

Diagnosis of Invasive pulmonary aspergillosis in critically ill patients

WEI-LUN LIU, MD
Director, Division of Critical Care Medicine
Fu Jen Catholic University Hospital,
New Taipei, Taiwan

1

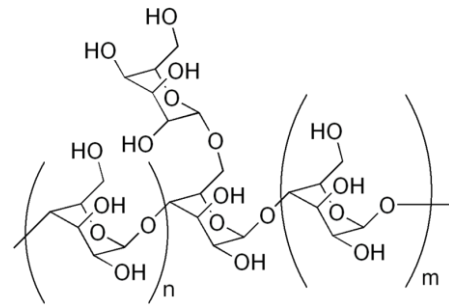
半乳糖甘露聚糖的應用

- 提煉自豆類的半乳糖甘露聚糖於食物中經常被當作安定劑 (stabilizer) 與增黏劑 (thickening agent) 使用。
- 半乳糖甘露聚糖是黴菌麴菌屬 (*Aspergillus*) 的細胞壁組成成分的一種。
- 冬蟲夏草成分的蟲草多糖 (*Cordyceps sinensis* polysaccharide) 屬於半乳糖甘露聚糖的一種。

Wikipedia

3

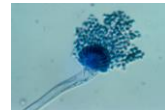
半乳糖甘露聚糖 (Galactomannan)



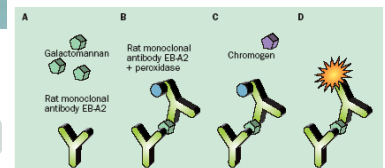
2

Galactomannan Evolution of Testing Methods

- a cell wall polysaccharide released by *Aspergillus* spp. during fungal growth in tissue.
- From hyphae, not conidia
- Help to discriminate from conidia contamination by PCR and culture

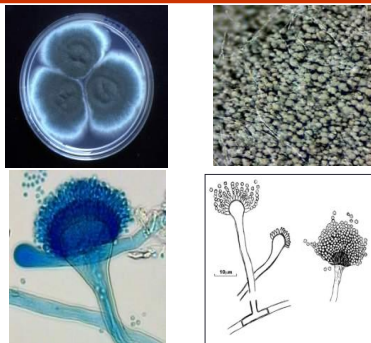


Mennink-Kersten et al.
Lancet Infect Dis 2004 4 349



4

Invasive aspergillosis : update on conventional diagnosis



Andreoni et al., Medical Mycology Atlas

5

Invasive aspergillosis : update on conventional diagnosis

Positive predictive value of a positive sputum culture for IA

Highly variable (15-77%)

Depends on host factors

allo BMT	60%
leukemia + neutropenia	70-80%
SOT	20-60%
HIV/AIDS	14-20%
Corticosteroids	20%

Perfect et al. (MSG), *Clin Infect Dis* 2001; 33: 1824

Yu et al., *Am J Med* 1986; 81: 249

Horvath and Dummer, *Am J Med* 1996; 100: 171

6

Invasive aspergillosis : update on conventional diagnosis

Yield of culture for molds in histopathologic positive samples

	n=	culture	positive
Autopsy samples	23	12	52%
Surgical or biopsy tissue	30	9	30%

Tarrand JJ et al., *Am J Clin Pathol* 2003; 119: 854

Invasive aspergillosis : update on conventional diagnosis

Culture : BAL is better than sputum (?)

Diagnostic yield of bronchoscopy specimen in histologically proven IPA			
	No. of cases	Bronchoscopy diagnostic	
Albeda 1984 ¹⁵	14	5	36 %
Treger 1985 ¹⁶	12	8	67 %
Kahn 1986 ¹⁷	27	13	48 %
Saito 1988 ¹⁸	9	0	0 %
Levy 1992 ¹¹	16	9	56 %
Mc Whinney 1993 ¹⁹	12	6	50 %
Saugier-Weber 1993 ²	10	3	30 %
Von Eiff 1995 ¹²	12	6	50 %
Horvath 1996 ⁶	29	11	38 %
Caillot 1997 ²⁰	18	8	45 %
Baron 1998 ⁴	13	8	61 %
Reichenberger 1999 ⁹	23	7	30 %
Overall	195	84	43 %

