EFISG ESCMID FUNGAL INFECTION STUDY GROUP

European Society of Clinical Microbiology and Infectious Diseases

hmm division of hygiene and medical microbiology

Non culture based diagnosis of aspergillosis

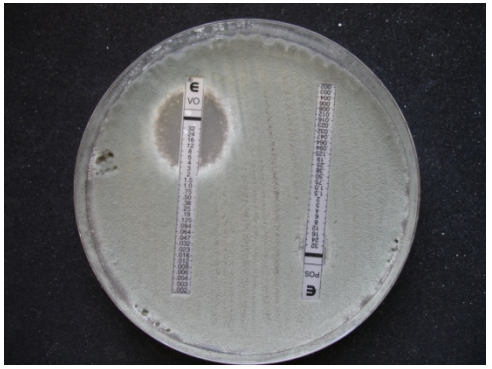
Cornelia Lass-Flörl

Innsbruck Medical University

AUSTRIA

Faculty disclosure

- Invited speaker: Pfizer, Gilead, MSD, Schering-Plough
- Consultant: Pfizer, Gilead, Schering-Plough
- Research Grants: Pfizer, Gilead, Schering-Plough



Discussion points

1. Aspergillosis appears in neutropenic as well as in non-neutropenic patients.
2. Realible diagnostic can improve outcome.
3. Conventional methods are insufficient.
4. PCR, galactomannan and ß-D-glucan tests are variable sensitive. Test „results“ - differ within the patient population.
5. Need of „combination“ of the various tests, no single method.
6. Multiple resistant (azole) strains are increasing.

Non cultured based methods

- Antigen detection
  - Galactomannan Antigen
  - ß-D-glucan
- PCR
  - Blood, serum
  - BAL, tissue
- Breath Tests
- Antibodies

Problem:
Lack of sensitive gold standard for comparison?

Shift from hematological to non-hematological patients!

57% in hematological malignancies

43% in non-neutropenic, non-hematological patients

Primary disease/underlying condition and case classification	No. (%) of patients, by outcome		
	Death (n = 63)	Recovery (n = 19)	Unknown (n = 6)
Primary disease/underlying condition			
Hematological malignancy			
All (n = 49)	29 (59)	16 (33)	4 (8)
Acute leukemia (n = 19)	8 (42)	11 (58)	0 (0)
Other hemopathy (n = 30)	21 (70)	5 (17)	4 (13)
Solid-organ transplantation (n = 10)	9 (90)	1 (10)	0 (0)
Chronic pulmonary disease (n = 18)	16 (89)	1 (5.5)	1 (5.5)
Vasculitis disease (n = 5)	5 (100)	0 (0)	0 (0)
Solid tumor (n = 3)	2 (67)	0 (0)	1 (33)
AIDS (n = 1)	1 (100)	0 (0)	0 (0)
Unknown (n = 2)	1 (50)	1 (50)	0 (0)
Case classification			
Proven IA (n = 12)	10 (83)	2 (17)	0 (0)
Probable IA (n = 52)	37 (71)	10 (19)	5 (10)
Possible IA (n = 24)	16 (67)	7 (29)	1 (4)
All cases of IA (n = 88)	63 (71.5)	19 (21.5)	6 (7)

Cornillet et al. Clin Infect Dis. (2006) 43 (5): 577-584.

Variable contribution of diagnostic tools according to the disease

	Culture Microscopy	Anti-Aspergillus antibodies	Aspergillus antigens	PCR	Imaging
Chronic aspergillosis	+	++	-	+	Radiography
Invasive aspergillosis	++	-	++	++	CT scan
Allergic aspergillosis	+/-	+	-	-/+	Radiography

Lass-Flörl et al. Expert Rev, submission

Invasive aspergillosis

Tests	Hematological malignancies
Sensitivity of culture	30-67%
Specificity of culture	72%
GM antigenemia Meta-analysis	58% -100%
ß1-D-glucan Meta-analysis	55%-68%
PCR Meta-analysis	54%-88%
Imaging : CT scan	Halo sign/ air- crescent

Antifungals decrease the sensitivity of diagnostic assays.
Prophylaxis and empirical antifungal strategies must be known to interpret the results.

