TIMM
Trends in Medical Mycology

EORTC / EFISG: Invasive aspergillosis management in 2017 and beyond

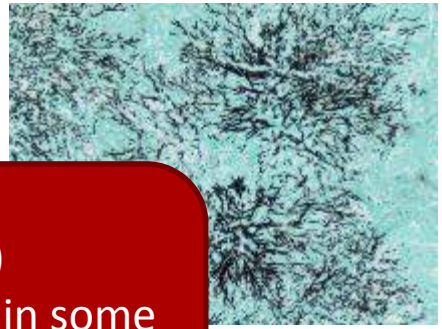
News from the lab

Ana Alastruey Izquierdo
Mycology Reference Lab Spain
Instituto de Salud Carlos III

Diagnosis of IA

Early diagnosis challenging and basic for improvement of survival

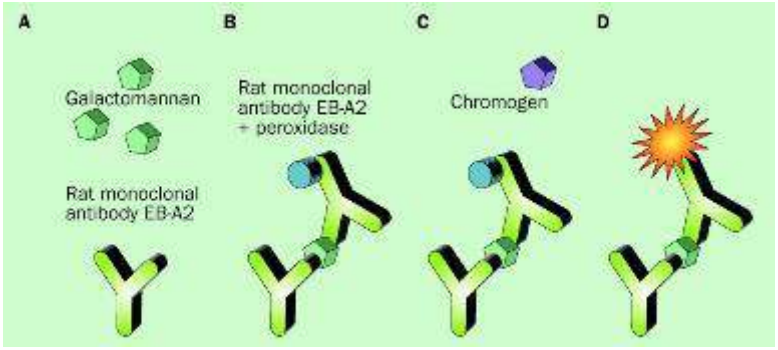
Gold standard → culture/direct sample/histopathology

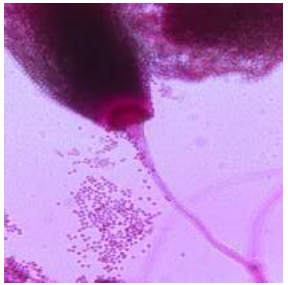


Biopsies difficult (critically ill)
Cultures low sensitivity and low PPV in some populations
Antifungal treatment

Galactomanan

- ✓ Enzyme-linked immunoassay
- ✓ Serum BAL, CSF
- ✓ Can be used for monitoring the infection





Mennink-Kersten Lancet Inf. Dis. 2004

GM for IA

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1999, p. 3223–3228 Vol. 37, No. 10
0095-1137/99/$04.00+0

Autopsy-Controlled Prospective Evaluation of Serial Screening for Circulating Galactomannan by a Sandwich Enzyme-Linked Immunosorbent Assay for Hematological Patients at Risk for Invasive Aspergillosis

JOHAN MAERTENS,[1] JAN VERHAEGEN,[2] HILDE DEMUYNCK,[1] PENELOPE BROCK,[3] GREGOR VERHOEF,[1] PETER VANDENBERGHE,[1] JOHAN VAN ELDERE,[2] LUDO VERBIST,[3] AND MARC BOOGAERTS[1]*

Departments of Haematology,[1] Paediatrics,[3] and Microbiology,[2] University Hospital Gasthuisberg, Leuven, Belgium

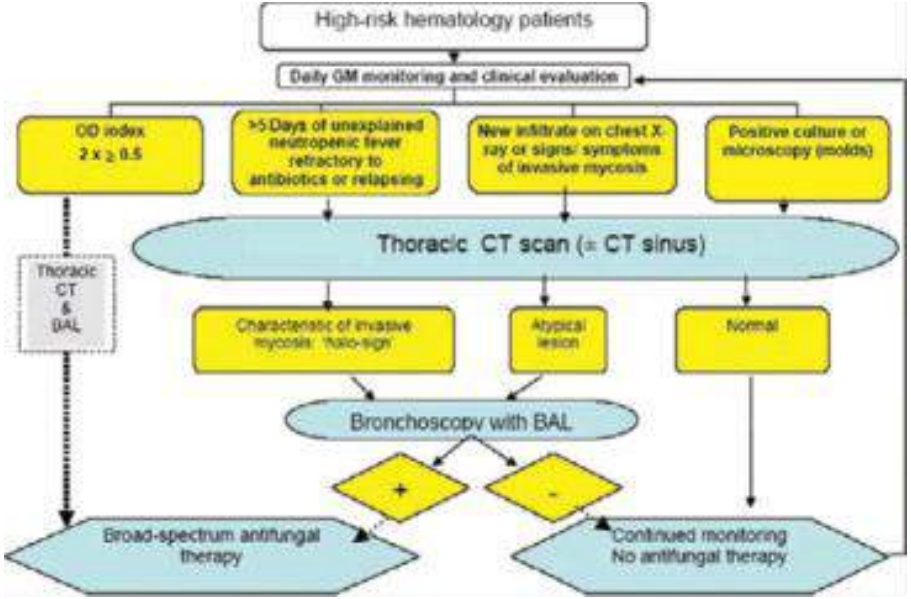
Sensitivity: 92.3%
Specificity: 95.4%

Bronchoalveolar Lavage Fluid Galactomannan for the Diagnosis of Invasive Pulmonary Aspergillosis in Patients with Hematologic Diseases

Johan Maertens,[1] Vincent Maertens,[1] Koen Theunissen,[2] Wouter Meersseman,[2] Philippe Meersseman,[2] Stef Meers,[1] Eric Verbeken,[3] Gregor Verhoef,[1] Johan Van Eldere,[4] and Katrien Lagrou[4]

Method	Sensitivity
GM-BAL	91.3%
Culture	50%
Microscopy	53.3%

Galactomanan



Maertens J et al. Clin Infect Dis. 2005;41(9):1242-1250.