Adapted from Reichenberger et al., *Bone Marrow Transplantation* 1999; 24: 1195

Aspergillosis

Antigen Tests:

Galactomannan

Patient group	Sensitivity (%)	Specificity (%)
Allo HSCT, neutropenia, all on steroids	96	99
Neutropenia, suspected IA, GVHD, steroids	100	93
Allo HSCT	81	89
Haematologic malignancy	80	82
Neutropenia, Cut Off 1.5	88	90

ELISA, LA; Cut off 0.5, except neutropenic group

Wheat L.J, *Transplant Infect Dis* (2006), 8:128

GM tests: Sensitivities and Specificities

N=27 trials, meta-analysis

Studies	Cases of proven IA			Cases of proven or probable IA		
	Pooled sensitivity TH/TP + FN (95% CI)	Pooled specificity TN/(TN + FP) (95% CI)		Pooled sensitivity TH/TP + FN (95% CI)	Pooled specificity TN/(TN + FP) (95% CI)	
All	163/229 0.71 (0.66-0.76)	360/360 0.99 (0.98-0.99)		266/467 0.61 (0.58-0.63)	289/305 0.95 (0.92-0.98)	
Studies limited to patients with hematological malignancy	106/162 0.70 (0.62-0.77)	257/260 0.92 (0.90-0.93)		177/204 0.58 (0.52-0.64)	232/242 0.97 (0.94-0.98)	
Studies limited to patients undergoing BMAT	49/60 0.82 (0.70-0.93)	72/843 0.86 (0.83-0.88)		33/49 0.66 (0.50-0.78)	17/26 0.65 (0.44-0.83)	
Studies limited to solid-organ transplant recipients	2/9 0.22 (0.03-0.63)	18/215 0.84 (0.78-0.88)		9/22 0.41 (0.21-0.64)	21/247 0.85 (0.80-0.89)	
Studies using EORTC/MSG criteria	74/116 0.64 (0.54-0.73)	254/269 0.99 (0.98-0.99)		211/264 0.80 (0.74-0.85)	262/262 0.99 (0.97-0.99)	
Studies not using EORTC/MSG criteria	89/113 0.79 (0.70-0.86)	105/118 0.89 (0.87-0.90)		39/53 0.74 (0.60-0.85)	211/237 0.89 (0.84-0.93)	
Studies involving pediatric population only	8/9 0.89 (0.51-1.00)	216/270 0.85 (0.85-0.89)		11/12 0.92 (0.82-1.00)	12/25 0.80 (0.36-0.91)	
Studies involving adult population only	58/93 0.62 (0.52-0.72)	1211/1398 0.87 (0.85-0.88)		102/140 0.73 (0.49-0.91)	802/889 0.90 (0.89-0.92)	
Studies of both pediatric and adult populations	75/93 0.75 (0.65-0.84)	1726/1875 0.92 (0.91-0.93)		92/196 0.47 (0.40-0.54)	1601/1701 0.94 (0.93-0.95)	
Studies using a cutoff value of 0.5 for defining positivity	3/11 0.27 (0.06-0.61)	27/341 0.79 (0.74-0.83)		69/67 0.79 (0.69-0.87)	493/571 0.86 (0.83-0.89)	
Studies using a cutoff value of 1.0 for defining positivity	65/107 0.79 (0.71-0.87)	1385/1598 0.87 (0.85-0.88)		103/159 0.65 (0.57-0.72)	1162/1242 0.94 (0.92-0.95)	
Studies using a cutoff value of 1.5 for defining positivity	75/111 0.68 (0.58-0.78)	1946/2116 0.92 (0.91-0.93)		78/161 0.48 (0.41-0.56)	1183/1247 0.95 (0.93-0.96)	

• Overall Sensitivity: 0.61, Specificity: 0.93

• Zygomycetes will not be detected.