Impact of neutropenia	< 100 PMN/L (n=18)	≥ 100 PMN/L (n=81)	P
Sensitivity GM	61%	19%	0.001

Reichenberger BMT 1999; Maertens JCM 1999; Pfeiffer CID 2006;
Cordonnier CMI 2009; Koo CID 2009; Mengoli Lancet ID 2009 ; White JCM 2010

Invasive aspergillosis

Tests	Solid organ recipients	COPD
Sensitivity of culture	40%-50%	83%
Specificity of culture	5-16% (Lung transplant)	22%
GM antigenemia	22%-60%	42%-48%
ß-D-glucan	Insufficient evaluation	
PCR	Insufficient evaluation	
Antibodies (precipitins)	?	+
Imaging	Mainly consolidation and nodules	

Decreased specificity
« but must not be trivialised »*

Transplant	GM antigenemia
Lung	22%-60%
Liver	56%

Bulpa ERJ 2007*; Singh CMR 2005; Cornelius JCM 2007; Pfeiffer CID 2006; Guinea CMI 2009; Meersseman CCM 2004; Cornillet CID 2006; Husain Transplantation 2007

Release of surrogate markers is a dynamic process!

Factors that influence GM performance

Site of infection

Microenvironment at site of infection (nutrients, pH, etc)

Molecule structure of released GM

Underlying condition / immunosuppression

Exposure to antifungals

Renal clearance, hepatic metabolism

Presence of GM antibodies

.....

Verweij P et al., Lancet Infect Dis 2004

Cuenca-Estrella M et al., JAC 2011

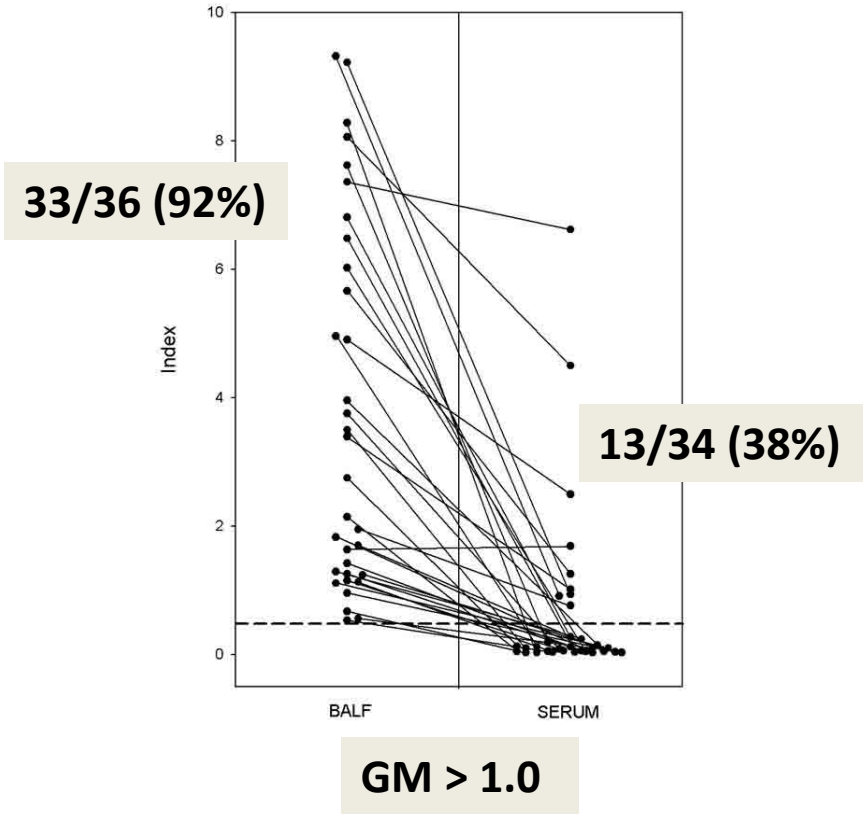
Aspergillus GM: Metaanalysis

- Twenty-seven studies 1966-2005
- Sensitivity 0.71 [95% CI, 0.68-0.74]
- Specificity 0.89 [0.88-0.90]

 in proven cases of invasive aspergillosis

„More useful in patients who have hematological malignancy or who have undergone hematopoietic cell transplantation than in solid-organ transplant recipients“

Pfeiffer CD et al, Clin Infect Dis 2006;42:1417-727



Aspergillus GM in BAL vs. Serum

Park et al., CID 2011

Antigen detection in bronchoalveolar lavage fluid for diagnosis of fungal pneumonia

Chadi A. Hage[a], Kenneth S. Knox[b], Thomas E. Davis[c] and Lawrence J. Wheat[d]

