DIAGNOSTIC STRATEGIES

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UNIVERSITY HOSPITALS LEUVEN

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EDITORIAL

Tackling Human Fungal Infections

FUNGI INFECT BILLIONS OF PEOPLE EVERY YEAR, YET THEIR CONTRIBUTION TO THE GLOBAL BURDEN of disease is largely unrecognized.

- Over 600 different fungi have been reported to infect humans
- Three issues require immediate attention
  - **Robust, rapid, simple, and cheap diagnostics are needed**
  - Safer and more effective antifungal drugs are needed
  - Fungal vaccines must be developed

Gordon D. Brown , David W. Denning , Stuart M. Levitz

www.sciencemag.org SCIENCE VOL 336 11 MAY 2012

Diagnostic tools in the lab

- Direct examination
- Culture-based methods
- Antigen detection
- PCR-based methods
- Susceptibility testing
- Therapeutic drug monitoring

Question

In a critically ill COPD patient with pulmonary infiltrates not responding to broad-spectrum antibiotics, a BAL is performed. I will request following tests for the diagnosis of a fungal infection:

1. Direct examination and fungal culture on BAL
2. Direct examination and fungal culture on BAL **AND** serum GM
3. Direct examination and fungal culture on BAL **AND** BAL GM
4. Direct examination and fungal culture on BAL **AND** serum 1-3-β-D-glucan (with or without serum or BAL GM)
5. Direct examination and fungal culture on BAL **AND** fungal PCR (with or without serum or BAL GM or serum 1-3-β-D-glucan)

Direct examination

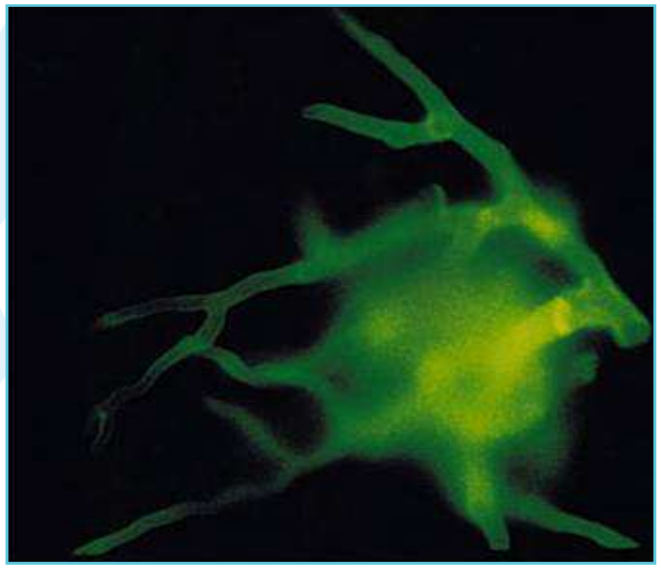
- Important!
  - Rapid, sufficient identification to guide management
  - ± 20% more diagnostic yield than culture for IA
- Obtaining optimal specimens is mandatory
  - Sensitivity of sputum samples is increased with increasing number of samples
  - BAL more representative sample from LRT
  - Presence of *Candida spp*. in BAL does not correlate with invasive lung infection

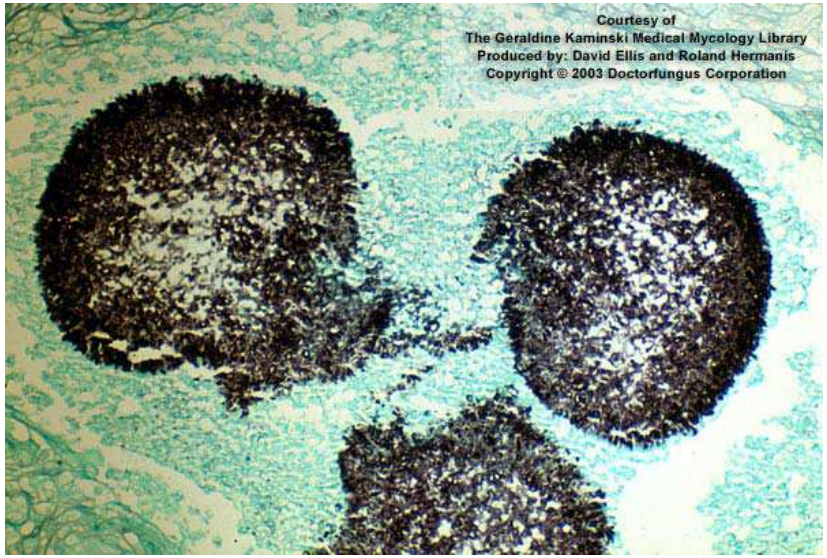
Levy H et al, Respir Med 1992; 86:243-248.
Reichenberger F et al, Bone Marrow Transplant, 1999; 24: 1195-1199.
Meersseman W et al, Am J Respir Crit Care Med 2008: 177: 27-34.

Direct examination

– Tissue

- Defines proven invasive fungal disease
- Should be taken whenever possible
- Sufficient material for **histological examination** (relation fungal elements to tissue structures) **and rapid direct microscopy** (less sensitive, optical brighteners recommended)

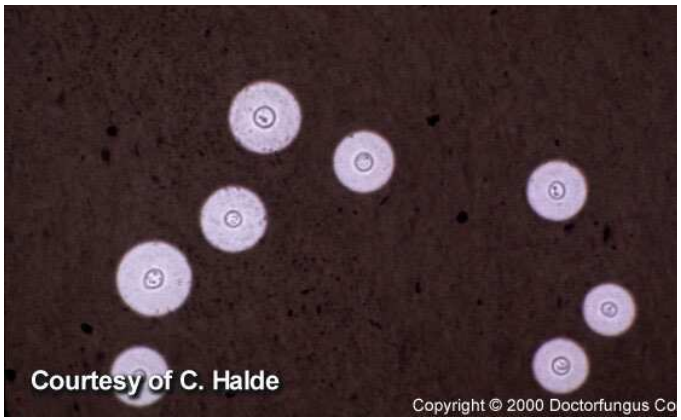




Arendrup MC et al., Bone Marrow Transplant 2012, 47: 1030-1045.

Direct examination

- CSF
  - Cryptococcal meningitis: sensitivity India ink ± 60% (higher in HIV positive patients), antigen test should be used!
  - Candida meningitis: Gram stain ± 40% sensitivity

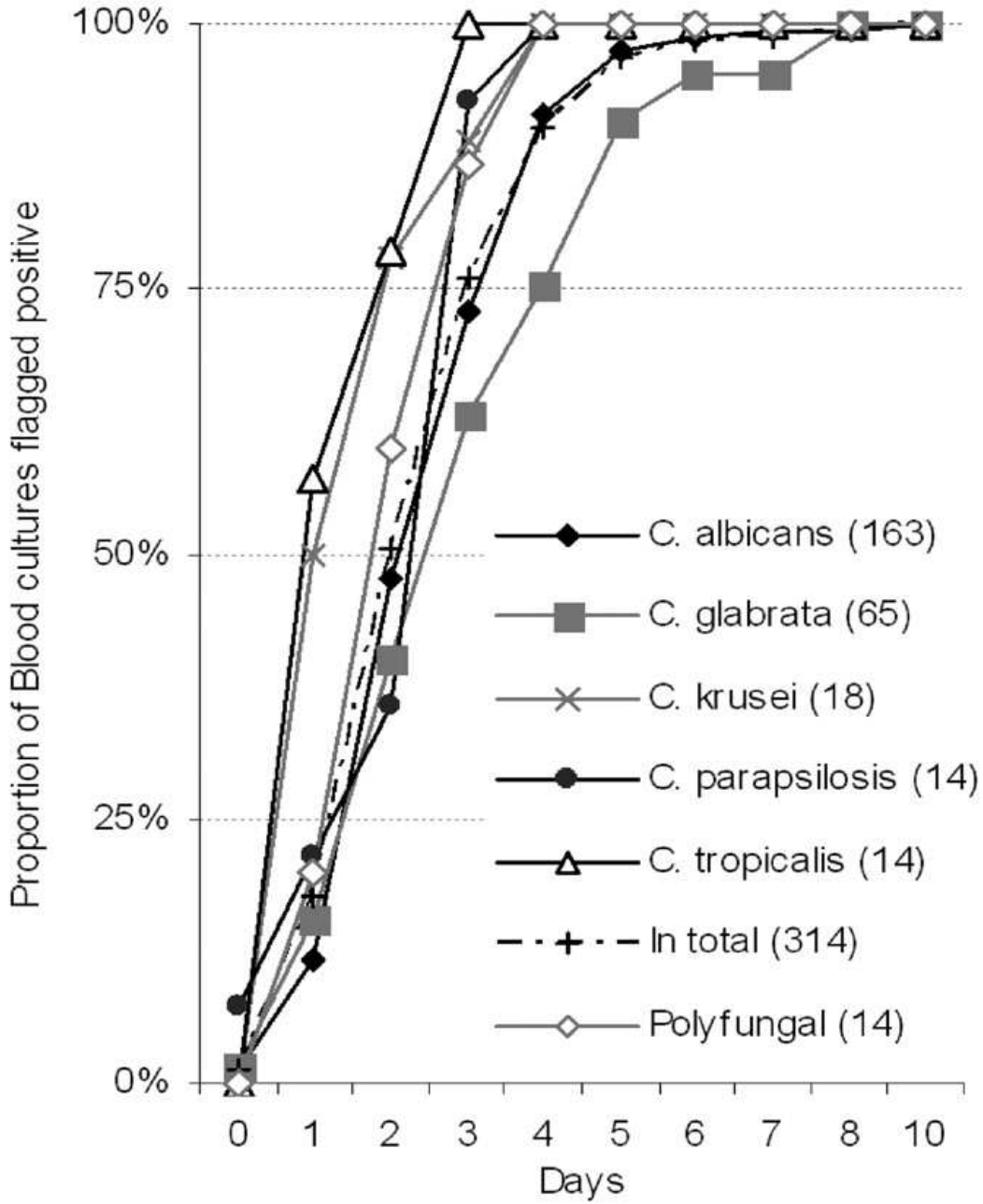


Antinori S et al, J Clin Microbiol 2005, 43: 5828-5829.
Sanches-Portocarrero J et al, Diagn Microbiol Infect dis 2000; 37: 169-179.

