MMTN
MEDICAL MYCOLOGY TRAINING NETWORK

Recent advances of fungal diagnostics and application in Asian laboratories

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ASIA FUNGAL WORKING GROUP an ISHAM working group

ISHAM INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

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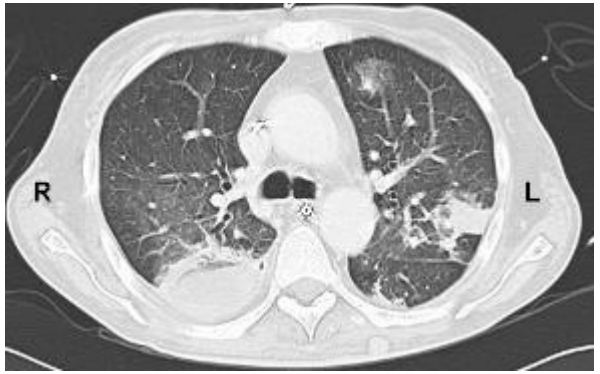
- **Mortality** due to invasive fungal infection
  - 97-100% if not treated
  - ~50% even after proper treatment
  - Why so poor outcome despite antifungal?

- **Can early therapy improve the outcome?**
- **Dilemma –** In absence of diagnosis, **Which** patient has fungal infection?

- **Clinical symptoms & signs not specific**
  - Occult in immunosuppressed patients, attenuated till late
  - How to distinguish from bacterial sepsis?



- **Imaging**
  - Findings subtle
  - Halo sign, air-crescent signs are absent in non-neutropenic

EMERGING INFECTIOUS DISEASES PERSPECTIVE Volume 23, Number 2—February 2017 P. 179

Delivering on Antimicrobial Resistance Agenda Not Possible without Improving Fungal Diagnostic Capabilities

David W. Denning, David S. Perlin, Eavan G. Muldoon, Arnaldo Lopes Colombo, Arunaloke Chakrabarti, Malcolm D. Richardson, Tania C. Sorrell

1. inaccurate diagnosis of **fungal sepsis** - resulting in inappropriate use of broad-spectrum antibacterial drugs

2. Most serious fungal infections are **'hidden'**, occurring as a consequence of other health problems such as asthma, AIDS, cancer, organ transplant & corticosteroid therapies

3. **Misdiagnosis** resulting in unnecessary antibacterial drugs

It is easy to advice - diagnose & then treat!

(*Candida* sepsis in ICUs)

- Blood culture **positivity ~50%**
- **Candida score, colonization index** – sampling for all colonization sites daily, impractical in clinical situation, not cost effective
- Indian study - **97% patients were colonized** with *Candida* species at any point of time during ICU stay
- **Ostrosky's rule** – easier to implement, but only 10% of those patients will develop proven or probable IC
- Do you know, **which patients to be treated with antifungal when predictive rules, candida score, blood culture fail**?

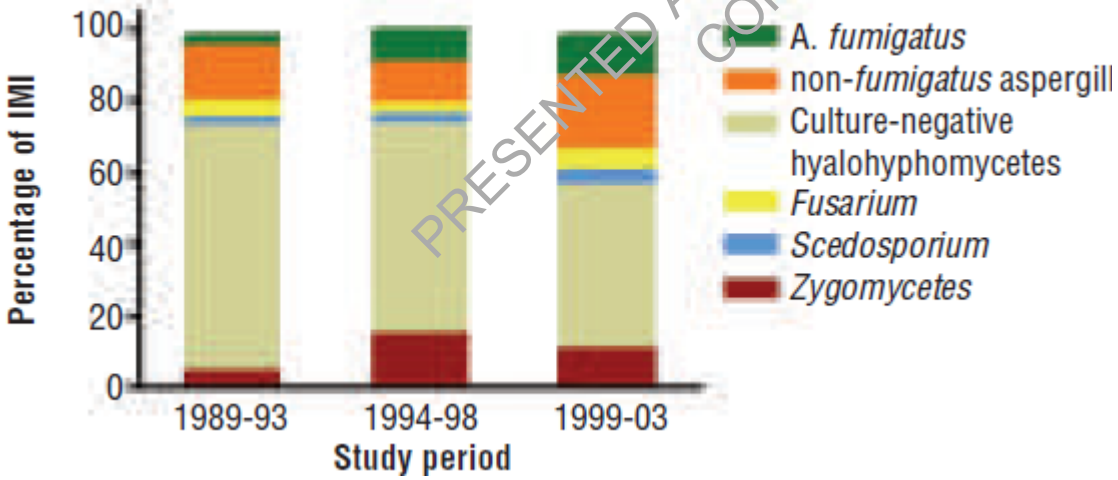
You may not get answer always

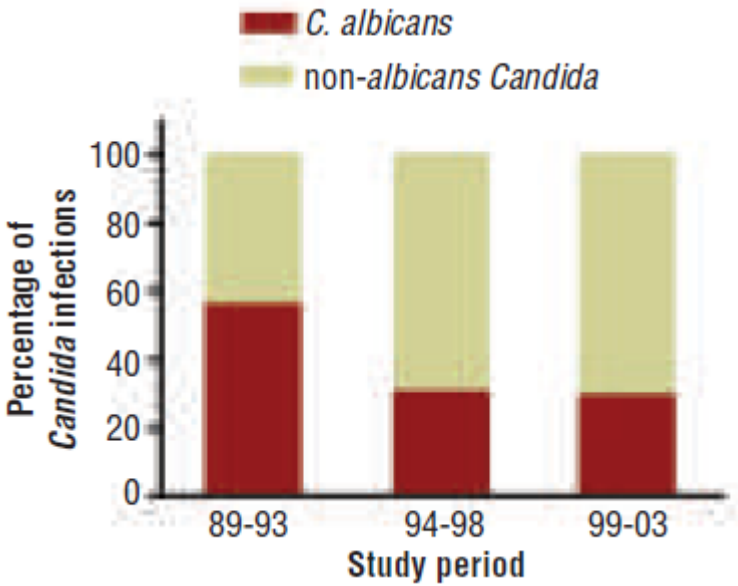
Haematologica. 2006 Jul;91(7):986-9.

Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: an autopsy study over a 15-year period (1989-2003)

Chamilos G[1], Luna M, Lewis RE, Bodey GP, Chemaly R, Tarrand JJ, Safdar A, Raad II, Kontoyiannis DP.

- 1,017 patients with haematological malignancies autopsied
  - 31% were found to have invasive fungal infections
  - **75% were not diagnosed before death**





Advances in diagnostics

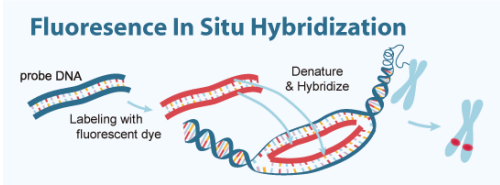
- In conventional techniques
- In culture & identification
- Biomarkers
- Nucleic acid detection
- Unmet needs & problems with present development
- In the pipeline
- Scenario in Asia

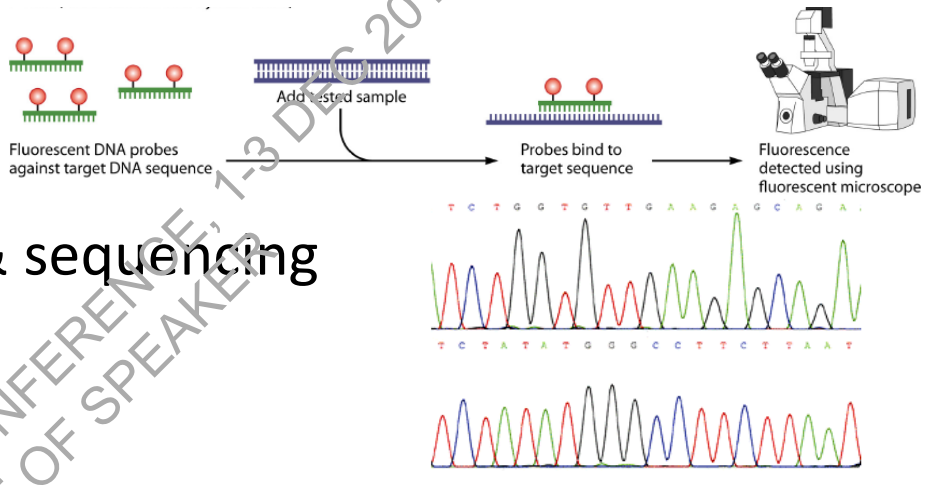
Laboratory diagnosis – some success

- Sample collection –
  - Improvement in invasive procedure (FNAC/lung biopsy), bronchoscopy
- Direct microscopy, culture & Histopathology –
  - Very important (especially PJP), can see mycelial fungi, takes few minutes
- Identification – important, as you can choose the drug
  - MALDI & sequencing – revolutionized
- Ag detection – excellent in Cryptococcus, Histoplasma (urine – 80-90% positive)

Identification of fungus in tissue

- Immunohistochemistry
- In situ hybridization
- Extraction of DNA from tissue & sequencing





JOURNAL OF MEDICAL MICROBIOLOGY

RESEARCH ARTICLE

Zaman *et al.*, *Journal of Medical Microbiology*

DOI 10.1099/jmm.0.000560

MICROBIOLOGY SOCIETY

Molecular diagnosis of rhino-orbito-cerebral mucormycosis from fresh tissue samples

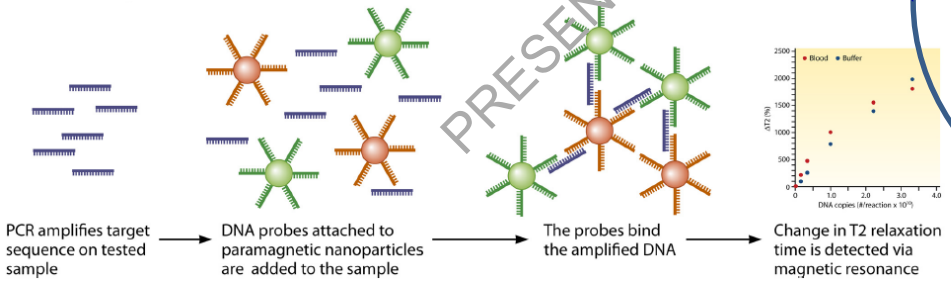
Kamran Zaman,[1] Shivaprakash Mandya Rudramurthy,[1] Ashim Das,[2] Naresh Panda,[3] Prasanna Honnavar,[1] Harsimran Kaur[1] and Arunaloke Chakrabarti[1,*]