Reduction in empiric treatment 35 → 7,7%

Utility of GM

- Other populations GM not performed well
  - ICU
  - COPD
  - AIDS
- Prophylaxis

Population	Sensitivity	Specificity
Neutropenic	96%	96%
HCT	70%	91%
Liver transplant	57%	94%
ICU	38%	100%
CGD	24%	95%

Adapted from Aguado GEMICOMED 2017

Maertens, Blood 01; Bretagne CID 98; Fortun, Transplant 01; Walsh, ICAAC 02

Galactomanan

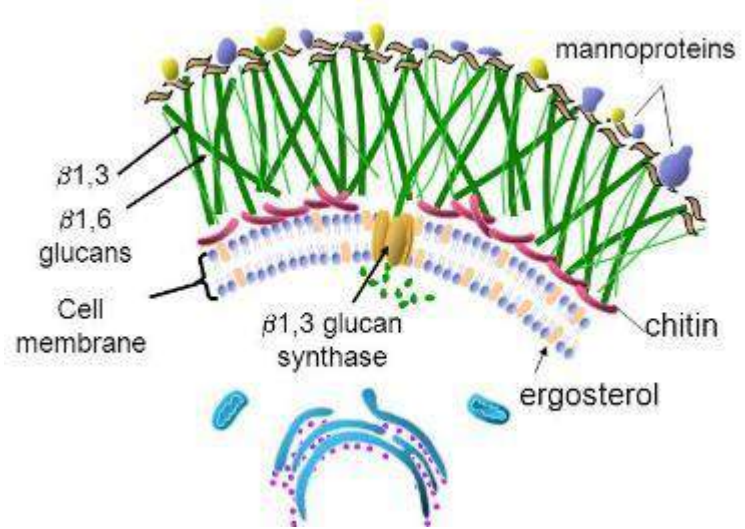
Table 2 Biological and epidemiological factors that influence the performance of GM detection in invasive aspergillosis[3]

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 IFD
Molecular structure of released galactomannan	Prevalence of IFD
Underlying condition/neutropenia/ level of immunosuppression	Cutoff for positivity
Renal clearance, hepatic metabolism	Laboratory experience
Circulating galactomannan antibodies	Nutritional factors (galactomannan-containing food)
Storage of clinical sample	Treatment with semi-synthetic β-lactam antibiotics
Pre-analytical treatment procedure	

ECIL recommendations for the use of biological markers for the diagnosis of invasive fungal diseases in leukemic patients and hematopoietic SCT recipients
O Marchetti, F Lamoth, M Mikulska, C Viscoli, P Verweij and S Bretagne and the European Conference on Infections in Leukemia (ECIL) Laboratory Working Groups

β-D glucan

- "pan fungal" (No Crypto no Mucorales)
- Sensitive
- Low specificity



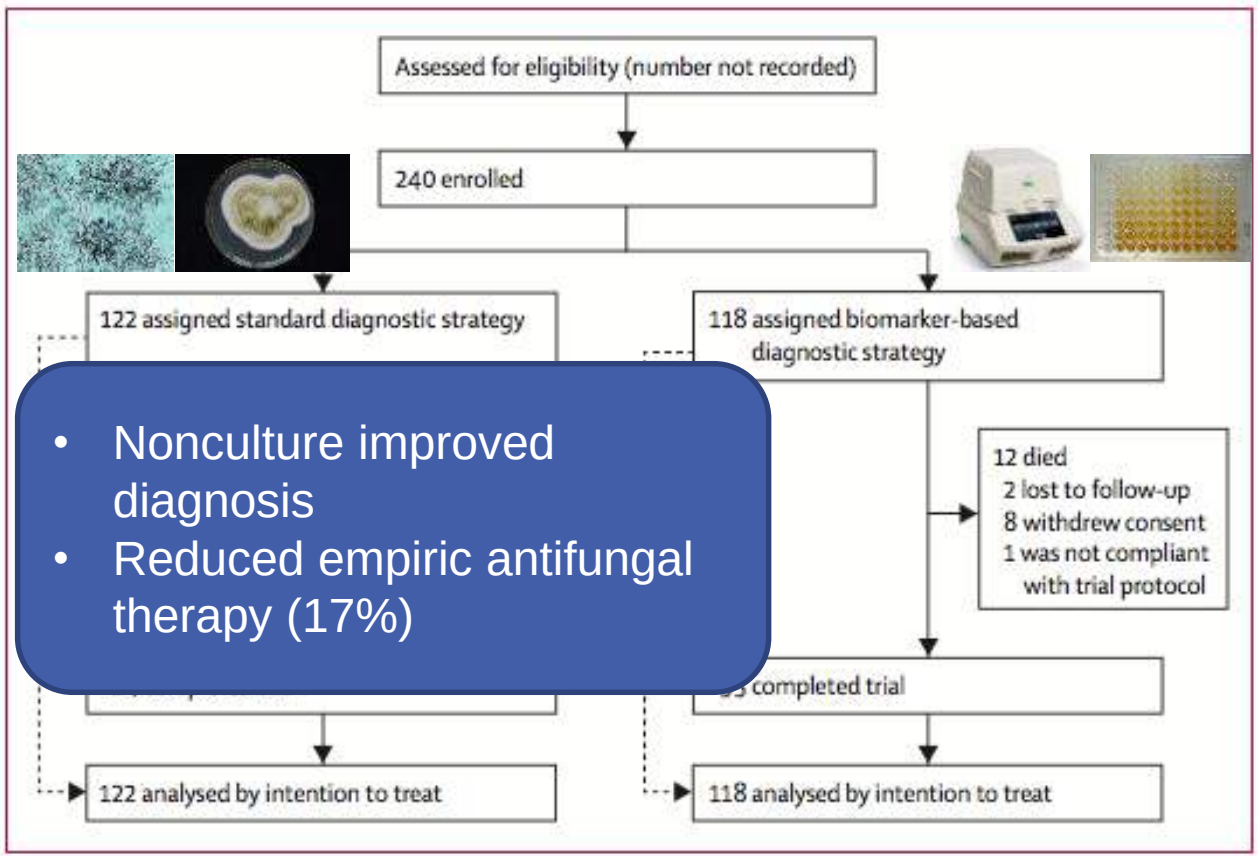
http://www.nammex.com/redefining-medicinal-mushrooms/

