İnvaziv Fungal Enfeksiyonlar:

Hızlı tanı mümkün mü?

Barış Otlu
İnönü Üniversitesi Tıp Fakültesi
Tıbbi Mikrobiyoloji Anabilim Dalı

Fungal Tanı Yöntemlerinin Gelişimi

- 1839 ve 1844 yılları arasında üç bağımsız çalışma cryptogam rapor edilmiş.

Charles-Phillipe Robin

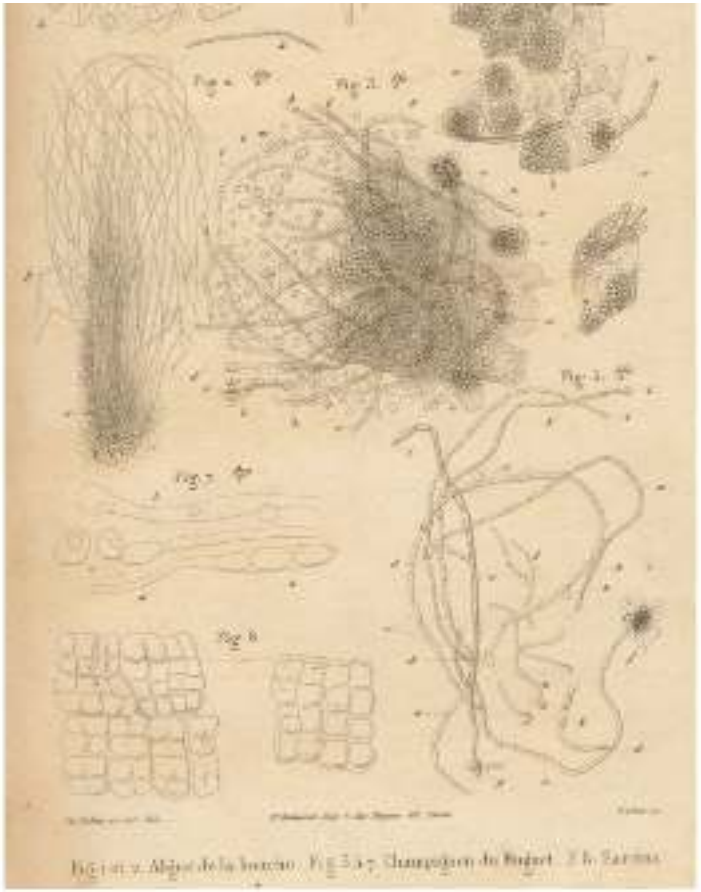


Figure 3. The first printed illustrations of *Candida* (*Oidium*) *albicans*: the thrush fungus (*champignon du muguet*). Plate I from

Fungal Tanı Yöntemlerinin Gelişimi

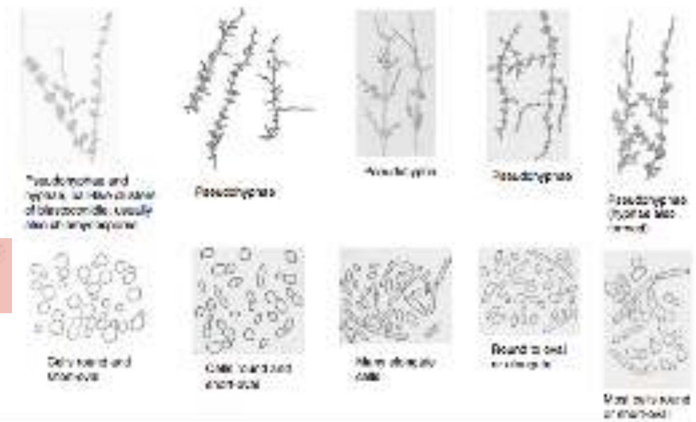
- 1950 'li yıllar kültür ve biyokimyasal yöntemlerin yükselişi.

Book Reviews

The Yeasts: A Taxonomic Study. J. Lodder and N. J. W. Kreger-Van Rij. Amsterdam: North-Holland Pub.; New York: Interscience, 1952. 713 pp. Illus.

Table 5. 1952: Differentiation of C. albicans from similar clinical isolates — results of Lodder and Kreger-van Rij [154]

	Candida albicans	*Candida guilliermondii*	*Candida krusei*	*Candida parapsilosis*	*Candida tropicalis*
Fermentation					
Galactose	+	−	−	+	+
Maltose	+	−	−	−	+
Sucrose	−	+	−	−	+
Aerobic utilization					
Galactose	+	+	−	+	+
Maltose	+	+	−	+	−
Ethanol	−	+	+	−	−
Appearance of filaments					
Appearance of budding cells					



Fungal Tanı Yöntemlerinin Gelişimi

- 1960 'lı yıllar *C. albicans* ve diğerleri.

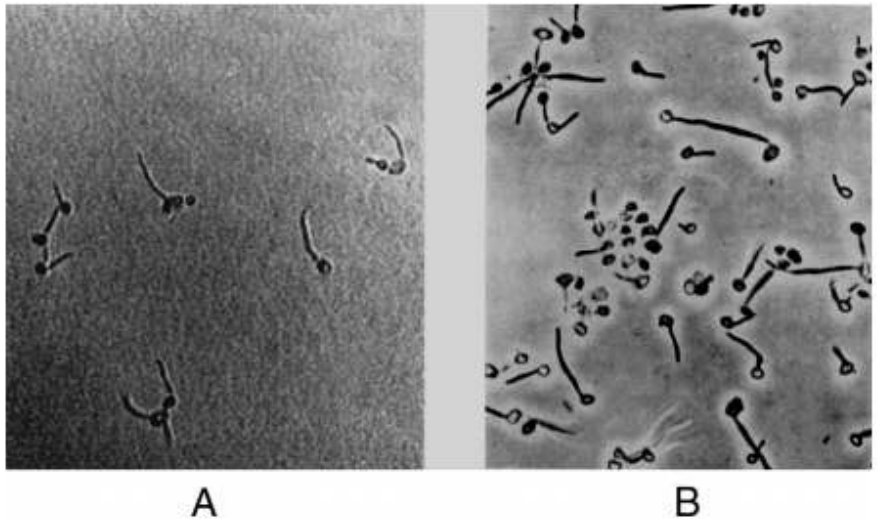


Figure 8. (a) Probably the earliest published photomicrograph of germ tube formation by *Candida albicans* (after incubation for 2 h in serum at 37 °C); from a 1960 paper of Taschdjian and her colleagues [264] in *American Medical Association Journal of Diseases of Children* **99**: 212. Copyright © (1960) American Medical Association. All Rights Reserved. (b) Germ tubes of *Candida albicans*, formed from individual yeast-like cells, incubated in bovine serum for 3 h at 37 °C. Photomicrograph published by Mackenzie [158] in 1962. Photomicrographs of germ tubes of *C. albicans* had been included in Donald Mackenzie's PhD thesis [157] in 1958

Fungal Tanı Yöntemlerinin Gelişimi

- 1970 'li yıllardan hızlı tanı arayışları artıyor.

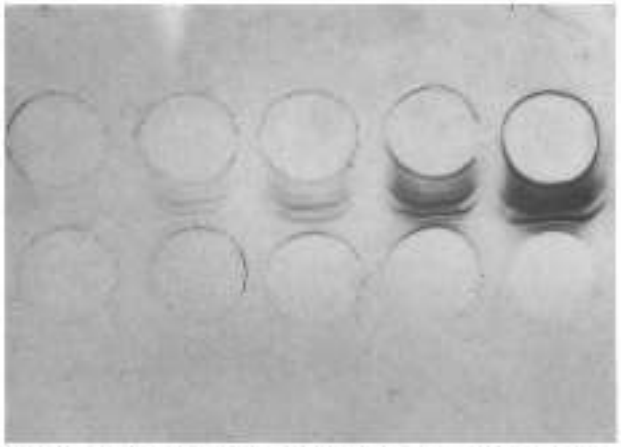
Mycopathologia et Mycologia applicata, vol. 51, 2–3, pag. 143–146, 1973

RAPID DIAGNOSIS OF PULMONARY MYCOSIS BY COUNTERELECTROPHORESIS

by

JUAN C. GALUSSIO., JULIO L. FRIDMAN., RICARDO NEGRONI

Laboratorio de Micología. Hospital Santojanni and Centro de Micología. Facultad de Medicina. Buenos Aires. Argentina.



Fungal Tanı Yöntemlerinin Gelişimi

- 1990 'lı yıllara kadar bir sonuç yok.

LABORATORY MEDICINE • VOL. 17, NO. 10, OCTOBER 1986

Rapid Methods for the Diagnosis of Fungal Infections

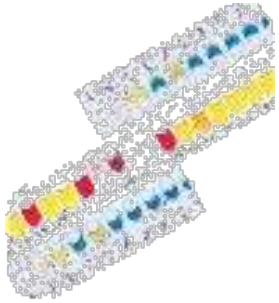
B. Laurel Elder, PhD, and Glenn D. Roberts, PhD

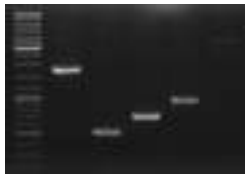
Rapid, accurate identification of fungal agents has become essential for the laboratory, particularly in those serving the increasingly frequent compromised patient. Rapid diagnosis can currently be achieved by using direct fungal smears whenever appropriate, by taking advantage of new fungal isolation systems, and by judicious selection of rapid identification tests for organism identification. In addition, knowledge of the fungi seen most frequently in each particular laboratory setting will allow the microbiologist to intensify efforts at identification for those organisms that are deemed most important or that occur most commonly.

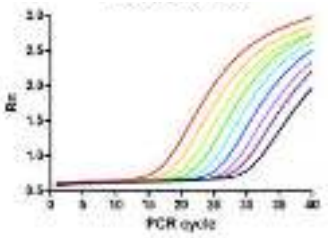
Commonly Available Identification Systems

Many commercially available yeast identification systems have been available to the microbiologist for a number of years. Most of these systems offer convenience to the laboratory by providing a panel of tests (primarily carbohydrate assimilations) that can be rapidly inoculated at one time. However, the majority of these systems continue to require 48 to 72 hours of incubation before a final identification is available, removing them from the category of "rapid tests." Several companies, however, have recently introduced identification systems that can be read in four to 24 hours, and may be useful as rapid identification systems. These systems have the advantage of identifying a wider spectrum of organisms than the individual tests previously discussed;

Mikrobiyolojik Tanı Yöntemleri










Mikrobiyolojik Hızlı Tanı Arayışı

- Geleneksel Tanı Yöntemleri

  düşük duyarlılığına rağmen (% 50-60) kan kültürü kandidemi tanısı için altın standart.

  C. tropicalis ve C. krusei için 19-22 saat, *C. albicans 39-60 saat*

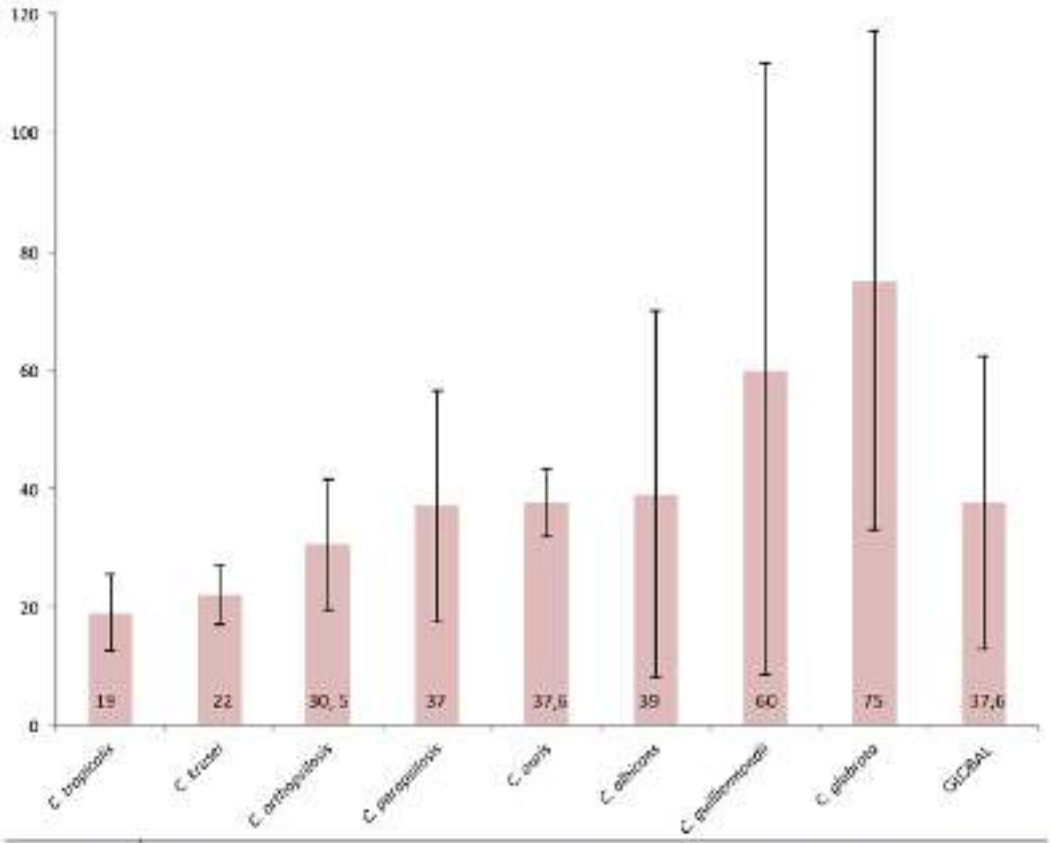


Figure 1 | Medium time of growth (hours) of different *Candida* species in blood cultures Data from 258 candidemia episodes in intensive care units at La Fe University Hospital

Geleneksel Yöntemlerdeki Durum

- Tanı için üremeyi, antimikrobiyal duyarlığın tespiti için üremenin inhibisyonun gözlenebilir hale getirilmesi zaman alıcı.

18-24 saat

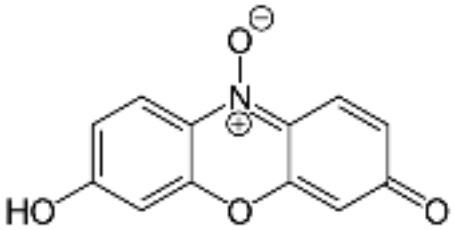
16-18 saat

8-10 saat

6-8 saat

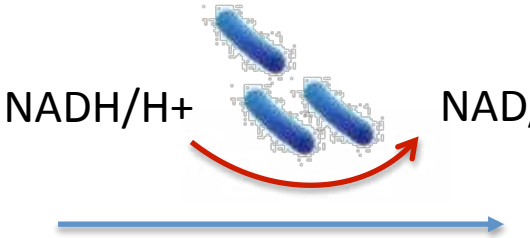
Kolorimetrik Yöntemler

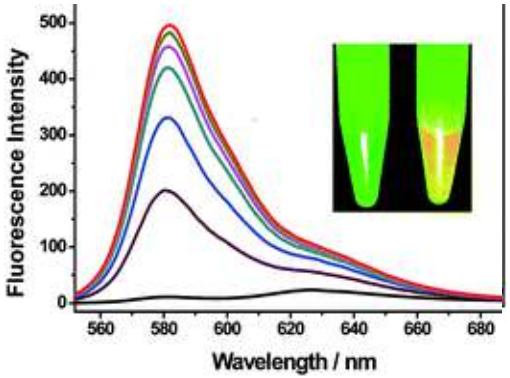
- Antibiyotik ile karşılaştırılan bakteri canlılığının kolorimetrik tespiti için sıklıkla resazurin kullanılmaktadır.

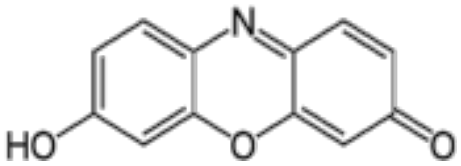


Resazurin

Mavi ve düşük floresans







Resafurin

pembe ve yüksek floresans

Kolorimetrik Yöntemler,

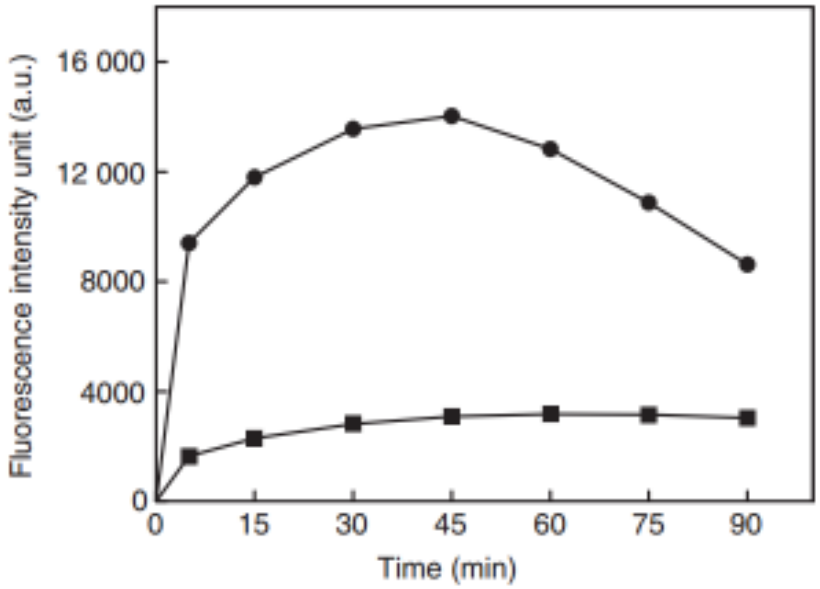
- Resazurin floresan temelli antifungal tespit yöntemi.

Letters in Applied Microbiology

Letters in Applied Microbiology ISSN 0266-8254

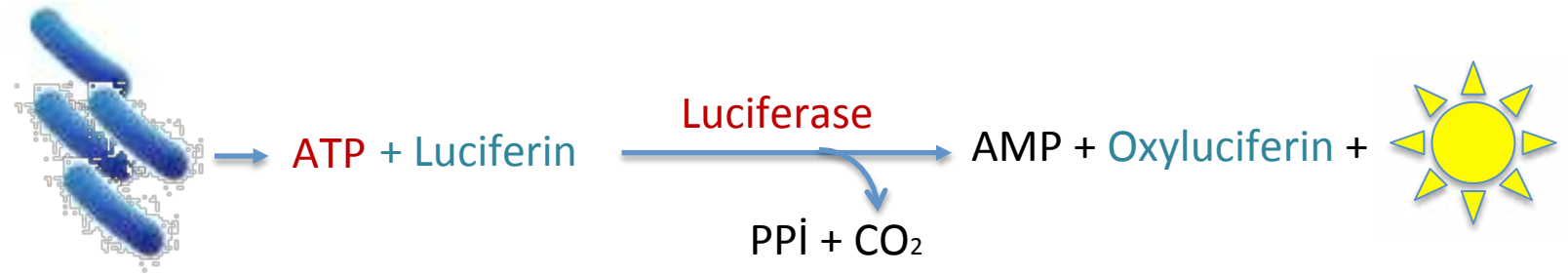
ORIGINAL ARTICLE

Simple fluorescence-based high throughput cell viability assay for filamentous fungi



ATP-biyoluminesans temelli yöntemler

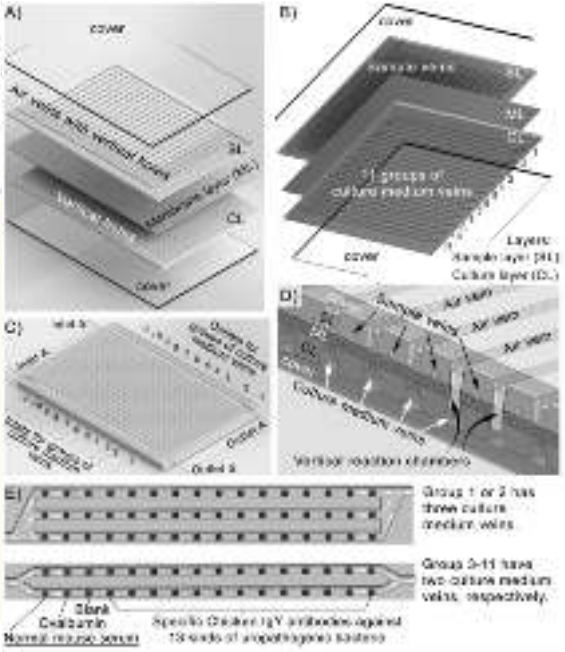
- ATP-biyoluminesans yönteminin hızlı direnç testlerinde kullanılması



