA, LSU rRNA, EF1α, β-tubulin, β-actin, etc.
- Practical uses:
  - Unusual fungal pathogens
  - Unusual clinical syndromes
  - Novel fungus discovery
  - Rapid identification of fastidious fungi
  - Quantification
  - Multiplex PCR (eg: with TB, PCP, etc)



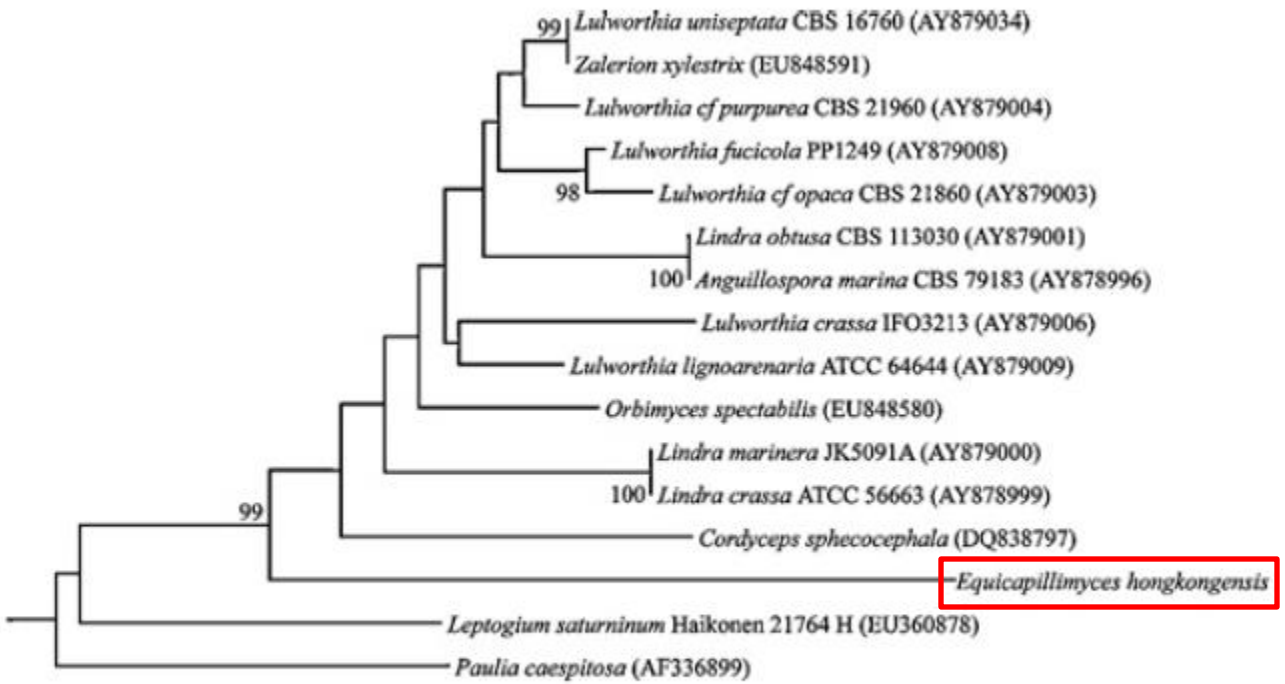
Unusual fungal pathogen: *Lasiodiplodia theobromae* pneumonia in liver transplant recipient (Woo PC et al., J Clin Microbiol. 2008;46:380-4.)







Novel clinical syndrome:
Brittle tail syndrome caused by *Equicapillimyces hongkongensis* gen. nov., sp. nov.