Success – fresh tissue (95%), formalin fixed tissue – 60%

T2 magnetic resonance nanoparticle mediated detection for *Candida*

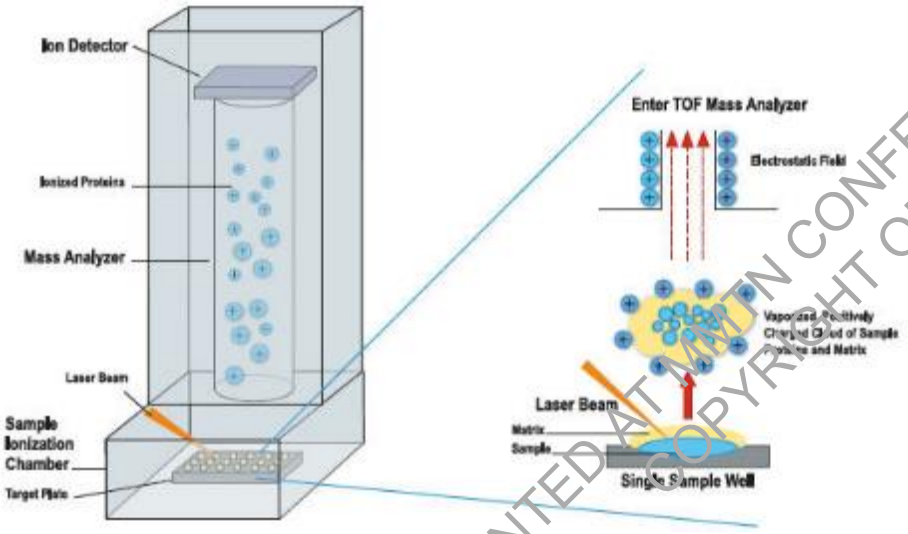
- Improved turnaround
  - BACTEC – 2.6d, T2 – 3-4h

- Identify only 5 *Candida* species
- Contamination
- Can't perform susceptibility
- Can't replace conventional
- Not available in developing countries



Beyda ND, *et al.* Diagn Microbiol Infect Dis 2013; 77: 324

MALDI-TOF-MS



- Identification within few minutes (yeast)
- Susceptibility testing
- Molecular typing

- Database needs improvement
- Not available in majority labs of developing countries

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry for the rapid identification of yeasts causing bloodstream infections

A. K. Ghosh, S. Paul, P. Sood, S. M. Rudramurthy, A. Rajbanshi, T. J. Jillwin and A. Chakrabarti

Clin Microbiol Infect 2015; 21: 372–378

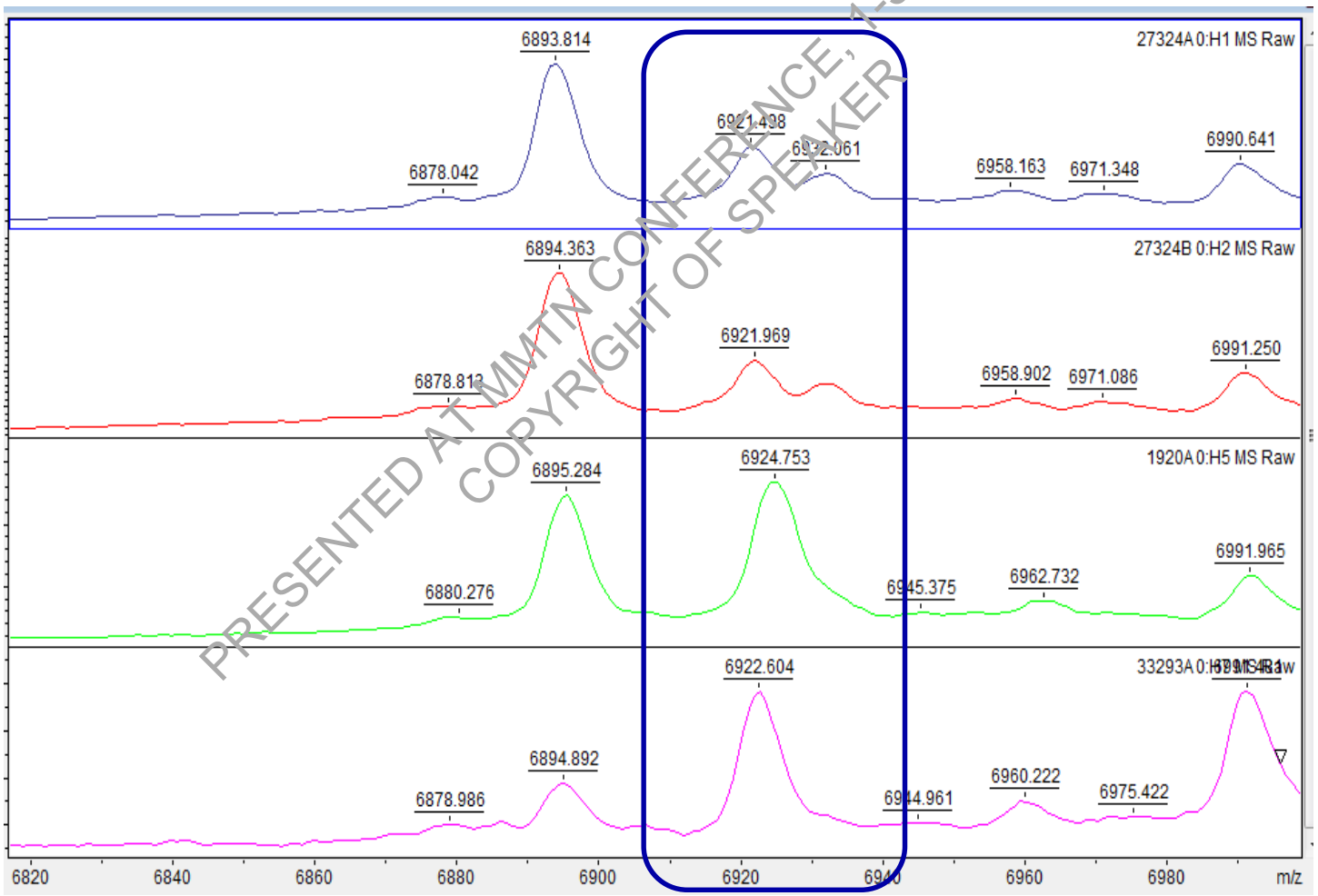
- 354 sequence yeast (standardization)
- 367 blind clinical yeast (validation)
- Database updated for *Candida auris, C. viswanathii, Kodamaea ohmeri* etc.

MALDI-TOF correctly identified 98.9% as compared to PCR-sequencing

Rapid detection of fluconazole resistance in *Candida tropicalis* by MALDI-TOF MS

Saikat Paul, Pankaj Singh, Shamanth A S,
Shivaprakash M. Rudramurthy, Arunaloke Chakrabarti
and Anup K Ghosh*

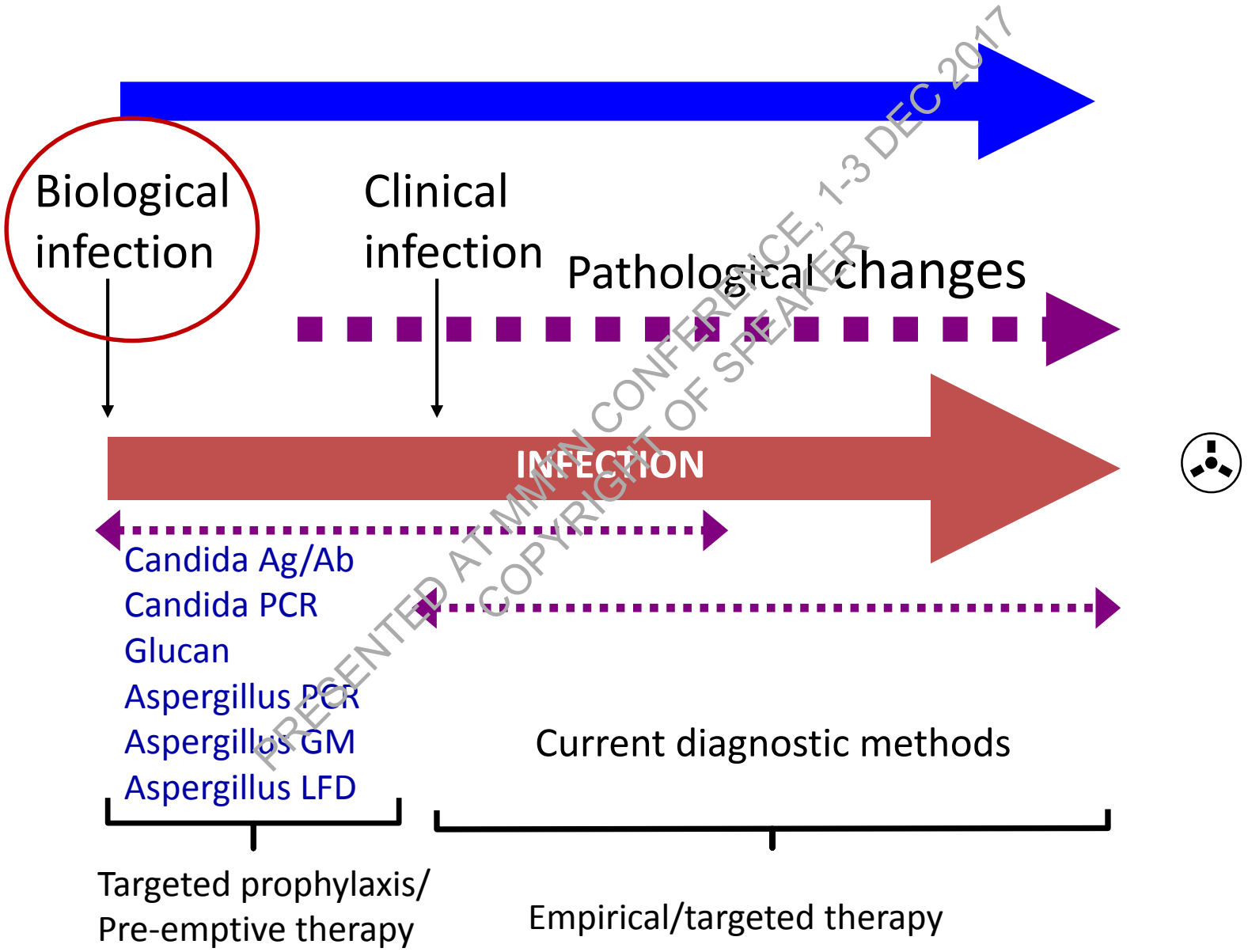
Medical Mycology, 2017, 0, 1–8
doi: 10.1093/mmy/myx042



"Okay—who put my lunch through the mass spectrometer..?"

http://www.lab-initio.com/250dpi/nz025.jpg

But what would be real success?