PCR

- Standardized
- Validation
- Variability
- EAPCRI --> DNA extraction

Biomarkers vs Standard

Multicenter randomized controlled trial



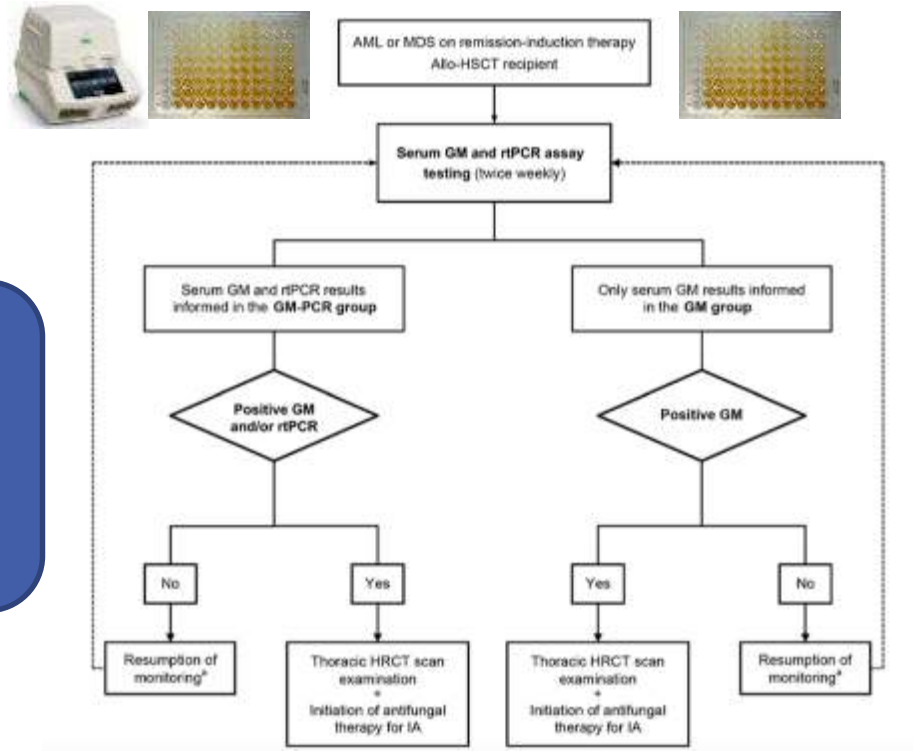
- Nonculture improved diagnosis
- Reduced empiric antifungal therapy (17%)

Morrissey et al. Lan. Infect. Dis. 2013

GM vs GM+PCR

Multi- center randomized study
PCRAga

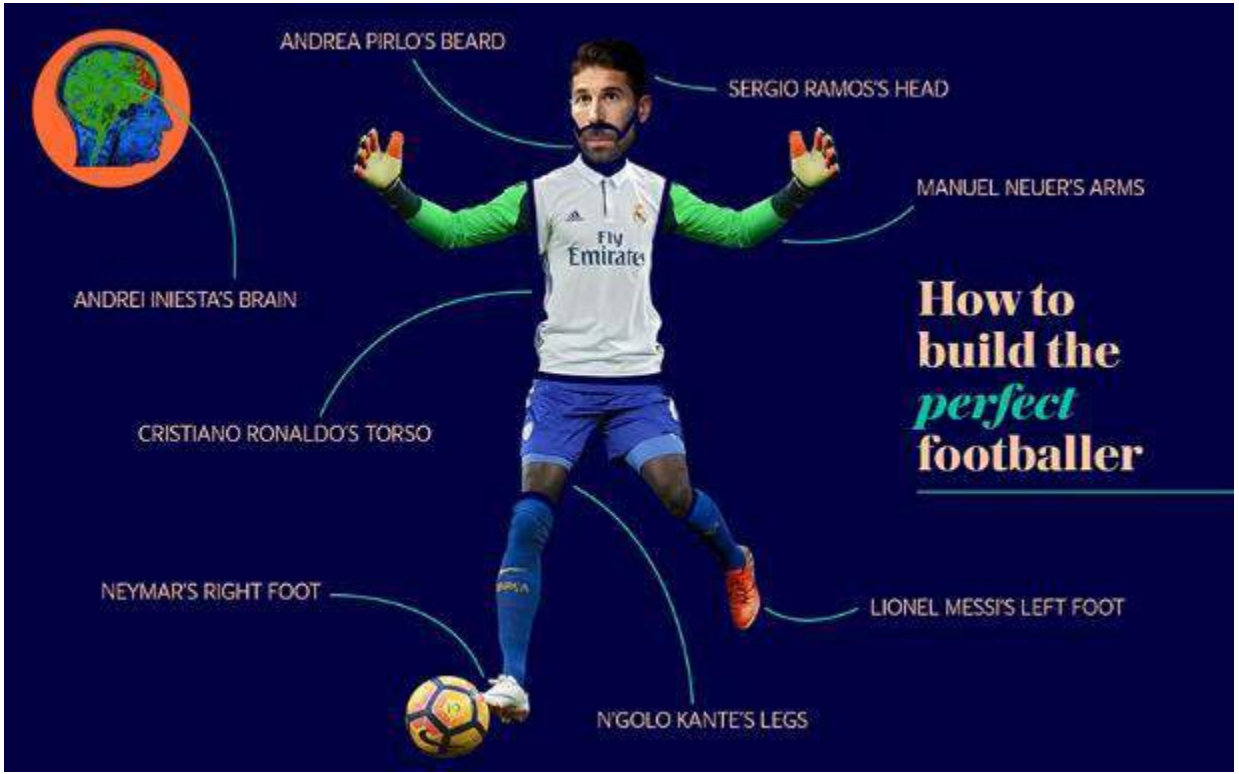
- ✓ Median to diagnosis GM-PCR (13 vs 20 days *P*= 0.022)
- ✓ Reduced empiric antifungal therapy (17% vs 29% *P*= 0.027)



Aguado et al. Clin Inf Dis 2015

No one is perfect

- Combinations



No one is perfect

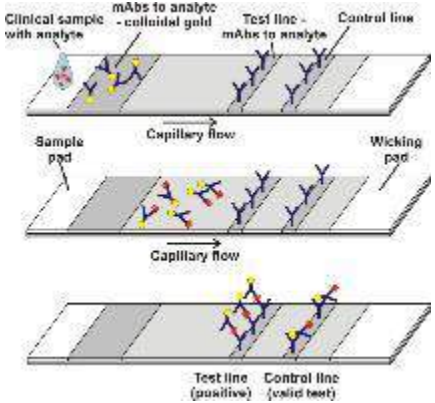
- Combinations
- New methods needed but…
- No reliable gold standard for validation
- EORTC/MSG
  - Proven: culture or histology ster
  - Probable: host + clinical + microb
  - Possible: host + (clinical or microbiological)

Miss classification possible
Source of variability

http://transplant-id.blogspot.rs/2015/12/2008-eortc-msg-criteria-invasive-fungal-disease-shortcomings.html

Lateral Flow assay

- Monoclonal Ab
- Detects extracelular glicoprotein
- Secreted during active growth of *Aspergillus* spp



PERFORMANCE CHARACTERISTICS

1. In haematological malignancy patients using serum EORTC Proven/probable IPA (*n* = 22) versus no IFD (*n* = 59)

Published study conducted at School of Medicine, Cardiff University, Wales, UK6. LFD compared to Bio-Rad Platelia GM-EIA and real-time PCR.

