

# Klinik Mikrobiyoloji Kongresi-2015

18-22 Kasım 2015

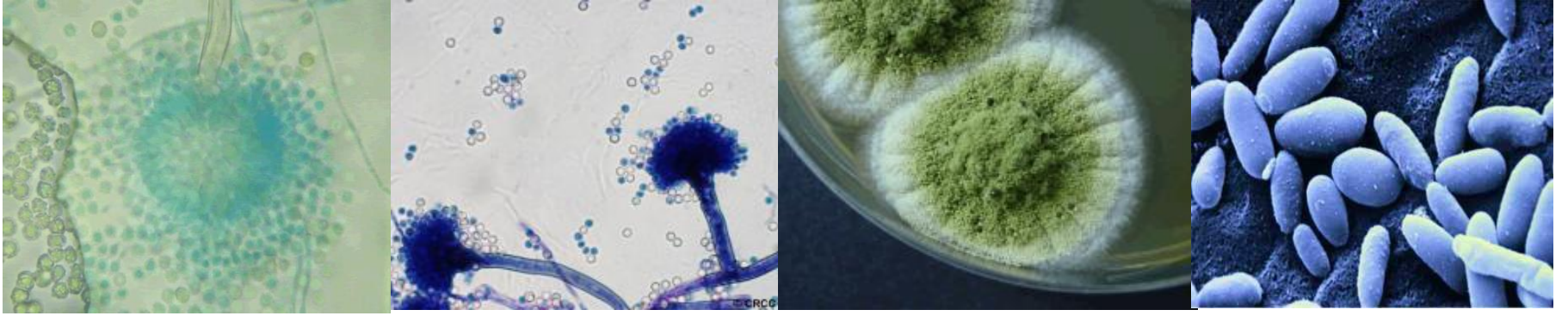
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**GÜLŞEN  
HAZIROLAN**

# TANIDA BİYO-BELİRTEÇLERİN DEĞERİ



**DR. GÜLŞEN HAZIROLAN**  
**Ankara Numune Eğitim ve Araştırma Hastanesi**

# Sunum Planı...

- *Candida* spp. : Biyo-belirteçler
- *Aspergillus* spp. : Biyo-belirteçler
- *Cryptococcus* spp. : Biyo-belirteçler
- Biyobelirteçlerinin tespitinde kullanılan moleküler testler



# İnvaziv Fungal Enfeksiyonlar-Etkenler

## Maya Mantarları

*Candida spp.*

*Cryptococcus neoformans*

*Trichosporon spp.*

*Blastoscizomyces capitatus*

*Malassezia spp.*

*Rhodotorula rubra*

*Saccharomyces cerevisia*

## Küf Mantarları

*Aspergillus spp.*

*Mucormycetes spp.*

*Fusarium spp.*

*Scedosporium apiospermum*

*Paecilomyces spp.*

*Trichoderma spp.*

*Acremonium spp.*

*Scopulariopsis spp.*



## EUROPEAN CONFERENCE on INFECTIONS in LEUKAEMIA

European Confederation of Medical Mycology



- CLSI M54-A 2012
- ECIL-3 –Classical Diagnostic Procedures 2012
- ECIL – Biological markers 2012
- ECIL 3- Mucormycosis 2013
- IDSA-ASM Guidelines 2013
- ESCMID – *Candida* 2012
- ESCMID-ECMM Mucormycosis 2013
- ESCMID-ECMM Rare Yeasts 2013
- ESCMID-ECMM Phaeohyphomycosis 2014
- ESCMID-ECMM Hyalohyphomycosis 2014

M54-A  
Vol. 32 No. 14

Principles and Procedures for Detection of  
Fungi in Clinical Specimens—Direct  
Examination and Culture; Approved  
Guideline



**IDSA**  
Infectious Diseases Society of America

# İnvaziv Fungal Enfeksiyonlar-Mikrobiyolojik Tanı

## KONVANSİYONEL TANI YÖNTEMLERİ

MİKROSKOBİ, KÜLTÜR

Halen mikroskopik inceleme ve kültürü elimine eden bir yöntem  $\emptyset$

Kan kültürü: 2-7 gün

İnvaziv Kandidiyazis: % 20-50

İnvaziv Aspergilloz: < % 5

Doku kültürü: duyarlılık % 40-70

BAL duyarlılık: %40-60

İdrar/BAL: kolonizasyon

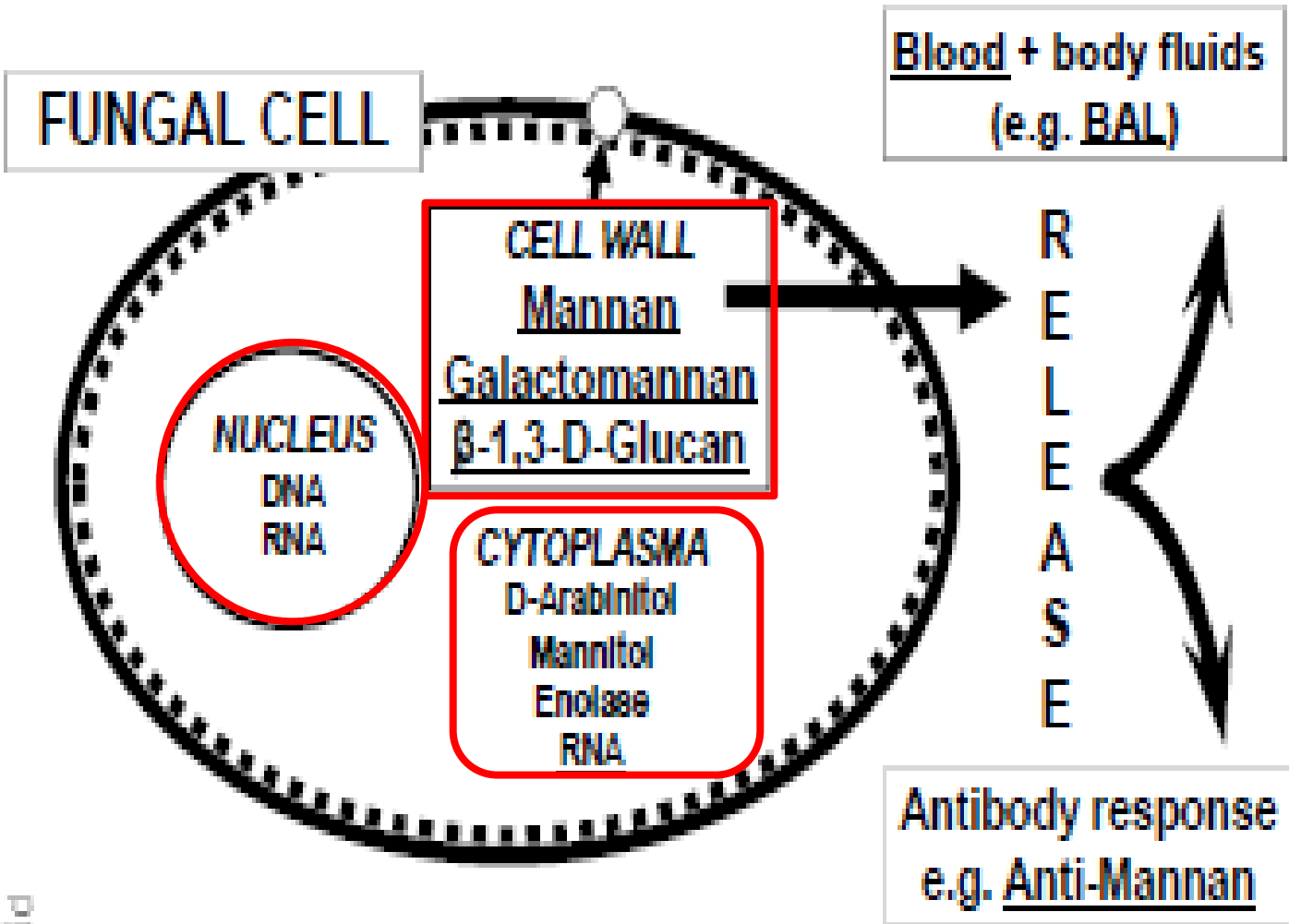
- Steril örnek alımındaki kısıtlamalar (nötropenik, trombositopenik)
- Var olan kültürel yöntemlerin zaman alıcı , duyarlılık düşük



# Biyo-belirteçler



PresenterMedia



### **Antijen:**

Sirkülasyon hızlı

İmmunkompleks, Kc'de Kupffer hücreleri

Ag testleri: POLİKLONAL AB

Çapraz reaksiyon, tekrarlanabilirlik düşük



**Duyarlılık**

### **Antikor:**

İmmünkompromize: Ab yükü düşük Yalancı (-)

Kolonizasyon: Yalancı(+)





## Biyo-belirteçler

- TANI...