Culture independent methods – proteomic vs. genomic approach

Proteomic approach

- Detection in clinical sample – promising, but success limited
- Limitation
  - presence of biomarker in pg
  - No scope of prior amplification before detection

Genomic approach

- Pre-amplification possible
- Higher sensitivity & specificity, low turn-around time
- GM released in active growth, PCR better in prophylaxis

Biomarker tests

Existing benchmark tests

- CRP & Procalcitonin ?
- Serum galactomannan
- BAL galactomannan
- Serum Beta-D gulcan

(Caution: may need 'expert' interpretation)

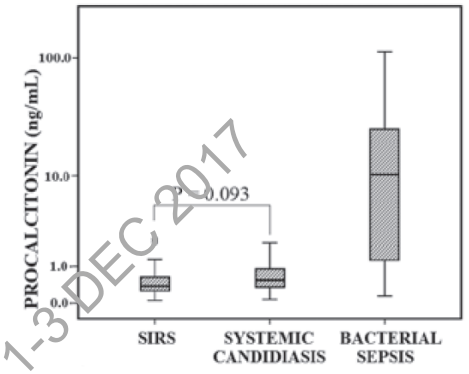
New biomarkers

- Aspergillus PCR
- Aspergillus GM + PCR
- Aspergillus Lateral flow
- BAL Beta-D glucan
- Mucorales PCR from blood
- Breath Volatile metabolites
- Many potential POCT

Procalcitonin, C-reactive protein and serum lactate dehydrogenase in the diagnosis of bacterial sepsis, SIRS and systemic candidiasis

Fabio Miglietta[1], Maria Letizia Faneschi[1], Giambattista Lobreglio[2], Claudio Palumbo[1], Adriana Rizzo[1], Marco Cucurachi[3], Gerolamo Portaccio[4], Francesco Guerra[2], Maria Pizzolante[2]

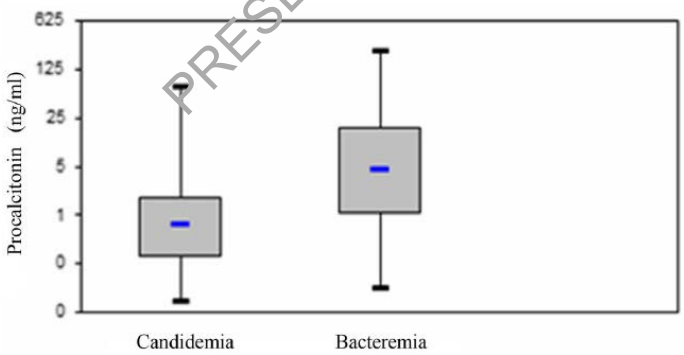
Le Infezioni in Medicina, n. 3, 230-237, 2015



Variables	*All patients (n=145)*	*SIRS (n=42)*	*P*	*Bacterial sepsis (n=70)*	*P*	*Systemic candidiasis (n=33)*
PCT (ng/mL)						
day 0	0.9 (0.4-9.4)	0.38 (0.26-0.64)	<0.001	10.2 (1.2825.3)	<0.001	0.55 (0.360.9)
day 2	0.6 (0.3-4.9)	0.28 (0.12- 0.5)	<0.001	4.9 (0.711.9)	0.001	0.5 (0.20.6)
CRP (mg/L)						
day 0	91.7 (55.7164)	68.6 (48.5139)	<0.001	128.6 (77-254.7)	<0.001	60.5 (54.4-96.5)
day 2	69.8 (52.3117)	58.5 (47.183)	0.001	99.9 (58-180.2)	0.046	67.4 (50-78.8)

Intern Emerg Med
DOI 10.1007/s11739-017-1627-7 Published online: 04 February 2017

Usefulness of procalcitonin in differentiating *Candida* and bacterial blood stream infections in critically ill septic patients outside the intensive care unit



Filippo Pieralli[1] · Lorenzo Corbo[1] · Arianna Torrigiani[2] · Dario Mannini[2] · Elisa Antonielli[1] · Antonio Mancini[1] · Francesco Corradi[2] · Fabio Arena[3] · Alberto Moggi Pignone[4] · Alessandro Morettini[2] · Carlo Nozzoli[1] · Gian Maria Rossolini[3,5,6,7]

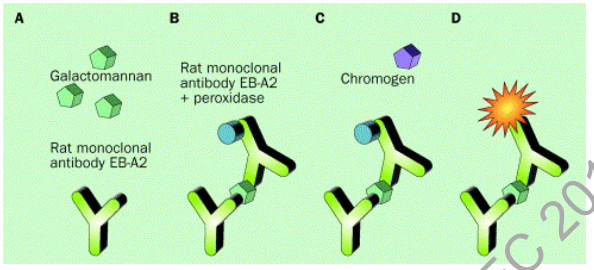
LDH can help in diagnosis of pneumocystis pneumonia

Variables	Clinical diagnosis			p-Value
	PJP (n = 19)	CAP (n = 18)	Other (n = 23)	
(1-3)-β-D-Glugan in serum (pg/mL)				
Median	183	29.8	52.8	
Mean (± SD)	240.8(± 185.7)	36.3 (±34.2)	67.3 (± 60.7)	<0.0001
LDH (U/L)				
Median	761	419	441	
Mean (± SD)	762.47 (± 433.18)	379.5(± 99.9)	442.6 (± 217.6)	0.003
Viral load (copies/mL)				
Median	62,609	3900	74,892	
Mean (± SD)	801,171 (± 2,194,157)	149,525 (± 270,015)	482,859 (± 135,562)	0.30
Lymphocyte T CD4+ (cell/mm^3)				
Median	40	230	97	
Mean (± SD)	73 (± 107)	303 (± 267)	217 (± 312)	0.001
Time since diagnosis of HIV infection (years)				
Median	0	15	11	
Mean (± SD)	4.94 (± 6.83)	14 (± 7.67)	10.7 (± 8.32)	0.001
Outcome				
Discharge	15	15	21	0.62
Death	4	3	2	
Mean time of hospitalization (in days)	22.9 (± 22.8)	13.4 (± 8.9)	16.1 (± 12.3)	0.31

PJP, *Pneumocystis jirovecii* pneumonia. PJP group included: *P. jirovecii* + community acquired pneumonia: 4 patients; *P. jirovecii* + *Mycobacterium* non-*tuberculosis*: 2 patients. CAP: community acquired pneumonia. Other: lower respiratory infection: 9 patients; tuberculosis: 5 patients; histoplasmosis: 2 patients; cryptococcosis: 1 patient; disseminated strongyloidiasis: 1 patient; nocardiosis: 1 patient; pulmonary embolism: 1 patient and undiagnosed: 3 patients.

Passos AL *et al*. Braz J Infect Dis 2017, July 28 (online)

Galactomannan



- GM for serum, BAL – FDA approved, can also be detected in CSF, urine
- But, many false positive and negative issues
- Better performance in patients undergoing intensive chemotherapy compared to solid-organ transplant patients

Patients	Sensitivity, % (95% CI)	Specificity, % (95% CI)
Haematological malignancy	58 (52–64)	95 (94–96)
HSCT	65 (60–78)	65 (44–83)
Solid organ transplant	41 (21–64)	85 (80–89)

- **Diagnosis-driven strategy**: GM monitoring every 3–4 days combined with clinical and microbiological evaluation and high-resolution CT imaging (**A II recommendation**)

1. Mennink-Kersten MA, et al. *Lancet Infect Dis* 2004;4:349–57;
2. Leeflang MM, et al. *Cochrane Database Syst Rev* 2008;8:CD007394.

Galactomannan in BAL

CHEST Original Research

LUNG INFECTION

Accuracy of BAL Galactomannan in Diagnosing Invasive Aspergillosis

A Bivariate Metaanalysis and Systematic Review

Ya-Ling Guo, MD; Yi-Qiang Chen, MD, PhD; Ke Wang, MD; Shou-Ming Qin, MD; Cong Wu, MD; and Jin-Liang Kong, MD, PhD

JCM JournalsASM.org

Diagnostic Accuracy of PCR Alone Compared to Galactomannan in Bronchoalveolar Lavage Fluid for Diagnosis of Invasive Pulmonary Aspergillosis: a Systematic Review

Tomer Avni,[a] Itzhak Levy,[b,c] Hannah Sprecher,[d,e] Dafna Yahav,[a] Leonard Leibovici,[a,c] and Mical Paul[a,c]

Medicine E, Beilinson Medical Center, Beilinson Hospital, Petah-Tikva, Israel[a]; Unit of Infectious Diseases, Schneider's Medical Center, Petah-Tikva, Israel[b]; Sackler Faculty of Medicine, Tel-Aviv University, Ramat Aviv, Israel[c]; Microbiology Laboratory, Rambam Health Care Campus, Haifa, Israel[d]; Bruce Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Haifa, Israel[e]; and Unit of Infectious Diseases, Rabin Medical Center, Beilinson Hospital, Petah-Tikva, Israel[f]

OPEN ACCESS Freely available online PLOS ONE

Systematic Review and Meta-Analysis of Detecting Galactomannan in Bronchoalveolar Lavage Fluid for Diagnosing Invasive *Aspergillosis*

Mingxiang Zou[1☯], Lanhua Tang[2☯], Shushan Zhao[2☯], Zijin Zhao[2], Luyao Chen[2], Peng Chen[3], Zebing Huang[4], Jun Li[1], Lizhang Chen[5], Xuegong Fan[4]

