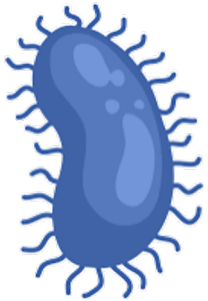
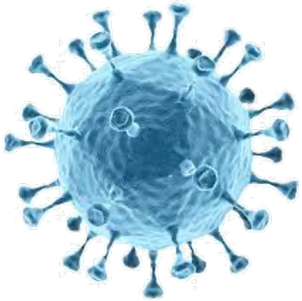
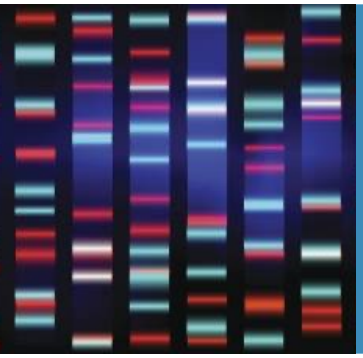
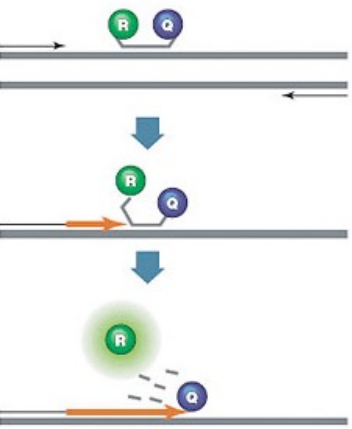






Bakteriyel ve viral enfeksiyonlar için yeni tanısal araçlar



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

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

- Mikroorganizmalarla ilk tanışma

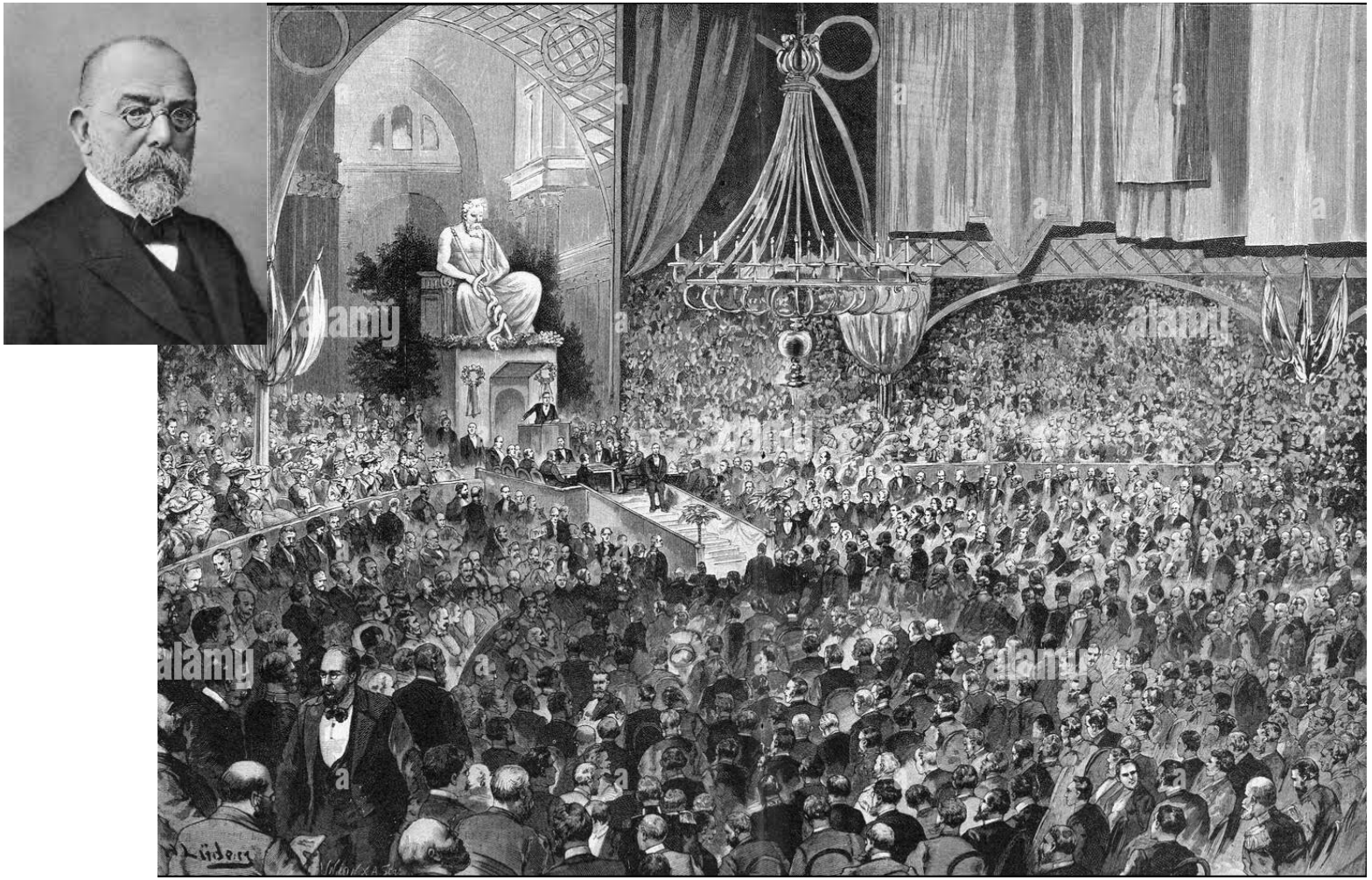
1680

1850’li Yıllara Kadar - Miasma Teorisi

- Ortaçağ'da ortaya çıkmış ve yüzyıllarca devam etmiştir.