ASSAY	SENSITIVITY	SPECIFICITY	NPV
LFD	81.82% (61.5-92.7)*	84.75% (73.5-91.8)*	92.59% (82.5-97.1)*
PCR	95.45% (78.2-99.2)*	72.88% (60.4-82.6)*	97.73% (88.2-99.6)*
GM-EIA	77.27% (56.6-89.9)*	81.36% (69.6-89.3)*	90.60% (79.8-95.9)*

* = 95% Confidence Intervals, NPV = Negative Predictive Value

2. In haematological malignancy patients and solid organ transplant recipients using BAL (*n* = 37; 27 HM, 10 SOT; EORTC probable IPA *n* = 12)

Published study conducted at Medical University of Graz, Austria4. LFD compared to Bio-Rad Platelia GM-EIA.

SENSITIVITY	SPECIFICITY	NPV
100%	81%	100%

NPV = Negative Predictive Value

GM levels in LFD negative BALs were significantly lower than in LFD positive BALs (n = 22; median <0.4 ODI; [IQR] <0.4-<0.4 vs. n = 17; **median 1.50 ODI**; [IQR] 0.72-11.33; p < 0.0001, Mann-Whitney U test). GM levels were also significantly lower in samples with weak LFD positives (n = 8; **median 0.97 ODI**; [IQR] <0.4-1.23), than in moderate or strong positive LFD samples (n = 9; **median 4.66 ODI**; [IQR] 2.8-19.3; p = 0.0012, Mann-Whitney U test).

BIBLIOGRAPHY

1. Thornton CR. (2013). Lateral-flow device for diagnosis of infection. Current Fungal Infection Reports, published online. DOI:10.1007/s12281-013-0136-x.
2. Thornton CR. (2008). Development of an immunochromatographic Lateral-Flow Device for Rapid Serodiagnosis of Invasive Aspergillosis. Clinical and Vaccine Immunology 15: 1095-1105.
3. Thornton CR, Johnson G, Agrawal S. (2012). Detection of invasive pulmonary aspergillosis in haematological malignancy patients by using lateral-flow technology. Journal of Visualized Experiments 61: e3721.
4. Hoenigl MW, Kaidl C, Duettmann W, Seeber K, Wagner J, Buzina W, Weiler A, Raggam RB, Thornton CR. (2012). Bronchoalveolar lavage lateral-flow device test for invasive aspergillosis diagnosis in haematological malignancy and solid organ transplant patients. Journal of Infection 65: 588-591.
5. White PL, Parr C, Thornton CR, Barnes RA. (2013). An evaluation of real-time PCR, galactomannan ELISA and a novel lateral-flow device for diagnosis of invasive aspergillosis. Journal of Clinical Microbiology 51: 1510-1516.
6. Hoda J, Schmidt T, Thornton CR, Kotter E, Bertz H. (2013). Comparison of a Novel Aspergillus Lateral-Flow Device and the Platelia® Galactomannan Assay for Diagnosis of Invasive Aspergillosis following Haematopoietic Stem Cell Transplantation. Infection, published online. DOI: 10.1007/s15010-013-0472-0.

Lateral Flow assay

- ✓ Serum (pretreatment) and BAL samples
- ✓ Fast and cheap
- ✓ Sensitivity 68% (52-81%) specificity 87% (80-92)
- ✓ Cross-reaction with *Penicillium*
- ✓ Fast (15 min) and cheap
- ✓ Lower sensitivity in prophylaxis and patients with hematological malignancies

Table 3 Sensitivity of BALF LFD for probable/proven IPA in patients with and without antifungal prophylaxis/therapy (information only available for a proportion of cases published).[a]

	BALF LFD sensitivity for IPA overall percentage (absolute numbers)
Overall	75% (50/67)
Under mold active systemic antifungals	56% (14/25)
Without mold active antifungals	86% (36/42)

Table 1 Per BALF sample performance of the BALF *Aspergillus* LFD for probable/proven invasive pulmonary aspergillosis versus no evidence for invasive pulmonary aspergillosis in different patient cohorts (percentage and absolute numbers)[a]

Patient group	Sensitivity	Specificity	PPV	NPV
Overall[b]	73% (83/113)	90% (498/552)	61% (83/137)	94% (498/528)
Solid organ transplantation	94% (15/16)	92% (89/97)	65% (15/23)	99% (89/90)
Intensive care unit	79% (26/33)	85% (176/206)	46% (26/56)	96% (176/183)
Respiratory diseases	78% (25/32)	91% (196/215)	57% (25/44)	97% (196/203)
Hematological malignancies	67% (36/54)	91% (126/139)	73% (36/49)	88% (126/144)

Hoenigl J et al. Infect. 2012

Hoenigl M et al. Journal of Clinical Microbiology 2014

Pan Z et al J Med Microbiol. 2015

Heldt Curr Fungal Infect Rep 2017

Lateral Flow assay

ISHAM
INTERNATIONAL SOCIETY FOR HUMAN AND ANIMAL MYCOLOGY

Medical Mycology, 2014, 52, 647–652
doi: 10.1093/mmy/myu019
Advance Access Publication Date: 17 June 2014
Short Communication

Short Communication

Serum and urine galactomannan testing for screening in patients with hematological malignancies

Wiebke Duettmann[1,2], Christoph Koidl[3], Katharina Troppan[2], Katharina Seeber[1], Walter Buzina[3], Albert Wölfler[2], Jasmin Wagner[1], Robert Krause[1] and Martin Hoenigl[1,4,*]

- ✓ Galactomannan in urine

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology

CrossMark

Urine Galactomannan-to-Creatinine Ratio for Detection of Invasive Aspergillosis in Patients with Hematological Malignancies

Frederike M. J. Reischies,[a] Reinhard B. Raggam,[b] Juergen Prattes,[a] Robert Krause,[a] Susanne Eigl,[c] Agnes List,[d] Franz Quehenberger,[e] Volker Strenger,[f] Albert Wölfler,[d] Martin Hoenigl[a,c,g]

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0.1)

PLOS ONE

OPEN ACCESS Freely available online

Detection of Urinary Excreted Fungal Galactomannan-like Antigens for Diagnosis of Invasive Aspergillosis