Critical Reviews in Microbiology

http://informahealthcare.com/mby
ISSN: 1040-841X (print), 1549-7828 (electronic)
Crit Rev Microbiol, Early Online: 1–11

DOI: 10.3109/1040841X.2013.804033

informa healthcare

REVIEW

Utility of bronchoalveolar lavage fluid galactomannan alone or in combination with PCR for the diagnosis of invasive aspergillosis in adult hematology patients: A systematic review and meta-analysis

Siow Chin Heng[1], Orla Morrissey[2], Sharon C-A Chen[3], Karin Thursky[4], Renee L Manser[4], Roger L Nation[1], David C-M Kong[1], and Monica Slavin[4]

Cut-off of 0.5

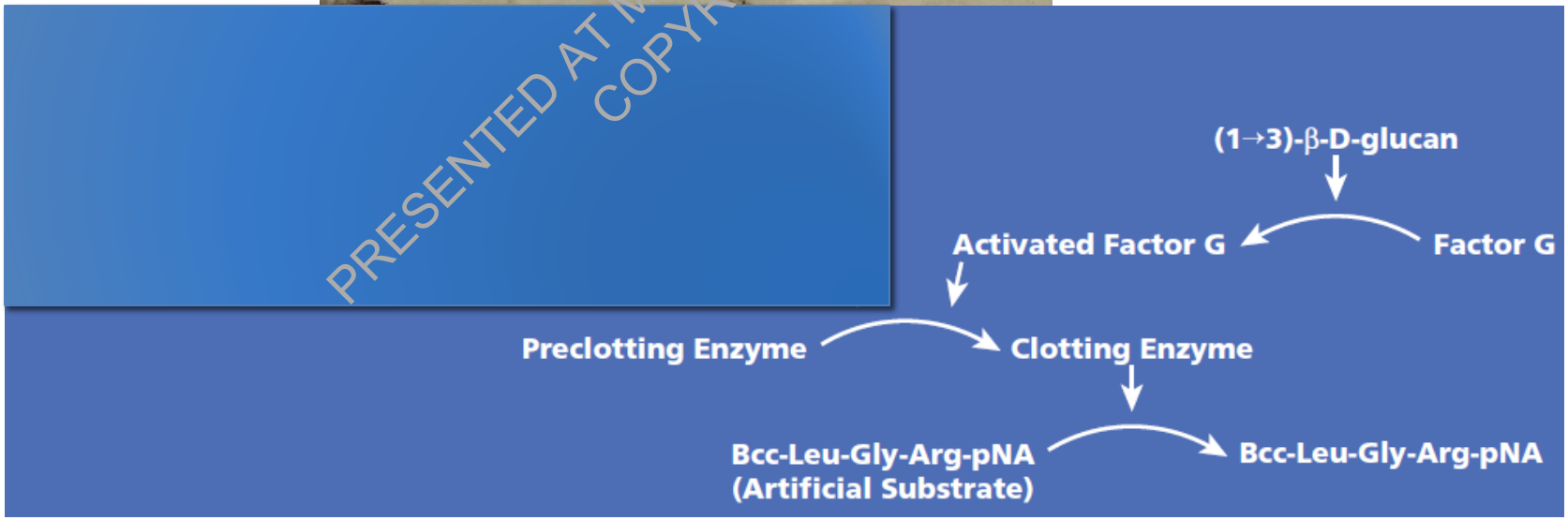
Sensitivity	Specificity	Ref.
90	94	Guo, *Chest* 2010
90	96.4	Avni, *JCM* 2011;4:665-70
87	94	Zou, *PlosOne* 2012; 7:e52833
92	98	Heng, *Crit Rev Microbiol* 2015;41:124-34 (haematological patients)

Pros & Cons of GM test

- FDA approved GM test in serum & BAL
- Detectable GM precedes clinical infection
- BAL GM precedes serum GM
- Good positive & negative predictive value in Haematology-Oncology
- Possibly we may use it also in CSF & urine
- Not yet standardized in ICU patients
- Limitation
  - Cross-reaction with some fungi (*Geotrichum, Penicillium, Histoplasma* etc.)
  - Variable turnaround time depending of number of specimens
  - False positive tests
  - Well-equipped laboratories & trained staff to perform the test

Amsden JR. Curr Fung Infect Rep 2015; 9:111

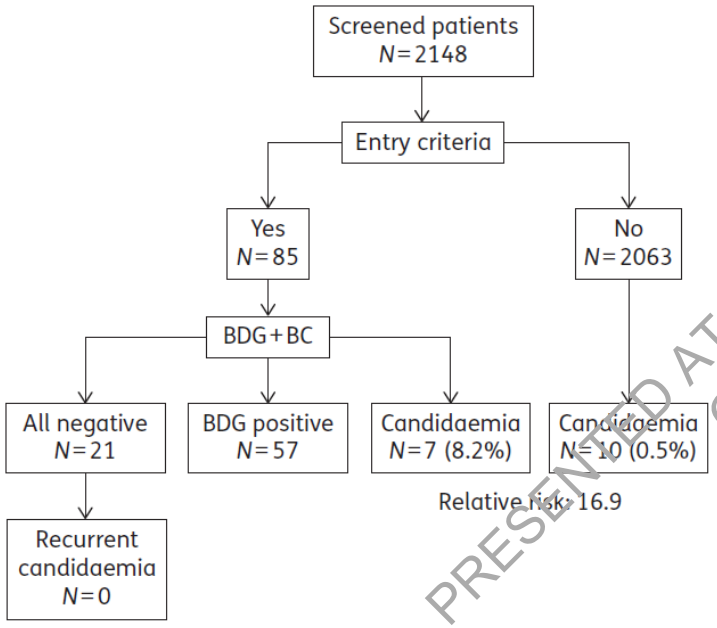
1,3-β-D-glucan detection



J Antimicrob Chemother 2016; **71**: 2628–2633

Discontinuation of empirical antifungal therapy in ICU patients using 1,3-β-D-glucan

Marcio Nucci[1]*, Simone A. Nouér[1], Patricia Esteves[2], Thais Guimarães[3], Giovanni Breda[4], Bianca Grassi de Miranda[3], Flavio Queiroz-Telles[4] and Arnaldo L. Colombo[2]



Factors related to false-positive BDG	Positive biomarker cohort (*N*=57), *n* (%)	Negative biomarker cohort (*N*=21), *n* (%)	*P*
Bacteraemia	11 (19.3)	6 (28.6)	0.38
Gram-positive	5 (8.7)	3 (14.3)	0.67
Gram-negative	5 (8.7)	3 (14.3)	0.67
anaerobe	1 (1.7)	0 (0)	1.0
use of β-lactam antibiotics	49 (86.0)	19 (90.5)	0.72
Infusion of albumin	7 (12.3)	1 (4.8)	0.44
Surgery	13 (22.8)	4 (19.0)	1.00

All 21 patients with baseline negative BDG discontinued anidulafungin on day 4. None developed candidaemia until day 30.

Conclusions: Early discontinuation of empirical echinocandin therapy in high-risk ICU patients based on consecutive negative BDG tests may be a reasonable strategy, with great potential to reduce the overuse of echinocandins in ICU patients.

The performance of BDG as per meta-analysis

References	Guidelines	Patients	Type of infection	Strength of recommendation
Marchetti et al. BMT 2012	The third European Conference on Infections in Leukemia (ECIL-3)	Hemato-oncological patients	Invasive fungal infections	B II
Ruhnke et al. Annals of Oncology 2012	Infectious Diseases Working Party of the German Society of Haematology and Oncology (AGIHO)	Hemato-oncological patients	Invasive fungal infections	B
Cuenca-Estrella et al. Clin Microbiol Infect 2012	European Society of Clinical Microbiology and Infectious Diseases (ESCMID)	ICU patients	Invasive candidiasis	II
Dellinger et al. Crit Care Med 2013	Surviving Sepsis Campaign for ICU patients	ICU patients	Invasive candidiasis	B II

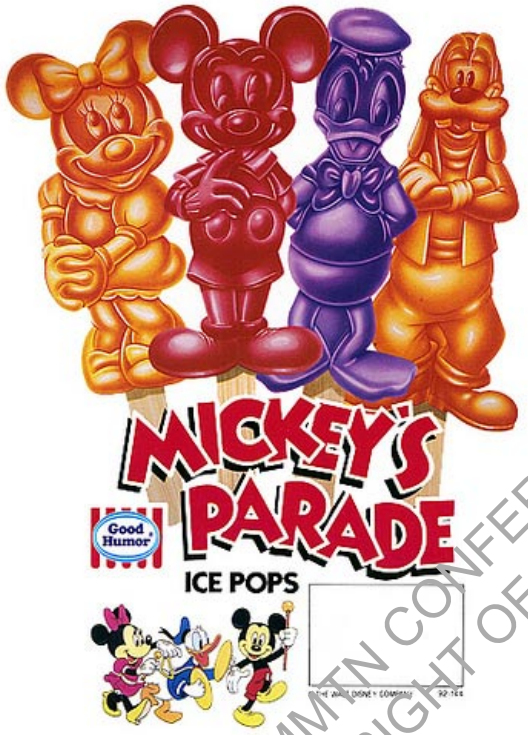
Furfaro E, *et al*. Curr Fung Infect Rep 2015; 9: 292