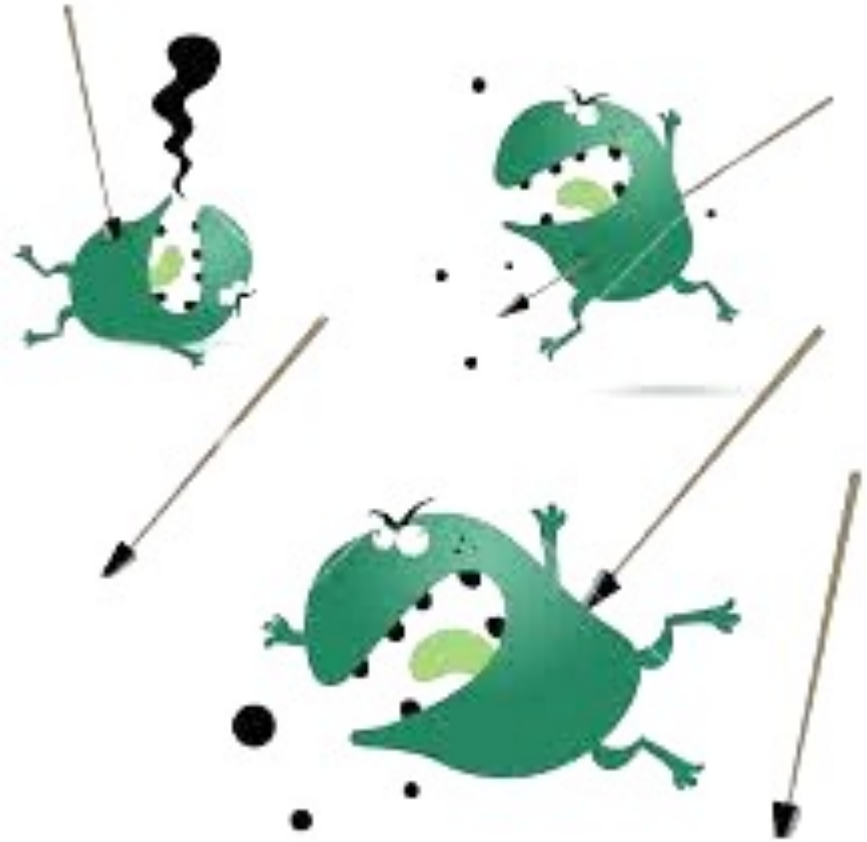
Berlin Dünya Tıp Kongresi -1890

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



Mikrobu Bul Mikrobu Yok Et!

- Bu tarihten sonra mikroorganizmalar ve insanoğlu arasındaki savaş başlamış ve "mikrobu bul, mikrobu yok et" sloganı yıllar boyu sürecek mücadelenin ana fikri olmuştur.



Mikrobiyolojik Tanı Yöntemlerinin Gelişimi

- Mikroskopi alanındaki gelişmeler

The Royal Society of Medicine.

October 20, 1913.

Sir Francis H. Champneys, Bt., M.D., President, in the Chair.

On Some of the Recent Advances in the Field of Microbiology; with Demonstrations of the Pure Cultures of various Spirochætes, of the Viruses of Rabies and Poliomyelitis, and of *Treponema pallidum* in the Brains of General Paralytics.

By Hideyo Noguchi, M.D., M.S., Sc.D.

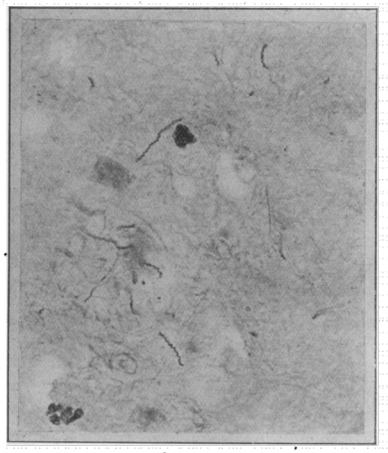


Fig. 8.

Treponema pallidum in the cortical layers of the brain of a general paralytic. Stained by the silver impregnation of Levaditi (with slight modification.) (× 1,100.)

Mikrobiyolojik Tanı Yöntemlerinin Gelişimi

- Kültür alanındaki gelişmeler

Mikrobiyolojinin annesi

Fanny Hesse 1850- 1934

«Bir mikrobiyolog için agarsız bir yaşam düşünülemez »

Geleneksel Yöntemlerdeki Durum

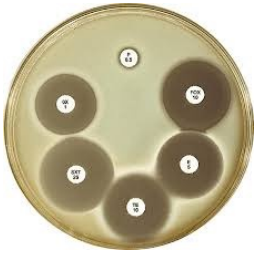
- Tanı için gözle görünür üremenin tespiti antimikrobiyal duyarlığın tespiti için de üremenin inhibisyonun gösterilmesi zaman alıcı.

ATP

metabolitleri

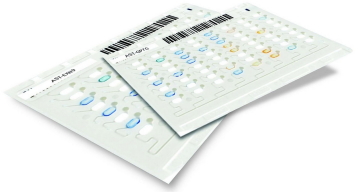
proteinleri

18-24 saat



16-18 saat

8-10 saat

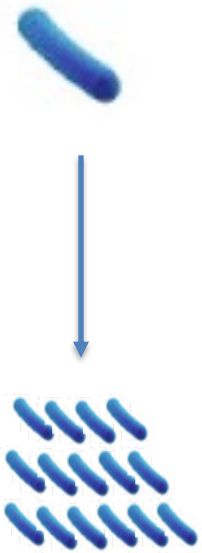


6-8 saat

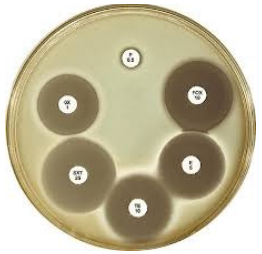
Geleneksel Yöntemlerdeki Durum

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

Tek bir bakteri hücresi

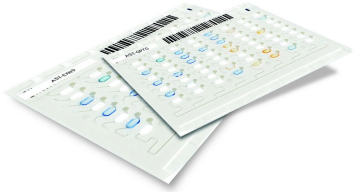


18-24 saat



16-18 saat

8-10 saat

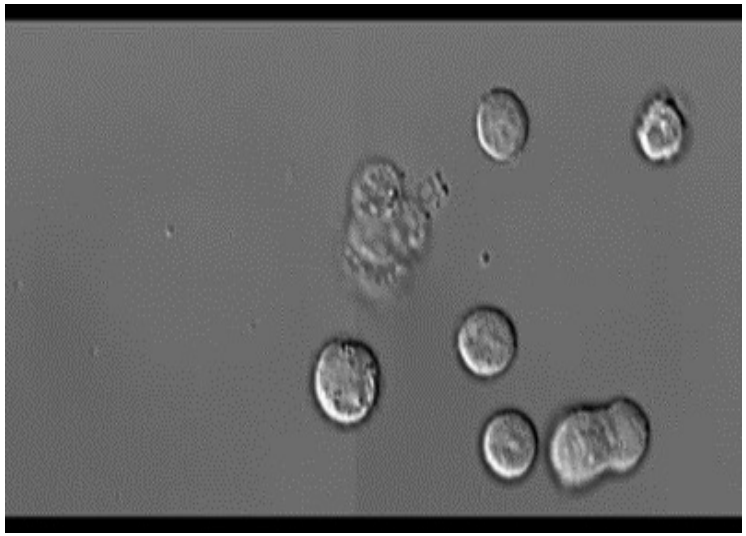


6-8 saat

Geleneksel Yöntemlerdeki Durum

- Antimikrobiyal duyarlılık;