Culture- based methods

- Important for species identification and susceptibility testing
- Blood culture: *Candida spp*, *Fusarium spp*, *Scedosporium spp*
- CSF
  - Highly sensitive for cryptococcal meningitis (98%), marker of response to treatment ($\leftrightarrow$ Ag test)
  - Sensitivity lower for CNS aspergillosis or candidiasis (parenchyma rather than meningeal involvement)
- Biopsies: homogenization reduces the culture yield of Mucorales
- Sensitivity BAL culture for IA no more than 50%

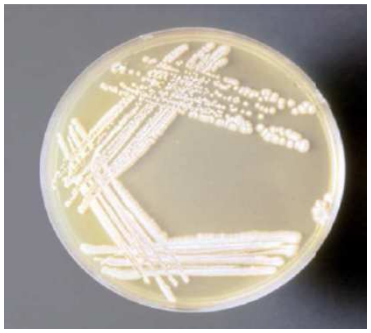
Time to blood culture positivity overall and by species



MC Arendrup et al, J Clin Microb 2011, 49: 3300-3308

Identification

YEASTS



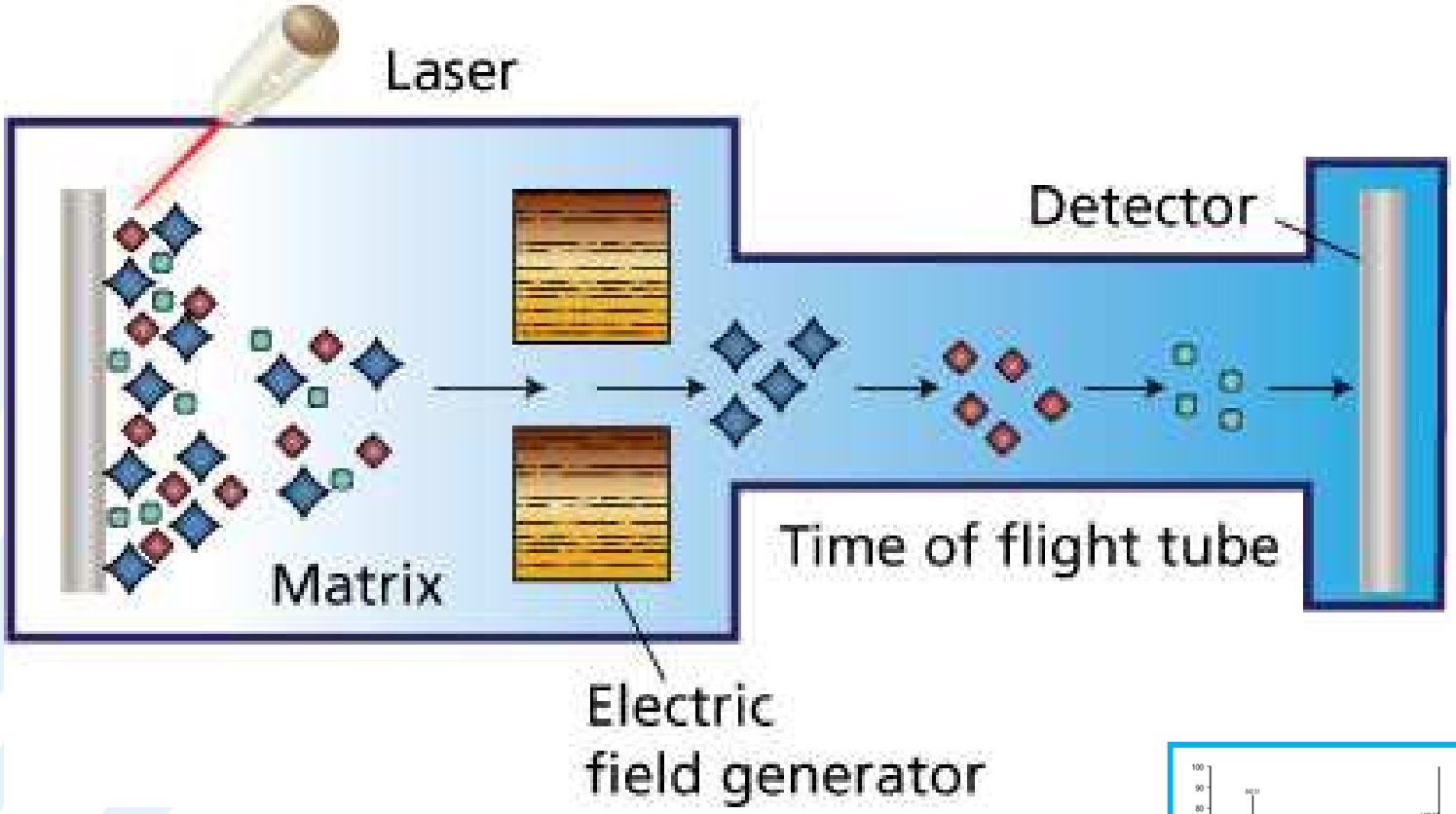
- Chromogenic agar
- Biochemical tests
- Fluorescence in-situ hybridization test (PNA-FISH)
- Maldi-TOF MS
- PCR based methods

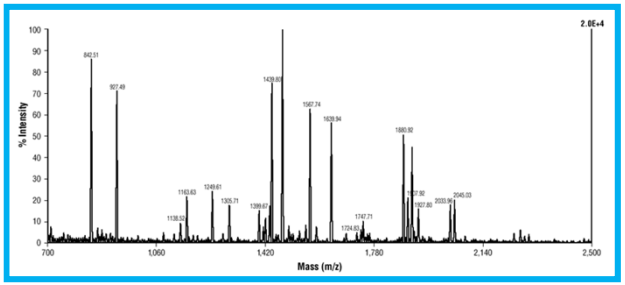
FILAMENTOUS FUNGI

- Microscopy
- PCR based methods
- Maldi-TOF MS

MALDI-TOF MS

Matrix-assisted laser desorption/ionization (MALDI)-Time of flight (TOF) Mass Spectrometry





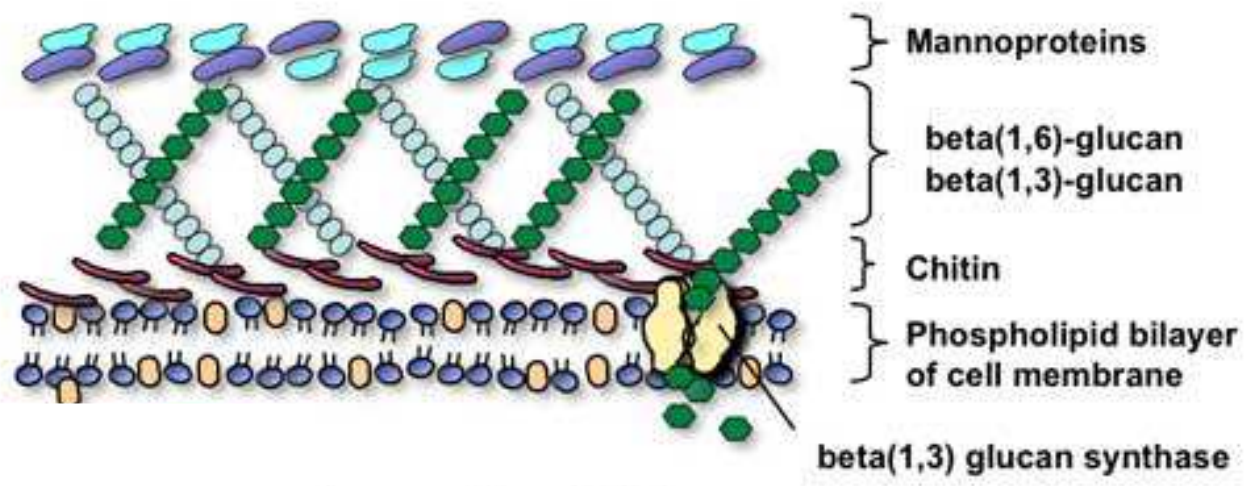
E. Vermeulen et al. Curr Fungal Infect Rep 2012, Epub ahead of print

Sequence-based identification: many new species

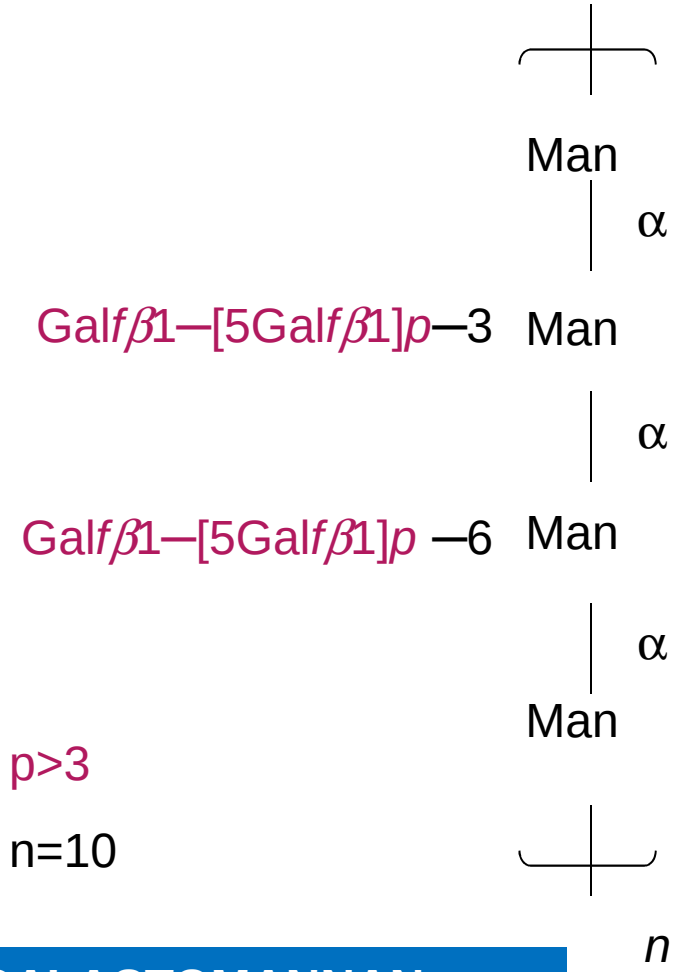
Species	Pathogenicity	Susceptibility
A. lentulus	Reported as causative pathogen in invasive aspergillosis Case reports of colonization in cystic fibrosis	- Reduced susceptibility to azole drugs - Reduced susceptibility to amphotericin B - Variable susceptibility to caspofungin.
A. udagawae	Reported as causative pathogen in invasive aspergillosis	- Reduced susceptibility to voriconazole - Reduced susceptibility to amphotericin B
N. pseudofisheri	Reported as causative pathogen in invasive aspergillosis Case reports of colonization in cystic fibrosis	- Reduced susceptibility to azole drugs - Variable susceptibility to amphotericin B
A. fumigatiaffinis	No clinical cases reported.	- Reduced susceptibility to azole drugs - Reduced susceptibility to amphotericin B
A. viridinutans	Reported as causative pathogen in chronic invasive aspergillosis, with unusual clinical features	- Reduced susceptibility to azole drugs - Reduced susceptibility to amphotericin B

Antigen detection

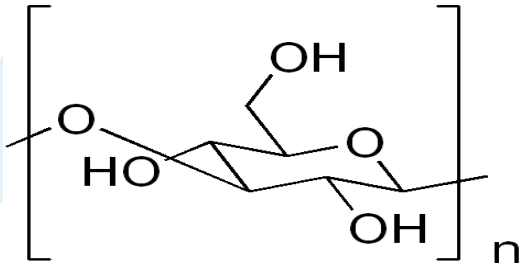
CAPSULE



CAPSULAR POLYSACCHARIDE ANTIGEN



CELL WALL



1,3-D-GLUCAN

GALACTOMANNAN

MANNAN

GALACTOMANNAN

- ***Aspergillus* spp.**(cross reactivity *Penicillium*, *Paecilomyces*, *Cryptococcus, Histoplasma*)
- Commercial kit: Platelia® Sandwich ELISA (BioRad)
- User friendly
  - Endpoint measurement
  - Samples not in duplicate

1-3-β-D-GLUCAN

- ***'Panfungal'***: *Aspergillus*, *Candida*, *Fusarium*, *Trichosporon*, *Pneumocystis jirovecii*, *Acremonium*, *Saccharomyces cerevisiae*), **NO detection of Mucorales and *Cryptococcus***
- Several commercial kits available (different cut-off values) : Fungitell™ (Glucatell ™) in US and Europe
- Not really user-friendly
  - Kinetic measurement
  - 1 NC and 5 ST in duplicate per run
  - Each sample in duplicate