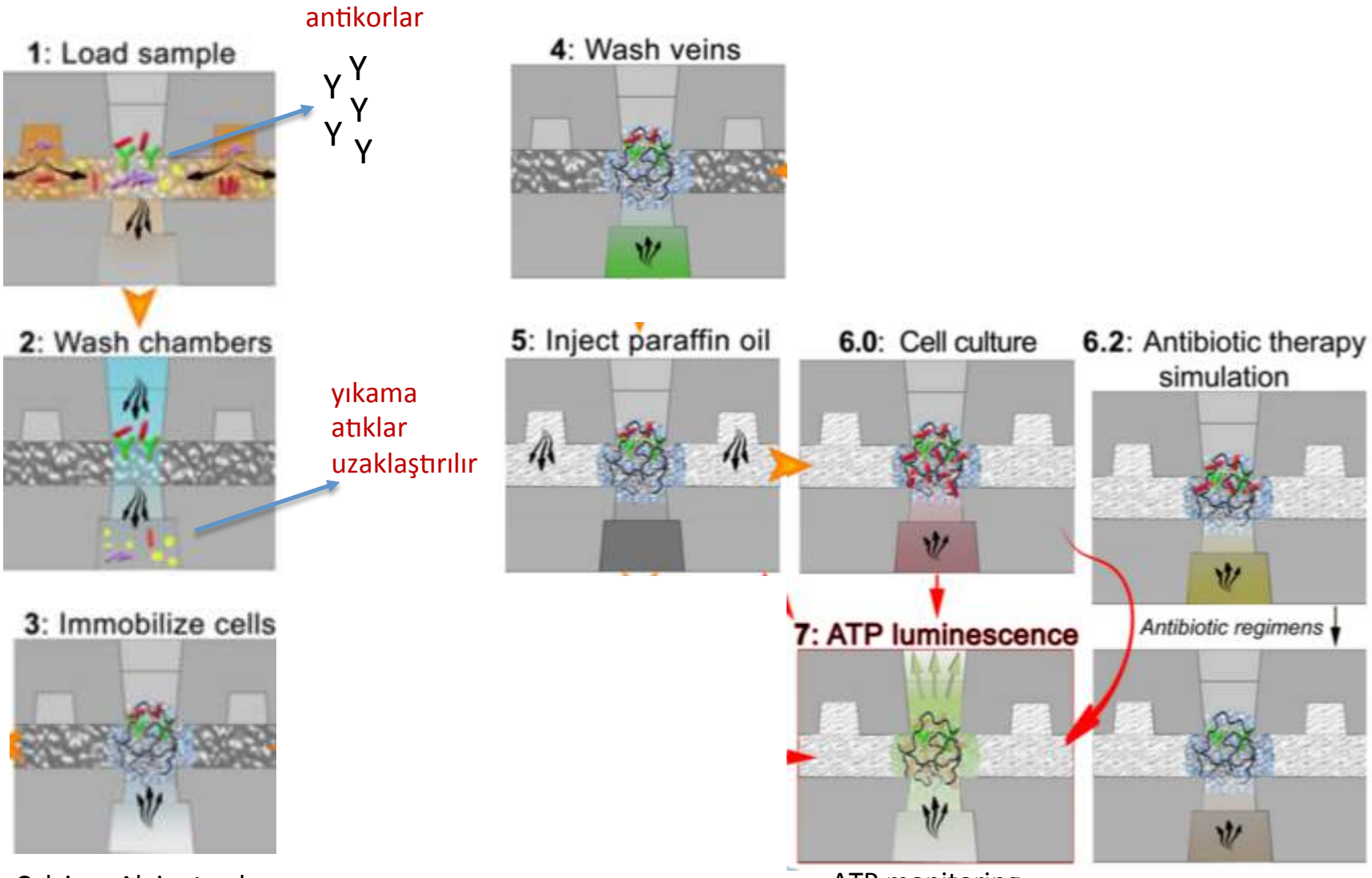
ATP-biyoluminesans temelli yöntemler

- 13 idrar yolu patojenini 20 dk da tespiti ve 3-6 saat arasında antimikrobiyal duyarlılık sonucu ATP-biyoluminesans yöntemi ile tespit edilebilmiş.

Rapid Identification and Susceptibility Testing of Uropathogenic Microbes via Immunosorbent ATP-Bioluminescence Assay on a Microfluidic Simulator for Antibiotic Therapy



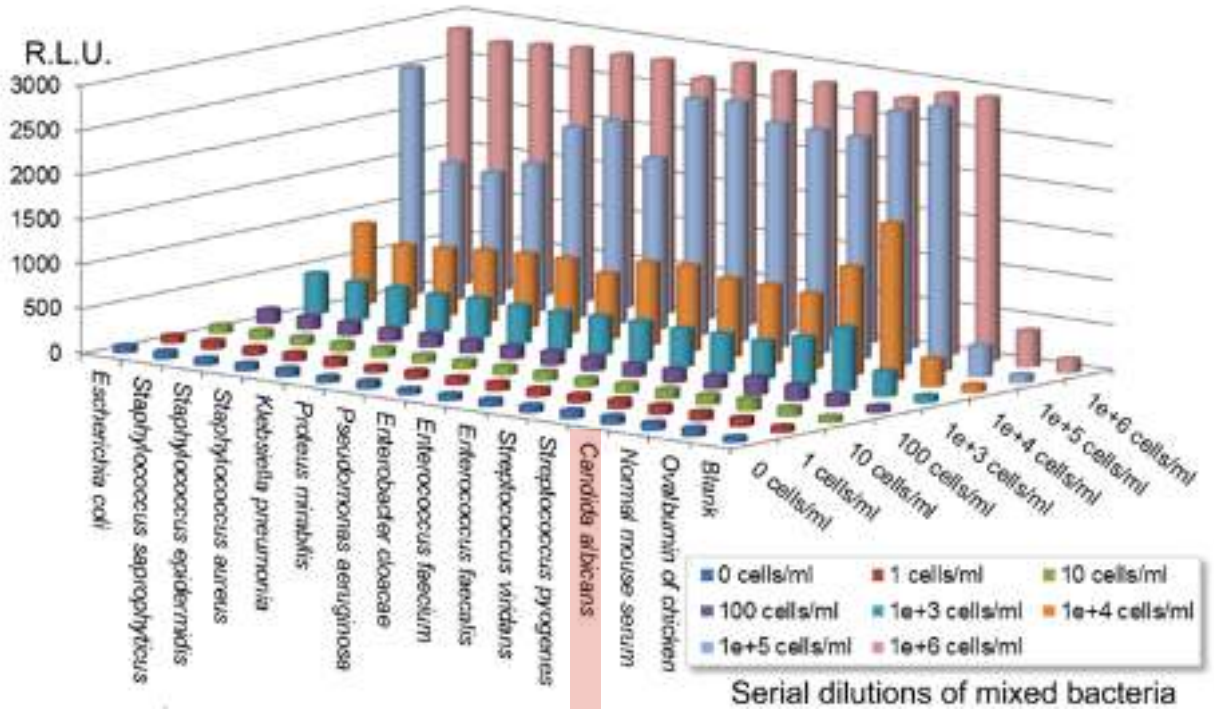
ATP-biyoluminesans temelli yöntemler



ATP-biyoluminesans temelli yöntemler

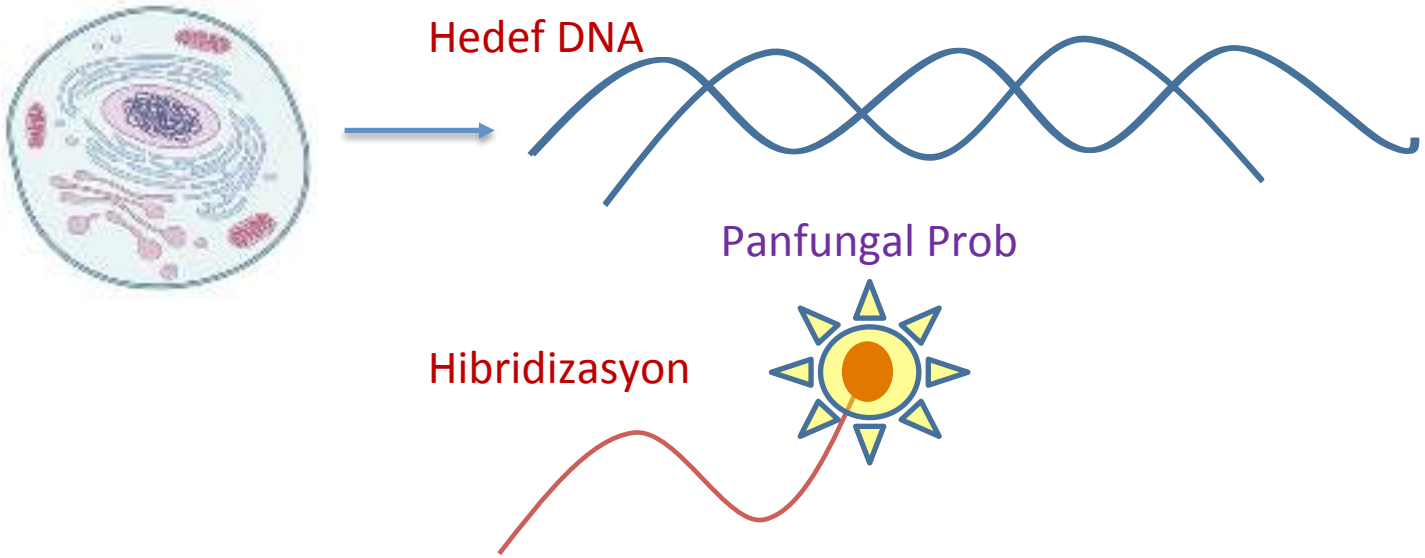
Rapid Identification and Susceptibility Testing of Uropathogenic Microbes via Immunosorbent ATP-Bioluminescence Assay on a Microfluidic Simulator for Antibiotic Therapy

Tao Dong*,†,‡,§ and Xinyan Zhao‡,§



Hibridizasyon Temelli Yöntemler

- 1970'li yıllardan itibaren geliştirilmeye başlandı.



Hibridizasyon Temelli Yöntemler

- PNA FISH ile kan kültür pozitifliğinden sonra ortalama;
  FISH yöntemi ile 5 saat içinde
  PCR-RFLP ile 20 saat, geleneksel yöntemlerle 6 günde sonuç alınabiliyor.

De Silva et al. Annals of Clinical Microbiology and Antimicrobials (2015) 14:8
DOI 10.1186/s12941-015-0065-5

ANNALS OF CLINICAL MICROBIOLOGY AND ANTIMICROBIALS

RESEARCH Open Access

Evaluation of fluorescence in situ hybridisation (FISH) for the detection of fungi directly from blood cultures and cerebrospinal fluid from patients with suspected invasive mycoses

Roberto Moreira De Silva Jr[1], João Ricardo De Silva Neto[2], Carla Silvana Santos[2], Hagen Frickmann[3], Sven Poppert[4], Kátia Santana Cruz[2], Daniela Koshikene[5] and João Vicente Braga De Souza[6,7*]

Abstract

The aim of this study was to evaluate the diagnostic performance of in-house FISH (fluorescence in situ hybridisation) procedures for the direct identification of invasive fungal infections in blood cultures and cerebrospinal fluid (CSF) samples and to compare these FISH results with those obtained using traditional microbiological techniques and PCR targeting of the ITS1 region of the rRNA gene. In total, 112 CSF samples and 30 positive blood cultures were investigated by microscopic examination, culture, PCR-RFLP and FISH. The sensitivity of FISH for fungal infections in CSF proved to be slightly better than that of conventional microscopy (India ink) under the experimental conditions, detecting 48 (instead of 46) infections in 112 samples. The discriminatory powers of traditional microbiology, PCR-RFLP and FISH for fungal bloodstream infections were equivalent, with the detection of 14 fungal infections in 30 samples. However, the mean times to diagnosis after the detection of microbial growth by automated blood culture systems were 5 hours, 20 hours and 6 days for FISH, PCR-RFLP and traditional microbiology, respectively. The results demonstrate that FISH is a valuable tool for the identification of invasive mycoses that can be implemented in the diagnostic routine of hospital laboratories.

Keywords: FISH, Invasive mycoses, CSF, Blood culture, rRNA, Hybridisation

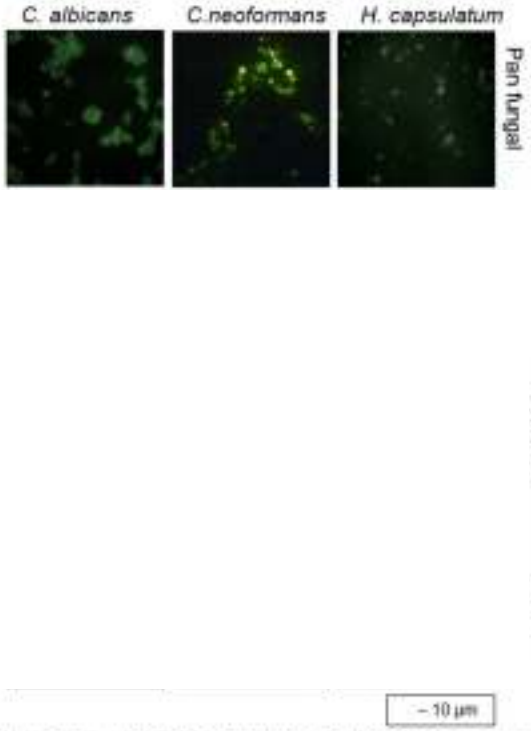
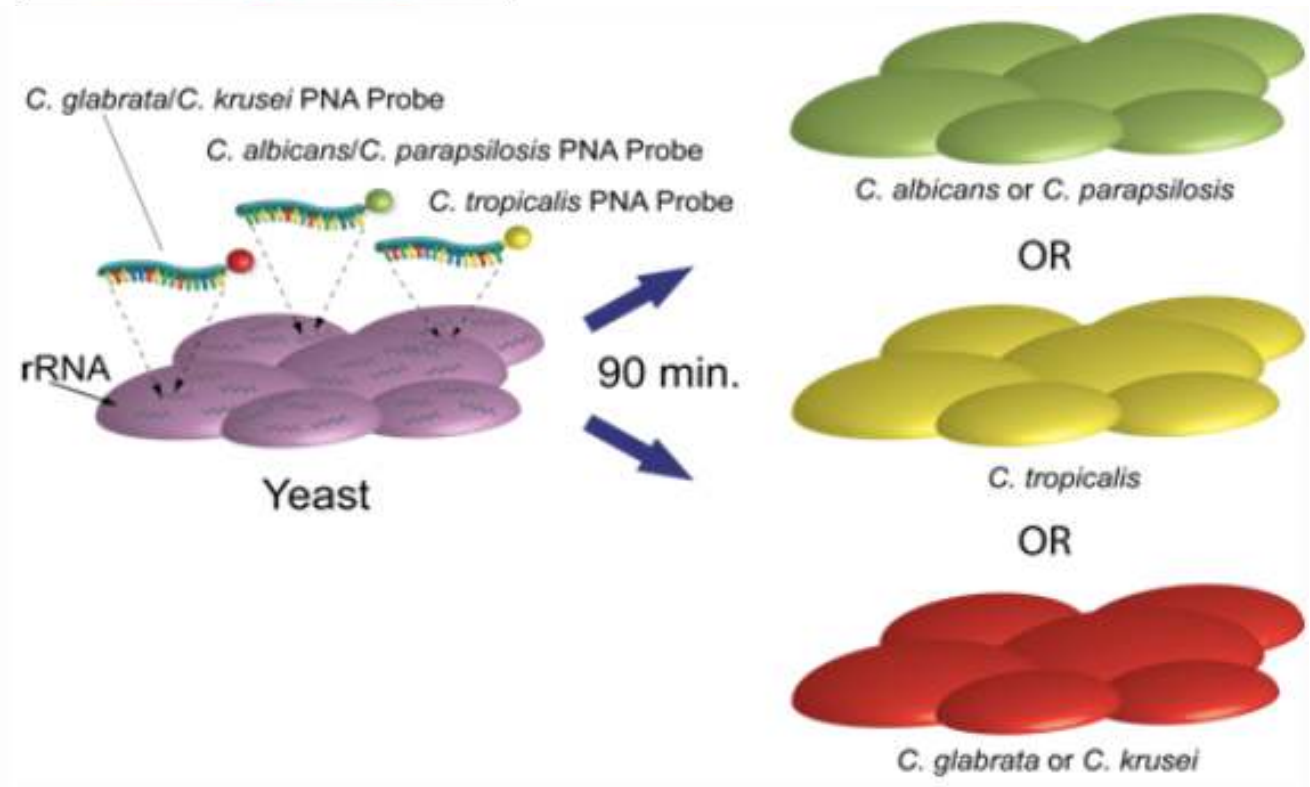


Figure 1 Fluorescence microscopy of microorganisms after FISH with various oligonucleotide probes. C. albicans, C. neoformans and H. capsulatum (vertical columns) were stained with FISH with probes Pan Fungal (green signal, specific for all fungi) and Cal3 specific for C. albicans). Cne 205 specific for C. neoformans and C. gattii, and Hcat specific for H. capsulatum, each Cy3 labelled (red signal).

Hibridizasyon Temelli Yöntemler

- Yeast Traffic Light® PNA FISH®



Hibridizasyon Temelli Yöntemler

- Yeast Traffic Light® PNA FISH®

  C. albicans, C. parapsilosis %100 duyarlılık ve özgüllük

JCM
Journals.ASM.org

Evaluation of PNA-FISH Yeast Traffic Light for Rapid Identification of Yeast Directly from Positive Blood Cultures and Assessment of Clinical Impact

N. R. H. Stone, R. L. Gorton, K. Barker, P. Ramnarain, C. C. Kibbler
Department of Microbiology, Royal Free Hospital, London, United Kingdom

The PNA-FISH Yeast Traffic Light assay was performed on 54 clinical isolates of yeasts inoculated into blood culture bottles. The assay showed high sensitivity (*Candida albicans/C. parapsilosis*, 100%; *C. glabrata/C. krusei*, 92.3%; *C. tropicalis*, 100%) and specificity (*C. albicans/C. parapsilosis*, 100%; *C. glabrata/C. krusei*, 94.8%; *C. tropicalis*, 100%). Case note review estimated a change in therapy in 29% of cases had the PNA-FISH result been available to the clinician.

Hibridizasyon Temelli Yöntemler

- Yeast Traffic Light® PNA FISH®; ortalama sonuç süresi 90 dakika idi.
  - 32 olguda (% 61.5) antifungal tedavide bir değişiklik yapılmadı
  - 11 olguda (% 21.2) kaspofunginden flukonazole geçiş ve
  - 4 olguda (% 7.7) flukonazoldan kaspofungine geçiş

} hastaların %40'ında tedaviye yön verilmiş

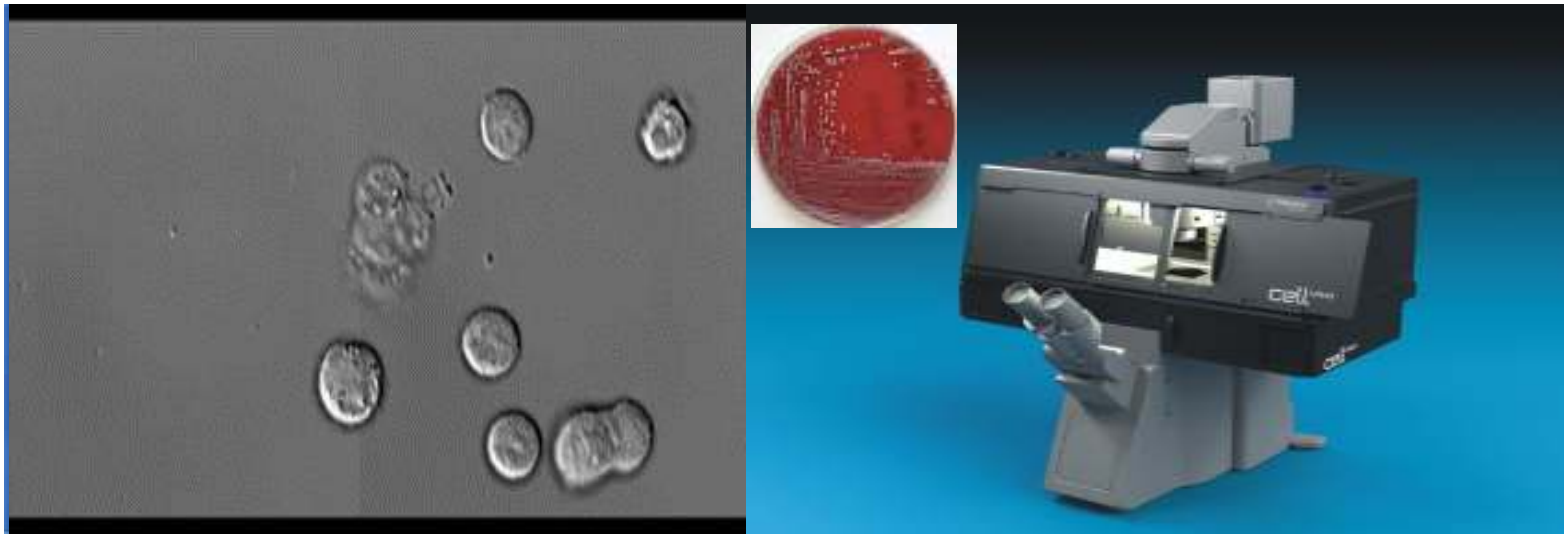
TABLE 2 Results summary by probe

Routine identification (AuxaColor 2)	No. of isolates by probe: Total	*C. albicans*/ *C. parapsilosis* (green)	*C. tropicalis* (yellow)	*C. glabrata*/ *C. krusei* (red)	Negative (no fluorescence)
C. albicans	17	17			
C. parapsilosis	10	10			
C. tropicalis	4		4		
C. krusei	4			4	
C. glabrata	8			8	
C. glabrata + *C. tropicalis*	1		1		
C. albicans + *C. tropicalis*	1	1	1		
C. guilliermondii	2				2
C. lusitaniae	1				1
C. pelliculosa	1				1
B. capitatus	1			1	
C. neoformans	2			1	1

Zaman Atlamalı Mikroskobik Yöntemler

- Zaman Atlamalı Mikroskobik Yöntemler

Hücrelerin canlı kalarak çoğalabildikleri bir ortamda, belirli zamana aralıklarında çekim yapabilen mikroskoplar.