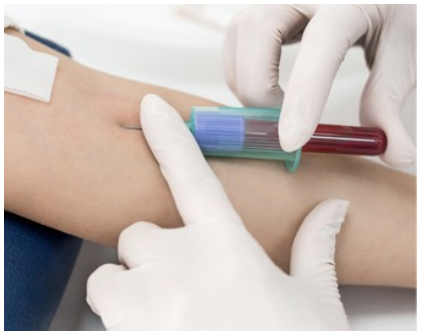
  mikroorganizma ile antibiyotik karşılaştırılmadan duyarlılık sonucundan bahsedilemez



Geleneksel Yöntemlerdeki Durum

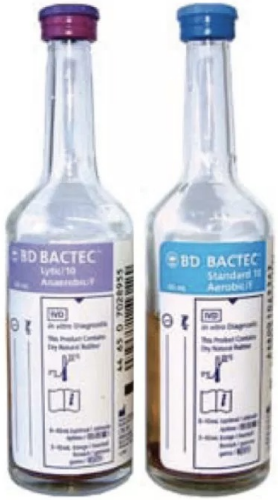
- Saatlerin önemli olduğu sepsisin tanısı
- Hızlı ve doğru başlangıç tedavisinin,

diğer tıbbi müdahalelere göre daha fazla yaşam kurtardığı gösterilmiştir.



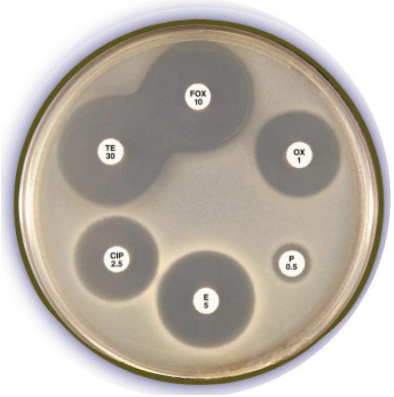
1 Gün

Pozitif Kan Kültür



1 Gün

1 Gün



Mikrobiyolojik Tanıda Yeni Arayışlar

- Hedef;
  - uygulaması kolay,
  - daha duyarlı,
  - daha hızlı,
  - aynı anda daha fazla hedefin gösterilebildiği ekonomik yöntemlerin geliştirilmesidir.

Moleküler Mikrobiyolojik Tanı

- Moleküler mikrobiyoloji, mikroskopla yapılan ilk gözlemlerle başlayan uzun bir sürecin doruk noktasıdır..

Mikrobiyolojinin babası

Neden Moleküler Mikrobiyolojik Tanı?

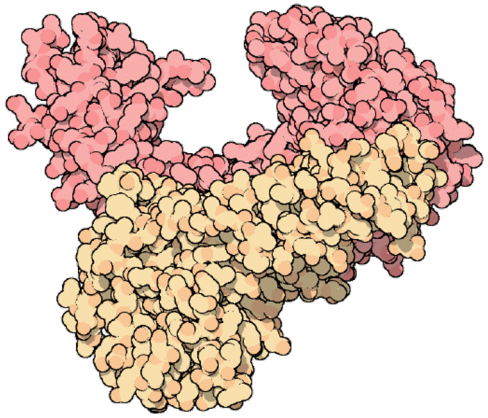
- Kültürde üretilemeyen etkenlerin tanısı için zorunluluk
  - Virüslerin tanısında ve tedavinin takibinde
  - Antiviral direncin tespitinde
- Zor ya da geç üreyen bakterilerin tansı için zorunluluk
  - Chlamydia spp., Neisseri spp., Mycoplasma spp.
  - Mycobacteriım spp., *Haemophilus influenzae*
- Kültürde üretilebilen etkenlerin tanısında hız için
  - Sendromik yaklaşım
- Antimikrobiyal direnç durumlarının hızla gösterilmesinde
  - Yaygın direnç genlerinin takibi
- Etkenlerin virülansının karakterizasyonunda
- Moleküler epidemiyoloji
  - Salgınların tespiti, izlenmesi
  - Korunma ve kontrol stratejilerinin geliştirilmesi

Moleküler Tanı Yöntemlerinin Gelişimi

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

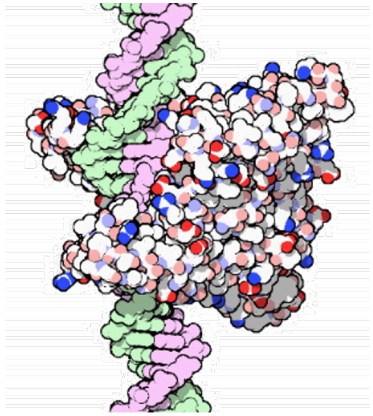
The Nobel Prize in Physiology or Medicine 1975
David Baltimore, Renato Dulbecco, Howard M. Temin

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".

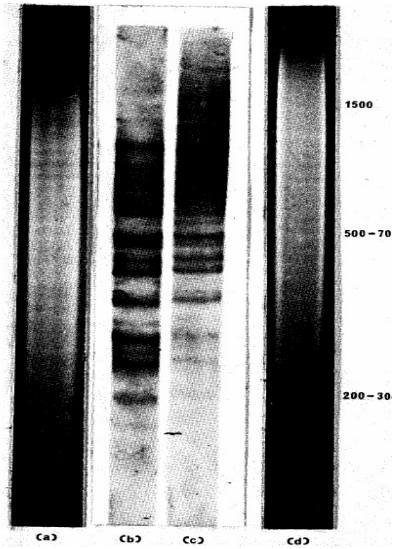


Moleküler Tanı Yöntemlerinin Gelişimi

- Bu gelişmeleri;

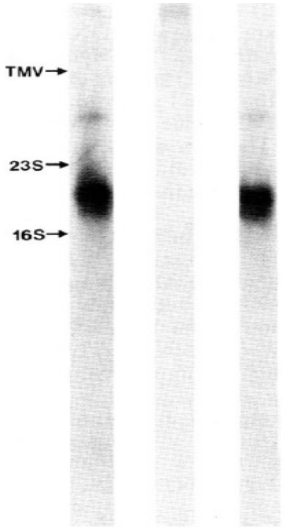
  hibridizasyon, blotlama ve DNA dizileme yöntemlerinin tanıtılması takip etmiştir.