Riskli hastalarda:

Uygunsuz antifungal kullanımı → % 57

Gereksiz antifungal kullanımı → %16

- **Ampirik antifungal tedavi kullanımını azaltmak**

*Munoz P. et al. Antifungal stewardship in daily practice and health economic implications. Mycoses 2015 58 (2):14-25.*

# Candida : Biyo-belirteçler

- ✓ Mannan antijen - anti mannan antikor
- ✓ D-arbinitol
- ✓ Enolaz, aldolaz, salgısal aspartik proteinaz
- ✓ Hif yapısına özgül *Candida* antijenleri
  - *Hyr 1* (Hyphally regulated protein 1)
  - *Ece 1* (Extent of cell elongation)
  - *Als 3* protein (Agglutinin like sequence)
  - *Hwp 1* protein (Hyphal wall protein)
- ✓ *Candida albicans* germ tüp antikor (CAGTA)
- ✓ Termolabil antijen (Cand-Tec)
- ✓  $\beta$  GLUKAN ANTİJEN  $\longrightarrow$  PANFUNGAL



# I- Mannan antijen - anti mannan antikor

## Mannan antijen:

- Pastorex Candida test (LA, Sanofi Diagnostic, Fransa)
- Platelia Antigen test (ELISA, Bio-Rad, Fransa)

## Anti-mannan antikor:

- Anti –Candida antibody kit (IgG Virotech, Almanya)
- IgG Biomerica (Biomerica, CA)
- SysCan3 (Rockeby Biomed, Avusturya)
- IgG Platelia ( Bio-Rad, CA)



**Platelia Candida Ag** : *C.albicans* mannopentoz monoklonal EBCA-1 ab

0.1ng/ml mannan

- Mannan ve Anti-mannan Ab testlerinin duyarlılığı *Candida* türüne göre de farklılık göstermekte
- Kan kültürü pozitifliğinden 2-15 gün önce pozitif sonuç alınabilmekte
- **ANCAK:** (-) sonuç enfeksiyonu eradike etmemekte!!!

Duyarlılık	Ag %	Ab %	Ag+Ab %
<i>C. albicans</i>	62	67	100
<i>C. glabrata</i>	58	83	83
<i>C. tropicalis</i>	70	60	80
<i>C. parapsilosis</i>	30	10	40
<i>C. krusei</i>	25	38	50

-3<sup>rd</sup> European Conference on Infection in Leukemia Non-invasive diagnostic procedures for yeast infections. September 25-29,2009, Juan-les-Pins- France.

-Kedzierska et al, Eur J Clin Microbiol Infect Dis (2007) 26:755

# Mannan antijen - anti mannan antikor

- Candida Ag

Duyarlılık:  
%58

Özgüllük  
%93

- Candida Ab

Duyarlılık:  
%59

Özgüllük  
%83

- Candida Ag+Ab

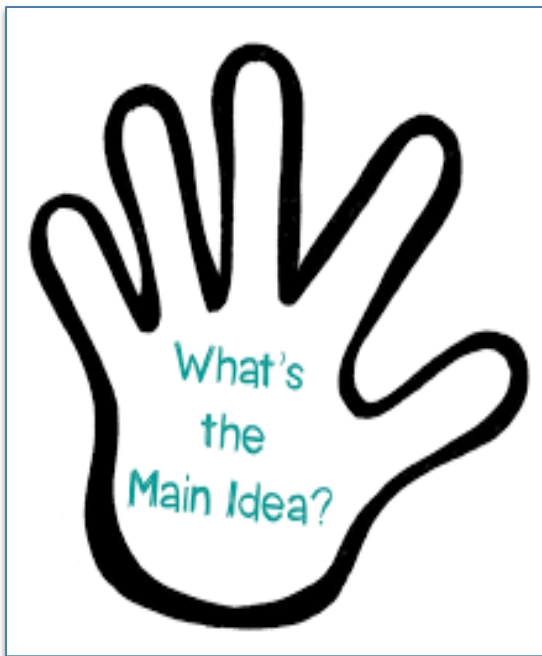
Duyarlılık:  
%83

Özgüllük  
%86

-L Ostrosky-Zeichner . *Invasive mycoses: diagnostic challenges.*  
*Am J Med.* 2012 Jan;125(1 ): 14-24.

-Mikulska et al. *The use of mannan antigen and anti-mannan antibodies in the diagnosis of invasive candidiasis: recommendations from the Third European Conference on Infections in Leukemia.* *Crit Care.* 2010;14:R222.

# Mannan antijen - anti mannan antikor



**İnvaziv kandidiyazis tanısında**

**Haftada iki**

**Mannan Ag+anti mannan Ab**

**Mannan Ag: SUT**

# (1,3) $\beta$ -D glukon (panfungal)



➤ *Candida, Aspergillus, Fusarium, Trichosporon, Acremonium*

➤ *Mucorales, Cryptococcus, Blastomyces dermatitis*

- **Fungitell (USA):** 60-80 pg/ml FDA onaylı (ABD ve Avrupa)
- **Fungitec-G (Japan):** 20 pg/ml
- **Wako (Japan):** 11 pg/ml
- **Maruha (Japan):** 11 pg/ml

Kitlerde kullanılan kromojenik sübstratlar farklı

USA: *Limulus polyphemus*

Japan: *Tachypleus tridentatus*

# (1,3) $\beta$ -D glukoz -kısıtlamalar

## Yalancı Pozitiflikler

- Hemodiyaliz
- Sirozlu hastalar
- Hemoliz, bilirubin, TG
- Kardiyopulmoner by-pass
- Abdominal cerrahi sonrası  
(*cerrahi tamponlar, gazlı bez %30*)
- İmmungloblin tedavileri
- Antitümör polisakkarit kullananlar  
(*lentinan, polisakkarit K, sizofilan*)
- Bakteriyemi, AB



Cins-türe özgü değil



# Candida: (1,3) $\beta$ -D glukan

## Kandidemi

%73-100 duyarlılık

%64-90 özgüllük

%73-97 NPD



## Meta-analiz

16:İnvaziv kandidiyazis

4 farklı beta glukan yöntemi

İki ardışık (+) > Tek (+) özgüllük



## Kandidemi

$\beta$ -D glukan: 1-2 hafta (ort: 10gün) önce

KK(+): 3hafta - 8 hafta



## Antifungal tedavinin takibi

Polyen grubu:  $\beta$ -D glukan seviyesinde artma  
Dolaşımdan geç temizlenme

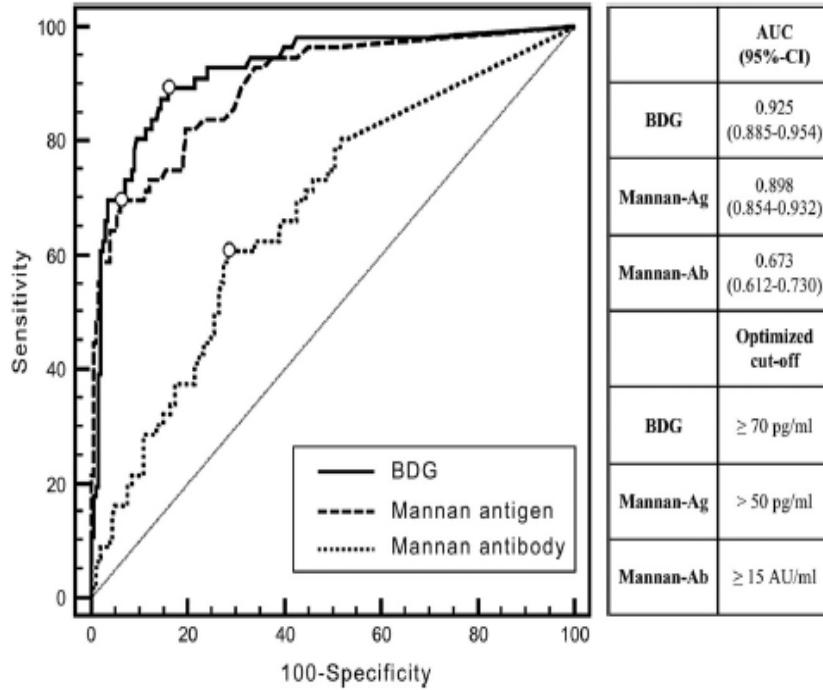


-Poissy J, et al. Presence of Candida cell wall derived polysaccharides in the sera of intensive care unit patients: relation with candidaemia and Candida colonisation. Crit Care. 2014 Jun 29;18(3):R135.

-Drosos E. Karageorgopoulos et al.  $\beta$ -D-Glucan Assay for the Diagnosis of Invasive Fungal Infections: A Meta-analysis. Clin Infect Dis. (2011) 52 (6):750-770

-Sims CR et al. Correlation of clinical outcomes with betaglucan levels in patients with invasive candidiasis. J Clin Microbiol 2012;50:2104-6

# Comparison of (1→3)-β-D-Glucan, Mannan/Anti-Mannan Antibodies, and Cand-Tec *Candida* Antigen as Serum Biomarkers for Candidemia



**Cand Tec Ag: duyarlılık %13 özgüllük %93.9**

**Ag: duyarlılık %58.9 özgüllük %97.5**

**Ab: duyarlılık %62.5 özgüllük %65**

**Kombine: duyarlılık %89.3 özgüllük %63**

**BG: duyarlılık %87.5 özgüllük % 85.5**

**BG+mannan: duyarlılık %89.3 özgüllük %85**

BDG > 80 pg/ml  
Mannan > 125 pg/ml  
Anti-mannan > 10 AU/ml

Cut-off düşürülünce: Duyarlılık ↑  
Özgüllük ↓

# Early diagnosis of candidemia in intensive care unit patients with sepsis: a prospective comparison of (1→3)- $\beta$ -D-glucan assay, *Candida* score, and colonization index

