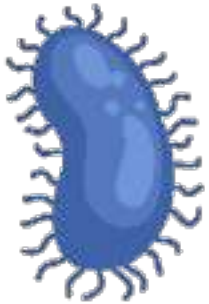
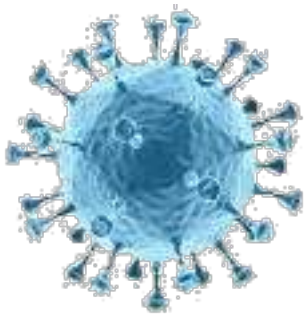
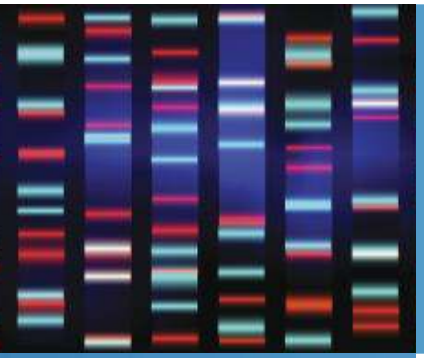






Mikrobiyolojik Tanı Sanatı:

Kadim Yöntemler, Yeni Teknolojiler ve Gelecek

Barış Otlu
İnönü Üniversitesi Tıp Fakültesi
Tıbbi Mikrobiyoloji Anabilim Dalı, Malatya.

Mikrobiyoloji Laboratuvarı

- Enfeksiyon etkenini tanımlamak, antimikrobiyal duyarlılığı belirlemek,
  - En geniş yelpazede mikrobiyal tanı
  - En doğru tanı
  - En hızlı tanı
  - En anlaşılır şekilde raporla
  - En ekonomik şekilde yap

Agrobacterium tumefaciens
Massilia timonae
Arthrobacter cumminsii
Globicatella sanguinis
Herbaspirillum huttiense
Myroides sp.
Providencia rettgeri
Leclercia adecarboxylata
Psychrobacter faecalis
Hafnia alvei
Kluyvera ascorbata
Cupriavidus gilardii

Mikrobiyolojik Tanı

- Son zamanlarda;

  hastanelerdeki mikrobiyoloji laboratuvarlarında sessiz bir devrim oluyor.

- Yeni yöntemlerle;
  - daha hızlı
  - daha doğru
  - daha duyarlı
  - daha kolay yorumlanabilir
  - otomasyon ve verimlilik

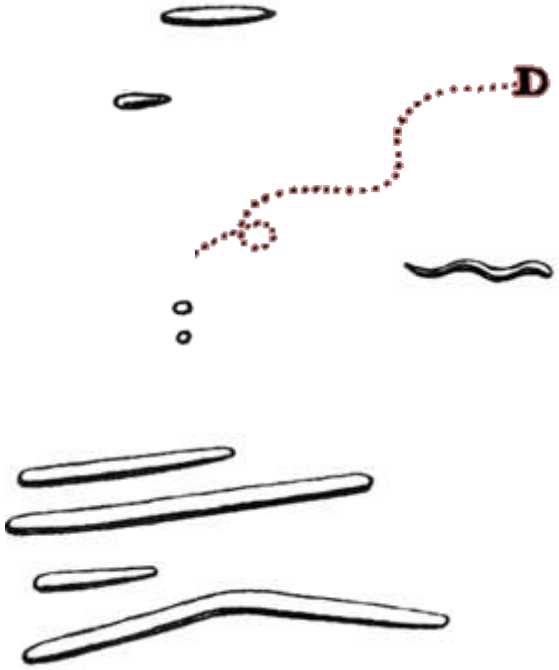
Mikroorganizmaların İlk Tanısı

- Mikroorganizmaların ilk tanısı

1674

mikrobiyolojinin babası

Animalcules / hayvancık



Mikroskopik organizmaların isim babası kim?

- Bu organizmaların isimleri çoktur ve düzenlenmelidir. "Mikrop" kelimesi, daha kısa olma avantajına sahip ve daha genel bir anlama sahip, ... , biz onu benimsiyoruz, ...

1878
mikrop kelimesi

Charles Sédillot

DES SÉANCES

DE L'ACADÉMIE DES SCIENCES.

CHIRURGIE. — *De l'influence des découvertes de M.* Pasteur *sur les progrès de la Chirurgie.* Note de M. C. SÉDILLOT.

« M. Pasteur a démontré que des organismes microscopiques, répandus dans l'atmosphère, sont la cause des fermentations attribuées à l'air qui n'en est que le véhicule et ne possède aucune de leurs propriétés.

» Ces organismes forment tout un monde, composé d'espèces, de familles et de variétés, dont l'histoire, à peine commencée, est déjà féconde en prévisions et en résultats de la plus haute importance.

» Les noms de ces organismes sont très-nombreux (¹) et devront être définis et, en partie, réformés. Le mot *microbe* (²) ayant l'avantage d'être plus court et d'une signification plus générale, et mon illustre ami, M. Littré, le linguiste de France le plus compétent, l'ayant approuvé, nous l'adoptons, sans néanmoins renoncer à ceux en usage, pour la désignation de variétés plus particulièrement étudiées.

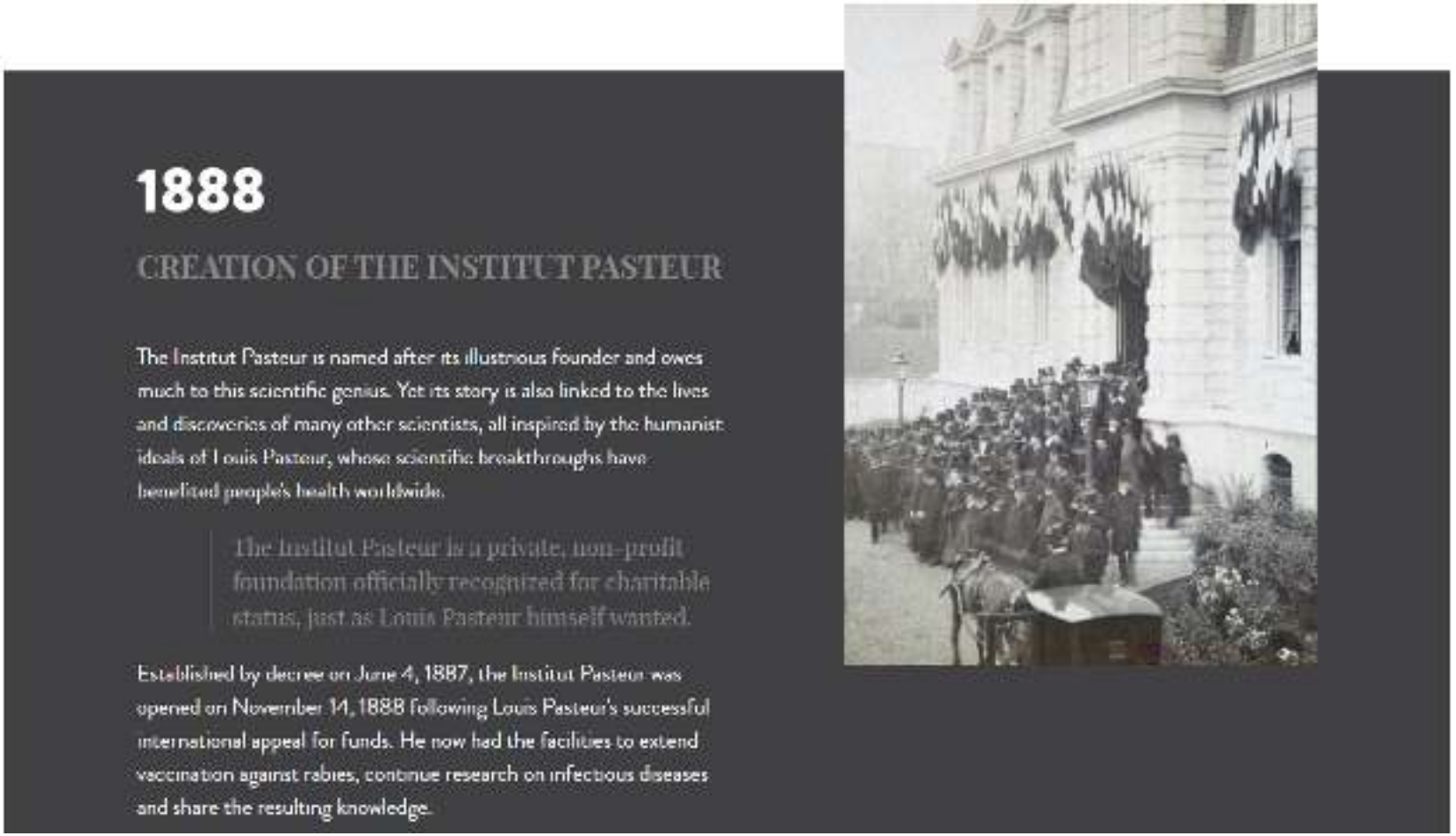
» La démonstration des microbes et de leur rôle, dont l'Académie a été juge et témoin, a jeté un rayon de vive lumière dans les obscurités et les fausses et contradictoires appréciations où s'égarait la Chirurgie.

Dès la plus haute antiquité la Médecine a compris et formulé l'influence prépondérante de l'air sur la vie, la santé et les maladies.

Mikrobiyoloji biliminin isim babası?

1880

mikrobiyoloji kelimesi



1890- Berlin Dünya Tıp Kongresi

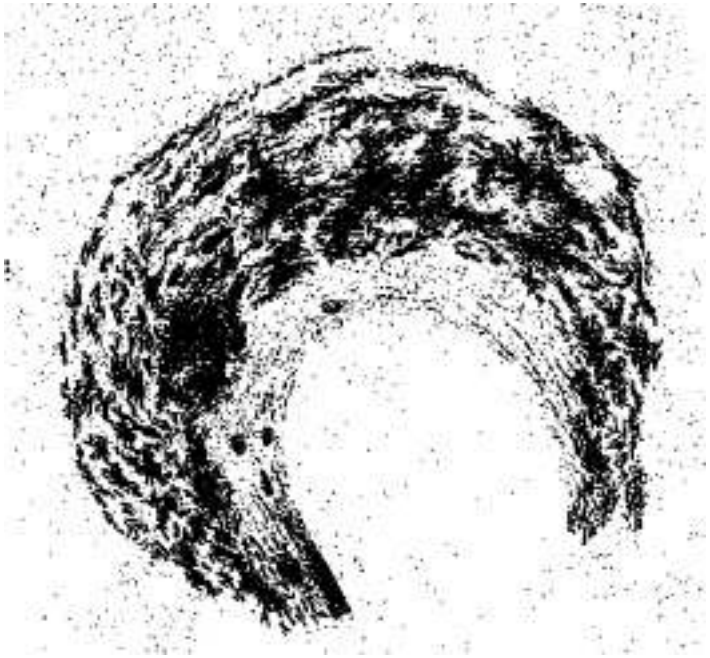
- Koch postülatı; bir mikroorganizma ve hastalık arasındaki nedensel ilişki kurmak için tasarlanmış dört kriteri yayınladı.

1893 - Bakteriyolojihane-i Şahane

Mikroskopik Tanın Gelişmi

- Mikroskopi alanındaki gelişmeler
- Ehrlich'in metilen mavisi yöntemini ve kahverengi bir zıt boya kullanarak tüberküloz dokusunda birkaç küçük basil saptadı.

WaltherHessen



Agarın hayatımıza girişi

- Kültür alanındaki gelişmeler

mikrobiyolojinin annesi

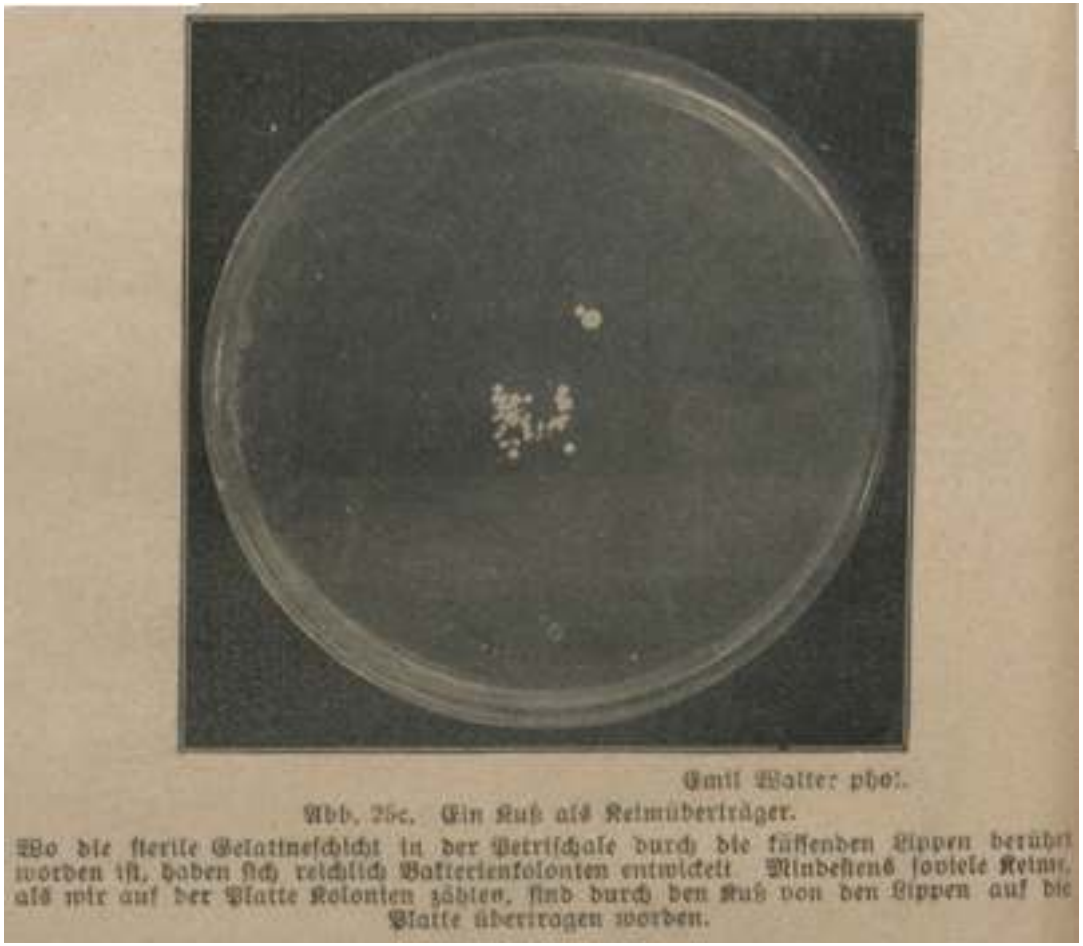
Fanny Hesse 1850- 1934

«Bir mikrobiyolog için agarsız bir düşünülemez »

Petri kabını bulunuşu

- 1887’, Robert Koch'un ekibinin üyesi diğer bir üyesi tıbbi bakteriyolog ve Richard Julius Petri tarafından tasarlandı.

Richard Petri



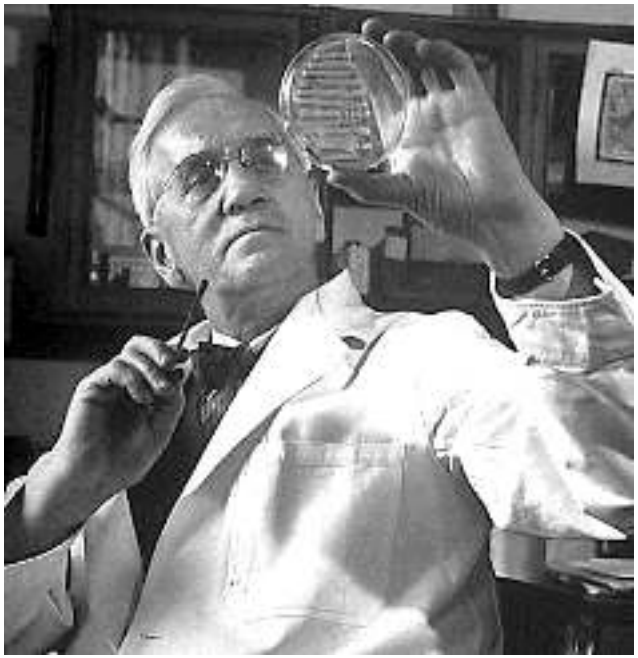
Emil Walter phot.

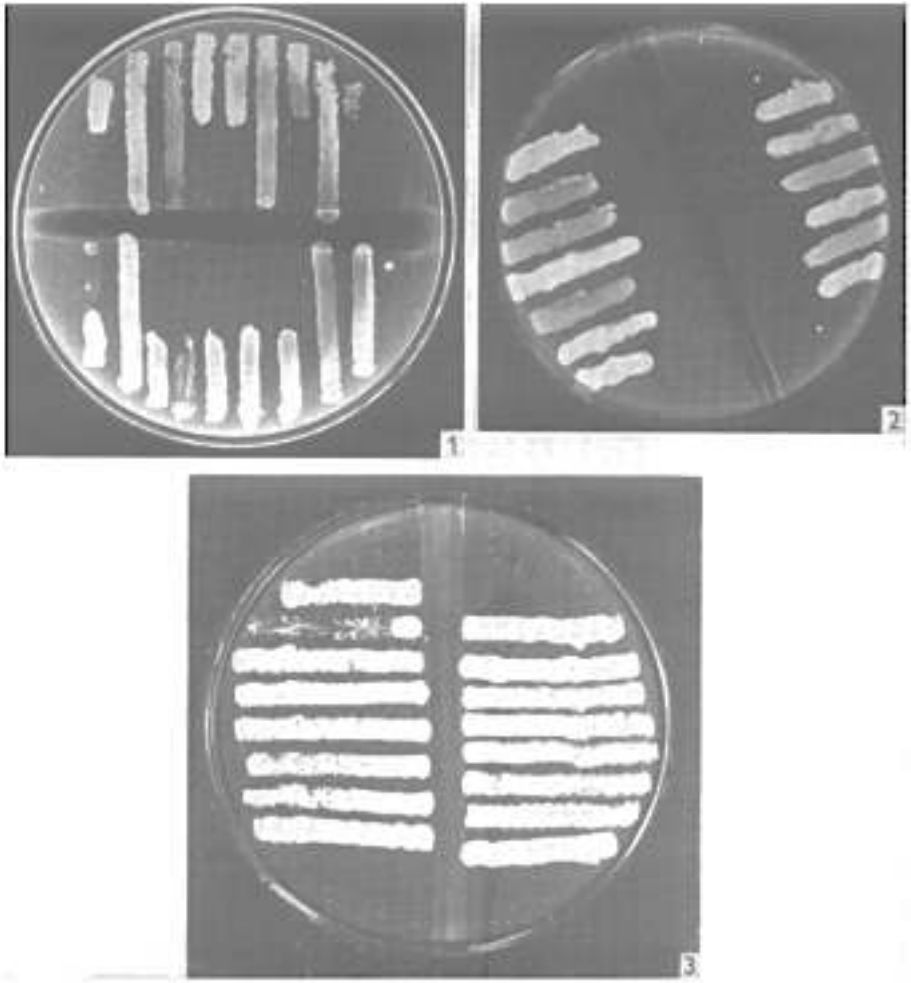
Abb. 26c. Ein Kuß als Keimüberträger.

Wo die sterile Gelatineschicht in der Petrischale durch die küssenden Lippen berührt worden ist, haben sich reichlich Bakterienkolonien entwickelt. Mindestens soviele Keime, als wir auf der Platte Kolonien zählen, sind durch den Kuß von den Lippen auf die Platte übertragen worden.

Alexander Fleming, Ne yapıyordu?

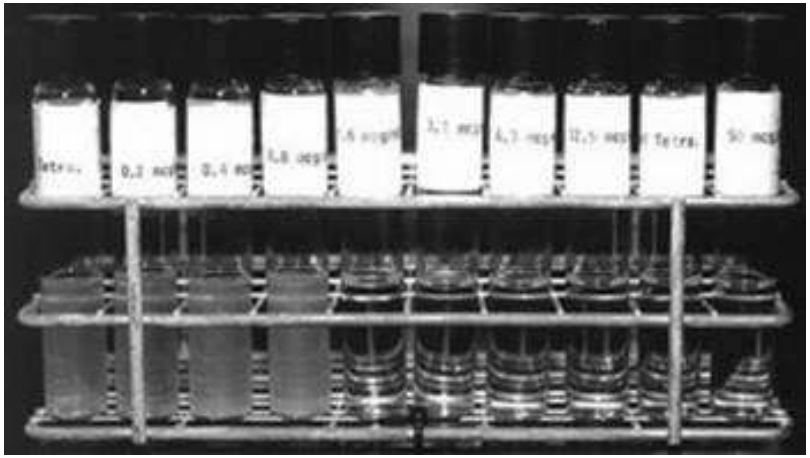
- 1924 “Ditch plate” tekniği, antiseptik duyarlılık testi





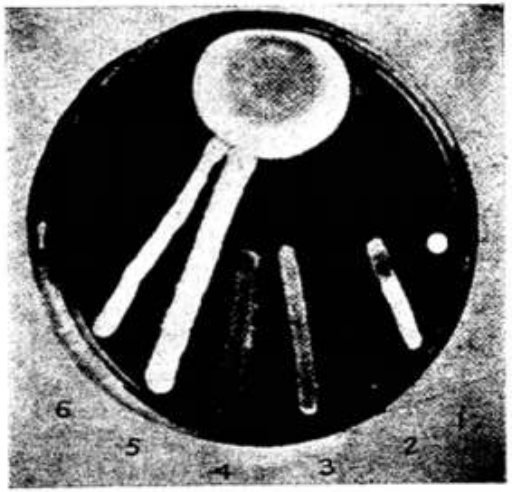
Alexander Fleming, Ne yapıyordu?

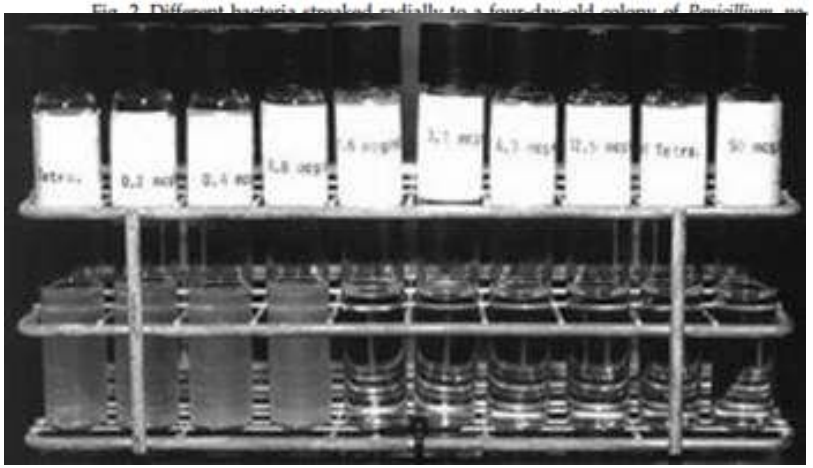
- 1924 “Broth dilution” tekniği ve antiseptikler için MIC tespiti



Alexander Fleming, Ne yapıyordu?

- Alexander Fleming
- 1929 penisilinin keşfi ve antimikrobiyal duyarlılık testi

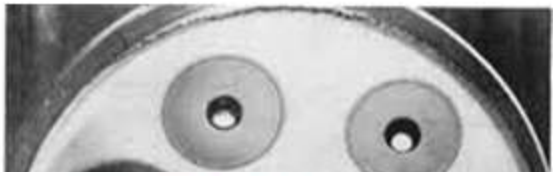




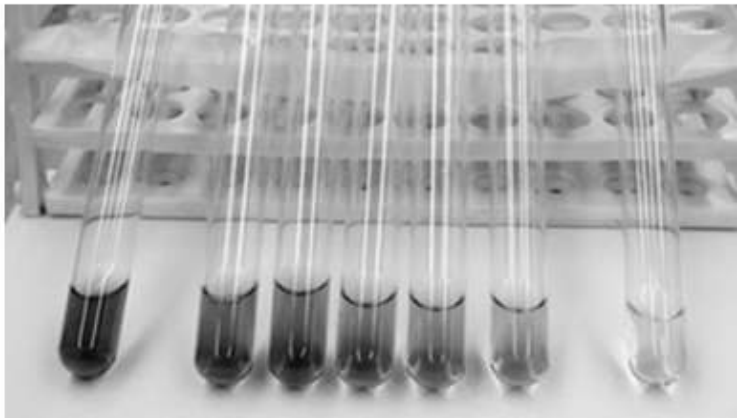
Antibiyotik Duyarlılık Testlerinin Gelişimi

- **1940** Antimikrobiyal emdirilmiş absorbent kağıtlar

- **1941** "Cup Plate" tekniği

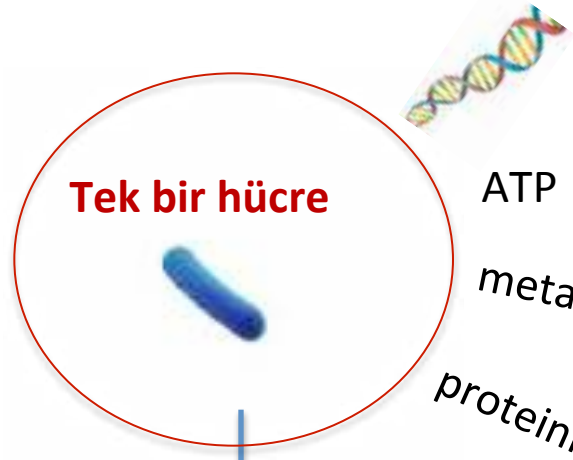


- **1942** "Broth dilution" tekniği ve pH indikatörü



Geleneksel Yöntemlerdeki Durum

- Tanı için gözle görünür üremenin tespiti antimikrobiyal duyarlığın tespiti için de üremenin inhibisyonun gösterilmesi zaman alıcı.



ATP

metabolitleri

proteinleri

18-24 saat

16-18 saat

8-10 saat

6-8 saat

Geleneksel Yöntemlerdeki Durum

- Saatlerin önemli olduğu sepsisin tanısı
- Hızlı ve doğru başlangıç tedavisinin,

diğer tıbbi müdahalelere göre daha fazla yaşam kurtardığı gösterilmiştir.

1 Gün

Pozitif Kan Kültür

1 Gün

1 Gün

İlk problem etkeni üretebilmek

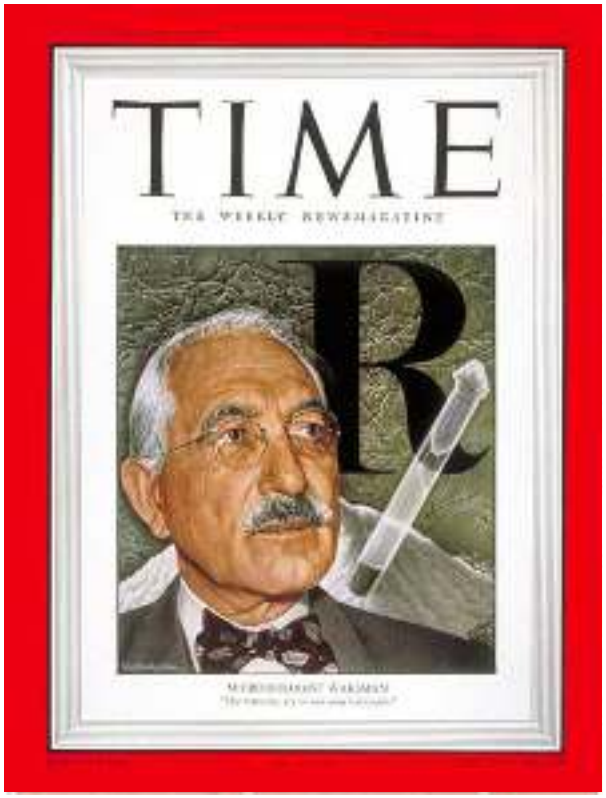
- Enfeksiyon etkenlerini üretemiyoruz.
- Bugün için klinik mikrobiyolojik tanı sık karşılaşılan kısıtlı sayıda mikroorganizma için optimize edilmiştir.
  - Sepsisli hastaların %40'ında,
  - İdrar yolu enfeksiyonlarında %25'inde etken mikroorganizma üretilemiyor.

ci Rep. 2021 Mar 26;11(1):6972. doi: 10.1038/s41598-021-86346-4.

N Engl J Med. 2015 Mar 12;372(11):1048-54. doi: 10.1056/NEJMra1410052.

İlk problem etkeni üretebilmek

- Waksman institute of microbiology;



Kültürü yapılamayan mikroorganizmalar

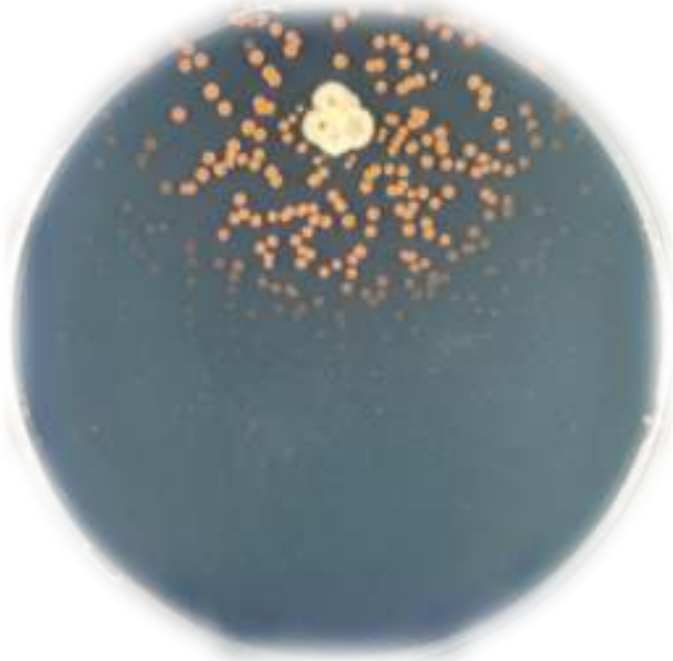
çeşitliliğin %99.9 oluşturuyor

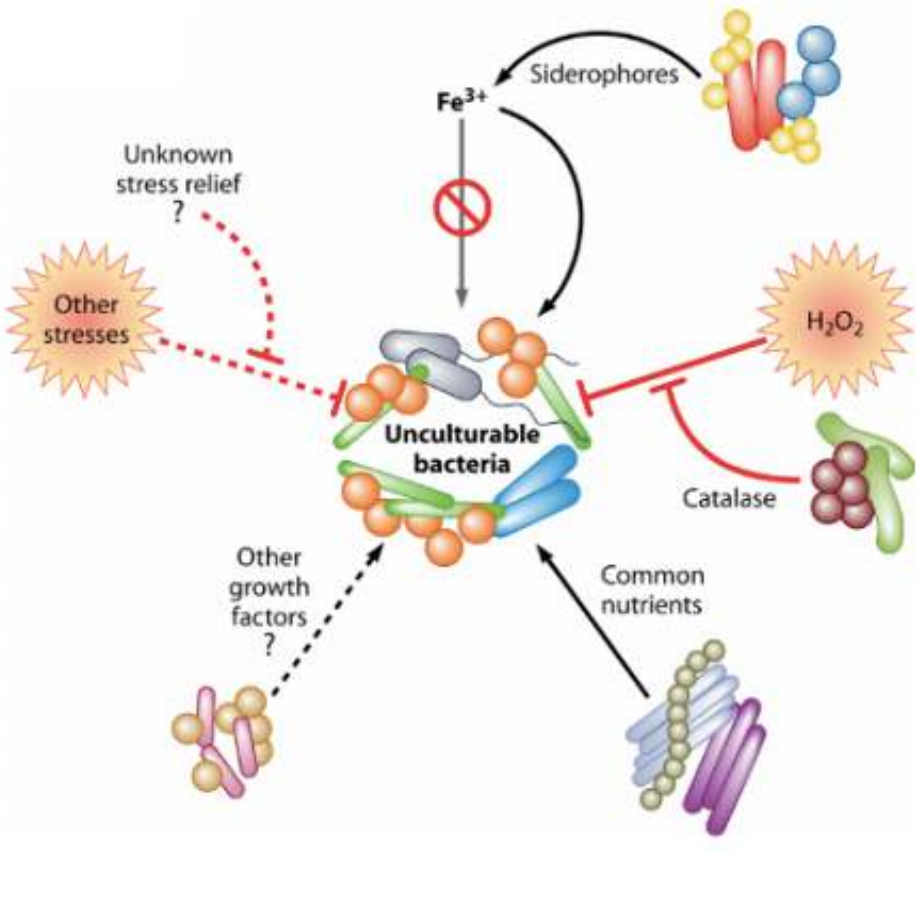
viable but non-culturable (VBNC) /

canlı ama üretilemez

Neden üremiyorlar ve nasıl ürerler

- Viable but non-culturable / canlı ama üretilemez





Stewart, E.J., 2012. Growing Unculturable Bacteria. J. Bacteriol. 194, 4151–4160. doi:10.1128/JB.00345-12

viable but non-culturable / canlı ama üretilemez

- Yeni nesil kültür sistemleri / kültür 2.0

ADVENTURES IN MICROBIOLOGY

Dogged researchers are designing technologies to find and grow microbes that have never before survived in the lab. By Amber Dance

Ledford, H. Promising antibiotic discovered in microbial 'dark matter'. *Nature* (2015). https://doi.org/10.1038/nature.2015.16675

viable but non-culturable / canlı ama üretilemez

- Patojen bakterileri de üretemiyoruz!

frontiers in MICROBIOLOGY

REVIEW ARTICLE
published: 02 June 2014
doi: 10.3389/fmicb.2014.00258

The importance of the viable but non-culturable state in human bacterial pathogens

Laam Li[1], Nilmini Mendis[1], Hana Trigui[1], James D. Oliver[2] and Sebastien P. Faucher[1]*

[1] Department of Natural Resource Sciences, Faculty of Agricultural and Environmental Sciences, McGill University, Ste-Anne-de-Bellevue, QC, Canada
[2] Department of Biology, University of North Carolina at Charlotte, Charlotte, NC, USA

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e-mail: sebastien.faucher2@mcgill.ca

Many bacterial species have been found to exist in a viable but non-culturable (VBNC) state since its discovery in 1982. VBNC cells are characterized by a loss of culturability on routine agar, which impairs their detection by conventional plate count techniques. This leads to an underestimation of total viable cells in environmental or clinical samples, and thus poses a risk to public health. In this review, we present recent findings on the VBNC state of human bacterial pathogens. The characteristics of VBNC cells, including the similarities and differences to viable, culturable cells and dead cells, and different detection methods are discussed. Exposure to various stresses can induce the VBNC state, and VBNC cells may be resuscitated back to culturable cells under suitable stimuli. The conditions that trigger the induction of the VBNC state and resuscitation from it are summarized and the mechanisms underlying these two processes are discussed. Last but not least, the significance of VBNC cells and their potential influence on human health are also reviewed.