Southern blotting



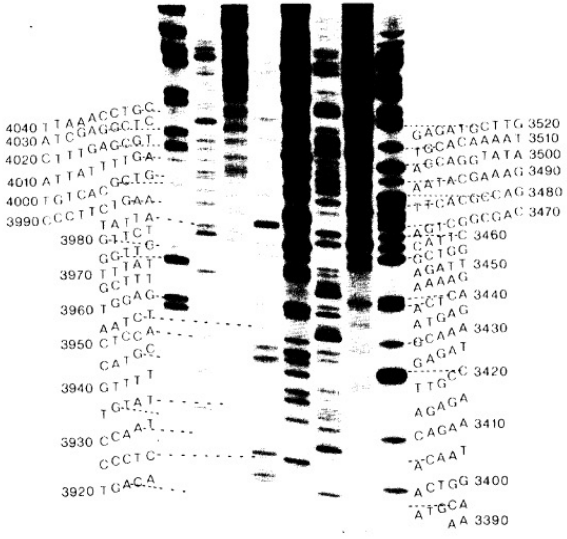
J Mol Biol. 1975 Nov 5;98(3):503-17.

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

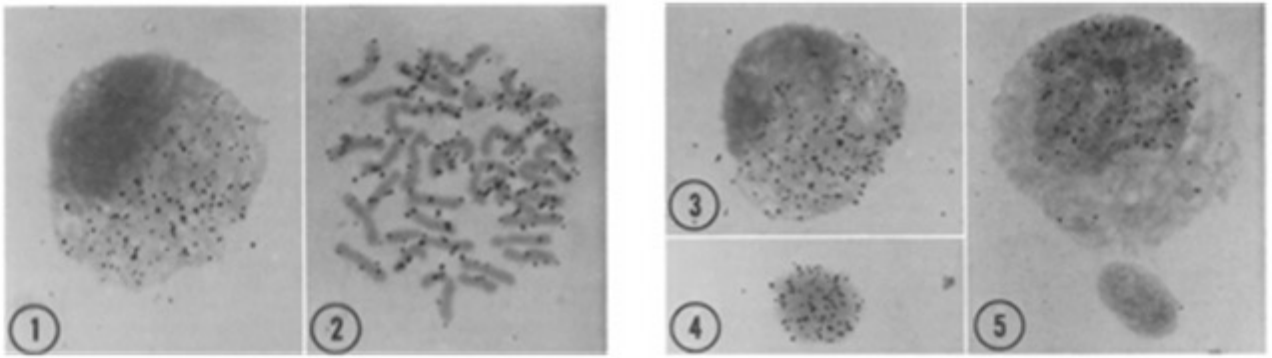
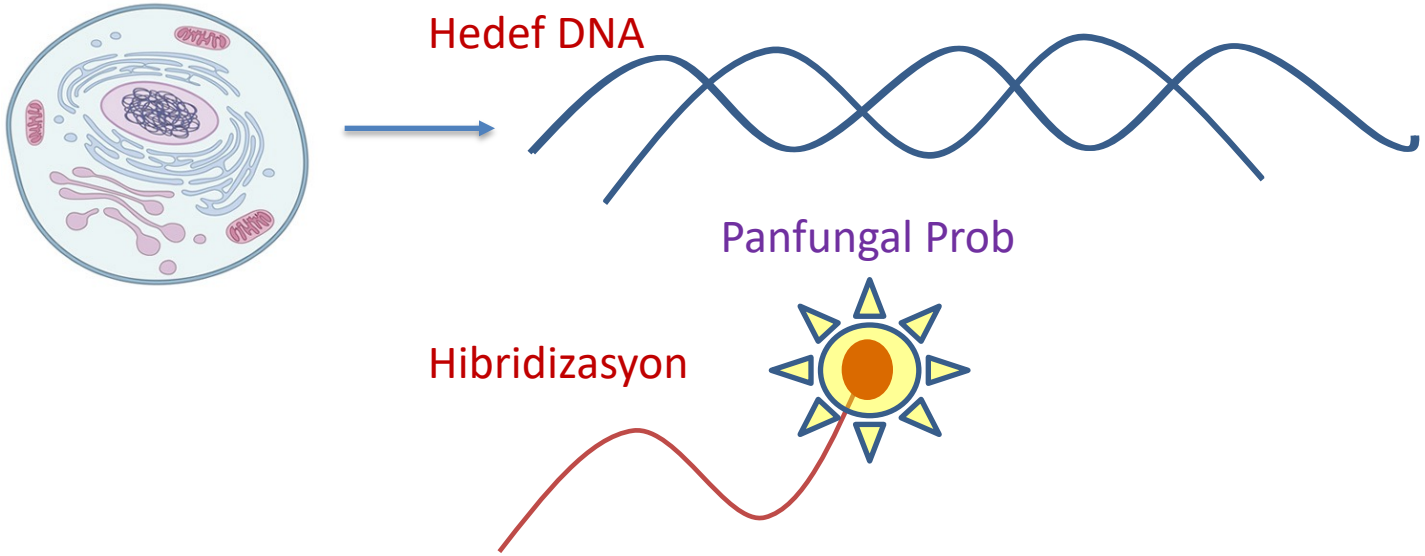


FIG. 1–4.—Autoradiographs of nuclei from the ovary of the toad *Xenopus* after cytological hybridization with radioactive *Xenopus* DNA which lacked the ribosomal cistrons. The DNA of these squash preparations was denatured *in situ*. The slide was then incubated with a solution of radioactive test DNA 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

- FDA tarafından onaylanmış ilk moleküler test, 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