Hibridizasyon Temelli Yöntemler

- Accelerate PhenoTest™ BC; direkt örnekten hızlı tanımlama ve duyarlılık

ACCELERATE
DIAGNOSTICS

Hibridizasyon Temelli Yöntemler

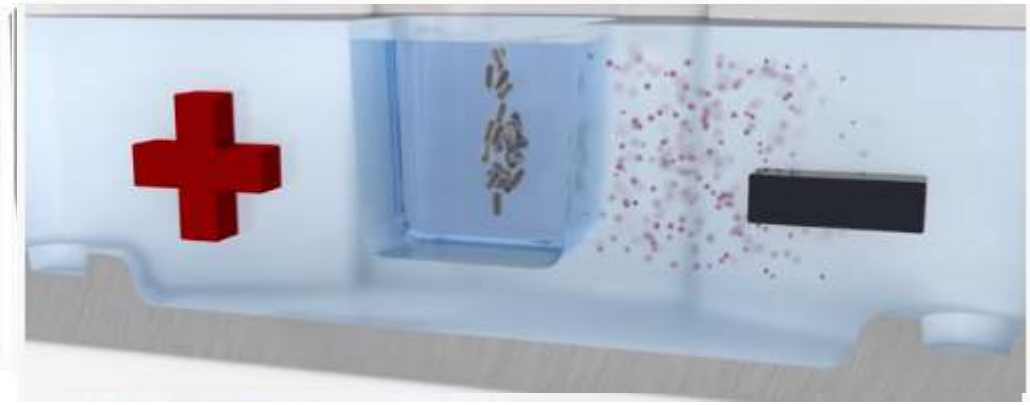
- Accelerate PhenoTest™ BC
- Örnekteki mikroorganizmayı saflaştırmak ve çoğaltmak

1 saat

8-48 saat

Hibridizasyon Temelli Yöntemler

- Accelerate PhenoTest™ BC
- Örnek muayene maddesinden mikroorganizmanın saflaştırılması için elektroforez ve filtreleme sistemi (elektrofiltrasyon).



Hibridizasyon Temelli Yöntemler

- Accelerate PhenoTest™ BC
- Mikroorganizmanın tanımlanması

1 saat

8-48 saat

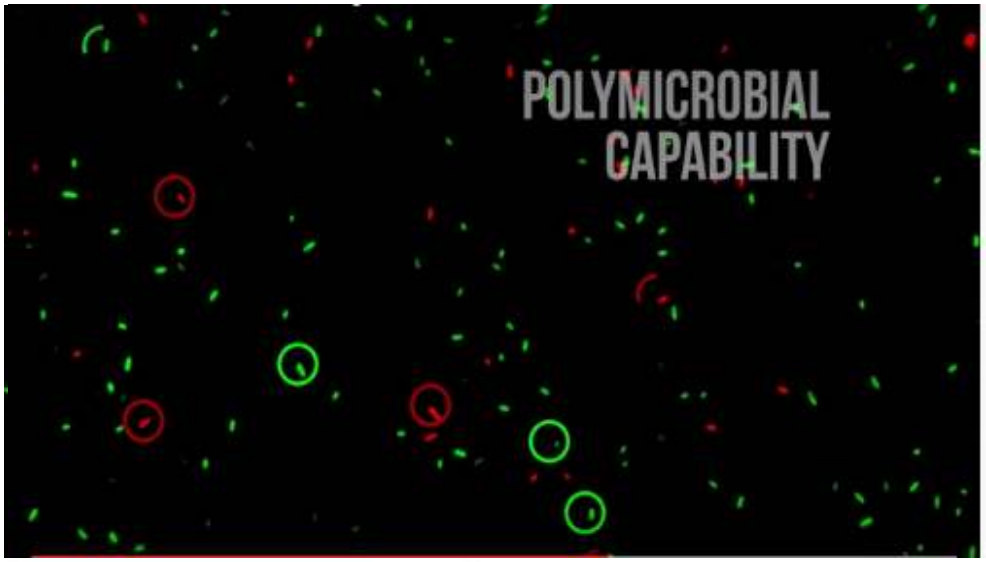
Hibridizasyon Temelli Yöntemler

- Accelerate PhenoTest™ BC

  Multiplexed Fluorescence in situ Hybridization (FISH)

Hibridizasyon Temelli Yöntemler

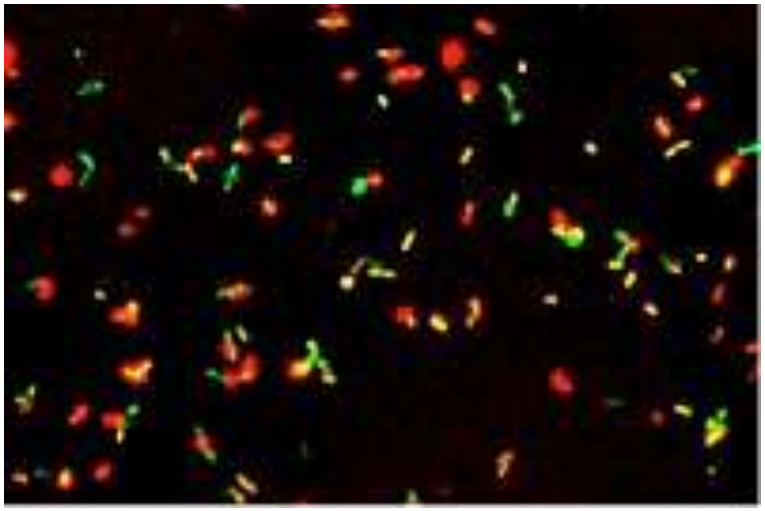
- Accelerate PhenoTest™ BC
- *Multiplexed Fluorescence in situ Hybridization* (FISH)



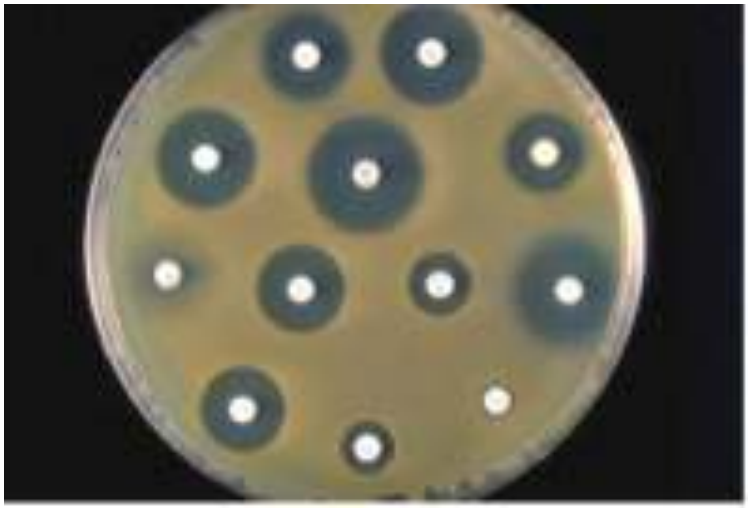
Hibridizasyon Temelli Yöntemler

- Accelerate PhenoTest™ BC
- Antimikrobial duyarlılıklarının belirlenmesi

4-5 saat



24-48 saat



Hibridizasyon Temelli Yöntemler

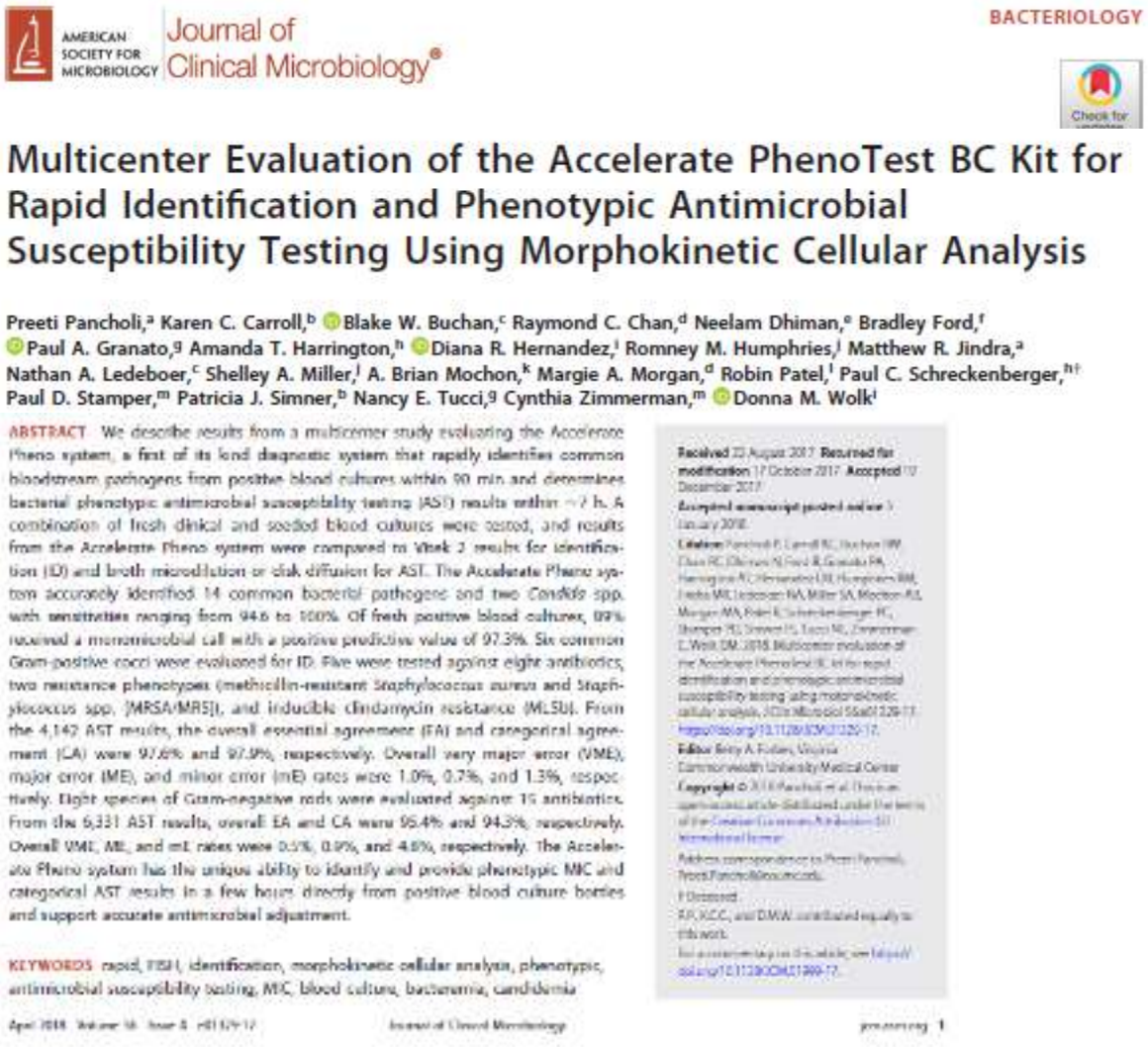
- Accelerate PhenoTest™ BC
- Antimikrobial duyarlılık testi; zaman-atlamalı mikroskop ile üreme/inhibisyonun takibi

Hibridizasyon Temelli Yöntemler

- Accelerate PhenoTest™ BC
- Antimikrobial duyarlılık testi; zaman-atlamalı mikroskop ile üreme/inhibisyonun takibi

Hibridizasyon Temelli Yöntemler

- Accelerate PhenoTest™ BC



AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

BACTERIOLOGY

Multicenter Evaluation of the Accelerate PhenoTest BC Kit for Rapid Identification and Phenotypic Antimicrobial Susceptibility Testing Using Morphokinetic Cellular Analysis

Preeti Pancholi,[a] Karen C. Carroll,[b] Blake W. Buchan,[c] Raymond C. Chan,[d] Neelam Dhiman,[e] Bradley Ford,[f] Paul A. Granato,[g] Amanda T. Harrington,[h] Diana R. Hernandez,[i] Romney M. Humphries,[j] Matthew R. Jindra,[a] Nathan A. Ledeboer,[c] Shelley A. Miller,[j] A. Brian Mochon,[k] Margie A. Morgan,[d] Robin Patel,[l] Paul C. Schreckenberger,[h] Paul D. Stamper,[m] Patricia J. Simner,[b] Nancy E. Tucci,[g] Cynthia Zimmerman,[m] Donna M. Wolk[i]

ABSTRACT We describe results from a multicenter study evaluating the Accelerate Pheno system, a first of its kind diagnostic system that rapidly identifies common bloodstream pathogens from positive blood cultures within 90 min and determines bacterial phenotypic antimicrobial susceptibility testing (AST) results within ~7 h. A combination of fresh clinical and seeded blood cultures were tested, and results from the Accelerate Pheno system were compared to Week 2 results for identification (ID) and broth microdilution or disk diffusion for AST. The Accelerate Pheno system accurately identified 14 common bacterial pathogens and two *Candida* spp. with sensitivities ranging from 94.6 to 100%. Of fresh positive blood cultures, 89% received a monomicrobial call with a positive predictive value of 97.3%. Six common Gram-positive cocci were evaluated for ID. Five were tested against eight antibiotics, two resistance phenotypes (methicillin-resistant *Staphylococcus aureus* and *Staphylococcus* spp. [MRSA/MRS]), and inducible clindamycin resistance (MLSi). From the 4,142 AST results, the overall essential agreement (EA) and categorical agreement (CA) were 97.6% and 97.9%, respectively. Overall very major error (VME), major error (ME), and minor error (mE) rates were 1.0%, 0.7%, and 1.3%, respectively. Eight species of Gram-negative rods were evaluated against 15 antibiotics. From the 6,331 AST results, overall EA and CA were 95.4% and 94.3%, respectively. Overall VME, ME, and mE rates were 0.5%, 0.9%, and 4.8%, respectively. The Accelerate Pheno system has the unique ability to identify and provide phenotypic MIC and categorical AST results in a few hours directly from positive blood culture bottles and support accurate antimicrobial adjustment.

KEYWORDS rapid, FISH, identification, morphokinetic cellular analysis, phenotypic, antimicrobial susceptibility testing, MIC, blood culture, bacteremia, candidemia

Received 22 August 2017 Returned for modification 17 October 2017 Accepted 17 December 2017

Accepted manuscript posted online 3 January 2018

Editor Betty A. Forbes, Virginia Commonwealth University Medical Center

Address correspondence to Preeti Pancholi, Preeti.Pancholi@osumc.edu.

† Deceased.

P.P., K.C.C., and D.M.W. contributed equally to this work.

For supplemental material for this article, see https://doi.org/10.1128/JCM.01999-17.

Hibridizasyon Temelli Yöntemler

- Accelerate PhenoTest™ BC

TABLE 1 Identification performance of Gram-positive bacteria, Gram-negative bacteria, and yeasts after reevaluation of DNA probe data with the 2017 Accelerate Pheno system software update[a]

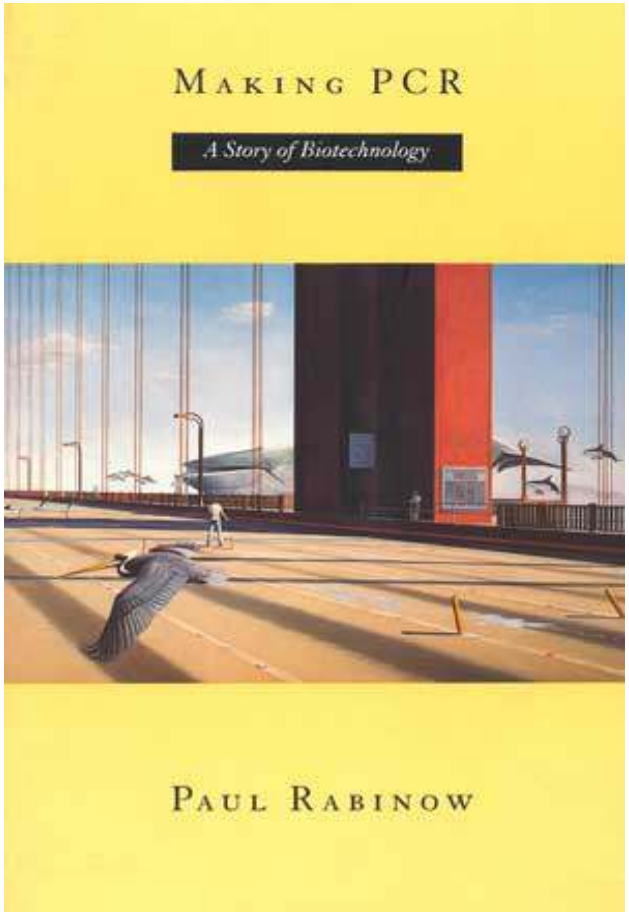
Probe (category and species)	No. of samples with the following result[b]: TP	FN	TN	FP	Sensitivity(%) (95% CI)[c]	Specificity (%) (95% CI)[c]
Gram-positive bacteria						
Staphylococcus aureus	242	5	1,643	19	98.0 (95.4–99.1)	98.9 (98.2–99.3)
CoNS[d]	264	15	1,589	28	94.6 (91.3–96.7)	98.3 (97.5–98.8)
Staphylococcus lugdunensis	77	2	1,857	1	97.5 (91.2–99.3)	100.0 (99.7–100)
Enterococcus faecium	109	4	1,809	9	96.5 (91.3–98.6)	99.5 (99.1–99.7)
Enterococcus faecalis	102	2	1,814	3	98.1 (93.3–99.5)	99.8 (99.5–99.9)
Streptococcus spp.	180	5	1,678	46	97.3 (93.8–98.8)	97.3 (96.5–98)
Total	974	33	10,390	106	96.7 (95.4–97.7)	99.0 (98.8–99.2)
Gram-negative bacteria						
Escherichia coli	148	2	1,771	2	98.7 (95.3–99.6)	99.9 (99.6–100)
Klebsiella spp.	126	3	1,790	6	97.7 (93.4–99.2)	99.7 (99.3–99.9)
Enterobacter spp.	108	2	1,822	4	98.2 (93.6–99.5)	99.8 (99.4–99.9)
Proteus spp.	88	1	1,838	6	98.9 (93.9–99.9)	99.7 (99.3–99.9)
Citrobacter spp.	95	1	1,768	8	99.0 (94.3–100)	99.6 (99.1–99.8)
Serratia marcescens	50	0	1,885	1	100.0 (92.9–100)	100.0 (99.7–100)
Pseudomonas aeruginosa	57	1	1,865	3	98.3 (90.9–99.9)	99.8 (99.5–100)
Acinetobacter baumannii	69	1	1,854	3	98.6 (92.3–99.9)	99.8 (99.5–100)
Total	741	11	14,593	33	98.5 (97.4–99.2)	99.8 (99.7–99.8)
Yeasts						
Candida albicans	44	1	1,827	7	97.8 (88.4–99.9)	99.6 (99.2–99.8)
Candida glabrata	49	1	1,818	8	98.0 (89.5–99.9)	99.6 (99.1–99.8)
Total	93	2	3,645	15	97.9 (92.7–99.4)	99.6 (99.3–99.8)
Overall	**1,808**	**46**	**28,628**	**154**	**97.5 (96.7–98.1)**	**99.5 (99.4–99.5)**