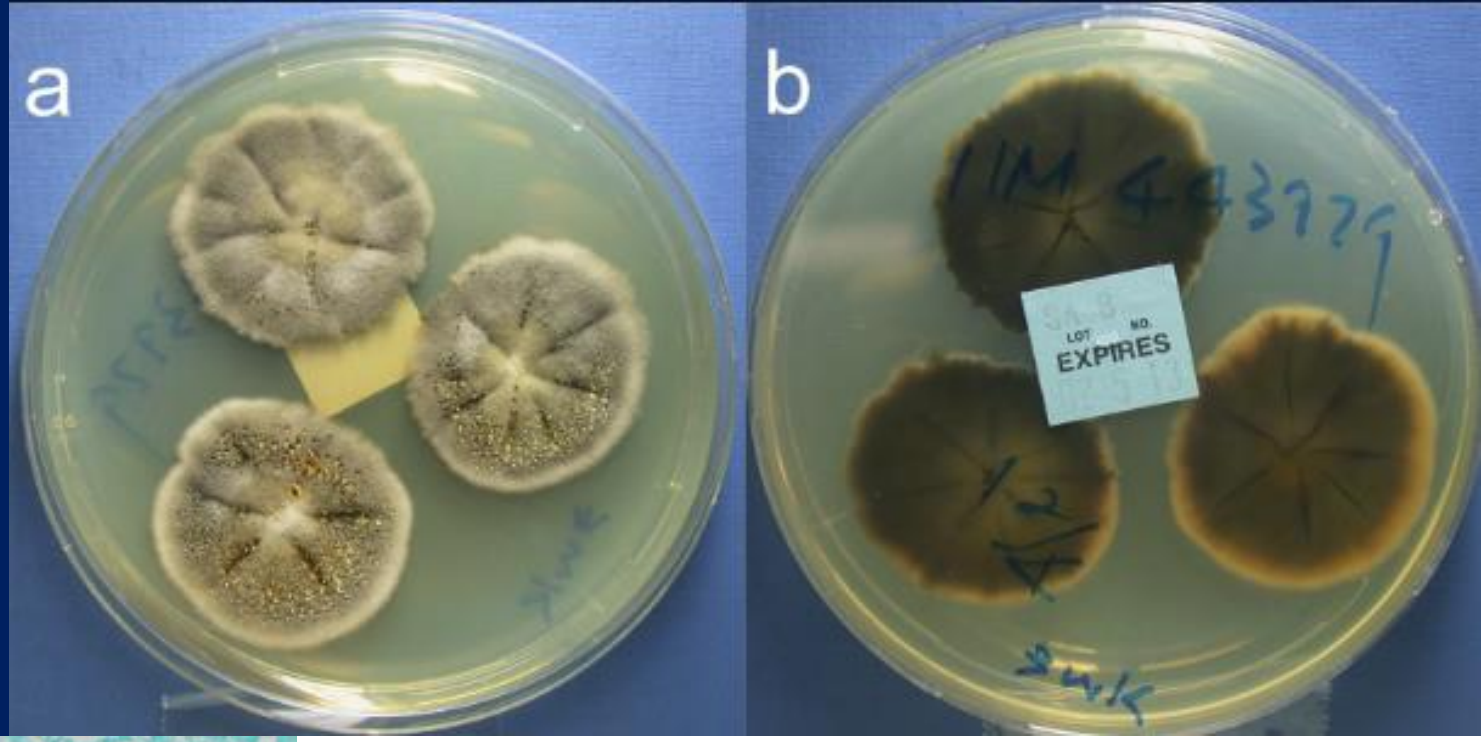


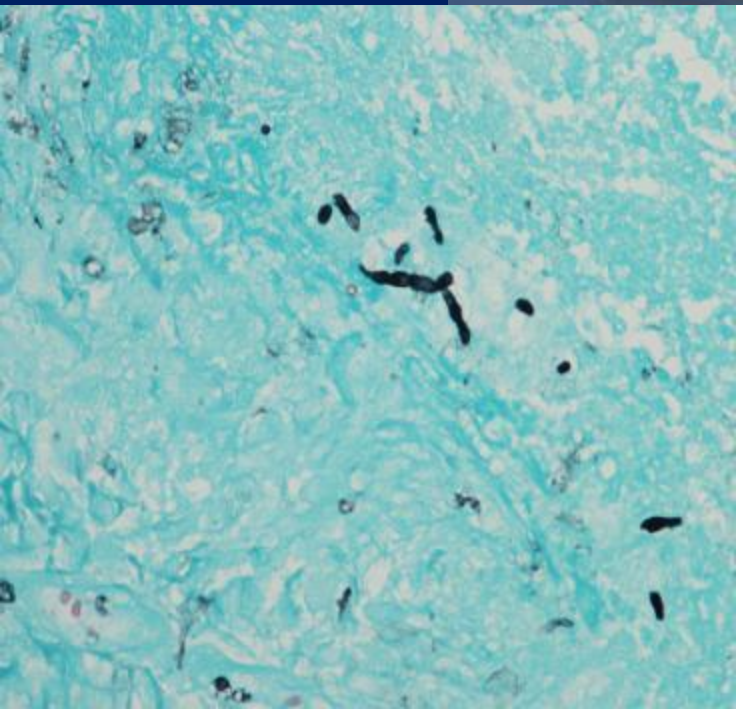
18S rRNA gene sequencing

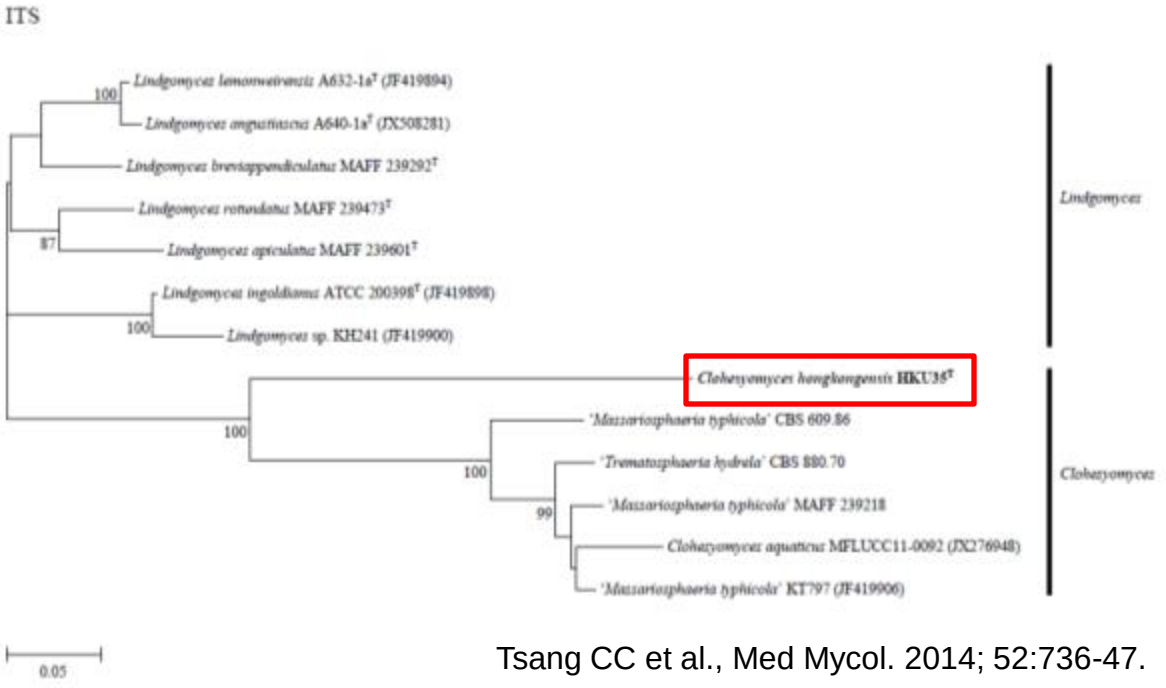
Wong SS et al., Vet Microbiol. 2012;155:399-408.

Discovery of novel fungi:

Invasive wound infection in a patient with IgG4-related sclerosing disease caused by a novel freshwater ascomycete, *Hongkongmyces pedis* sp. nov.







Tsang CC et al., Med Mycol. 2014; 52:736-47.

Rapid identification of fastidious fungi

JCM Journals.ASM.org

Use of Nasopharyngeal Aspirate for Diagnosis of *Pneumocystis* Pneumonia

Kelvin K. W. To,[a,b,c,d] Sally C. Y. Wong,[d] Ting Xu,[d] Rosana W. S. Poon,[d] Ka-Yi Mok,[d] Jasper F. W. Chan,[d] Vincent C. C. Cheng,[d] Kwok-Hung Chan,[d] Ivan F. N. Hung,[a,e] Kwok-Yung Yuen[a,b,c,d]