Simon F. Dufresne[1,5*], Kausik Datta[1*], Xinming Li[3,6], Ekaterina Dadachova[3], Janet F. Staab[1], Thomas F. Patterson[4], Marta Feldmesser[5], Kieren A. Marr[1,2]

1 Johns Hopkins University School of Medicine, Baltimore, Maryland, United States of America, 2 The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins Medical Institutions, Baltimore, Maryland, United States of America, 3 Albert Einstein College of Medicine, Bronx, New York, United States of America, 4 University of Texas Health Science Center and South Texas Veterans Healthcare System, San Antonio, Texas, United States of America, 5 Département de Microbiologie et Immunologie, Université de Montréal, Montréal, Québec, Canada, 6 China Medical University, Shenyang, People's Republic of China

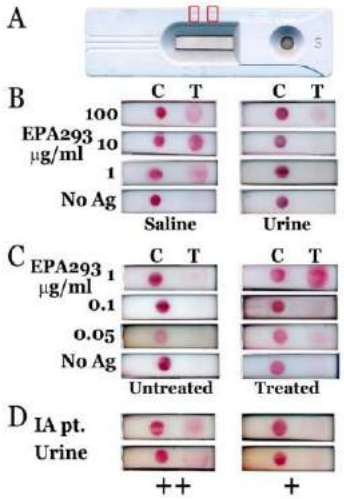
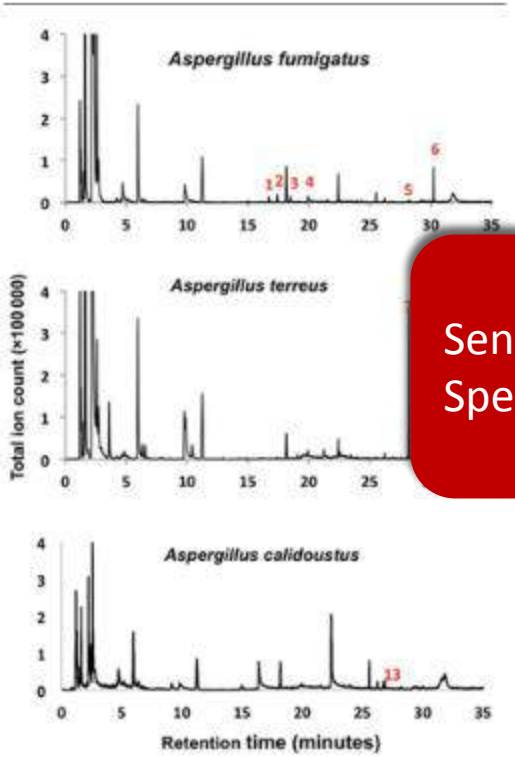


Figure 5. MAb476-based Lateral flow immunochromatographic assay device (LFD) detects Galf antigen in simulated and human urine samples. *A*, appearance of the assembled LFD; subsequent panels show scanned images of the reaction window. *B*, Comparison of urine and normal saline (NS) as diluent for EPA293 in LFD. *C*, Detection of EPA293 in simulated samples after concentration (5–10 folds, 3 KDa MWCO) and desalting (7 KDa MWCO), compared to no treatment. *D*, Detection of Galf-containing antigen in processed clinical urine samples; 2 patients have intermediate (++) positive signal and 2 have weak (+) positive signal.
doi:10.1371/journal.pone.0042736.g005

Duettmann et al. Med Mycol. 2014;52.
Reischies et al. J Clin Microbiol. 2016c;54(3):771–4.
Dufresne SF, et al. PLoS One. 2012;7(8)
Heldt Curr Fungal Infect Rep 2017

Volatile organic compounds



Sensitivity: 94%
Specificity: 93%

Table 3. Breath Aspergillus Metabolite Signature by the Reference Standard and Test Parameters

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)		

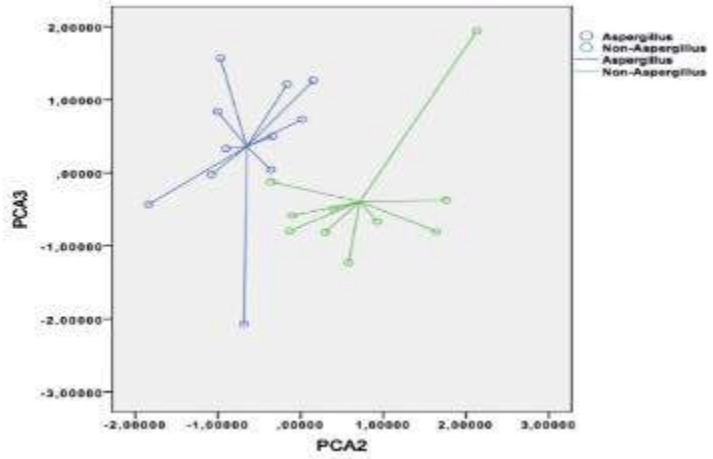
Koo S et al. Clinical Infectious Diseases 2014