**BG $\geq$ 80pg/ml AUC 0.98**

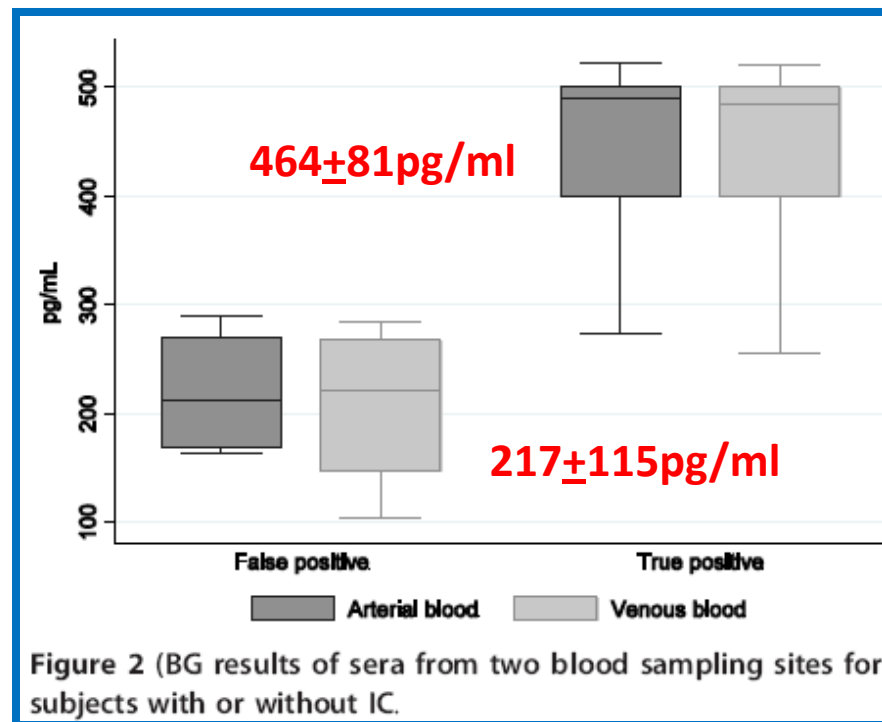
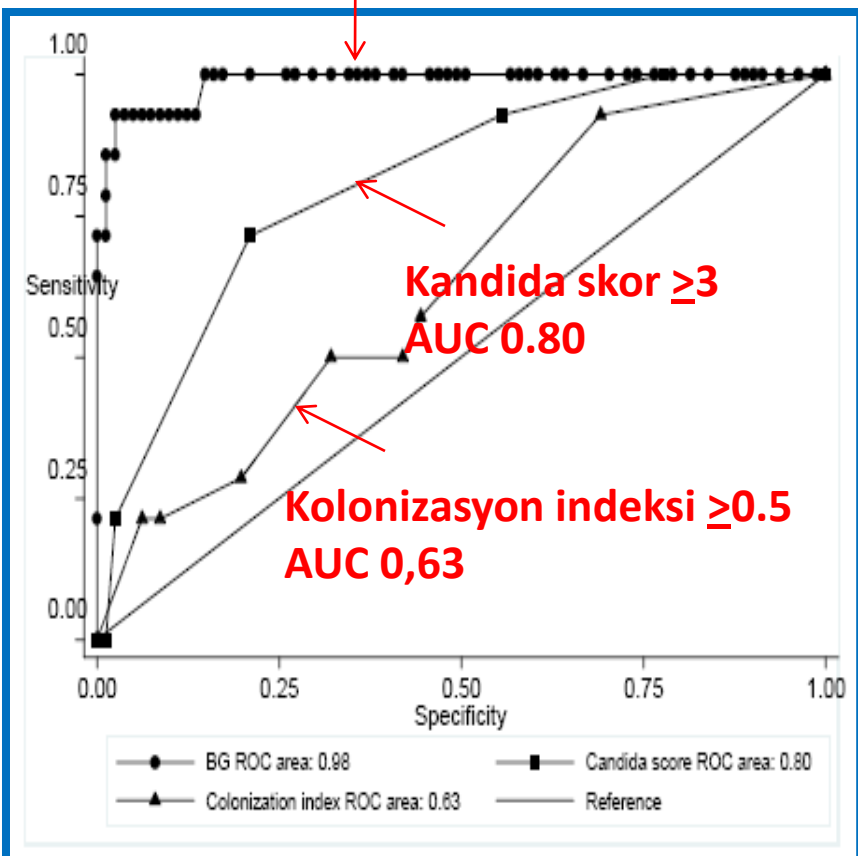


Figure 2 (BG results of sera from two blood sampling sites for subjects with or without IC.

SVK: [Mannan] ↑

SVK: Fungal yük - *Candida* kolonizasyonu

SVK- periferik kan: Farklı [Mannan]

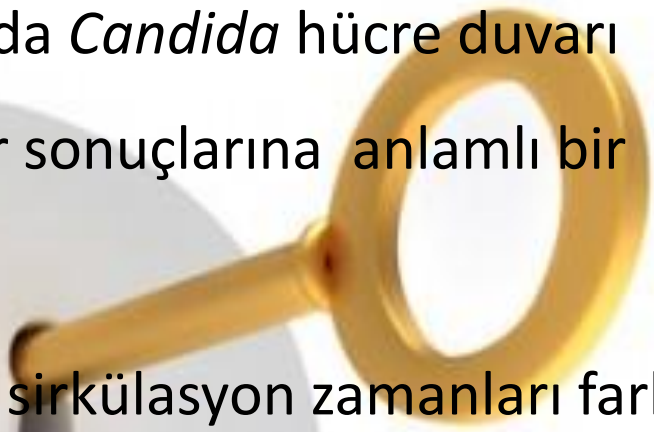
SVK İLİŞKİLİ ENF ??????

# Kandidemi ve/veya histopatolojik kanıtlı invaziv kandidiyazis

Antigen detected	Method used	Antibody used <sup>a</sup>	No. positive for antigen/total no. of subjects <sup>b</sup> (%)	
			Patients with proven invasive candidiasis	Controls <sup>c</sup>
Enolase	Dot immunobinding assay	Polyclonal Ab	28/39 (71.8)	0/40 (0)
Enolase	Double-sandwich liposomal immunoassay	Polyclonal Ab	18/24 (75.0)	6/146 (4.1)
$\beta$ -Glucan	Limulus test		27/32 (84.4)	5/40 (12.5)
Heat-labile antigen	Cand-Tec assay	Polyclonal Ab	30/39 (76.9) <sup>+</sup> 16/39 (41.0) <sup>++</sup>	5/40 (12.5) <sup>+</sup> 2/40 (5.0) <sup>++</sup>
Heat-labile antigen	Cand-Tec assay	Polyclonal Ab	21/32 (66.0) <sup>+</sup> 14/32 (44.0) <sup>++</sup>	
Heat-labile antigen	Cand-Tec assay	Polyclonal Ab	16/33 (49.0) <sup>+</sup> 2/83 (6.0) <sup>++</sup>	
Mannan	EIA	Polyclonal Ab	6/29 (20.7)	0/14 (0)
Mannan	ELISA	Polyclonal Ab	16/19 (84.2)	2/177 (1.1)
Mannan	ELISA	Polyclonal Ab	43/58 (74.1)	0/151 (0)
Mannan	LA <sup>d</sup> (Pastorex)	MAb	3/12 (25.0)	0/60 (0)
Mannan	EIA (ICON)	Polyclonal Ab	13/19 (68.4)	8/95 (8.4)
Mannan	Dot immunobinding assay	MAb	10/15 (67.0)	4/57 (7.0)
Mannan	LA (Pastorex)	MAb	10/39 (25.6)	0/40 (0)
Mannan	EIA	MAb	18/43 (41.8)	3/150 (2)
Mannan	LA (Pastorex)	MAb	12/43 (27.9)	0/150 (0)

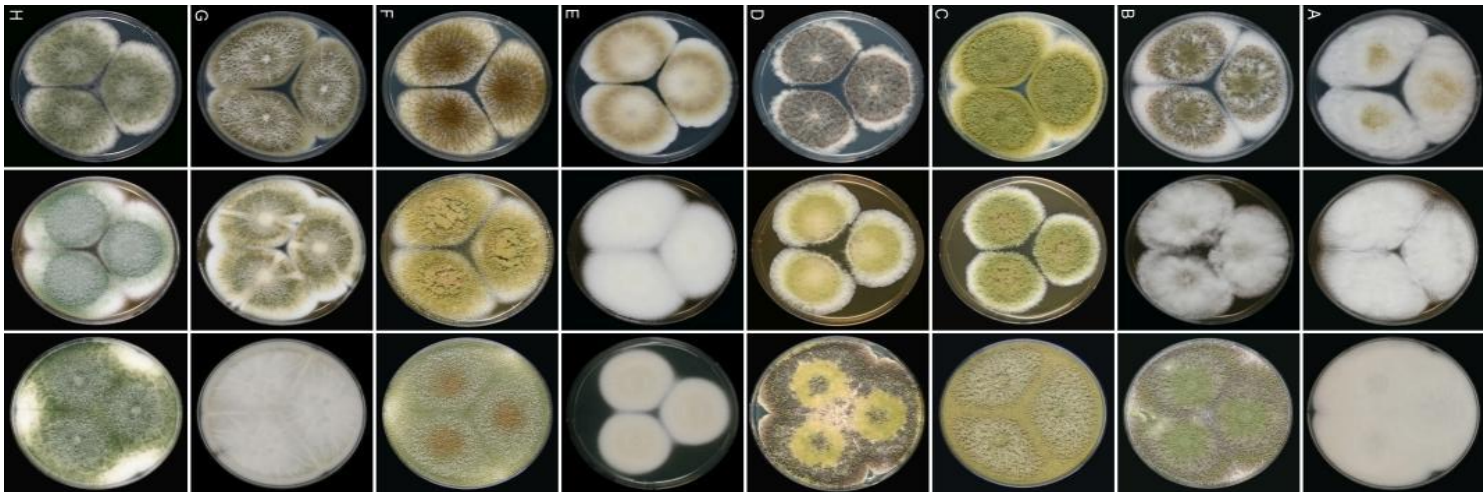


## KİLİT MESAJLAR:

- ✓ İnvazif kandidiazis tanısında serumda *Candida* hücre duvarı polisakkaritlerinin tespiti kan kültür sonuçlarına anlamlı bir yardımcı
  - ✓  $\beta$  glukan ve mannan: kinetikleri ve sirkülasyon zamanları farklı
  - ✓ BG erken tespit, özgüllük düşük
  - ✓ Mannan geç tespit, özgüllük yüksek
  - ✓ BG+manna preempirik tedavi için anlamlı
  - ✓ BG+manna takibi tedavi takibinde çok anlamlı değil, ancak yükselme relaps habercisi
- 