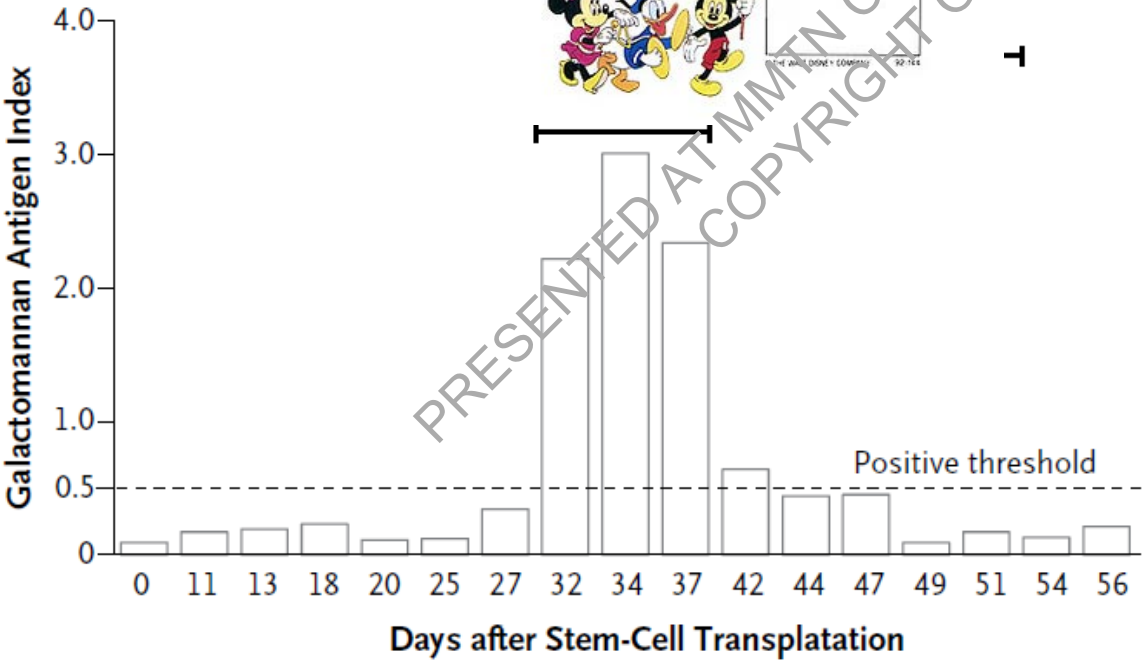
- Pan-fungal marker except Mucor & possibly Cryptococcosis
- Positive before clinical symptoms; Helps to monitor therapy
- Good performance in suspected *Pneumocystis* & *Candida* infection
- False positivity, difficulty to test, cost

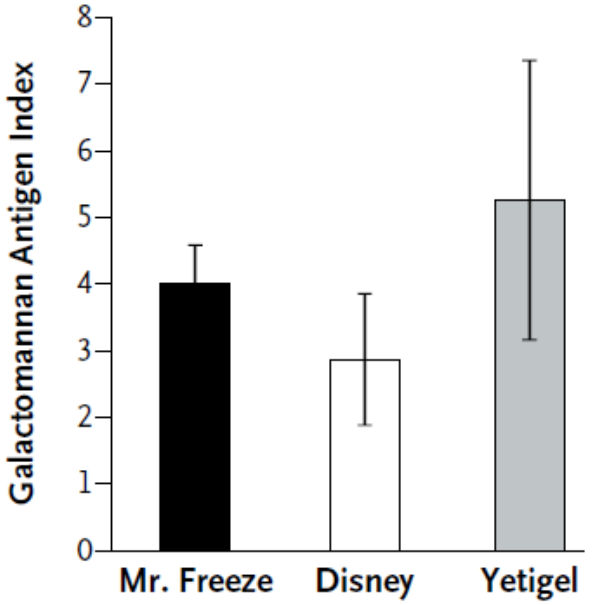
False positivity of bio-marker tests

	Beta-D-glucan	Galactomannan
Medication	i.v. amoxycillin-clavulanate or ampicillin-sulbactam	Piperacillin-Tazobactum Other beta-lactam antibiotics
Infusion	i.v. immunoglobulin Cellulose filter for i.v. infusion Albumin	Plasmalyte (electrolyte infusion) i.v. solution with sodium gluconate
Medical intervention	Hemodialysis with cellulose filter Gauze packing in serosal surface	Enteral feeding with soybean proteins
Other infections	*Pneumocystis* infection	*Penicillium* spp., *Histoplasma capsulatum, Geotrichum, Neosartoria, Bifidobacterium*

Fischer BT. Curr Fungal Infect Rep. 2013; 7: 7–14







Nucleic acid detection - Real challenge in clinical sample

- PCR based detection assay - Real time PCR or qPCR
- Large number of PCR protocols published over 20 years, but absence of consensus standardized technique
- PCR is not included in EORTC/MSG guideline

Comparison with virology

- Different protocol published for viruses, but this does not hamper acceptance of PCR in diagnostic virology
- For viruses – we deal with $>10^3$

Challenges in fungal PCR

- Too few fungal DNA in sample
- PCR inhibitors – heparin, haemoglobin, lactoferrin
- Contamination is a big issue - environment
  - 10-20% tube may have *Aspergillus* DNA contamination
  - 18% commercial tubes with anticoagulant have fungal DNA

Recommendation EAPCRI

- Serum may be used, plasma best – blood volume >3ml
  - Elution in small volume
  - Mechanical lysis better than enzymatic lysis of cell wall
  - Internal control, ITS target

Diagnosis of aspergillosis – comparison GM/BDG/PCR

White PL *et al.* Clin Infect Dis 2015; 61: 1293

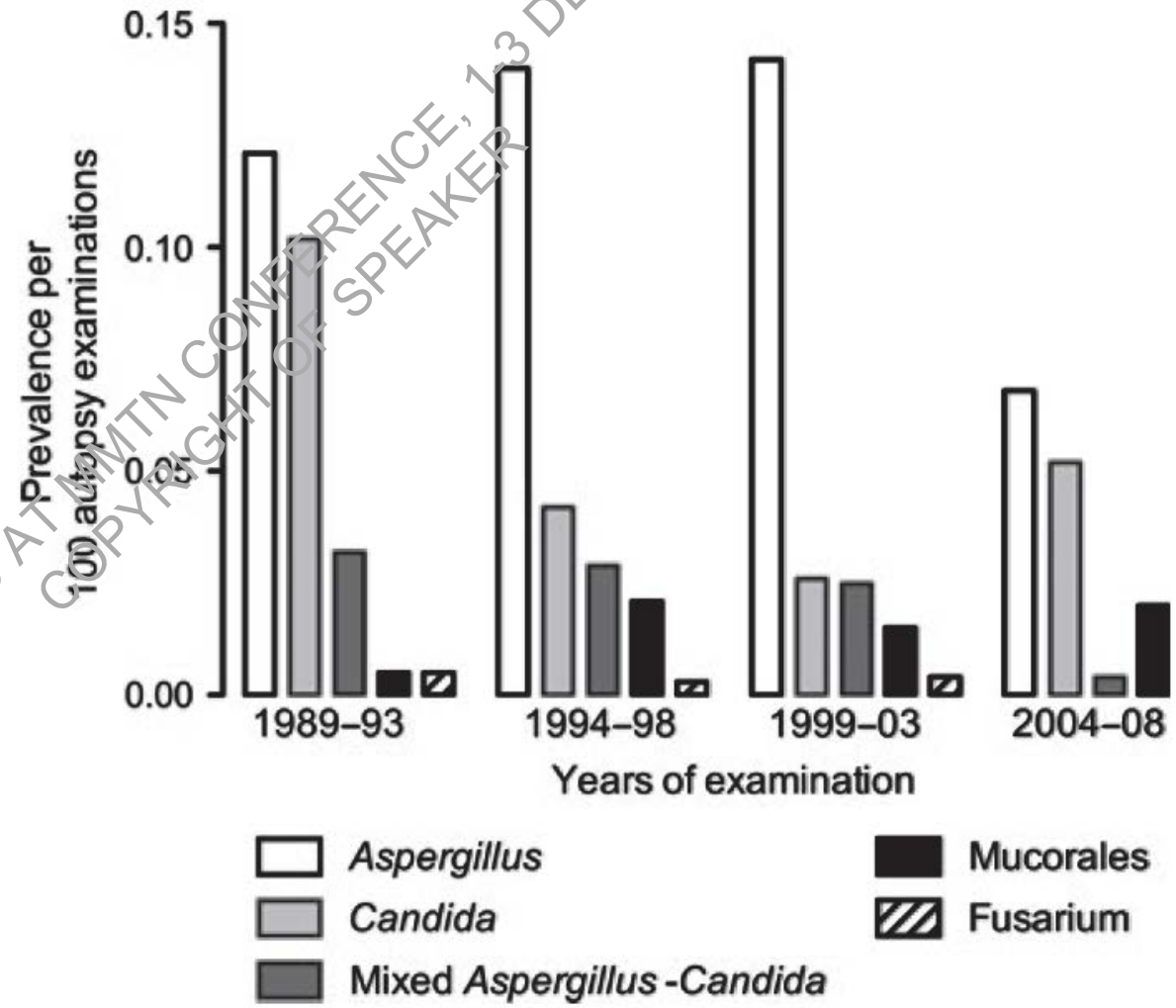
Characteristic	GM-EIA	B-D-glucan	PCR
Methodological recommendation	Single commercial assay with SOP: **Platelia Aspergillus antigen** (BioRad)	5 commercial assays: **Fungitell** (Associates of Cape Cod) **Fungitec G-Test MK** (Seikagaku Corporation) **B-G Star** (Maruha Corporation **B-Glucan Test Wako** (Wako Pure Chemicals) **Dynamiker Fungus** (1–3)-β-D-Glucan Assay (Dynamiker Biotechnology Co, Ltd)	**Pathonostics Aspergenius, Roche Septifast, Myconostica MycAssay, Ademtech Mycogenie, Renishaw Fungiplex,** Procedural recommendations for DNA extraction **(EAPCRI)**
Quality control	Internal – BioRad Proficiency panel	No	Independent – QCMD & EAPCRI Panels
Sensitivity %	Blood: 79.3 BAL: 83.6–85.7	Blood: IA: 56.8–77.1	Blood: 84–88 BAL: 76.8–79.6
Specificity %	Blood: 80.5–86.3 BAL: 89.0–89.4	Blood: 81.3–97.0	Blood: 75–76 BAL: 93.7–94.5
False positive	Yes	Yes	Yes
False negative	Yes	Yes	Yes
Clinical utility	Yes	Limited	yes

Current diagnostics: consensus

Infection	Culture/ Histo	Biomarker (Ab)	Biomarker (Ag)	Response to Rx
Aspergillosis	Yes -invasive	No	GM/BDG/PCR	Increasing evidence
Cryptococcosis	Routine	No	Ag/PCR	Yes (CSF Ag)
Histoplasmosis	Culture - delay	Limited	Ag	Yes (Ag)
Mucormycosis	Yes - invasive	No	Investigational	No
Other moulds	Yes –invasive	No	Investigational	No
Candidaisis	Routine	Investigational (anti-mannan)	PCR/mannan/BDG	No

Improvement in diagnosis
(MD Anderson autopsy data on haematological malignancy)

- 84% of IFI were diagnosed post-mortem during 1989-93
- 49% of IFI were diagnosed post-mortem during 2004-08
- Improvement in aspergillosis diagnosis due to pre-emptive approach or introduction of molecular tests



Lewis RE *et al*. Mycoses 2013; 56: 638

Interpretation of non-culture diagnostic tests

- If blood culture is negative due to low level of candidemia, beta-glucan & PCR assays unlikely to make diagnosis reliably
- If a patient in low-risk group (ICU admission), positive result does not help, but negative result excludes the disease
- If a patient in high-risk group (repeated ileal leak or pancreatitis), a positive result increases the likelihood of invasive candidiasis
- Temptation – shorter turn around time & early therapy
- We tend to believe - non-culture diagnostic tests can identify blood culture negative primary or secondary deep-seated candidiasis
- Two high positive results are compelling
- Similarly multiple negative results are compelling

Are we ready with *Candida* biomarkers?