CID 2006;42:1417-27

OD cutoff and performance

OD cutoff	Sensitivity	Specificity
0.5	0.79	0.86
1.0	0.65	0.94
1.5	0.48	0.95

CID 2006;42:1417-27

Cochrane systematic review

- an overall sensitivity of 78% (61%–89%)
- an overall specificity of 81% (72%–88%)
- using a GM optical density index (GM-ODI) of 0.5 as a cutoff.

Leeflang MM, Debets-Ossenkopp YJ, Visser CE, et al. Galactomannan detection for invasive aspergillosis in immunocompromised patients. *Cochrane Database Syst Rev* (Online) 2008; (4):CD007394.

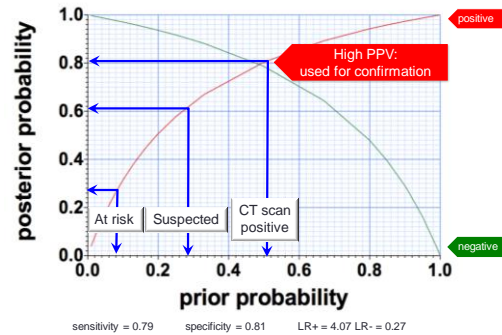
BAL galactomannan testing

- more sensitive (60% -100%) and specific (82% - 100%) than serum galactomannan.
- Two cutoff BAL galactomannan optical density (OD) indices have been proposed - ≥ 0.5 [5] and ≥ 1.0 [6-10]
- the amount of sterile saline instilled per BAL varied (40 ml to 150 ml)

Hsu et al. BMC Infectious Diseases 2010, 10:44

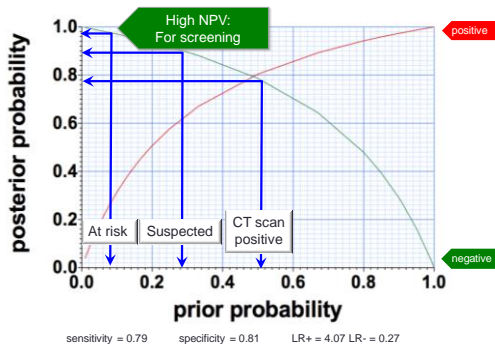
13

Post-test probabilities for serum/plasma GM (1)



14

Post-test probabilities for serum/plasma GM (2)



15

Galactomannan and Computed Tomography-Based Preemptive Antifungal Therapy in Neutropenic Patients at High Risk for Invasive Fungal Infection: A Prospective Feasibility Study

Johan Maertens,¹ Koen Theunissen,² Gregor Verhoet,³ Johnny Verschakelen,² Katrien Lagrou,⁴ Eric Verheken,⁵ Alexander Wilmet,⁶ Jan Verhaegens,⁷ Marc Boogaerts,⁸ and Johan Van Eldere⁹

Departments of ¹Hematology, ²Radiology, ³Microbiology, ⁴Pathology, and ⁵Medical Intensive Care, University Hospital Ghent, Ghent, Belgium

Results. Neutropenic fever developed in 117 episodes, of which at least 41 episodes (35%) satisfied existing criteria for empirical antifungal therapy. However, our protocol-driven preemptive approach reduced the rate of antifungal use for these episodes from 35% to 7.7% (a 78% reduction) and led to the early initiation of antifungal therapy in 10 episodes (7.3%) that were clinically not suspected of being IFI. No undetected cases of invasive aspergillosis were identified; 1 case of zygomycosis was missed. Breakthrough candidemia was diagnosed by conventional culture techniques and was treated successfully. With use of a preemptive approach, the 12-week survival rate for patients with IFI was 63.6% (it was 63.1% for those with invasive aspergillosis).

Conclusion. Preemptive therapy based on enzyme immunoassay and HRCT reduced the exposure to expensive and potentially toxic drugs and offered effective antifungal control, but it failed to detect non-*Aspergillus* IFI.