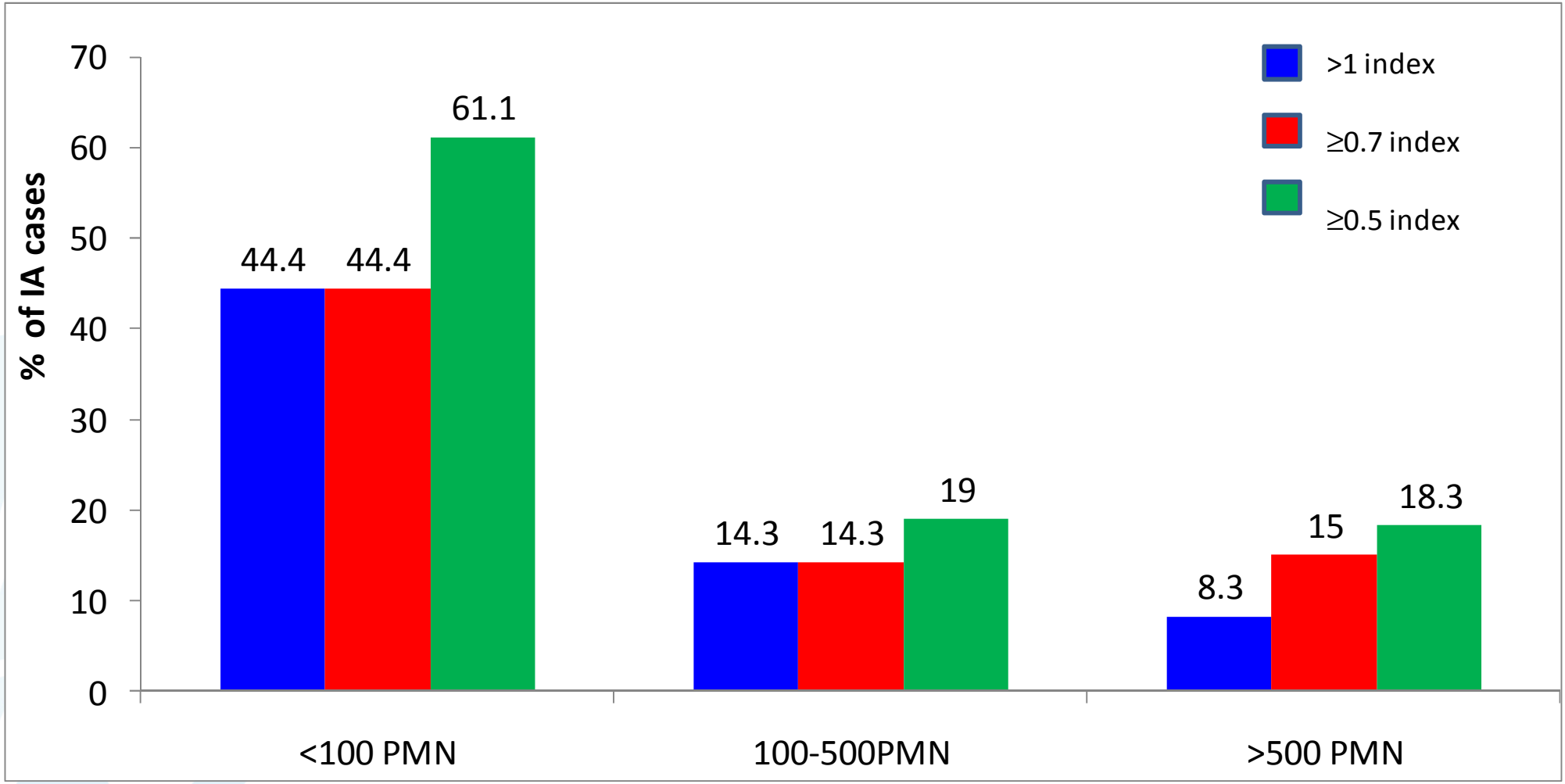
GALACTOMANNAN

1-3-β-D-GLUCAN

CAUSES FOR FALSE POSITIVITY

GALACTOMANNAN	1-3-β-D-GLUCAN
β-lactam antibiotics	β-lactam antibiotics
Galactomannan containing food	Hemodialysis/hemofiltration
Blood product conditioning fluids	Blood components: immunoglobulin or albumin preparations
Gluconate (Plasma-Lyte)	Surgical gauzes
	Bacterial infections
	Mucositis *(Candida colonization of the gut*)

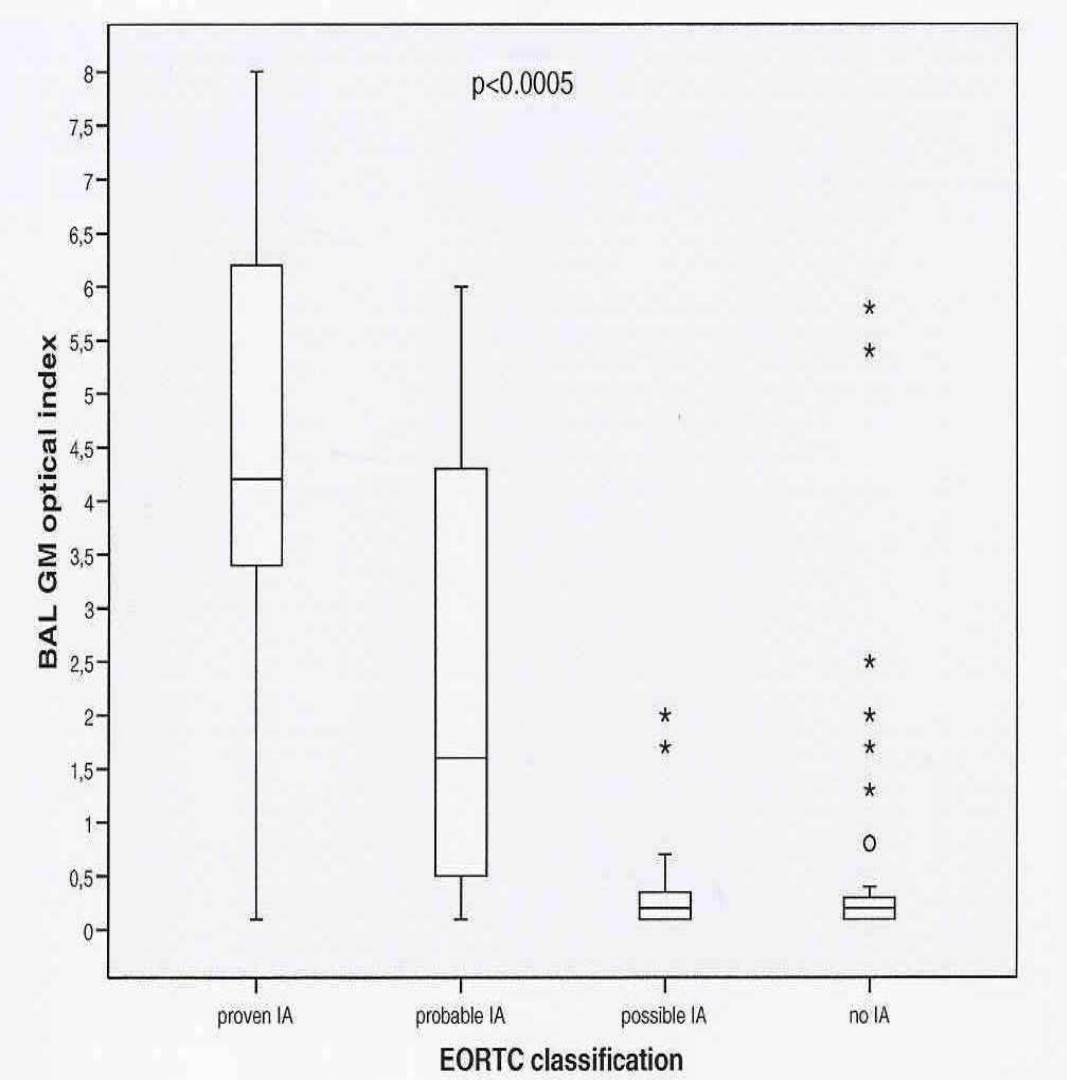
Serum galactomannan assay: good sensitivity in neutropenic patients only!



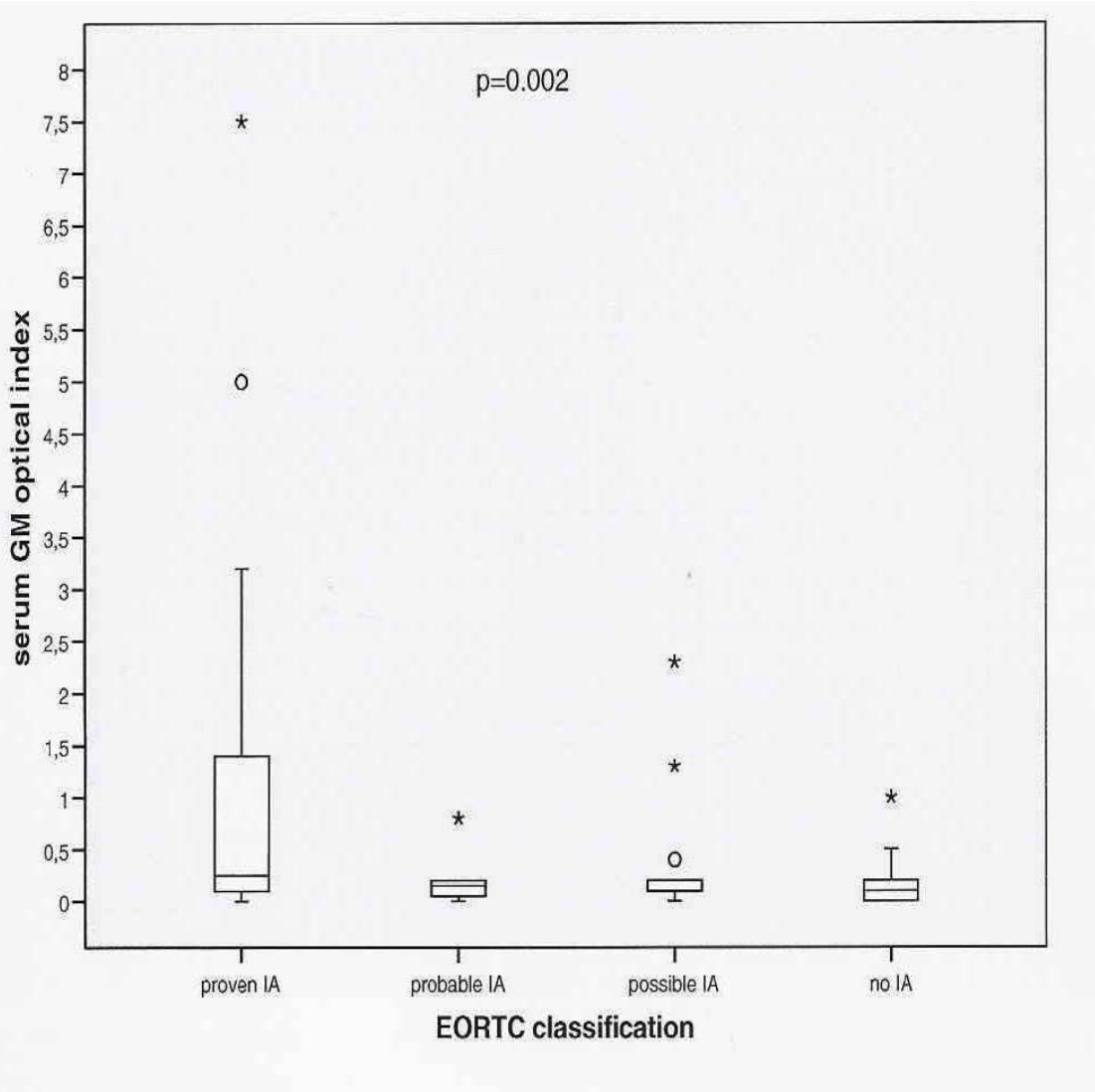
Cordonnier et al, CMI 2008, 15: 81-86.