Comparison of methods for diagnosis of aspergillosis

Parameter	Microscopy	Culture	Serum GM	BAL GM	Reference
Sensitivity	53%	50%	55%	100%	[3••]
Specificity	NA	NA	NA	88%	CO 1.0
Sensitivity	40%	69%	40%	100%	[4]
Specificity	NS	100%	100%	73/98%	CO 0.5/1.0
Sensitivity	58%	69%	58%	100%	[5]
Specificity	NS	NS	NS	93%	CO 2.0
Sensitivity	55%	69%	72%	58%	[6]
Specificity	NS	NS	NS	96%	CO 0.5
Sensitivity	50%	40%	25%	100%	[7]
Specificity	93%	93%	97%	84/91%	CO 0.5/1.0
Sensitivity	29%	57%	79%	64%	[11]
Specificity	NS	NS	NS	100%	CO 0.5
Sensitivity	NS	NS	78%	78%	[12]
Specificity	NS	NS	100%	92%	CO 0.98

BAL, bronchoalveolar lavage; CO, cut-off, GM, galactomannans; NS, not significant.

Cutoff ⇧ : specificity does increase!

Current Opinion in Pulmonary Medicine 2011, 17:167–171

Use Aspergillus GM AG

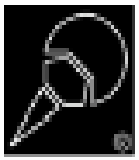
- Monitoring antigenemia HSCT
  - Always verify in second specimen
  - Serial screening (2-3 samples per week)
  - Reduction in treatment
- Workup suspected IA
  - BAL superior to serum
- Monitoring treatment?
  - Prospective study in progress
  - Candine influence GM-values?
    - A paradoxical increase in circulating *Aspergillus* antigen was observed during treatment with caspofungin. (Klont, CID 2006)
    - Batches of Cancidas (caspofungin) infusion solutions tested were GM positive
    - GM detection in Ecalta/Eraxis (anidulafungin) was variable in different batches
    - Mycamine (micafungin) gave negative test results.
    - The median GM indices were 4.4, 0.85, and <0.5 caspofungin, anidulafungin, and 100 mg micafungin. Steinmann et al., JCM, 2010

(1→3)-Beta-D-Glucan Detection Reagent Kit

GLUCATELL™

For Research Use Only

Manufactured by:



ASSOCIATES OF
CAPE COD
INCORPORATED

704 Main St. • Falmouth, MA 02540

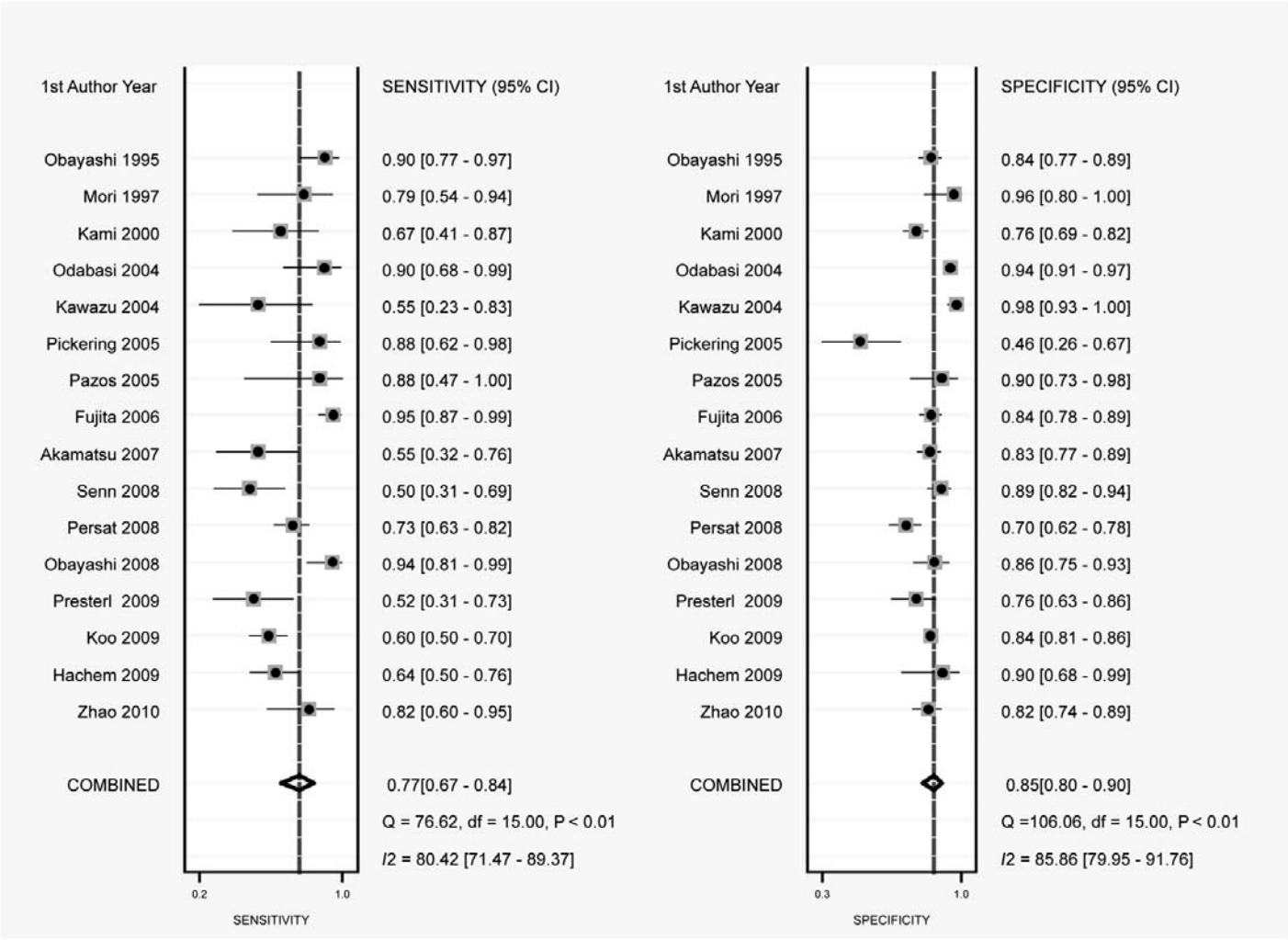
Glucatell(R), a Horseshoe Crab blood-based reagent, measures (1,3)-beta-D-glucan present in patient serum. (1,3)-beta-D-glucan is a fungal wall compound that is shed into the blood during the course of fungal infections. Glucatell(R) detects beta-glucan in the serum of patients.