Leon et al, Crit Care 2016; 20: 149

Single or combined biomarker screening in prospective ICU cohort (candidiasis incidence, 13%)
Patients with (medical or surgical) severe abdominal condition, & expected ICU stay ≥7 days

	Colonization			Infection	
	Not colonized	Low-grade colonized	High-grade colonized	Intra-abdominal candidiasis	Candidemia
	N = 48	*N* = 130	*N* = 24	*N* = 20	*N* = 11
BDG ≥ 80 pg/mL, no. (%)	16/46 (34.8)[a]	50/124 (40.3)[a]	17/24 (70.8)[b]	15/20 (75.0)[b]	8/10 (80.0)[b]
BDG ≥ 100 pg/mL, no. (%)	16/46 (34.8)[a]	45/125 (36.0)[a]	14/24 (58.3)[a,b]	13/20 (65.0)[b]	8/10 (80.0) [b]
BDG ≥ 200 pg/mL, no. (%)	10/47 (21.3)[a]	20/128 (15.6)[a]	11/24 (45.8)[b]	10/20 (50.0)[b]	8/10 (80.0)[b]
CAGTA positive, no. (%)	10/47 (21.3)[a]	44/128 (34.4)[a]	17/24 (70.8)[b]	8/20 (40.0)[a,b]	8/10 (80.0)[b]
Mannan-Ag positive, no. (%)	10/48 (20.8)[a]	40/127 (31.5)[a]	15/24 (62.5)[b]	8/20 (40.0)[a,b]	5/10 (50.0)[a,b]
Mannan-Ab positive, no. (%)	6/48 (12.5)	12/128 (9.4)	4/24 (16.7)	5/20 (25.0)	3/11 (27.3)
C-PCR positive, no. (%)	14/23 (60.9)	37/54 (68.5)	6/8 (75.0)	12/14 (85.7)	9/11(81.8)

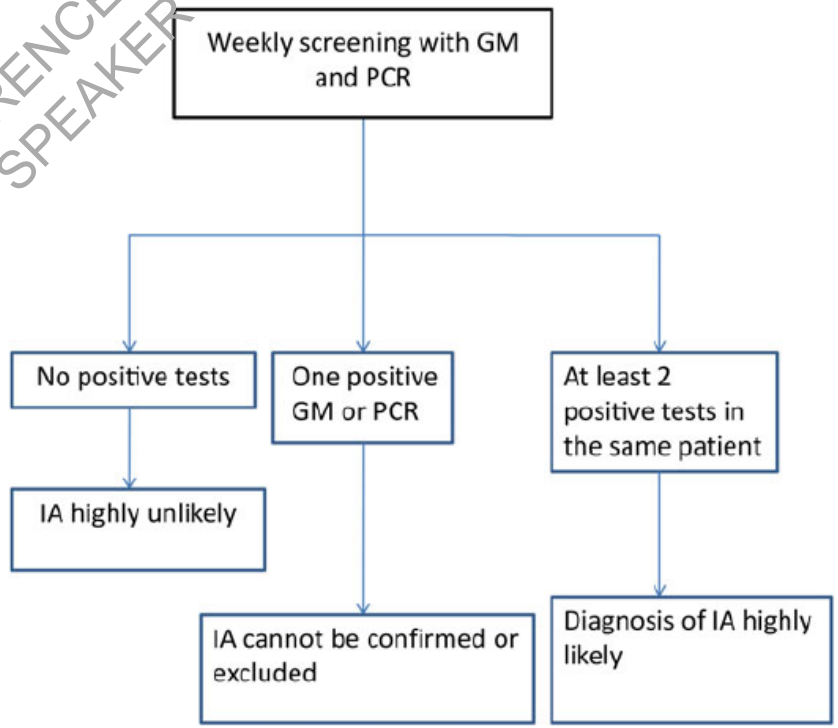
- Single assays are highly nonspecific (≈80% of positive results are false)
- Positive *Candida albicans* germ tube antibody & ß-D-glucan in a single blood sample or ß-D-glucan positivity in two consecutive blood samples allowed discriminating invasive candidiasis
- **Sensitivity still low in very high risk (≈30% of cases missed)**
- **A negative test does not rule out candidiasis in a high-risk patient**

Galactomannan and Polymerase Chain Reaction–Based Screening for Invasive Aspergillosis Among High-Risk Hematology Patients: A Diagnostic Meta-analysis

Marios Arvanitis,[1,2,3] Theodora Anagnostou,[1,2] and Eleftherios Mylonakis[1,2]

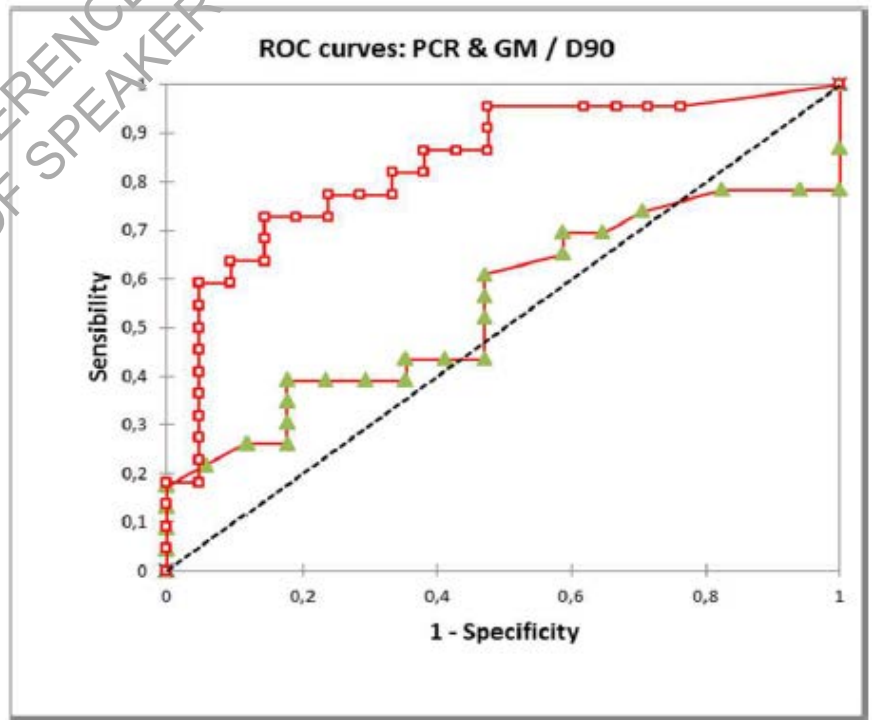
Clin Infect Dis 2015; 61: 1263

Test	Sensitivity, % (95% CI)	Specificity, % (95% CI)
PCR	84 (71–92)	76 (64–85)
2 PCRs	57 (40–72)	93 (87–97)
GM	92 (83–96)	90 (81–95)
2 GMs	62 (48–74)	95 (91–97)
GM or PCR	99 (96–100)	64 (49–77)
GM and PCR	68 (54–80)	98 (94–100)



GM + PCR better than GM alone

- 941 patients, 5146 serum samples
- 51 patients – proven/probable IA
- PCR – 66.7% sens., 98.7% spec.
- GM – 78.4% sens., 87.5% spec.
- PCR+GM – 88.2% sens.



Imbert *et al.* Clin Microbiol Infect 2016, 22: 562.e1-8

Biomarkers may monitor therapy

- 18 centres (US & Belgium) – 47 patients with IA (9 proven + 38 probable)
- GM & BDG – twice weekly for six weeks

	GM+BDG (mean z-score)		BDG[a] (pg/mL)		GM[a] (ng/mL)		GMI[a]	
Response	**Baseline to W-2**	**Baseline to W-6**	**Baseline to W-2**	**Baseline to W-6**	**Baseline to W-2**	**Baseline to W-6**	**Baseline to W-2**	**Baseline to W-6**
Week 6								
R, Mean (N)	-0.10 (25)	-0.12 (25)	929 (24)	693 (24)	0.48 (25)	0.29 (25)	0.27 (25)	0.18 (25)
NR, Mean (N)	0.24 (22)	0.31 (22)	2174 (20)	999 (20)	1.49 (20)	1.53 (21)	0.85 (20)	0.83 (21)
Mean Difference	0.34	0.43	1245	306	1.01	1.24	0.58	0.65
90% CI	-0.17, 0.84	-0.11, 0.97	-1825, 4315	-1080, 1694	-0.06, 2.09	-0.01, 2.49	0.09, 1.07	0.06, 1.23
***P* value**	**0.13**	**0.09**	**0.25**	**0.36**	**0.06**	**0.05**	**0.03**	**0.03**
Week 12								
R, Mean (N)	-0.12 (27)	-0.16 (27)	873 (26)	656 (26)	0.56 (25)	0.29 (26)	0.33 (25)	0.19 (25)
NR, Mean (N)	0.42 (14)	0.55 (14)	3555 (12)	1607 (12)	1.91 (14)	2.19 (14)	1.05 (14)	1.17 (14)
Mean Difference	0.54	0.71	2682	951	1.35	1.89	0.72	0.98
90% CI	-0.01, 1.10	0.12, 1.3	-1083, 6447	-752, 2655	0.09, 2.62	0.44, 3.35	0.13, 1.29	0.30, 1.66
***P* value**	**0.05**	**0.02**	**0.12**	**0.18**	**0.04**	**0.02**	**0.02**	**0.01**

GM: Galactomannan, BDG: Beta-D-Glucan, GMI: Galactomannan Optical Density Index, W: Week, R: Responder, NR: Non-responder, N: Number, CI: Confidence Interval.