BAL galactomannan assay: no influence of neutropenia in ICU patients

BAL



SERUM



Meersseman et al., Am J Respir Crit Care Med 2008, 177: 27-34.

BAL galactomannan assay: no influence of neutropenia in ICU patients

BAL culture/microscopy and serum GM remained negative in 11/26 (42%) proven cases

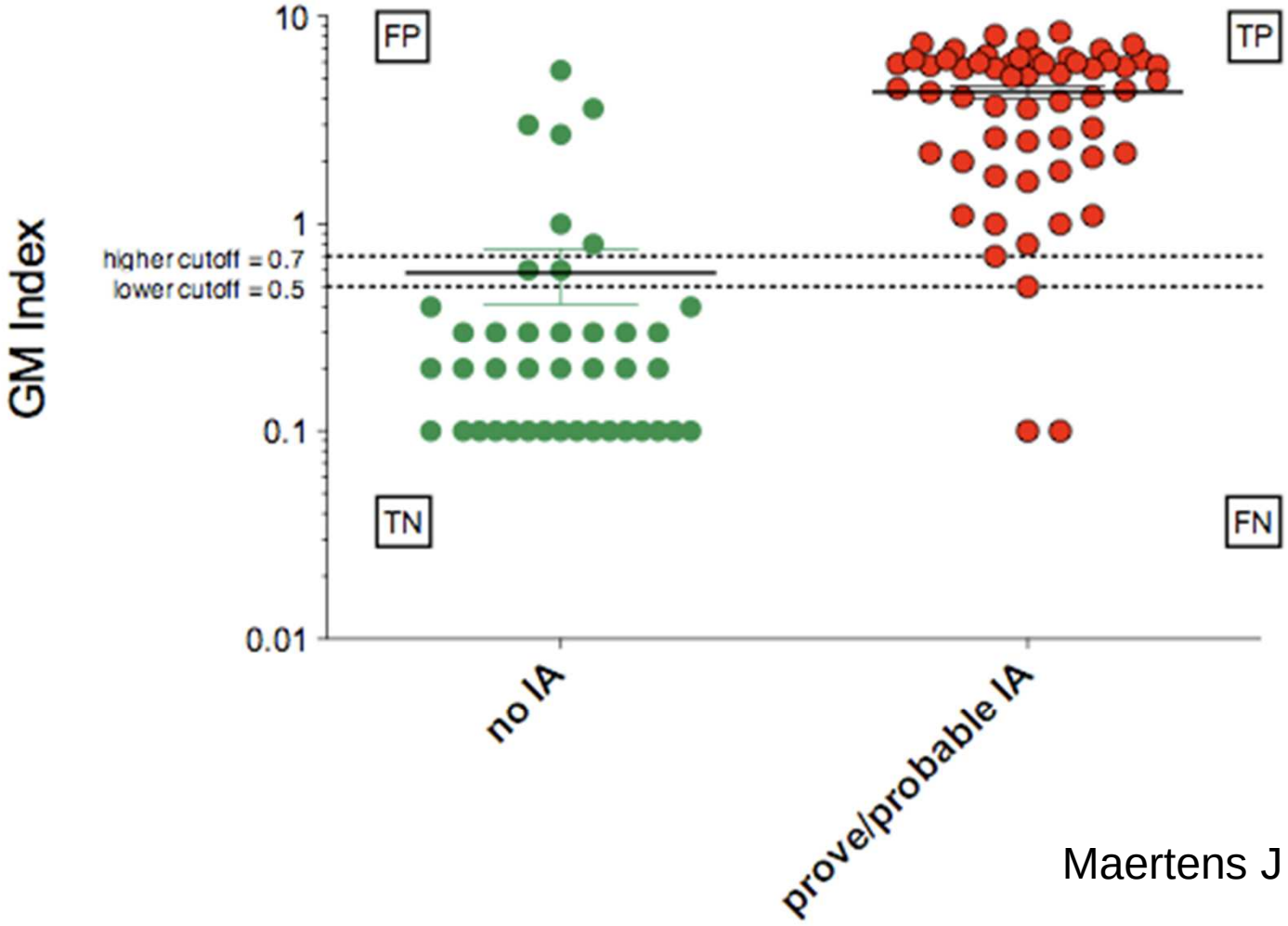
Sensitivity of GM detection on BAL = 100% (≥ 0.5) in proven cases if second BAL is taken into account

GM detection on BAL performed equally well in neutropenic and non-neutropenic patients

58% of proven cases had a positive culture and/or direct examination

Meersseman et al., Am J Respir Crit Care Med 2008, 177: 27-34.

BAL galactomannan assay: no influence of neutropenia in hematological patients



Jan 2005 – Sept 2008
58 proven/probable IA
41 controls

Maertens J et al., CID, 2009, 49: 1688-1693.

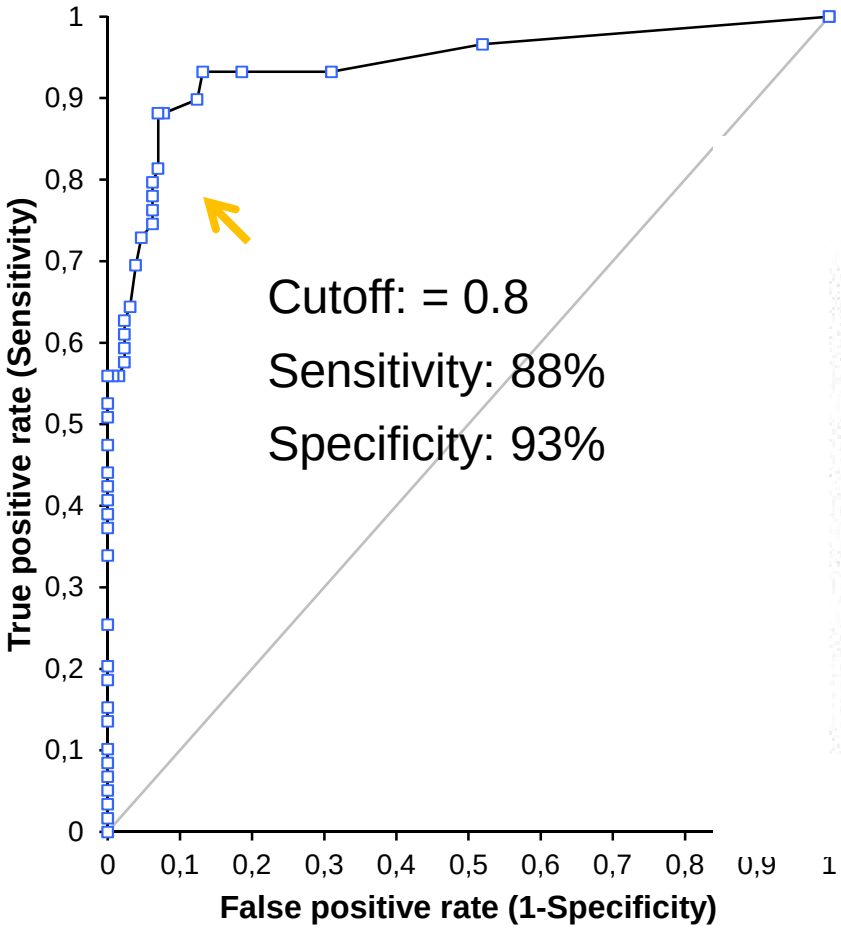
	Neutropenic	non-Neutropenic	P-value
BAL, 1.0	100%	94.7%	0.99
Serum, 0.5	90%	36.8%	0.008

Question

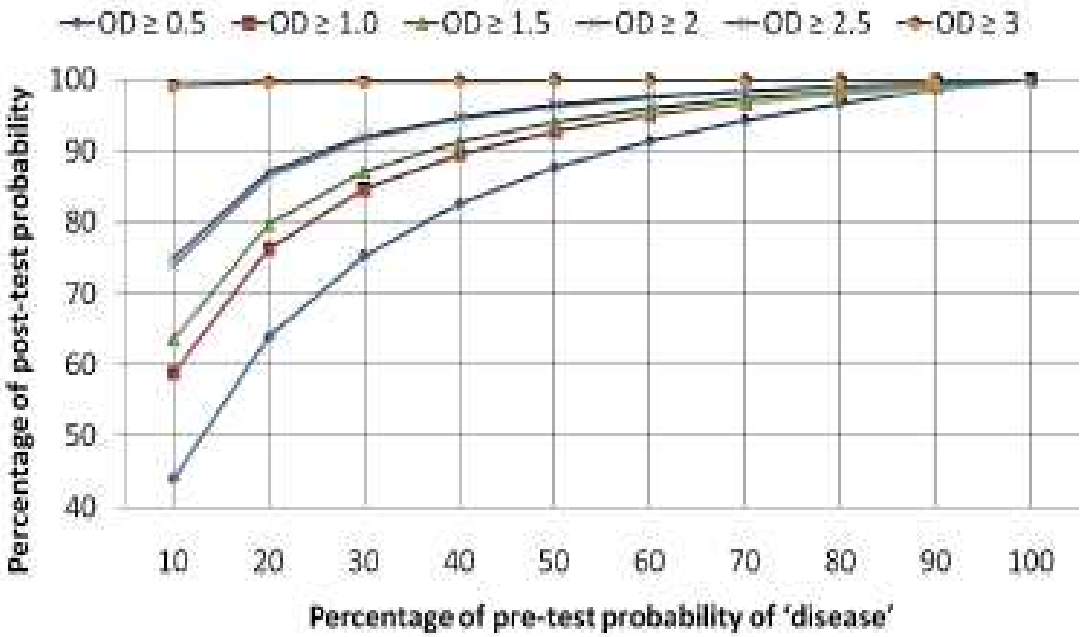
In a critically ill COPD patient with pulmonary infiltrates not responding to broad-spectrum antibiotics and a BAL galactomannan OD index of 4.5, the probability of invasive aspergillosis is:

1. 0-20%
2. 20-40%
3. 40-60%
4. 60-80%
5. 80-100%

Performance of BAL GM testing: mixed population of at-risk patients



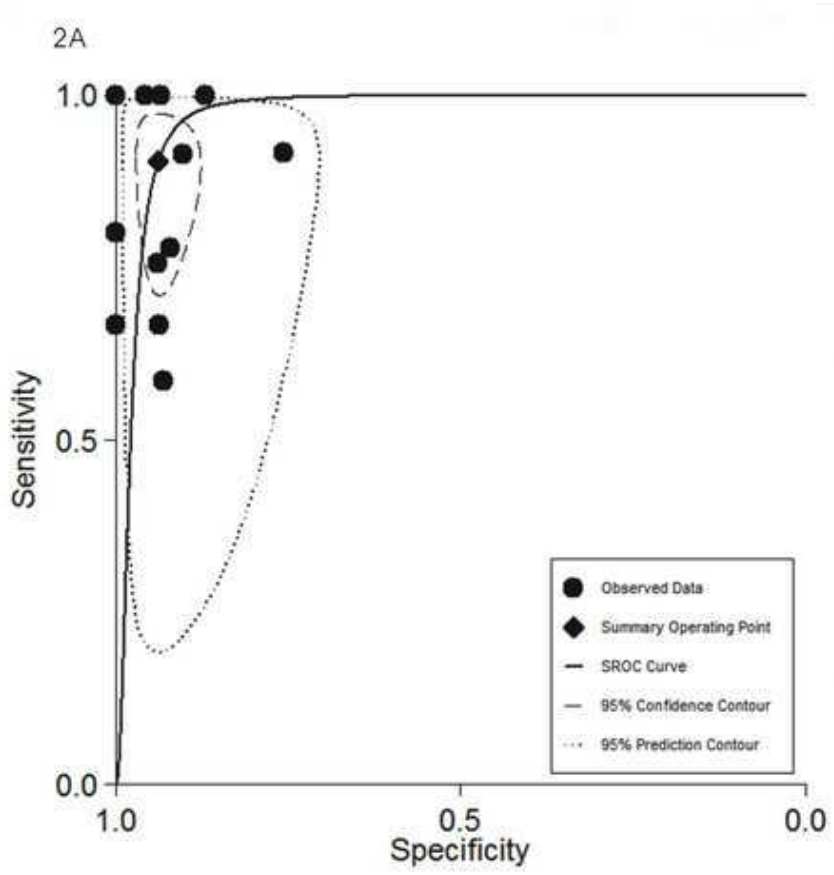
59 proven and probable cases



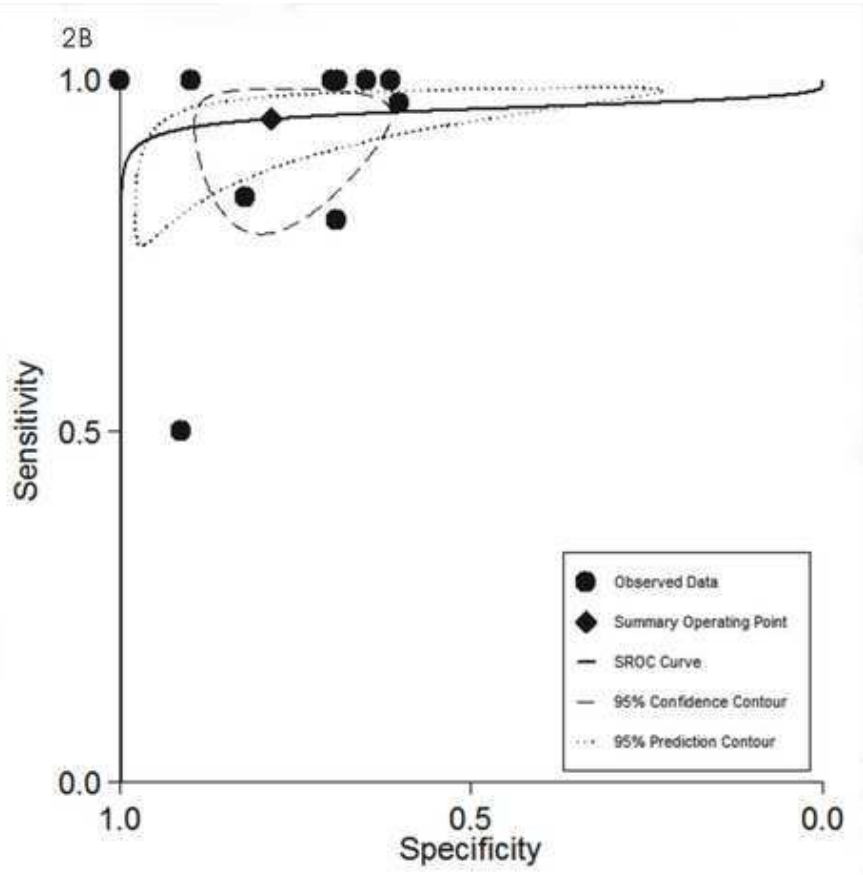
J. D'Haese et al., JCM, 2012, 50: 1258-1259.

BAL galactomannan: meta-analysis

Proven and Probable



Proven



13 studies (proven and probable):

Pooled sensitivity = 90% (79%- 96%)
Pooled specificity = 94% (90%- 96%)

Ya-Ling Guo et al., Chest, May 7, 2010

Interaction piperacilin tazobactam: is the story over?

J Antimicrob Chemother. 2012 Jul;67(7):1746-8. Epub 2012 Apr 11.

Piperacillin/tazobactam (Tazocin™) seems to be no longer responsible for false-positive results of the galactomannan assay.

Mikulska M, Furfaro E, Del Bono V, Raiola AM, Ratto S, Bacigalupo A, Viscoli C.

Division of Infectious Diseases, San Martino University Hospital and University of Genoa, Genoa, Italy. m.mikulska@unige.it

Abstract

OBJECTIVES: Galactomannan (GM) testing is extremely useful for diagnosing invasive aspergillosis in high-risk patients, but false-positive results have been reported in patients treated with piperacillin/tazobactam. The aims of this study are to test if the recent piperacillin/tazobactam (Tazocin™; Pfizer) preparation still contains GM, and if serum GM positivity in haematopoietic stem cell transplant (HSCT) recipients receiving piperacillin/tazobactam can be attributed to this treatment.

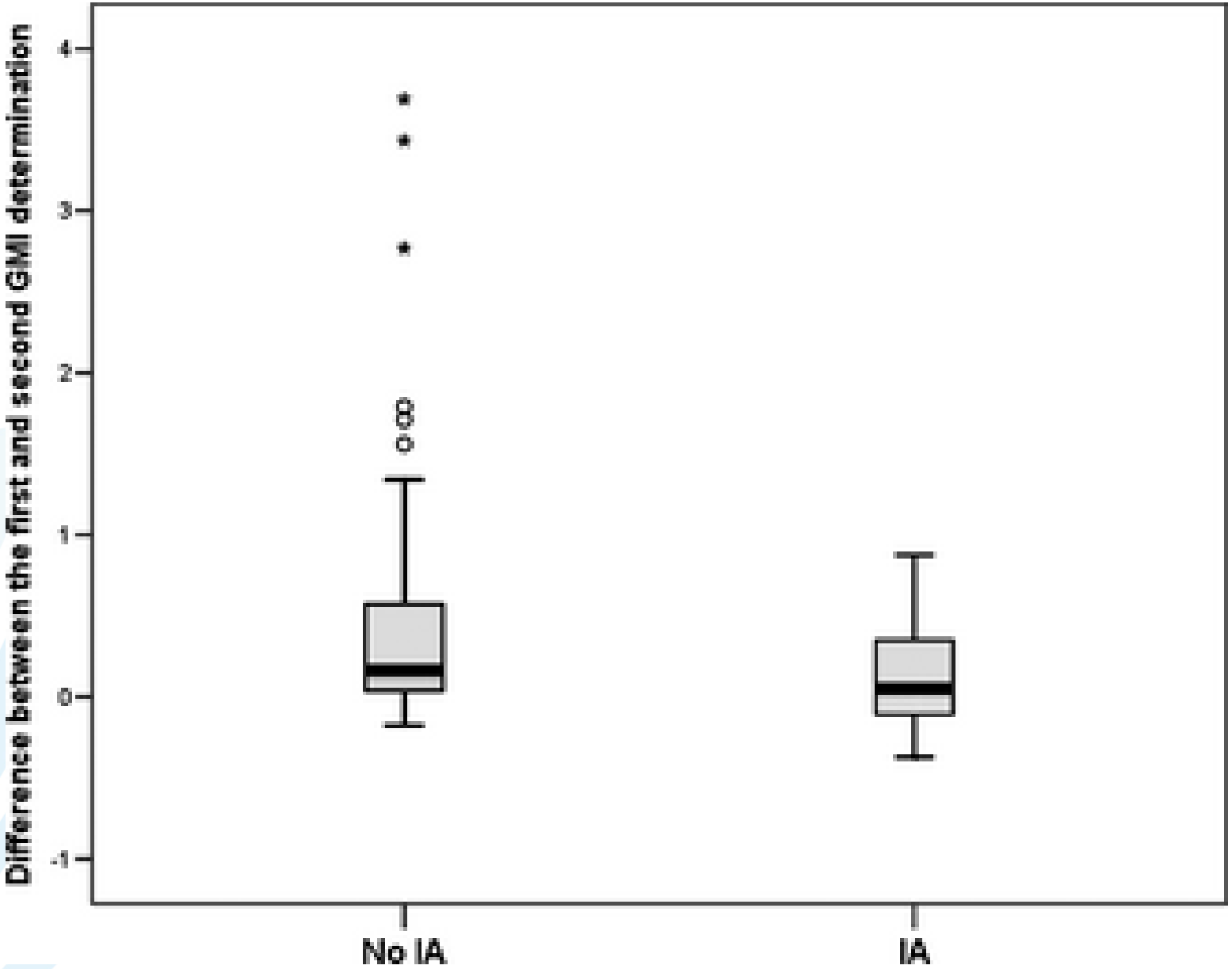
PATIENTS AND METHODS: Serum samples obtained from 1 October 2009 to 31 October 2010 from HSCT recipients for GM testing were analysed. The difference in the rate of positive results (defined as GM ≥ 0.5) in patients receiving and not receiving piperacillin/tazobactam was evaluated. Piperacillin/tazobactam vials from randomly selected batches were tested.

RESULTS: Of 1606 samples drawn in the absence of piperacillin/tazobactam therapy, 25 (1.6%) tested positive for GM versus 10 of 394 samples (2.5%) drawn while on piperacillin/tazobactam (P = 0.18). The median GM result of samples drawn on piperacillin/tazobactam was slightly higher than that of samples drawn in the absence of piperacillin/tazobactam (0.141 versus 0.122; P < 0.001). All 90 piperacillin/tazobactam vials from 30 randomly selected batches tested negative for GM, with a median GM value of 0.057 (range: 0.011-0.320).

CONCLUSIONS: Although some residual GM might still be present in piperacillin/tazobactam, currently available brand piperacillin/tazobactam preparations seem no longer responsible for false-positive GM results.

Since introduction of new brand Tazocin EFTM, Pfizer in 2006?

GM: testing the same sample twice?