State Key Laboratory for Emerging Infectious Diseases,[a] Carol Yu Centre for Infection,[b] Research Centre of Infection and Immunology,[c] Department of Microbiology,[d] and Department of Medicine,[e] The University of Hong Kong, Pokfulam, Hong Kong Special Administrative Region, China

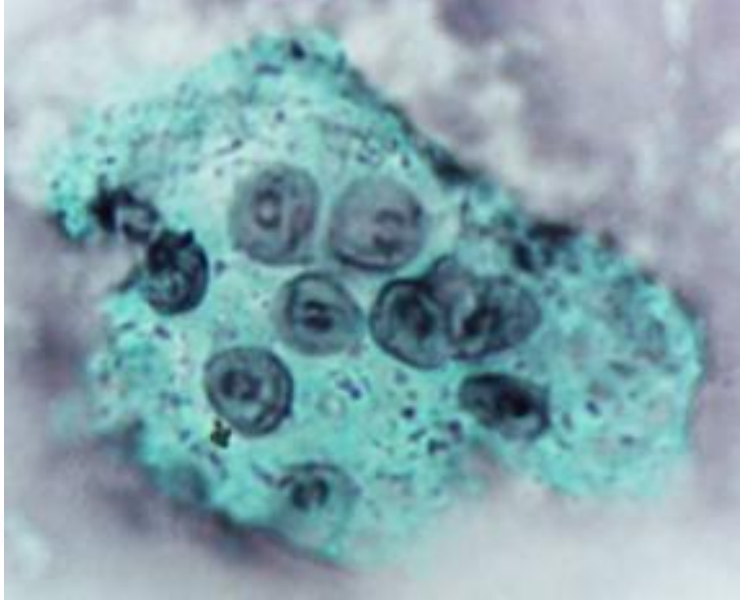


TABLE 2 Performance of LSU-qPCR in NPA specimens for the diagnosis of PCP, with microscopic examination of bronchoscopic specimens as the gold standard

LSU-qPCR in NPA specimens	No. of specimens (microscopic examination of bronchoscopic specimens using methenamine silver staining)		
	Positive	Negative	Total
Positive	15	*4	19
Negative	0	98	98
Total	15	102	117