# *Aspergillus* : Biyo-belirteçler

- Galaktomannan antijen tespiti
- (1,3)  $\beta$ -D glukon antijen tespiti
- *Aspergillus* özgül glikoprotein antijen tespiti



# Aspergillus : Biyo-belirteçler 1976



## Diagnosis of invasive aspergillosis

SIR,—Opportunist fungal infections in recipients of organ transplants, open heart surgery patients, and others<sup>1-10</sup> are becoming an increasing problem. Aspergillosis is particularly troublesome because the infecting fungus is seldom cultured, the disease progresses very rapidly, and consequently diagnosis is most commonly made post mortem.<sup>2,3-8</sup>

Where treatment (amphotericin B) is given it is often unsuccessful, although there is evidence that early diagnosis would improve its effectiveness.<sup>3</sup>

Detection of fungal antigens or metabolites in patients' serum would aid the early diagnosis of infection, and monitoring serum antigen or metabolite levels might also assist in determining the effectiveness or required duration of treatment. Cryptococcal antigen detection is a routine test for the diagnosis of cryptococcosis, and detection of candida metabolites in serum shows promise as a means of rapidly diagnosing systemic candidiasis.<sup>7</sup>

We are currently looking for circulating aspergillus antigens and metabolites in the sera of infected animals which may find application in the improved diagnosis of systemic human infections. Because of the poor live diagnosis rate and, in absolute terms, the rarity of the disease in humans the difficulty we face is obtaining sera from known positive cases of invasive aspergillosis. If any readers have any such sera or would be willing to co-operate in storing sera from possible cases until diagnosis were made we would very much like to hear from them.

L O WHITE  
M D RICHARDSON  
R C WARREN

Department of Microbiology  
University of Birmingham,  
Birmingham B15 2TT

# ***Aspergillus* : Biyo-belirteçler 1979**

Mycopathologia vol. 67.2: 83-88, 1979

## **DETECTION OF CIRCULATING ANTIGEN OF ASPERGILLUS FUMIGATUS IN SERA OF MICE AND RABBITS BY ENZYME-LINKED IMMUNOSORBENT ASSAY**

M. D. RICHARDSON\*, L. O. WHITE\*\* & R. C. WARREN\*

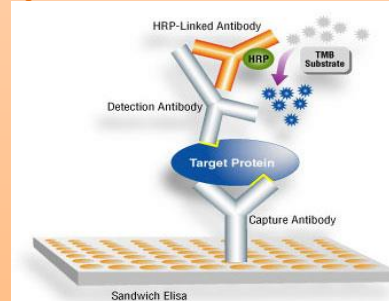
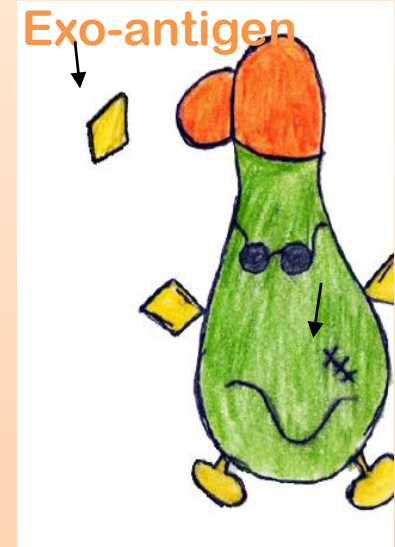
\*Department of Microbiology, University of Birmingham, Birmingham B15 2TT, England.

\*\*Department of Medical Microbiology, Southmead General Hospital, Westbury-on-Trym, Bristol, BS10 5NB, England.



# Aspergillus : Galaktomannan

- *Aspergillus*, *Penicillium*, *Paecilomyces* türleri için önemli bir hücre duvarı komponenti
- **Ekzoantigen** (ExA) olarak da ortama salınabilir
  - Çekirdek (mannan):  $\alpha$  (1,2) ve  $\alpha$ (1,4) mannoz (mannan)
  - Yan zincir (galaktofuran)
- Latex Aglütinasyon veya **ELISA** testi ile serum ,plazma, BAL, BOS, idrar
- 2003 FDA Platelia Bio-Rad, Fransa
- 2008 EORTC/MSG “ probable IA’ ’ yüksek olasılıklı İA
- ELISA testinde fare EB-A2 monoklonal antikoları ile GM yan zinciri **(1→5)-β-D-galactofuranoz** tespit edilir



<b>Antijen</b>	<b>Galaktomannan</b>	<b>(1,3)-<math>\beta</math>-D-glukan</b>
<b>Yöntem</b>	'Sandwich' ELISA Lateks agglütinasyon	Kolorimetrik
<b>Ticari Kit</b>	Bio-rad (Fransa) Pastorex Aspergillus	Fungitell (ABD) Fungitec-G MK (Japonya) Wako (Japonya) Maruha (Japonya)
<b>Sınır değeri (serum)</b>	<u>0.5</u> -1.5	Fungitell 80 pg/mL
<b>BAL</b>	0.5-1	Diğerleri 11-20 pg/mL
<b>Üreticinin önerdiği klinik örnekler</b>	Serum, BOS, BAL	Serum
<b>Erken Tanı</b>	5-8 gün	3-10 gün
<b>Serum Duyarlılık</b>	%29-100	%47-98
<b>Özgüllük</b>	%20-100	%86-98
<b>BAL Duyarlılık</b>	%76-100	%50-93
<b>Özgüllük</b>	%56-100	%55-73
<b>Tdv takibi</b>	+	∅
<b>Maliyet</b>	1300 Usd/110 test	900 Euro/96 test

# Aspergillozis

**Antijen Tests:**

**Galaktomannan**

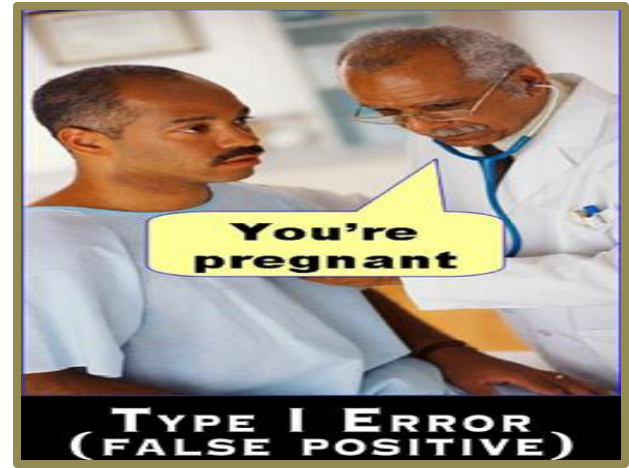
Altta yatan hastalık, etkenin invazyonu, etkenin türü, örnek sıklığı



Hasta grubu	Duyarlılık (%)	Özgüllük (%)
Hematolojik Malignensi	70	92
HSCT	82	86
SOT	22	84

*Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. Clin. Infect. Dis. 2006;42(10):1417–1427*

# Galaktomannan



- Anti-aspergillus antikor varlığı
- Antifungal tdv, düşük fungal yük
- Etkenin invazyonuna (apse, kavite)
- Testin cut-off değerlerinin düşürülmesi yalancı negatifliği azaltmaktadır

- GM çeşitli besinlerde (tahıllar, pirinç, konserve, besin katkı maddeleri) bulunabilir
- **Beta-laktam antibiyotiklerde (pip/tazo, amox/clav)**
- Barsaklardan translokasyon
- *Bifidobacterium* türleri(süt ve süt ürünleri) çocuklardaki yüksek yalancı pozitiflik
- *Penicillium* , *Histoplasma*, *Blastomycosis X*

# Piperasillin Tazobaktam Alanlarda GM Yalancı Pozitifliği

TABLE 2 Studies of galactomannan positivity in specimens from patients receiving piperacillin-tazobactam and in lots of piperacillin-tazobactam<sup>a</sup>