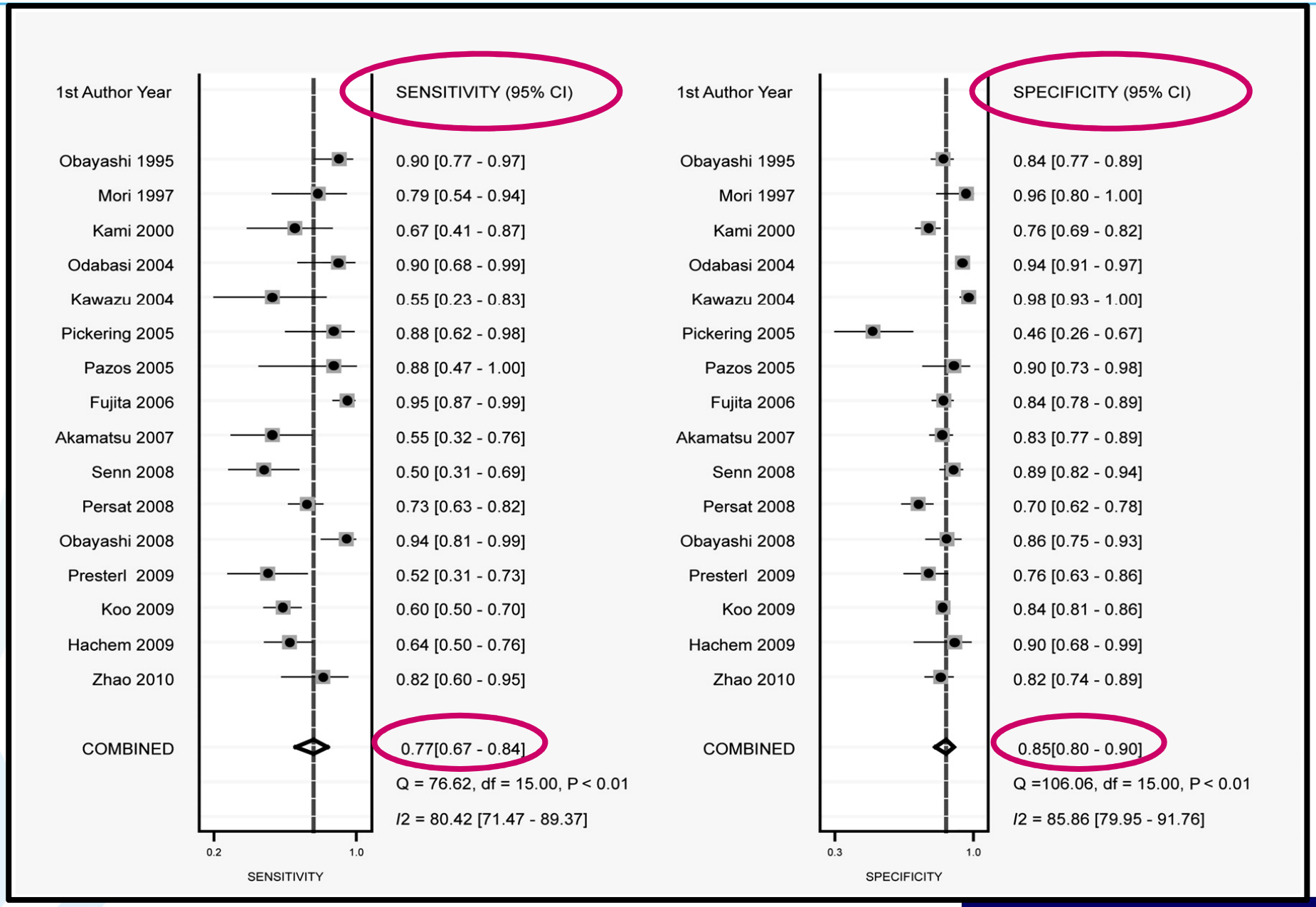
Furfaro E et al., Transpl Infect Dis 2012, Apr 8

Urine GM testing

- Well suited for point-of-care testing
- Novel IgM monoclonal antibody that recognizes GM-like antigens from *Aspergillus* and other molds
- Inhibitor removal and concentration needed
- Proof-of-concept: antigenuria shown in guinea pigs and humans

SF Dufresne et al., PLOS One, 2012; e42736

Forest plot of the pooled sensitivity and specificity of measuring serum or plasma (1→3)-β-D-glucan levels for the diagnosis of proven or probable invasive fungal infections



Karageorgopoulos D E et al. Clin Infect Dis. 2011;52:750-770

Clinical Infectious Diseases

(1→3)-β-D-glucan and invasive candidiasis in surgical ICU patients

No. of positive BG samples	Sensitivity and specificity (%)			
	Proven (*n* = 3)		Proven plus probable (*n* = 9)	
	Sensitivity	Specificity	Sensitivity	Specificity
1	100	50	91	57
2	100	59	66	73
≥3	100	67	63	73

- Samples collected twice weekly from 57 patients
- Cutoff of ≥ 80 pg/mL (Fungitell kit)
- Author defined diagnostic criteria for proven and probable invasive candidiasis
- 25% rate of false positivity during the first 3 ICU days

JF Mohr et al., JCM 2011, 49: 58-61.

(1→3)-β-D-glucan and invasive aspergillosis in medical ICU patients

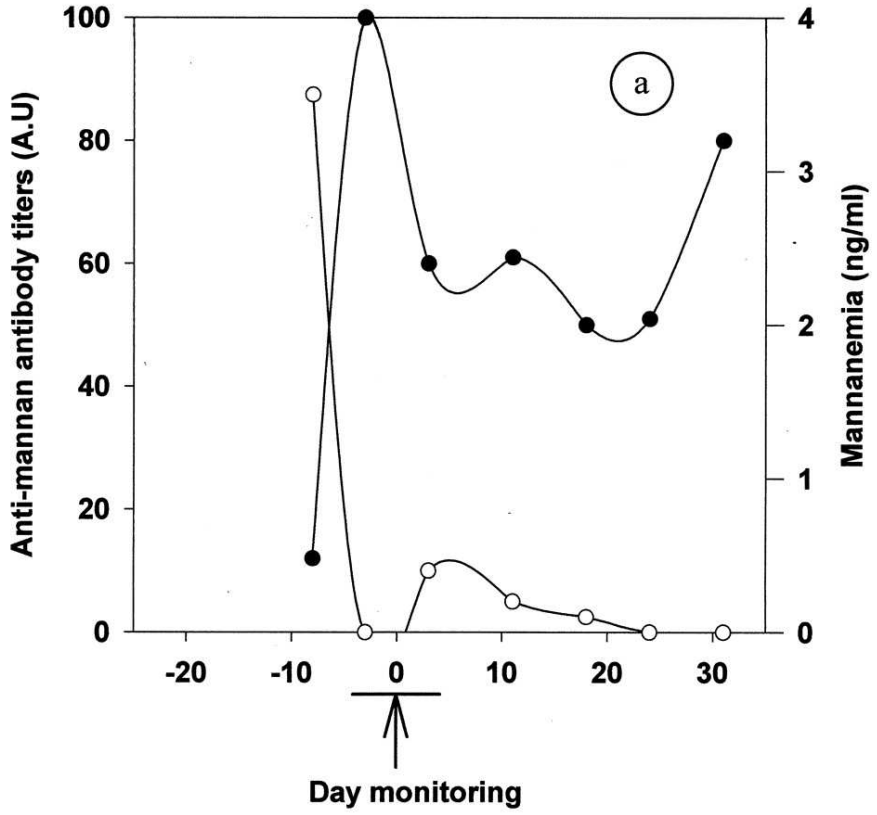
- Only one serum sample per patient (day±1 BAL GM was tested)
- 14 proven IA, 33 without IA
- Best performance at cutoff of 140 pg/mL:
  sensitivity: 85.7%, specificity: 69.7%
  specificity only 36.4% at cutoff of 80 pg/mL
- No difference in BG levels in patients colonized with *Candida*
- BAL GM alone has same sensitivity than combined use of serum BG and serum/BAL GM
- Test properties are insufficient to use the test as a diagnostic tool for IA in this population

G. De Vlieger et al., JCM 2011, 49: 3783-3787.

DIAGNOSIS OF INVASIVE CANDIDIASIS

MANNAN AND ANTI-MANNAN

Mannan: immunodominant surface antigen of the *Candida* cell wall, that is released during infection



-●- anti-mannan
-○- mannan

Mannan detection is inversely correlated to the presence of anti-mannan antibodies

B. Sendid et al., JCM, 1999, 1510-1517

Platelia *Candida* Ag Plus and Platelia *Candida* Ab Plus (BioRad)

Days of neutropenia	Assay	Sn	Sp
< 15	Ag-Plus	7/10 (70%)	2/5 (40%)
	Ab-Plus	3/10 (30%)	3/5 (60%)
	BG	5/9 (55%)	4/5 (80%)
>15	Ag-Plus	6/11 (55%)	11/25 (44%)
	Ab-Plus	7/11 (64%)	23/25 (92%)
	BG	7/11 (64%)	23/25 (92%)

Significant correlation between presence of superficial candidiasis (oropharyngeal and oesophagitis) and positive *Candida* Ag Plus and Ab Plus

No difference in levels of circulating mannan between superficial infected controls and systemic patients.

Verduyn Lunel FM et al, Medical Mycology, 2011, Epub.

PCR-based diagnostics

- Studies focused on *Aspergillus*, *Candida* or panfungal
- Many publications, important technical differences
- Minimal Information for Publication of Quantitative real-time PCR experiments
  (Bustin et al, 2009, Clin Chem; 55:611-622).

Detection of Aspergillus DNA

- European *Aspergillus* PCR Initiative (launched in 2006)

- Main advantage whole blood (↔ serum): not restricted to the detection of freely circulating DNA, most data
- DNA load in many samples close to the detection limit of even highly sensitive PCR protocols
- Efficiency of PCR is limited by the extraction procedure, critical stage for whole blood testing are:
  - ≥ 3 mL whole blood
  - RBC and WBC lysis before fungal lysis (inhibition!)
  - Fungal lysis by bead beating
  - Elution volume < 100 µL

White L. et al, J Clin Microbiol, 2010, 48: 3753-3755

Detection of Aspergillus DNA

- Recommendations for serum testing:
  White P.L. et al., JCM 2011, 49: 3842-3848
- Serum extraction easier, faster and less labor-intensive
- External Quality Control program available (QCMD)
- Also possible on BAL fluid, CSF, tissue
- Optimal specimen not known yet

Detection of *Candida* DNA on whole blood

- Systematic review and meta-analysis
  - 54 studies with 4,694 patients, 963 proven/probable or possible IC
  - 100% sens/spec for candidemia versus healthy controls
  - PCR positivity rates in patients with proven/probable IC 85% versus 38% for blood cultures
  - Attractive method for early diagnosis of *Candida spp*.
  - Effect on clinical outcomes should be investigated

T. Avni et al., J Clin Microbiol 2011;49:665-70.

- Pilot program QCMD for external quality control

Commercial assays

- May improve inter-laboratory reproducibility:
  - SeptiFast kit (Roche Diagnostics)
  - MycXtra® + MycAssay™ (Myconostica)
  - VYOO® (SIRS-Lab, Jena)
  - Prove-it™ Sepsis (Mobidiag)
  - SepsiTest™ (Molzym)
  - …….
- Expensive, technically complex assays,…
- Validation needed in specific clinical settings at high risk of invasive fungal disease.

ECIL recommendations for the use of biological markers

STRONG EVIDENCE	Galactomannan detection in serum (A II) Cryptococcus antigen in serum and CSF (AII)
MODERATE EVIDENCE	(1→3)-β-D-glucan detection in serum (BII) Combined mannan/anti-mannan testing for hepatosplenic candidiasis (BIII) and candidemia (CII)
NO RECOMMENDATIONS	PCR

Need for randomized controlled studies to define clinical utility in guiding patients' management.

Role and cost-effectiveness in providing a more rapid and accurate diagnosis of IFI.

- Early initiation of appropriate antifungal therapy
- Reducing the empirical use of antifungal drugs in patients without IFI

O. Marchetti et al., Bone Marrow Transplantation 2012, 47: 846-854.

Points to consider before and during test implementation…

- Test with high sensitivity:
  a negative result rules out the diagnosis
- Test with high specificity:
  a positive result rules in the diagnosis
- Focus on tests that have an impact on patient management (tests that generate large shifts from pretest to posttest probability)
- Include cost and turn around time into the decision process
- Define clearly the use of the test

Clinical algorithm to diagnose IPA in ICU patients

Proven invasive pulmonary aspergillosis (idem EORTC/MSG criteria)

Putative invasive pulmonary aspergillosis (all four criteria must be met)

1. Aspergillus-positive lower respiratory tract specimen culture (= entry criterion)
2. Compatible signs and symptoms
3. Abnormal medical imaging by portable chest X-ray or CT scan of the lungs
4. Either 4a or 4b

   4a. Host risk factors

   4b. Semiquantitative Aspergillus-positive culture of BAL fluid (+ or ++), without bacterial growth together with a positive cytological smear showing branching hyphae

SI Blot et al., Am J Respir Crit Care Med 2012, 186, 55-64.