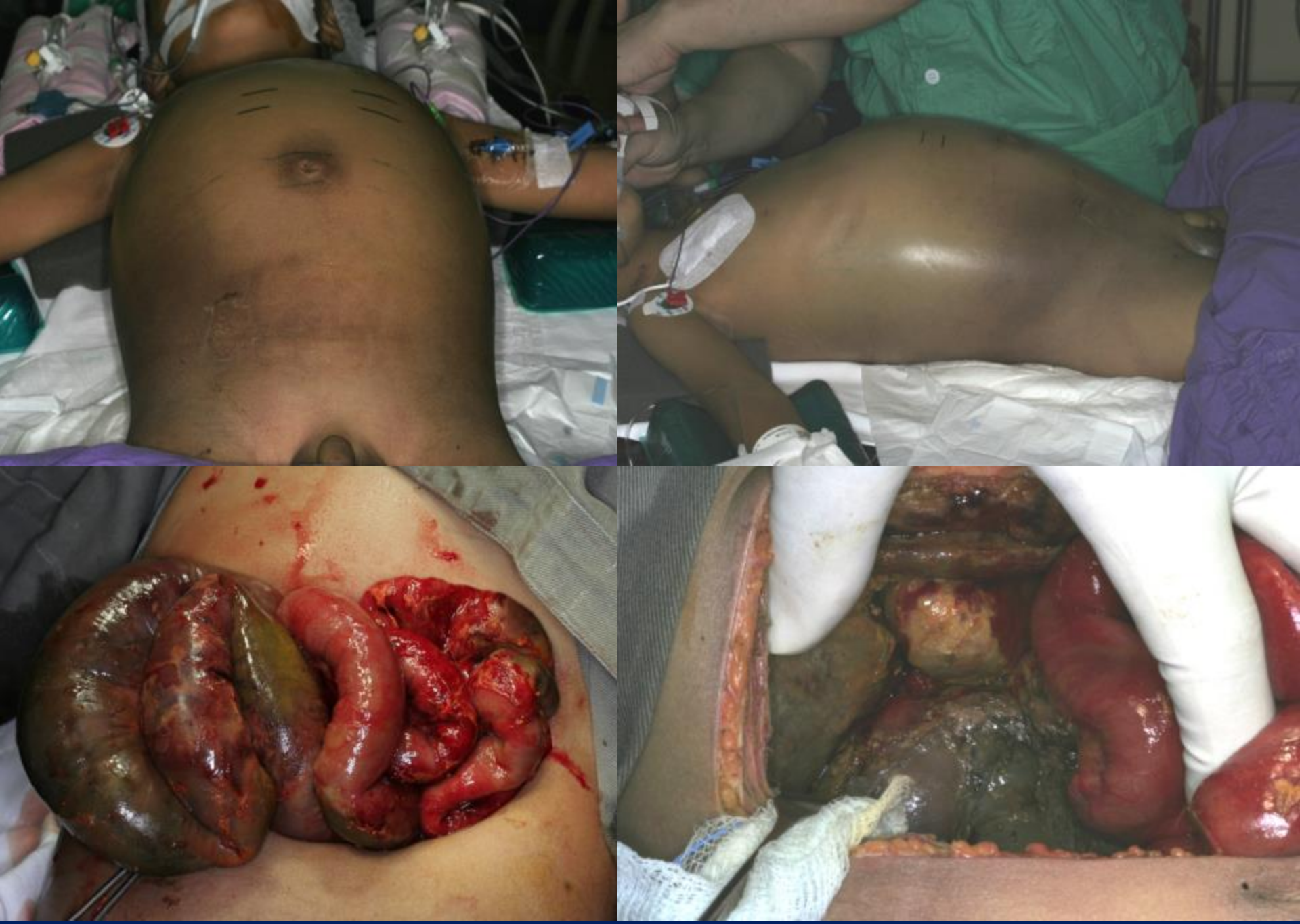
Assay	Method	Company	Targets	Results	Specimen	TAT	FDA-Approved/ Cleared
Yeast Traffic Light	PNA FISH	AdvanDx, Inc, Woburn, MA, USA	26S rRNA for *Candida* spp	Qualitative, with speciation of most *Candida* spp	Blood culture bottles positive for growth	2–3 h	Yes
Multiplex xTAG Fungal ASR Assay	Multiplex PCR and bead-based flow cytometry	Luminex Corp, Austin, TX, USA	23 clinically significant fungi (yeasts and molds)	Qualitative, with detection to the species level when possible	Respiratory specimens; blood culture bottles positive for growth	5–6 h post extraction	No
Aspergillus Real-Time PCR Panel	Real-time PCR	Viracor-IBT Laboratories, Lee's Summit, MO, USA	18S rRNA and ITS1 for *Aspergillus* spp	Qualitative, with detection of *Aspergillus* spp, *Aspergillus fumigatus*, or *Aspergillus terreus*	BAL; bronchial washing	Within 8–12 h of specimen receipt at Viracor	No
Candida Real-time PCR Panel	Real-time PCR	Viracor-IBT Laboratories, Lee's Summit, MO, USA	ITS1 for *Candida* spp	Qualitative, with detection of *Candida albicans* and/or *Candida tropicalis*; *Candida glabrata* and/or *Candida krusei*; and *Candida parapsilosis* complex	Plasma; serum	Same day of specimen receipt at Viracor	No
PLEX-ID Broad Fungal Assay	Multiplex PCR and mass spectrometer	Abbott Ibis Biosciences, Abbott Park, IL, USA	Up to 75 fungi	Qualitative, unique organism identification	BAL; blood	Within 6 h, or 1 working day	No
MycAssay *Aspergillus*	Real-time PCR	Myconostica Ltd, Cambridge, UK	18S rRNA for *Aspergillus* spp	Qualitative	Serum; BAL	3 h	No
SeptiFast	Real-time PCR	Roche Molecular Diagnostics, Pleasanton, CA, USA	5 species of *Candida* and *A fumigatus*	Qualitative	Blood	6 h	No