Manufacturer	Patient at risk for invasive aspergillosis	No. of GM-positive patients or specimens/ total no. of patients or specimens (%) from:		P value	No. of GM-positive lots/total no. of PTZ lots (%)	Yr of publication (reference)
		Patients receiving PTZ	Patients not receiving PTZ			
Not stated	Not stated	Not reported	Not reported	NA	4/not reported	2003 (4)
Not stated	Hematologic malignancies	Not reported	Not reported	NA	3/4 (75)	2004 (6)
Not stated	HSCT recipient	17/23 (73.9) <sup>b</sup>	4/36 (11.1) <sup>b</sup>	P < 0.001	12/15 (80)	2004 (7)
Not stated	Not stated	5/13 (38.5) <sup>b</sup>	0/13 (0) <sup>b</sup>	P < 0.001	Not performed	2004 (8)
Wyeth	Not at risk	6/135 (4.4) <sup>b</sup>	Not reported	NA	Not performed	2010 (9)
Pfizer	HSCT recipients	10/394 (2.5) <sup>c</sup>	25/1,606 (1.6) <sup>c</sup>	P = 0.18	<u>0/90 (0)</u>	2012 (10)
Mylan	Hematologic malignancies, solid organ tumors	7/20 (35.0) <sup>b</sup>	10/27 (37.0) <sup>b</sup>	P = 0.870	Not performed	2012 (11)
Wyeth Pfizer	Hematologic malignancies, solid organ tumors	17/34 (50) <sup>b</sup>	13/53 (24.5) <sup>b</sup>	P = 0.027	<u>0/4 (0)</u>	2012 (11)
Fresenius	Hematologic malignancies, solid organ tumors	10/32 (31.2) <sup>b</sup>	12/41 (29.3) <sup>b</sup>	P = 0.941	Not performed	2012 (11)
Multiple	Not at risk	1/27 (3.7) <sup>b</sup>	Not performed	NA	<u>0/32 (0)</u>	2014 (this study)

Son yıllarda Avrupa ve ABD GM yalancı pozitif(+) çok düşük oranda

ABD: 5 firma, 32 lot GM tespit edilmiyor

## Comparison of Galactomannan Enzyme Immunoassay Performance Levels when Testing Serum and Plasma Samples

P. Lewis White,<sup>a</sup> Tim Jones,<sup>a</sup> Katie Whittle,<sup>a</sup> Joanne Watkins,<sup>a</sup> Rosemary A. Barnes<sup>b</sup>

TABLE 1 Clinical performance of galactomannan EIA when testing plasma and serum samples<sup>a</sup>

Parameter	Performance comparison for each pair of sample types with:			
	Plasma testing		Serum testing <sup>b</sup>	
	Proven/probable IA vs no IFD	Possible IA vs no IFD	Proven/probable IA vs no IFD	Possible IA vs no IFD
Sensitivity (%; 95% CI)	6/7 (85.7; 48.7-97.4)	<u>4/10 (40; 16.8-68.7)</u>	6/7 (85.7; 48.7-97.4)	<u>0/10 (0; 0-27.8)</u>
Specificity (%; 95% CI)	41/48 (85.4; 72.8-92.8)	41/48 (85.4; 72.8-92.8)	38/48 (79.2; 65.7-88.3)	38/48 (79.2; 65.7-88.3)
PPV (%; 95% CI)	6/13 (46.2; 23.2-70.9)	10/17 (36.4; 15.2-64.6)	6/16 (37.5; 18.5-61.4)	0/10 (0; 0-27.8)
NPV (%; 95% CI)	41/42 (97.6; 87.7-99.6)	41/47 (87.2; 74.8-94.0)	38/39 (97.4; 86.8-99.6)	38/48 (79.2; 65.7-88.3)

# Galaktomannan-Örnek seçimi-BAL

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

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### Abstract

**Background:** Bronchoalveolar lavage (BAL) galactomannan (GM) assay has been used for diagnosing invasive *aspergillosis* (IA). We aimed to derive a definitive estimate of the overall accuracy of BAL-GM for diagnosing IA.

**Methods and Results:** We undertook a systematic review of thirty diagnostic studies that evaluated the BAL-GM assay for diagnosing IA. PubMed and CBM (China Biological Medicine Database) databases were searched for relevant studies published in all languages up until Feb 2012. The pooled diagnostic odds ratio (DOR) and summary receiver operating characteristic (SROC) were constructed for each cutoff value. Additionally, pooled sensitivity (SEN), specificity (SPE), and positive and negative likelihood ratios (PLR and NLR, respectively) were calculated for summarizing overall test performance. Thirty studies were included in this meta-analysis. The summary estimates of pooled DOR, SEN, SPE, PLR, and NLR of the BAL-GM assay (cutoff value 0.5) for proven or probable IA were 52.7 (95% confidence interval (CI) 31.8–87.3), 0.87 (95% CI 0.79–0.92), 0.89 (95% CI 0.85–0.92), 8.0 (95% CI 5.7–11.1) and 0.15 (95% CI 0.10–0.23) respectively. The SROC was 0.94 (95% CI 0.92–0.96). Compared with cutoff value of 0.5, it has higher DOR, SPE and PLR, and similar SEN and NLR with cutoff value of 1.0, which indicated the optimal cutoff value might be 1.0. Compared with BAL-GM, serum GM has a lower SEN and higher SPE, while PCR displays a lower SEN and a similar SPE.

**Conclusion:** With the optimal cutoff value of 1.0, the BAL-GM assay has higher SEN compared to PCR and serum GM test. It is a useful adjunct in the diagnosis of proven and probable IA.

Meta –analiz

16 çalışma

Serum-BAL GM

	Duyarlılık % (%95 Güvenlik aralığı)	Özgüllük % (%95 Güvenlik aralığı)
Serum	65 (54–75)	95 (90–97)
BAL	85 ( 72–92)	86 (78–92)

# Detection of Galactomannan in Bronchoalveolar Lavage Fluid Samples of Patients at Risk for Invasive Pulmonary Aspergillosis: Analytical and Clinical Validity

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TABLE 2 Performance characteristics of the Platelia galactomannan enzyme immunoassay in bronchoalveolar lavage fluid

OD index cutoff	Value for the parameter (95% CI) <sup>a</sup>								
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	+LR	-LR	DOR	EOR	
≥0.5	93.2 (85.7-97.2)	86.8 (83.4-88.6)	76.4 (70.2-79.6)	96.6 (92.7-98.6)	7.0 (5.1-8.5)	0.07 (0.03-0.17)	90.5 (29.9-270.3)	2.0 (1.1-4.4)	
≥0.80	86.4 (77.4-92.7)	90.7 (86.6-93.6)	81 (72.5-86.8)	93.6 (89.3-96.5)	9.29 (5.75-14.3)	0.15 (0.07-0.26)	62.15 (22.0-183.9)	0.65 (0.53-0.87)	
≥1.0	79.7 (71.6-85.2)	93.8 (90.1-96.3)	85.5 (76.8-91.4)	91 (87.4-93.5)	12.8 (7.2-23.3)	0.21 (0.15-0.31)	59.2 (23-152.3)	0.26 (0.27-0.21)	
≥1.5	72.9 (65-77.9)	95.3 (91.7-97.6)	87.8 (78.2-93.8)	88.5 (85.1-90.6)	15.6 (7.8-33.1)	0.28 (0.22-0.38)	55 (20.5-146.5)	0.13 (0.16-0.08)	
≥2.0	62.7 (55.6-66)	97.7 (94.4-99.2)	92.5 (81.9-97.3)	85.1 (82.3-86.4)	26.9 (9.9-80)	0.38 (0.34-0.41)	70.6 (21-233.5)	0.04 (0.07-0.01)	
≥2.5	59.3 (52.1-62.6)	97.7 (94.4-99.2)	92.1 (81-97.2)	84 (81.2-85.3)	25.5 (9.3-76)	0.41 (0.37-0.5)	61.2 (18.3-201.8)	0.03 (0.06-0.01)	
≥3.0	55.9 (50-55.9)	100 (97.6-100)	100 (90.6-100)	83.2 (81.2-83.2)	∞ (21-∞)	0.44 (0.44-0.5)	∞ (41.5-∞)	0 (0.02-0)	
≥4.0	47.5 (42.2-47.5)	100 (97.6-100)	100 (88.9-100)	80.6 (78.7-80.6)	∞ (17.5-∞)	0.52 (0.52-0.59)	∞ (29.6-∞)	0 (0.02-0)	

<sup>a</sup> Values are for proven and probable cases versus controls ( $n = 188$ ). CI, confidence interval; OD, optical density; PPV, positive predictive value; NPV, negative predictive value; +LH, positive likelihood ratio [sensitivity/(1 - specificity)]; -LH, negative likelihood ratio [(1 - sensitivity)/specificity]; DOR, diagnostic odds ratio {[sensitivity/(1 - sensitivity)]/[(1 - specificity)/specificity]}; EOR, error odds ratio {[sensitivity/(1 - sensitivity)]/[specificity/(1 - specificity)]}.