Keywords: VBNC, stress, resuscitation, virulence, human pathogens, biofilm, antibiotic

viable but non-culturable / canlı ama üretilemez

- *Vibrio cholerae*, *E. coli*, *Campylobacter jejuni*, *Salmonella* spp., *Listeria monocytenenes*, *Yersinia enterocolitica*

Viable but Nonculturable Bacteria

Jvo Siegrist
Microbiology Focus Edition 1.4

IN MANY SPECIMENS, MORE BACTERIA ARE PRESENT THAN WE CAN DETECT WITH COMMON CULTURAL METHODS.

The expression "viable but nonculturable" (VNC) bacteria, describes cells that cannot normally be cultured. However this makes little sense, when one considers that the demonstration of culturability remains the best practically acceptable definition of viability. So a better explanation of the status of these bacteria would be "not immediately culturable". In most cases the non-spore-forming bacteria is in a survival state (e.g., resting, dormancy, quiescence, or debilitation) and the metabolic pathways are still active but the organism are not growing. According to the latest VNC definition, VNC cells are regarded as viable and potentially replicative, but the methods required for resuscitation are beyond our current knowledge. With special media or with certain supplements it has been shown that it is possible to recover them. VNC bacteria have often undergone a treatment like heating, drying, setting under high osmotic pressure (high salt content) or contact with inhibiting chemicals. The end result of the treatment is sensitive cells or sub-lethally damaged cells, which can mean the loss of some ribosomes, damaged enzymes, cell membranes and other problems causing malfunctions in cells.

In the recent years species of *Vibrio cholerae, E. coli, Campylobacter jejuni, Salmonella* spp., *Listeria monocytenenes* and *Yersinia enterocolitica* have been reported to enter the viable but nonculturable (VNC) state [1-10].

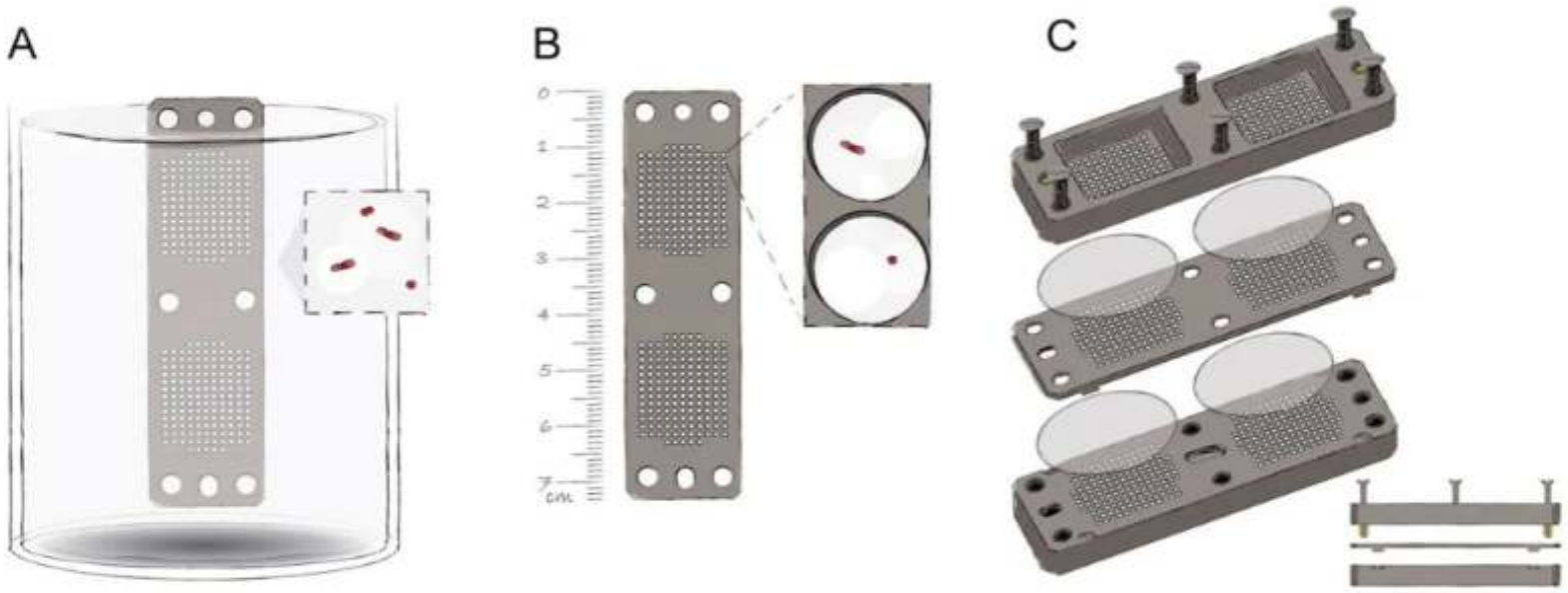
viable but non-culturable / canlı ama üretilemez

- İdrarda *E. coli* suşları, hücre duvarını hedefleyen bir antibiyotik ile tehdit sırasında duvarlı halden L-formuna kolayca geçer.
- Antibiyotiğin kesilmesini takiben sonra etkili bir şekilde duvarlı duruma geri dönerler.

OPEN

Possible role of L-form switching in recurrent urinary tract infection

Katarzyna M. Mickiewicz[1]*, Yoshikazu Kawai[1], Lauren Drage[1], Margarida C. Gomes[2], Frances Davison[1]



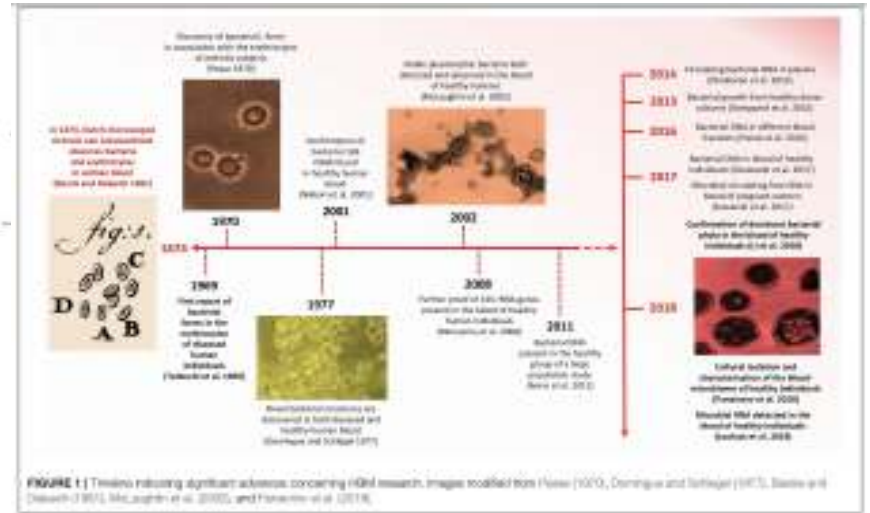
viable but non-culturable / canlı ama üretilemez

- Kan örneğinde hücresiz bakteriyel DNA (cfDNA) metagenomik dizileme ile patojen tespit edilebilir.

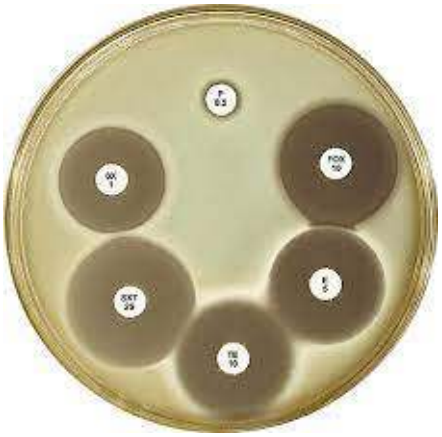
The Healthy Human Blood Microbiome: Fact or Fiction?

*Diego J. Castillo[1], Riaan F. Rifkin[1,2], Don A. Cowan[1] and Marnie Potgieter[1]**

[1] *Department of Biochemistry, Genetics and Microbiology, Centre for Microbial Ecology and Genomics, University of Pretoria, Pretoria, South Africa,* [2] *Human Origins and Palaeo Environmental Research Group, Department of Anthropology and Geography, Oxford Brookes University, Oxford, United Kingdom*



Antimikrobiyal Duyarlılığı Belirlemek için Kullanılan Testler



Önemli Soru?

- Direnç her zaman başarısızlığı öngörür mü?

- Duyarlılık her zaman tedaviye olumlu yanıtı mı gösterir?

Sefotaksim ile yapılan faz III klinik çalışması

- 8 saatte bir 2 g intravenöz (iv) dozda sefotaksim ile tedavi edilen, çeşitli monomikrobik Gram-negatif enfeksiyonları olan hastalar

TABLE 1. Correlation of disease outcome with the results of MIC determinations in patients with infection who were treated with cefotaxime[a] (16)

Cefotaxime MIC (μg/ml)	Category[b]	Number of patients	% Cured or improved	% Eradication
≤4	S	1003	94	91
8	S	273	90	86
16	I	151	77	75
32	I	70	84	71
64	R	19	64	50

[a] All patients had defined monomicrobic infections and were treated with intravenous cefotaxime alone, typically at a dosage of 2 g q8h.
[b] Susceptibility categories: S, susceptible; I, intermediate; R, resistant.

Sefoperazonun Faz III çalışmaları

- Sefoperazonun güvenlik ve klinik etkinliği Amerika Birleşik Devletleri, Avrupa, Güney ve Latin Amerika ve Japonya'da yürütülen klinik çalışmalarda değerlendirilmiştir

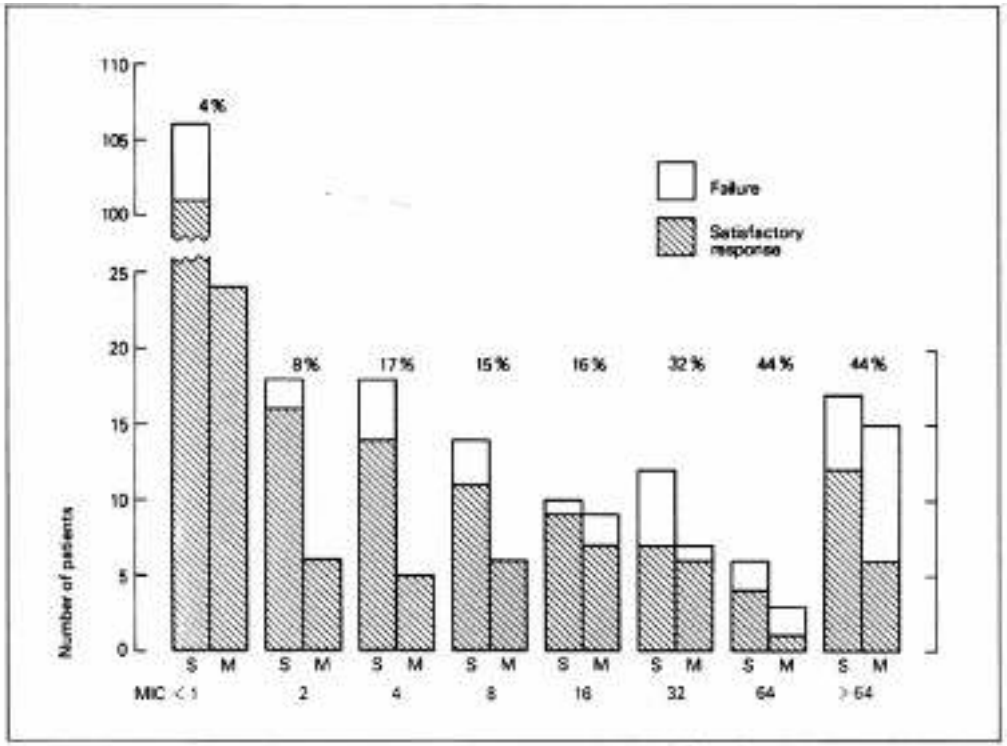


Fig. 1. Relationship between failure rate and minimum inhibitory concentration [MIC] [μg/ml] for the causative pathogen treated with cefoperazone.

Bars represent the number of patients with an infection due to a pathogen susceptible to the MIC of cefoperazone as indicated on the abscissa. The numbers in % represent the corresponding failure rate. S means infections associated with a single pathogen. M stands for mixed infections and represents the organism with the highest MIC isolated from such an infection, the organisms with a lower MIC being neglected.

Duyarlılık testi ne zaman yüksek prediktif değere sahip?

- Doğru tanımlanmış tek bir mikrobiyal etkenin neden olduğu
- parenteral uygulanabilen tek bir antimikrobiyal ile tedavi mümkün olduğunda
- ilacın enfeksiyon bölgesine penetrasyonunun tahmin edilebilir olduğunda
- bağışıklık sistemi baskılamamış hastalara uygulandığı

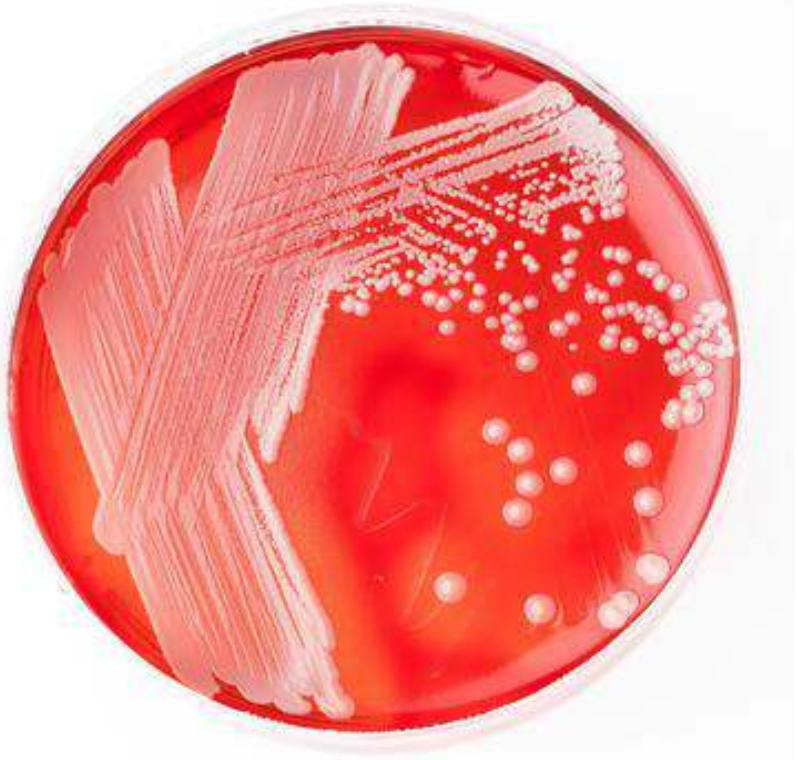
Laboratuvarda in vitro duyarlılık testlerinin yapıldığı

ASM ournal of Clinical Microbiology Vol. 49, No. 9_Supplement,

Peki neden?

- Antimikrobiyal duyarlılık;

  mükemmel optimize edilmiş besiyerlerinde üretilen, saf kültürler



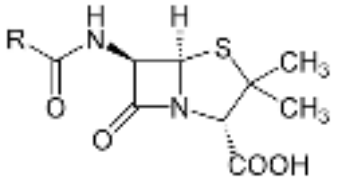
Peki neden?

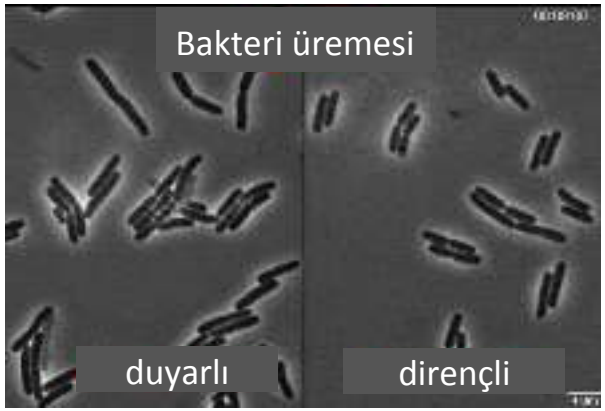
- Antimikrobiyal duyarlılık;

  mükemmel optimize edilmiş besiyerlerinde üretilen, saf kültürler

E. coli

Antibiyotik

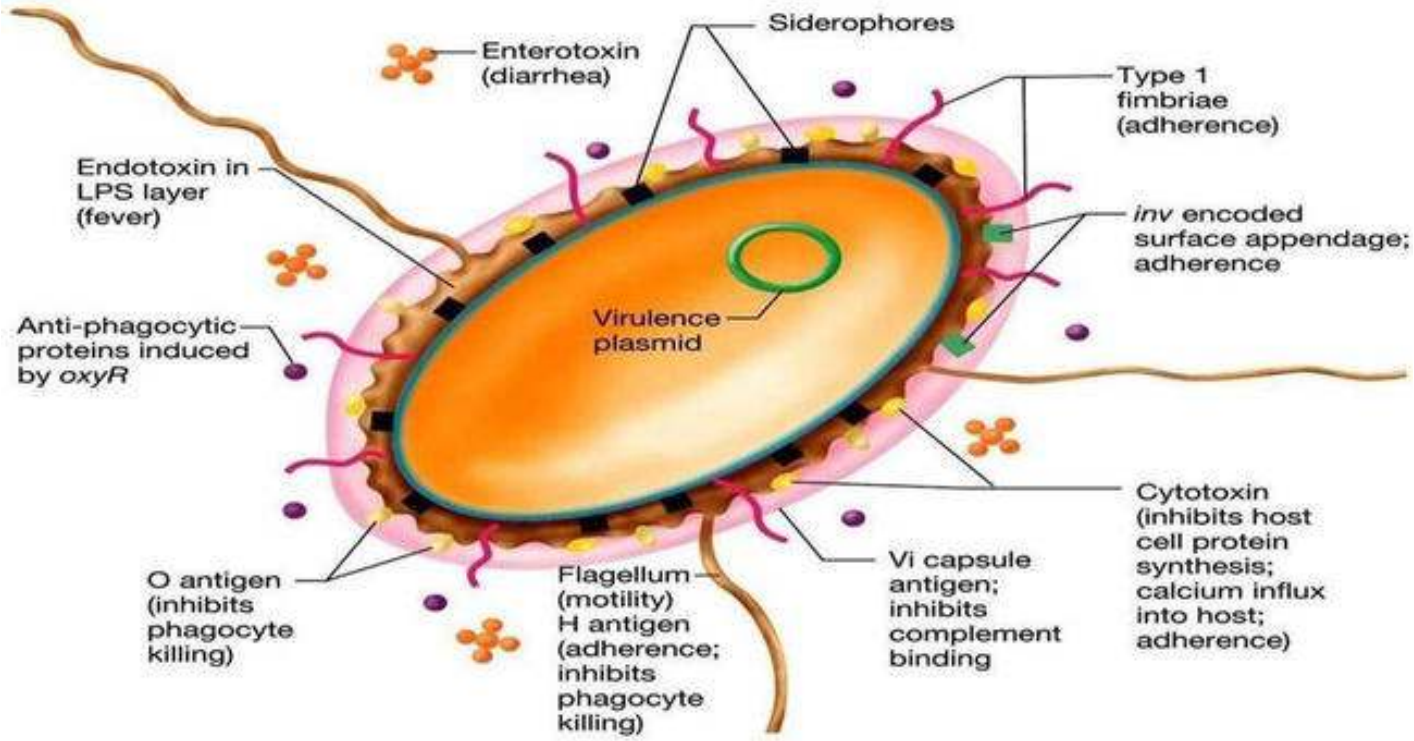




- Bu koşullar altında belirlenen aktivitenin değerlendirilmesi, kombinasyon tedavisi alan hastalarda veya polimikrobiyal enfeksiyonlu hastalarda sonucu güvenilir bir şekilde tahmin edebilir mi?

Bakteriyel Virülans Faktörleri ?

- Enfeksiyonun sonucunu belirleyen bir diğer önemli belirleyici de bakteriyel virülans faktörlerinin varlığı veya yokluğudur.



Konak Faktörleri

- Diğer bir önemli neden in vitro duyarlılık testlerinin en büyük dezavantajı konak faktörü bulunmamasıdır!!

Bağışıklık sistemi

Beslenme yönetimi

sitokinler

Albumin

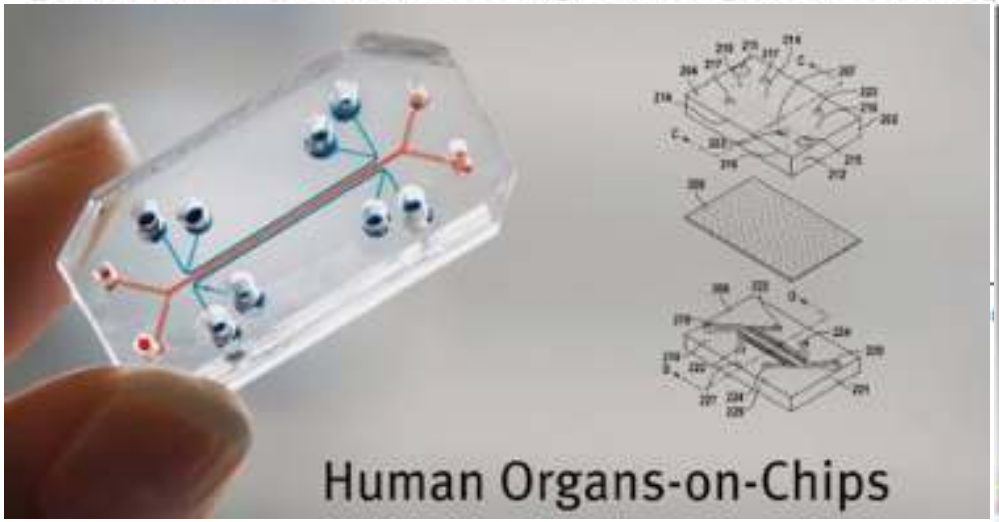
Kompleman etkisi

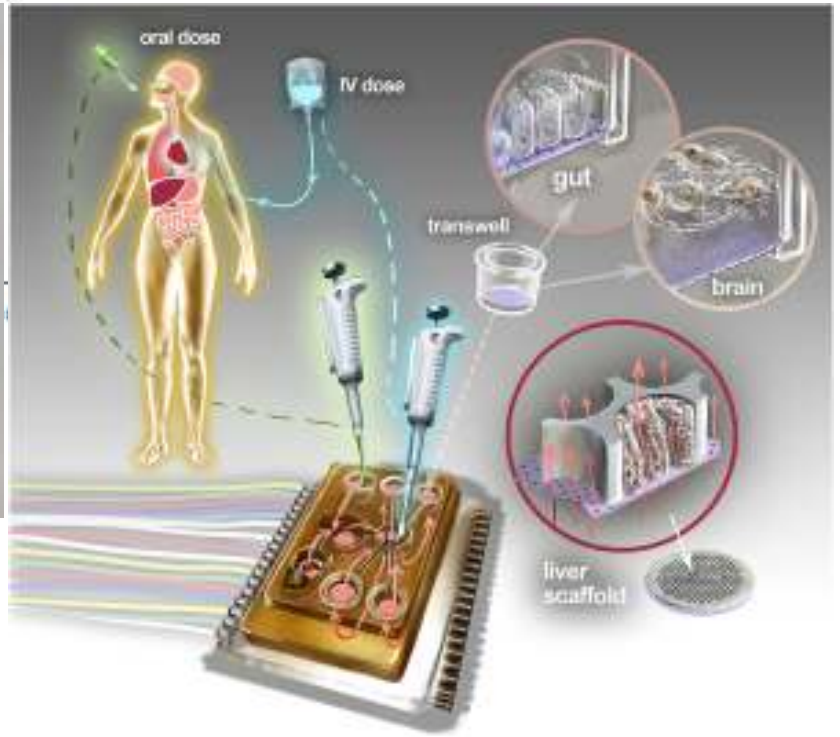
Mikrobiyatının etkisi

Kanser ilaçları için durum farklı

Progress in Molecular Biology and Translational Science
Volume 187, Issue 1, 2022, Pages 41-91

Chapter Three - From organ-on-chip to body-on-chip: The next generation of microfluidics platforms for *in vitro* drug efficacy and toxicity



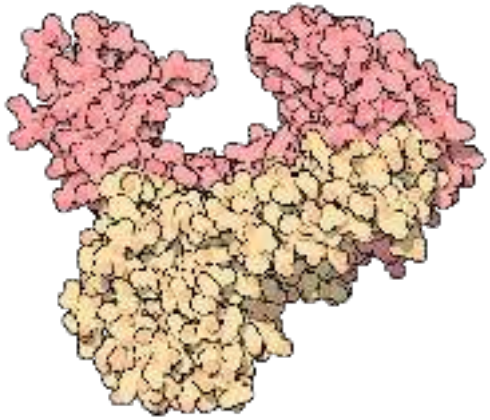


Moleküler Tanı Yöntemlerinin Gelişimi

- 1970'li yıllarda revers transkriptaz ve restriksiyon endonükleaz'ların keşfi

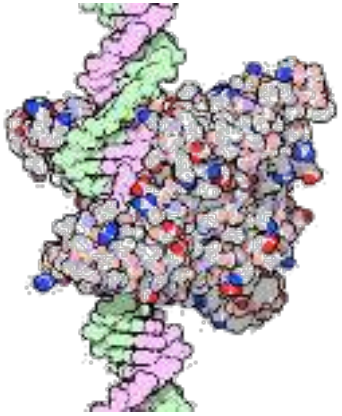
The Nobel Prize in Physiology or Medicine 1975
David Baltimore, Renato Dulbecco, Howard M. Temin

The Nobel Prize in Physiology or Medicine 1975 was awarded jointly to David Baltimore, Renato Dulbecco and Howard Martin Temin "for their discoveries concerning the interaction between tumour viruses and the genetic material of the cell".



The Nobel Prize in Physiology or Medicine 1978
Werner Arber, Daniel Nathans, Hamilton O. Smith

The Nobel Prize in Physiology or Medicine 1978 was awarded jointly to Werner Arber, Daniel Nathans and Hamilton O. Smith "for the discovery of restriction enzymes and their application to problems of molecular genetics".

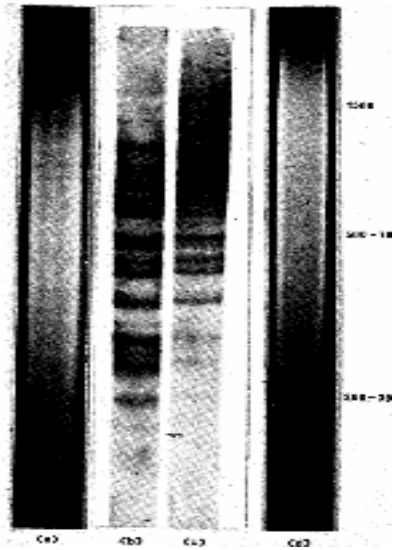


Moleküler Tanı Yöntemlerinin Gelişimi

- Bu gelişmeleri;

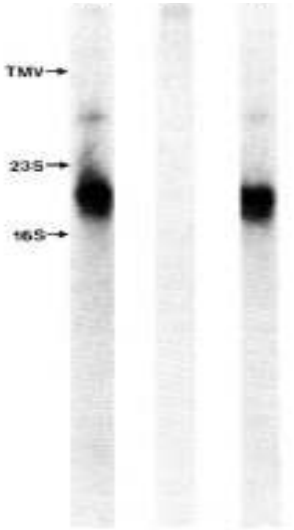
  hibridizasyon, blotlama ve DNA dizileme yöntemlerinin tanıtılması takip etmiştir.

Southern blotting



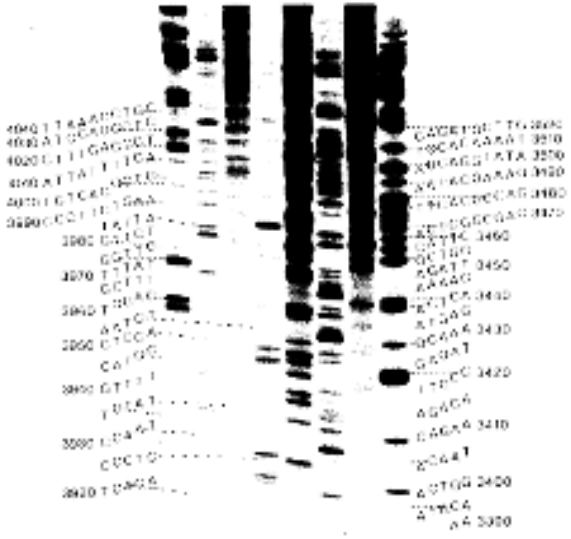
J Mol Biol. 1975 Nov 5;98(3):503-17.

Northern blotting



Proc Natl Acad Sci U S A. 1977 Dec;74(12):5350-4.

DNA dizileme



Proc Natl Acad Sci U S A. 1977 December; 74(12): 5463–5467.