ß-D-glucan and galactomannan in comparison

Diagnosis	ß-D-glucan	Galactomannan
Aspergillosis	+	+
Fusariosis	+	-
Zygomycosis	-	-
Candidämie	+	-

Yoshida, Med Myc 2006

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.



Clinical Infectious Diseases

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

Pro and cons:
the ß-D-glucan test in clinical routine

1. No difference between yeasts and molds; detects a number of different fungi, but no idea of which one (Acremonium, Aspergillus, Coccidioides immitis, Fusarium, Histoplasma capsulatum, Pneumocystis jiroveci, Saccharomyces cerevisiae, Sporothrix schenckii, and Trichosporon) (Chandrasekar et al., 2009)
2. **No considerable difference in the sensitivity of BDG testing for the detection of invasive Candida or Aspergillus infections**.
3. The sensitivity of BDG measurement seems to be lower than the specificity, 77% compared with 85%.
4. **The diagnostic performance of BDG testing for IFIs evaluated in this meta-analysis seems to be similar to that of galactomannan detection used for the diagnosis of invasive aspergillosis.**
5. Pitfalls: false-positive test result in ICU or surgical settings, as many medical products contain glucan (Chandrasekar et al., 2009)
6. **Assay technically difficult (need glucan free tubes) and expensive.**
7. **Timing and frequency of testing, cutoffs?**