Summary

Table 1 Advantages of the current methodologies for fungal diagnosis

Culture **(conventional)**	Galactomannan	(1→3)-β-D-glucan	Real-time PCR	**MALDI-TOF-MS**
Simple and cheap It allows identification of the fungus and antifungal susceptibility testing	Non-invasive method Useful for early diagnosis; reproducible methodology	Non-invasive method Useful for early diagnosis; reproducible methodology	Non-invasive method Useful for early diagnosis; reproducible methodology	**MALDI-TOF-MS (yeasts > molds: fungal colonies; direct blood culture)** **Advantages:** **-Rapid TAT** **-Only requires single colony** **-Automated, high throughput** **-Broad applicability to bacteria & fungi** **-Revisable database** **-Minimal consumables** **-Cost-effective** **-ST** **Disadvantages:** **-ID limited by database** **-Diff. b/w related organisms (eg: *E. coli* & *Shigella* sp.)**
High rate of isolation in blood cultures for *Fusarium* spp.; a recently improved strategy still needs to be tested in multiple laboratories for other molds	Greater values of sensitivity and specificity for mold diagnosis, particularly when non-*fumigatus* *Aspergillus* spp. are involved **Concerns:** **1. Cross-reactivity** **2. Frequency of tests** **3. Cut-off value** **4. Antifungal Rx** **5. Serum and BAL**	Broad coverage of fungal species; it can be used for screening of patients with possible mold diseases Useful in patients under antifungal therapy **Other uses:** **PCP (HIV / non-HIV)** **Negative in:** ***Cryptococcus* sp.** **Zygomycetes** ***Basidiomyces dermatitidis***	Broad coverage of fungal species; it can be used for screening of patients with possible mold diseases Useful in patients under antifungal therapy **Concerns:** **1. Standardization** **2. Expertise / facilities** **3. Cost**	

Patel R et al., Clin Infect Dis. 2013, 57:564-72.
Beirao F et al., Eur J Clin Microbiol Infect Dis. 2013, 32:3-9.