Clinical algorithm to diagnose IPA in ICU patients

POSITIVE PREDICTIVE VALUE AND NEGATIVE PREDICTIVE VALUE OF THE CLINICAL ALGORITHM TO DIAGNOSE PROBABLE INVASIVE PULMONARY ASPERGILLOSIS ACCORDING TO ITS ASSUMED PREVALENCE

		Assumed Prevalence of IPA in ICU Patients with *Aspergillus*-Positive Endotracheal Aspirate Cultures (%)			
		20	30	40	50
All histopathology-controlled patients (n = 115)	PPV	37	50	61	70
	NPV	97	95	92	89
Immunocompromised patients* (n = 70)	PPV	27	38	49	59
	NPV	100	100	100	100
Patients with COPD receiving prolonged corticosteroid therapy (n = 30)	PPV	45	59	69	77
	NPV	100	100	100	100
Nonimmunocompromised patients (n = 45)	PPV	44	57	68	76
	NPV	91	85	79	71

SI Blot et al., Am J Respir Crit Care Med 2012, 186, 55-64.

SUSCEPTIBILITY TESTING

Question

For the treatment of a patient with candidemia, I

1. Do not rely on in vitro susceptibility testing
2. Rely on in vitro susceptibility testing
3. Rely on in vitro susceptibility testing (except for *Candida glabrata* and fluconazole)
4. Rely on in vitro susceptibility testing (except for ‘the fluconazole susceptible species’)
5. Have a different approach than 1, 2, 3 or 4

Interpretative breakpoints (CLSI)

Drug Resistance Updates 13 (2010) 180–195

Contents lists available at ScienceDirect

Drug Resistance Updates

journal homepage: www.elsevier.com/locate/drup

Wild-type MIC distributions, epidemiological cutoff values and species-specific clinical breakpoints for fluconazole and *Candida*: Time for harmonization of CLSI and EUCAST broth microdilution methods

M.A. Pfaller[a,*], D. Andes[b], D.J. Diekema[a], A. Espinel-Ingroff[c], D. Sheehan[d], The CLSI Subcommittee for Antifungal Susceptibility Testing

Adjusted clinical breakpoints for should be more sensitive for detecting emerging resistance among **Candida species** and provide consistency with EUCAST clinical breakpoints.

C. albicans, C. parapsilosis, C. tropicalis: S ≤ 2 mg/L; SDD 4 mg/L; R ≥ 8 mg/L
C. glabrata: SDD ≤ 32 mg/L , R ≥ 64 mg/L

Previous interpretative breakpoints for echinocandins (CLSI)

caspofungin, micafungin, anidulafungin

S: MIC ≤ 2 µg/mL
NS: MIC > 2 µg/mL

Of 18 candidiasis cases refractory to echinocandins and with fks mutations, **28%** (caspofungin), **58%** (anidulafungin) and **66%** (micafungin) had MICs in the S category using CBP of ≤2µg/ml

Pfaller M et al., Drug Resist. Updates, 2011, 14:164-76

New interpretative breakpoints for echinocandins (CLSI)

C. albicans, C. tropicalis, C. krusei: S ≤ 0,25 mg/L, R ≥ 1 mg/L

C. parapsilosis: S ≤ 2 mg/L, R ≥ 8 mg/L

C. glabrata: S ≤ 0,12 mg/L, R ≥ 0,5 mg/L (anidulafungin and caspofungin)
S ≤ 0,06 mg/L, R ≥ 0,25 mg/L (micafungin)

Pfaller M et al., Drug Resist. Updates, 2011, 14:164-76

UZ LEUVEN

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

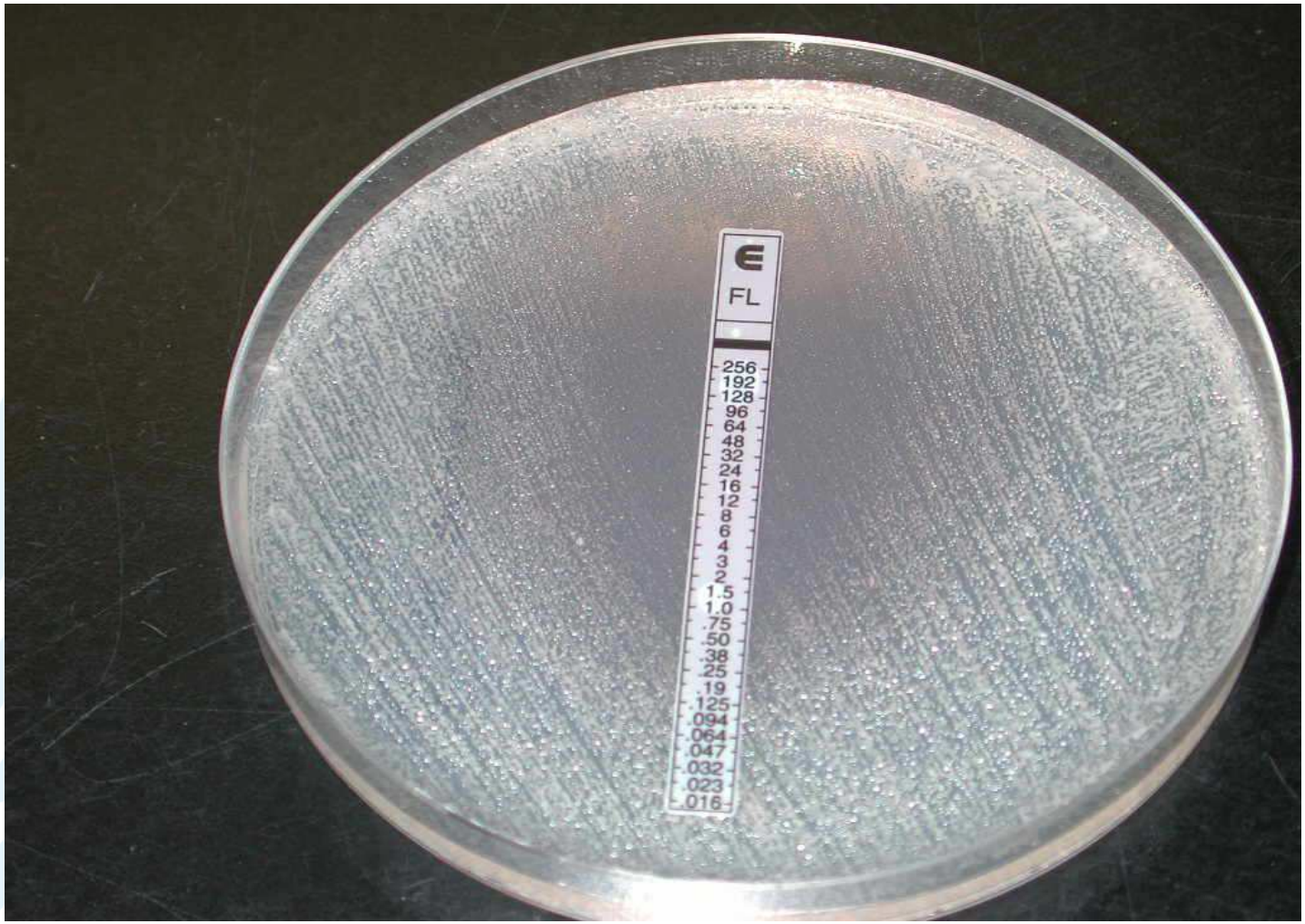
Clinical breakpoints Candida spp. EUCAST

(valid from 2012-03-05)

Antifungal agent	MIC breakpoint (mg/L)							
	C. albicans		C. glabrata		C. parapsilosis		C. tropicalis	
	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >
Amphotericin B	1	1	1	1	1	1	1	1
Anidulafungin	0.03	0.03	0.06	0.06	-	-	0.06	0.06
Fluconazole	2	4	IE	IE	2	4	2	4
Posaconazole	0.06	0.06	IE	IE	0.06	0.06	0.06	0.06
Voriconazole	0.12	0.12	IE	IE	0.12	0.12	0.12	0.12