- Virüs eklenmiş kombine nazal ve boğaz sürüntülerinde yaklaşık 20 dakikada tespit edebilen hızlı bir floresan in situ hibridizasyon (FISH) protokolü

www.nature.com/scientificreports

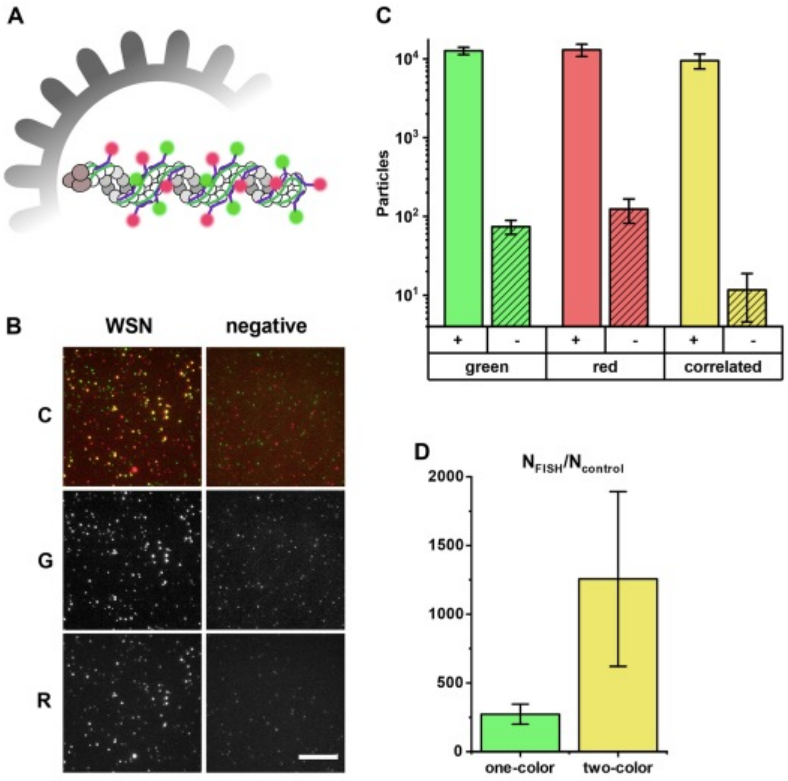


Figure 5. Increased signal-to-noise ratio with dual-color FISH. (**A**) Schematic depiction of the NA segment in an influenza particle labelled with two probe sets carrying spectrally distinct fluorophores. (**B**) Representative image of a dual-labelled WSN sample. Left column: diluted virus culture supernatant (10^6 PFU/mL). Right

Hibridizasyon Temelli Yöntemler

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

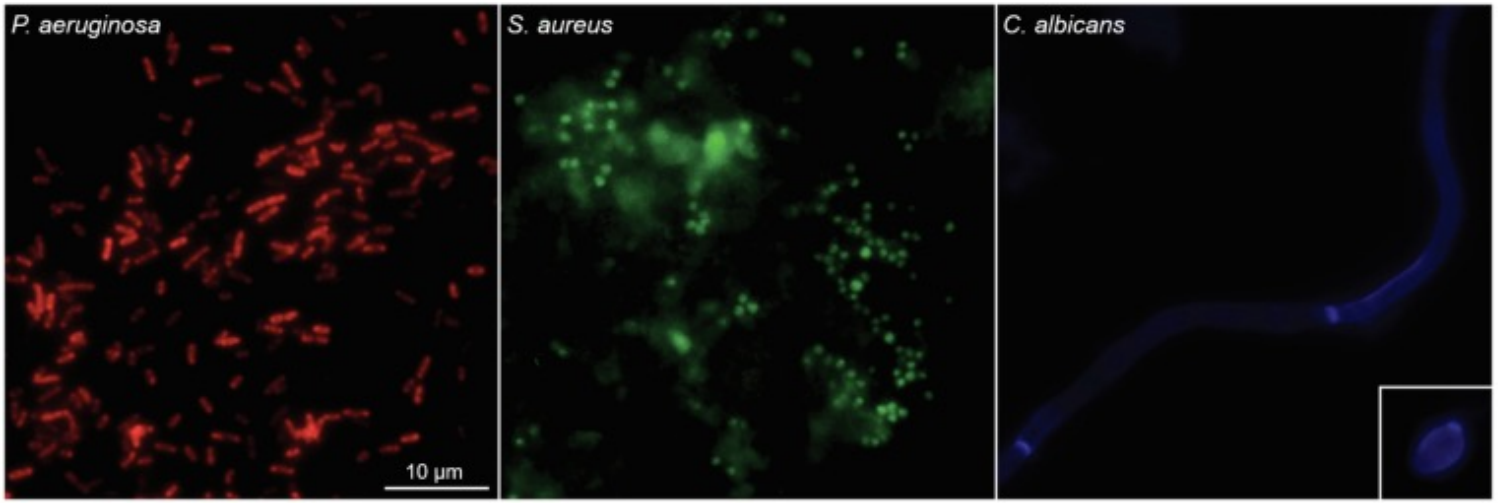
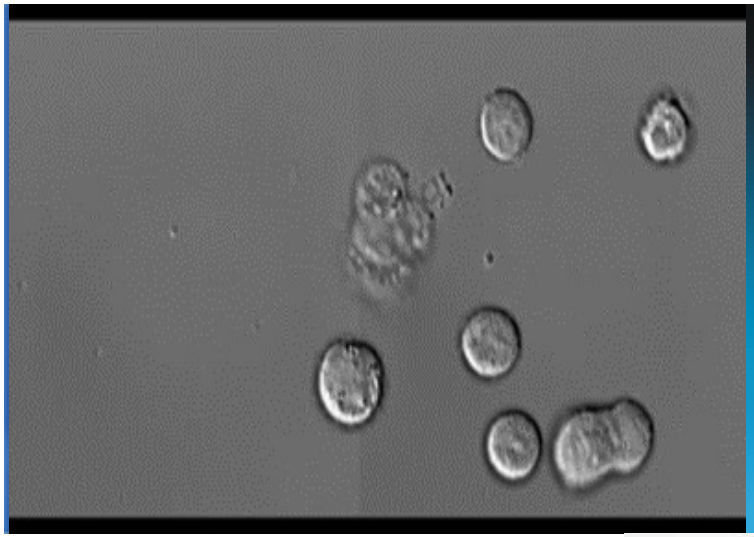


Figure 2. Pathogenic organisms identified from swabs of partial-thickness burn wounds. Each panel represents an individual channel and location for visualization of the microorganisms. *Pseudomonas aeruginosa* was clearly seen in the red channel as bright clusters of rods with minimal staining of the 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.