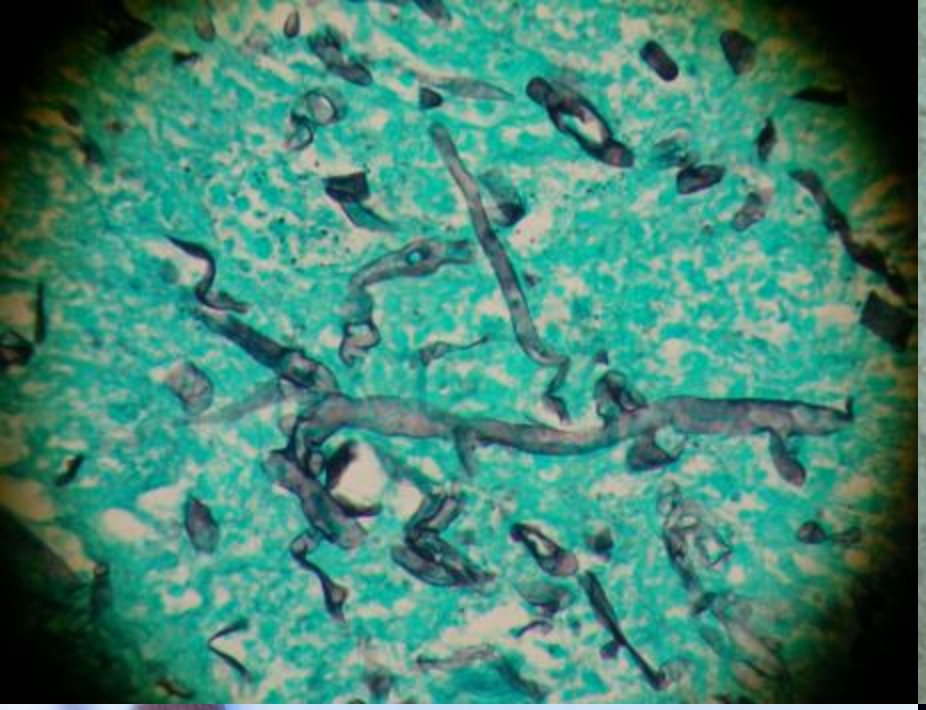


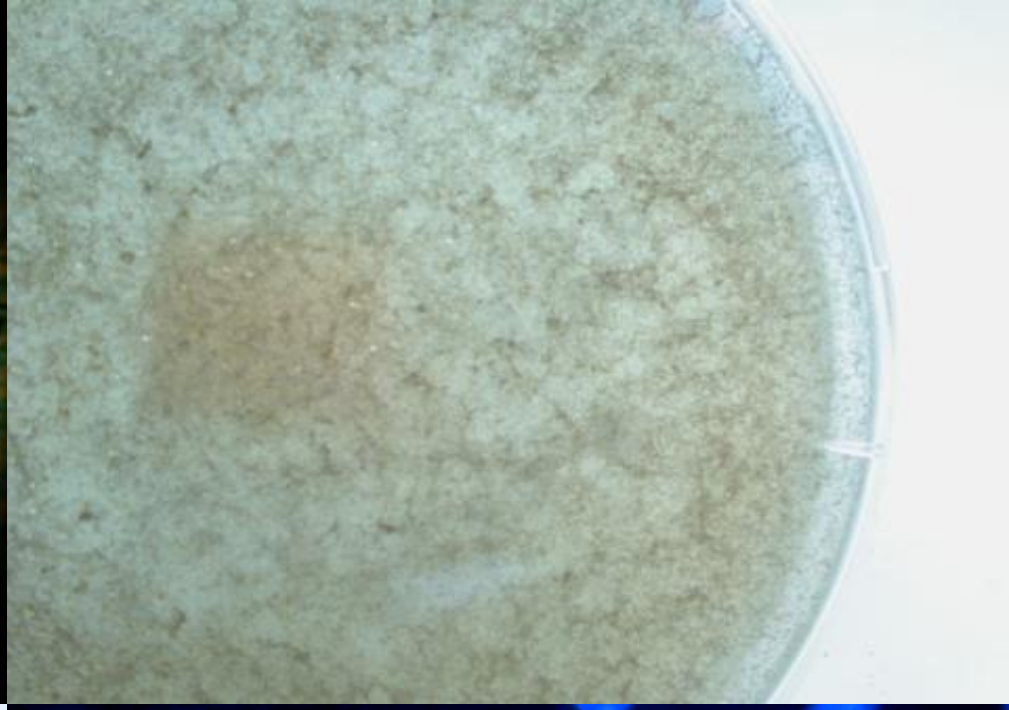
14 Jan 2009

Courtesy of Dr. Patrick Chung, Dept of Surgery, QMH

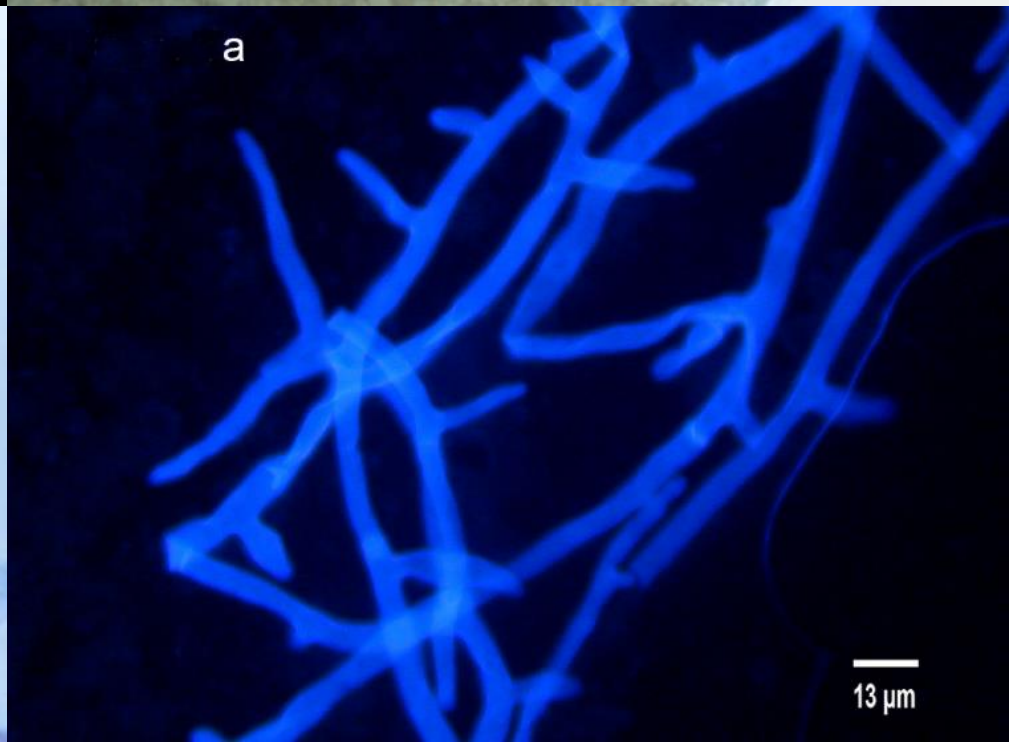
Case (type)[a]	Sex/age (ward)[b]	Hemic malignancy (chemotherapy)[c]	Symptoms and key blood tests[d]	Abdominal CT and laparotomy findings[e]	Histopathological findings[f]	Antifungal regimen and clinical outcome[g]
1 (I)	M/6 (C6)	ALL (V, D, Met, Leu, and Dexa)	Abdominal pain; ANC: 0.34; ALT: 13; Cr: 44	CT: distended bowel loops at central abdominal region, swollen R psoas muscle with abscess formation; moderate R hydronephrosis; laparotomy: necrotic small, large bowel, R posas and paraspinal muscles	Extensive involvement of all the tissues by fungal elements with pronounced angiotrophism with thrombosis, invasion of the blood vessel wall by fungal hyphae, and extensive full-thickness infarction of the bowel walls	Posa, AmB, Caspo 3 days and Des 27 days after symptom onset; died 36 days after symptom onset
2 (I)	M/11 (K8N)	AML (Met, Ida, Cyt and E)	RLQ pain; ANC: 0.05; ALT: 9; Cr: 48	CT: swollen appendix measuring up to 2.5 cm with thickened wall of 7 mm; laparotomy: infarction and perforation of paracecal appendix and adjacent omentum	Transmural infarction of the appendix with an abundant amt of fungal organisms suggestive of *Mucorales* by their branching at right angles and invasion of blood vessel walls	Posa, AmB, Caspo, Des 1 day after symptom onset; fever and abdominal pain resolved 16 days after therapy
3 (I)!	M/57 (E3)	DLBCL (V, D, Met, A, and Dexa)	Abdominal pain; ANC: 0.26; ALT: 152; Cr: 126	CT: intraperitoneal gas suggestive of perforated bowel; laparotomy: two perforations at cecum with indurated edge	Mucosal infiltration and necrosis due to fungal element	Fluconazole; died 8 days after symptom onset
4 (M)	M/35 (J8N)	Fanconi's anemia/MDS (TBI, ATG, F, and Cyc)	Diarrhea; ANC: 0.48; ALT: 57; Cr: 67	CT: segmental thickening of proximal jejunal wall	NA	Posa, AmB, anidulafungin 1 day after symptom onset; died 44 days after symptom onset due to complications of BMT