All information available on the internet: http://www.eucast.org/

Etest


E
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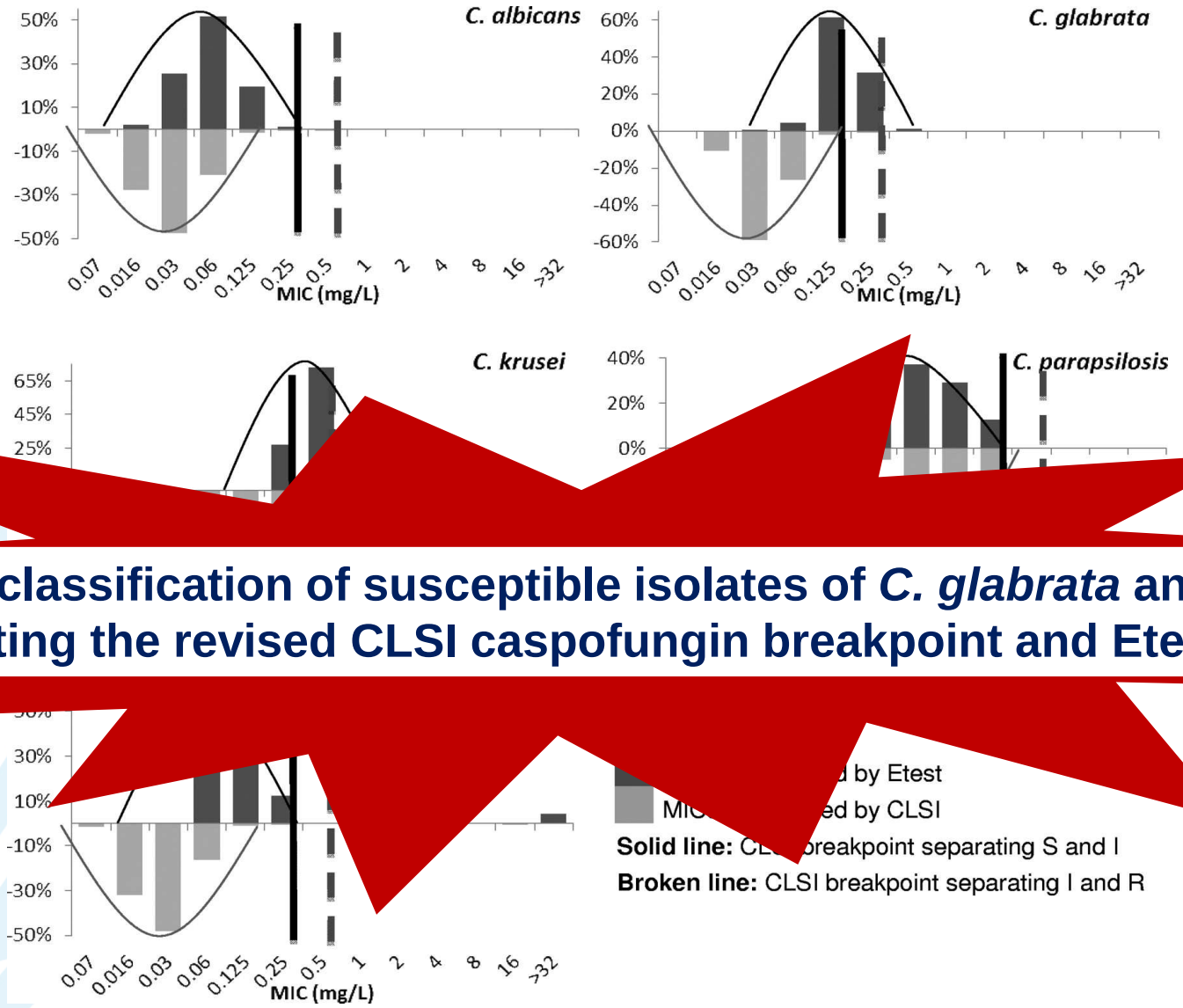
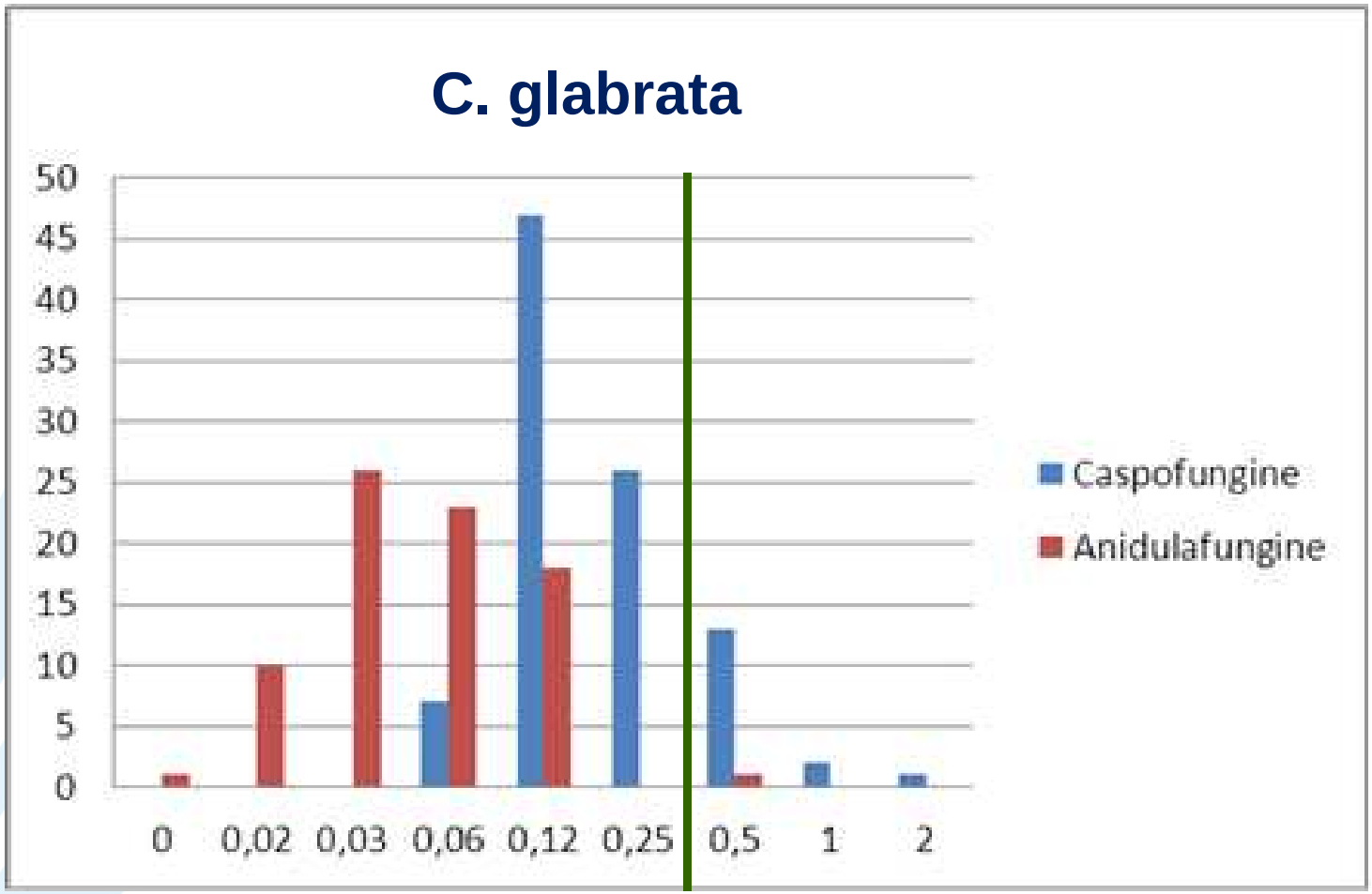
Caspofungin MIC distributions obtained by Etest (above the x axis) compared to caspofungin MICs obtained by CLSI M27-A3 (below the x axis)



Risk of misclassification of susceptible isolates of *C. glabrata* and *C. krusei* when adopting the revised CLSI caspofungin breakpoint and Etest

Arendrup M C et al. Antimicrob. Agents Chemother. 2012;56:3965-3968

Same problem for Sensititre….



Data UZ Leuven, January 2011- June 2012

Azole resistance in *Aspergillus*: a growing public health menace

"A 'perfect storm' combining extensive antifungal fungal exposure ... along with a highly efficient, evolutionarily perfected dispersal system, has led to our current situation."

David W Denning[†1,2] & David S Perlin[3]

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[2]The Mycology Reference Centre, Manchester; Manchester Academic Health Science Centre; University Hospital of South Manchester, Southmoor Road, Manchester, M23 9LT, UK

[3]Public Health Research Institute, New Jersey Medical School-UMDNJ, Newark, NJ, USA

[†]Author for correspondence: Tel.: +44 161 291 5811/5818 ▪ Fax: +44 161 291 5806 ▪ david.denning@manchester.ac.uk

Editorial

Future Microbiology

Future Medicine, 2011, 6, 1229-1232

Emerging azole resistance

4,5% resistance in CF patients
Mortensen, JCM, 2011

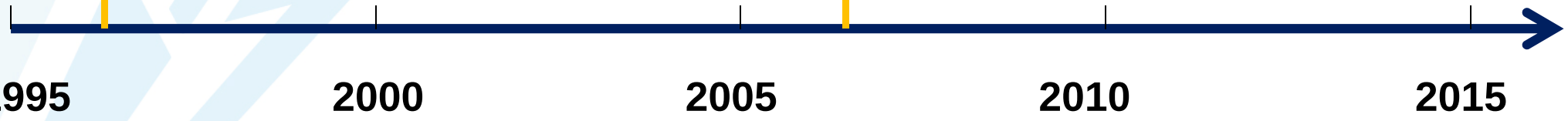
Few sporadic resistant isolates in Sweden, Spain France and UK

Resistant isolates in Denmark, Norway, Belgium, USA, China, Canada, India, …

5,8% resistance Artemis (mainly China)
Lockhart S, AAC, 2011

Itraconazole resistance in 2 cases
Denning, AAC, 1997

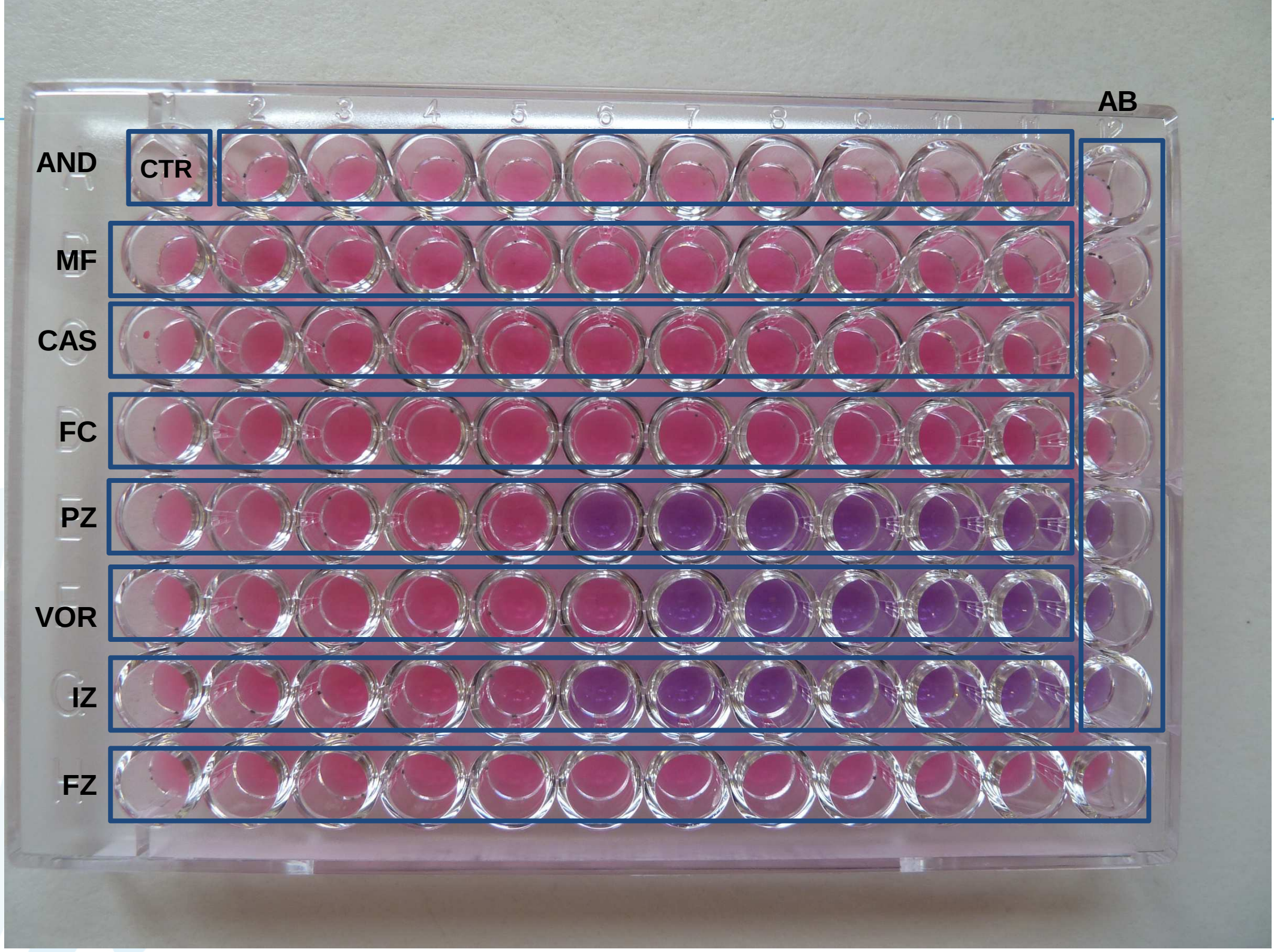
Many more resistant isolates in Nijmegen (7%) and Manchester (15-20%)
Bueid, JAC, 2010
Verweij, NEJM, 2007;J. Van der Linden, EID, 2011



Clinical breakpoints Aspergillus spp. EUCAST

(valid from 2012-03-05)

Antifungal agent	MIC breakpoint (mg/L)							
	A. fumigatus		A. flavus		A. niger		A. terreus	
	S ≤	R >	S ≤	R >	S ≤	R >	S ≤	R >
Amphotericin B	1	2	IE	IE	1	2	-	-
Itraconazole	1	2	1	2	IE	IE	1	2
Voriconazole	IP	IP	IP	IP	IP	IP	IP	IP
Posaconazole	0.12	0.25	IE	IE	IE	IE	0.12	0.25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PER YEAR

14.000 FUNGAL CULTURES

3.000 POSITIVE FUNGAL CULTURES