Hibridizasyon Temelli Yöntemler

- Accelerate PhenoTest™ BC

Amplifikasyon Temelli Yöntemler

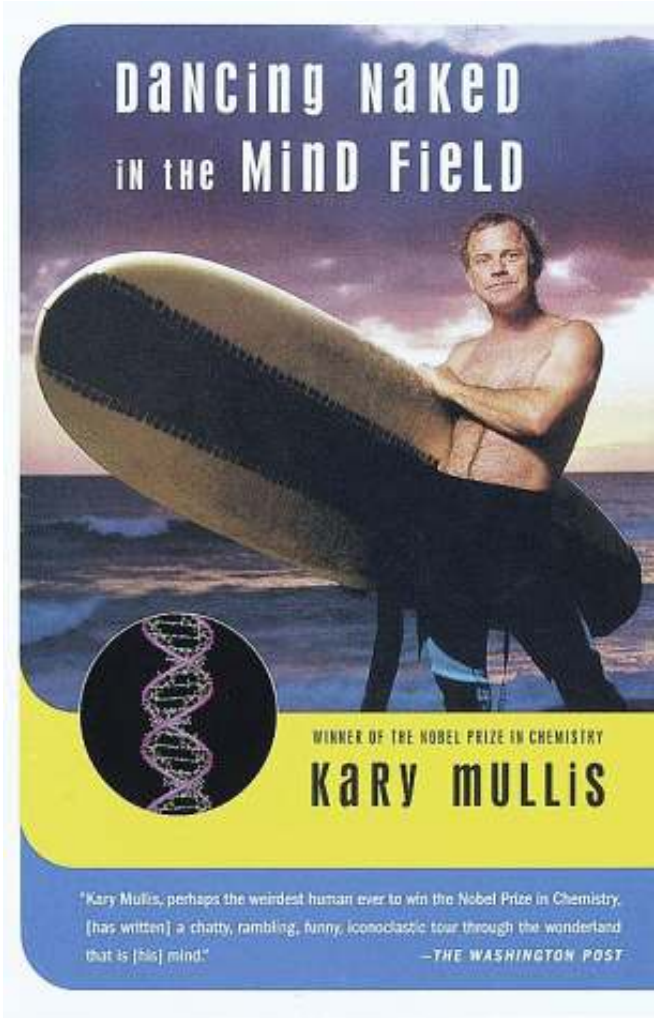
- *Polymerase Chain Reaction* (PCR)



- *PCR*
- *Multiplex PCR*
- *Nested PCR*
- *Seminetested PCR*
- *Broad Range PCR*
- *Hot Start PCR*
- *Touchdown PCR*
- *Reverse Transcription PCR*

Amplifikasyon Temelli Yöntemler

- *Polymerase Chain Reaction* (PCR)



DNA Amplification for Direct Detection of HIV-1 in DNA of Peripheral Blood Mononuclear Cells

CHIN-YIH OU,* SHIRLEY KWOK, SHEILA W. MITCHELL, DAVID H. MACK, JOHN J. SNINSKY, JOHN W. KREBS, PAUL FEORINO, DONNA WARFIELD, GERALD SCHOCHETMAN

By means of a selective DNA amplification proviral sequences of the human immun directly in DNA isolated from periphe persons seropositive but not in DNA isolat the virus. Primer pairs from multiple re achieve maximum sensitivity of provirus d 100% of DNA specimens from seropositi was isolated by coculture, but in none of t seronegative, virus culture–negative perso ed in 64% of DNA specimens from seroj men. This method of DNA amplification days, whereas virus isolation takes up to used to complement or replace virus isolat 1 infection.

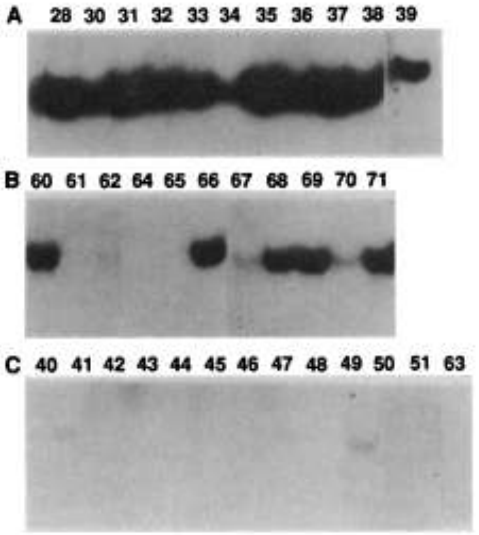


Fig. 1. (**A** to **C**) Representative DNA amplification analysis of peripheral blood lymphocyte DNA from HIV-1–seropositive and seronegative persons (see Table 1). DNA samples were amplified for 35 rounds with the primer pair SK68/69 (Table 2) representing a conserved gp41 region, restricted with BstN I and fractionated in a 30% polyacrylamide gel. The detailed experimental procedures are described in (*21*).

Amplifikasyon Temelli Yöntemler

- *Polymerase Chain Reaction* (PCR)

Detection of *Pneumocystis carinii* Sequences by Polymerase Chain Reaction: Animal Models and Clinical Application to Noninvasive Specimens

KAZUHIRO KITADA,[1] SHINICHI OKA,[2] SATOSHI KIMURA,[2] KAORU SHIMADA,[2] TADAO SERIKAWA,[3] JUNZO YAMADA,[3] HAJIME TSUNOO,[4] KOHJI EGAWA,[1] AND YOSHIKAZU NAKAMURA[1]*

Departments of Tumor Biology[1] and Infectious Diseases,[2] The Institute of Medical Science, The University of Tokyo, P.O. Takanawa, Tokyo 108, Institute of Laboratory Animals, Faculty of Medicine, Kyoto University, Kyoto 606,[3] and Meiji Institute of Health Science, Odawara 250,[4] Japan

Received 13 March 1991/Accepted 21 June 1991

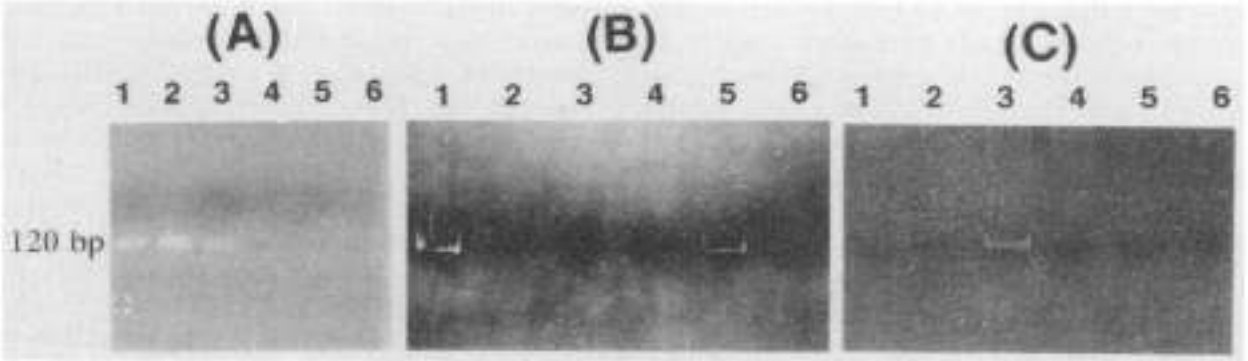


FIG. 3. PCR tests of clinical specimens. Experimental procedures and conditions are as described in Materials and Methods. (A) Lung autopsy. Lanes 1 through 3, patients diagnosed with *P. carinii* pneumonia; lanes 4 through 6, patients unrelated to *P. carinii* pneumonia. (B) Lung biopsy. Lane 1, positive control (*P. carinii*-infected mouse lung); lane 2, negative control (water); lanes 3 through 6, needle aspirates from individual AIDS patients. (C) Blood samples. Blood (0.5 ml) from individuals was used in the reaction. Lane 1, patient 2; lane 2, patient 1 on 19 March 1990; lane 3, the patient 1 on 2 April 1990; lanes 4 through 6, three individual healthy human controls.

Amplifikasyon Temelli Yöntemler

- *Polymerase Chain Reaction* (PCR)

SolGent Co., Ltd.

Item Name

About SolGent / Molecular Diagnosis / Molecular Research / Service / Customer Service

Molecular Diagnosis

Core Technology

Diagnostic Kit

ZIKA virus

Catalogue &Brochure

DiaPlexC™ Fungi Detection Kit (Conventional)

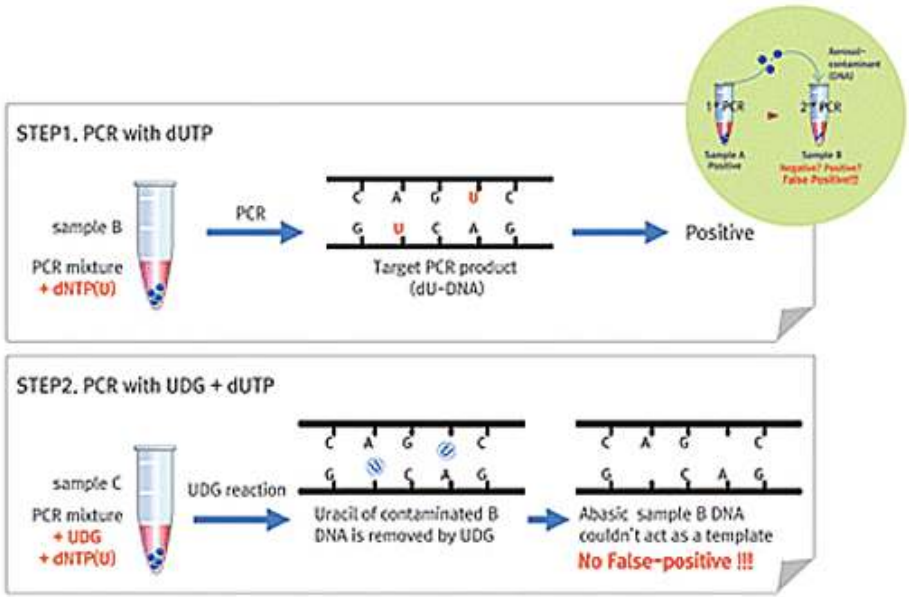
The DiaPlexC™ Fungi Detection Kit is designed to detect 16 species of Fungi present in cell cultures using the Multiplex PCR technology which can detect multiple specific target genes in a single PCR.

Pathogen Information

Fungi are one of the major ubiquitously found contaminants in the laboratory. It can contaminate any kind of biological related materials and spoil them. In the laboratory, cell cultures, culture media, broth, serum and plasma are all highly susceptible to the fungal contamination unless strict aseptic measures are applied. Even when excellent aseptic technique is applied, it is essential to monitor and test to confirm results are free from contamination. In the absence of antibiotics they will grow rapidly, however, if antibiotics are routinely used low levels of fungal infections may develop that might be more difficult to observe.

Amplifikasyon Temelli Yöntemler

- *Polymerase Chain Reaction* (PCR)

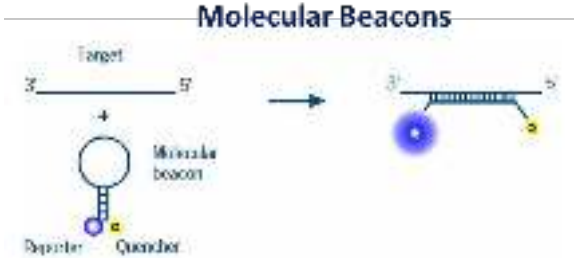


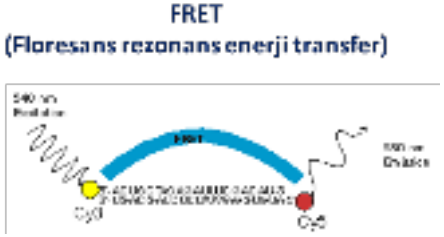
At the pre-PCR stage, dTTP is substituted with dUTP and the UDG is included in the reaction mix. All other reaction components remain the same. During the PCR, DNA polymerase substitutes dU for dT in the growing DNA strand. In the final product, there is now dU instead of dT in the DNA sequence. Before any new sample is processed, it is first exposed to the UDG enzyme. If the UDG comes across any U-containing DNA strands, the Us are cleaved, leaving the strand with gaps. Following heating in the next PCR, the abasic strands fall apart and cannot be amplified. The use of UDG provides the added advantage of a hot start by degrading all PCR products made prior to the first full cycle.

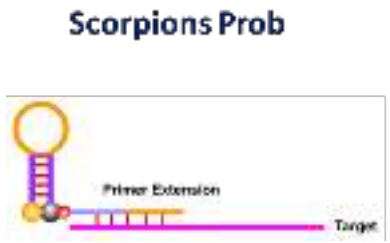
Amplifikasyon Temelli Yöntemler

- *Real-time* PCR,

  PCR'ın keşfinden sonraki en önemli gelişme.







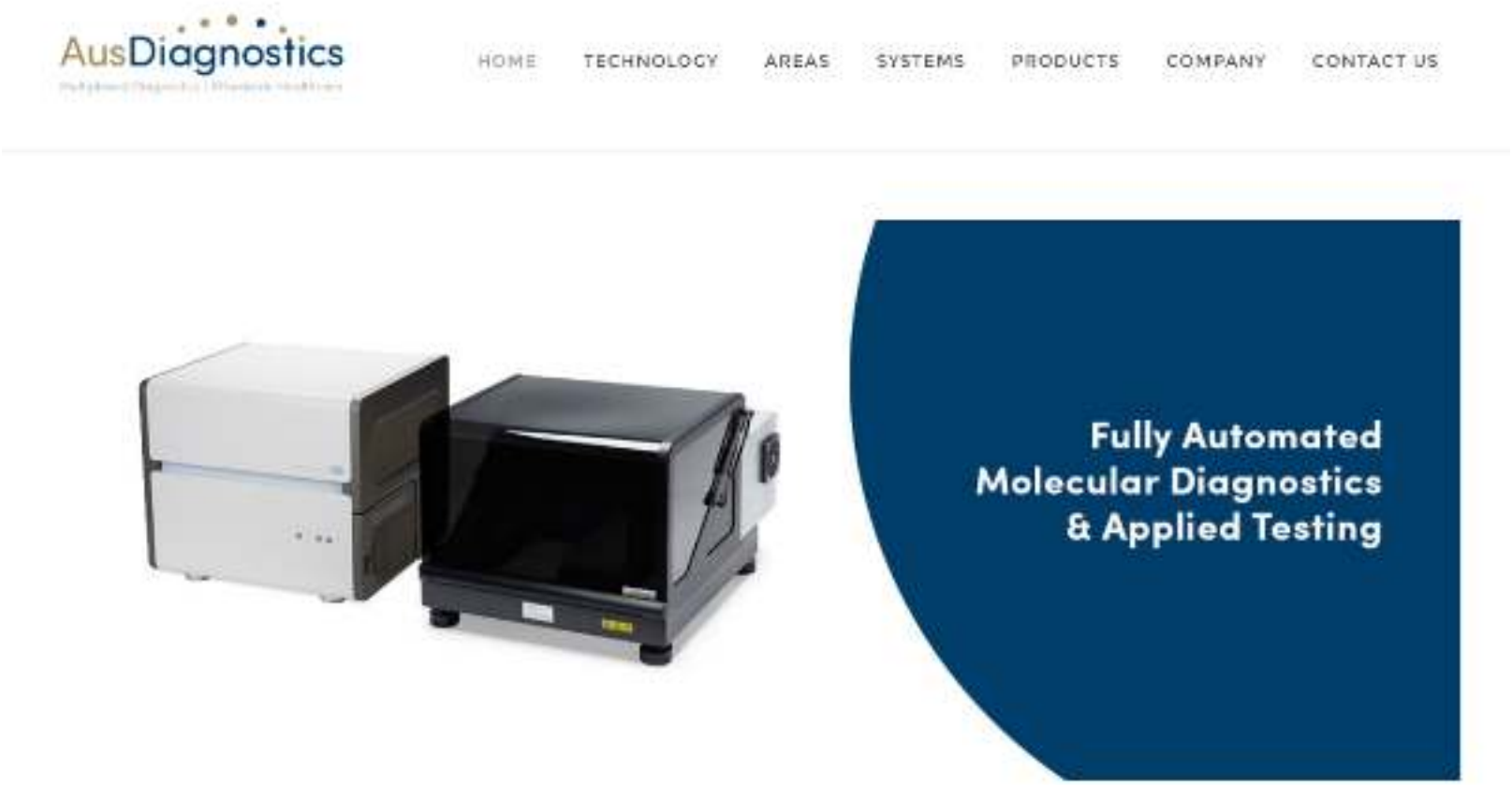
Amplifikasyon Temelli Yöntemler

- *Real-time* PCR
  - Real-time PCR cihazları laboratuvarlarda yerini aldılar.

Amplifikasyon Temelli Yöntemler

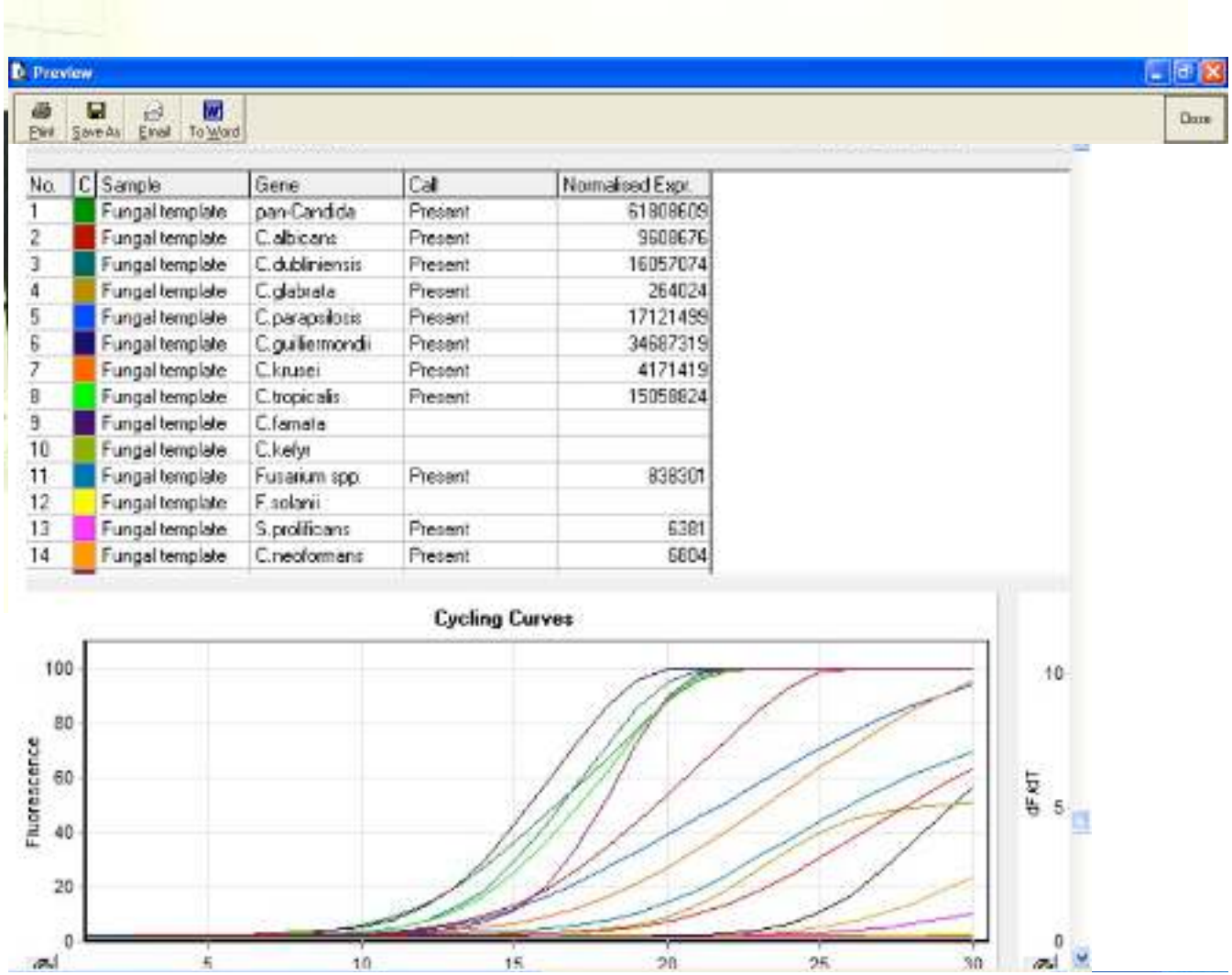
- *Real-time* PCR

direkt örnekten tanı için multiplex Real-time PCR



Amplifikasyon Temelli Yöntemler

- *Real-time* PCR



Amplifikasyon Temelli Yöntemler

- *Real-time* PCR

66 örneğin 61'inde (%92) uyumlu sonuç elde edilmiş.

JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2010, p. 811–816 Vol. 48, No. 3
0095-1137/10/$12.00 doi:10.1128/JCM.01650-09

Comparison of Whole Blood, Serum, and Plasma for Early Detection of Candidemia by Multiplex-Tandem PCR▽

Anna Lau,[1,2] Catriona Halliday,[1,3] Sharon C.-A. Chen,[1,3] E. Geoffrey Playford,[4] Keith Stanley,[5] and Tania C. Sorrell[1,2]*

Centre for Infectious Diseases and Microbiology,[1] Westmead Millennium Institute,[2] and Centre for Infectious Diseases and Microbiology Laboratory Services, Institute of Clinical Pathology and Medical Research, Sydney West Area Health Service,[3] Westmead, NSW, Princess Alexandra Hospital, Brisbane, QLD,[4] and AusDiagnostics Pty. Ltd., Alexandria, NSW,[5] Australia

Diagnostics) at 55°C for 1 h to overnight. DNA was extracted according to the manufacturer's instructions with a final elution volume of 60 μl.

(ii) Serum and plasma. Plain and EDTA-coated blood collection tubes were centrifuged at 1,258 × *g* for 10 min to gather cells; the serum and plasma, respectively, were then removed. DNA was extracted from a 1-ml sample by using the protocol for whole blood on a nucliSENS easyMAG instrument (bioMérieux, Baulkham Hills, New South Wales, Australia) with a final elution volume of 60 μl.

MT-PCR amplification and detection. Master mix reagents and 72-well gene discs containing lyophilized primers were prepared by AusDiagnostics Pty. Ltd. (Alexandria, New South Wales, Australia). The fungal ribosomal DNA (rDNA) internal transcribed spacer 1 (ITS1) and ITS2 regions, elongation factor 1-alpha (EF1-α), and β-tubulin gene loci were used to design primers specific for the 10 most frequently encountered *Candida* species in Australia (3): *C. albicans*, *C. dubliniensis*, *C. famata*, *C. glabrata*, *C. guilliermondii*, *C. kefyr*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis* complex (*C. parapsilosis*, *C. metapsilosis*, and *C. orthopsilosis*), and *C. tropicalis*, as well as *Yarrowia lipolytica*, *Cryptococcus neoformans* complex (*C. neoformans* var. *grubii*, *C. neoformans* var. *neoformans*, and *Cryptococcus gattii*), *Saccharomyces cerevisiae*, *Fusarium solani*, *Fusarium* spp., and *Scedosporium prolificans*. The primer sequences are not shown due to commer-

assay for each sample always tested positive.

MT-PCR analysis of whole blood in EDTA-coated tubes, serum, and plasma samples from patients with documented candidemia. The results of MT-PCR with whole-blood, serum, and plasma samples relative to the time of collection of the first blood sample that flagged positive by culture are illustrated in Fig. 1. Fungal DNA was detected an average of 2.2 days (range, 0.5 to 8 days) before the blood cultures signaled positive for 52/74 (70%) patients, enabling the detection of fungemia as well as *Candida* species identification to be expedited by up to 4 days (the time required for the blood culture to signal positive and the time for species identification by use of the API 32C system [bioMérieux]). For 21/39 patients (54%), *Candida* DNA was detected an average of 1.5 days (range, 0.5 to 4.5 days) prior to the time of collection of the first positive blood culture sample ($T < 0$). Although the sample numbers were small, the MT-PCR assay performed better with serum

Amplifikasyon Temelli Yöntemler

- *Real-time* PCR
  - *Candida auris*

Tired of MRSA and CPE? The new superbug has arrived and it's a yeast!

November 9, 2016 Andreas Voss (@AVIPNL) antimicrobial resistance, Uncategorized
#candida, antifungals, Candida auris

All of a sudden, *Candida auris* seems to become the "new" global super villain. Antimicrobial Resistance and Infection Control recently published the first, large European outbreak of *C. auris* in London with 50 cases (Schelenz et al.) and CDC just published the first 17 US cases (Vallabhaneni et al.). While I believe that *C. auris* deserves our full attention, as it is a multi-resistant yeast, with increased MICs to all three major classes of antifungals, likes to evades traditional diagnostic methods, seems to be difficult to eradicate from the hospital environment, and

Amplifikasyon Temelli Yöntemler

- *Real-time* PCR
  - *Candida auris*

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Identification Method	Organism *C. auris* can be misidentified as
Vitek 2 YST	*Candida haemulonii* *Candida duobushaemulonii*
API 20C	*Rhodotorula glutinis* (characteristic red color not present) *Candida sake*
BD Phoenix yeast identification system	*Candida haemulonii* *Candida catenulata*
MicroScan	*Candida famata* *Candida guilliermondii* *Candida lusitaniae* *Candida parapsilosis*
RapID Yeast Plus	*Candida parapsilosis*

Amplifikasyon Temelli Yöntemler

Araştırma Konusu

- *Real-time* PCR
  - *Candida auris*

Journal of Clinical Microbiology®

MYCOLOGY

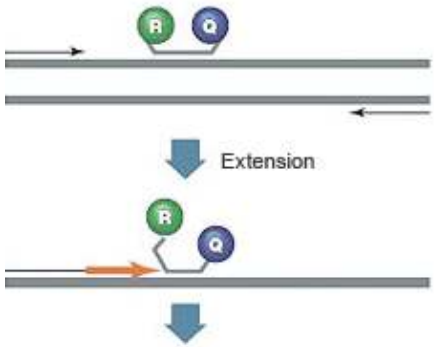
Development and Validation of a Real-Time PCR Assay for Rapid Detection of *Candida auris* from Surveillance Samples

L. Leach,[a] Y. Zhu,[a] S. Chaturvedi[a,b]

[a]Mycology Laboratory, Wadsworth Center, New York State Department of Health, Albany, New York, USA
[b]Department of Biomedical Sciences, School of Public Health, University at Albany, Albany, New York, USA

ABSTRACT *Candida auris* is an emerging multidrug-resistant yeast causing invasive health care-associated infection with high mortality worldwide. Rapid identification of *C. auris* is of primary importance for the implementation of public health measures to control the spread of infection. To achieve these goals, we developed and validated a TaqMan-based real-time PCR assay targeting the internal transcribed spacer 2 (ITS2) region of the ribosomal gene. The assay was highly specific, reproducible, and sensitive, with the detection limit of 1 *C. auris* CFU/PCR. The performance of the *C. auris* real-time PCR assay was evaluated by using 623 surveillance samples, including 365 patient swabs and 258 environmental sponges. Real-time PCR yielded positive results from 49 swab and 58 sponge samples, with 89% and 100% clinical sensitivity with regard to their respective culture-positive results. The real-time PCR also detected *C. auris* DNA from 1% and 12% of swab and sponge samples with culture-negative results, indicating the presence of dead or culture-impaired *C. auris*. The real-time PCR yielded results within 4 h of sample processing, compared to 4 to 14 days for culture, reducing turnaround time significantly. The new real-time PCR assay allows for accurate and rapid screening of *C. auris* and can increase effective control and prevention of this emerging multidrug-resistant fungal pathogen in health care facilities.

KEYWORDS *Candida auris*, surveillance samples, TaqMan chemistry, assay validation, real-time PCR assay

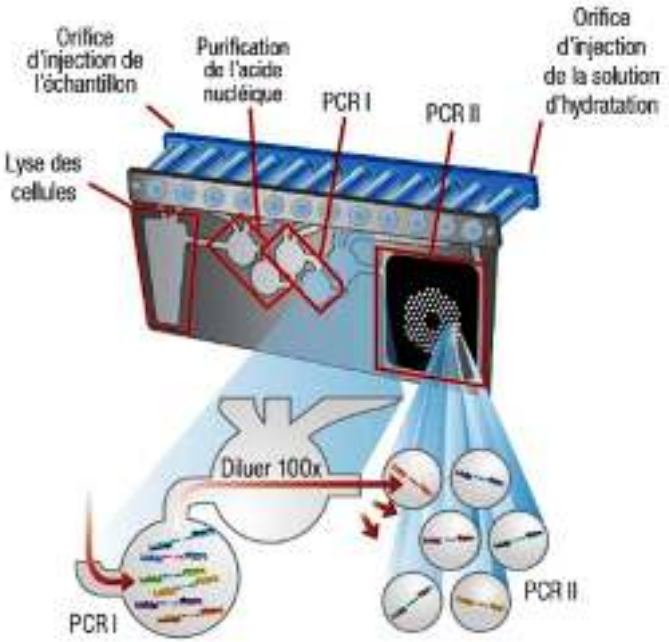


MATERIALS AND METHODS

***C. auris* real-time PCR assay.** The multiple alignment of the *ITS2* genes from all four clades of *C. auris* and closely related pathogenic yeasts revealed a region highly specific for *C. auris* (see Fig. S1 in the supplemental material). Primers and a probe were designed from this region using the PrimerQuest program (Integrated DNA Technologies, Coralville, IA). Primers and a probe were also designed for an inhibition control from a *bicoid* gene. Sequences for primers and probes for *C. auris ITS2* gene were as follows: V2424F (CAURF), 5′-CAGACGTGAATCATCGAATCT-3′; V2425P (CAURP), 5′-/56-carboxyfluorescein (FAM)/AATCTTCGC/ZEN/GGTGGCGTTGCATTCA/3IABkFQ/-3′; and V2426 (CAURR), 5′-TTTCGTGCAAGC TGTAATTT-3′. Those for the *bicoid* gene were as follows: V2375 (BICF), 5′-CAGCTTGCAGACTCTTAG-3′; V2384 (BICP), 5′/Cy3/AACGCTTTGACTCCGTCACCCA/3AbRQSp/-3′; and V2376 (BICR), 5′-GAATGACTCG CTGTAGTG-3′. All primers and probes were obtained from Integrated DNA Technologies.

Amplifikasyon Temelli Yöntemler

- *Real-time* PCR
  - Yüksek multipleks kapasiteye sahip cihazlar (Biofire, Filmarray)



Amplifikasyon Temelli Yöntemler

- *Real-time* PCR
  - Yüksek multipleks kapasiteye sahip cihazlar. Biofire, Filmarray

Gram-Positive Bacteria

Enterococcus
Listeria monocytogenes
Staphylococcus
Staphylococcus aureus
Streptococcus
Streptococcus agalactiae
Streptococcus pneumoniae
Streptococcus pyogenes

Gram-Negative Bacteria

Acinetobacter baumannii
Haemophilus influenzae
Neisseria meningitidis
Pseudomonas aeruginosa
Enterobacteriaceae
Enterobacter cloacae complex
Escherichia coli
Klebsiella oxytoca
Klebsiella pneumoniae
Proteus
Serratia marcescens

Yeast

Candida albicans
Candida glabrata
Candida krusei
Candida parapsilosis
Candida tropicalis

Antibiotic Resistance Genes

mecA – methicillin resistant
vanA/B – vancomycin resistant
KPC – carbapenem resistant

Amplifikasyon Temelli Yöntemler

- *Real-time* PCR

Rapid Detection of Bloodstream Pathogens in Liver Transplantation Patients With FilmArray Multiplex Polymerase Chain Reaction Assays: Comparison With Conventional Methods

B. Otlu[a,*], Y. Bayindir[b], F. Ozdemir[c], V. Ince[c], S. Cuglan[a], M. Hopoglu[b], Y. Yakupogullari[a], C. Kizilkaya[d], C. Kuzucu[a], B. Isık[a], and S. Yilmaz[c]

Gram negative bacteria	Gram positive bacteria	Yeast	Antimicrobial resistance genes
Acinetobacter baumannii *Haemophilus influenzae* *Neisseria meningitidis* *Pseudomonas aeruginosa* *Enterobacter cloacae* complex *Escherichia coli* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Proteus spp.* *Serratia marcescens*	*Enterococcus spp.* *Listeria monocytogenes* *Staphylococcus spp.* *Staphylococcus aureus* *Streptococcus spp.* *Streptococcus agalactiae* *Streptococcus pneumoniae* *Streptococcus pyogenes*	*Candida albicans* *Candida glabrata* *Candida krusei* *Candida parapsilosis* *Candida tropicalis*	*mecA* - methicillin resistance gene *vanA/B* - vancomycin resistance gene KPC - carbapenem resistance gene

Amplifikasyon Temelli Yöntemler

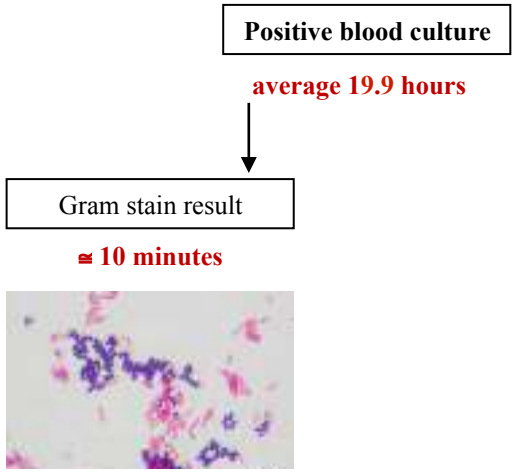
- *Real-time* PCR
- Ortalama kan kültür pozitiflik süresi: 19.9 saat

Positive blood culture

average 19.9 hours

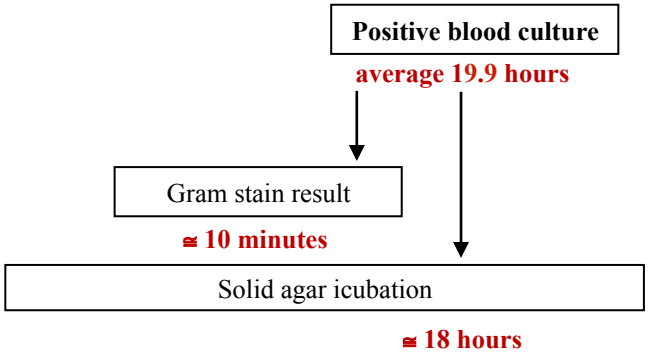
Amplifikasyon Temelli Yöntemler

- *Real-time* PCR
- Gram boyama sonucu



Amplifikasyon Temelli Yöntemler

- *Real-time* PCR
- Kültür Sonucu

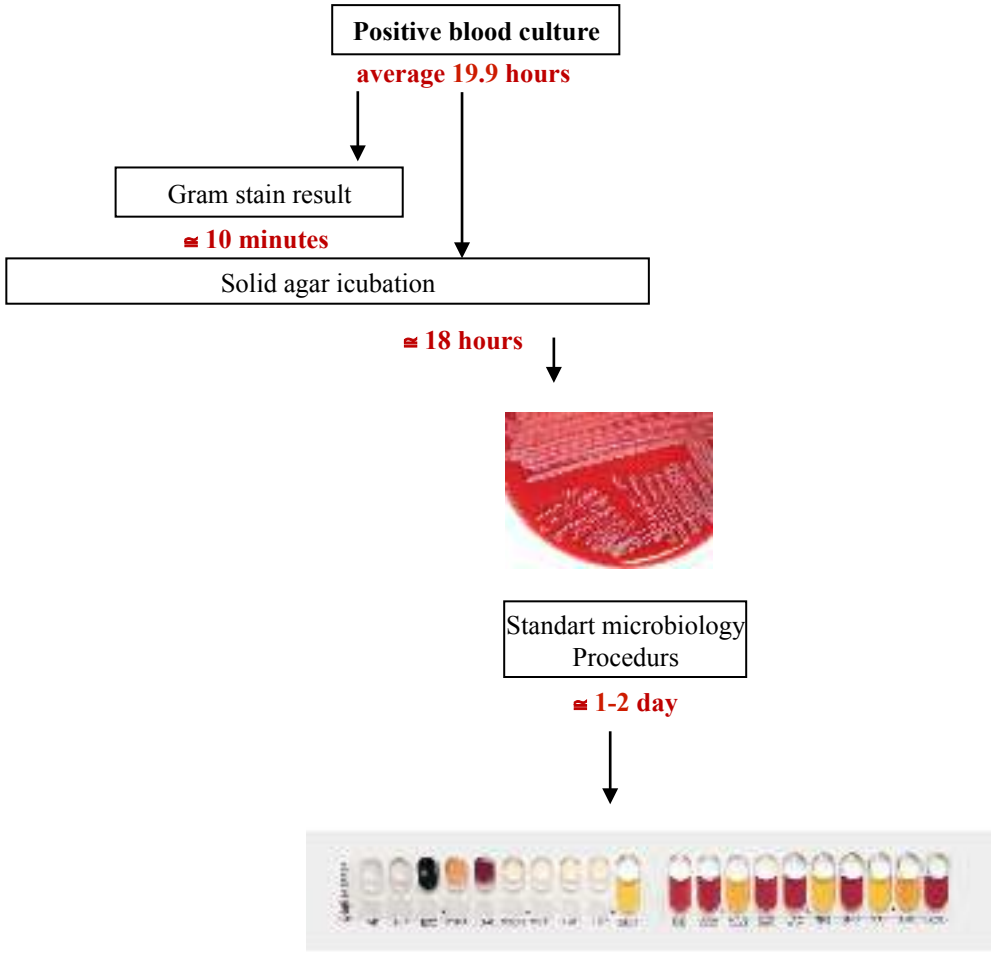


Saf kültür

Subkültür?