Zaman Atlamalı Mikroskobik Yöntemler

- Zaman Atlamalı Mikroskobik Yöntemler

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



Zaman Atlamalı Mikroskobik Yöntemler

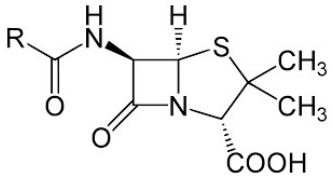
mikroorganizma ile antibiyotik

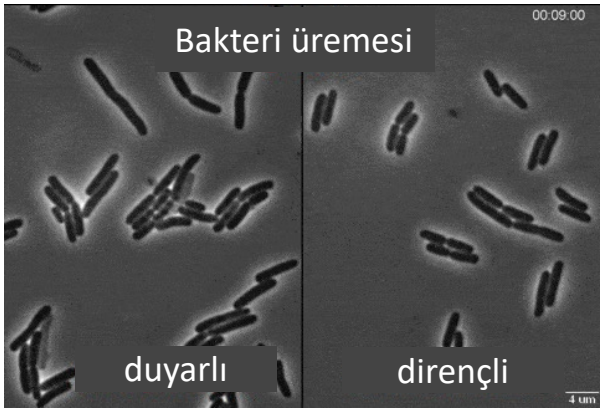
karşılaştırılmadan duyarlılık sonucundan

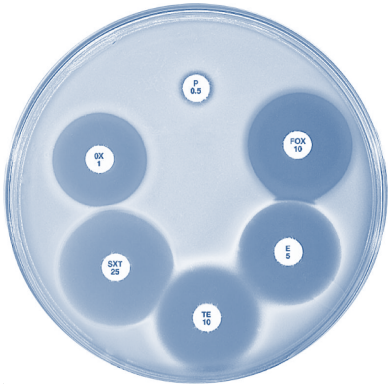
bahsedilemez

E. coli

Antibiyotik







Zaman Atlamalı Mikroskobik Yöntemler

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

JCM
Journals.ASM.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 g dimensional (2D) picture. (c) 2D picture of *S. a* microscope.

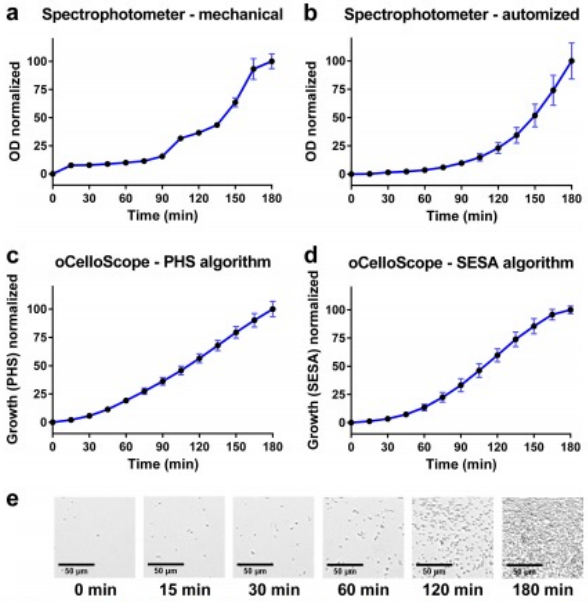
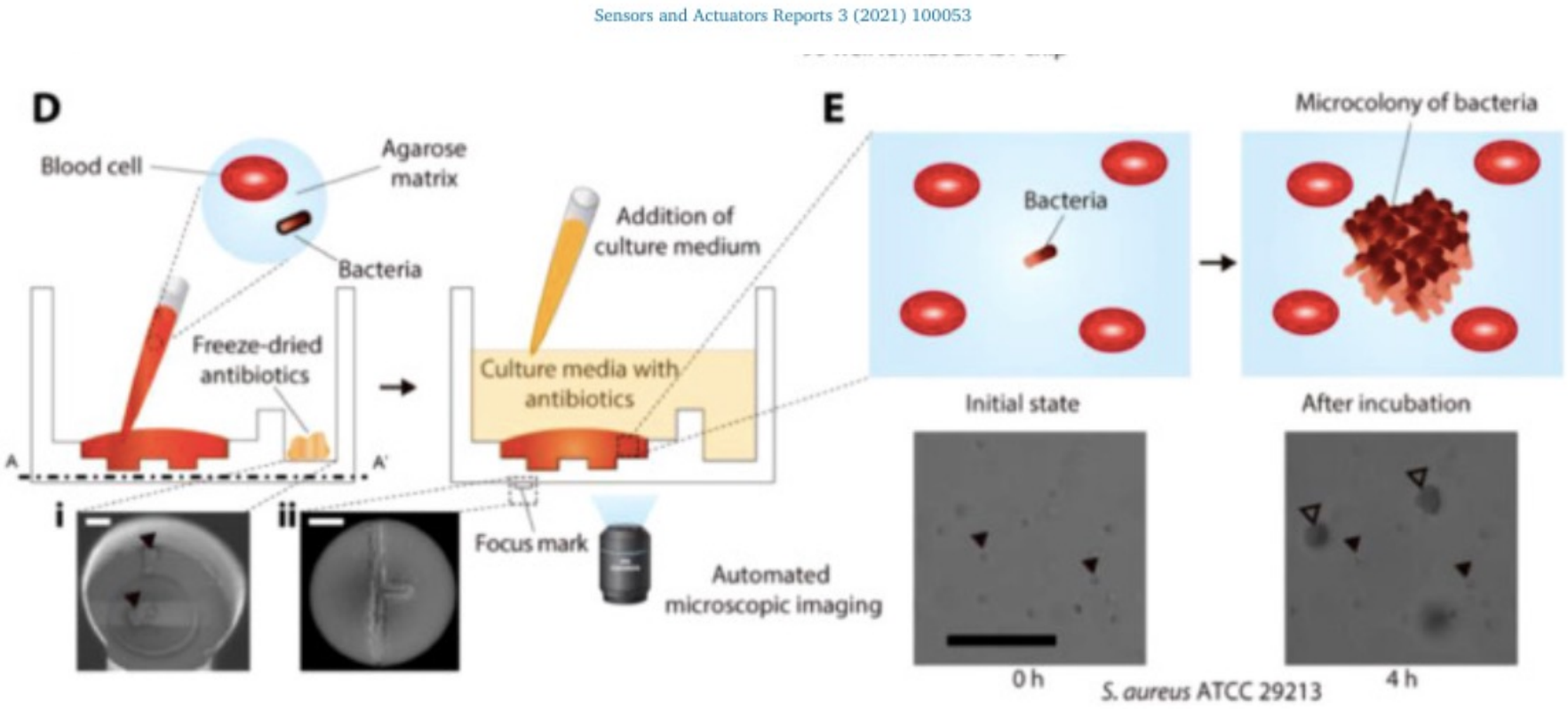