Reduce the rate of antifungal use: 35% → 7.7%
Early treatment: 7.3%

Maertens J, et al. Clinical Infectious Diseases 2005; 41:1242-50

16

A. fumigatus, Invasiveness

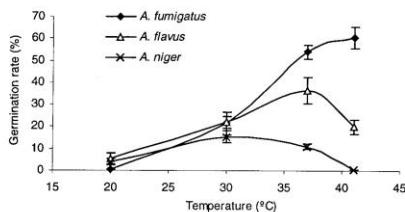


FIG. 3. Percentage of germination by 5-day-old conidia of the three tested *Aspergillus* species after incubation for 8 h in RPMI 1640 medium at different temperatures. Each point represents the mean value \pm standard error of the mean for all tested strains of each *Aspergillus* species.

Araujo R, Rodrigues AG. J. Clin. Microbiol., 42 (2004), pp. 4335-4337

17

TABLE 3. Performances of GM enzyme immunoassay and BG test for patients infected with different organisms (per sample)

Test and organism	Sensitivity (%)	Specificity (%)	PPV (%) ^a	NPV (%) ^a
GM enzyme immunoassay				
<i>A. fumigatus</i> (n = 69)	13	99	90	66
Non- <i>fumigatus Aspergillus</i> species (n = 39)	49	99	95	86
Other mold (n = 77)	6	99	83	62
BG test				
<i>A. fumigatus</i> (n = 69)	61	88	75	79
Non- <i>fumigatus Aspergillus</i> species (n = 39)	64	88	64	88
Other mold (n = 76)	47	88	72	72

^a PPV, positive predictive value; NPV, negative predictive value.

18

False-positive Aspergillus Antigenemia

- (1) cross-reaction from an existing non-Aspergillus fungal infection (*Histoplasma*, *Penicillium marneffeii*, *Trichosporon*, or *Cryptococcus*)
- (2) intravenous administration of fungal-derived products as betalactam (Piperacillin/tazobactam, amoxicillin/clavulanate....) or gluconate-containing PlasmaLyte solutions
- (3) poor postextraction management of samples in the laboratory
- (4) enteric absorption of ingested GM present in thickening gums or bacterial antigens (this has been proposed but not proven). In these cases, the relationship with mucositis is controversial.

Clinical Infectious Diseases 2012;55(4):e22–e27

19

False positive rates are higher:

- pediatric patients,
- patients with graft-versus-host disease (GVHD)
- those taking dietary GM or fungus-derived antibiotics, such as piperacillin-tazobactam (PIPC/TAZ).

High incidence of false-positive *Aspergillus* galactomannan test in multiple myeloma

Yasuo Mori,^{1,2} Yoji Nagasaki,^{1,2} Kenjiro Kamezaki,¹ Katsuto Takenaka,¹ Hiromi Iwasaki,² Naoki Harada,¹ Toshihiro Miyamoto,¹ Yasunobu Abe,² Nobuyuki Shimono,¹ Koichi Akashi,^{1,2} and Takanori Teshima²

Yasuo Mori, et al. American Journal of Hematology 2010

21

False-positive Aspergillus Antigenemia Due to Blood Product Conditioning Fluids

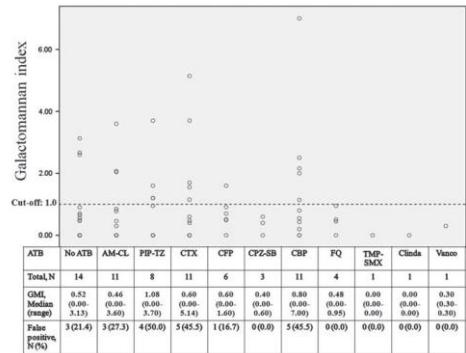
Table 2. Galactomannan Optical Density Index Readings of Blood Products