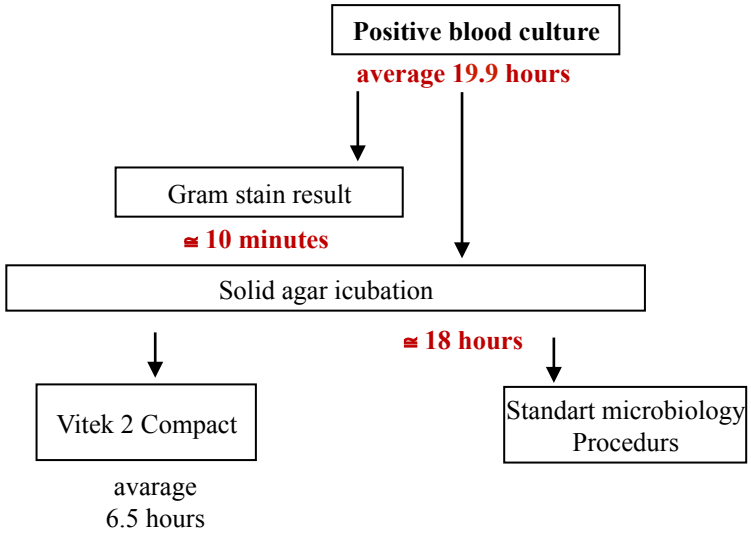
Amplifikasyon Temelli Yöntemler

- *Real-time* PCR
- Kültür Sonucu ve klasik yöntemlerle tanımlama



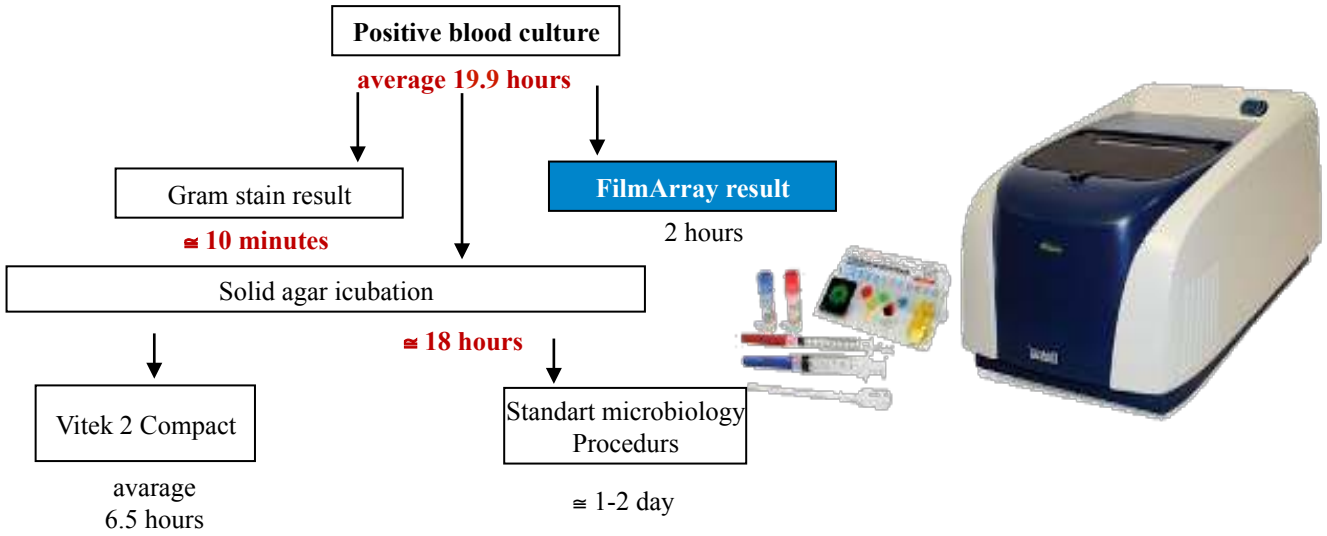
Amplifikasyon Temelli Yöntemler

- *Real-time* PCR



Amplifikasyon Temelli Yöntemler

- *Real-time* PCR



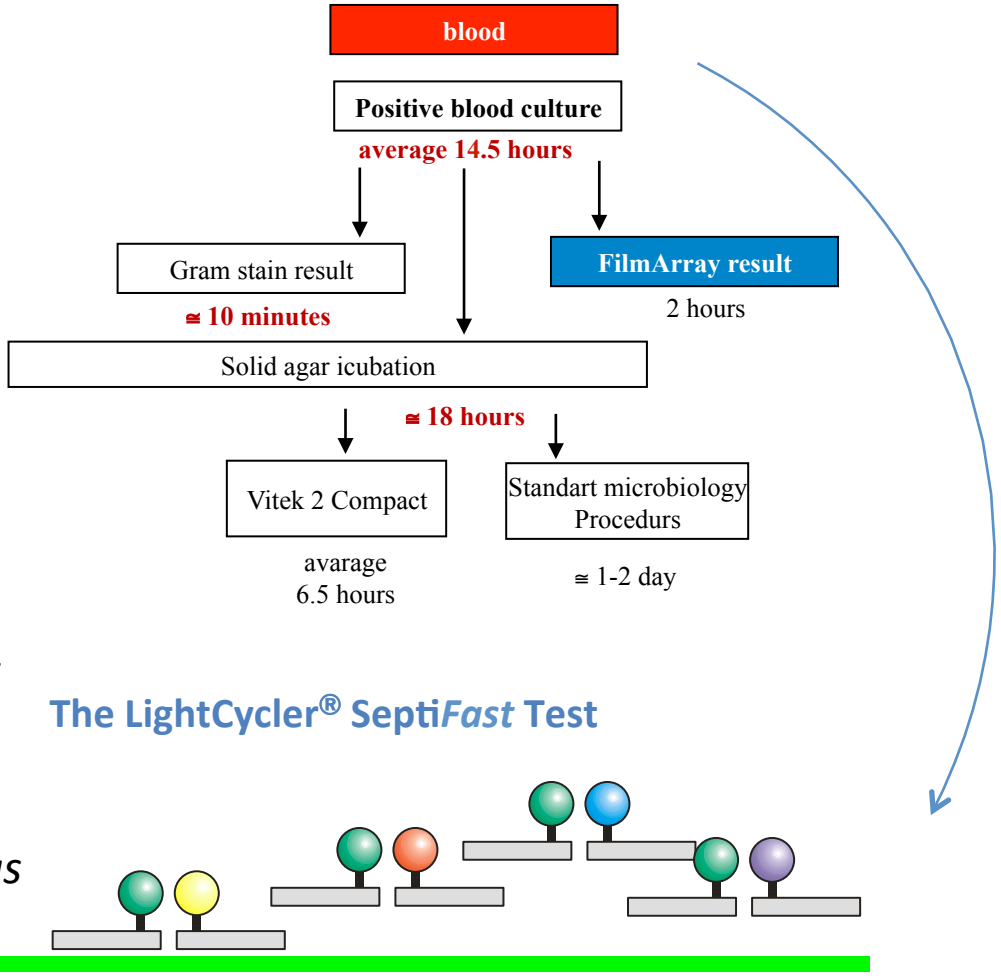
Amplifikasyon Temelli Yöntemler

- *Real-time* PCR

Sample No	Time to blood positvity (hours)	Gram stain from positive bottle	FilmArray Identification	Identification after solid agar incubation (18 hours)		
				Standart microbiology methods / Time to result (day)	Vitek 2 Compact / Time to result (hours)	Vitek MS
1	11.5	Gr - bacilli	*E. cloace complex*	*Enterobacter spp.* / 4	*E. cloace complex* / 5	*E. cloace*
2	8.2	Gr - bacilli	*E. coli, KPC* (-)	*E. coli / 1*	ND[b]	ND[b]
3	10.2	Gr - bacilli	*K. pneumoniae , P. aeruginosa*	*K. pneumoniae* /1	*K. pneumoniae* / 4.15	*K. pneumoniae*
4	25.2	Gr - bacilli	*K. pneumoniae*	*K. pneumoniae* / 1	*K. pneumoniae* / 5	*K. pneumoniae*
5	8.1	Gr - bacilli	*K. pneumoniae, Streptococcus spp*	*K. pneumoniae* / 1	*K. pneumoniae* /4.45	*K. pneumoniae*
6	7.2	Gr + cocci	*A. baumannii*	*Acinetobacter spp.* / 4	*A. baumannii complex* /6	*A. baumannii complex*
7	10.1	Gr + cocci	*A. baumannii*	*Acinetobacter spp* . /1	*A. baumannii complex* /6	*A. baumannii complex*
8	100.8	Gr - bacilli	*K. pneumoniae*	*K. pneumoniae* / 1	*K. pneumoniae* / 6	*K. pneumoniae*
9	10.4	Gr - bacilli	*E. coli, KPC* (-)	*E. coli* / 1	ND[b]	ND[b]
10	12.1	Gr + cocci	*Enterococcus spp., Van A/B* (-)	*Enterococcus spp.* / 1	*E. faecium* / 6	*E. faecium*
11	18.6	Gr + cocci	*Enterococcus spp., Van A/B* (-)	*Enterococcus spp.* / 2	*E. faecium* / 6	*E. faecium*
12	14.1	Gr + cocci	*Not detected*	*Micrococcus spp.*/ 2	*E. cloacae* / 5	*E. cloacae*
13	15.5	Gr - bacilli	*Staphylococcus spp., MecA (-)*	*Morganella spp.*/ 2	*Morganella morgani* / 5.45	*Morganella morgani*
14	11.4	Gr + cocci	*Enterococcus spp., Van A/B* (-) *Staphylococcus spp., MecA (-)*	Contamination with skin flora	ND[c]	ND[c]
15	38.6	Gr + cocci	*A. baumannii* *Enterococcus spp., Van A/B* (-)	Contamination with skin flora	ND[c]	ND[c]
16	12.1	Gr - bacilli	*E. cloacae complex, KPC (-)*	*Enterobacter spp.,* carbepenemase (+) / 2	*E. cloacae complex* / 5.15	*E. cloacae*
17	9.7	Gr + cocci	*A. baumannii*	*Acinetobacter spp.*/ 1	*A. baumannii complex* / 6	*A. baumannii complex*
18	10.6	Gr - bacilli	*E. coli, KPC* (-)	*E. coli,* ESBL (+)/1	ND[b]	ND[b]
19	71.1	Yeast	*C. tropicalis*	*Canida spp.* / 1	*C. tropicalis* / 18.15	*C. kefyr*[d]
20	9.4	Gr - bacilli	*E. coli, KPC* (-)	*E. coli* / 1	ND[b]	ND[b]
21	12.4	Gr - bacilli	*Not detected*	*Morganella spp. /1*	*Morganella morgani* / 5.45	*Morganella morgani*
22	8.2	Gr - bacilli	*K. pneumonia* KPC(-), Enterococcus *Van A/B*(-)	*K. pneumonia,* carbepenemase (+) / 1	*K. pneumonia*	*K. pneumonia*
23	11.1	Gr - bacilli	*E. coli, KPC* (-)	*E. coli,* ESBL (+), carbepenemase (+) / 1	ND[b]	ND[b]
24	14.4	Gr - bacilli	*E. coli, KPC* (-)	*E. coli,* ESBL (+), carbepenemase (+) / 1	ND[b]	ND[b]
25	10.1	Gr + cocci	*A. baumannii*	*Acinetobacter spp.*/ 1	*A. baumannii complex* / 8	*A. baumannii complex*
26	17.4	Gr - bacilli	*P. aeruginosa*	*P. aeruginosa* / 1	*P. aeruginosa* / 4.45	*P. aeruginosa*
27	52.1	Gr - bacilli	*K. pneumoniae , KPC* (-)	*K. pneumoniae* / 1	*K. pneumoniae* / 4.45	*K. pneumoniae*
28	49.4	Gr - bacilli	*K. pneumoniae KPC* (-)	*K. pneumoniae* / 1	*K. pneumoniae* / 4	*K. pneumoniae*

Amplifikasyon Temelli Yöntemler

- *Real-time* PCR
  daha hızlı olanabilir mi?



Fungi

- *Candida albicans*
- *Candida tropicalis*
- *Candida parapsilosis*
- *Candida krusei*
- *Candida glabrata*
- *Aspergillus fumigatus*

Amplifikasyon Temelli Yöntemler

- *Real-time* PCR, toplam 1006 hasta verisi

HEALTH TECHNOLOGY ASSESSMENT

VOLUME 19 ISSUE 35 MAY 2015
ISSN 1366-5278

Rapid detection of health-care-associated bloodstream infection in critical care using multipathogen real-time polymerase chain reaction technology: a diagnostic accuracy study and systematic review

Geoffrey Warhurst, Graham Dunn, Paul Chadwick, Bronagh Blackwood, Daniel McAuley, Gavin D Perkins, Ronan McMullan, Simon Gates, Andrew Bentley, Duncan Young, Gordon L Carlson and Paul Dark

Setting: Critical care departments within NHS hospitals in the north-west of England.

Participants: Adult patients requiring blood culture (BC) when developing new signs of systemic inflammation.

Main outcome measures: SeptiFast real-time PCR results at species/genus level compared with microbiological culture in association with independent adjudication of infection. Metrics of diagnostic accuracy were derived including sensitivity, specificity, likelihood ratios and predictive values, with their 95% confidence intervals (CIs). Latent class analysis was used to explore the diagnostic performance of culture as a reference standard.

Results: Of 1006 new patient episodes of systemic inflammation in 853 patients, 922 (92%) met the inclusion criteria and provided sufficient information for analysis. Index test assay failure occurred on 69 (7%) occasions. Adult patients had been exposed to a median of 8 days (interquartile range 4–16 days) of hospital care, had high levels of organ support activities and recent antibiotic exposure. SeptiFast real-time PCR, when compared with culture-proven bloodstream infection at species/genus level, had better specificity (85.8%, 95% CI 83.3% to 88.1%) than sensitivity (50%, 95% CI 39.1% to 60.8%). When compared with pooled diagnostic metrics derived from our systematic review, our clinical study revealed lower test accuracy of SeptiFast real-time PCR, mainly as a result of low diagnostic sensitivity. There was a low prevalence of BC-proven pathogens in these patients (9.2%, 95% CI 7.4% to 11.2%) such that the diagnostic rule-in utility than was apparent using conventional analyses of diagnostic accuracy.

Conclusion: SeptiFast real-time PCR on blood samples may have rapid rule-in utility for the diagnosis of health-care-associated bloodstream infection but the lack of sensitivity is a significant limiting factor. Innovations aimed at improved diagnostic sensitivity of real-time PCR in this setting are urgently required. Future work recommendations include technology developments to improve the efficiency of pathogen DNA extraction and the capacity to detect a much broader range of pathogens and drug resistance genes and the application of new statistical approaches able to more reliably assess test performance in situation where the reference standard (e.g. blood culture in the setting of high antimicrobial use) is prone to error.

Study registration: The systematic review is registered as PROSPERO CRD42011001289.

Funding: The National Institute for Health Research Health Technology Assessment programme. Professor Daniel McAuley and Professor Gavin D Perkins contributed to the systematic review through their funded roles as codirectors of the Intensive Care Foundation (UK).

Amplifikasyon Temelli Yöntemler

- *Real-time* PCR, toplam 1006 hasta verisi

HEALTH TECHNOLOGY ASSESSMENT

VOLUME 19 ISSUE 35 MAY 2015

Rapid detection of health-care-associated bloodstream infection in critical care using multipathogen real-time polymerase chain reaction technology: a diagnostic accuracy study and systematic review

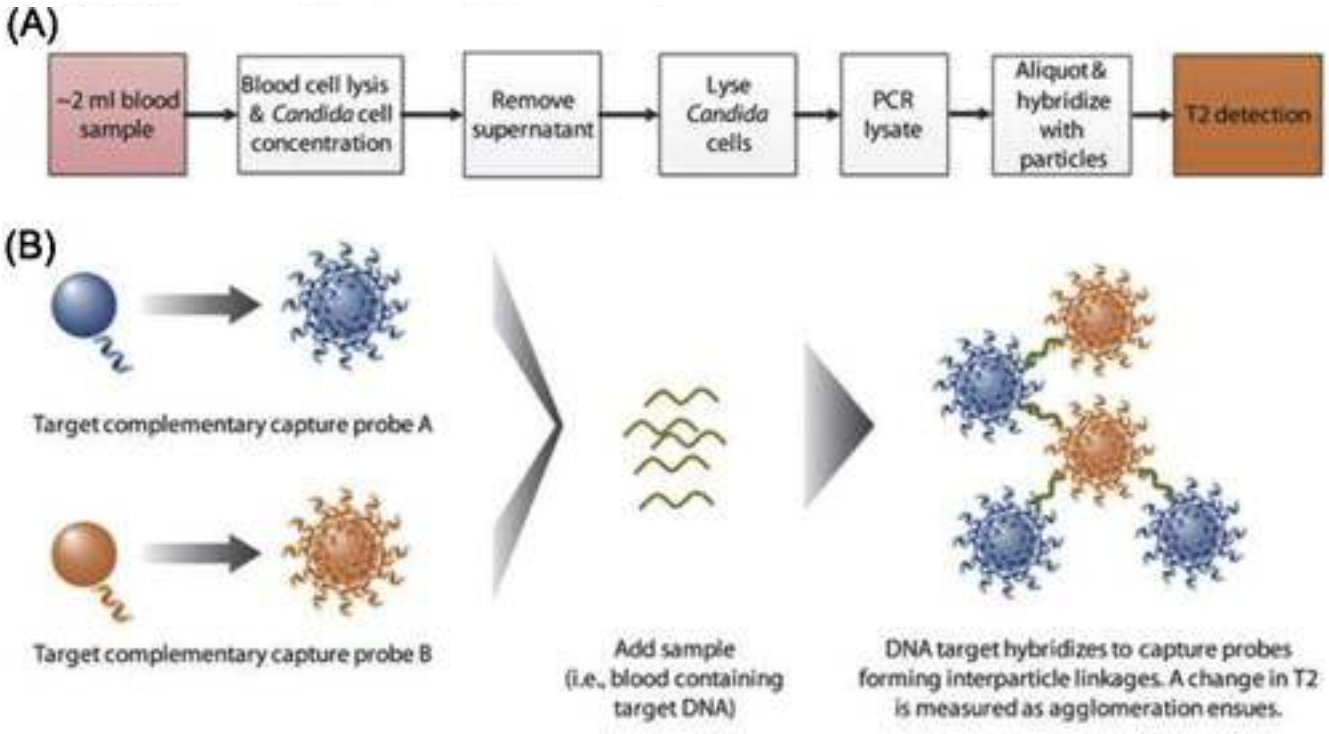
Geoffrey Warhurst, Graham Dunn, Paul Chadwick, Bronagh Blackwood, Daniel McAuley, Gavin D Perkins, Ronan McMullan, Simon Gates, Andrew Bentley, Duncan Young, Gordon L Carlson and Paul Dark

TABLE 14 Pathogens detected: BC (*continued*)

Pathogen species	Number of cases detected		
	BC only	PCR only	BC and PCR
Fungi			
Candida albicans	2	9	2
Candida tropicalis	0	1	0
Candida parapsilosis	0	0	0
Candida glabrata	3	2	0
Candida krusei	0	3	0
Aspergillus fumigatus	0	1	0

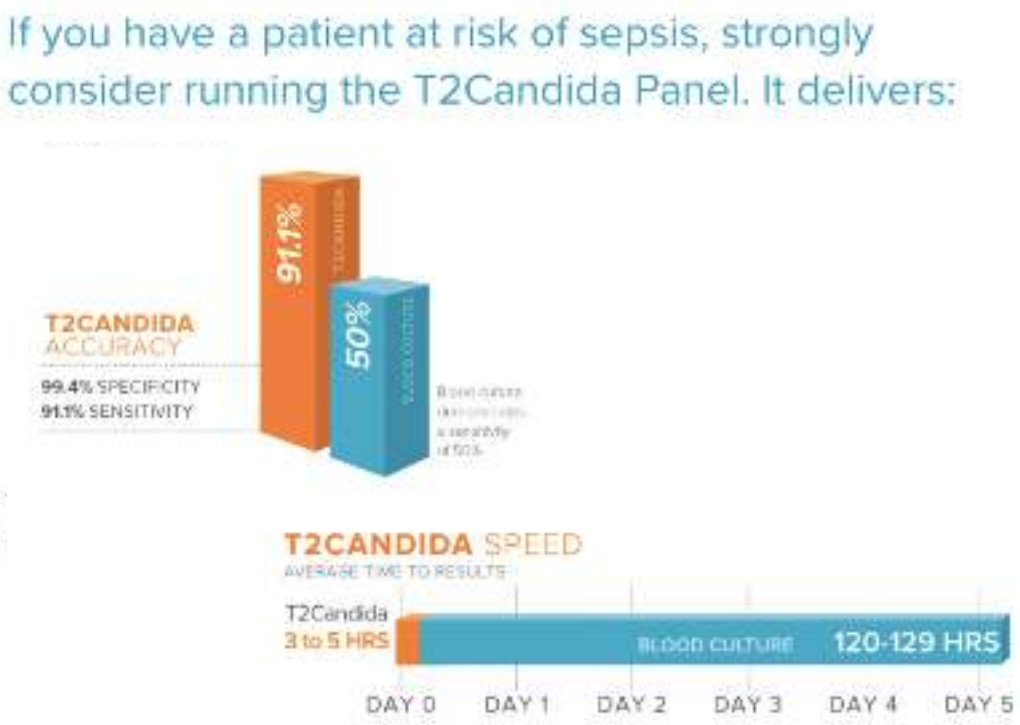
Amplifikasyon Temelli Yöntemler

- *T2 Candida Panel- magnetic resonance*



Amplifikasyon Temelli Yöntemler

- *T2 Candida Panel- magnetic resonance*



Amplifikasyon Temelli Yöntemler

- *T2 Candida Panel- magnetic resonance*

Update in Micology

Elisa Ibáñez-Martínez[1]
Alba Ruiz-Gaitán[1]
Javier Pemán-García[1,2]

Update on the diagnosis of invasive fungal infection

[1]Instituto de Investigación Sanitaria La Fe, Valencia.
[2]Servicio de Microbiología, Hospital Universitari i Politècnic La Fe, Valencia

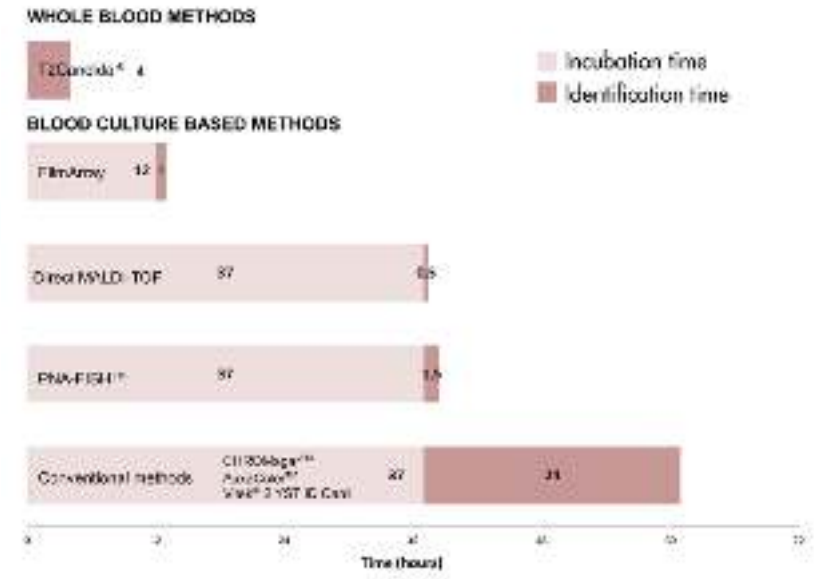


Figure 2 Graphic representation of time (hours) to yeast identification depending on employed technique.

Amplifikasyon Temelli Yöntemler

- *T2 Candida Panel- magnetic resonance*

1 yıl takip, 5100 hastada tanı, yaklaşık 6 milyon dolar daha az harcama

RESEARCH ARTICLE

For reprint orders, please contact: reprints@futuremedicine.com

The economic impact of rapid *Candida* species identification by T2Candida among high-risk patients

Sara Pinar Bilir*[1], Cheryl P Ferrufino[1], Michael A Pfaller[2,3] & Julie Munakata[1]

ABSTRACT **Introduction:** This study estimates the cost–effectiveness and hospital budget impact of rapid candidemia identification using T2Candida, a novel diagnostic panel with same-day species-specific results. **Materials & Methods:** A 1-year decision-tree model estimates hospital costs (2013 US$) and effects (candidemia-related deaths) for faster diagnostics versus blood culture (BC), accounting for disease prevalence, distribution of *Candida* species, test characteristics (sensitivity/specificity/time to result), antifungal medication and differential length-of-stay and mortality by appropriate treatment timing. **Results:** The model estimates a hospital with 5100 annual high-risk patients could possibly save $5,858,448 with T2Candida versus BC, a 47.6% decrease in candidemia diagnosis and treatment budget ($1149/patient tested), while averting 60.6% of candidemia-related mortality. **Conclusion:** Hospitals may observe lower candidemia-related inpatient costs and mortality with rapid *Candida* diagnosis.