<0,5= %93,2 olasılıkla İPA değildir

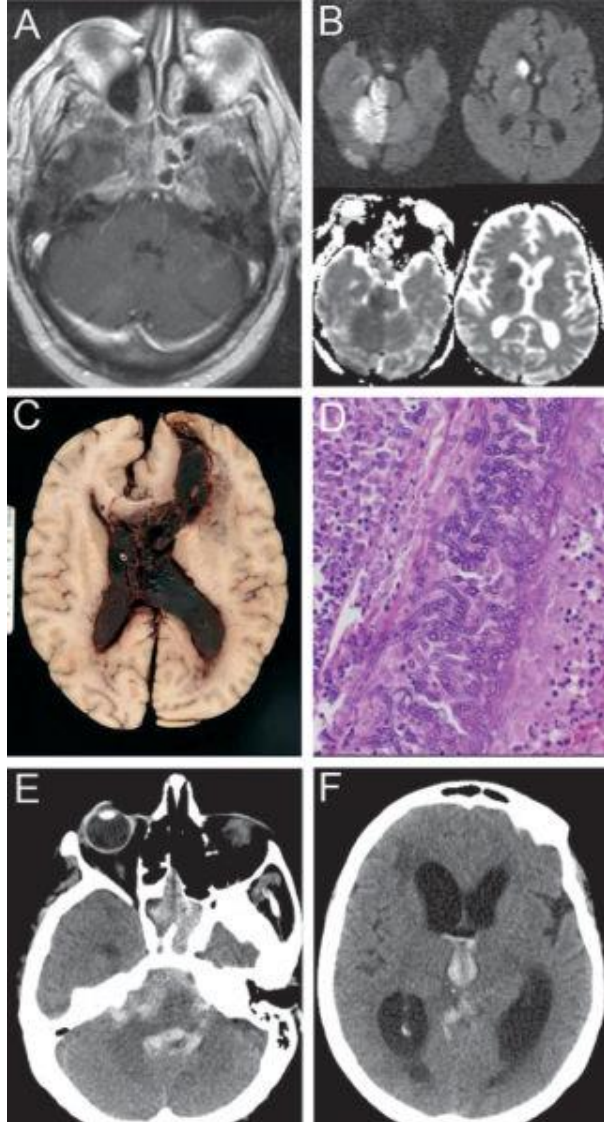
>3= %100 olasılıkla İPA'dır

Cut-off yükseldikçe: Duyarlılık ↓  
Özgüllük ↑



# Galaktomannan-Örnek seçimi-BOS

## Serebral Aspergillozda Galaktomannan



Duyarlılık %80

Özgüllük %100

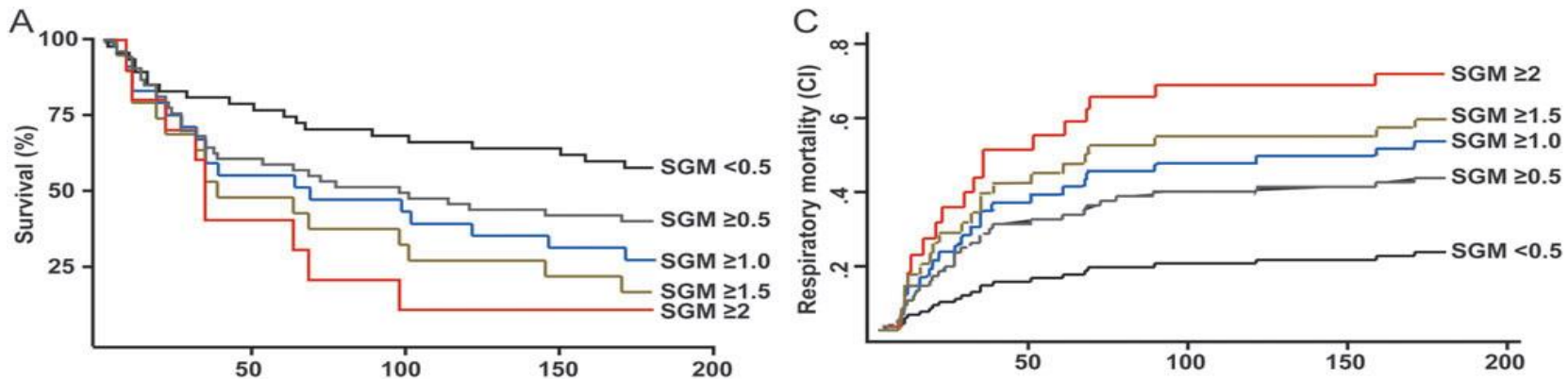
	Serum GM	BOS GM
Antinori <i>et al.</i>	2.5	7.4
Van de Beek <i>et al.</i> (Olgu 1)	0.3	6.4
(Olgu 2)	0.06	2.7

-Antinori, *et al. J Infect* 2013 (3): 218-238

-Van de Beek, *et al. Neurology*, 2008(10): (24pt2)

# Galaktomannan indeksi ile İA'de klinik sonuç arasındaki ilişki

- Klinik sonucun tahmininde kullanılabilen bir biyo-belirteç
- Serum GMI mortalite oranının göstergesi



GMI	42-d Overall Mortality			180-d Overall Mortality		
	N (%)	HR (95% CI)	PValue	N (%)	HR (95% CI)	PValue
<0.5	1/7 (14.3)	1.0	.14	3/7 (42.9)	1.0	.11
0.5–0.99	8/24 (33.3)	2.76 (.34–22.09)		12/24 (50)	1.37 (.39–4.86)	
1.0–1.99	10/20 (50)	4.97 (.64–38.91)		15/20 (75)	2.75 (.79–9.52)	
$\ge 2.0$	4/6 (66.7)	6.98 (.78–62.82)		5/6 (83)	3.35 (.80–14.11)	

-Fisher CE et al. Clin Infect Dis 2013 57:1001–1004.

-Mikulska M et al. Clin Infect Dis 2013 57:1786–1787.

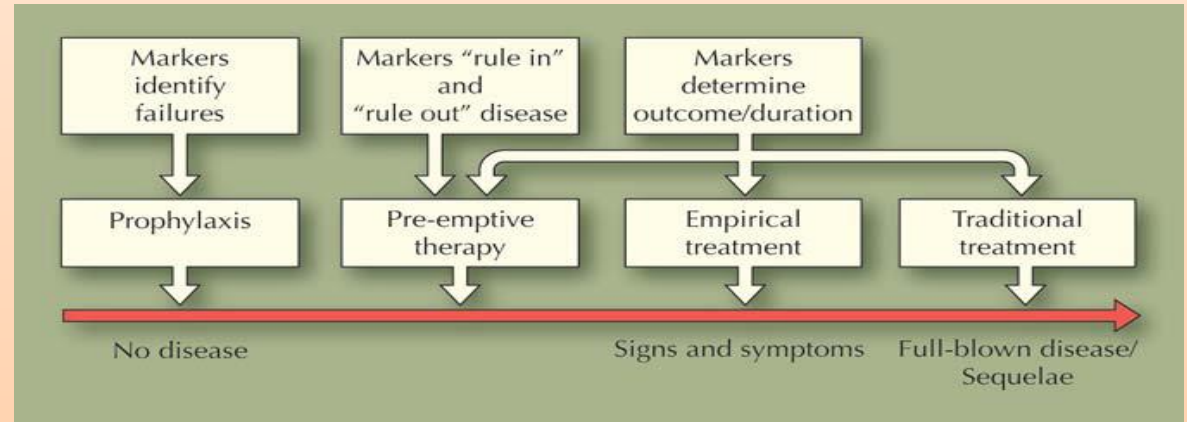
# Galaktomannan indeksinin tedaviye etkisi

117 nütropenik ateş epizot: 41 epizot : %35 (ampirik)

GALAKTOMANNAN



%7,7 (pre-ampirik)



Ampirik antifungal kullanımında %78 lik azalma

Ancak *Aspergillus* dışı İFi kaçırıyor.

*Maertens J, et al. Galactomannan and computed tomography-based preemptive antifungal therapy in neutropenic patients at high risk for invasive fungal infection: a prospective feasibility study. Clin Infect Dis. 2005 Nov 1;41(9):1242-50.*

# Galaktomannan Ag Tespiti ECIL-3, 2012

☀ Prospektif : İA gelişme riski >%5-10 olan nütropenik hastalarda kullanımı önerilmektedir (serum **AII**, plasma **CIII**)

☀ 3-4 günde bir GM takibi önerilmektedir (AII)

☀ İA'da serum GM düzey takibi, antifungal tedavinin başarısı ve prognoz açısından bilgi verebilir



# Aspergillozis: (1,3) $\beta$ -D glukun

**%55-95**

**%77-96**

Nötropenik hastalarda tanı değeri GM 

GM *A. fumigatus* (%13) < *A. fumigatus* dışı (%49)

$\beta$ -D glukun *A. fumigatus* ve *A. fumigatus* dışı (%88)

**İA TARAMA-TANI: HaftaX2**

**Duyarlılık azalmakta, özgüllük artmakta**

**SUT:Ø**



## GM ve (1,3) $\beta$ -D glukun kinetiđi

Hematolojik malignansi  
İnvazif aspergilloz



	GM	$\beta$ -D glukun
Ateş	4	5 (4-6)
Klinik septomlar	9 (4-15)	10.7 (4-21)
Pulmoner HRCT	7.2 (1-5)	9.3 (1-21)
AF tedavi başlanması	12.5 (1-23)	14 (4-25)

## Aspergillozis : Galaktomannan - (1,3) $\beta$ -D glukuan



Duyarlılık % 87.5  
Özgüllük % 89.6



Duyarlılık % 87.5  
Özgüllük % 89.6



Duyarlılık % 87.5  
Özgüllük % 100

**İA riskli hastalarda her iki teste kullanılabilir**

**Kombine, PPD: %100, Testlere bağlı yalancı (+) ekarte ediliyor**

# Aspergillozis

## Aspergillus spesifik glikoprotein antijen tespiti

### Lateral Flow Device (LFD):

### İmmunkromotografi

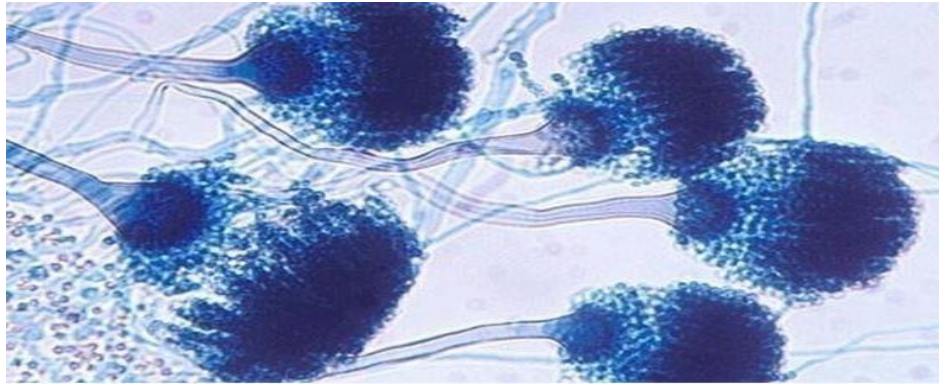
*Aspergillus* tarafından salgılanan ekstrasellüler glikoproteine karşı oluşturulmuş JF5 IgG3 monoklonal Ab kullanmakta.