Moleküler Tanı Yöntemlerinin Gelişimi

- 1970'ler radyoaktif işaretli hibridizasyon problarının keşfi

MOLECULAR HYBRIDIZATION OF RADIOACTIVE DNA TO THE DNA OF CYTOLOGICAL PREPARATIONS

By Mary Lou Pardue and Joseph G. Gall

KLINE BIOLOGY TOWER, YALE UNIVERSITY

Communicated by Norman H. Giles, August 13, 1969

Abstract.—A method is presented for detecting the cellular location of specific DNA fractions. The technique involves the hybridization of a radioactive test

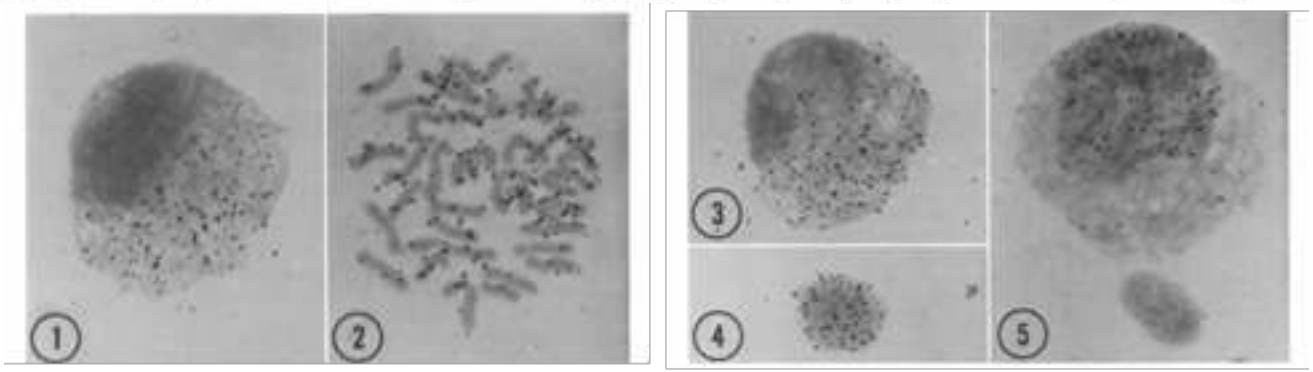
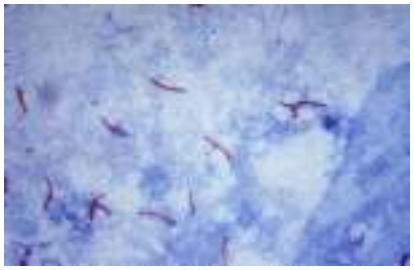
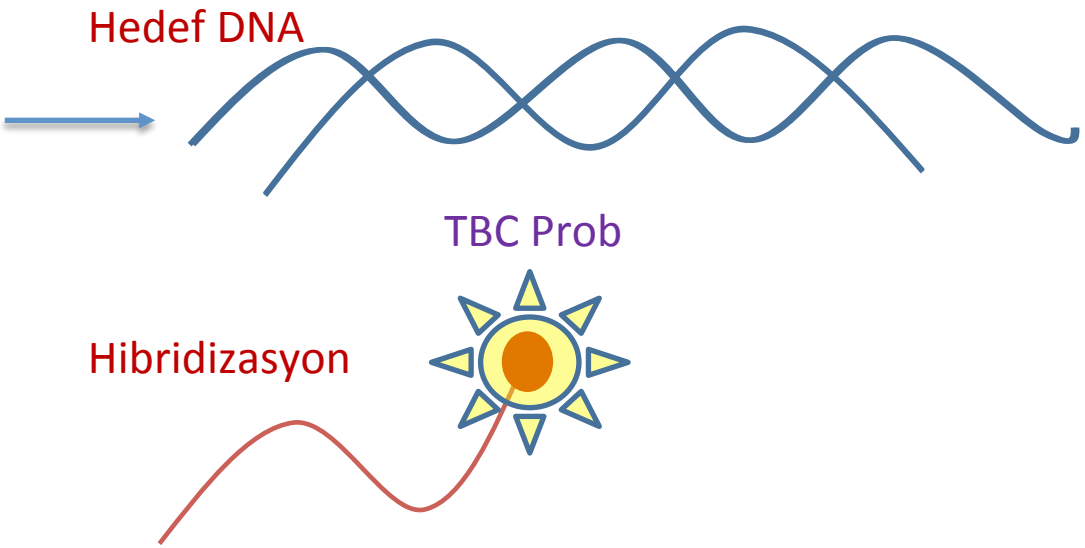


FIG. 1–4.—Autoradiographs of nuclei from the ovary of the toad *Xenopus* after cytological hybridization with radioactive *Xenopus* DNA which lacked the ribosomal cistrons. The DNA of these squash preparations was denatured *in situ*. The slide was then incubated with a solution of radioactive test DNA from which the rDNA had been removed by separation on a CsCl density gradient. The specific activity of the test DNA was 130,000 cpm/μg. The slides were stained with Giemsa.

Hibridizasyon Temelli Yöntemler

- 1980'li yıllardan itibaren mikrobiyal tanı için geliştirilmeye başlandı.





Moleküler Tanı Yöntemlerinin Gelişimi

- 1990 FDA tarafından onaylanmış ilk moleküler tanı testi, hibridizasyon temelli *Chlamydia trachomatis* ve *Neissseria gonorrhoeae*'nin tespiti için geliştirilmiştir (Gen-Probe PACE).

JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1989, p. 632–635
0095-1137/89/040632-04$02.00/0

Vol. 27, No. 4

Evaluation of a Prototype DNA Probe Test for the Noncultural Diagnosis of Gonorrhea

PAUL A. GRANATO[1,2]* AND MARYANN ROEFARO FRANZ[3]

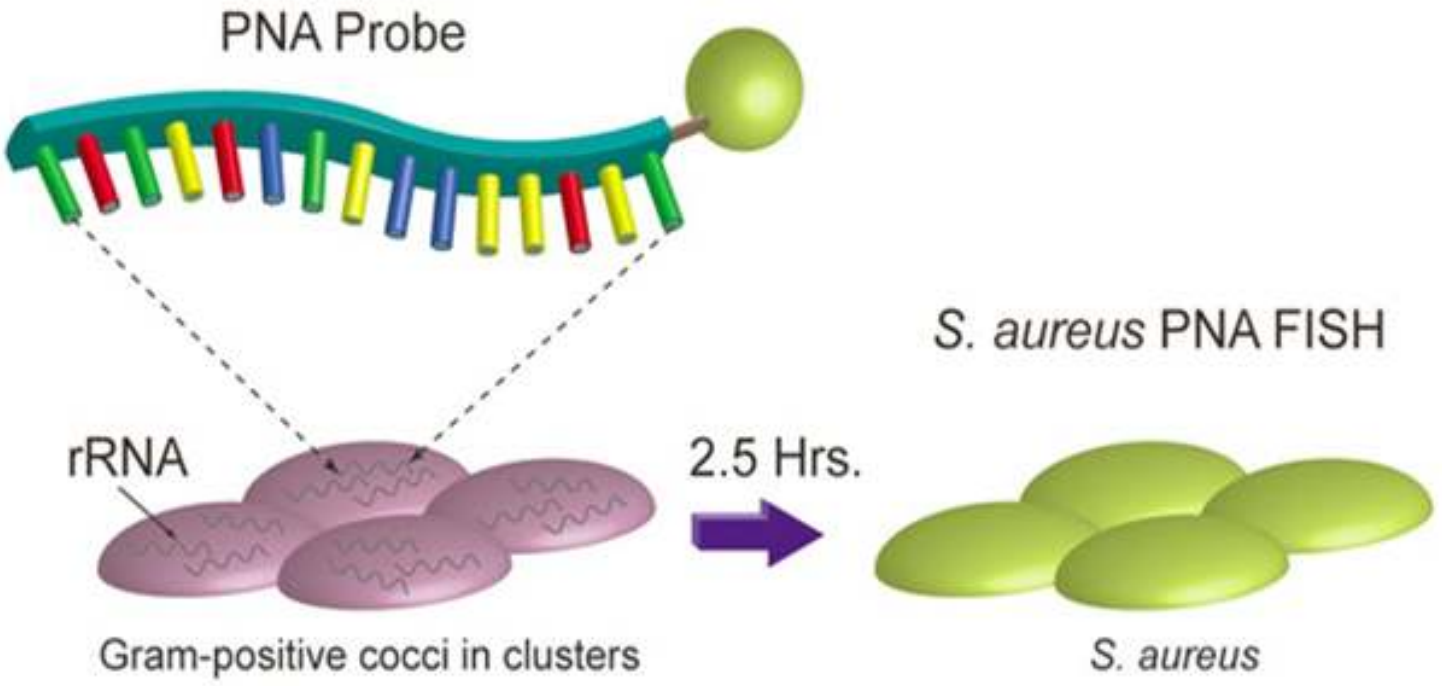
Department of Pathology, Crouse Irving Memorial Hospital,[1] and Departments of Pathology and Microbiology, SUNY Health Science Center at Syracuse,[2] Syracuse, New York 13210, and Department of Pathology, Community-General Hospital, Syracuse, New York 13215[3]*

Received 27 September 1988/Accepted 15 December 1988

A prototype, nonisotopic, chemiluminescent DNA probe test called the Gen-Probe PACE (Probe Assay-Chemiluminescence Enhanced) system for *Neisseria gonorrhoeae* (Gen-Probe, San Diego, Calif.) was compared with conventional Martin-Lewis culture medium in JEMBEC plates for the laboratory diagnosis of gonorrhea. This 2-h noncultural assay is based upon the use of an acridinium ester-labeled DNA probe. The rRNA-directed DNA probe hybridizes with the target rRNA, and the hybridized probe is separated from the unhybridized probe through the use of magnetic microparticles. The esterified acridinium is hydrolyzed from the hybridized probe by the addition of an alkaline hydrogen peroxide solution, resulting in the production of visible light which is measured in a luminometer. The amount of light generated is directly proportional to the amount of gonococcal target rRNA present in the sample. A total of 407 clinical specimens (203 urethral and 204 endocervical) were collected from high-risk walk-in patients attending a sexually transmitted disease clinic. Separate patient specimens were collected for culture on Martin-Lewis medium in JEMBEC plates and for DNA probe assay. Statistical analysis of the overall comparative results showed that the DNA probe assay had a sensitivity, specificity, and positive and negative predictive values of 93, 99, 97, and 99%, respectively, in a patient population with a gonococcal disease prevalence of 21%. The results of this comparative study showed that the prototype chemiluminescent DNA probe assay is a rapid and reliable noncultural alternative for the laboratory diagnosis of gonorrhea.

Hibridizasyon Temelli Yöntemler

- Peptit nükleik asit probları (PNA FISH) kullanılarak bulaşıcı hastalıkların hızlı ve doğru teşhisini sağlayan yeni bir teşhis tekniğidir.



Hibridizasyon Temelli Yöntemler

- Yanık yaralarında kültüre dayalı olmayan FDA onaylı PNA-FISH problarını kullanan tanımlama tekniği ile patojenler 2-3 saat içerisinde tanımlanmış

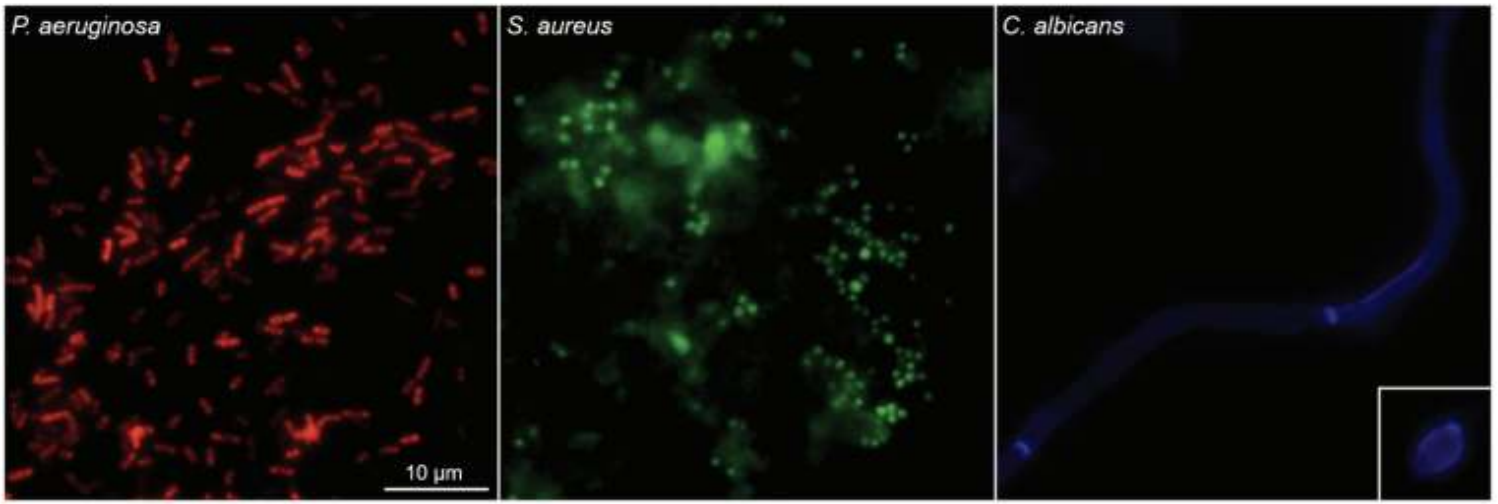
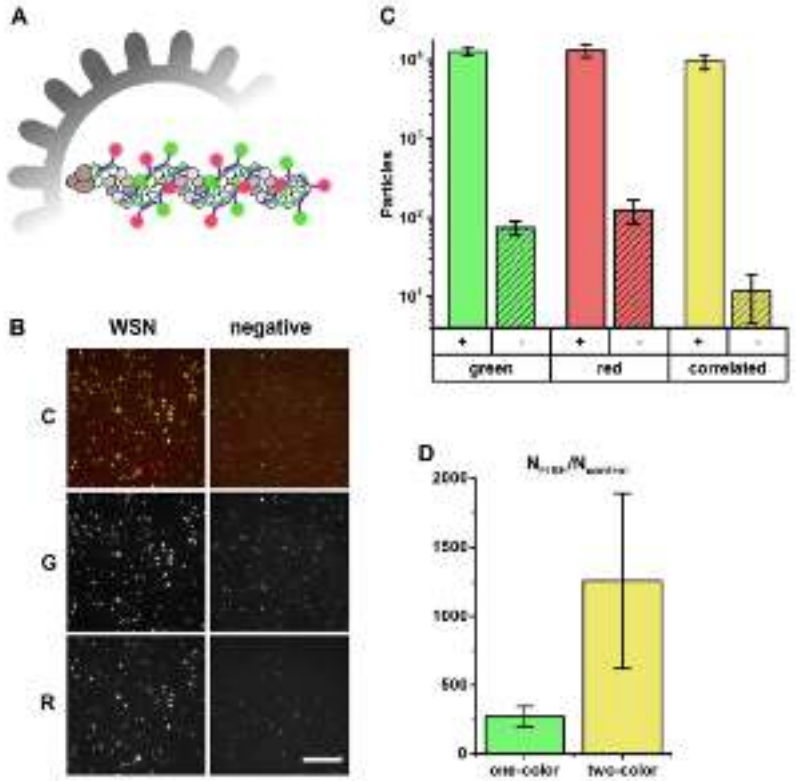


Figure 2. Pathogenic organisms identified from swabs of partial-thickness burn wounds. Each panel represents an individual channel and location for visualization of the microorganisms. *Pseudomonas aeruginosa* was clearly seen in the red channel as bright clusters of rods with minimal staining of the tissue debris. In the green channel, there were several clusters of *Staphylococcus aureus* that could be distinguished from the autofluorescent nature of the tissue debris. Tissue debris autofluorescence was also seen in the blue channel (not shown in panel), but *Candida albicans* was still clearly identifiable in both hyphae and bud form (inset, same scale). All images were taken with 63× objective. Single scale bar applies to all panels.

Hibridizasyon Temelli Yöntemler

- SARS-Cov2 yaklaşık 20 dakikada tespit edebilen hızlı bir floresan in situ hibridizasyon (FISH) protokolü

www.nature.com/scientificreports



Zaman Atlamalı Mikroskobik Yöntemler

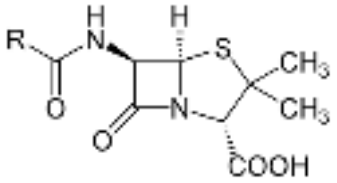
Zaman Atlamalı Mikroskobik Yöntemler

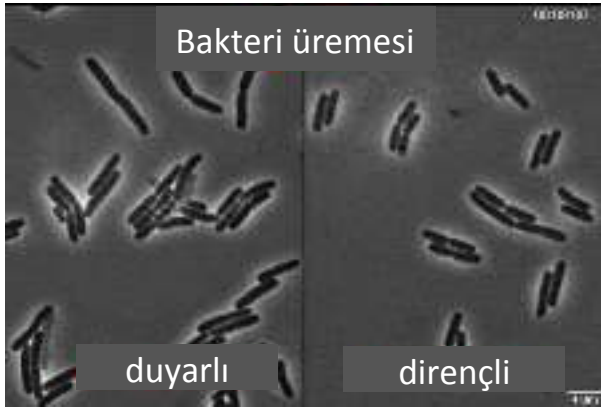
- Antimikrobiyal duyarlılık;

  mikroorganizma ile antibiyotik karşılaştırılmadan duyarlılık sonucundan bahsedilemez

E. coli

Antibiyotik





Zaman Atlamalı Mikroskobik Yöntemler

- Mikroorganizmalar üzerine antibiyotik etkisi 6 – 30 dakikada tespit edilebiliyor.

JCM
JournalsASM.org

Real-Time Optical Antimicrobial Susceptibility Testing

Fredborg et al.

FIG 1 The oCelloScope detection system. (a)
detection principle. A volume of 50 µl of a
dimensional (2D) picture. (c) 2D picture of *S.*
microscope.

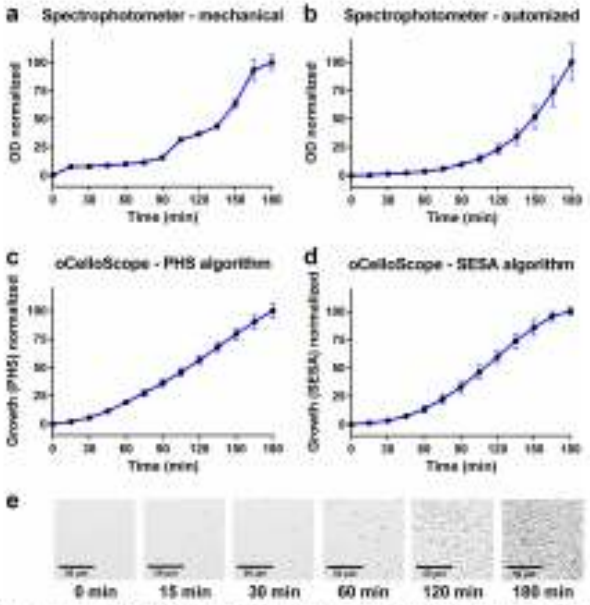
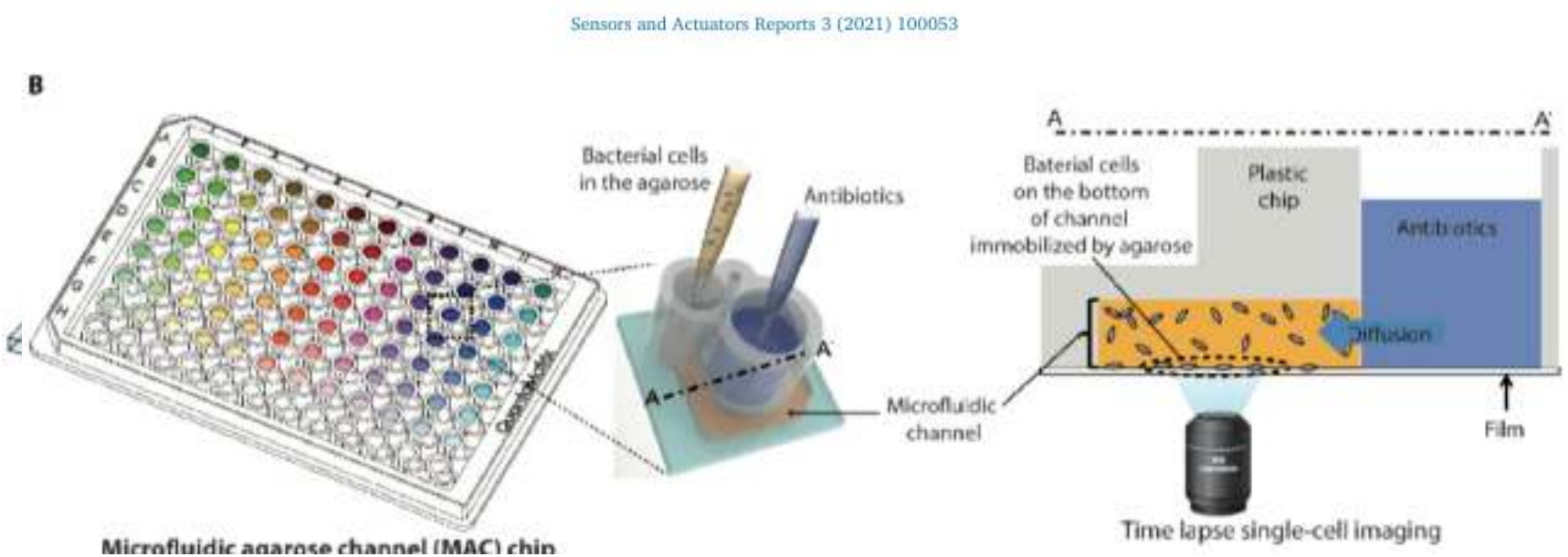


FIG 2 Bacterial growth of *S. aureus* measured by the oCelloScope and traditional OD measurements. (a) Growth was measured by optical density in a standard laboratory spectrophotometer with one cuvette. The absorbance was measured at 600 nm. (b) Growth was measured by optical density (absorbance, 655 nm) using a standard laboratory plate reader with a 96-well plate. (c) Growth was measured by optical density using the oCelloScope pixel histogram summation (PHS) algorithm. (d) Growth was measured by the oCelloScope segmentation and extraction of surface area (SESA) algorithm. (e) Pictures taken by the oCelloScope showing bacterial growth at different time points. All experiments were done as eight replicates, and standard deviations are shown as error bars on the curves. Scale bar, 50 µm.

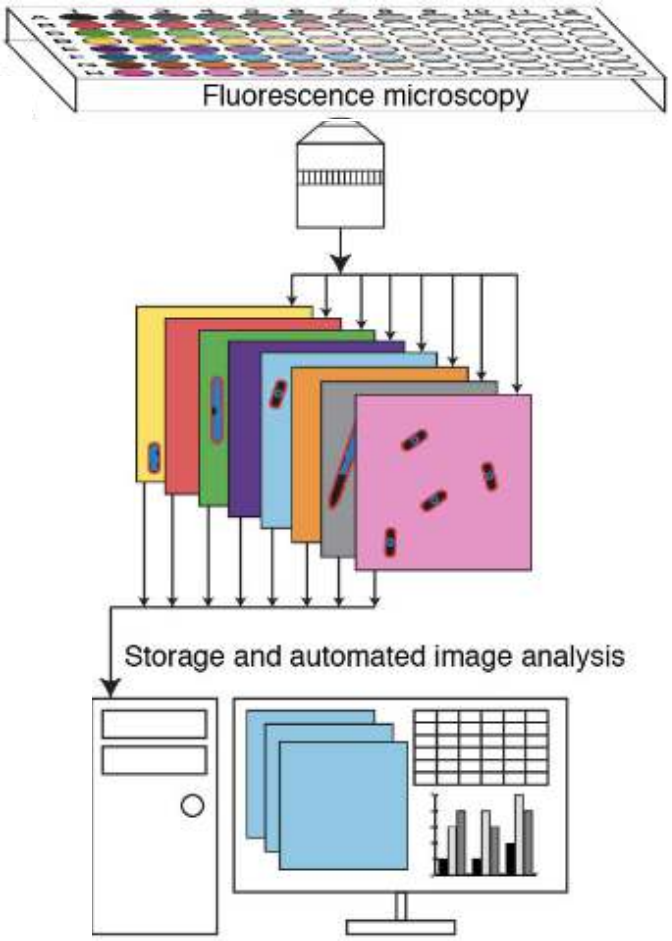
Zaman Atlamalı Mikroskobik Yöntemler

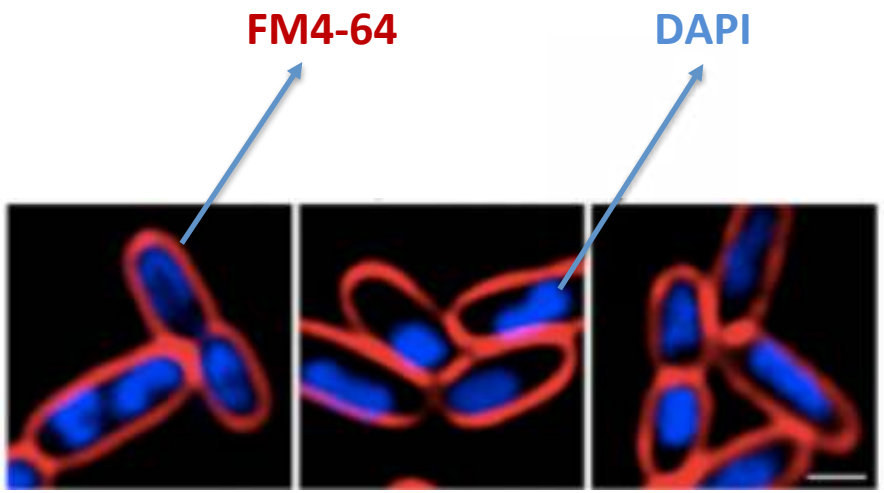
- Gelişmiş makine veya derin öğrenme algoritmaları ile entegre olan sistemler yakın gelecekte yaygınlaşacağı ön görülüyor.



Floresans Mikroskobik Yöntemler

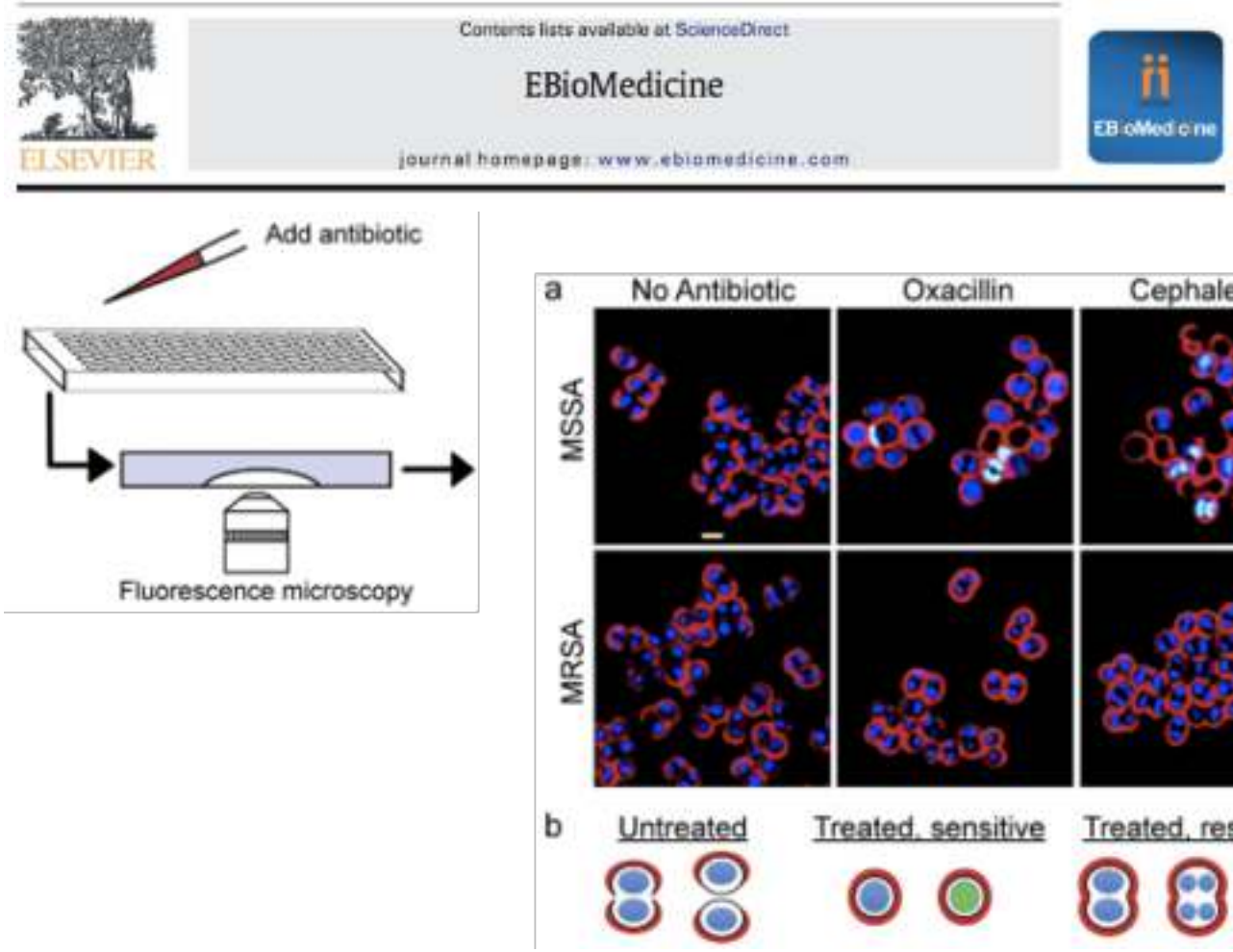
- *Bacterial cytological profilling* (BCP), bakterilerin morfolojik analizi ile antibiyotik duyarlılık





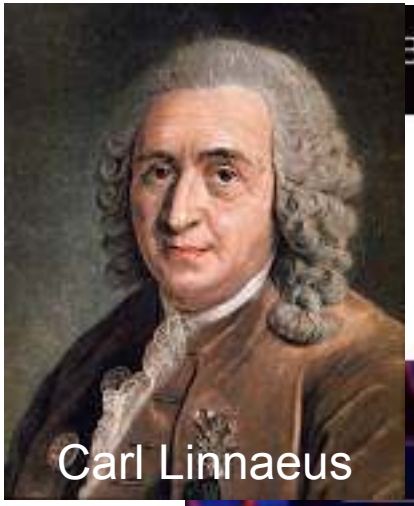
Floresans Mikroskobik Yöntemler

- *Bacterial cytological profiling*, %100 doğrulukla 1-2 saat içinde metisilin 30 dakikada daptomisin direnci tespiti



Floresans Mikroskobik Yöntemler

- Mikrobiyal hastalıkları ve ortaya çıkan ilaca dirençli bakteriyel enfeksiyonları tedavi etmek için devrim niteliğinde yeni bir yaklaşım sunar.


Carl Linnaeus

BIOSCIENCE

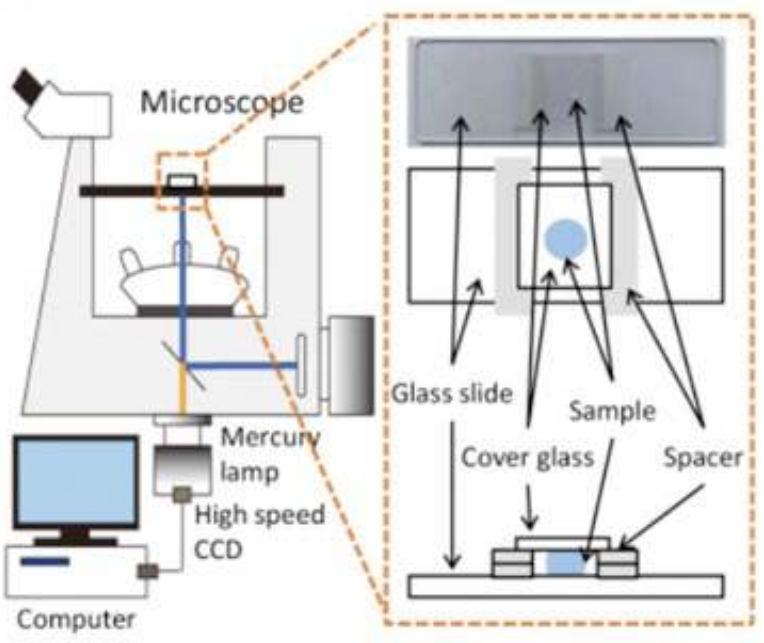
ABOUT US TECHNOLOGY SERVICES PIPELINE CONTACT

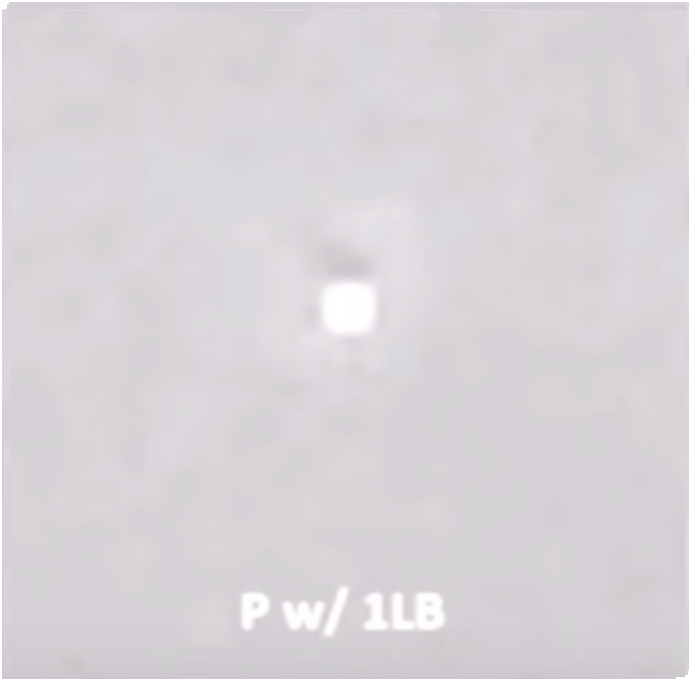
Linnaeus is using its proprietary technology to develop a pipeline of molecules to address the growing problem of microbial infection and drug resistance. Linnaeus also continues to establish partnerships with collaborators for the discovery and development of novel therapeutics.