Amplifikasyon Temelli Yöntemler

- *T2 Candida Panel- magnetic resonance*

Bu Yöntemler Klinik Kullanıma Hazır mı?

Current Fungal Infection Reports
https://doi.org/10.1007/s12281-018-0313-1

ADVANCES IN DIAGNOSIS OF INVASIVE FUNGAL INFECTIONS (S CHEN, SECTION EDITOR)

CrossMark

PCR-Based Methods for the Diagnosis of Invasive Candidiasis: Are They Ready for Use in the Clinic?

Clinical Performance of Candida PCR

There is a large body of literature on PCR-based methods for diagnosing invasive candidiasis. The interpretation of

Table 1 Performance characteristics of an ideal diagnostic test for invasive candidiasis

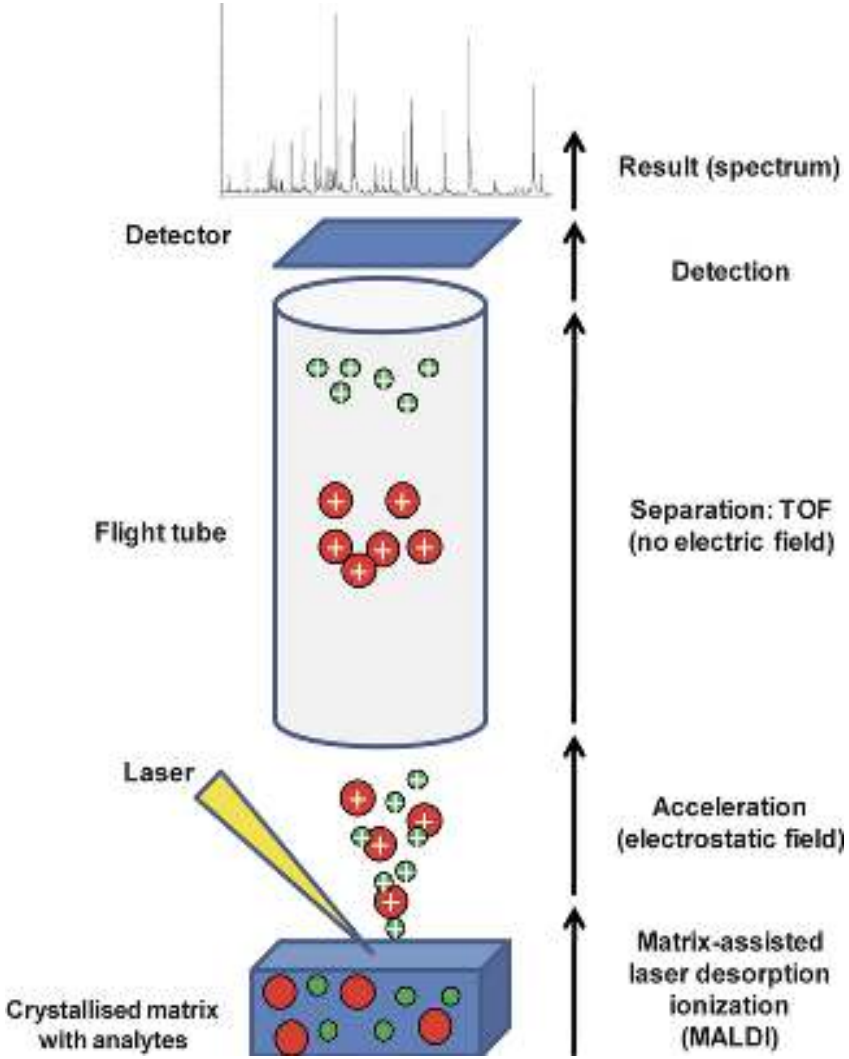
- Blood-based assay
- Requires low-volume samples
- Rapid turn-around
- Minimal labor and laboratory technician time
- Cost-effective
- Sensitive and specific for both bloodstream and deep-seated infections
- Provides species identification
- Multiplex capabilities
- Capacity for detection of antifungal resistance
- Provides diagnostic and prognostic information (e.g., predicts outcomes of infection)

Kütle Spektrometrik Yöntemler

- MALDI-TOF matrix-assisted laser desorption ionization time-of-flight mass spectrometry

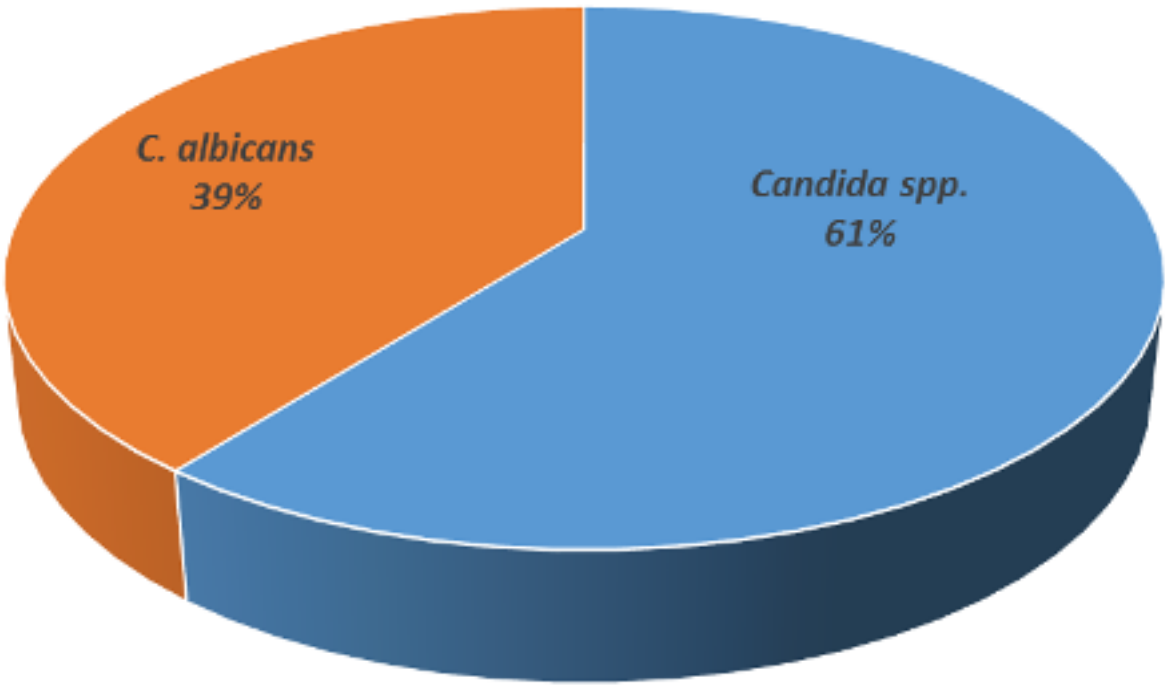
Kütle Spektrometrik Yöntemler

- MALDI-TOF matrix-assisted laser desorption ionization time-of-flight mass spectrometry



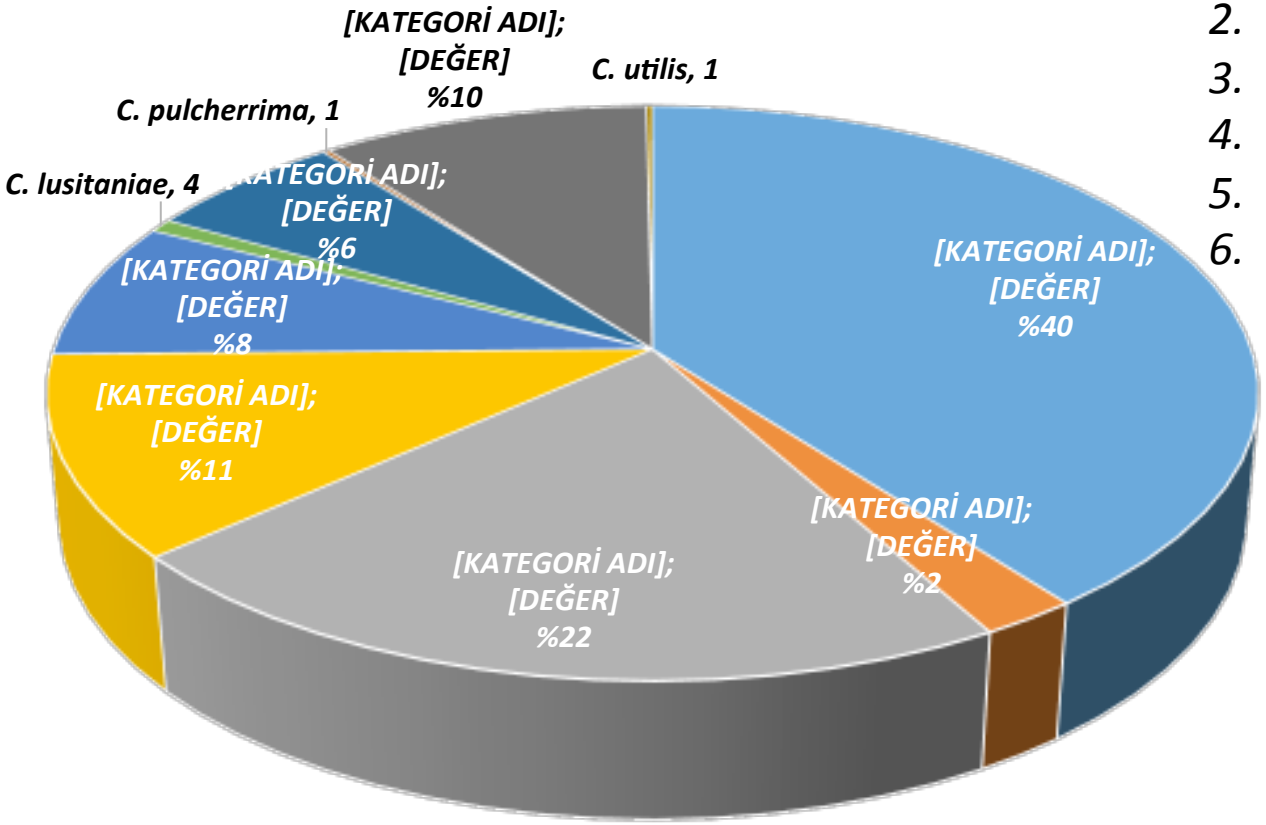
Kütle Spektrometrik Yöntemler

- MALDI-TOF
- Etkenlerin dağılımı; 01.01.2015-29.11.2016



Kütle Spektrometrik Yöntemler

- MALDI-TOF
- Etkenlerin dağılımı



1. *C. albicans*
2. *C. glabrata*
3. *C. keyfr*
4. *C. tropicalis*
5. *C. krusei*
6. *C. parapsilosis*

- MALDI-TOF

Recommendations for Identification of *Candida auris*

Reporting

Healthcare facilities or laboratories that suspect they have a patient with *C. auris* infection should contact state or local public health authorities and CDC (candidaauris@cdc.gov) immediately for guidance.

How to identify *Candida auris*

Diagnostic devices based on matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) can differentiate *C. auris* from other *Candida* species, but not all the reference databases included in MALDI-TOF devices allow for detection. Currently, accurate identification of *C. auris* can be performed using Bruker Biotyper brand MALDI-TOF using their "research use only" databases or the updated FDA-approved CA system database and VITEK (MALDI-TOF) MS RUO (with Saramis Ver 4.14 database and Saccharomycetaceae update).

Molecular methods based on sequencing the D1-D2 region of the 28s rDNA or the Internal Transcribed Region (ITS) of rDNA also can identify *C. auris*.

Kütle Spektrometrik Yöntemler

- MALDI-TOF- Direkt Örnekten

Direkt olarak pozitif kan kültür şişesinden tanımlama için çeşitli protokoller geliştirildi.

JCM
Journals.ASM.org

Direct MALDI-TOF Mass Spectrometry Assay of Blood Culture Broths for Rapid Identification of *Candida* Species Causing Bloodstream Infections: an Observational Study in Two Large Microbiology Laboratories

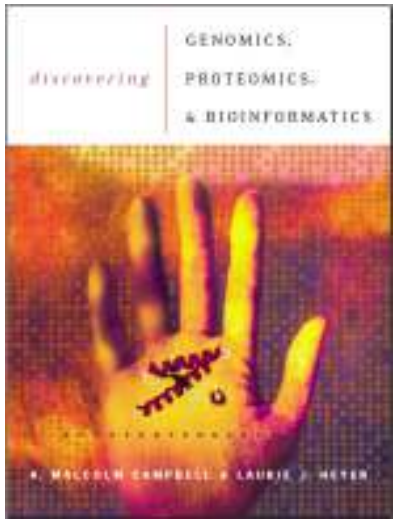
TABLE 1 Performances of Bruker Biotyper for direct identification of yeasts from blood culture bottles with culture-based identification as reference

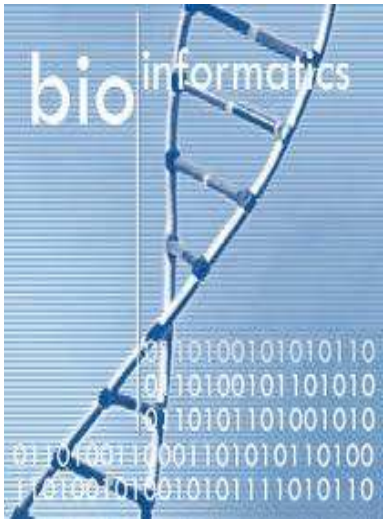
Comparison method ID	No. of isolates		
	Total tested	Concordant ID[a]	% Sensitivity (95% CI)[b]
Candida albicans	195	187	95.9 (91.8–98.1)
Candida famata	1	0	NT
Candida glabrata	26	22	84.6 (64.3–94.9)
Candida guilliermondii	10	6	60.0 (27.4–86.3)
Candida krusei	8	6	75.0 (35.6–95.5)
Candida lusitaniae	2	1	NT
Candida parapsilosis	69	65	94.2 (85.1–98.1)
Candida tropicalis	32	28	87.5 (70.1–95.9)
Rhodotorula glutinis	1	0	NT
Rhodotorula mucilaginosa	2	0	NT
Total	346	316	91.3 (87.7–93.9)

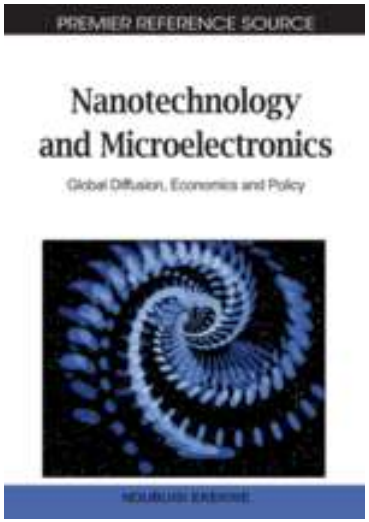
[a] Species identification furnished by the Bruker Biotyper was concordant with that of the comparison method.
[b] NT, not tested. Sensitivity was not calculated when <5 isolates were found.

Yeni Gelişmeler

- Genomik, biyoinformatik ve mikroelektronik alanında yaşanan hızlı gelişmelerin en göze çarpan sonuçları,
  - Biyosensörlerdir ve
  - DNA mikroçip teknolojileri



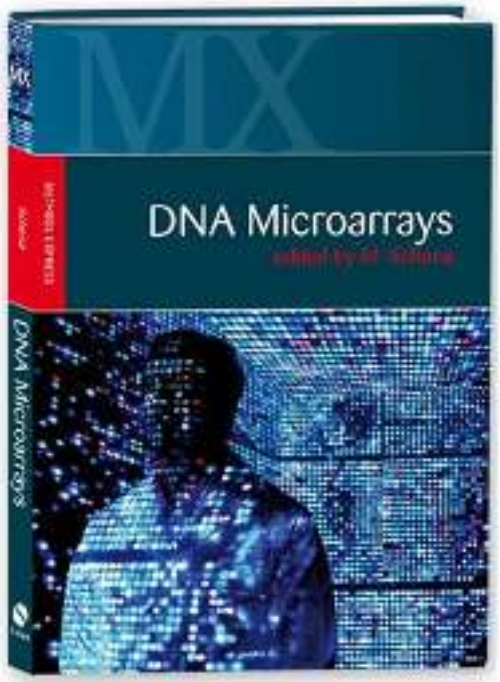




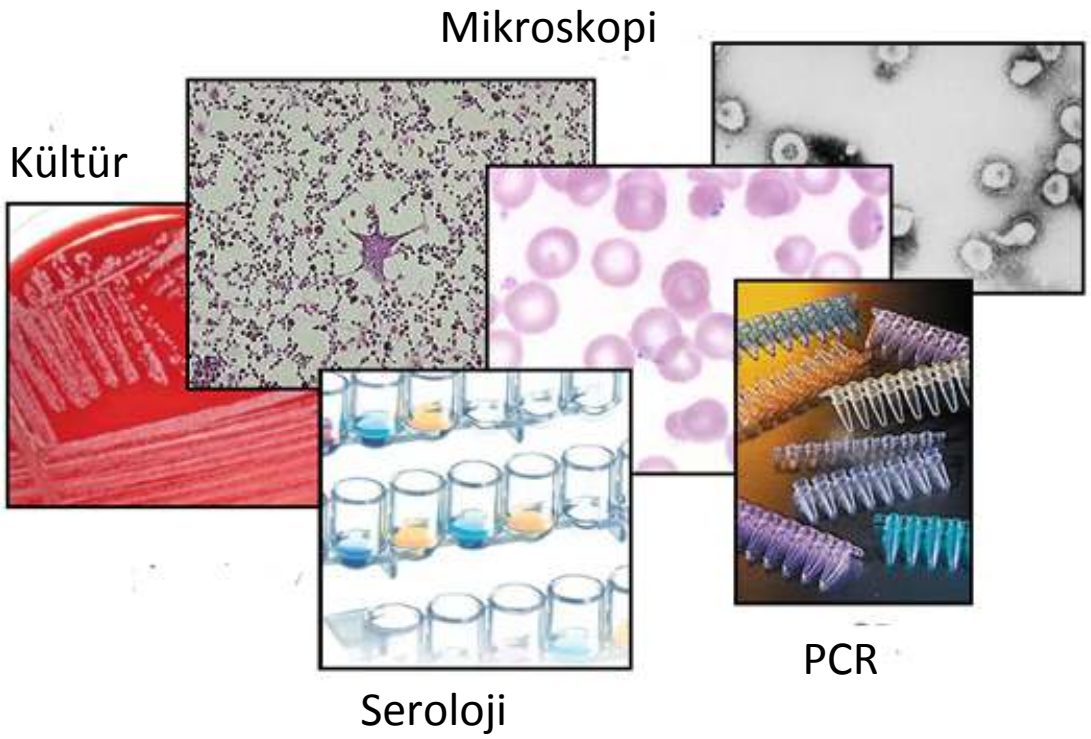
DNA Mikroçip Temeli Yöntemler

- DNA mikroçip teknolojileri, çevresel ve

  klinik örneklerden mikroorganizmaların tanısı için giderek artan oranda kullanılmaktadır.



Geleneksel Mikrobiyolojik Tanı Yöntemleri

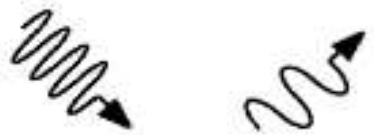


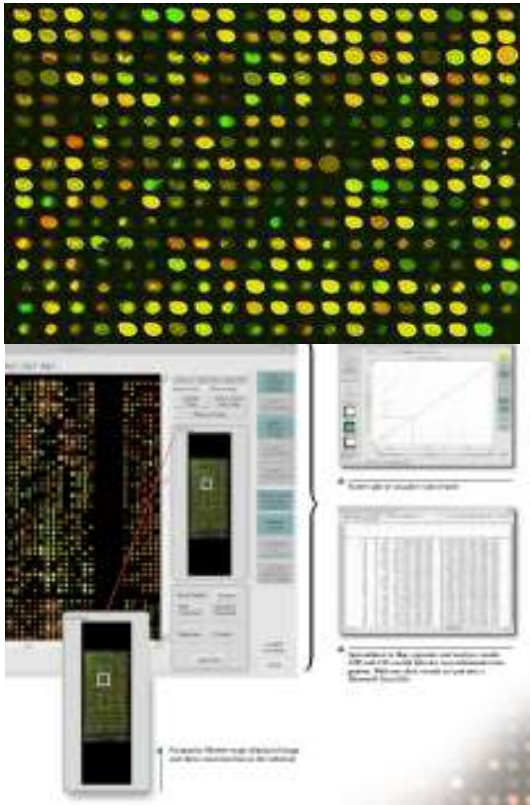
DNA Mikroçip Temeli Yöntemler

- PCR ile elde edilen floresanla işaretli amplikonların

  çok sayıda farklı oligonükleotid prob içeren katı yüzeylerde, kendisine uyan probla

  hibridize olması temeline dayanmaktadır.