FIG 2 Bacterial growth of *S. aureus* assessed by the oCelloScope and traditional OD measurements. (a) Growth was measured by optical density in a standard laboratory spectrophotometer with one cuvette. The absorbance was measured at 600 nm. (b) Growth was measured by optical density (absorbance, 655 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 to different time points. All experiments were done as eight replicates, and standard derivations are shown as error bars on the curves. Scale bar, 50 µm.

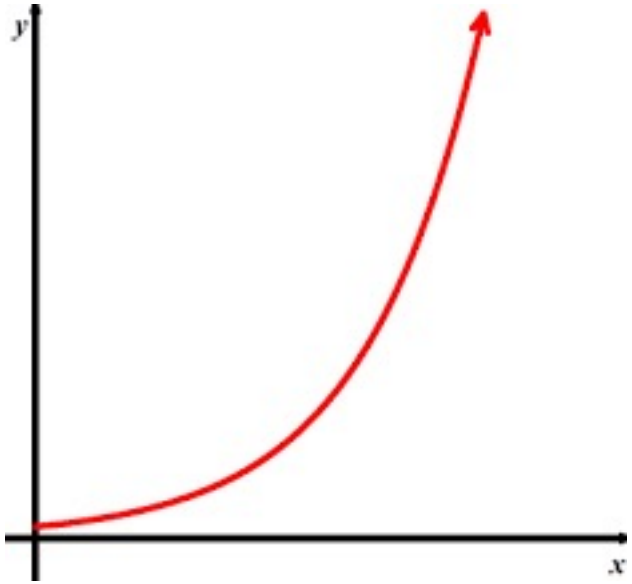
Zaman Atlamalı Mikroskobik Yöntemler

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



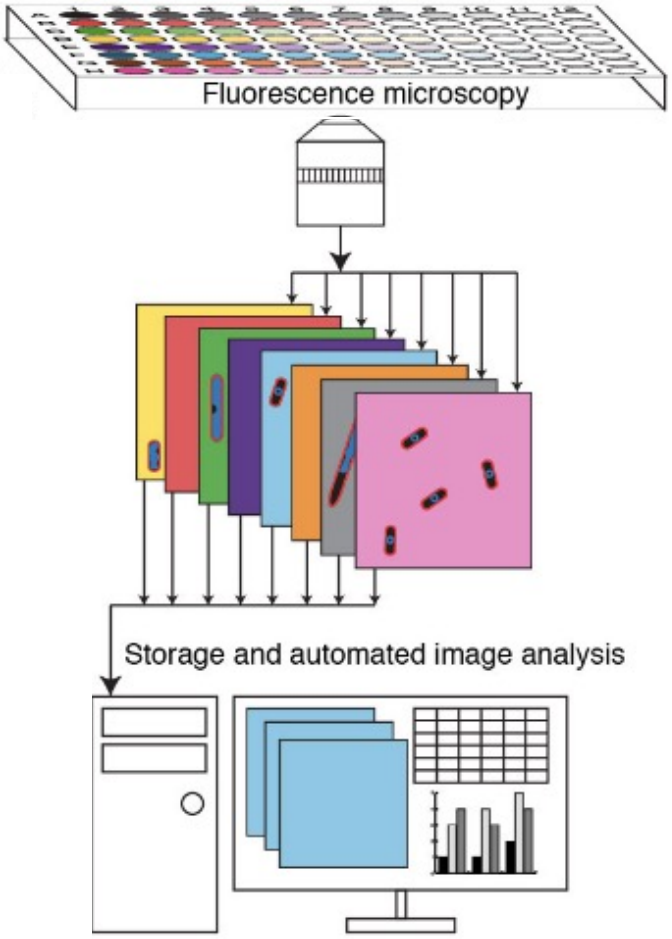
Zaman Atlamalı Mikroskobik Yöntemler

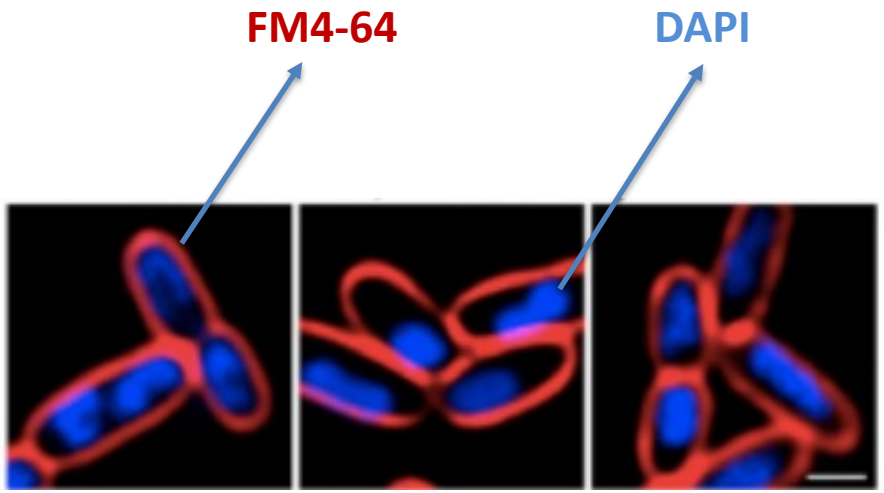
- Üssel artışın inanılmaz gücü!