Karageorgopoulos, CID, 2011

Fungal PCRs

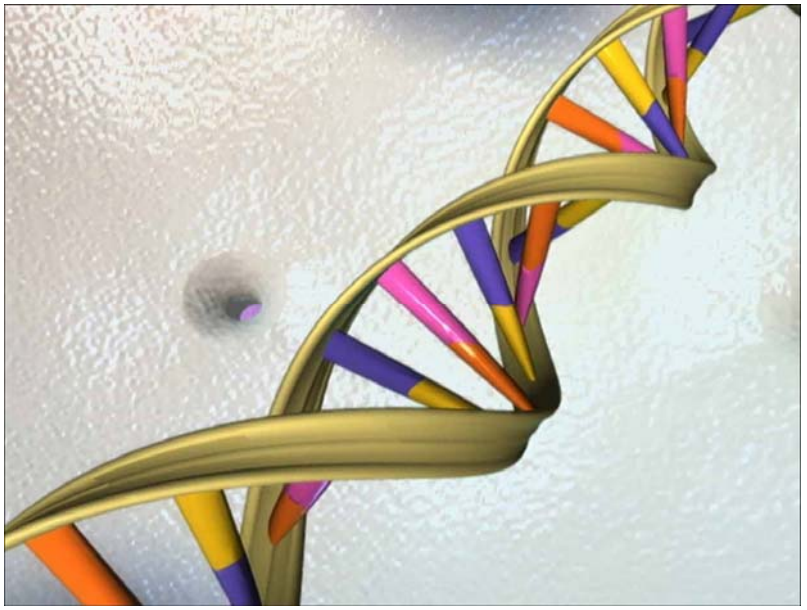
In house PCRs

– blood

– serum, plasma

– airway specimens

– tissue



Commercial PCRs

– blood

– airway specimens

Format

– PCR, PCR-ELISA, real-time PCR, nested PCR, multiplex PCR followed by microarray,…..

(In house) - PCRs: some pitfalls and variable sensitivity/specificity

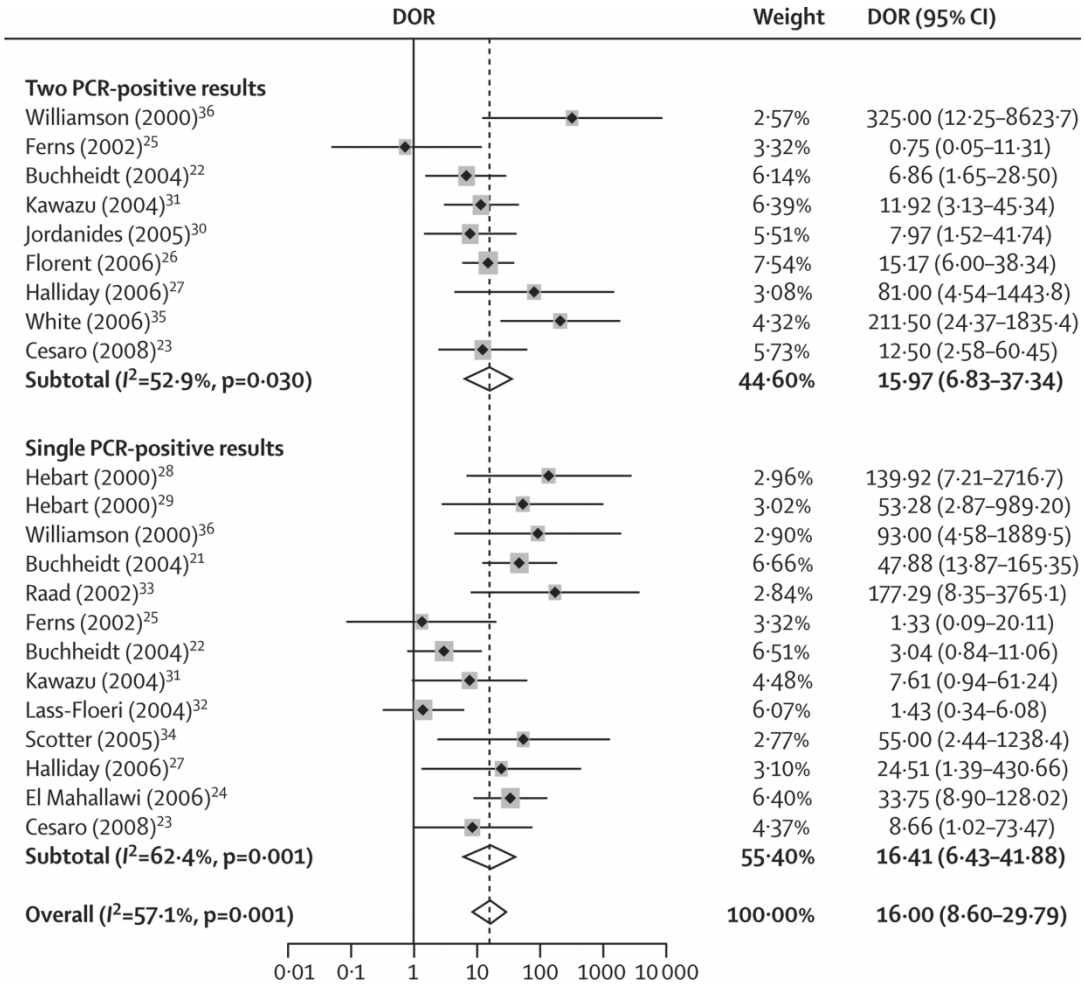
False positives due to contamination ? Cross reactivity?
False negatives ?
Other factors affect PCR assay performance & study interpretation.
Which assay, which specimen - lack of knowledge!
No validation!