PROGRAM	TARGET	LIBRARY SCREENING	BCP FOR MOA	LEAD SELECTION	LEAD OPTIMIZATION	PRECLINICAL
Our BCP platform identifies novel chemical scaffolds hitting new targets in drug resistant Gram negative pathogens	*E. coli*	→	→	→		
	A. baumannii	→	→	→		
	P. aeruginosa	→	→	→		
	K. pneumoniae	→				

Optikal Difüzometri

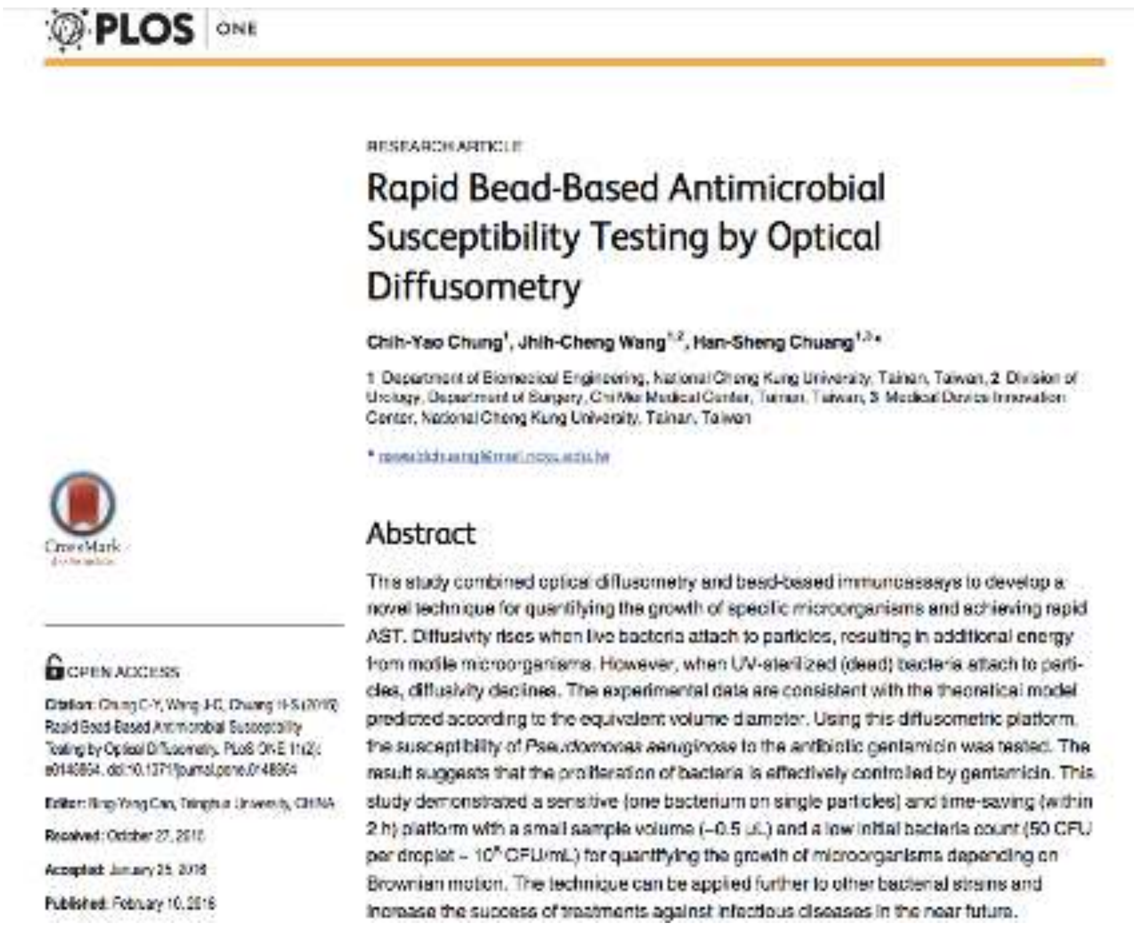
- Parçacıkların difüzyon katsayılarını ölçmek için tasarlanmış bir sistemdir.





Optikal Difüzometri

- Optikal difüsometri ve *bead-based immunoassays* yöntemlerinin kombine edildiği yöntemde, *P. aeruginosa*'da gentamisin direnci iki saat içerisinde tespit edilebilmiştir.


PLOS ONE

RESEARCH ARTICLE

Rapid Bead-Based Antimicrobial Susceptibility Testing by Optical Diffusometry

Chih-Yao Chung[1], Jhih-Cheng Wang[1,2], Han-Sheng Chuang[1,3]*

1 Department of Biomedical Engineering, National Cheng Kung University, Tainan, Taiwan, 2 Division of Urology, Department of Surgery, Chi Mei Medical Center, Tainan, Taiwan, 3 Medical Device Innovation Center, National Cheng Kung University, Tainan, Taiwan

* oswaldchuang@mail.ncku.edu.tw

OPEN ACCESS

Citation: Chung C-Y, Wang J-C, Chuang H-S (2016) Rapid Bead-Based Antimicrobial Susceptibility Testing by Optical Diffusometry. PLoS ONE 11(2): e0148864. doi:10.1371/journal.pone.0148864

Editor: Bing-Yang Cao, Tsinghua University, CHINA

Received: October 27, 2015

Accepted: January 25, 2016

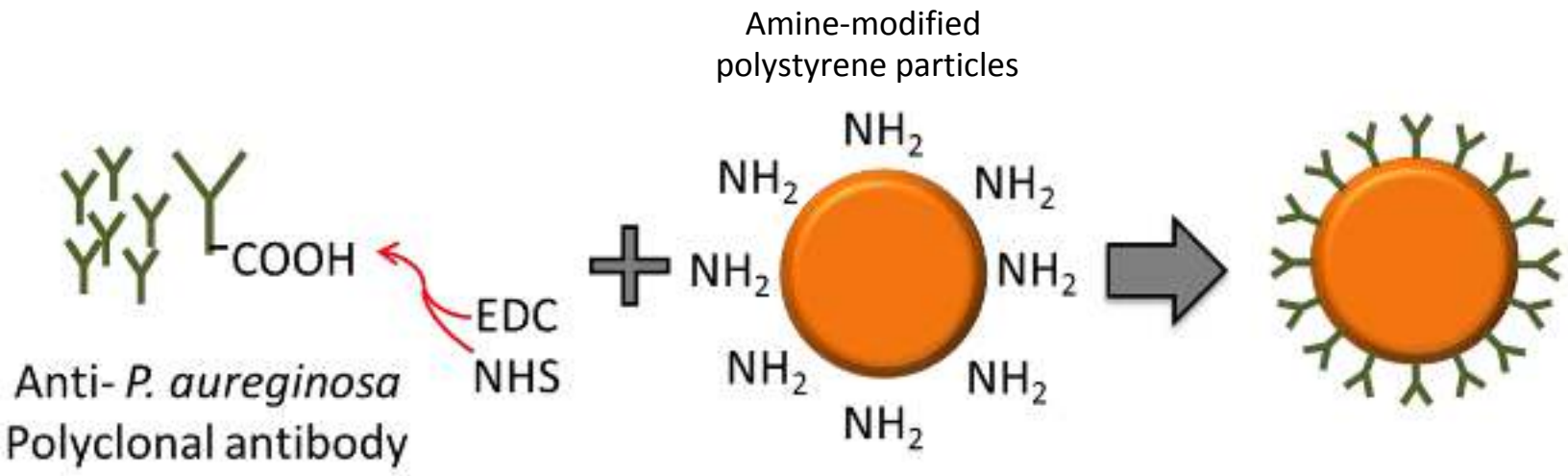
Published: February 10, 2016

Abstract

This study combined optical diffusometry and bead-based immunoassays to develop a novel technique for quantifying the growth of specific microorganisms and achieving rapid AST. Diffusivity rises when live bacteria attach to particles, resulting in additional energy from motile microorganisms. However, when UV-sterilized (dead) bacteria attach to particles, diffusivity declines. The experimental data are consistent with the theoretical model predicted according to the equivalent volume diameter. Using this diffusometric platform, the susceptibility of *Pseudomonas aeruginosa* to the antibiotic gentamicin was tested. The result suggests that the proliferation of bacteria is effectively controlled by gentamicin. This study demonstrated a sensitive (one bacterium on single particles) and time-saving (within 2 h) platform with a small sample volume (~0.5 μL) and a low initial bacteria count (50 CFU per droplet ~ 10^5 CFU/mL) for quantifying the growth of microorganisms depending on Brownian motion. The technique can be applied further to other bacterial strains and increase the success of treatments against infectious diseases in the near future.

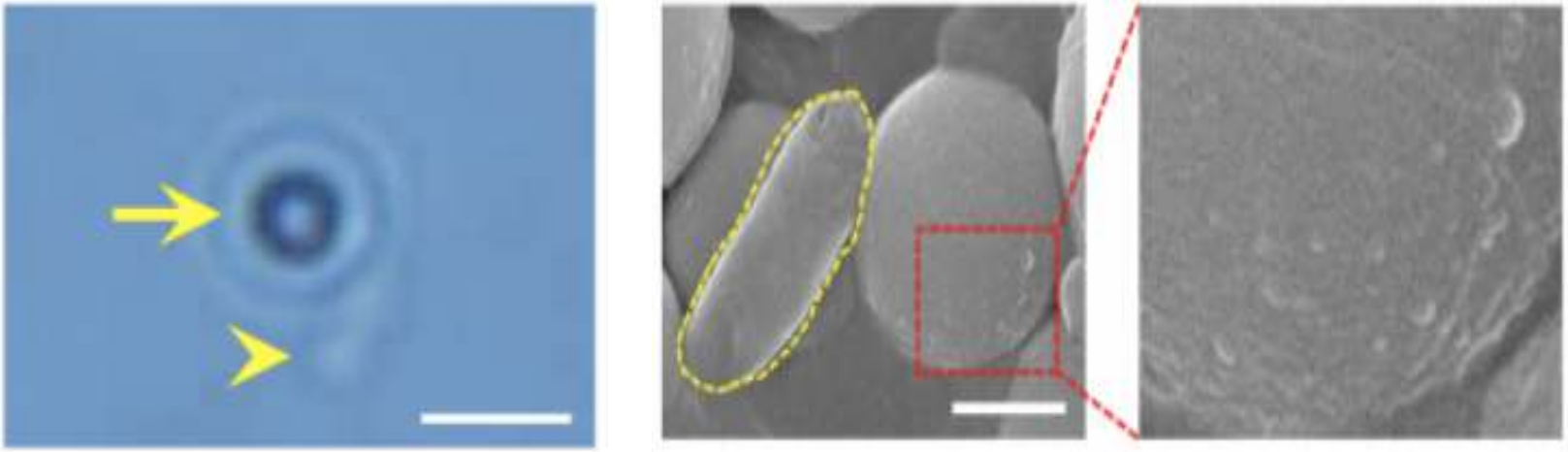
Optikal Difüzometri

- Antikorun aktive edilmesinde EDC-NHS kimyasalları kullanılarak antikorun polystyrene matrikse bağlanması.



Optikal Difüzometri

- Boncuklar üzerinde yakalanmış *P. aeruginosa*.



Optikal Difüzometri

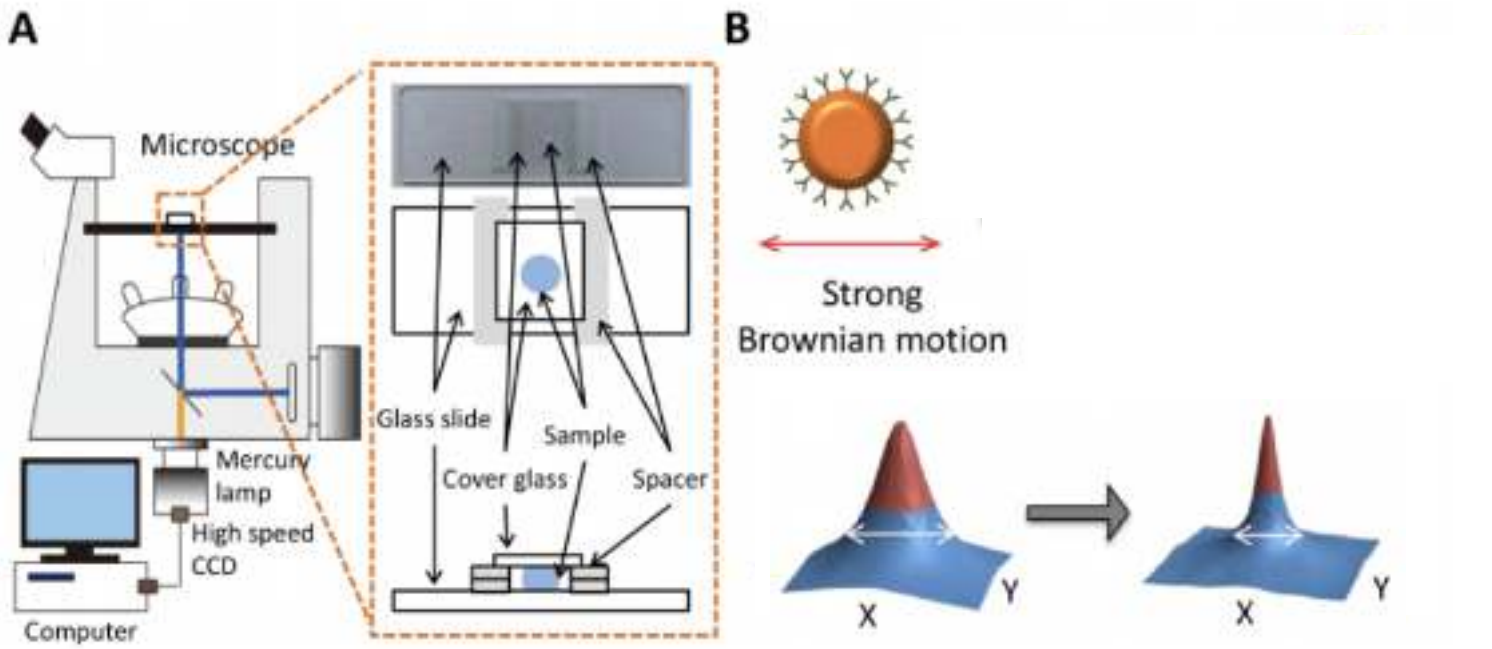
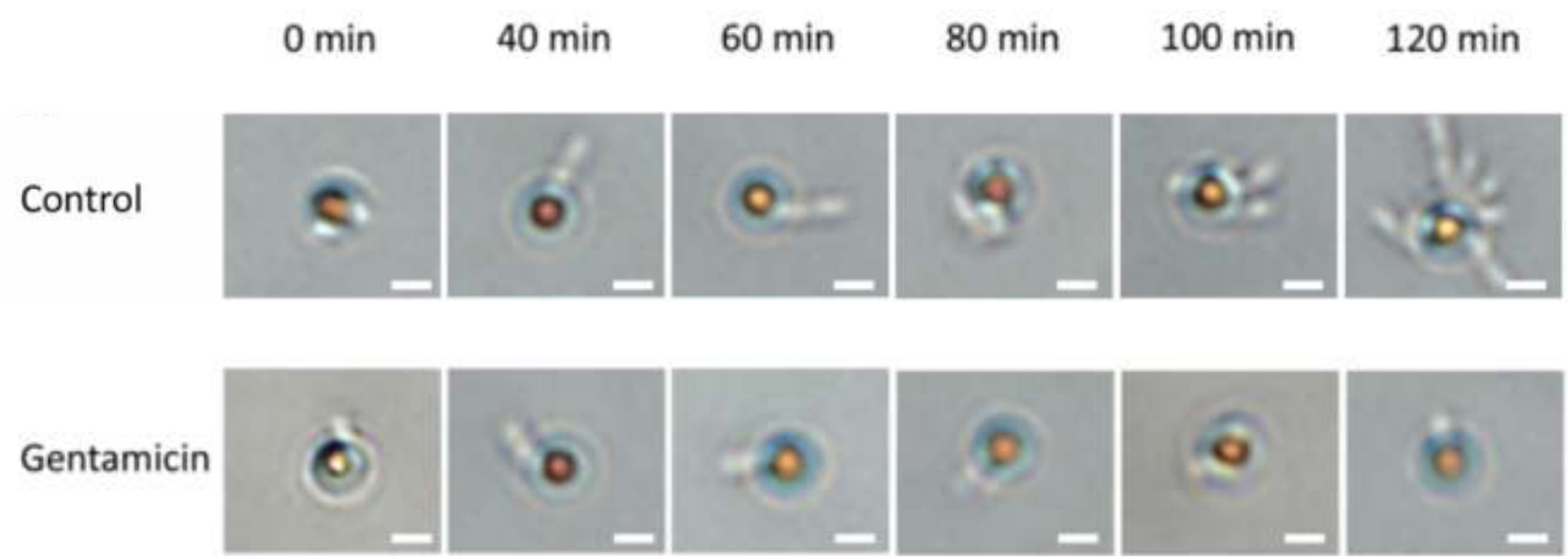


Fig 1. The optical diffusometric platform. (A) Schematic of the optical diffusometry. (B) The relationship of Brownian motion and the particle size change due to the bacterium-particle binding. The corresponding diffusivity values are derived from the cross-correlation algorithm. A large particle diameter results in a narrow correlation peak.

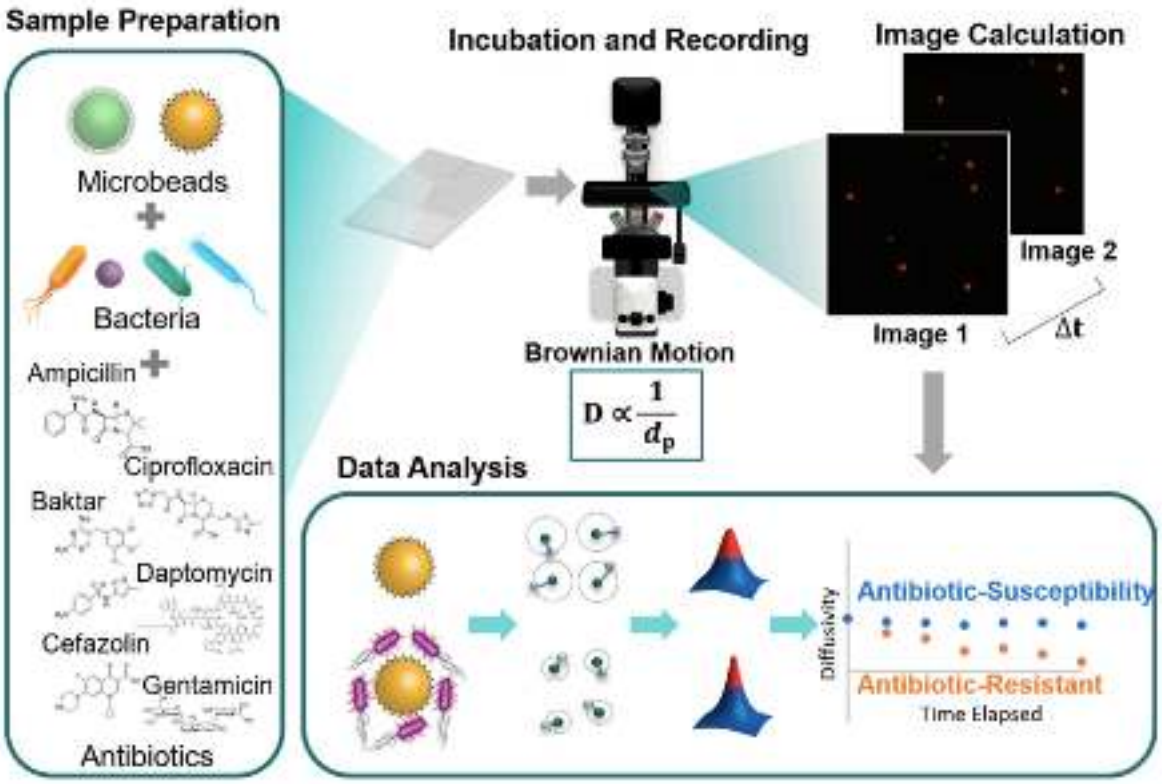
doi:10.1371/journal.pone.0148864.g001

Optikal Difüzometri



Optikal Difüzometri

- *E. coli* , *P. aeruginosa* , *K. pneumoniae* ve *S. aureus*, altı farklı antibiyotik için tespit süresi 40 dakika



Hibridizasyon Temelli Yöntemler

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

Faster sepsis treatment requires faster diagnostics.

The Accelerate Pheno™ system delivers phenotypic antibiotic susceptibility results along with microbial identification directly from positive blood cultures — critical information to select the best drug, for the specific pathogen, at the appropriate dose — 40 hours faster on average, than current methods used in most labs today.

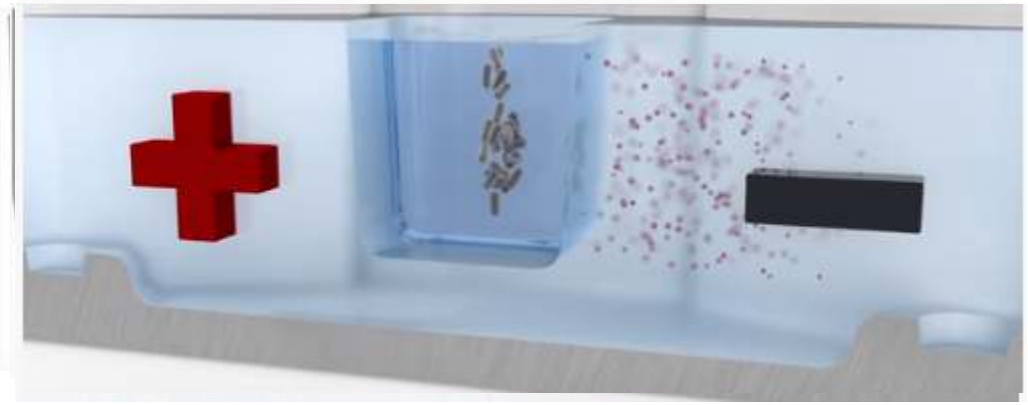
Hospitals are now dramatically shortening the time to get patients on the best antibiotic therapy while also freeing up time for laboratory technicians.

This earlier infection intelligence equips hospital teams to reduce adverse effects of antibiotic overuse — and gain ground against sepsis morbidity and mortality — by ensuring patients receive optimal, individualized sepsis treatment as quickly as possible.

Hibridizasyon Temelli Yöntemler

- Accelerate PhenoTest™ BC

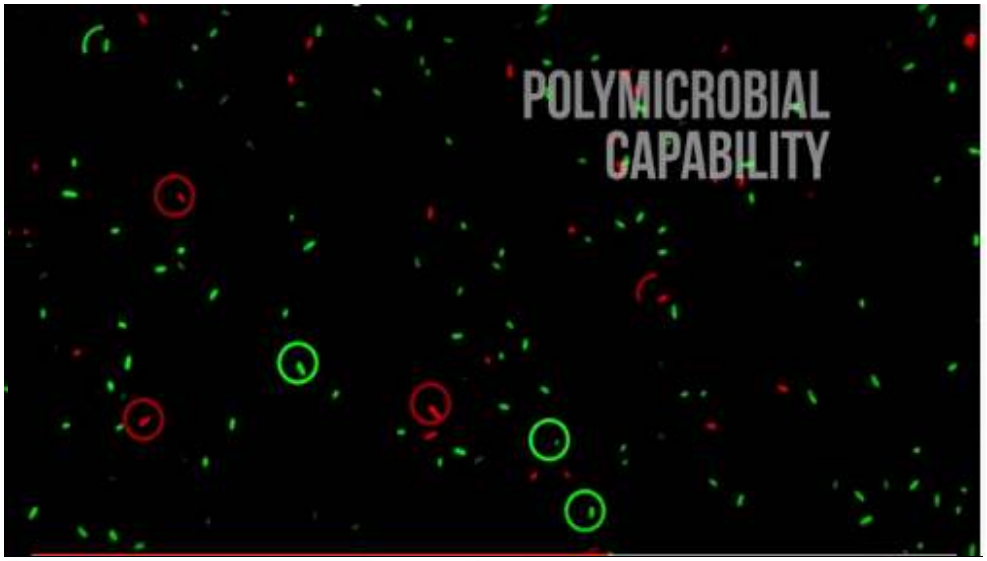
  Örnek muayene maddesinden mikroorganizmanın saflaştırılması için elektroforez ve filtreleme sistemi (elektrofiltrasyon).



Hibridizasyon Temelli Yöntemler

- Accelerate PhenoTest™ BC

  Multiplexed Fluorescence in situ Hybridization (FISH)



Hibridizasyon Temelli Yöntemler

- Accelerate PhenoTest™ BC

  Antimikrobial duyarlılık testi;

  zaman-atlamalı mikroskop ile üreme/inhibisyonun tespiti.

Hibridizasyon Temelli Yöntemler

- Accelerate PhenoTest™ BC

  Antimikrobiyal duyarlılık testi;

  zaman-atlamalı mikroskop ile üreme/inhibisyonun tespiti.

Hibridizasyon Temelli Yöntemler

- Accelerate PhenoTest™ BC

We Cut the Time Clinicians Have to Wait for AST by > 75%

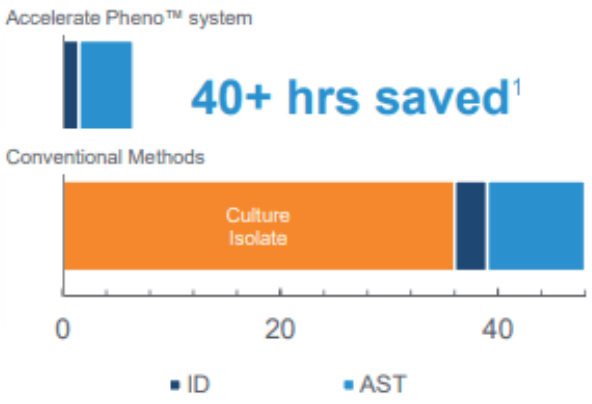
Novel Tech Creates High Barriers to Entry

- Custom Machine Learning Algorithms
- Custom CUDA Computing Platform
- Proprietary Sample Prep Automation
- Patented Optics and Chemistry

AST and pathogen identification *days* faster

ACCELERATE *pheno*™

The only FDA cleared solution for quantitative AST directly from positive blood cultures



Hibridizasyon Temelli Yöntemler

- Accelerate PhenoTest™ BC

Pioneering work to tackle sepsis at hospital trust

HAMPSHIRE Hospitals has become the first trust in the UK to adopt new state-of-the-art technology which helps to identify a life-threatening condition earlier.

New diagnostic equipment means that staff can rapidly identify the cause of sepsis, which affects 260,000 people in the UK every year with the early detection potentially saving lives, helping clinicians to provide targeted and more effective treatment sooner.

Consultant microbiologist and clinical lead for microbiology and infection at Hampshire Hospitals NHS Foundation Trust, Nick Cortes, said: "This is an incredibly exciting time as we are always looking to explore innovative ways to improve patient care through diagnostics.

A member of staff at Basingstoke and North Hampshire Hospital using the new technology

Hibridizasyon Temelli Yöntemler

- Accelerate PhenoTest™ BC

Faster Antibiotic Susceptibility Testing Results Improve Patient Care, Quality in the ICU

Perhaps most importantly, the system also helped reduce the rates of sepsis mortality—from 14% in February 2017, to only 4% in September 2017, which is a remarkable decrease.

"The Accelerate Pheno system provided fast, reliable results while significantly improving turnaround time in blood culture diagnostics," concluded Chirca, noting that as the implementation of ASPs in treating infections increases to comply with new Centers for Medicare & Medicaid Services regulations, the importance of quick pathogen identification and susceptibility will increase as well.

Polymerase Chain Reaction (PCR) Keşfi

- 1983 yılında polimeraz zincirleme tepkimesinin keşfedilmesi ile moleküler biyoloji alanındaki gelişmeler ivme kazanmıştır.