5 (I)	M/38 (K20N)	ALL (Clo)	RLQ pain; ANC: 0.03; ALT: 833; Cr: 94	CT: bowel wall thickening of the terminal ileum, cecum, and proximal ascending colon; laparotomy: swollen cecum and terminal ileum; acute gangrenous appendicitis	Extensive invasion of the terminal ileum, cecum, appendix, and omentum by fungal elements composed of aseptate hyphae; fungi stain with Grocott silver stain and Periodic acid-Schiff-digested stain; morphologically consistent with *Mucorales*	Posa, AmB, Caspo 3 days after symptom onset; died 28 days after symptom onset
6 (C)	M/38 (J8N)	AML (B and Cyc)	Asymptomatic; ANC: 6.54; ALT: 37; Cr: 68	CT: no abnormal bowel dilatation or bowel wall thickening	NA	Posa 5 days after isolation of *Rhizopus sp.* in stool; stable
7 (C)	M/50 (J8N)	Precursor T-ALL (TBI + Cyc)	Asymptomatic; ANC: 2.73; ALT: 60 Cr: 67	CT: no abnormal bowel dilatation or bowel wall thickening	NA	Posa 2 days after isolation of *Rhizopus sp.* in stool; stable
8 (I)!	F/42 (K20N)	NK cell lymphoma (A)	Abdominal distention; ANC: 3.72; ALT 12; Cr 79	CT: moderate amt of ascites, thickened peritoneal surface with omental cake suggestive of lymphomatous infiltration; laparotomy: thinning of cecal wall without perforation; inflamed omentum with adhesion to pelvic cavity	Extensive involvement of small intestine, stomach, urinary bladder, omentum, mesentery, large intestine, kidney, lung, liver, spleen, and pancreas with branching aseptate fungal elements	Voriconazole; died 23 days after symptom onset; *Rhizopus sp.* isolated in peritoneal fluid 1 day after patient succumbed
9 (C)	M/73 (K20N)	DLBCL (Rit, Cyc, Epi, V, P)	Asymptomatic	CT: no abnormal bowel dilatation or bowel wall thickening	NA	No antifungal agent given; stable
10 (C)	M/66 (J8N)	Mantle cell lymphoma (Cyc, Car, E)	Asymptomatic	Not done	NA	Posa 8 days after isolation of *Rhizopus sp.* in stool; stable
11 (C)	F/55 (J8N)	B-cell lymphoma (Cyc, Car, E)	Asymptomatic	Not done	NA	Posa 2 days after isolation of *Rhizopus sp.* in stool; stable
12 (M)	F/59 (K20N)	AML (Cyt, D)	Diarrhea	CT: mural thickening at cecum and terminal ileum	NA	Posa, AmB, Caspo 1 day after symptom onset; stable