Mab *Aspergillus* spp. oldukça spesifik

*Candida* , *Fusarium*, *Scedosporium*, *Mucormycetes*

10-15 dk, ekipman gerektirmiyor

HM, SOT : NPD





**Performance of lateral flow device and galactomannan for the detection of *Aspergillus* species in bronchoalveolar fluid of patients at risk for invasive pulmonary aspergillosis**

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Anne E. Kizy<sup>2</sup>, Linoj Samuel<sup>3</sup>, Hui Jiang<sup>4</sup>,  
Christopher R. Thornton<sup>5</sup>, Mayur Ramesh<sup>2</sup>  
and George Alangaden<sup>2</sup>

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Issue



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# LFD- PERFORMANS?

İPA BAL-GM  
BAL- LFD

İPA şüpheli 311 BAL olgu prospektif

HM ve HM dışı (SOT, Steroid Kullanan, Solid Tm, Diğer)

96 hasta: 89 HM dışı

HM: GM=LFD

HM dışı: LFD>GM

Duyarlılık  
%66

Özgüllük  
GM %52  
LFD %94



A microscopic image showing numerous bright, circular oocysts of Cryptosporidium against a dark background. Each oocyst has a distinct, thick, white outer wall and a darker, granular inner core. The oocysts are scattered across the field of view, with some appearing in small groups and others in isolation.

**İnvaziv Kriptokokoz, Biyo- belirteçler**

## *C. neoformans* LATEKS ANTİJEN TESTLERİ (LCATS)

**Crypto-LA** (*International Biological Labs, Inc., Cranburg, NJ, USA*)

**Myco-Immune** (*American Microscan, Mahwah, NJ, USA*)

**Immy Latex-Crypto Antigen** (*Immuno-Mycologics, Inc., Norman, Okla, USA*)

**Calas-Cryptococcal Atigen Latex Agglutination System** (*Meridian Bioscience Inc., Cincinnati, USA*)

**Pastorex Cryptococcus Test** (*Sanofi Diagnostic Pasteur*)

**Liken Latex Test** (*Liken Tokyo*)



# Kriptokokal tanı testlerinin BOS örneklerinde performansı

Tanı testi	Örnek sayısı	Duyarlılık (%)	Özgüllük (%)	PPD	NPD
CRAG LFA	666	99.3	99.1	99.5	98.7
CRAG latex (Meridian)	279	97.8	85.9	92.6	95.5
CRAG latex (Immy)	749	97.0	100.0	100.0	75.8
Çini Mürekkebi	805	<u>86.1</u>	97.3	98.2	80.2
BOS kültürü	806	<u>90.0</u>	100.0	100.0	85.3
•100 µl	524	94.2	100.0	100.0	91.2
•10 µl	282	82.4	100.0	100.0	75.8

Boulware, D.R et al. Multisite validation of cryptococcal antigen lateral flow assay and quantification by laser thermal contrast. *Emerg. Infect. Dis.* 2014, 20, 45–53.

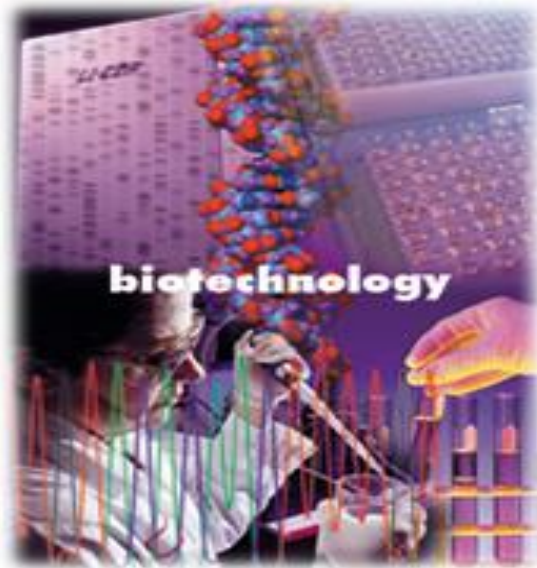
# Fungal Biyo-belirteçler: MOLEKÜLER YÖNTEMLER



# DNA Teknolojisi

## DNA probları

Southern Hibridizasyon  
In-situ Hibridizasyon  
Microarray  
Macroarray  
Reversed line blot



## PCR primerleri

Panfungal PCR  
Multiplex PCR  
Nested PCR  
Real Time PCR  
PCR Fingerprinting  
AFLP  
PCR-RFLP

# Fungal Biyo-belirteçler: MOLEKÜLER YÖNTEMLER

- **Üniversal fungal primerler**

- Çok kopyalı gen bölgeleri:

→ rDNA : 18S, ITS1/2, 5.8S, 28S, 5S, IGS

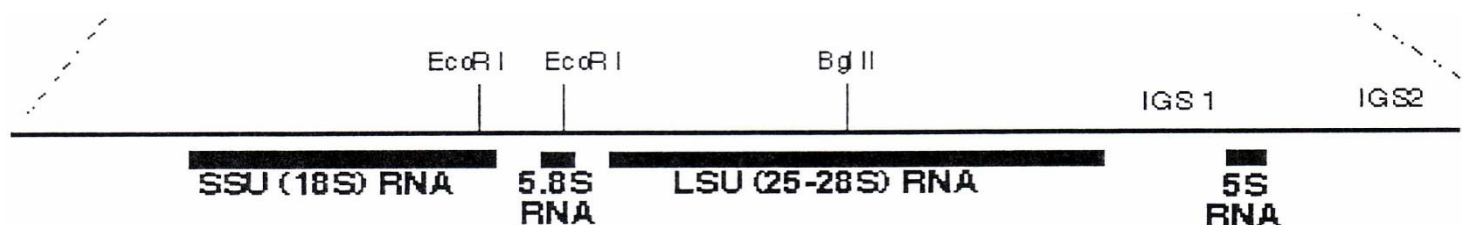
- Tek kopyalı gen bölgeleri:

→ Aktin, Alkalin Proteaz (*ALP*), Kitin Sentaz , Lanosterol -  $\alpha$  - demetilaz (*LIA1*), Secreted Aspartic Protease (*SAP*), Beta glukon sentataz, Histon.

- **Cins veya tür spesifik primerler**

→ 18S, ITS1/2, 28S rDNA, Mitochondrial DNA, Histone

*Candida, Cryptococcus, Aspergillus*



# Fungal Biyo-belirteçler: MOLEKÜLER YÖNTEMLER

Çok sayıda çalışma var: İCE-İAE

DM ve kültüre göre duyarlılıkları yüksek

Tam kan, serum, plazma, doku, BAL, BOS  
ABD: % 5, Ülkemizde: epidemiyolojik, araştırma

DNA ekstraksiyonu için optimum yöntem  
onay almış standart test yöntemi yok



-Arendrup MC, Fisher BT, Zaoutis TE. Invasive fungal infections in the paediatric and neonatal population: diagnostics and management issues. *CMI*. 2009; 15; 613-624.

-Xavier MO, Oliveria FM, Severo LC. Laboratory diagnosis of pulmonary mycose. *J Bras Pneumol*. 2009; 35; 907-919



# Fungal Biyo-belirteçler: MOLEKÜLER YÖNTEMLER

## KISITLAMALAR

- Standardizasyon
- Kontaminasyon
- Kolonizasyon-infeksiyon ayırımı
- Proflaktik tedavinin DNA salınımına etkisi
- Tedaviye yanıt olmaksızın negatifleşme



“The honest package you will never see.”

# Candida



- 20 çalışma
- Serum/tam kan
- İnvaziv Kandidiyazis
  
- Duyarlılık %56.2- 100
- Özgüllük %54-100

- Kan kültürü %38
- PCR: %85

Derin doku kandidiyazis KK (+) %17, Beta glukan (+) %62, PCR(+) %88

## Pozitif kan kültürü şişesinden

Yöntem	Firma	Method	Patojen	Tespit limiti (CFU/ml)	Süre (h)
Luminex xTAG Fungal ASR	Luminex, TX, ABD	Multipleks PCR ve Flow sitometri	<i>Candida</i> ve <i>Cryptococcus</i> dahil 23 fungal etken	-	5-6
PNA-FISH	AdvanDX, MA, ABD	PNA problemleri ile Floresans temelli hibridizasyon	5 <i>Candida</i> spp.	-	1.5
FilmArray	İdaho Tecno, UT, ABD	Multipleks PCR	5 <i>Candida</i> spp.	-	1
Prove-it-sepsis	Mobidiag, Helsinki, Finlandiya	Multipleks PCR-Microarray hibridizasyon	<i>Candida</i> ve <i>Cryptococcus</i> dahil 13 fungal etken	-	3-5

### PNA-FISH

Duyarlılık %99 Özgüllük %100 PPD %100  
2. jenerasyon 30 dk  
Konvansiyonel yöntemlerden 24-48 saat önce