The Nobel Prize in Chemistry 1993

The Royal Swedish Academy of Sciences awards this year's Nobel Prize in Chemistry to

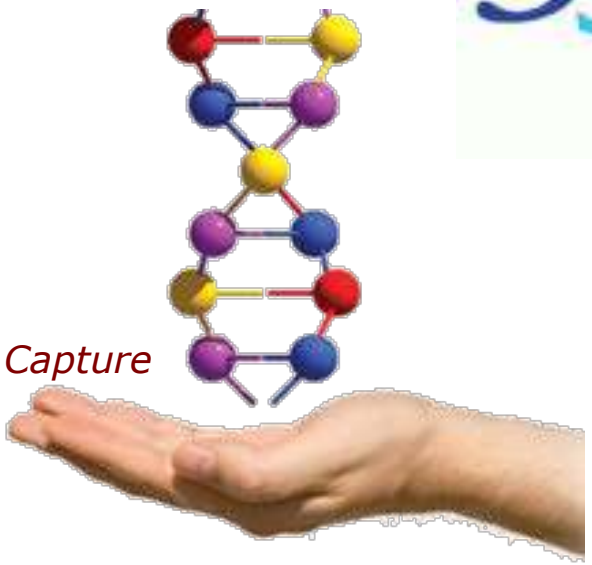
Kary B. Mullis
USA, for his invention of the polymerase chain reaction (PCR) method

tanısal moleküler mikrobiyoloji

Moleküler Tanı Yöntemlerinin Gelişimi

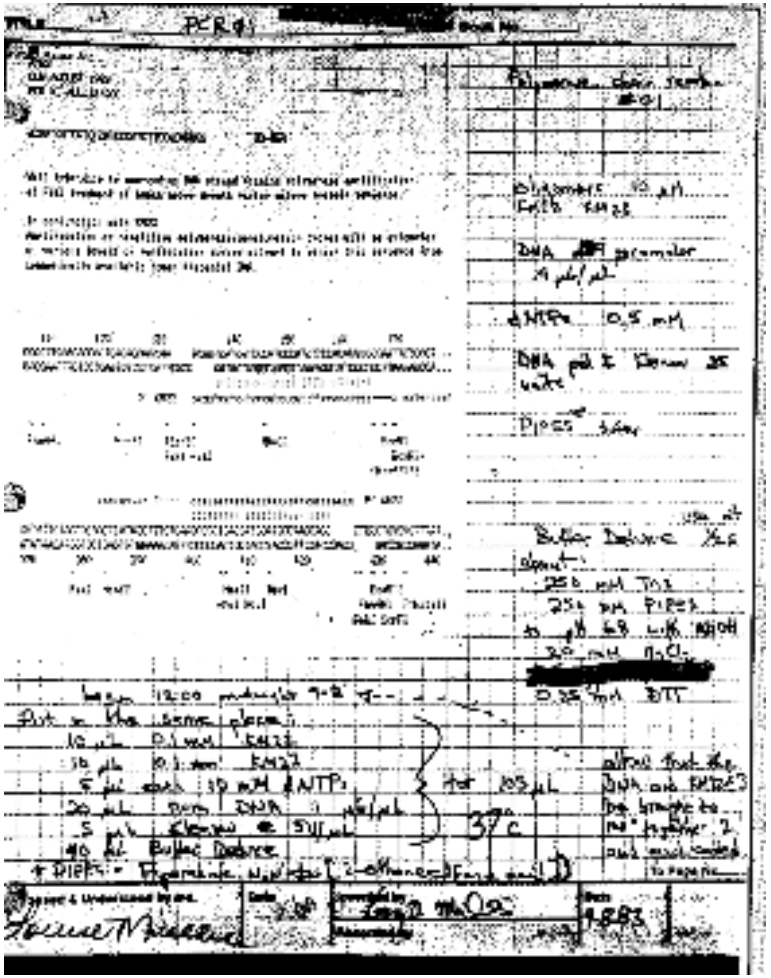
PCR

- TMA
- LAMP
- NASBA
- *bDNA*
- *Hybrid Capture*

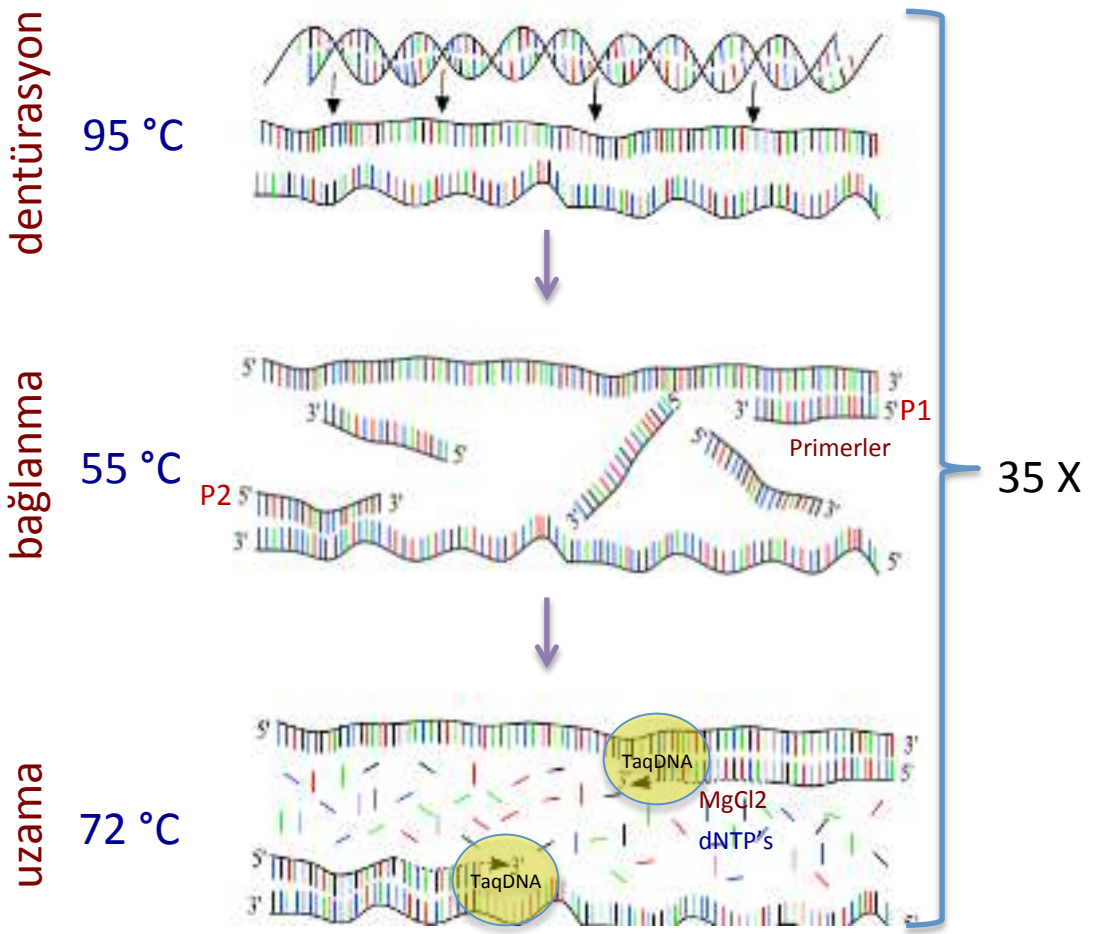


1983, Polymerase Chain Reaction (PCR) Keşfi

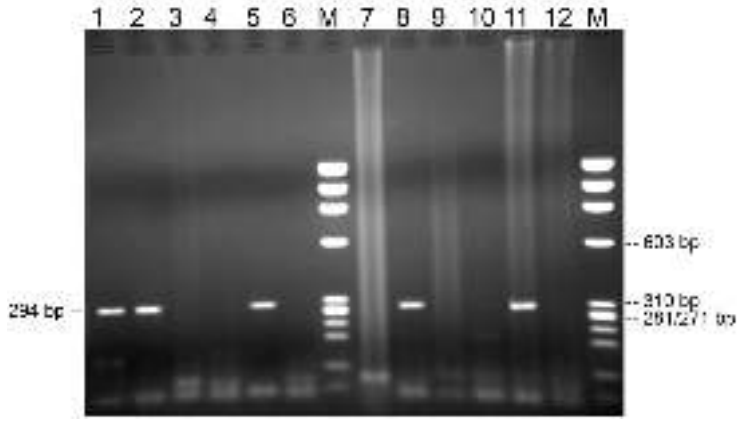
- İlk Polimeraz Zincir Reaksiyonu. Kary B. Mullis'in defterinden 8 Eylül 1983'te mor kapaklı bir tüpte 'PCR01' için bir araya getirilen reaktifleri gösteren sayfa



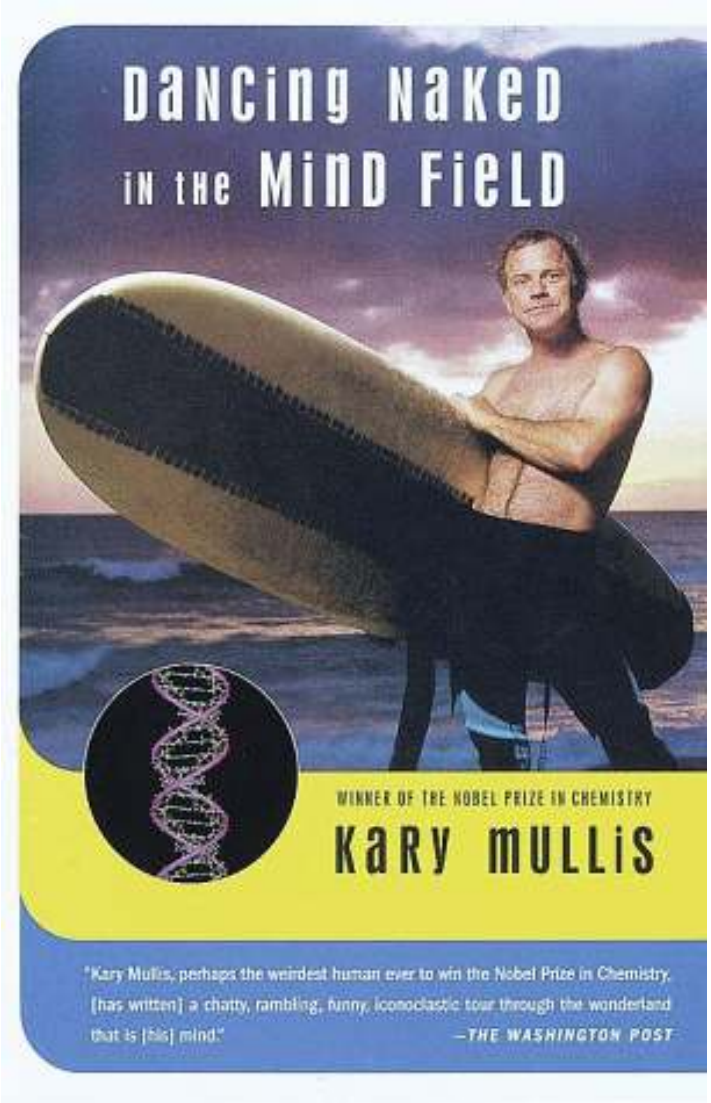
Polymerase Chain Reaction (PCR)



EtBr



Polymerase Chain Reaction (PCR) Keşfi



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 technique called polymerase chain reaction, proviral sequences of the human immunodeficiency virus (HIV-1) were identified directly in DNA isolated from periphe persons seropositive but not in DNA isola the virus. Primer pairs from multiple re achieve maximum sensitivity of provirus d 100% of DNA specimens from seroposit was isolated by coculture, but in none of t seronegative, virus culture–negative perso ed in 64% of DNA specimens from sero men. This method of DNA amplification days, whereas virus isolation takes up to used to complement or replace virus isolat 1 infection.

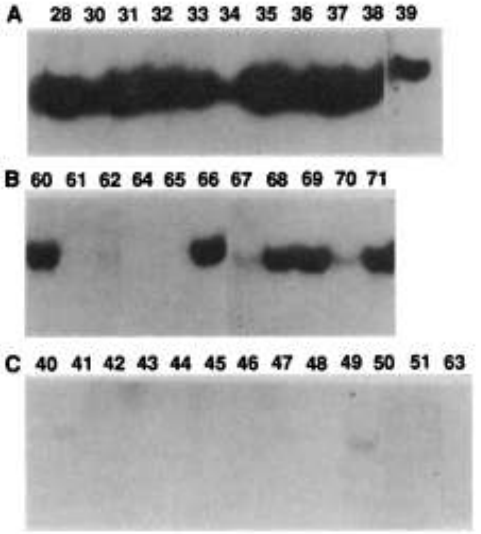


Fig. 1. (**A** to **C**) Representative DNA amplification analysis of peripheral blood lymphocyte DNA from HIV-1–seropositive and seronegative persons (see Table 1). DNA samples were amplified for 35 rounds with the primer pair SK68/69 (Table 2) representing a conserved gp41 region, restricted with BstN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

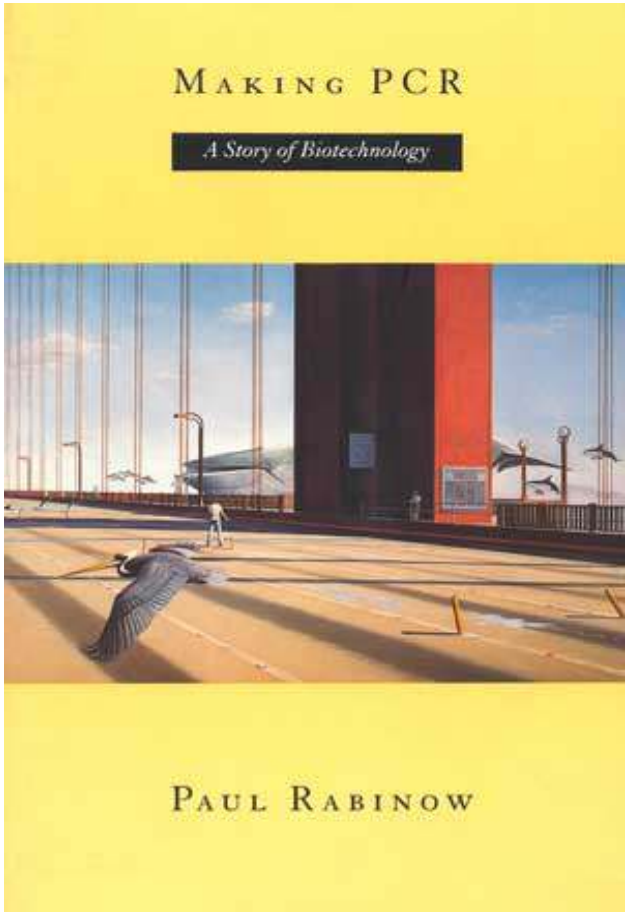
Isı döngü cihazlarındaki gelişmeler

- Isı döngü cihazlarındaki gelişmeler

40 cycle PCR in 20 minutes

Amplifikasyon Temelli Yöntemler

- *Polymerase Chain Reaction* (PCR)

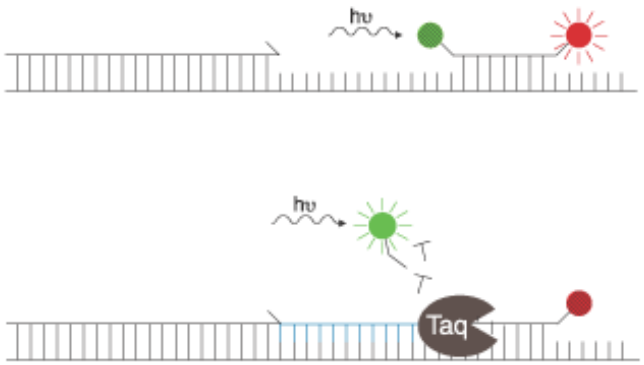


- *PCR*
- *Multiplex PCR*
- *Nested PCR*
- *Seminetested PCR*
- *Broad Range PCR*
- *Hot Start PCR*
- *Touchdown PCR*
- *Reverse Transcription PCR*

Real-time PCR'ın Keşfi-1992

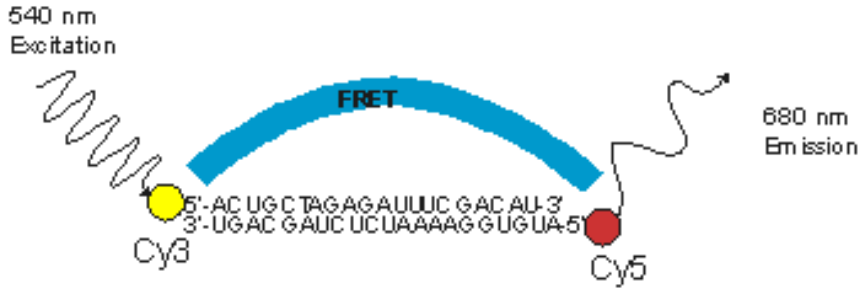
- Real-time PCR yönteminde kullanılan problar

TaqMan Prob



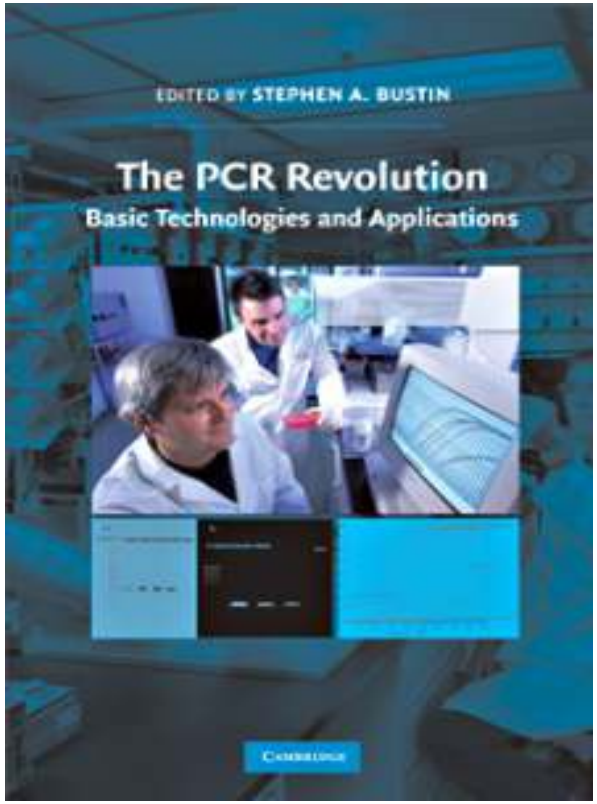
Molecular Beacons

FRET
(Floresans rezonans enerji transfer)



Scorpions Prob

Real-time PCR



Foreword

Russell Higuchi

Advances in science and technol
state of the art can be obsolete i
earns a Nobel prize today will b
underestimate the rate of change.
and technologists can be made t
career. Faced with this, what do s
In the Stephen Sondheim play
neering artist, George Seurat, the
"passing on" to posterity are "cl
the children part but I wondere
this context. As evidenced by u
and Sciences" – a holdover I bel
and art did not used to be so far apart. I believe that beautiful, inventive think-
ing can be art, and good science
as PCR.
When I first heard of PCR, I t
heard from Kary Mullis his idea
Kary proposed the Hot Start (a
aficionado) – art.
Nonetheless, I did find myself t
better will come along." More th
proven to be the case, as PCR has
this book, is still increasingly usef
was the first to put into practice,
However, something better will
parallel sequencing of clonally am
of a human genome a week. We a
human genome a day. If ways ca
cost effectively among large num
guess at what sequences might b
that is there and at what frequenc
So the question: If its use is s
in general, "usefulness" is not a

"Bugün Nobel Ödülü kazanan bir çalışma, bundan on yıl sonra bir bitirme tezi olacak"

İnanıyorum ki;
güzel ve yaratıcı bir düşünce sanat olabilir ve PCR gibi iyi bilim güzelliklerle, yaratıcı düşünce ile doludur. …PCR'ı ilk duyduğumda sanat olduğunu düşündüm. ..Kary Mullis'ten termostabil bir enzimi kullanma fikrini duyduğumda bunun sanat olduğunu düşündüm.

Amplifikasyon Temelli Yöntemler

- Yeni yaklaşımlar
  - *İzolasyondan itibaren otomatize sistemler*

Moleküler Tanı Yöntemlerinin Gelişimi

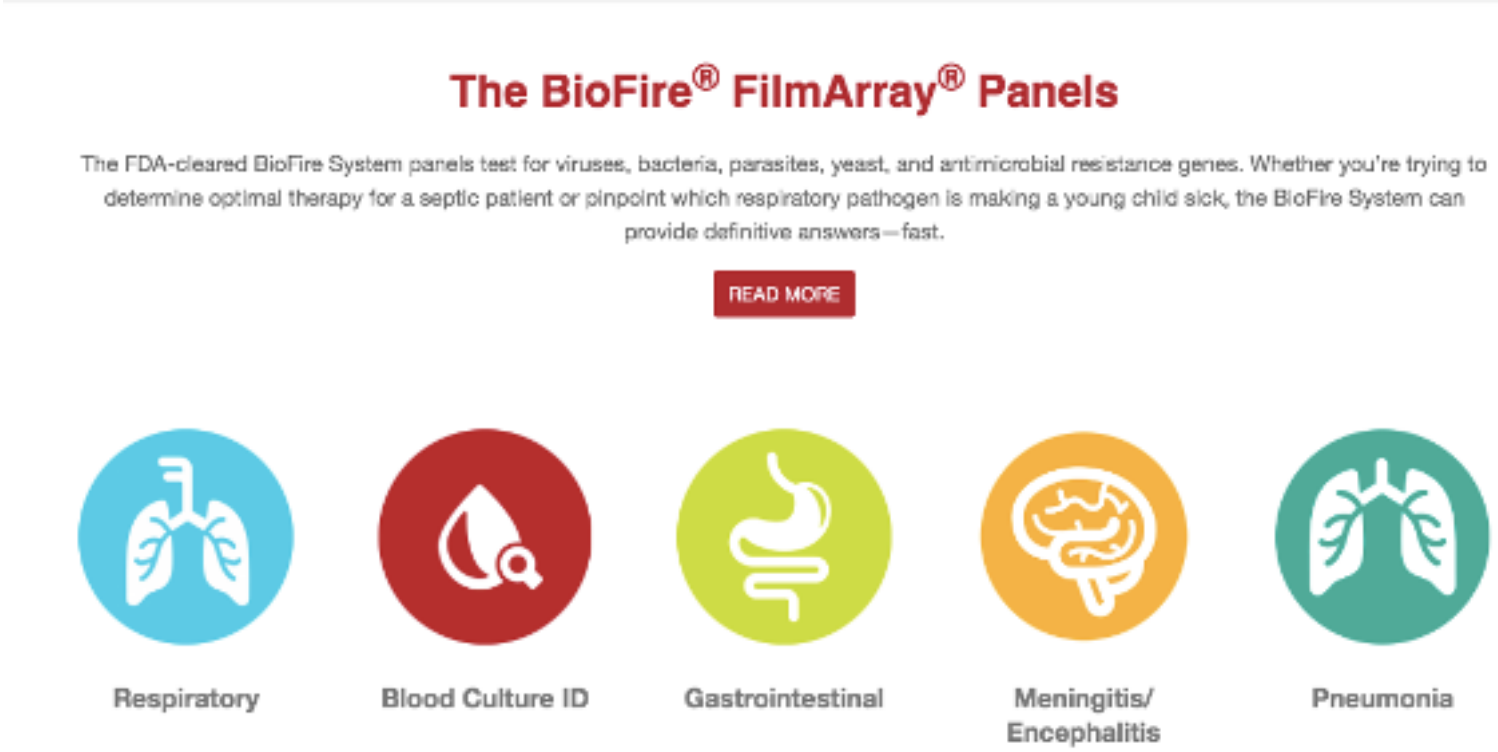
- Sendromik Testler

  Yüksek multipleks kapasiteye sahip sistemlerin üretilmesiyle

  onlarca mikroorganizmayı aynı anda taramak mümkün hale geldi.

Moleküler Tanı Yöntemlerinin Gelişimi

- Sendromik Testler



Moleküler Tanı Yöntemlerinin Gelişimi

1 Test. 27 Targets. All in about an hour.

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

Moleküler Tanı Yöntemlerinin Gelişimi

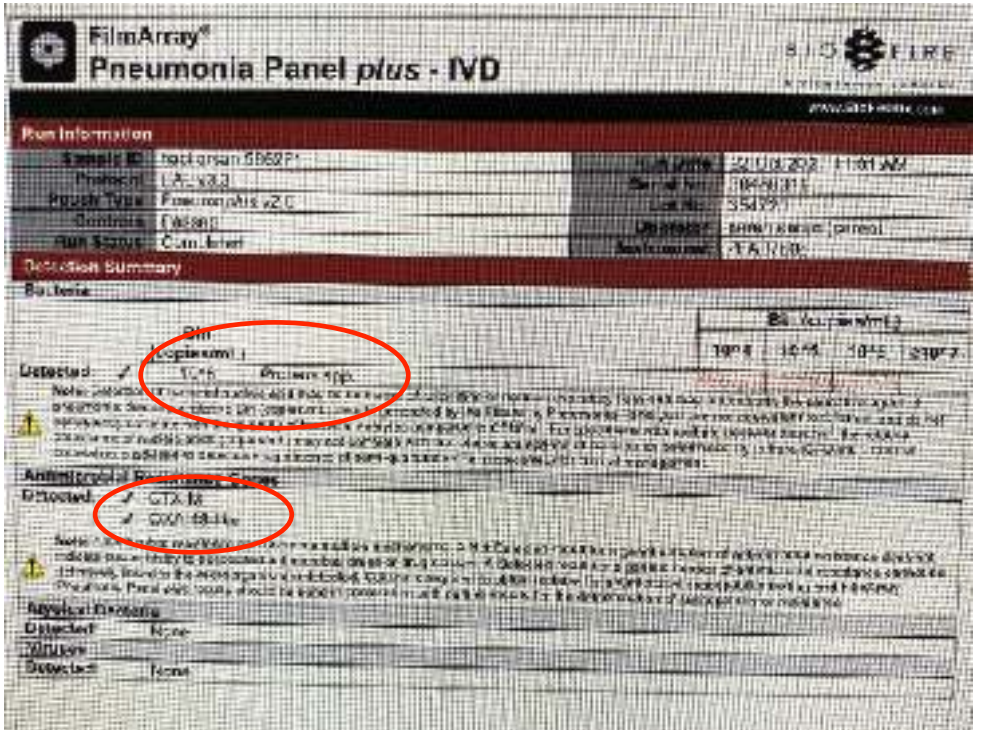
Rapid Detection of Bloodstream Pathogens in Liver Transplantation Patients With FilmArray Multiplex Polymerase Chain Reaction Assays: Comparison With Conventional Methods

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

Moleküler Tanı Yöntemlerinin Gelişimi

- Sendromik Testler



Amplifikasyon Temelli Yöntemler

- *GeneXpert Sistemi*

  Moleküler tanı alanında yeni bir yaklaşım; nükleik asit izolasyonundan itibaren otomatize sistemler.

« Previous Release | Next Release »

Cepheid

Jul 28, 2015

World's Most Portable Molecular Diagnostics System Unveiled at AACC

GeneXpert Omni to Further Decentralize Critical TB, Virology and Ebola Tests

SUNNYVALE, Calif. and GENEVA, July 28, 2015 /PRNewswire/ -- Cepheid (Nasdaq: CPHD) and FIND today unveiled the GeneXpert® Omni, the world's most portable molecular diagnostics system enabling unprecedented access to accurate, fast and potentially life-saving diagnosis for patients suspected of TB, HIV and Ebola in even the most remote areas of the world.

Point-of-Care PCR 2.0

Posted on **October 20, 2015**

- **Ubiquitome Quickens Pace of POC Apps for Its Freedom4**
- **Cepheid Unveils its POC Diagnostics System**
- **Hopkins Crew Brews "Coffee Mug-Sized" Gizmo for Fully Automated Chlamydia Testing**

Amplifikasyon Temelli Yöntemler

- Yeni yaklaşımlar

Elektrokimyasal Real-Time PCR

analytical chemistry

ARTICLE

pubs.acs.org/ac

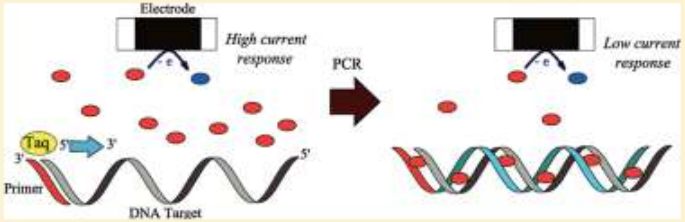
Real-Time Electrochemical PCR with a DNA Intercalating Redox Probe

Thibaut Defever, Michel Druet, David Evrard, Damien Marchal,* and Benoit Limoges*

Laboratoire d'Electrochimie Moléculaire, UMR CNRS 7591, Université Paris Diderot, 15, rue Jean-Antoine de Baïf, 75205 Paris Cedex 13, France

Supporting Information

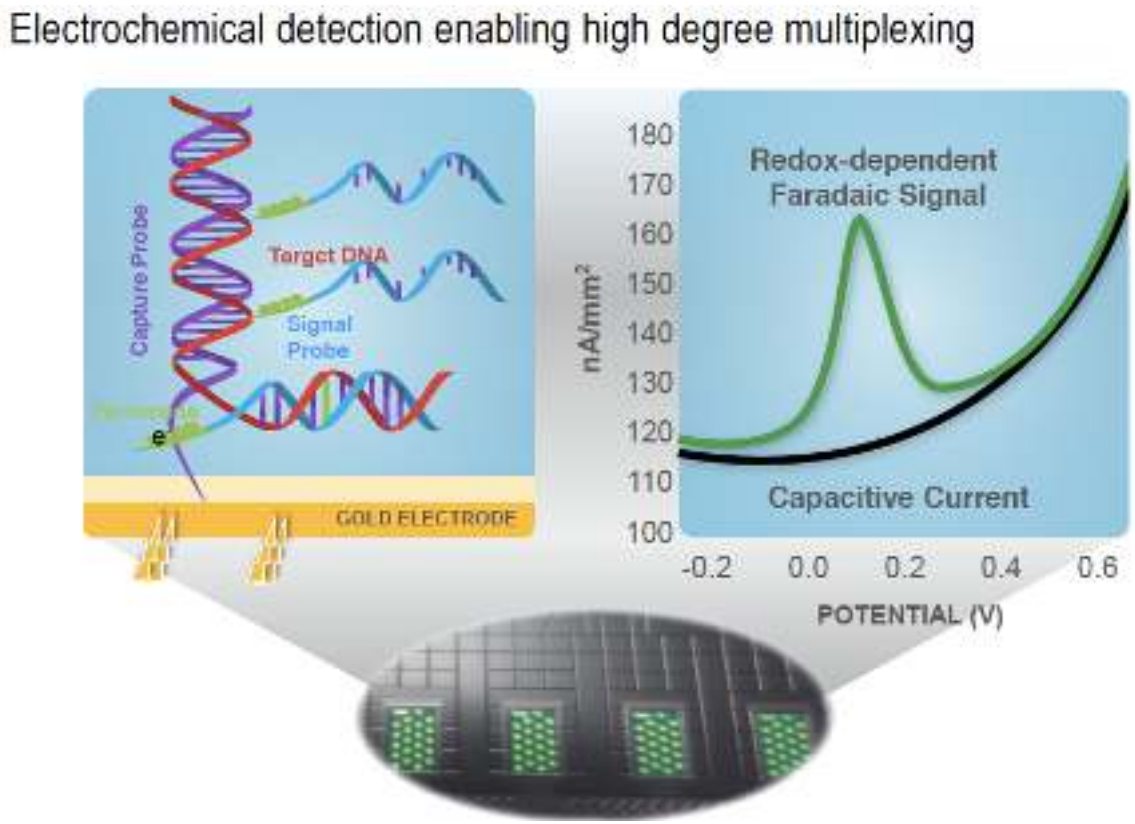
ABSTRACT: The proof-of-principle of a nonoptical real-time PCR method based on the electrochemical monitoring of a DNA intercalating redox probe that becomes considerably less easily electrochemically detectable once intercalated to the amplified double-stranded DNA is demonstrated. This has been made possible thanks to the finding of a redox intercalator that (i) strongly and specifically binds to the amplified double-stranded DNA, (ii) does not significantly inhibit PCR, (iii) is chemically stable under PCR cycling, and (iv) is sensitively detected by square wave voltammetry during PCR cycling. Among the different DNA intercalating redox probes that we have investigated, namely, methylene blue, $Os[(bpy)_2phen]^{2+}$, $Os[(bpy)_2DPPZ]^{2+}$, $Os[(4,4'\text{-dimethyl-bpy})_2DPPZ]^{2+}$ and $Os[(4,4'\text{-diamino-bpy})_2DPPZ]^{2+}$ (with bpy = 2,2′-bipyridine, phen = phenanthroline, and DPPZ = dipyrido[3,2-a:2′,3′-c]phenazine), the one and only compound with which it has been possible to demonstrate the proof-of-concept is the $Os[(bpy)_2DPPZ]^{2+}$. In terms of analytical performances, the methodology described here compares well with optical-based real-time PCRs, offering finally the same advantages than the popular and routinely used SYBR Green-based real-time fluorescent PCR, but with the additional incomes of being potentially much cheaper and easier to integrate in a hand-held miniaturized device.



Defever T et all. Real-Time Electrochemical PCR with a DNA Intercalating Redox Probe. Anal Chem. 2011 Jan 31.

Amplifikasyon Temelli Yöntemler

- Yeni yaklaşımlar
  - *Elektrokimyasal Real-Time PCR*



Amplifikasyon Temelli Yöntemler

Roche

Media Investors

About Roche Solutions Innovation | Stories

Media Releases

Roche signs definitive merger agreement with GenMark Diagnostics, Inc., to access novel technology to test for broad range of pathogens with one patient sample

Amplifikasyon Temelli Yöntemler

We are Biomeme:
A smartphone-based DNA detection platform.
No lab necessary.