PCR

- Contaminated blood collection tubes
- >500 tubes, 17 different brands
- 0-18% were Aspergillus PCR positive

Harrison, Diagn Microbiol Infect. 2010

Two positive results increase specificity and decrease sensitivity in Aspergillus PCR



Single test no value!

Mengoli et al. 2009, Infect Dis Lancet

Name	Affigene Aspergillus Tracer	MycAssay Aspergillus	Aspergillus spp. Q-PCR Alert Kit	MycoReal Aspergillus
Company	Cepheid	Myconostica	Nanogen	Inogenetix
IVD accredited	yes	yes	yes	no
Detection	Genus Aspergillus	Genus Aspergillus	Genus Aspergillus	A. fumigates, A. terreus, A. flavus, A. niger, A. nidulans and others
Species identification	no	no	no	yes
Sample materials	full blood, serum, plasma	BAL, sputum	BAL, sputum	blood, liquor, BAL, puncture specimen, tissues, paraffin
DNA extraction kits	no kit/protocol	MycXtra	EXTRAcell	protocol
Technology	Scorpions (FAM) amplification graphs	Molecular Beacons (FAM) amplification graphs	TaqMan-MGB (FAM) amplification graphs	HybProbes (LC640, 705) amplification and melting graphs
Target	18S-rRNA-gene	18S-rRNA-gene	18S-rRNA-gene	ITS-region
Internal control	plasmid (ROX)	plasmid (HEX)	beta-globin-gene (VIC)	plasmid (LC610)
PCR-platform	Mx3000P and Mx300P iQ and iQ5 Rotor-Gene 3000	Light Cycler 2.0 ABI 7500, SmartCycler MX3000	ABI 7500	LightCycler 2.0
Analysis	automatically	automatically	manually	manually
Sensitivity	0,54 genome equivalents/µl	1,3 copies of genome/PCR	10 copies of target/PCR	3 CFU/PCR
Specificity	cross reaction (Penicillium)	cross reaction (Penicillium)	cross reaction (Penicillium)	specific for Aspergillus

PCR in invasive pulmonary aspergillosis

MycAssay Aspergillus (Myconostica)

Laboratory result	ABPA	CPA	IPA	Normals
Culture positive for Aspergillus spp.	0/19	7/42 (16.7%)	20/22 (90.9%)	0/11
Culture positive for A fumigatus	0/19	7/42 (16.7%)	10/22 (45.5%)	0/11
qPCR positive for Aspergillus spp	15/19 (78.9%)	30/42 (71.4%)	21/22 (95.5%)	4/11 (36.4%)
A. fumigatus CYP51A mutation detected directly from qPCR-positive sample	6/8 (75%)	12/24 (50%)	NT[a]	NT[a]

Denning et al., CID 2011

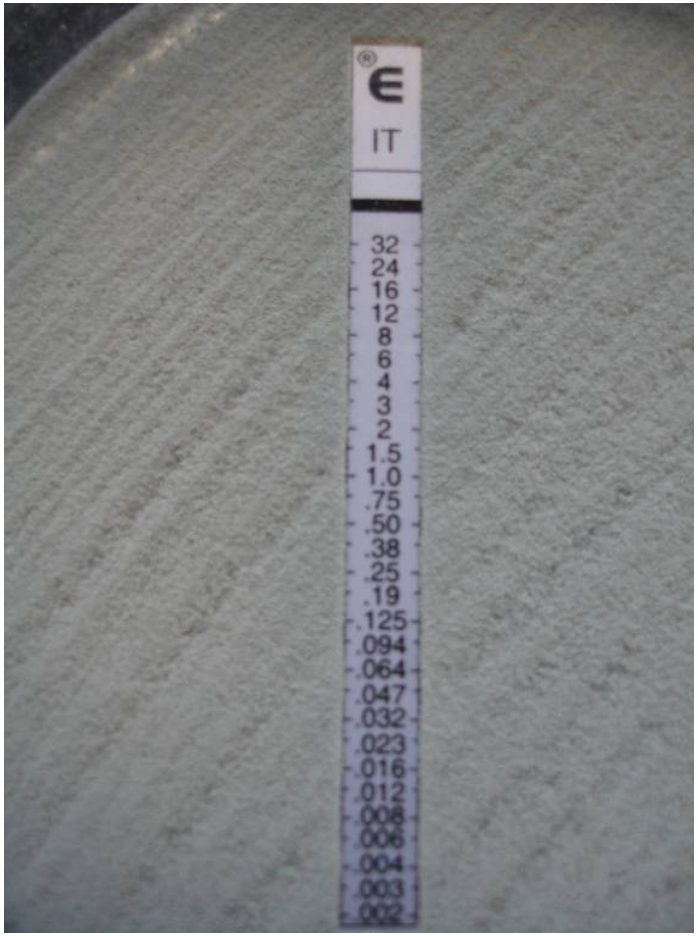
Detection of *Aspergillus* in Lung and other Tissue Samples using the FXG™: RESP (Asp +) real-time PCR kit