Electronic nose technology in COPD

11 COPD
A.fumigatus +

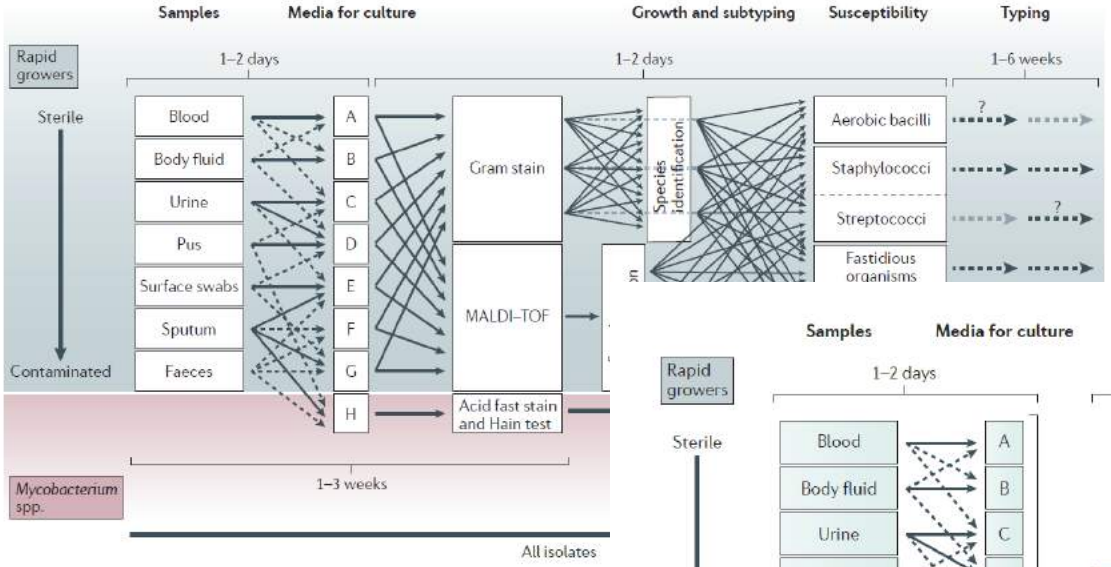
10 COPD
A.fumigatus
-

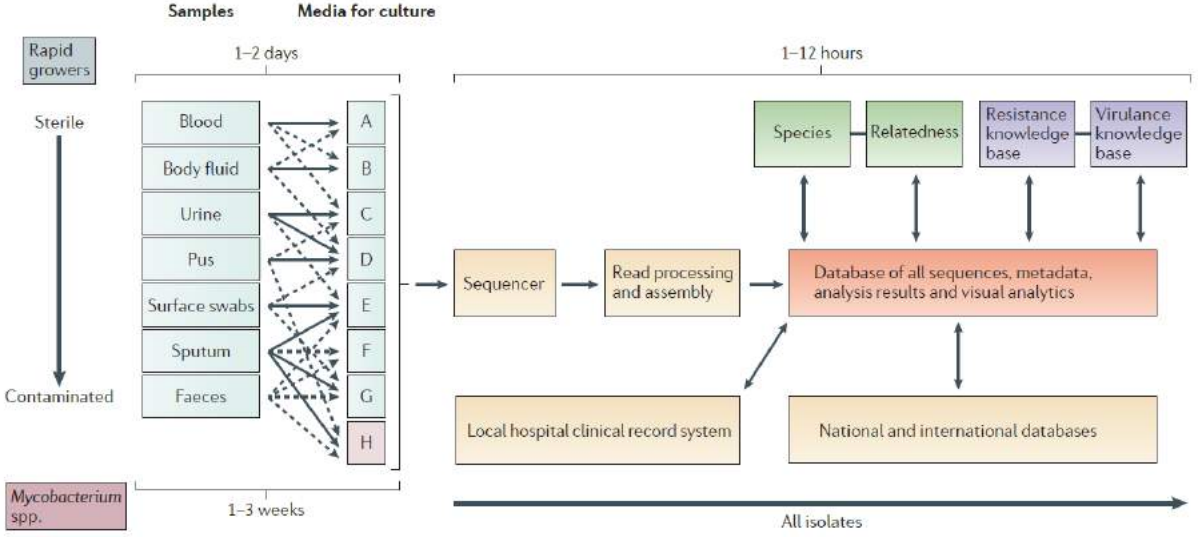
Sensor data reduced to 2 PC
Cross-validity accuracy 90.5%
Sensitivity: 91%
Specificity: 90%



Roe of Rohe et al. ECCMID 2017 #P1477

NGS





Didelot et al Nat Rev Genet. 2012

NGS

Application of next generation sequencing in clinical microbiology and infection prevention

Ruud H. Deurenberg[a], Erik Bathoorn[a,1], Monika A. Chlebowicz[a,1], Natacha Couto[a,1], Mithila Ferdous[a,1], Silvia García-Cobos[a,1], Anna M.D. Kooistra-Smid[a,b,1], Erwin C. Raangs[a,1], Sigrid Rosema[a,1], Alida C.M. Veloo[a,1], Kai Zhou[c,1], Alexander W. Friedrich[a], John W.A. Rossen[a,*]

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[b] Certe, Department of Medical Microbiology, Groningen, The Netherlands
[c] State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital of Medicine School, Zhejiang University, Hangzhou, China

R.H. Deurenberg et al. / Journal of Biotechnology 243 (2017) 16–24

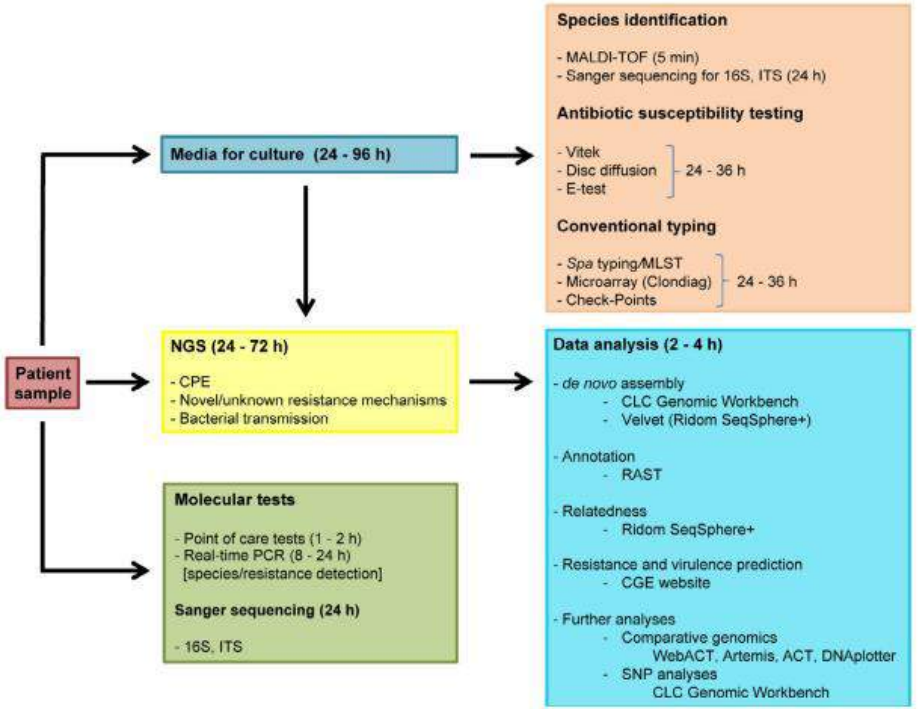


Fig. 1. A schematic overview of the general workflow of diagnostic procedures including NGS in our laboratory.