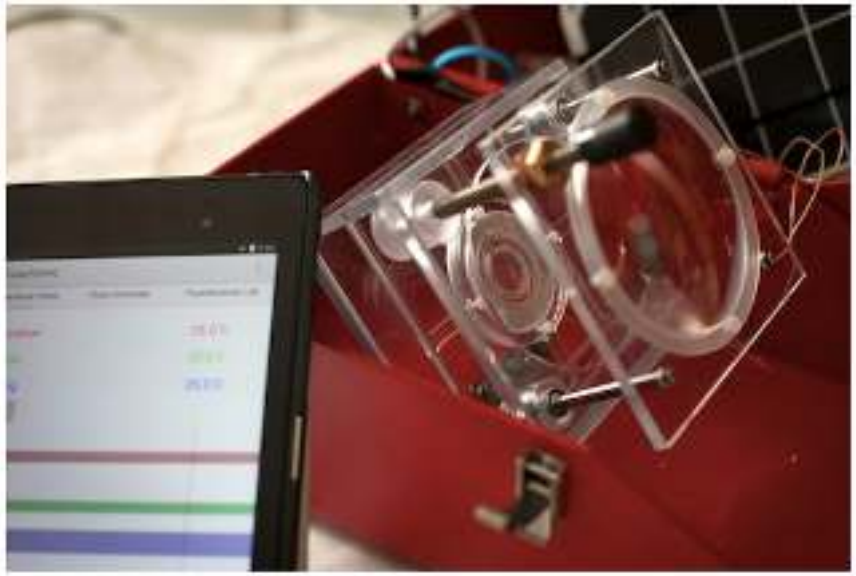
Amplifikasyon Temelli Yöntemler

Biomeme Sample Prep for Children

Amplifikasyon Temelli Yöntemler

Amplifikasyon Temelli Yöntemler

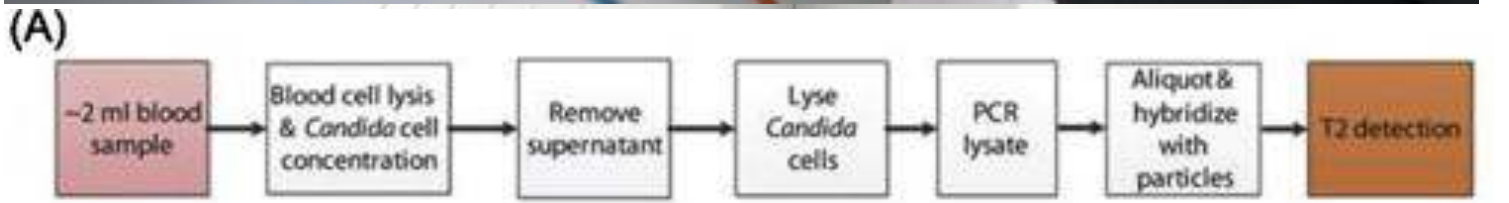
Sun And Smartphone Power

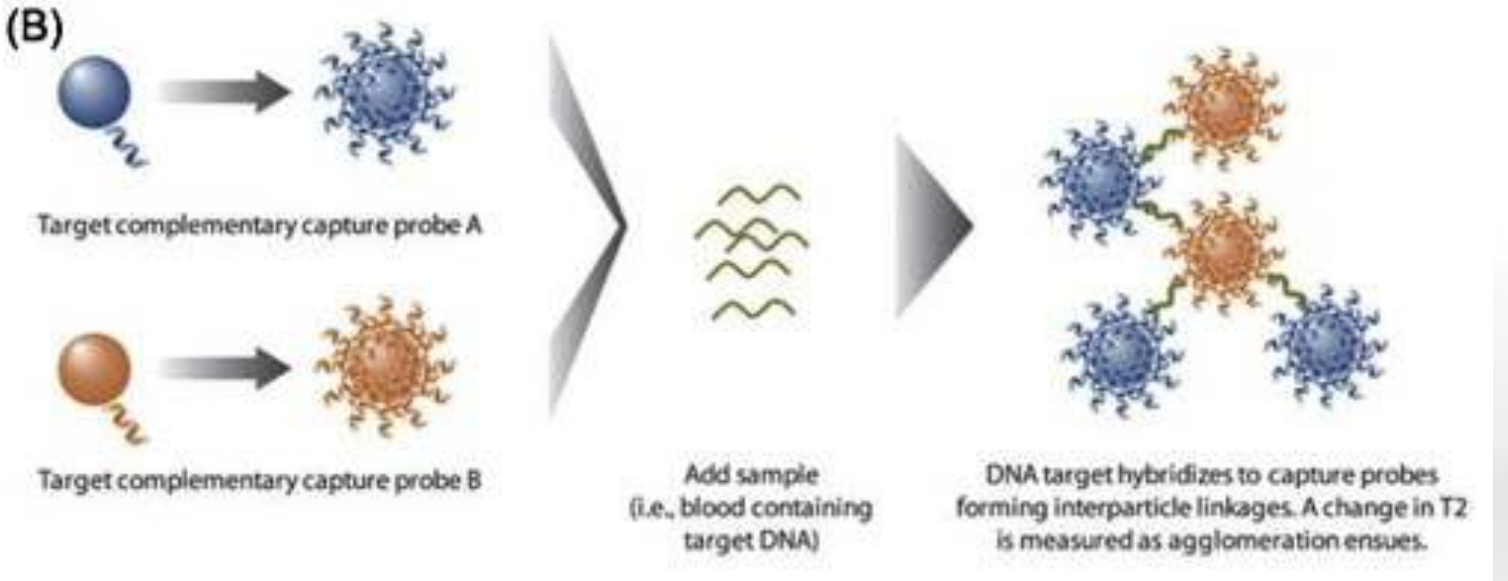


Other authors of the study, "Solar Thermal Polymerase Chain Reaction for Smartphone-Assisted Molecular Diagnostics," include Matthew Mancuso, a doctoral candidate in Cornell's Department of Biomedical Engineering; Zhengda Lu, M.Eng. '13; and Gunkut Akar, a researcher in pathology and laboratory medicine at Weill Cornell Medical College.

Amplifikasyon Temelli Yöntemler

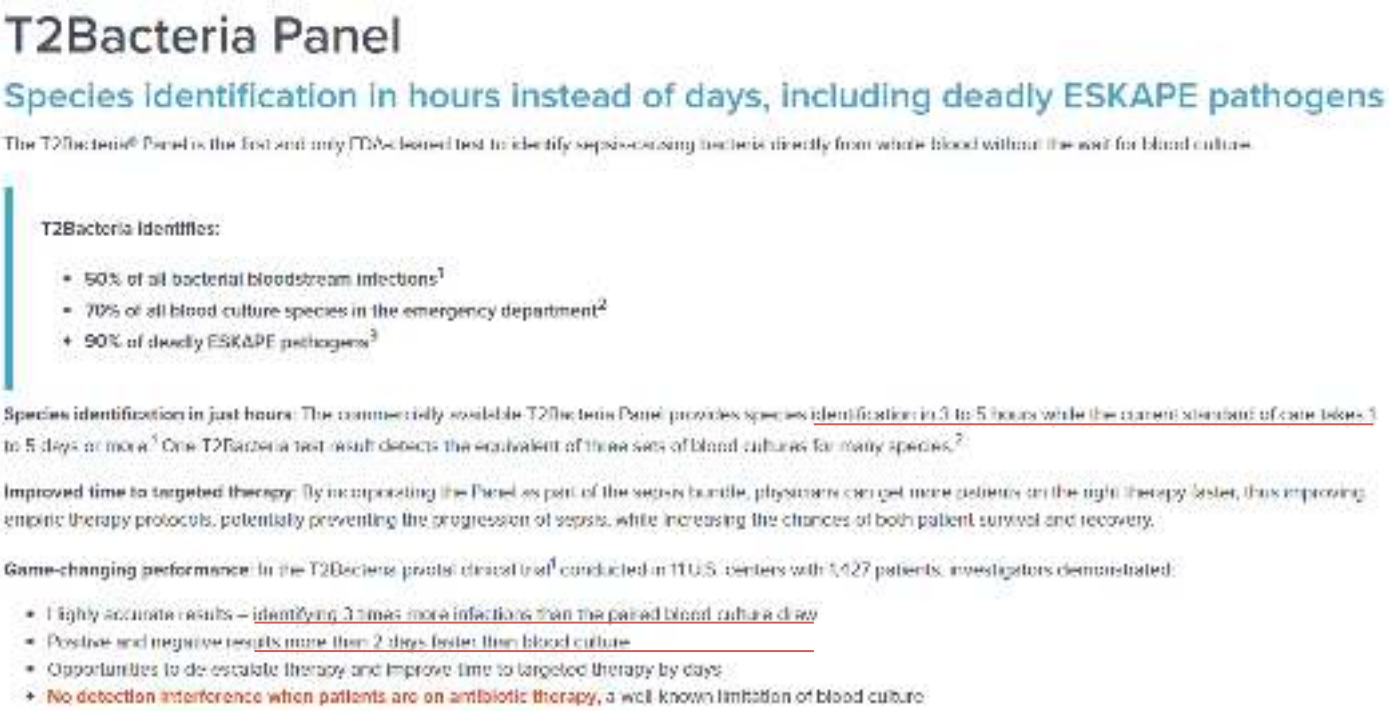
- *T2 Biosystem - magnetic resonance*





Amplifikasyon Temelli Yöntemler

- 2019'da FDA , T2Resistance Paneli için panelin dirençli enfeksiyonları hızla belirleme amacını yansıtan «çığır açan cihaz» tanımı yaptı.
- Kan kültür sistemlerinde 3 kat daha duyarlı ve 2 gün daha hızlı



T2Bacteria Panel

Species identification in hours instead of days, including deadly ESKAPE pathogens

The T2Bacteria® Panel is the first and only FDA-cleared test to identify sepsis-causing bacteria directly from whole blood without the wait for blood culture

T2Bacteria identifies:

- 50% of all bacterial bloodstream infections[1]
- 70% of all blood culture species in the emergency department[2]
- 90% of deadly ESKAPE pathogens[3]

Species identification in just hours: The commercially available T2Bacteria Panel provides species identification in 3 to 5 hours while the current standard of care takes 1 to 5 days or more.[1] One T2Bacteria test result detects the equivalent of three sets of blood cultures for many species.[2]

Improved time to targeted therapy: By incorporating the Panel as part of the sepsis bundle, physicians can get more patients on the right therapy faster, thus improving empiric therapy protocols, potentially preventing the progression of sepsis, while increasing the chances of both patient survival and recovery.

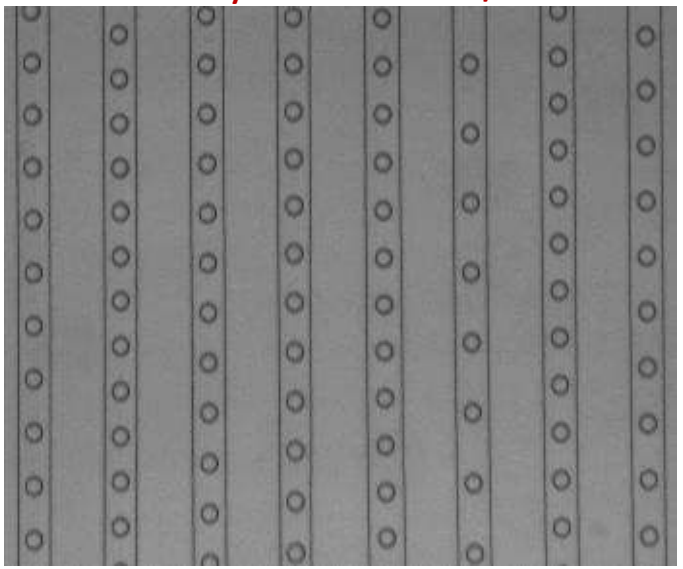
Game-changing performance: In the T2Bacteria pivotal clinical trial[1] conducted in 11 U.S. centers with 1,427 patients, investigators demonstrated:

- Highly accurate results – identifying 3 times more infections than the paired blood culture assay
- Positive and negative results more than 2 days faster than blood culture
- Opportunities to de-escalate therapy and improve time to targeted therapy by days
- No detection interference when patients are on antibiotic therapy, a well known limitation of blood culture

Dijital PCR

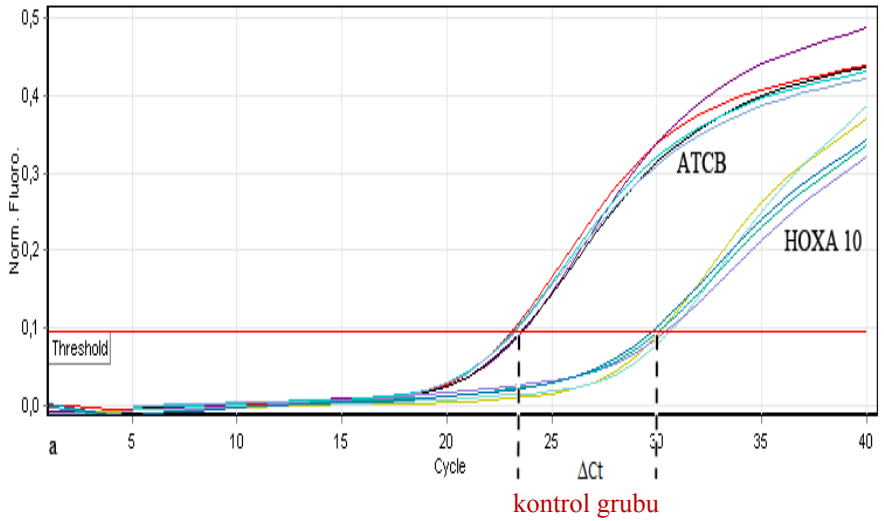
- Real time PCR yetersiz duyarlılık, yetersiz kantitasyon
  - Emülsiyon PCR, Damlacık PCR

- Saniyede 80,000 PCR reaksiyonu aynı anda 8 kanalda
  5 milyon damlacık/dk

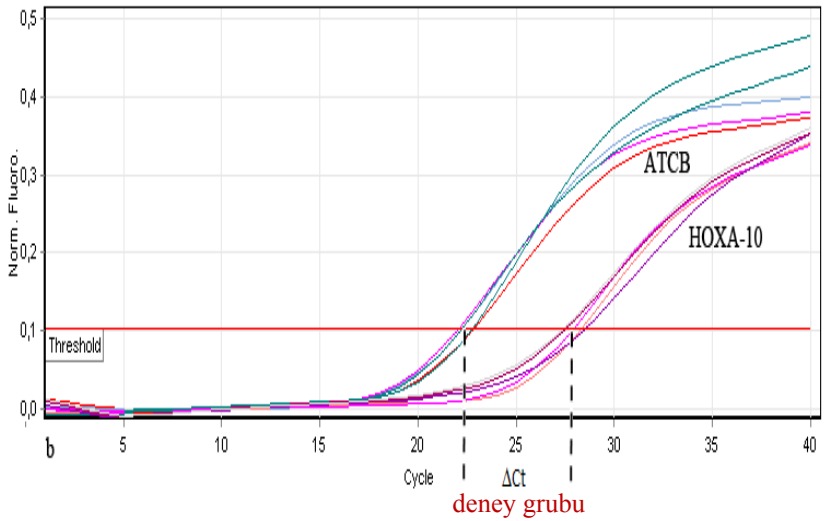


Dijital PCR

- Real time PCR Bağıl Kantitasyon



kontrol grubu



deney grubu

Kontrol Grubu: ΔCt = Ct (hedef gen) - Ct (ATCB)

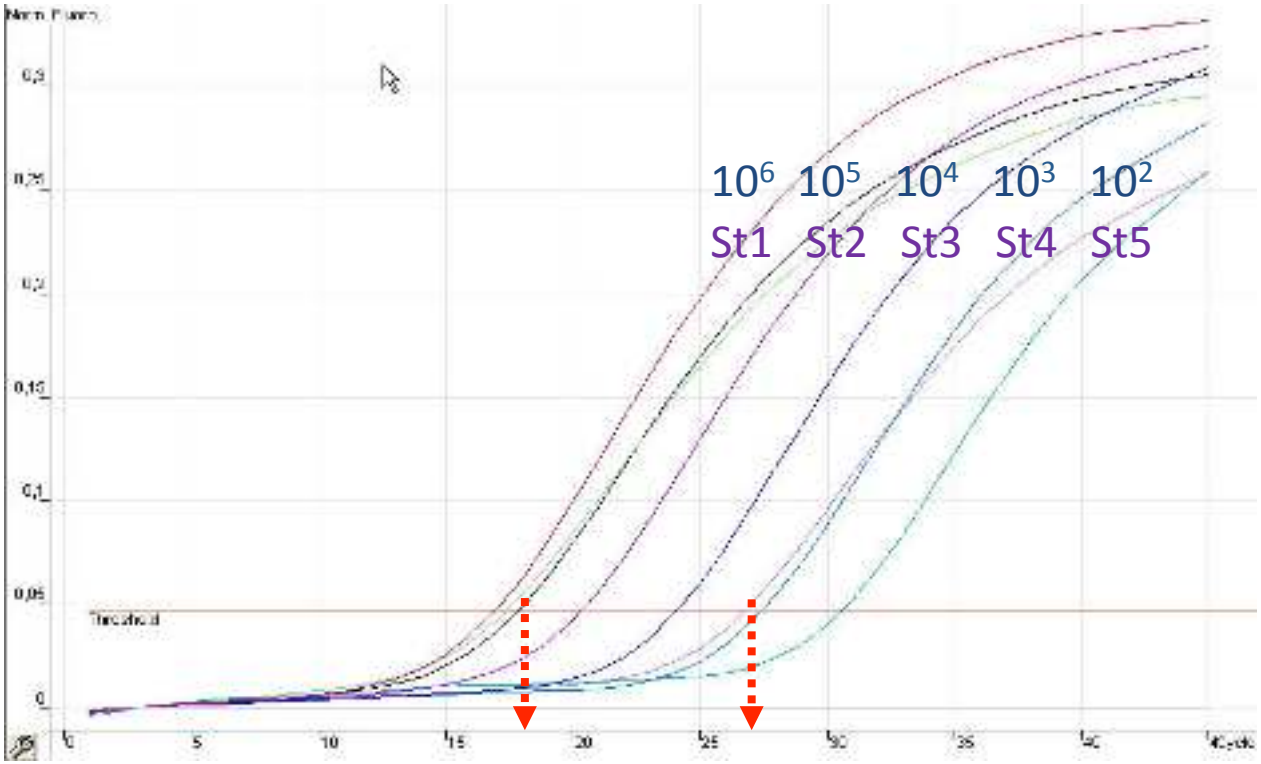
Deney Grubu: ΔCt = Ct (hedef gen) - Ct (ATCB)

$\Delta\Delta Ct = \Delta Ct$ (deney grubu) - ΔCt (kontrol grubu)

$Ratio = 2^{-\Delta\Delta CT}$

Dijital PCR

- Real time PCR Mutlak Kantitasyon



Real-time PCR ile kantitasyon

- HBV de tedavini takibi için mutlak kantitasyon yetersiz

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.423

World J Gastroenterol 2009 January 28; 15(4): 423-430
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

Juan-Ramón Larrubia, PhD, Series Editor

DNA-guided hepatitis B treatment, viral load is essential, but not sufficient

Relationship between Viral Load and Hepatic Histopathology in Patients with Chronic Hepatitis B

Kronik Hepatit B Tanılı Hastalarda Viral Yük ile Karaciğer Histopatolojisi İlişkisi

Damla Akdağ[1], Tansu Yamazhan[1], Hüsnü Pullukçu[1], Meltem Işıkgöz Taşbakan[1], Raika Durusoy[2]

[1]Ege University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, İzmir, Turkey
[2]Ege University Faculty of Medicine, Department of Public Health, İzmir, Turkey

ABSTRACT

Objectives: It is not always possible to determine a clear relationship between the DNA level of hepatitis B virus (HBV) and histology. In this study, we aimed to determine the relationship between HBV-DNA level and liver histopathology in patients with chronic hepatitis.

Materials and Methods: Between 2008 and 2016, 361 patients diagnosed with chronic HBV infection were retrospectively examined with age, sex, hepatitis B e antigen status, alanine aminotransferase (ALT) and HBV-DNA levels and liver biopsy scores according to modified Ishak criteria. Patients were divided into five groups (10^3, 10^3-10^4, 10^4-10^5, 10^5-10^6, ≥10^6) based on their HBV-DNA level (IU/mL) and upon histopathological evaluation, hepatic injury was divided into two groups - mild and moderate/severe- according to Ishak score (grade 1-6: mild, 7-18: moderate/severe and stage 0-2: mild, 3-6: moderate/severe) to investigate the statistical relationship between HBV-

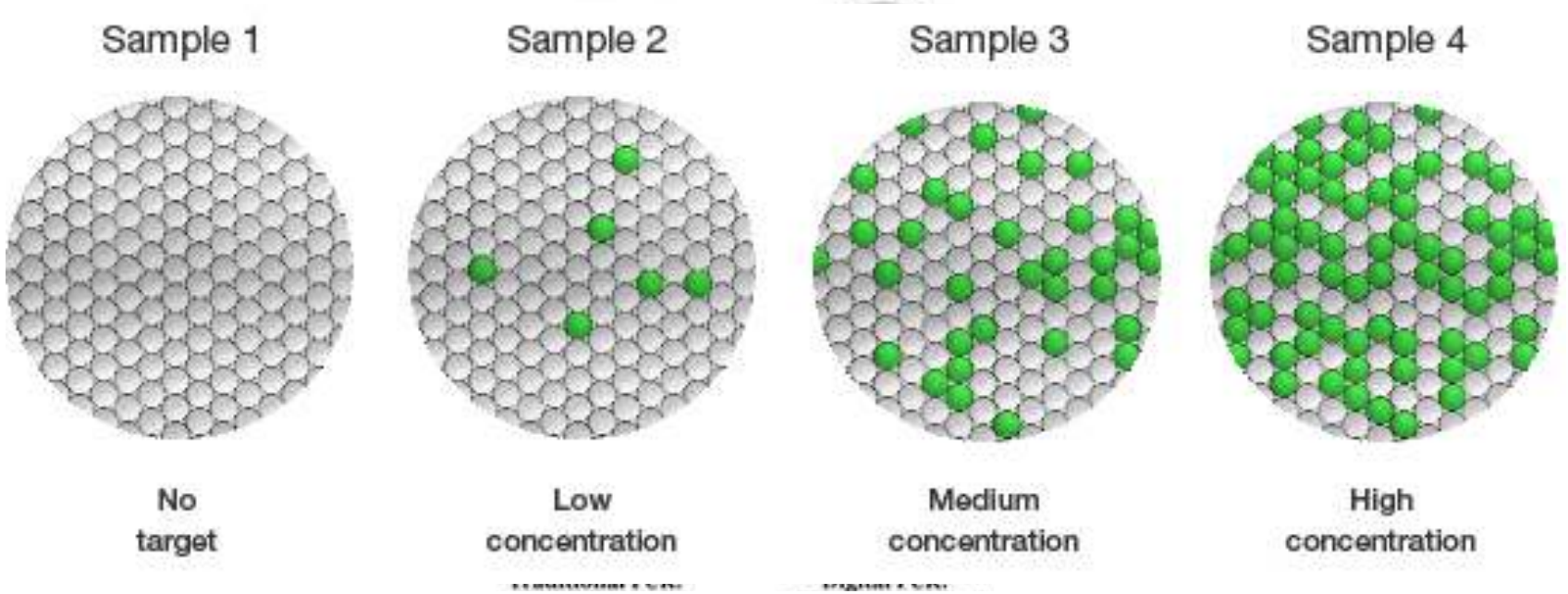
ÖZ

Amaç: Hepatit B virüs (HBV)-DNA düzeyi ile histoloji arasında her zaman net bir ilişki saptamak mümkün olmamaktadır. Bu çalışmada kronik hepatit tanılı hastalarda HBV-DNA düzeyi ile karaciğer histopatolojisi arasındaki ilişkinin ortaya konması amaçlanmıştır.

Gereç ve Yöntemler: 2008-2016 yılları arasında kronik hepatit B tanılı 361 hastanın; yaş, cinsiyet, hepatit B e antijeni durumu, alanin aminotransferaz (ALT) ve HBV-DNA düzeyleri ile modifiye Ishak kriterlerine göre karaciğer biyopsi skorları retrospektif olarak incelenmiştir. HBV-DNA düzeyi ile grade/stage skorları arasındaki istatistiksel ilişkinin araştırılması açısından hastalar, HBV-DNA düzeyine göre 5 grubu (<10^3, 10^3-10^4, 10^4-10^5, 10^5-10^6, ≥10^6/L, histopatolojik değerlendirmede ise grade: 1-6 hafif, 7-18 orta/yüksek; stage 1-2 hafif, 3-6 orta/yüksek olmak üzere olmak üzere 2'şerli gruplara ayrılmıştır. Analizlerde çapraz tablo ve

Dijital PCR

- Emülsiyon PCR, Damlacık PCR
  - Direkt Kantitasyon



Dijital PCR

scientific reports

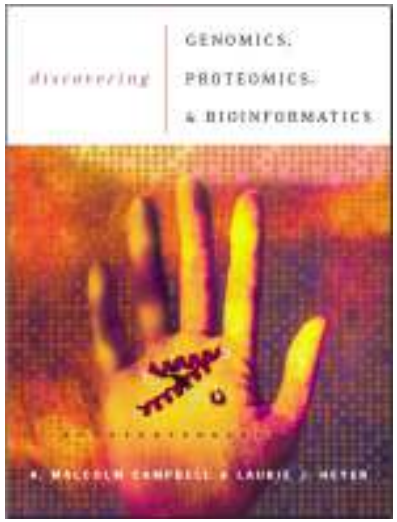
OPEN

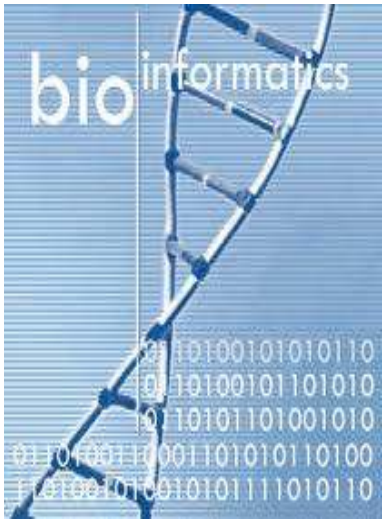
Droplet digital PCR assay provides intrahepatic HBV cccDNA quantification tool for clinical application

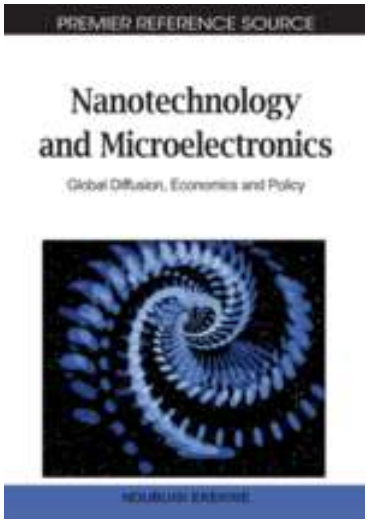
Sanae Hayashi[1,7], Masanori Isogawa[1], Keigo Kawashima[1], Kyoko Ito[1], Natthaya Chuaypen[2], Yuji Morine[3], Mitsuo Shimada[3], Nobuyo Higashi-Kuwata[4], Takehisa Watanabe[7], Pisit Tangkijvanich[2], Hiroaki Mitsuya[4,5,6] & Yasuhito Tanaka[1,7]✉

The persistence of covalently closed circular DNA (cccDNA) poses a major obstacle to curing chronic hepatitis B (CHB). Here, we used droplet digital PCR (ddPCR) for cccDNA quantitation. The cccDNA-specific ddPCR showed high accuracy with the dynamic range of cccDNA detection from 10^1 to 10^5 copies/assay. The ddPCR had higher sensitivity, specificity and precisely than qPCR. The results of ddPCR correlated closely with serum HB core-related antigen and HB surface antigen (HBsAg) in 24 HBV-infected human-liver-chimeric mice (PXB-mice). We demonstrated that in 2 PXB-mice after entecavir treatment, the total cccDNA content did not change during liver repopulation, although the cccDNA content per hepatocyte was reduced after the treatment. In the 6 patients with HBV-related hepatocellular carcinoma, ddPCR detected cccDNA in both tumor and non-tumor tissues. In 13 HBeAg-negative CHB patients with pegylated interferon alpha-2a, cccDNA contents from paired biopsies were more significantly reduced in virological response (VR) than in non-VR at week 48 ($p = 0.0051$). Interestingly, cccDNA levels were the lowest in VR with HBsAg clearance but remained detectable after the treatment. Collectively, ddPCR revealed that cccDNA content is stable during hepatocyte proliferation and persists at quantifiable levels, even after serum HBsAg clearance.

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







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.

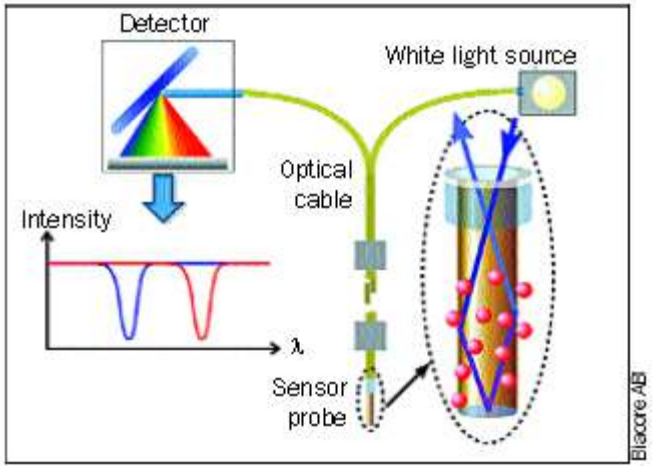
Figure 2. Fiber-optic, fully automated biosensor performs four immunoassays simultaneously in 5 to 10 minutes and shows the results on an LCD screen in words.

novics, 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.

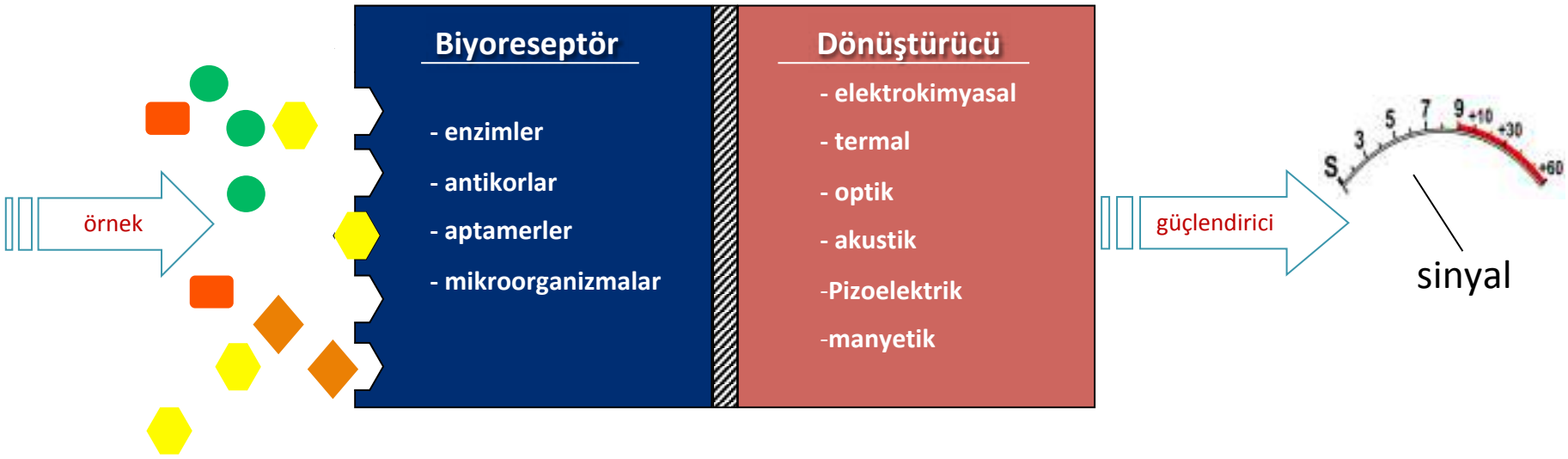
be seeking, says Frances Light of the Naval Research Laboratory (NRL).