DNA Mikroçip Temeli Yöntemler

- Sepsiste mikroçip temelli hızlı testler

DNA Mikroçip Temeli Yöntemler

Prove-it™ Sepsis workflow

Sample: DNA extract from positive blood culture

1. PCR amplification (1,5 hours)

Accurate DNA-based identification of sepsis-causing bacteria and fungi simultaneously

- Identification of over 60 bacteria, *mecA, vanA and vanB* resistance markers and 13 fungi in a single assay
- Based on PCR amplification followed by microarray detection
- Automated software for result detection and analysis
- Faster results: assay time only 3 hours
- Easy to adapt into laboratory routine
- CE-IVD marked

Prove-it® Sepsis CE IVD

DNA Mikroçip Temeli Yöntemler

- Kan kültüründen toplam tespit süresi 3 saat
- 388 örneğin karşılaştırmalı analizinde fungal tanıda duyarlılık %99, özgüllük %98

JCM
Journals.ASM.org

Accurate and Rapid Identification of *Candida* spp. Frequently Associated with Fungemia by Using PCR and the Microarray-Based Prove-it Sepsis Assay

TABLE 3 Speciation results of the clinically analyzed clinical fungal isolates obtained using the fungal oligonucleotide microarray[a]

Fungal species	No. of specimens	No. of specimens positive by microarray	Identification by microarray	Confirmatory test results by:	
				Sequence analysis	Conventional methods and other technology
Candida albicans	53	48	*Candida albicans*		
		1	Pan-yeast	*Candida glabrata*	
		1	Pan-yeast		*Candida dubliniensis*
Candida glabrata	31	31	*Candida glabrata*		
Candida parapsilosis	22	22	*Candida parapsilosis*		
Candida tropicalis	15	14	*Candida tropicalis*		
		1	*Candida krusei*, *Candida glabrata*		*Candida krusei*, *Candida glabrata*
Candida krusei	8	7	*Candida krusei*		
		1	*Candida krusei*, *Candida glabrata*		*Candida krusei*, *Candida glabrata*
Candida guilliermondii	4	4	*Candida guilliermondii*		
		1	*Candida guilliermondii*, *Candida lusitaniae*		*Candida guilliermondii*, *Candida lusitaniae*
Candida lusitaniae	2	2	*Candida lusitaniae*		
		1	Pan-yeast	*Candida rugosa*	
Candida kefyr	6	5	Pan-yeast		
		1	*Candida guilliermondii*	*Candida guilliermondii*	
Candida famata	1	1	Pan-yeast		
Candida norvegensis	1	1	Pan-yeast		
Candida dubliniensis	1	1	Pan-yeast		
Saccharomyces cerevisiae	6	6	Pan-yeast		
Cryptococcus albidus	1	1	Negative		
Cryptococcus neoformans	2	0	Negative		
Trichosporon asahii	1	0	Negative		
Trichosporon inkin/mucoides	2	0	Negative		
Trichosporon sp.	3	0	Negative		
		1	Pan-yeast		*Trichosporon asahii*
Trichosporon beigelii	1	0	Negative		
Unidentified yeast species	1	1	Pan-yeast	Could not be clarified	Could not be clarified
Total	189	151			

[a] Organisms included 1 *Rhodotorula mucilaginosa*, 1 *Saccharomyces cerevisiae*, 1 *Candida famata*, and 2 *Cryptococcus* species.

Biyosensör Temeli Yöntemler

- Mikroelektronik alanındaki gelişmeler ve biyolojik moleküllerin olağanüstü duyarlılıktaki yanıt verme kapasitelerinin keşfedilmesi, biyosensör teknolojisinin hızla gelişmesine neden olmuştur

NEWS by Jennifer Ouellette

Biosensors: Microelectronics marries biology

For decades, scientists have sought to couple biomolecules with electronic detection devices for sensing applications. These biosensors have been slow to penetrate commercial markets, however, because they are not as fast as more-established sensing methods, are often bulky and are expensive to manufacture. The development of increasingly innovative biosensors, including multichannel DNA probe arrays and the possibility of integrating living cells on chips is making the technology more attractive to researchers, physicians, and industry. As a result, biosensors are at the forefront of a multidisciplinary science that marries the biological world and the electronic world.

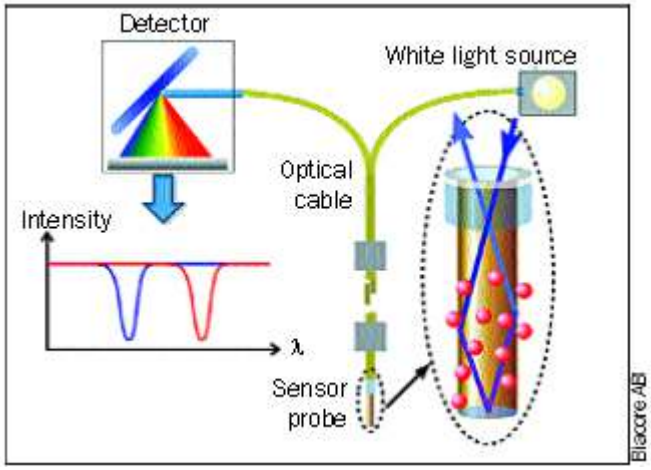
Figure 2. Fiber-optic, fully automated biosensor performs four immunoassays simultaneously in 5 to 10 minutes and shows the results on an LCD screen in words.

nostics, environmental monitoring, and food processing," says S. J. Allcock, head of biosensor development at Cranfield Biotechnology Center in Cranfield, England. Transducers used in biosensors can also take many forms, depending on the parameters being measured. The most widely used biosensors measure electrochemical effects, but other types can be used to measure thermometric, piezoelectric, acoustic, magnetic, or optical responses.

be seeking, says Frances Light of the Naval Research Laboratory (NRL).

Growth of applications

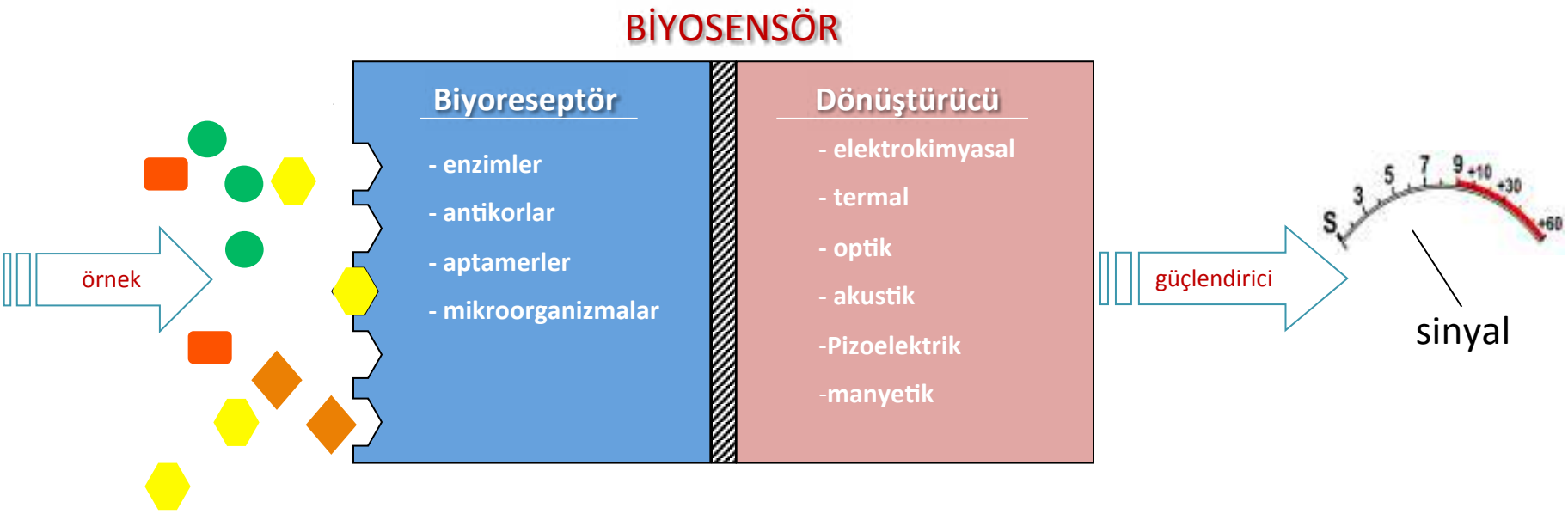
The first biosensors, comprised of enzymes immobilized on oxygen electrodes, were reported in the 1960s. Their subsequent development led to the commercialization of devices for the measurement of glu-



Biyosensör Temeli Yöntemler

- Bu dönüştürücülerden bazıları;

biyolojik etkileşimi gerçek zamanlı olarak gösterebilirken bazıları ikincil elementlerin varlığına (fosfataz veya işaretli antikorlar) ihtiyaç duyarlar.



Biyosensör Temeli Yöntemler

- Mantarların tanısında biyosensör mantarlar

SCIENCE ADVANCES | RESEARCH ARTICLE

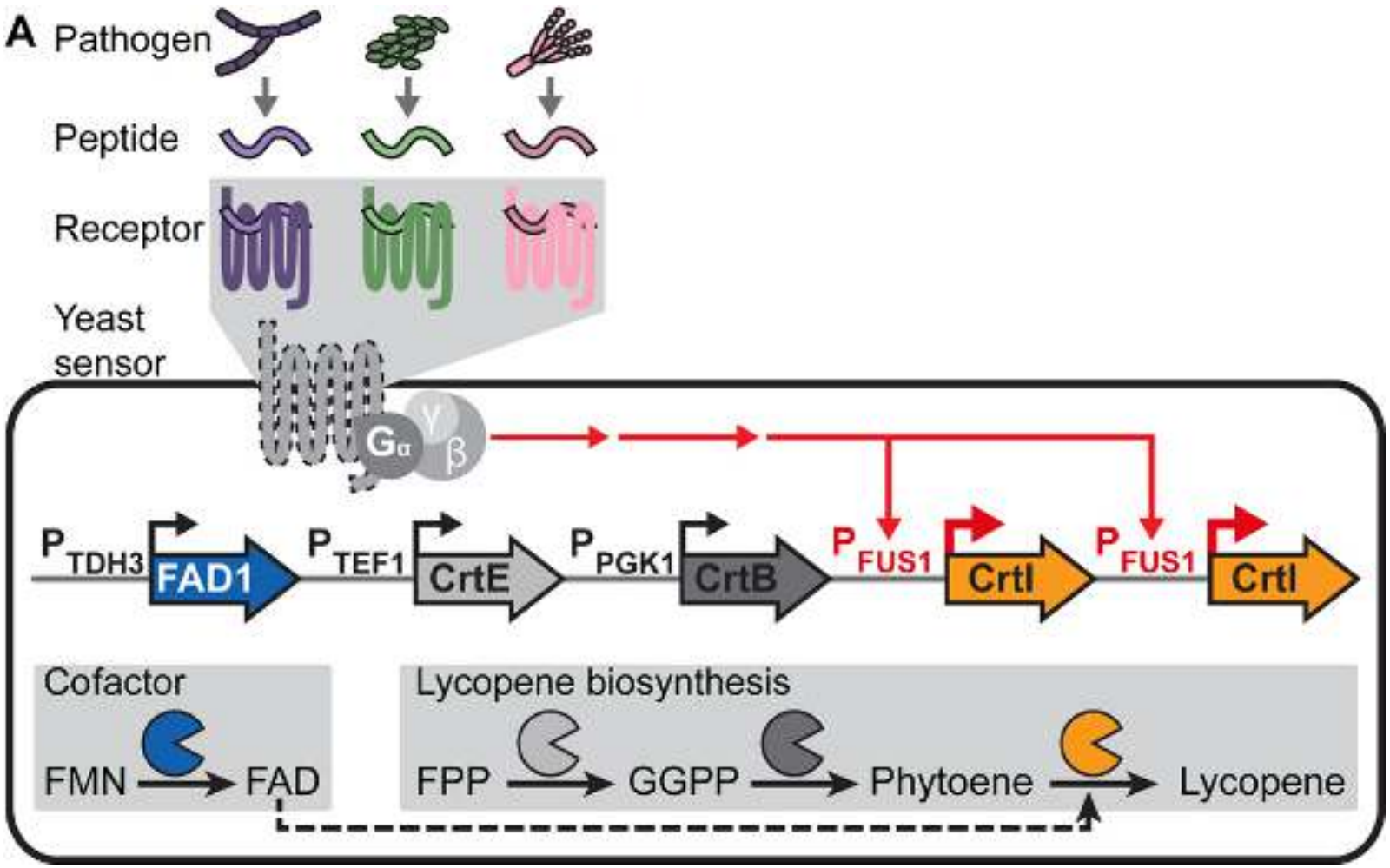
HEALTH AND MEDICINE

A modular yeast biosensor for low-cost point-of-care pathogen detection

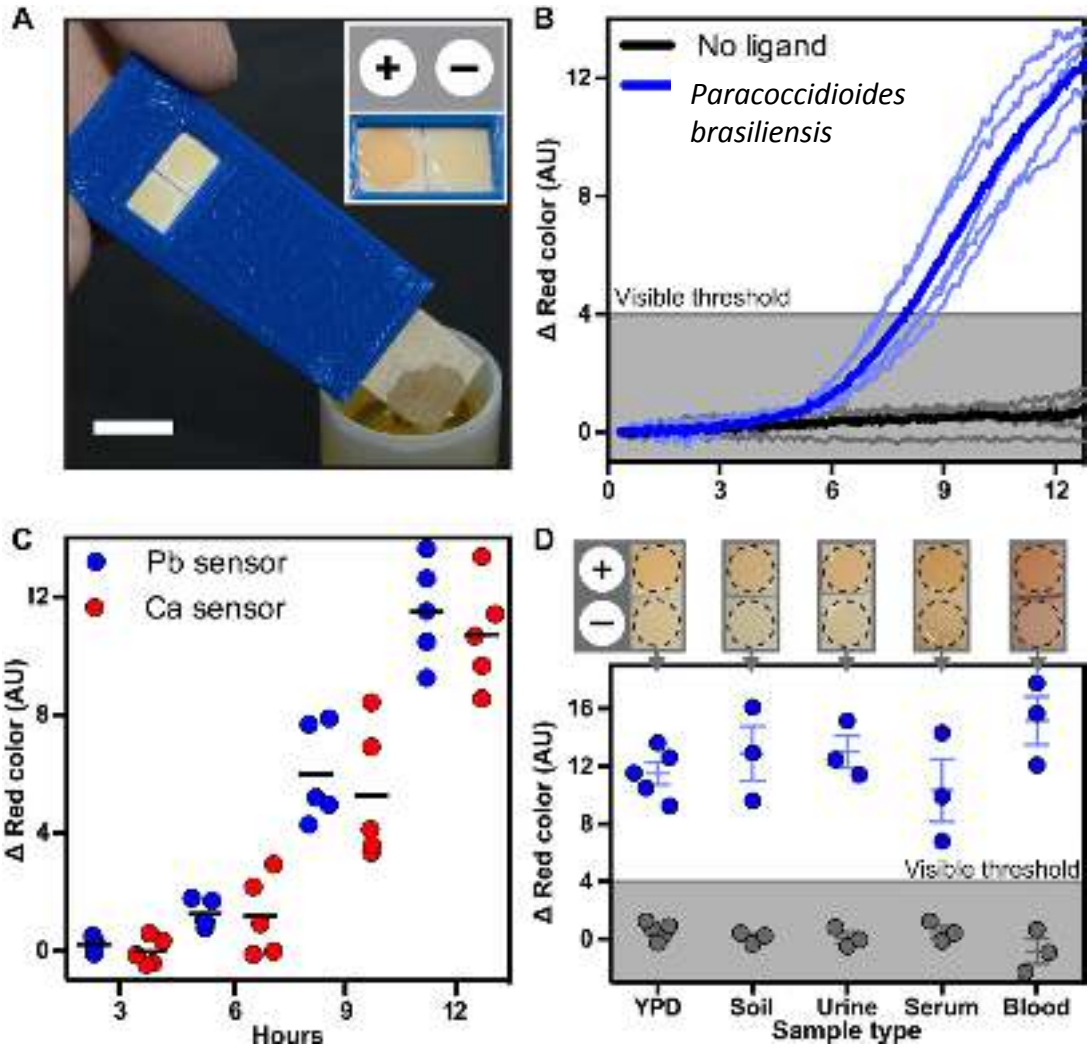
Nili Ostrov,[1*†] Miguel Jimenez,[1*‡] Sonja Billerbeck,[1*] James Brisbois,[1] Joseph Matragrano,[1] Alastair Ager,[2,3] Virginia W. Cornish[1,4§]

The availability of simple, specific, and inexpensive on-site detection methods is of key importance for deployment of pathogen surveillance networks. We developed a nontechnical and highly specific colorimetric assay for detection of pathogen-derived peptides based on *Saccharomyces cerevisiae*—a genetically tractable model organism and household product. Integrating G protein–coupled receptors with a visible, reagent-free lycopene readout, we demonstrate differential detection of major human, plant, and food fungal pathogens with nanomolar sensitivity. We further optimized a one-step rapid dipstick prototype that can be used in complex samples, including blood, urine, and soil. This modular biosensor can be economically produced at large scale, is not reliant on cold-chain storage, can be detected without additional equipment, and is thus a compelling platform scalable to global surveillance of pathogens.

Biyosensör Temeli Yöntemler



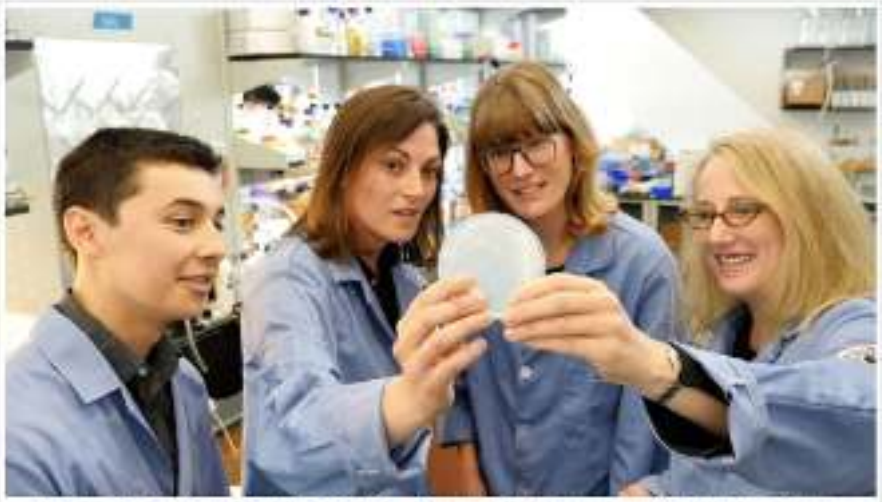
Biyosensör Temeli Yöntemler



Biyosensör Temeli Yöntemler

Yeast's Newest Trick: Detecting Deadly Pathogens

By Carl Engelking | June 28, 2017 1:00 pm



Researchers in the Cornish lab at Columbia University examine baker's yeast, modified to detect pathogens, growing in a petri dish. *(Credit: Courtesy of Columbia University Office of Communications and Public Affairs)*

"While at an early stage of implementation, these biosensors can be immediately adopted in the clinic to shorten the time required for diagnosis of fungal pathogens from blood cultures," researchers wrote in their study, which was published Wednesday in the journal *Science Advances*.

The new biosensor can detect a variety of pathogens in blood, plasma, soil, water and urine. *(Credit: Courtesy of Columbia University Office of Communications and Public Affairs)*

Moleküler Mikrobiyolojik Teknikler

- Moleküler hızlı tanı yöntemlerine harcanan

  her 1 USD harcama 5 USD olarak geri dönüyor.

New Technology for Detecting Multidrug-Resistant Pathogens in the Clinical Microbiology Laboratory

Lance R. Peterson*† and Gary A. Noskin*†
*Northwestern Memorial Hospital and †Northwestern University Medical School, Chicago, Illinois, USA

Northwestern Memorial Hospital instituted in-house molecular typing to rapidly assess microbial clonality and integrated this typing into an infection control program. We compared data on nosocomial infections collected during 24 months before and 60 months after implementing the new program. During the intervention period, infections per 1,000 patient-days fell 13% (p=0.002) and the percentage of hospitalized patients with nosocomial infections decreased 23% (p=0.000006). In our hospital, the percentage of patients with nosocomial infections is 43% below the U.S. rate. Our typing laboratory costs approximately $400,000 per year, a savings of $5.00 for each dollar spent.