### Luminex, FilmArray

Duyarlılık %100  
Özgüllük %99

## Direkt tam kan

İnvaziv Kandidiyazis: PCR duyarlılık %95 özgüllük %92

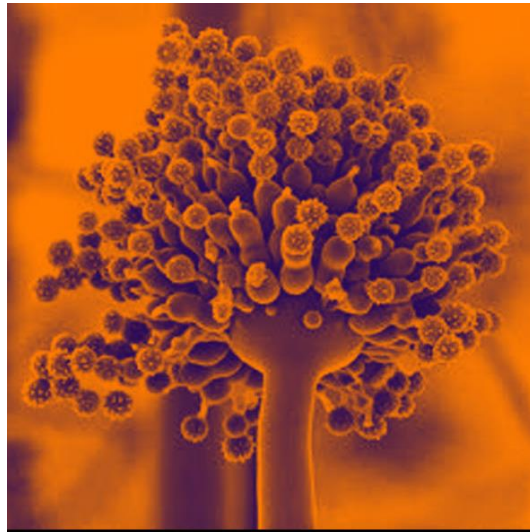
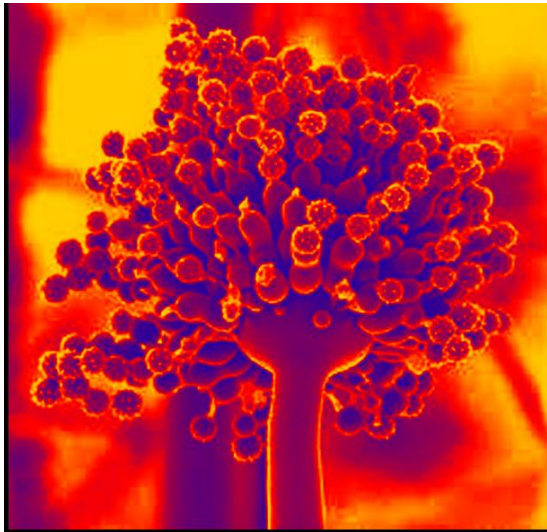
Yöntem	Firma	Method	Patojen	Tespit limiti (CFU/ml)	Süre (h)
SeptiTest	Molzym, Almanya	Geniş spektrumlu PCR	5 <i>Candida</i> spp. ve <i>Cryptococcus</i>	20-40	8-12
Magicpleks Sepsis Real-Time Test	Seegene Inc, Güney Kore	Multipleks Real time PCR	6 <i>Candida</i> spp	-	3-4
Real-Time PCR panel	Quest Diag. NJ, ABD	Multipleks Real time PCR	5 <i>Candida</i> spp.	1-350	6
Real-Time PCR panel	Viracor-IBT, MO, ABD	Real-Time PCR	5 <i>Candida</i> spp.	<1	6
<u>LightCycler</u> Septifast Test	Roche, NJ, ABD	Multipleks Real time PCR	5 <i>Candida</i> spp.	30-100	6

Duyarlılık %61  
Özgüllük %99

-Chang, S.S et al. Multiplex PCR system for rapid detection of pathogens in patients with presumed sepsis: a systematic review and meta-analysis. *PLoS ONE* 2013, 8, e62323.

-Micheal Pfaller Application of Culture-Independent Rapid Diagnostic Tests in the Management of Invasive Candidiasis and Cryptococcosis. *J Fungi* 2015, 1(2), 217-251

# Aspergillozis- PCR



# Aspergillus - PCR standardizasyon çalışmaları

JMD  
CME Program

Related Commentary on page 297

*Journal of Molecular Diagnostics, Vol. 8, No. 3, July 2006*  
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and the Association for Molecular Pathology  
DOI: 10.2353/jmoldx.2006.050120

## A Consensus on Fungal Polymerase Chain Reaction Diagnosis?

18S, 28S

*A United Kingdom-Ireland Evaluation of Polymerase Chain Reaction Methods for Detection of Systemic Fungal Infections*

LightCycler (Roche, UK)

Corbett Rotor-Gene ( Corbett,Research LTD, UK)

TaqMan (Applied Biosystems, UK)

10-5000 konidya/ml *Candida* –*Aspergillus*, *Aspergillus* DNA

Duyarlılık, Özgüllük, PPD, NPD

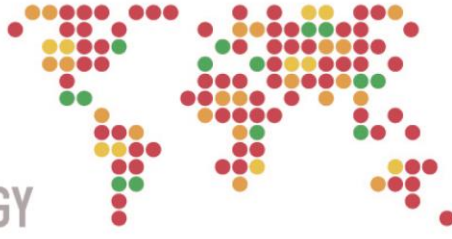
28S>18S



Rotor-Gene  
% 100 Duyarlılık  
%100 PPD



TaqMan  
% 100 Duyarlılık  
%100 NPD

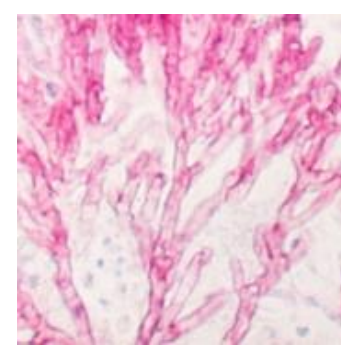


## European Aspergillus PCR Initiative (EAPCRI) çalışma grubu Önerileri

- ❖ Tüm öneriler EDTA tam kan
- ❖ En az 3ml kan ekstrakte edilmeli
- ❖ Fungal hücrelerin lizisi cam boncuklarla yapılmalı
- ❖ Her örnek iki kere analiz edilmeli, uyumsuz ise DNA ekstraksiyonu tekrar
- ❖ PCR internal kontrol mutlaka yapılmalı
- ❖ DNA ekstraksiyonu ve PCR için mutlaka negatif kontrol yapılmalı
- ❖ Elüsyon hacmi < 100 µl
- ❖ Tüm kullanılan ürünler olası bir fungal kontaminasyon açısından taranmalı



# İnvaziv Aspergilloz-PCR



## Örnek seçimi önemli

Plazma-Tam kan

Serum -Tam kan

## Örnek sayısı önemli

Tek PCR -Çift PCR pozitif

Tek PCR (-) sonuç: İA ekarte etmekte

İA tanısı için çift PCR(+) çünkü duyarlılık tek PCR(+) den yüksek

### PCR - İA

- Duyarlılık %43-100
- Özgüllük %64-100

### PCR İA -BAL

- Duyarlılık %36-100
- Özgüllük %70-100

### PCR İA- BOS

- Duyarlılık % 100
- Özgüllük % 93



# İnvaziv Aspergilloz-PCR

Sadece tarama- tedavi yanıtı izlenebilir mi ?

DNA: miçelyal yıkım

AF tedavi: miçelyal yıkım, PCR ürün artış

İA fare modellerinde

Posakonazol, Kaspofungin: PCR duyarlılığı azaltmakta

Amfoterisin B: PCR duyarlılığı etkilenmiyor

**BAL PCR duyarlılığı tek AF kullanımdan etkilenmiyor**

Hastaların çoğunluğu *Candida* türlerine özgü tek AF ile profilaksi altında

-McCulloch E, et al. Antifungal treatment affects the laboratory diagnosis of invasive aspergillosis. *J. Clin. Pathol.* 2012 65:83-6

-Reinwald M, et al. Therapy with antifungals decreases the diagnostic performance of PCR for diagnosing invasive aspergillosis in bronchoalveolar lavage samples of patients with haematological malignancies. *J. Antimicrob. Chemother.* 2012 67:2260–2267

# İnvaziv Aspergilloz-PCR

**Mortalite:** Ardışık PCR (+) yüksek mortalite

**Ampirik tedavi:** 2 (+) PCR → HM:%37 azalama



6 merkez, 240 HM

Biyo-belirteçler (GM-PCR)

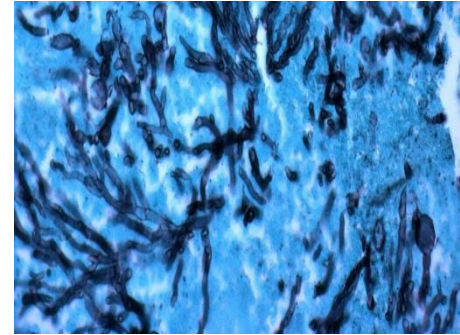
Ampirik tedavide

↓  
%17 azalma

*Morrissey et al. Galactomannan and PCR versus culture and histology for directing use of antifungal treatment for invasive aspergilloz in high-risk haematology patients: a randomised controlled trial. Lancet Infect. Dis. 2013 13:519-528.*

# İnvaziv Aspergilloz: PCR - GM

- İA BAL PCR - GM performansları aynı anlamlı fark yok
- İA Serum PCR-GM performansları aynı anlamlı fark yok
- **Kombine kullanım yüksek duyarlılık (%97)**
- **Özgüllük azalmıyor (%97.5)**



## SONUÇ...



- I. İFi tanısında altın standart yöntemin duyarlılığı düşük, yeterince hızlı değil
- II. Hızlı ve doğru tanımlama yapabilecek yöntemler: **Biyo- Belirteçler**
- III. Erken tanıya yardımcı , ampirik tedaviyi azaltıyor, erken-etkin tedavi
- IV. **GM** testleri (plazma, serum, BAL, BOS) invazif aspergilloz tanısında EORTC/MSG kriterlerine girmiştir
- V. **Beta-glukan** testleri (serum) İFi tanısında EORTC/MSG kriterlerine girmiştir
- VI. Moleküler yöntemler: standardizasyon ve validasyon eksikliği EORTC/MSG kriterlerine girmemiştir

*İlginiz İin TeŖekkür Ederim...*