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



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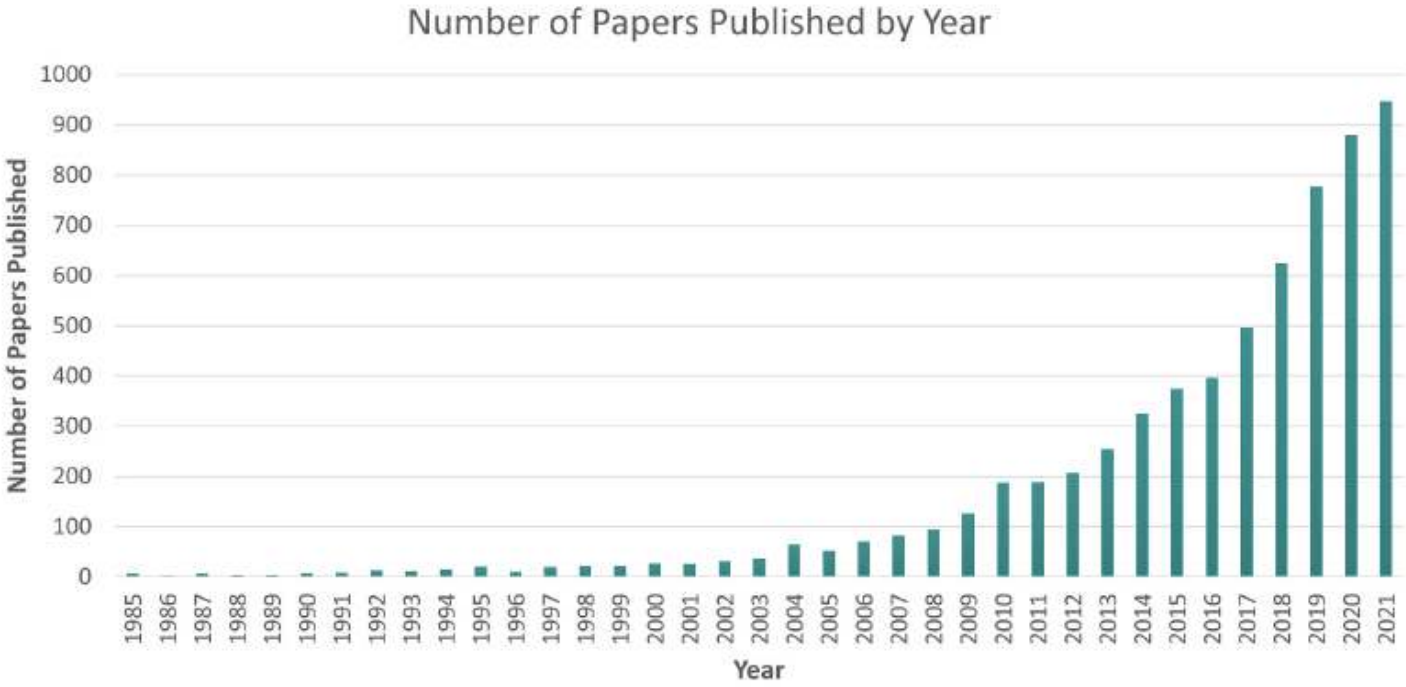

Fig. 1. Annual number of papers published using the following keywords: biosensor + clinical + diagnosis. www.pubmed.ncbi.nlm.nih.gov, accessed on 31st December 2021.

Biyosensör Temeli Yöntemler

Biyosensör Temeli Yöntemler

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BIO X

Solutions for Hospital-Acquired Infections

BIO-X® offers a structured approach to partnering with innovators from academia, clinics and biotech SME's to develop proof of concept, proof of mechanisms or proof of hypothesis for new life science products and services. The BIO-X program offers selected project teams tailor-made process support and financing, up to 1 million SEK per year for up to two years. We are currently looking for
healthcare for projects seeking to
diagnostics and other solutions to
infections.

The recent BIO-X Call for proposals for
fighting hospital acquired infections gene
in academic research, clinics as well as

- **Rapid and sensitive diagnostic bench-top system for detection of hospital-acquired infections**
  A fully automated microfluidic benchtop system for rapid, sensitive and decentralised detection of hospital-acquired infections, based on magnetic bioassays.
- **Antibacterial polymers for prevention of surgical site and wound infections**
  Antibacterial and biocompatible polymers for prevention of surgical site infections and wound infections.
- **Sampling device for simultaneous transportation and enrichment of multidrug-resistant bacteria samples**
  A sampling device for simultaneous transportation and broth enrichment of multidrug-resistant bacteria to increase sensitivity and shorten the screening process.
- **Cranioplasty implant for large skull defects limiting hospital-acquired infections**
  A bio-ceramic implant for use in cranioplasty of large skull defects limiting bacterial infections related to replacement of the skull bone.

- **Biosensors for early diagnostics of hospital-acquired infections**
  An optical biosensor platform technology for early diagnosis of infectious agents on a multitude of surfaces, *eg* on medical devices, in patients' wounds, etc.

Antimicrobial surface technology for covalently coating medical grade materials for reducing hospital acquired infections; in this instance, single use silicon based devices used in ventilatory support.

- **Antifungal coating for medical devices**
  Antifungal coating technology to prevent re-growth of fungal hyphae and prevent biofilm formation on medical devices.
- **Ultrafast lab-on-a-chip system for microbial detection**
  An antibody-based ultrafast, sensitive, lab-on-a-chip system for microbial

Biyosensör Temeli Yöntemler

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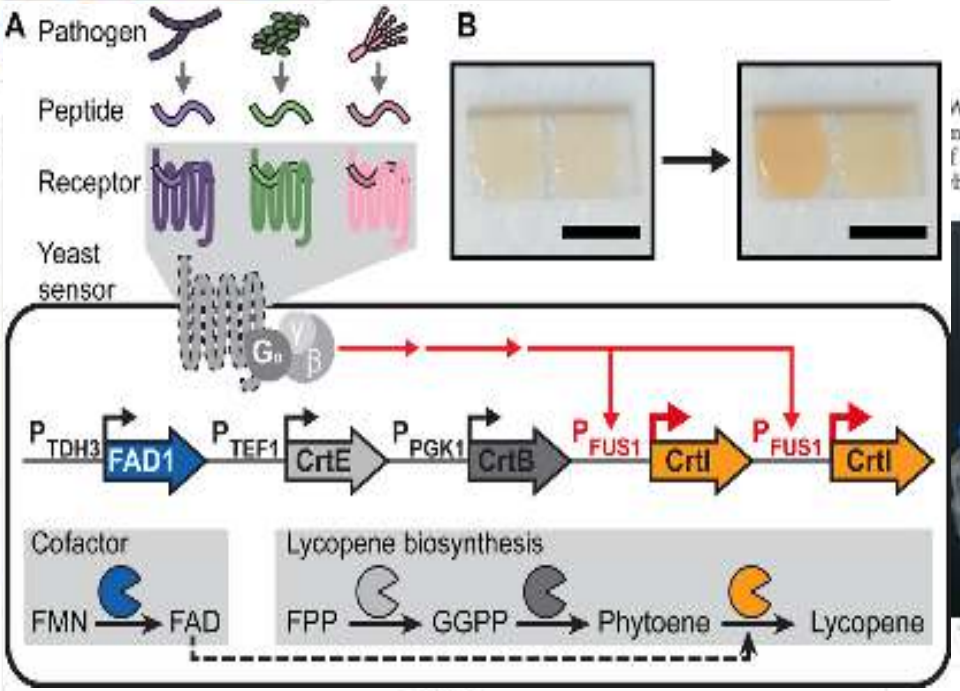
Yeast's Newest Trick: Detecting Deadly Pathogens

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



While at an early stage of implementation, these biosensors can be nmediately adopted in the clinic to shorten the time required for diagnosis f fungal pathogens from blood cultures," researchers wrote in their study, hich 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)

Biyosensör Temeli Yöntemler

FDA-Tarafından Önerilen Testler

- Sitokinler

Sepsis Sensed on Needle-shaped Substrates in 2.5 Minutes

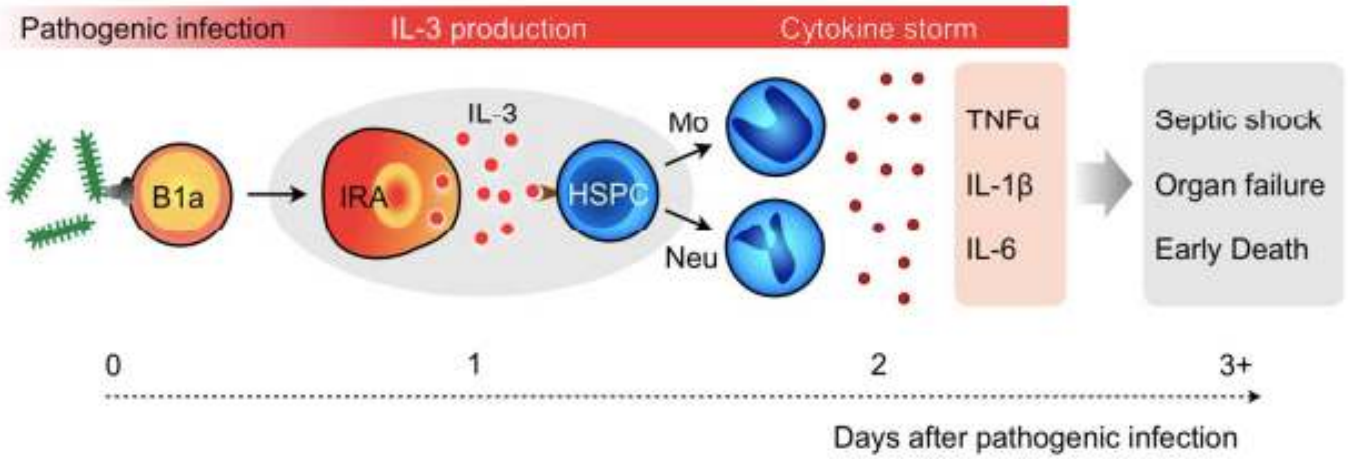
NEWS Feb 19, 2019 | Original story from the University of Strathclyde Glasgow

Image credit: the University of Strathclyde Glasgow

Using a microelectrode, a biosensor device is used to detect if one of the protein biomarkers of sepsis– interleukin-6 – is present in the bloodstream. IL-6 is a molecule secreted by the immune system and the levels of it in the blood increase in many of those who have the condition.

Biyosensör Temeli Yöntemler

- IL-3

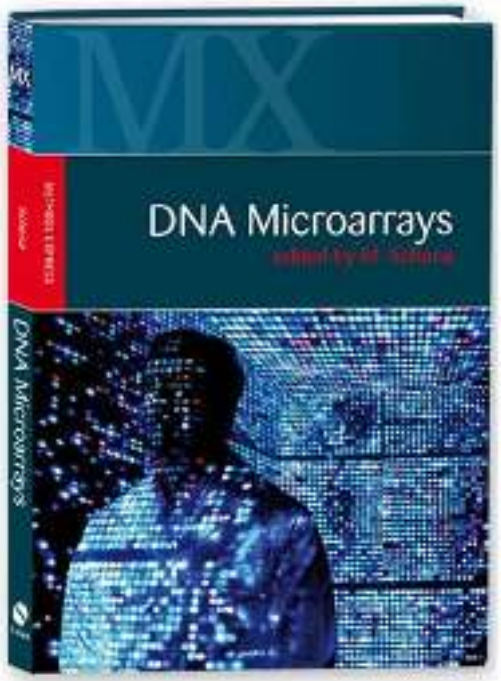


Scheme 1. IL-3 mediated mechanism of sepsis. Peritoneal B1a cells are activated by pathogens and give rise to IL-3+ B cells (IRA, innate response activator). IL-3 acts on hematopoietic stem progenitor cells (HSPC) to promote the emergency generation of inflammatory leukocytes that are released into the circulation. This leads to an uncontrolled cytokine storm, multiple organ failure, and septic shock that may result in death.

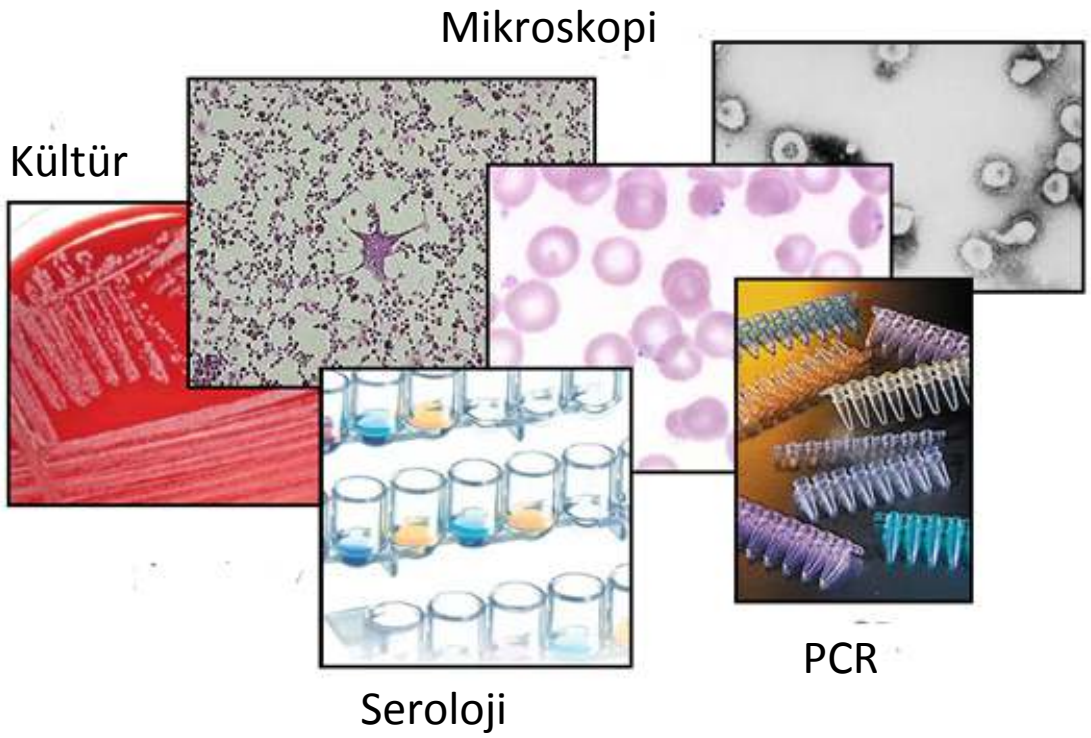
DNA Mikroçip Teknolojileri

- DNA mikroçip teknolojileri, çevresel ve

  klinik örneklerden mikroorganizmaların tanısı için giderek artan oranda kullanılmaktadır.



Geleneksel Mikrobiyolojik Tanı Yöntemleri

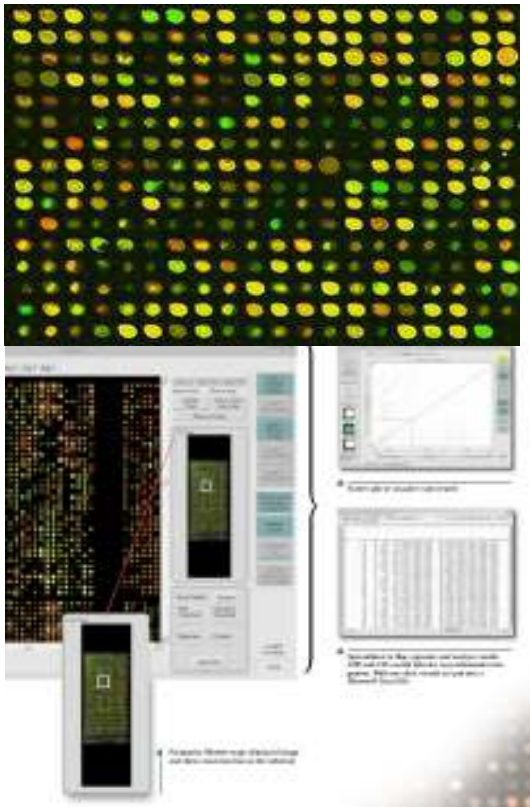


DNA Mikroçip Teknolojileri

- PCR ile elde edilen floresanla işaretli amplikonların

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

  hibridize olması temeline dayanmaktadır.

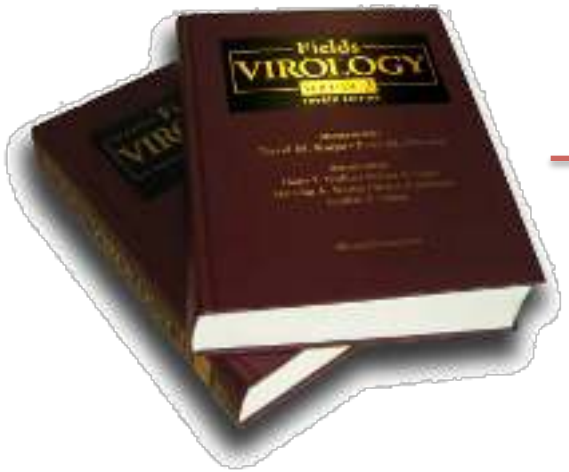


DNA Mikroçip Teknolojileri

- **Virochip** 1500 virüse ait 36.000 prob içermektedir.

Using a Pan-Viral Microarray Assay (Virochip) to Screen Clinical Samples for Viral Pathogens

Eunice C. Chen[1], Steve A. Miller[1], Joseph L. DeRisi[1,2], Charles Y. Chiu[1,2]
[1]Department of Laboratory Medicine, University of California, San Francisco
[2]Division of Infectious Diseases, University of California, San Francisco



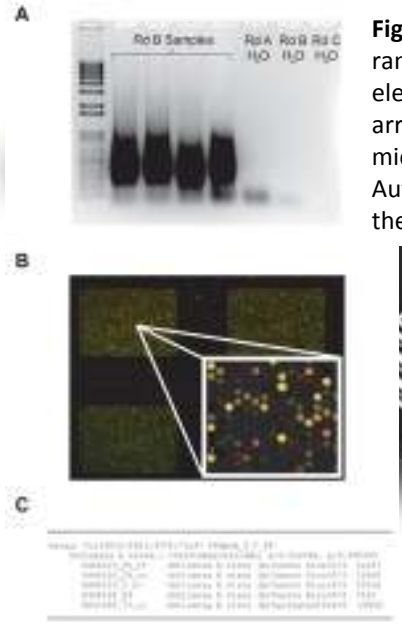


Figure 2. Steps in the Virochip assay. After amplification by random PCR, a smear of 200 - 1000 bp can be visualized by gel electrophoresis **(A)**. **(B)** Three Virochip microarrays out of the 8 arrays / glass slide are shown, with a small region of one microarray blown-up in the inset on the bottom right corner. **(C)** Automated microarray viral analysis using E-Predict revealing the presence of influenza A virus in the clinical sample.

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)

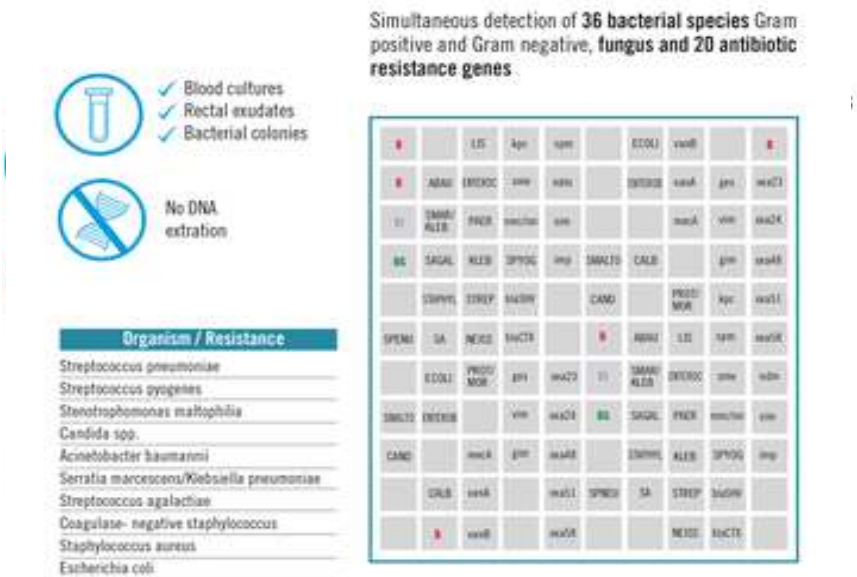
Accurate DNA-based identification of sepsis-causing bacteria and fungi simultaneously

- 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

- DNA ters hibridizasyon ile geçirgen membran üzerine lekeleme temeline dayanan otomatik platform: *DNA-Flow technology*



Kütle Spektrometrik Yöntemler

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

A Brief History of MALDI-TOF

It all began with two German scientists.

Michael Karas and Franz Hillencamp were the pioneers of matrix-assisted laser desorption ionization, or MALDI. They discovered in 1985 that alanine could be more easily ionized if mixed with tryptophan and irradiated with a 266 nm pulse.The tryptophan absorbed energy, helping ionize the non-absorbing alanine.

In 1987, Japanese engineer Koichi Tanaka combined cobalt particles in glycerol with a 337 nm nitrogen laser to ionize incredibly large proteins such as carboxypepbidase-A.

Tanaka, who went on to receive a Nobel Prize, was able to demonstrate that, with the proper combination of a laser wavelength and a matrix, proteins could be ionized in a laboratory setting.

The time-of-flight (TOF) mass spectrometer, which is often paired with the MALDI technique in order to determine the mass-to-charge ratio of ions, was first reportedly used by A. E. Cameron and D. F. Eggers Jr of the Y-12 National Security Complex in 1948.

Kütle Spektrometrik Yöntemler

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

2002

Koichi Tanaka is awarded Nobel Prize in chemistry for mass spectrometric analyses of biological macromolecules.

FDA onayı ile klinik mikrobiyolojik tanıya girdi

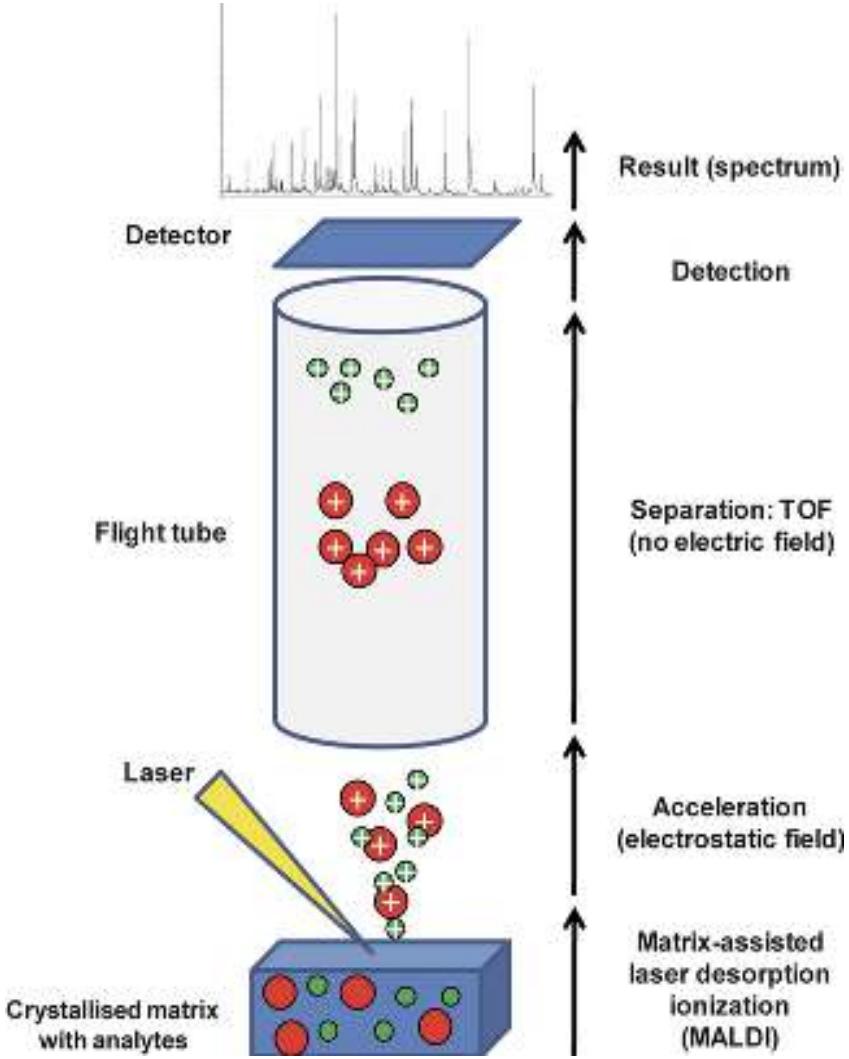
Günümüzde 2000'e yakın bakteri ve mantar tanımlanabiliyor

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- Direkt Örnekten

  Son yıllarda steril vücut örneklerinden kültürsüz olarak direkt tanımlama yapılmaya başlandı. Örnek tipleri;

  - Pozitif Kan Kültür Şişelerinden
  - İdrar
  - BOS
  - Peritoneal-plevral aspirat
  - Eklem sıvısı
  - Kist sıvısı

Kütle Spektrometrik Yöntemler

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

EUCAST

Kan Kültürü Şişelerinden Doğrudan Hızlı Antibiyotik Duyarlılık Testi (HADT)

EUCAST, pozitif kan kültürü şişelerinden doğrudan kısa süreli inkübasyonlu (4, 6 ve 8 saat) ADT için önerilerini yayımlamıştır. Bunun için aşağıda anlatılan yöntem izlenmelidir:

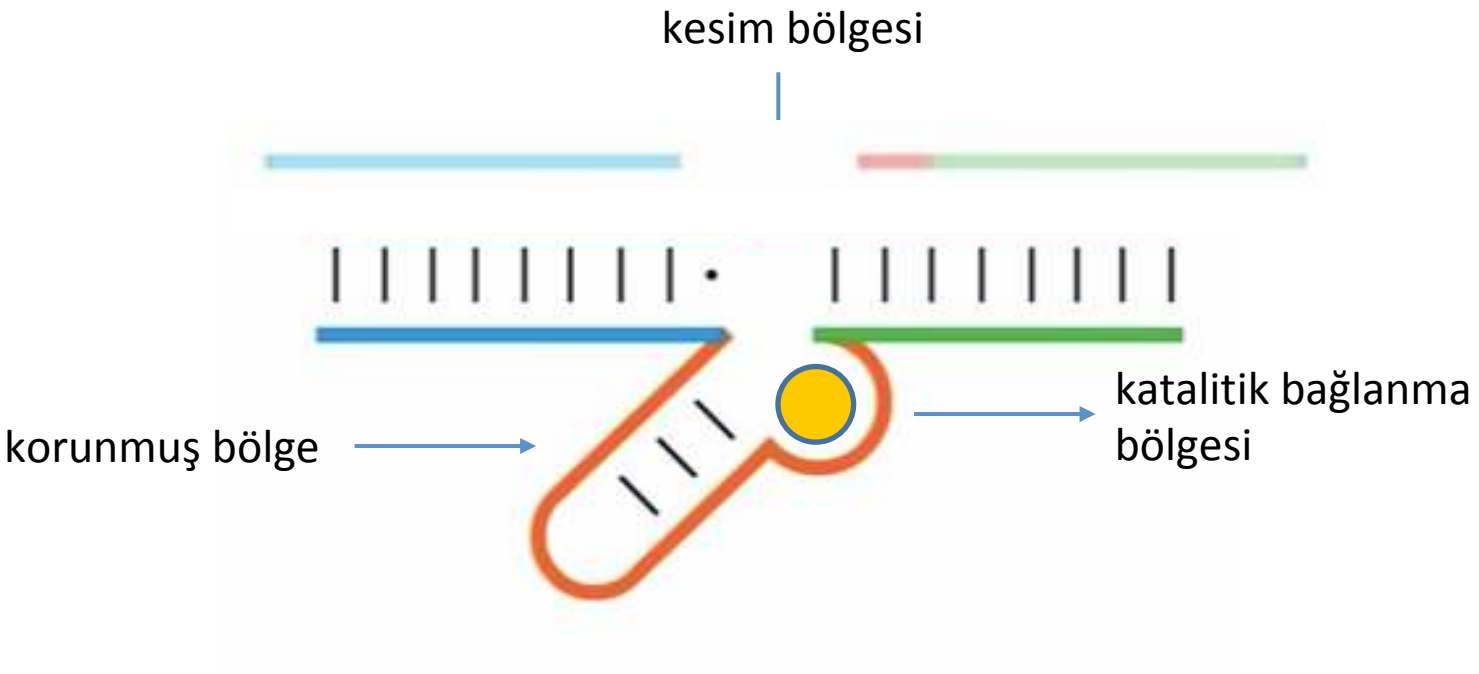
Yöntem – Pozitif kan kültürü şişelerinden doğrudan EUCAST hızlı antibiyotik duyarlılık testi (HADT, RAST)

EUCAST HADT yöntemi, EUCAST standart disk difüzyon yöntemine dayanmakla birlikte, inokülum değiştirilmiş, inkubasyon süresi kısaltılmış, okuma açıklamaları değiştirilmiş ve özgül HADT sınır değerleri tanımlanmıştır.

Not. Yöntem SADECE HADT uygulamak üzere ve ADT plaklarının SADECE maksimum 8 saatlik inkübasyonu için valide edilmiştir (geçerli kılınmıştır). Daha uzun süre inkübasyon gerektiğinde EUCAST standart disk difüzyon yöntemi kullanılmalıdır.

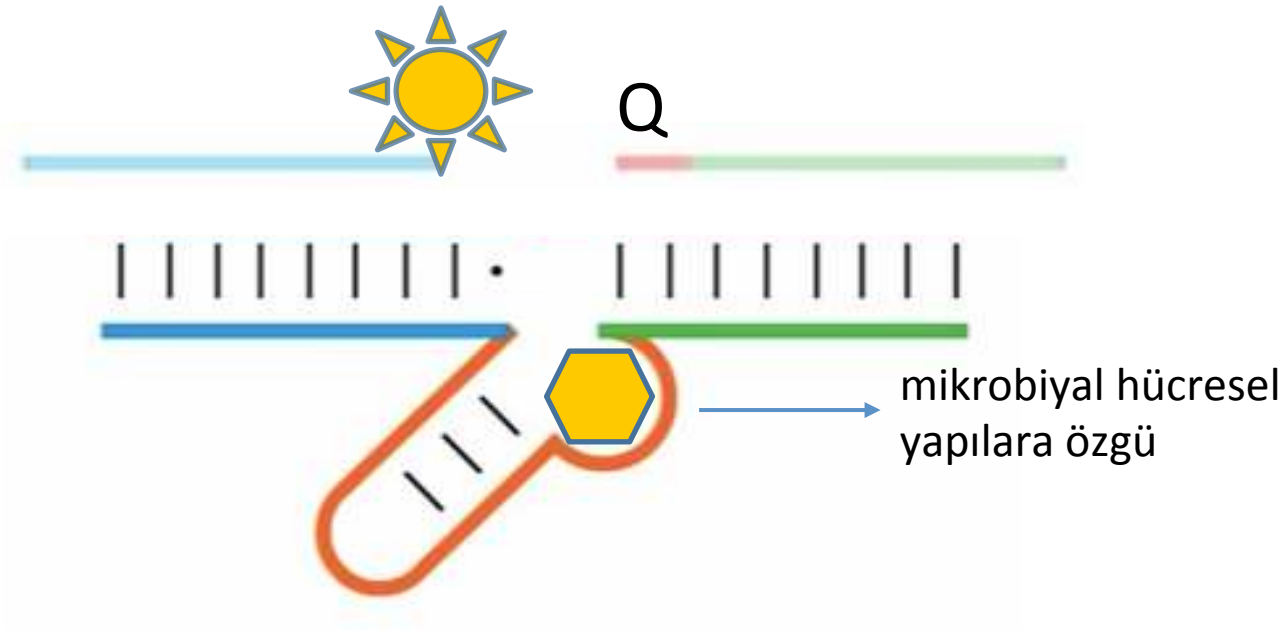
Yeni Yaklaşımlar

- Katalitik aktivite gösteren DNA molekülü; DNAzyme



Yeni Yaklaşımlar

- Katalitik aktivite gösteren DNA molekülü; DNAzyme



(FS: 50-ACTCTTCCTAGCF-rA-QGGTTCGATCAAGA-30 (F-Fluorescein-dT, rA-Riboadenosine, Q-Dabcyl-dT)) and acatalytic sequence (RFD-EC1: 50-CACGGATCCTGACAAGGATGTGTGCGTTGTCGAGACCTGCGACCGGAACACTACACTGTGTGGGATGGATTTCTTTACAGTTGTGTGCAGCTCCGTCCG-30)

Yeni Yaklaşımlar

- Katalitik aktivite gösteren DNA molekülü; DNAzyme

nature COMMUNICATIONS

ARTICLE

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DOI: 10.1038/ncomms6427 OPEN

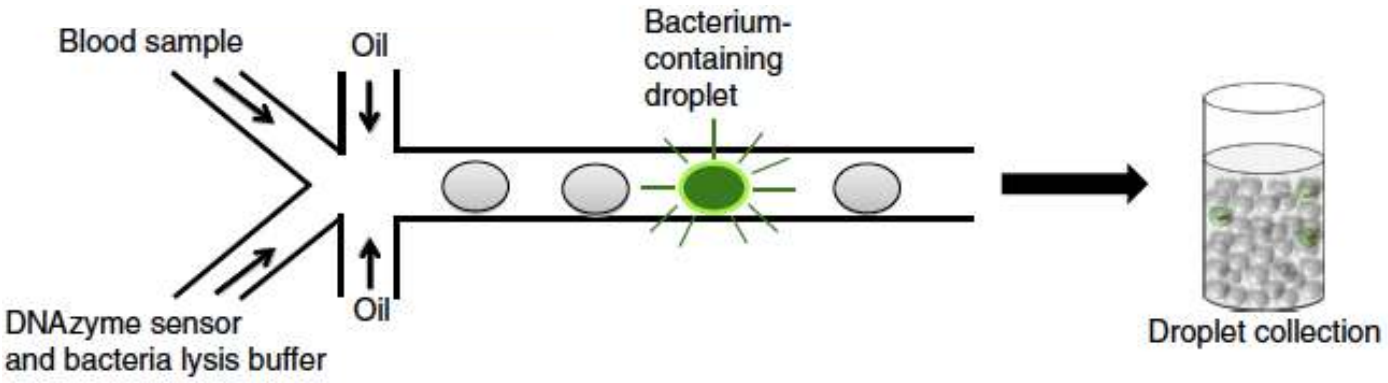
Rapid detection of single bacteria in unprocessed blood using Integrated Comprehensive Droplet Digital Detection

Dong-Ku Kang[1,2,3,4,5,*], M. Monsur Ali[1,2,3,4,5,*], Kaixiang Zhang[1,2,3,4,5,6], Susan S. Huang[7], Ellena Peterson[8], Michelle A. Digman[5,9,10], Enrico Gratton[5,9] & Weian Zhao[1,2,3,4,5]

Blood stream infection or sepsis is a major health problem worldwide, with extremely high mortality, which is partly due to the inability to rapidly detect and identify bacteria in the early stages of infection. Here we present a new technology termed 'Integrated Comprehensive Droplet Digital Detection' (IC 3D) that can selectively detect bacteria directly from milliliters of diluted blood at single-cell sensitivity in a one-step, culture- and amplification-free process within 1.5–4 h. The IC 3D integrates real-time, DNAzyme-based sensors, droplet microencapsulation and a high-throughput 3D particle counter system. Using *Escherichia coli* as a target, we demonstrate that the IC 3D can provide absolute quantification of both stock and clinical isolates of *E. coli* in spiked blood within a broad range of extremely low concentration from 1 to 10,000 bacteria per ml with exceptional robustness and limit of detection in the single digit regime.