Product	Blood Collection Bag Manufacturer	Number of Samples Tested	GM-ODI Median (range)
Packed red cells	Macopharma ^a	4	0.18 (0.11–0.28)
	Fenwal ^a	2	0.22–0.30
	Fresenius Kabi ^b	13	0.95 (0.31–4.43)
Frozen fresh plasma	Fresenius Kabi ^b	18	>5.00 (>5.00)
Pooled platelets ^c	Fresenius Kabi ^b	19	>5.00 (2.90–>5.00)
Apheresis platelets ^d	Haemonetics ^e	10	0.25 (0.09–0.47)

Clinical Infectious Diseases 2012;55(4):e22–e27

20

False-positive serum and BAL *Aspergillus* galactomannan



Scandinavian Journal of Infectious Diseases, 2010; 42: 461–468

22

Journal of Antimicrobial Chemotherapy (2008) 62, 1109–1112
doi:10.1093/jac/dkn308
Advance Access publication 21 July 2008

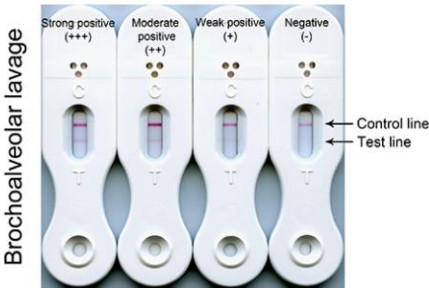
JAC

False positivity of the *Aspergillus* galactomannan Platelia ELISA because of piperacillin/tazobactam treatment: does it represent a clinical problem?

We conclude that the clinical relevance of false-positive GM results during piperacillin/tazobactam treatment is small if samples are collected prior to infusion and if a cut-off level of >0.7 is used.

23

jove Journal of Visualized Experiments
Video Article
Detection of Invasive Pulmonary Aspergillosis in Haematological Malignancy Patients by using Lateral-flow Technology
Christopher Thornton¹, Gemma Johnson², Samir Agrawal³



24

Lateral Flow Device test in ICU Patients

	Present study population	Gray	Woodruff	Vincent	Marxheim
Number of ICU patients	140 (15)	73 (5)	114 (5)	10 (1)	10 (1)
Sex					
Male	87 (65.4%)	45 (61.7%)	25 (22.2%)	6 (60%)	8 (80%)
Female	46 (34.6%)	18 (24.9%)	21 (18.7%)	7 (70.2%)	2 (20%)
Median age (range)	60 (19 to 85)	68 (19 to 83)	58 (26 to 85)	55 (41 to 76)	55.5 (40 to 75)
Type of ICU admission (n(%))					
Medical	111 (79.3%)	60 (82.1%)	55 (48.3%)	10 (100%)	10 (100%)
Neurosurgery	9 (6.4%)	5 (7.0%)	1 (1.1%)	—	—
Elective surgery	20 (14.3%)	—	4 (3.5%)	—	—
Emergency surgery	9 (6.4%)	4 (5.5%)	4 (3.5%)	—	—
Trauma	11 (7.9%)	1 (1.4%)	1 (0.9%)	—	—
ICU classification					
Intensive care (ICU) patients	45	—	2 (1.8%)	—	—
Non-ICU (NICU) patients	101 (72.1%)	65	97	10	10
Positive IPH (ICU patients)	21 (20)	6 (5)	6 (5)	—	—
No IPH (NICU patients)	104 (75)	59 (50)	91 (78)	10 (100)	—
Producible or proven IPH versus no IPH		LTD	Conventional BAL culture		
Sensitivity	87% (10/23)	87% (10/23)	87% (10/23)		
Specificity	87% (88/101)	88% (88/101)	88% (88/101)		
PPV	44% (10/23)	40% (10/25)	40% (10/25)		
NPV	96% (88/92)	96% (88/92)	96% (88/92)		
DOR (95% CI)	17.6 (5.3 to 58.3)	5.9 (2.1 to 16.3)	—		

Egli S, et al. Critical Care 2015; 19: 178.
Thornston CL. Clin Vaccine Immunol 2008;15:1005.
Thornston Car et al. 2012; J Am Fam 23:3721.