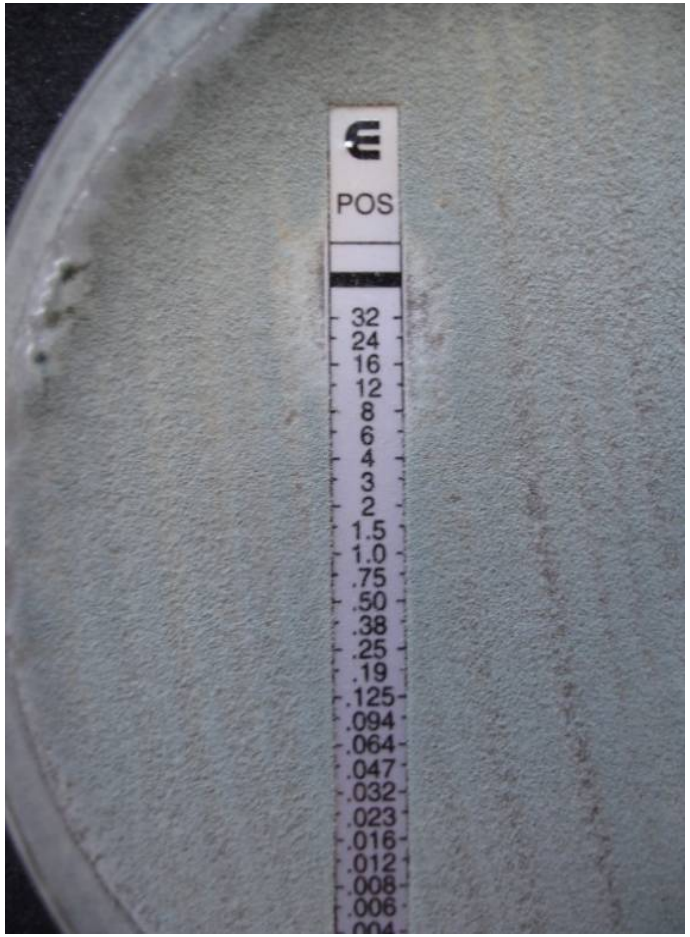
Conventional Methods		FXG: RESP (Asp +) positive	FXG: RESP (Asp +) negative
Microscopy	Septate hyphae	8	**0**
	Non-septate hyphae	0	2
	No hyphae	0	4
Culture	Aspergillus positive	8	0
	Aspergillus negative	0	8

100% sensitivity and specificity against culture.