Yeni Yaklaşımlar

- Katalitik aktivite gösteren DNA molekülü; DNAzyme



Yeni Yaklaşımlar

- Katalitik aktivite gösteren DNA molekülü; DNAzyme

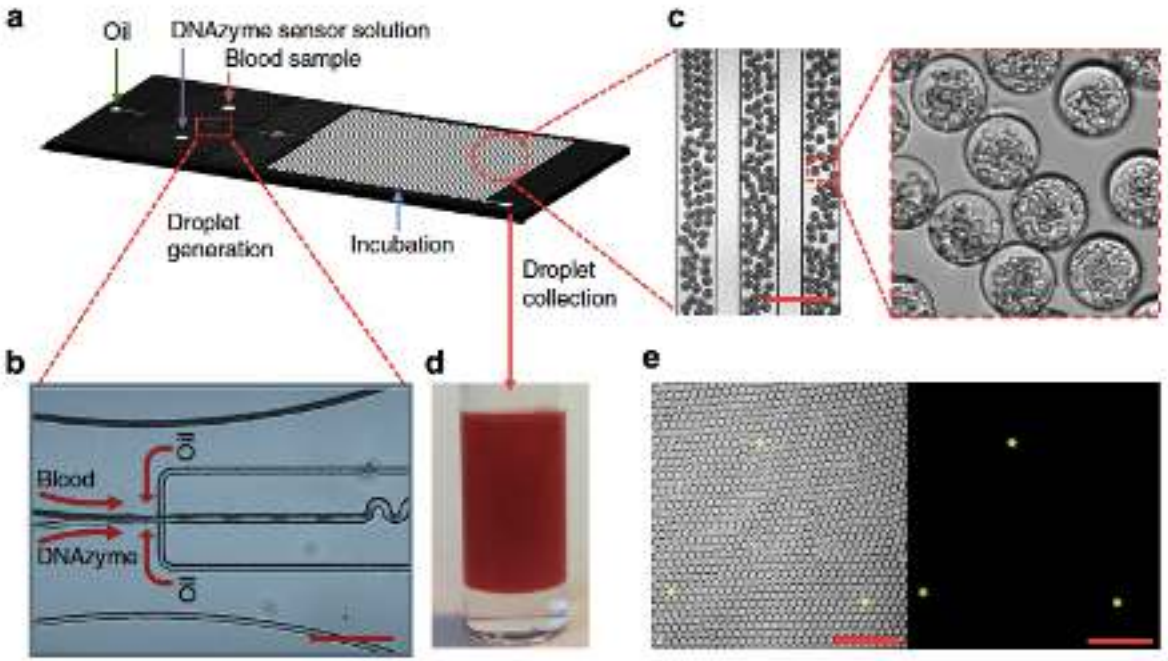
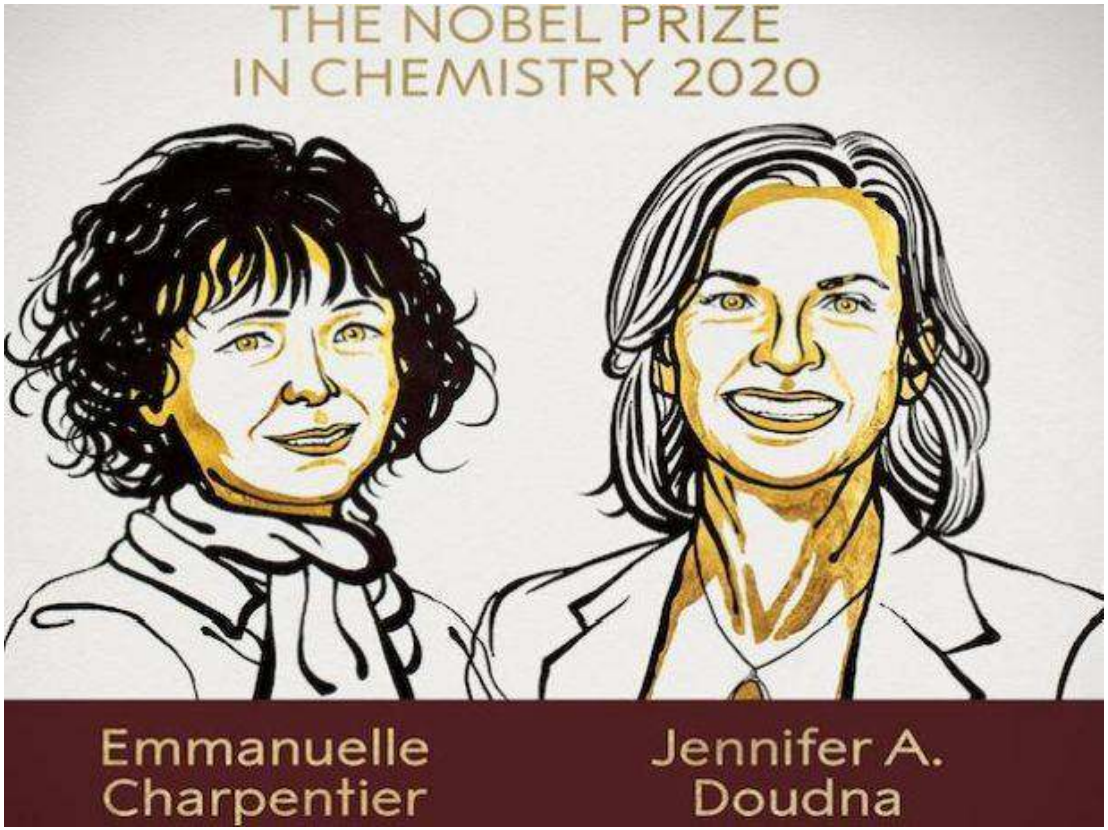


Figure 3 | Workflow of microencapsulation. (**a**) Layout of the droplet-based microfluidic device. Devices were designed with three inlets; one for oil and the other two for blood samples and DNAzyme/bacterial lysis buffer. (**b**,**c**) Representative microscopy images showing uniform 30 µm droplets containing 10% blood and sensor solution are being generated using flow focusing. Scale bar, 200 µm. In **c**, blood contents especially red blood cells are clearly visible in droplets. (**d**) Droplets collected in the cuvette used for 3D particle counter experiments. (**e**) Representative fluorescence microscope images demonstrate DNAzyme sensors (250 nM) light up the droplets that contain single *E. coli* K12 in 10% blood after 3-h reaction. Left panel: overlay of fluorescence and brightfield. Right panel: fluorescence. Scale bar, 200 µm.

Yeni Yaklaşımlar

- CRSPR Cas sistemi ve mikrobiyolojik tanı



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Yeni Yaklaşımlar

- CRSPR Cas sistemi ve mikrobiyolojik tanı

CellPress

Cell Host & Microbe
In Translation

Cutting-Edge Infectious Disease Diagnostics with CRISPR

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https://doi.org/10.1016/j.chom.2018.05.016

Three recent *Science* articles (Chen et al., 2018; Gootenberg et al., 2018; Myhrvold et al., 2018) describe the use of CRISPR-Cas technology to develop point-of-care diagnostics that directly detect viruses from clinical samples. These tests could radically transform approaches to diagnosing infectious diseases at the bedside and in the field.

Yeni Yaklaşımlar

- CRSPR Cas sistemi ve mikrobiyolojik tanı

India's Feluda COVID-19 test cheaper, faster alternative to RT-PCR, say scientists

PTI • Last Updated: Sep 28, 2020, 06:42 PM IST

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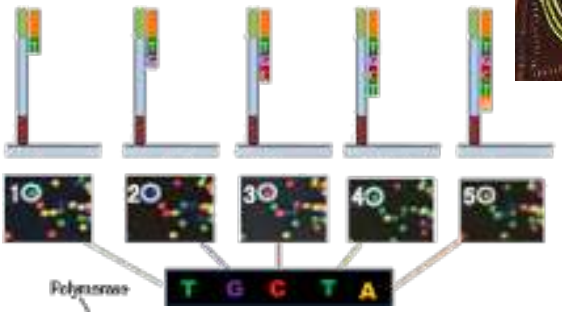
Synopsis

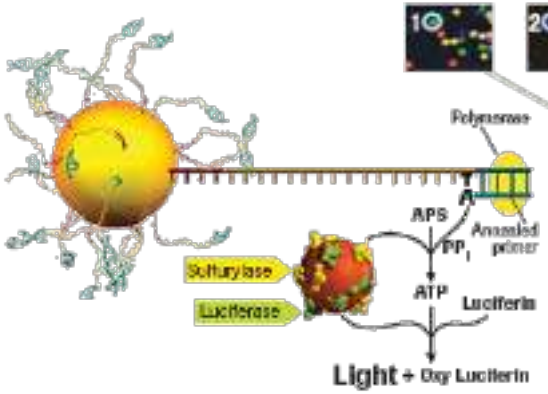
Named after Satyajit Ray's famed detective, the Feluda test, which is priced at Rs 500 and can deliver a result in 45 minutes, is able to differentiate SARS-CoV-2 from other coronaviruses even if genetic variations between them are minute.

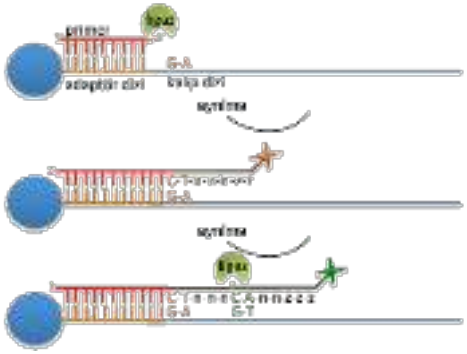
The CRISPR Feluda test is the world's first diagnostic test to deploy a specially adapted Cas9 protein, derived from Francisella novicida bacteria, to successfully detect the virus that causes COVID-19, the researchers said.

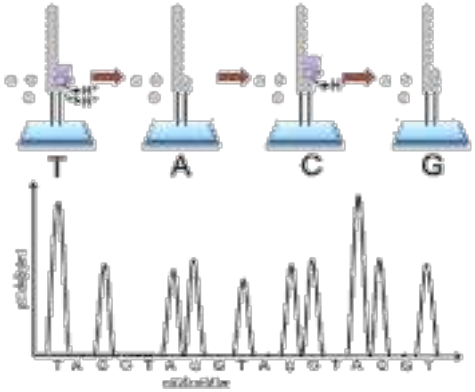
More accurate than a rapid antigen test and almost as quick, India's CRISPR 'Feluda' COVID-19 test that changes colour on detection of the SARS-CoV-2 virus could be a cheaper, faster and simpler alternative to an RT-PCR diagnosis, say scientists.

Yeni Nesil Dizileme Sistemleri

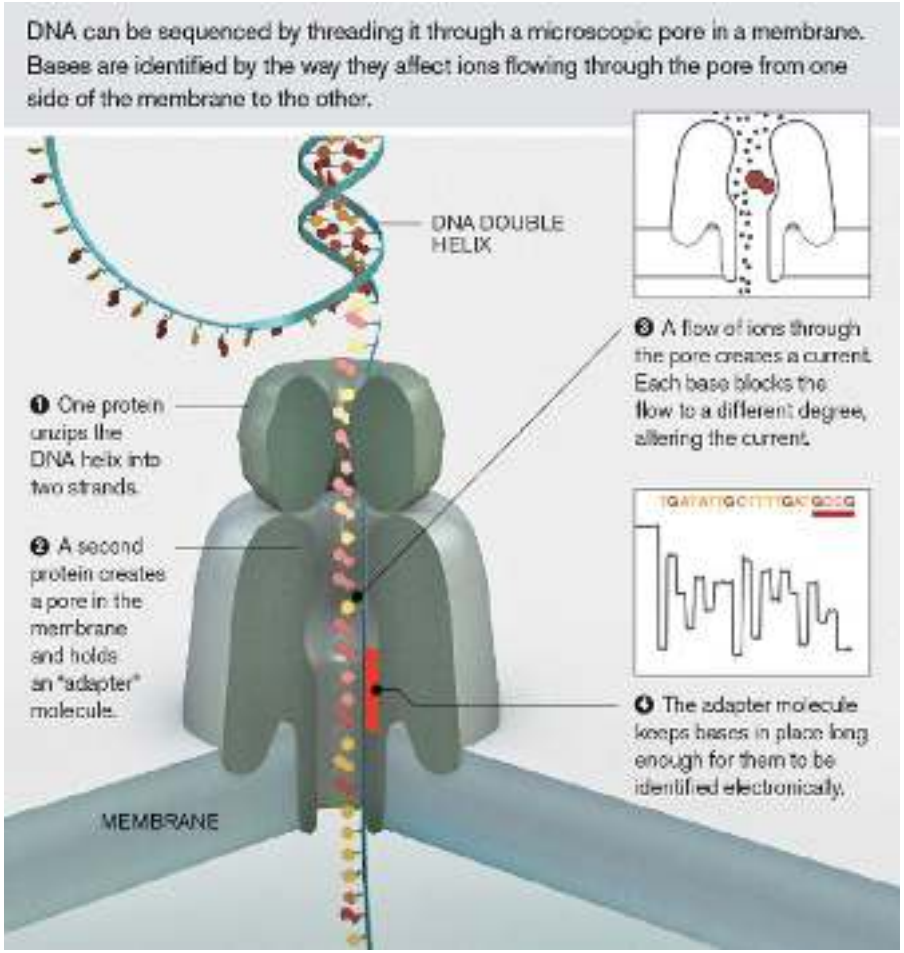






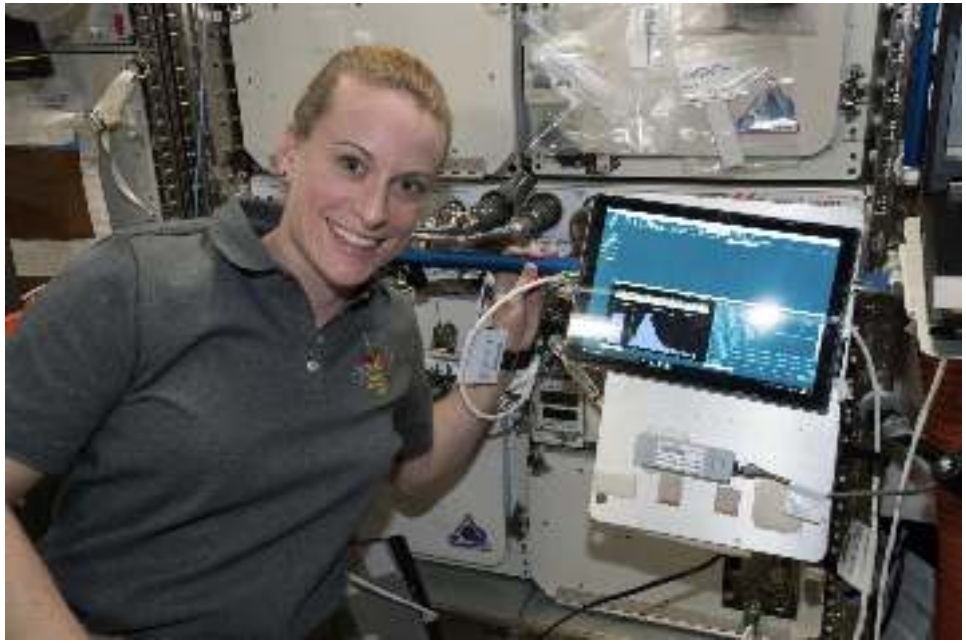


Oxford Nanopore Technology



My Twitter feed just exploded. Oxford Nanopore, long the sleeper project to watch in the field of mapping DNA, just announced two products that could dramatically change the field of DNA sequencing: a new DNA sequencer that may be able to handle a human genome in 15 minutes, and a USB thumb drive DNA sequencer that can read DNA directly from blood with no prep work.

Oxford Nanopore Technology

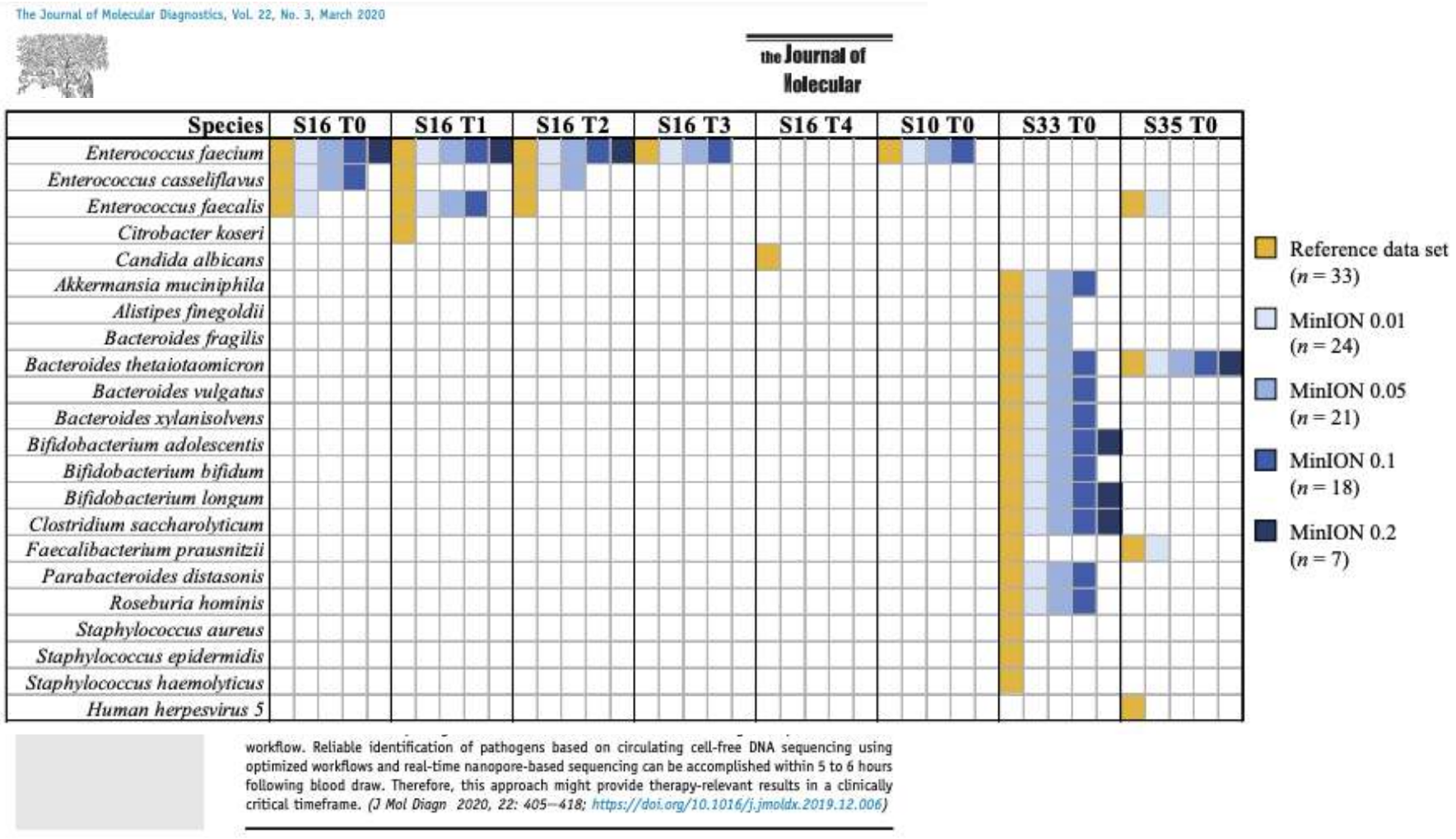


Oxford Nanopore Technology

- Uluslararası Uzay İstasyonunda yaşayan dört yeni tür bakteri bulundu

Oxford Nanopore Technology

- 239 sepstik tablodaki hastanın %90'nında 5-6 saat içerisinde etken tespit edilebilmiş.



Antimikromiyal Yönetişim Programları

The Role of the Microbiology Laboratory in Antimicrobial Stewardship Programs

Table 1
Features of select rapid diagnostic assays currently used in clinical practice

Technology	Manufacturer	Specimen	Organisms	Resistance Markers	Time Required (h)	FDA Cleared
PNA FISH	AdvanDx, Inc, Woburn, MA	Blood	*Staphylococcus aureus*/coagulase-negative staphylococci, *Enterococcus faecalis*/other *Enterococcus* spp, *Escherichia coli*/*Klebsiella pneumoniae*/*Pseudomonas aeruginosa*, *Candida albicans*/*C parapsilosis*/*C tropicalis*/*C glabrata*/*C krusei*	*mecA*[*]	0.3–1.5	Yes[*]
qPCR	BD GeneOhm, Inc, Sparks, MD; Cepheid, Sunnyvale, CA; Roche Molecular Systems, Inc, Indianapolis, IN	Blood, wounds	*S aureus*	*mecA*/SCCmec	1–2	Yes
MALDI-TOF MS	Bruker Daltonics, Inc, Billerica, MA; bioMerieux, Inc, Durham, NC	All body sites	Large number of organisms including bacteria and yeast	None	0.2	No
Nucleic acid microarray BC-GP	Nanosphere, Inc, Northbrook, IL	Blood	*Staphylococcus* spp, *S aureus*, *S epidermidis*, *S lugdunensis*; *Streptococcus* spp, *S pneumoniae*, *S pyogenes*, *S agalactiae*, *S anginosus* group; *E faecalis*, *E faecium*, *Listeria* spp	*mecA*, *vanA*, *vanB*	2.5	Yes
Nucleic acid microarray BC-GN	Nanosphere, Inc, Northbrook, IL	Blood	*Escherichia coli*/*Shigella* spp, *Klebsiella pneumonia*, *Klebsiella oxytoca*, *P aeruginosa*, *Serratia marcescens*, *Acinetobacter* spp, *Proteus* spp, *Citrobacter* spp, *Enterobacter* spp	KPC, NDM, CTX-M, VIM, IMP, OXA	2.5	Yes
Multiplex nucleic acid amplification test	BioFire, Inc, Salt Lake City, UT	Blood	*Enterococcus* spp, *Listeria monocytogenes*, *Staphylococcus* spp, *S aureus*, *Streptococcus* spp, *S agalactiae*, *S pyogenes*, *S pneumoniae*, *A baumannii*, *Haemophilus influenzae*, *Neisseria meningitides*, *P aeruginosa*, *E cloacae* complex, *E coli*, *K oxytoca*, *K pneumoniae*, *S marcescens*, *Proteus* spp, *Enterobacteriaceae* spp, *C albicans*, *C parapsilosis*, *C tropicalis*, *C glabrata*, *C krusei*	*mecA*, *vanA*, *vanB*, KPC	1	Yes

[*] Probe for *mecA* gene has been evaluated in a recent clinical trial, but not yet FDA approved.

Paradigma Değişiminin Zirvesi

- Transplantasyon İnfeksiyonları için Gelişen Mikrobiyolojik Tanı:

  Bir Paradigma Değişiminin Zirvesinde

Review

Emerging Microbiology Diagnostics for Transplant Infections: On the Cusp of a Paradigm Shift

Marwan M. Azar, MD,[1] David C. Gaston, MD, PhD,[1] Camille N. Kotton, MD,[2] and Maricar F. Malinis, MD[1,3]

Abstract. In light of the heightened risk for infection associated with solid organ and hematopoietic stem cell transplantation, rapid and accurate microbiology diagnostics are essential to the practice of transplant clinicians, including infectious diseases specialists. In the last decade, diagnostic microbiology has seen a shift toward culture-independent techniques including single-target and multiplexed molecular testing, mass-spectrometry, and magnetic resonance-based methods which have together greatly expanded the array of pathogens identified, increased processing speed and throughput, allowed for detection of resistance determinants, and ultimately improved the outcomes of infected transplant recipients. More recently, a newer generation of diagnostics with immense potential has emerged, including multiplexed molecular panels directly applicable to blood and blood culture specimens, next-generation metagenomics, and gas chromatography mass spectrometry. Though these methods have some recognized drawbacks, many have already demonstrated improved sensitivity and a positive impact on clinical outcomes in transplant and immunocompromised patients.

(*Transplantation* 2020;104: 1358–1384).

Paradigma Değişiminin Zirvesi

- Transplantasyon İnfeksiyonları için Gelişen Mikrobiyolojik Tanı:

  Bir Paradigma Değişiminin Zirvesinde

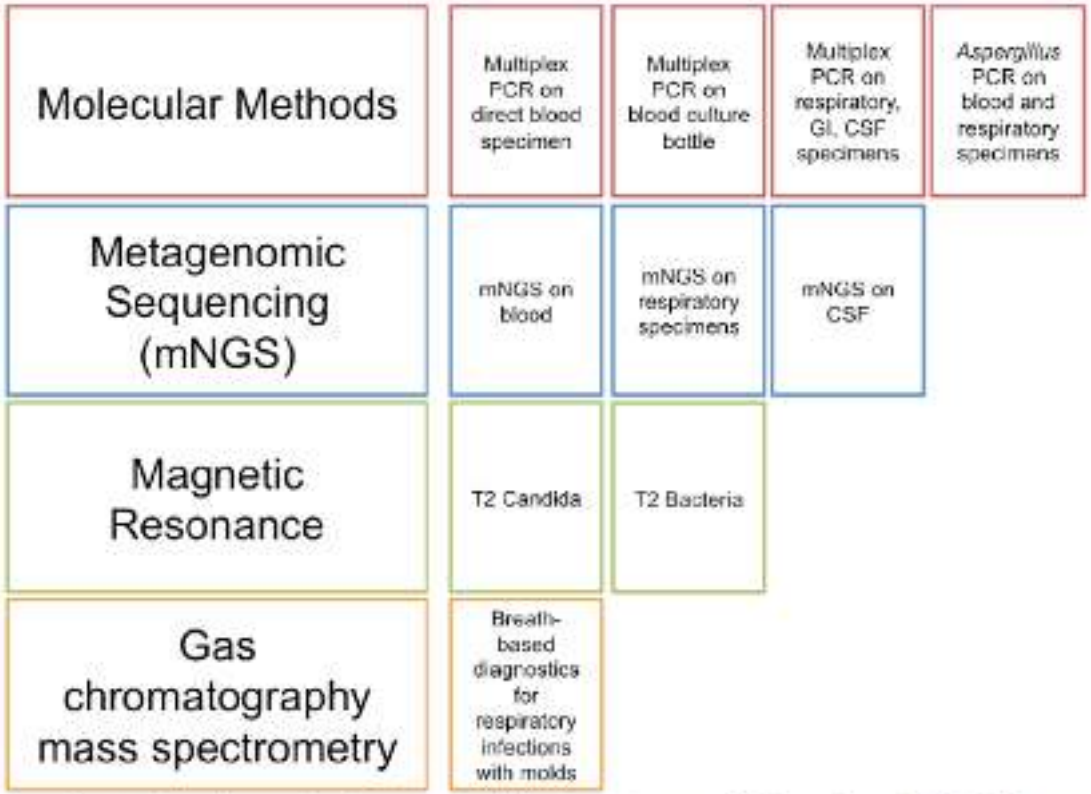


FIGURE 1. Overview of emerging diagnostics for transplant infectious diseases. CSF, cerebrospinal fluid; GI, gastrointestinal; PCR, polymerase chain reaction.

Antimikromiyal Yönetişim Programları

The Role of the Microbiology Laboratory in Antimicrobial Stewardship Programs

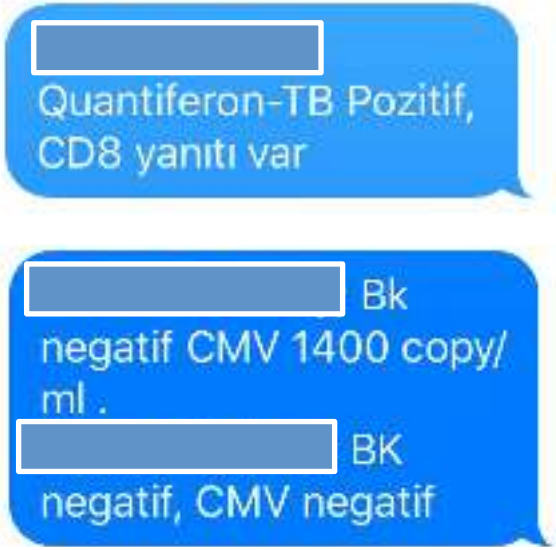
Edina Avdic, PharmD, MBA[a,*], Karen C. Carroll, MD[b]

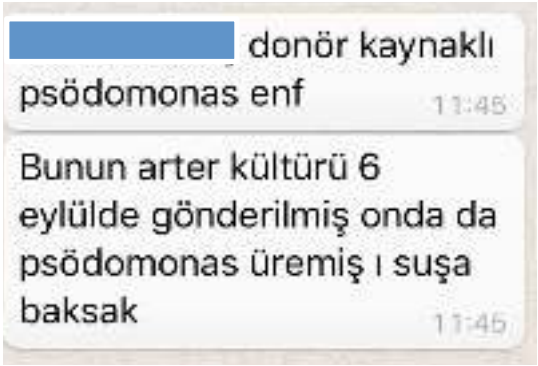
Med Clin N Am 102 (2018) 883–898
https://doi.org/10.1016/j.mcna.2018.05.003
medical.theclinics.com

- Mikrobiyolojik veriden özellikle faydalanacak ve mikrobiyolog tarafından hızlı protokollere yönlendirilecek (fast-track protokoller uygulanacak) hastaların sistematik seçimi
- Özel öneme sahip klinik örnekler için laboratuvar çalışma prosedürünün hızlandırılması
- Bazı önemli patojenlerin hızlı taranması
- Mikrobiyolojinin kırmızı telefon hattı: pozitif ve negatif test sonuçlarının hızlı bildirimi

Antimikromiyal Yönetişim Programları

- Hızlı tanı kadar sonucun da hızla ulaştırılmasına ihtiyaç var.
- Bu da ancak rutin dışındaki yöntemlerle mümkün oluyor.





Quo Vadis? Nereye Gidiyor Bu Mikrobiyolojik Tanı?

- Dünya Sağlık Örgütü, her yıl 1.000.000 dan fazla ölümün çoklu ilaca dirençli enfeksiyonların doğrudan bir sonucu olduğunu tahmin ediyor.