Cheng VC et al., J Clin Microbiol. 2009;47:2834-43.





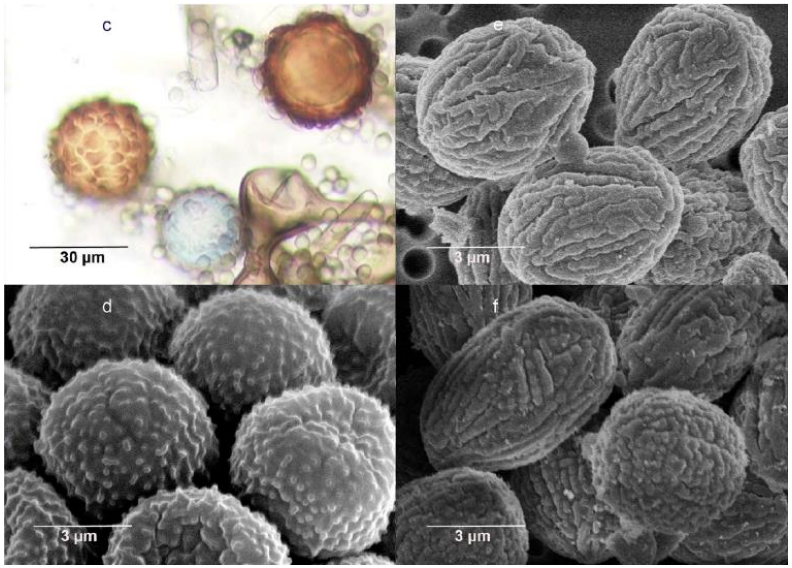

100 µm


a
13 µm

Outbreak of intestinal mucormycosis in hematology/BMT patients due to *Rhizopus microsporus* (contaminated allopurinol tablets)

TABLE 3. Screening of food and drug samples for *Rhizopus microsporus* group

Item (n^a)	Description of sample (total no. and site of collection)[b]
Fresh fruits (2)	Apple (1 from kitchen)
	Orange (1 from kitchen)
Ready-to-eat food (179)	Additives (14 packs from kitchen),
	Biscuits (70 packs from kitchen and 12 packs from convenience store)*
	Cakes (5 from kitchen and 12 from convenience store)
	Cereals (3 packs from kitchen)
	Macaroni (1 pack from kitchen)
	Raisins (1 pack from kitchen)
	Sandwiches (3 from kitchen and 38 from convenience store)*
	Juice (2 bottles from kitchen and 1 bottle from convenience store)
	Milk (15 bottles from kitchen and 2 bottles from convenience store)
Drugs (150)	Acyclovir tablets (3 vials from wards, 14 vials from pharmacy)
	Allopurinol tablets (5 vials from wards, 11 vials from pharmacy)*
	Bactidol mouthwash (11 bottles from wards)
	Chinese herbal medicine (6 bottles from patients)
	Ciprofloxacin tablets (5 vials from pharmacy)
	Cotrimoxazole tablets (4 vials from wards, 6 vials from pharmacy)
	Dexamethasone tablets (6 vials from pharmacy)
	Esomeprazole (3 vials from wards, 21 vials from pharmacy)
	Famotidine tablets (1 vial from ward, 8 vials from pharmacy)
	Gastrocaine (1 vial from ward)
	Itraconazole tablets (1 vial from ward, 5 vials from pharmacy)
	Itraconazole syrup (1 vial from ward, 1 vial from pharmacy)
	Lactulose (1 vial from ward)
	Paracetamol (3 vials from wards, 11 vials from pharmacy)
	Phenytoin (2 vials from wards, 11 vials from pharmacy)
	Potassium syrup (2 vials from wards)
	Prednisolone (2 vials from wards, 5 vials from pharmacy)



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Laboratory Diagnosis of Invasive Fungal Infection in Transplant Recipients: Theory & Practice

Jasper F. W. Chan
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The University of Hong Kong

Workshop on infections in transplant recipients: prevention, control and management
Centre for Health Protection / Hospital Authority
4 February 2015