AF resistance

Delayed diagnosis

High mortality rates

Empiric and prophylactic treatment

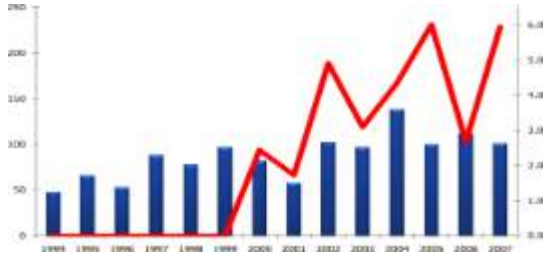
Resistance

Agricultural use of azoles

Shift in the epidemiology

secondary R

AF resistance *Aspergillus*



Snelders et al., PLOS 2008 Vol. 5:11

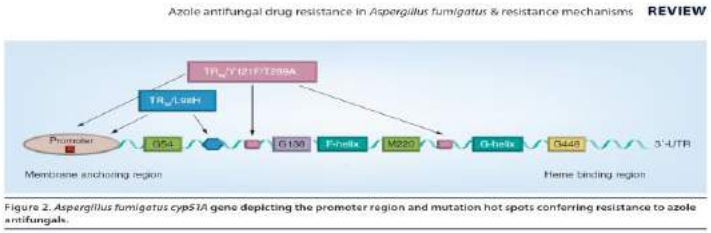
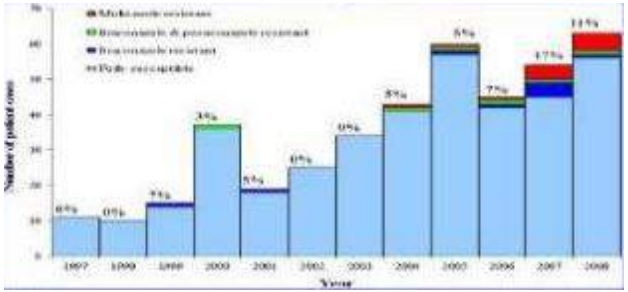


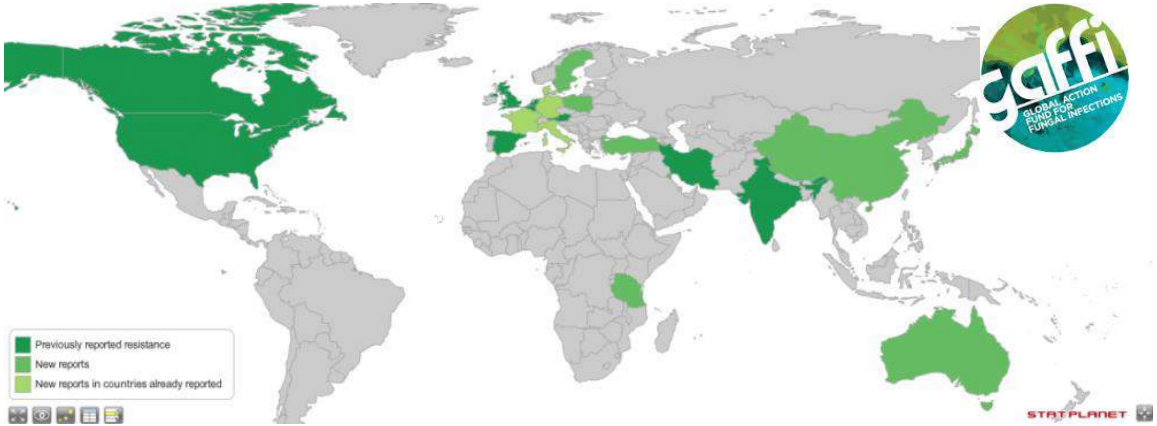
Figure 2. *Aspergillus fumigatus cyp51A* gene depicting the promoter region and mutation hot spots conferring resistance to azole antifungals.

Chowdhari Future Micro 2015

Secondary resistance

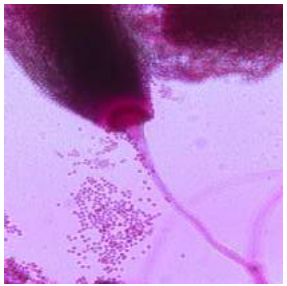


Howard et al., EID 2009, Vol. 15, No. 7



http://www.gaffi.org/uk-calls-for-agricultural-fungicide-restraint-to-reduce-azole-resistance-in-aspergillus/

Aspergillus cryptic species



11%

15%

Table 1. *Aspergillus* sp … demiological surveys from Spain and the U. S.[22]

Species	Section	Transnet N isolates	Transnet %	FILPOP N isolates	FILPOP %
A. fumigatus	Fumigati	139	63.8	156	56.1
A. lentulus	Fumigati	4	1.8	3	1.1
A. udagawae	Fumigati	3	1.4	0	0.0
N. pseudofischeri	Fumigati	1	0.5	1	0.4
A. viridinutans	Fumigati	0	0.0	1	0.4
A. fumigatiafinis	Fumigati	0	0.0	1	0.4
A. flavus	Flavi	29	13.3	27	9.7
A. alliaceus	Flavi	0	0.0	3	1.1
A. terreus	Terrei	11	5.0	26	9.4
A. carneus	Terrei	0	0.0	1	0.4
A. tubingensis	Nigri	6	2.8	22	7.9
A. niger	Nigri	13	6.0	21	7.6
A. calidoustus	Usti	6	2.8	4	1.4
A. insuetus	Usti	0	0.0	1	0.4
A. keveii	Usti	0	0.0	1	0.4
A. sydowii	Versicolores	2	0.9	1	0.4
A. versicolor	Versicolores	3	1.4	0	0.0
E. quadrilineata	Nidulantes	1	0.5	0	0.0
A. nidulans	Nidulantes	0	0.0	8	2.9
A. westerdijkiae	Circumdati	0	0.0	1	0.4
Total		218	100	278	100