Neofytos D, *et al*. PLoS ONE 2015; 10: e0129022. doi:10.1371

Commercial platforms for diagnosis of Candidiasis

- Detection from clinical samples
  - Biomarkers – fungal antigen detection – beta-D-glucan
    - Fungitell assay (Cape Cod, USA)
  - PCR based methods from whole blood (Serum)
    - SeptiFast (Roche)
    - Fungiplex *Candida* – RT-PCR (Bruker)
    - T2 magnetic resonance (T2 Biosystem)
- Identification from culture
  - *Candida* PNA FISH (AvanDx)
  - FilmArray multiplex PCR (BioFire Dx, BioMerieux)
  - MBT Sepsityper (MALDI-TOF) (Bruker)

Commercial platforms for diagnosis of Aspergillosis

- Detection from clinical samples
  - Biomarkers – fungal antigen detection – Galactomannan
    - Platelia *Aspergillus* (Bio-RAD)
  - PCR based methods from whole blood (Serum)
    - Pathonostics Aspergenius
    - Roche Septifast
    - Myconostica MycAssay
    - Ademtech Mycogenie
    - Renishaw Fungiplex

New techniques

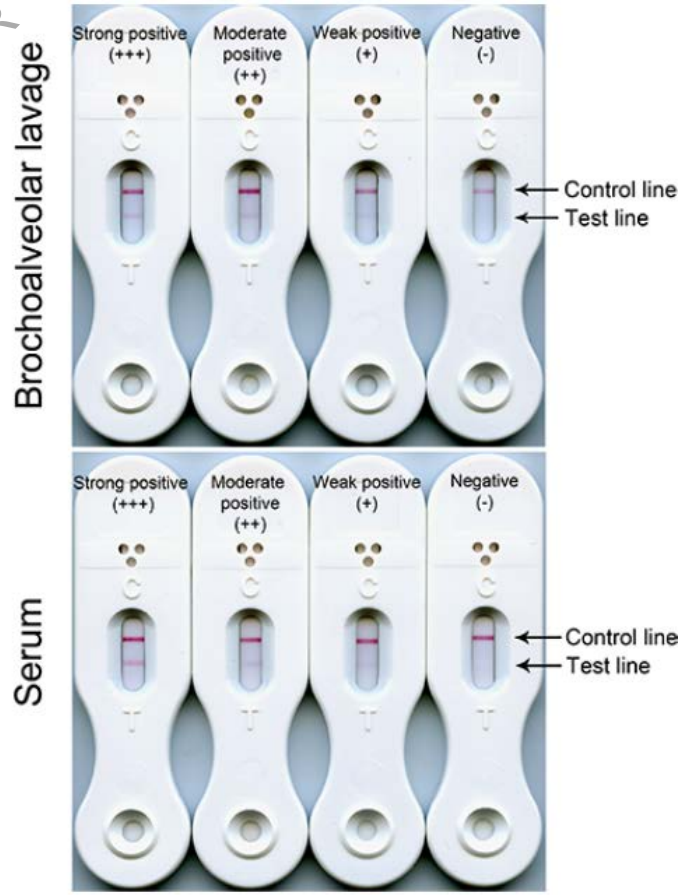
POCT tests

Lateral flow assay

- Cryptococcosis – well standardized
- Used in many laboratories, cost effective

- *Aspergillus* specific extracellular glycoprotein
- Secreted during active growth of fungi
- Mab (JF5) developed
- Lot of variability in sensitivity & specificity

- **Use of test with BAL fluid >> serum**
- Most promising in non-neutropenic patients
- Use in combination with PCR +/- GM

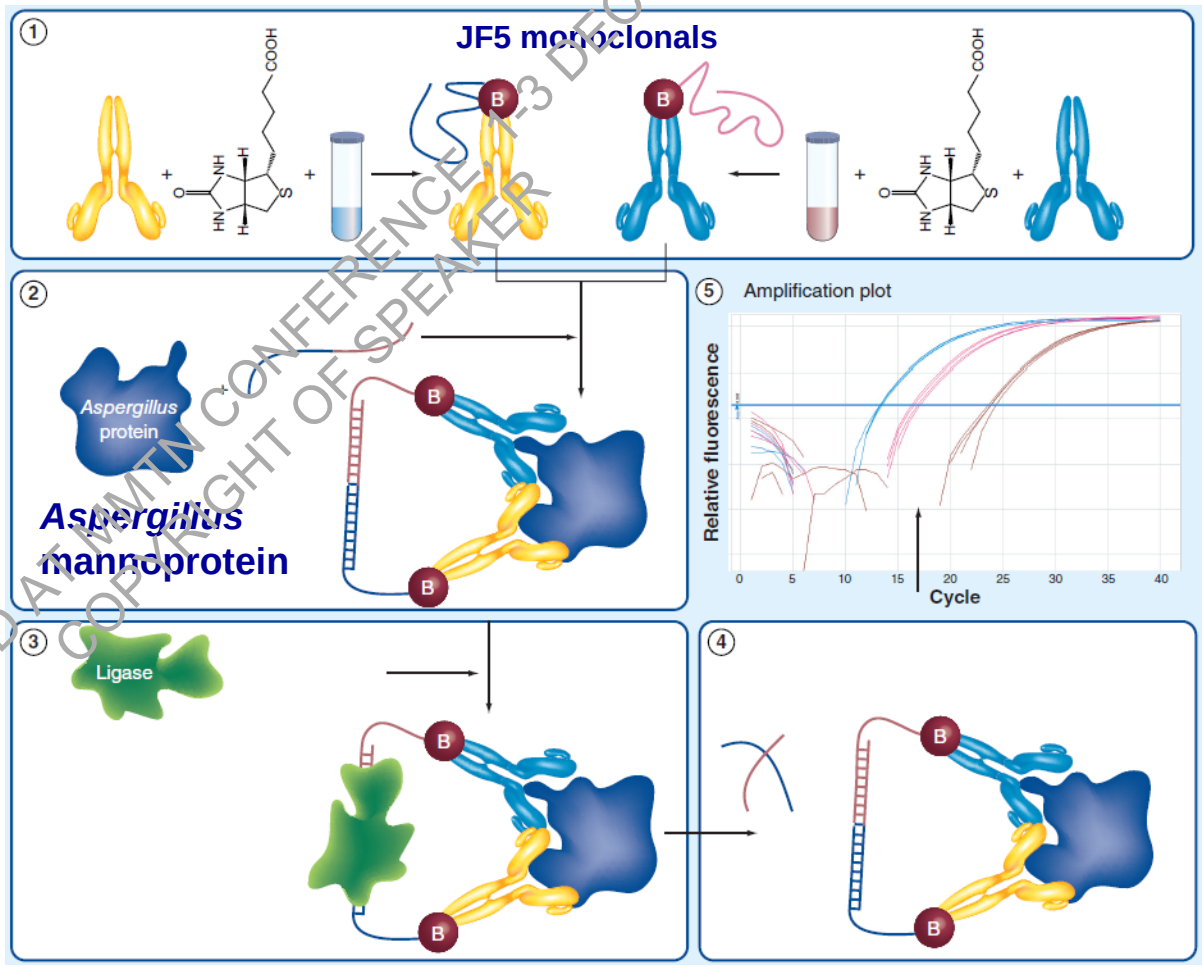


Serum specificity tests

Proximity ligation assay for the early detection of invasive aspergillosis

G. Johnson[1], M. Shannon[1], C. Thornton[1], S. Agrawal[2], C. Lass-Flörl[3], W. Mutschlechner[3], S. Bustin[1]

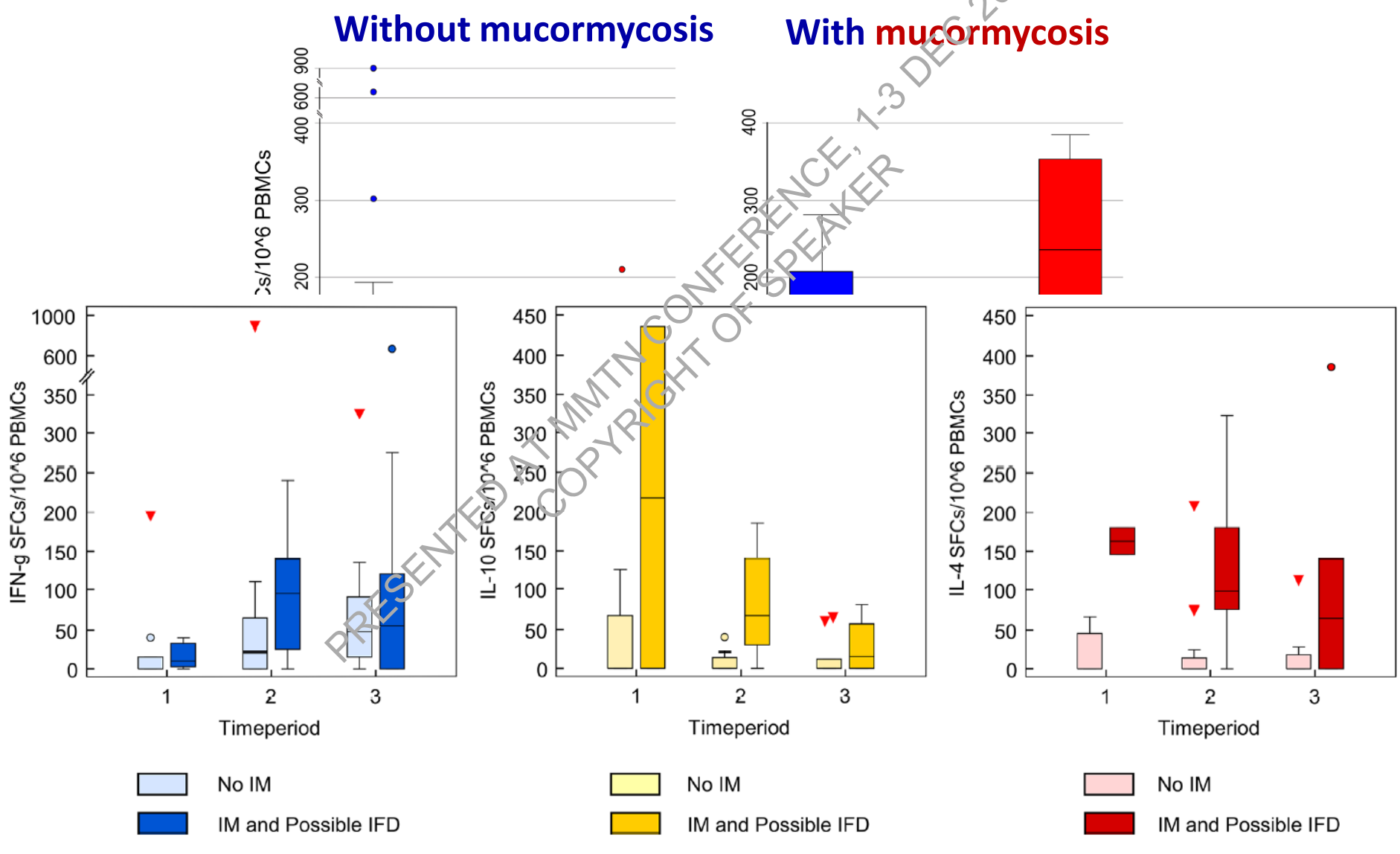
- PLA 10-100 fold higher sensitivity to GM
- 1000 fold higher sensitivity to lateral flow assay (LFD)
- No cross reaction with other fungal species