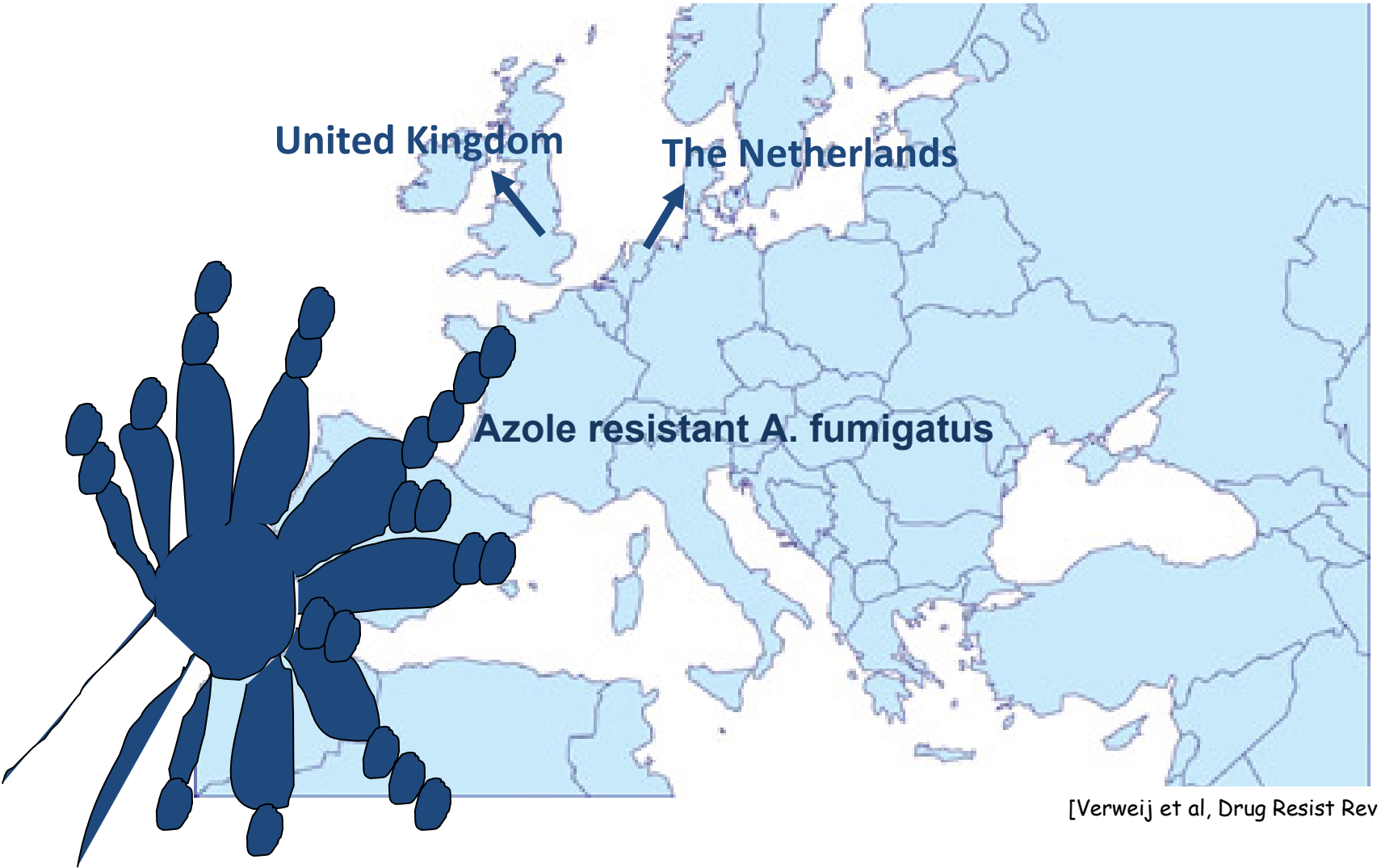
Lass-Flörl et al. submission

High-frequency of Triazole Resistance Found In Nonculturable *Aspergillus fumigatus* from Lungs of Patients with Chronic Fungal Disease!





Increasing resistance rates have been found since 2004 in the Netherlands and the UK, with 20% of patients in Manchester in 2009 having **triazole-resistant** isolates.



[Verweij et al, Drug Resist Rev 2009;12:141-7]

Laboratory result	ABPA	CPA	IPA	Normals
Culture positive for Aspergillus spp.	0/19	7/42 (16.7%)	20/22 (90.9%)	0/11
Culture positive for A fumigatus	0/19	7/42 (16.7%)	10/22 (45.5%)	0/11
qPCR positive for Aspergillus spp	15/19 (78.9%)	30/42 (71.4%)	21/22 (95.5%)	4/11 (36.4%)
A. fumigatus CYP51A mutation detected directly from qPCR-positive sample	6/8 (75%)	12/24 (50%)	NT[a]	NT[a]

Denning DW et al. Clin Infect Dis. 2011;52:1123-1129

Clinical Infectious Diseases

Novel diagnostics: progress toward a breath test for invasive Aspergillus fumigatus

- GC/MS to identify Aspergillus-derived volatile organic compounds in the head space of cultures. A promising diagnostic marker molecule is 2PF (2-Pentyl-furan).

- 2PF was found in the breath of patients with lung disease who were colonized or infected with A. fumigatus. 2 cases were investigated.

- Unanswered questions
  - how much 2PF, if any, is produced by extensive lung inflammation
  - whether food containing high levels of 2PF can cause false positive breath tests either from contamination in the mouth or gastrointestinal absorption and subsequent excretion in the breath.

Chambers et al., Med Myc 2011

Conclusion

1. Variable contribution of tools according to disease
2. Galactomannan: superior in BAL
3. ß-D-glucan: similar to GM,
4. PCR: commercial tests overcome lack of quality control, Myconostica assay good performance
5. Pro and con‘s in non-cultur assays are similar
6. Need of „combination“ of the various tests,

   no single method.

Thank you very much for your attention!