Balajee et al, JCM 2009

Alastruey-Izquierdo et al. AAC 2013

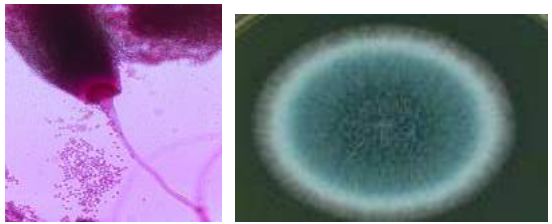
Alastruey-Izquierdo et al. ANYAS 2012

Aspergillus cryptic species

	n	AMB	ICZ	VCZ	PCZ	CPF	MCF	ANF
A. lentulus	26	**3**	**2.3**	**3.4**	**0.23**	1.6	0.1	0.1
N. hiratsukae	9	1.7	0.9	1.1	0.16	0.11	0.03	0.03
N. pseudofischerii	6	0.25	**4**	**2.51**	**0.22**	0.86	0.03	0.03
A. fumigatiaffiinis	6	**4.8**	**5**	**3.1**	**0.4**	0.22	0,03	0,03
N. udagawae	5	**2**	0.6	**2.3**	**0.25**	0.3	0.03	0.03
A. viridinutans	3	0,7	**16**	**4**	**0,25**	5,66	0,06	0,09
A. tubingensis	22	0.11	0.42	0.76	0.09	0.3	0.05	0.03
A. calidoustus	19	0.9	**8.6**	**6.2**	**6.8**	0.5	0.04	0.04
A. insuetus	2	0.7	**11.3**	**8**	**2.8**	5.6	1.4	0.9
A. keveii	1	0,25	**16**	**16**	**16**	16	16	16
A. alliaceus	30	**19.25**	0.2	0.5	0.11	12.15	3.8	1.9

Data from Mycology Reference Lab, CNM, ISCIII

Screening for azole resistance in the lab



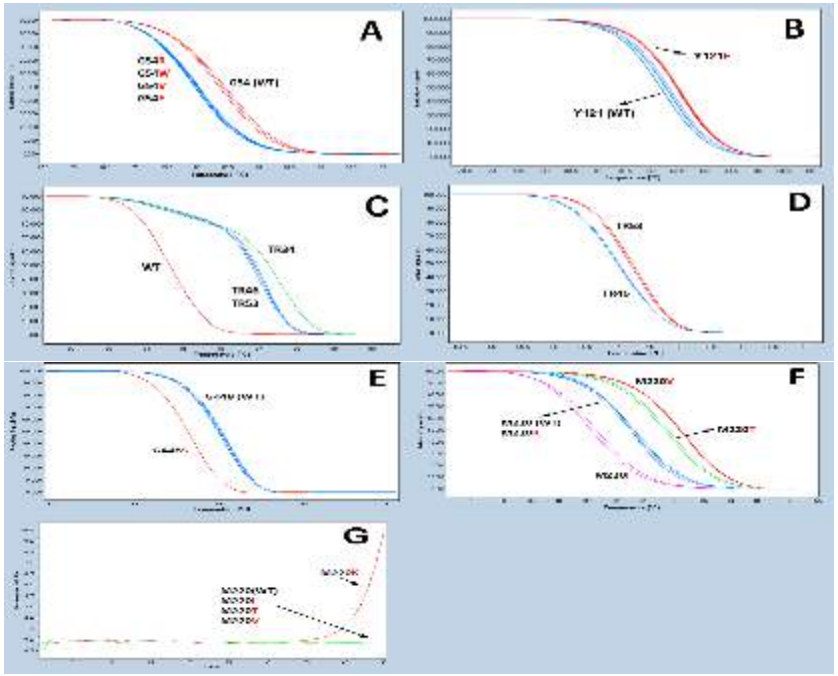
40 WT and 39 Azole R mutants
3 labs/2 readers
Good agreement

Meletiadis et al. ECCMID 2017 # P1750

Sensitivity & specificity based on presence of *CYP51A* mutations & growth in >1 azole containing agar well

Centre	Balis Plates - Observer 1		PV Plates - Observer 1		Balis Plates - Observer 2		PV Plates - Observer 2	
	Sens.	Spec.	Sens.	Spec.	Sens.	Spec.	Sens.	Spec.
ITC/VRC/PSC								
Overall	99%	99%	99%	98%	99%	100%	99%	98%
ITC/VRC								
Overall	99%	99%	99%	98%	99%	100%	98%	98%
ITC/PSC								
Overall	88%	99%	88%	98%	87%	100%	89%	98%
VRC/PSC								
Overall	85%	99%	85%	100%	89%	100%	88%	100%

Azole resistance detection



Bernal-Martinez AAC 2017

PATHONOSTICS

AsperGenius® a multiplex Real Time PCR assay for the detection of Aspergillus fumigatus and identification of azole resistance markers

AsperGenius

Species multiplex

- *Aspergillus fumigatus*
- *Aspergillus terreus*
- *Aspergillus species*
- Internal Amplification Control (IAC)

Resistance multiplex

- L98H
- Tandem repeat 34
- T289A
- Y121F

Azole resistance detection

J Antimicrob Chemother 2013; **68**: 1497–1504
doi:10.1093/jac/dkt071 Advance Access publication 5 March 2013

Journal of Antimicrobial Chemotherapy

Azole resistance in *Aspergillus fumigatus* from bronchoalveolar lavage fluid samples of patients with chronic diseases

Yanan Zhao[1]†, Christen R. Stensvold[2]†, David S. Perlin[1] and Maiken C. Arendrup[2]*

Table 4. Mutations in the *CYP51A* gene of *A. fumigatus* detected directly from BAL fluid samples

No. of samples	Amino acid substitutions caused by non-synonymous mutations	Polymorphisms caused by synonymous mutations	Culture/azole susceptibility
Samples carrying single or multiple non-synonymous mutations			
1	N425S		*A. fumigatus*/susceptible
1	M172V[a], F46Y[a]		*A. fumigatus*/susceptible
1	L206P, L210P	G89G	negative/NA
1	K67E	G89G	*Penicillium* species/POS=1, VRC=4, ITC>4
1	Y107C	D70D, G89G	negative/NA
1	**M220V**[b]	G89G	*A. fumigatus*/susceptible
1	N33D, K80E	G89G	ND/NA
1	F41S, E66G	D70D, G89G	ND/NA
1	**P216L**[c]	G89G	negative/NA
Samples carrying synonymous mutations only			
1		G89G, F495F	ND/NA
4		D70D, G89G	3 ND; 1 negative/NA
1		V44V, G89G	negative/NA
1		G89G, F165F	*A. fumigatus*/susceptible
38		G89G	one resistant *A. fumigatus* isolate; POS=1, VRC=2, ITC>4

Summary

- ✓ No perfect diagnostic test nor diagnosis algorithm for IA
- ✓ Combination of tests
- ✓ Future promises: LFD / VOCs
- ✓ Azole resistance emerging
- ✓ Detection methods available
- ✓ Treatment options should be adapted to local situations
- ✓ AFST, epidemiological studies
- ✓ New antifungals

Thanks for your attention

TIMM
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