Biomark Med 2014; 8: 429-51; 25th ECCMID congress, Copenhagen, 2015

Mucorales-Specific T Cells in Patients with Hematologic Malignancies

Potenza L, et al. (2016) PLoS ONE 11(2): e0149108



Electronic Nose Cyranose ®

Electronic Nose Technology for Detection of Invasive Pulmonary Aspergillosis in Prolonged Chemotherapy-Induced Neutropenia: a Proof-of-Principle Study

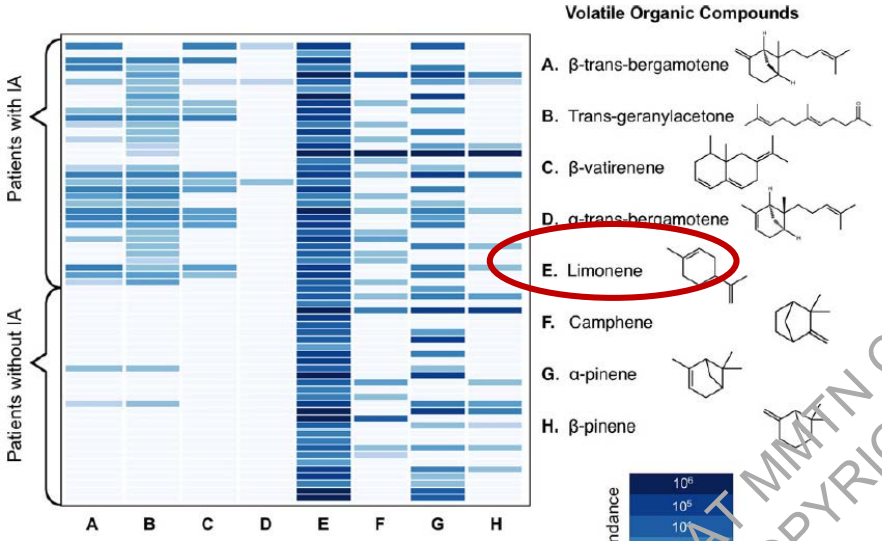
Highly effective in invasive aspergillosis in neutropenic patients

Sensitivity 100%
Specificity 83%

De Heer K. *J.Clin.Microb.* 2013;51:1490-5

A Breath Fungal Secondary Metabolite Signature to Diagnose Invasive Aspergillosis

Sophia Koo,[1,2,3,a] Horatio R. Thomas,[1,3,a] S. David Daniels,[1] Robert C. Lynch,[1] Sean M. Fortier,[1] Margaret M. Shea,[1] Preshious Rearden,[4] James C. Comolli,[4] Lindsey R. Baden,[1,2,3] and Francisco M. Marty[1,2,3]

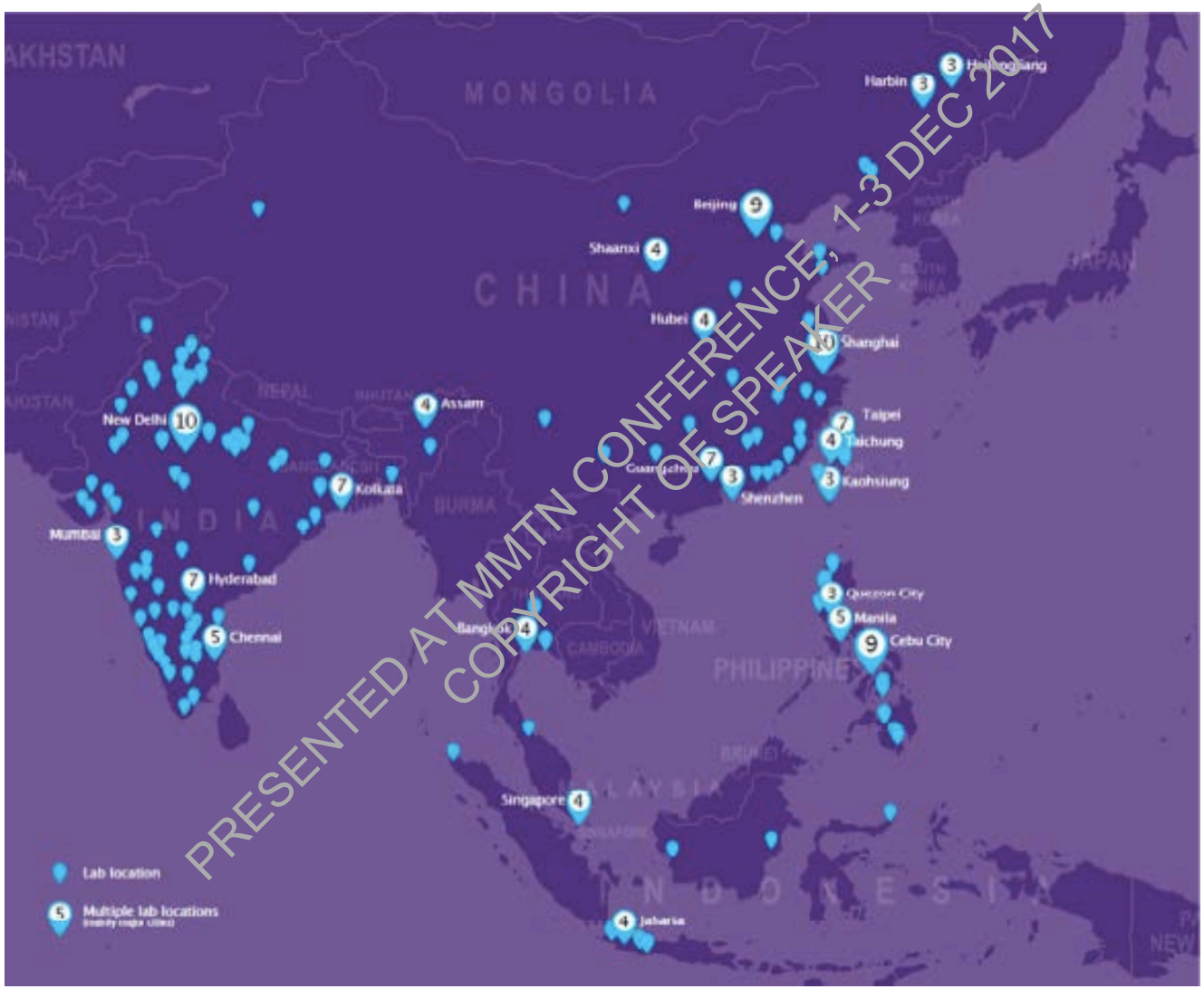


Clinical Infectious Diseases® 2014;59(12):1733–40

Parameter	Invasive Aspergillosis[a]	Other Pneumonia	Total Patients
Aspergillus metabolite signature[b] +	32	2	34
Aspergillus metabolite signature –	2[c]	28	30
Total patients	34	30	64
Test parameters			
Sensitivity (95% CI)	0.94 (.81–.98)		
Specificity (95% CI)	0.93 (.79–.98)		
Positive likelihood ratio (95% CI)	14.1 (3.69–54.0)		
Negative likelihood ratio (95% CI)	0.063 (.02–.24)		

CYRANOSE 320

Present scenario in Asian countries; 241 laboratories surveyed



Chindamporn *et al*. Med Mycol 2017 (accepted)

Present scenario in Asian countries

Tests	Overall n=241 (%)	China n=71 (%)	India n=104 (%)	Indonesia n=11 (%)	Philippines n=26 (%)	Singapore n=4 (%)	Taiwan n=18 (%)	Thailand n=7 (%)
Crypto Ag	65.2	66.7	58.3	50.0	75.0	100	100	50.0
Histo Ag	2.6	5.0	2.7	0	0	0	0	0
Candida Ag	14.8	43.8	7.1	22.2	0	0	0	0
GM	**22.8**	25.4	26.9	9.1	11.5	25.0	27.8	14.3
BDG	**10.0**	25.4	3.8	0	3.8	0	0	14.3
PCR	**37.8**	43.8	46.2	0	1 lab	0	0	0
TDM	**38.1**	58.3	16.7	0	0	0	0	0

Almost no access biomarker tests in Indonesia, Philippines, Thailand

Chindamporn *et al*. Med Mycol 2017 (accepted)

Summary

Thank you!

- Areas of interest – detection of fungi in blood & tissue, rapid identification of fungi, & antifungal drug resistance in clinical samples
- Proteomic approach – MALDI, biomarkers - promising
- Genomic approach – more promising, but majority are in house & not standardized
- EAPCRI is a bold initiative, but commercial closed system required
- New initiatives – genetic susceptibility, POCT (lateral flow, proximity ligation assay, microarray, nano technology, T2)
- **Asian laboratories** – investment required, LFA – cheaper option, need to develop reference lab with availability of all biomarker tests