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

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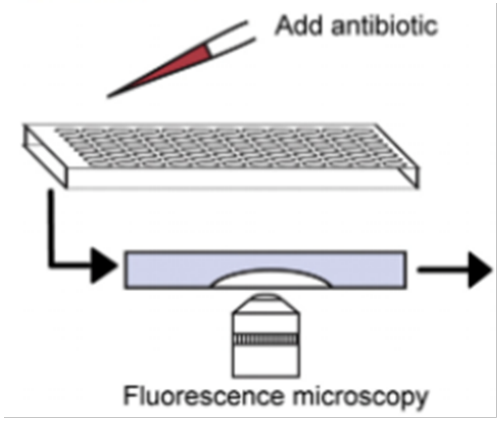
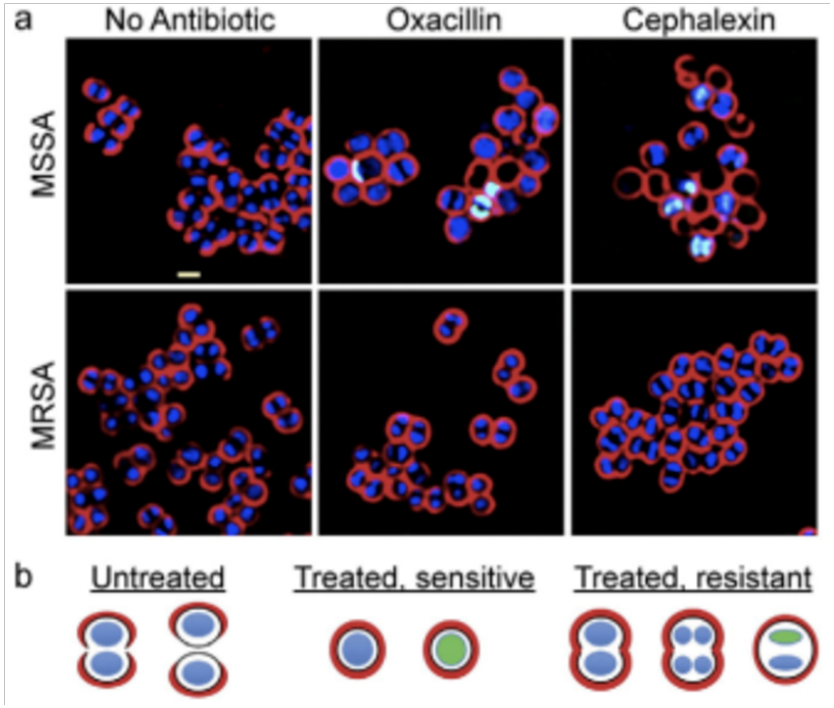
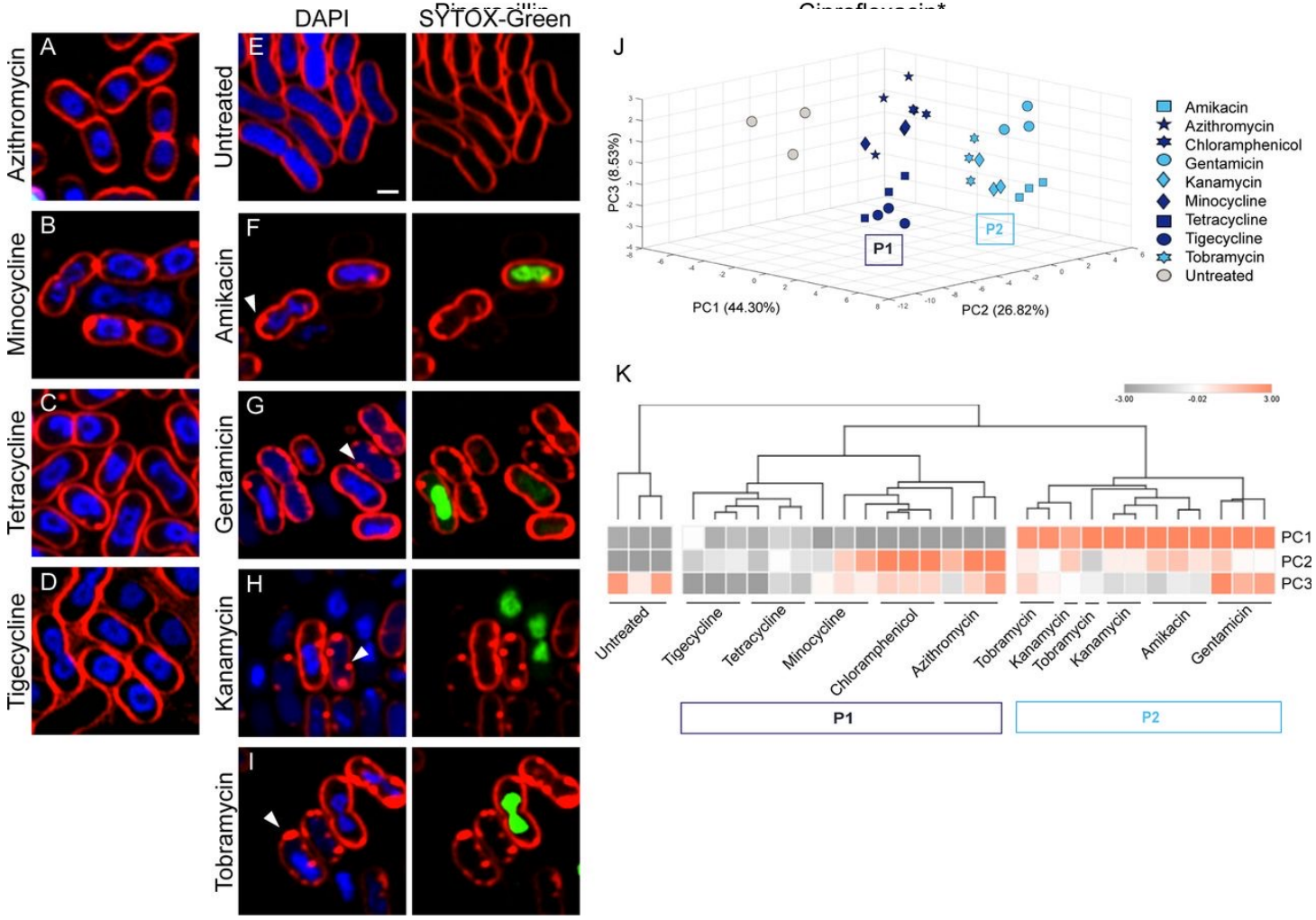
journal homepage: www.ebiomedicine.com





Floresans Mikroskobik Yöntemler

- A. baumanii için BCP platformunun altı ana antibiyotik sınıfı arasında ayrım yapabiliyor.

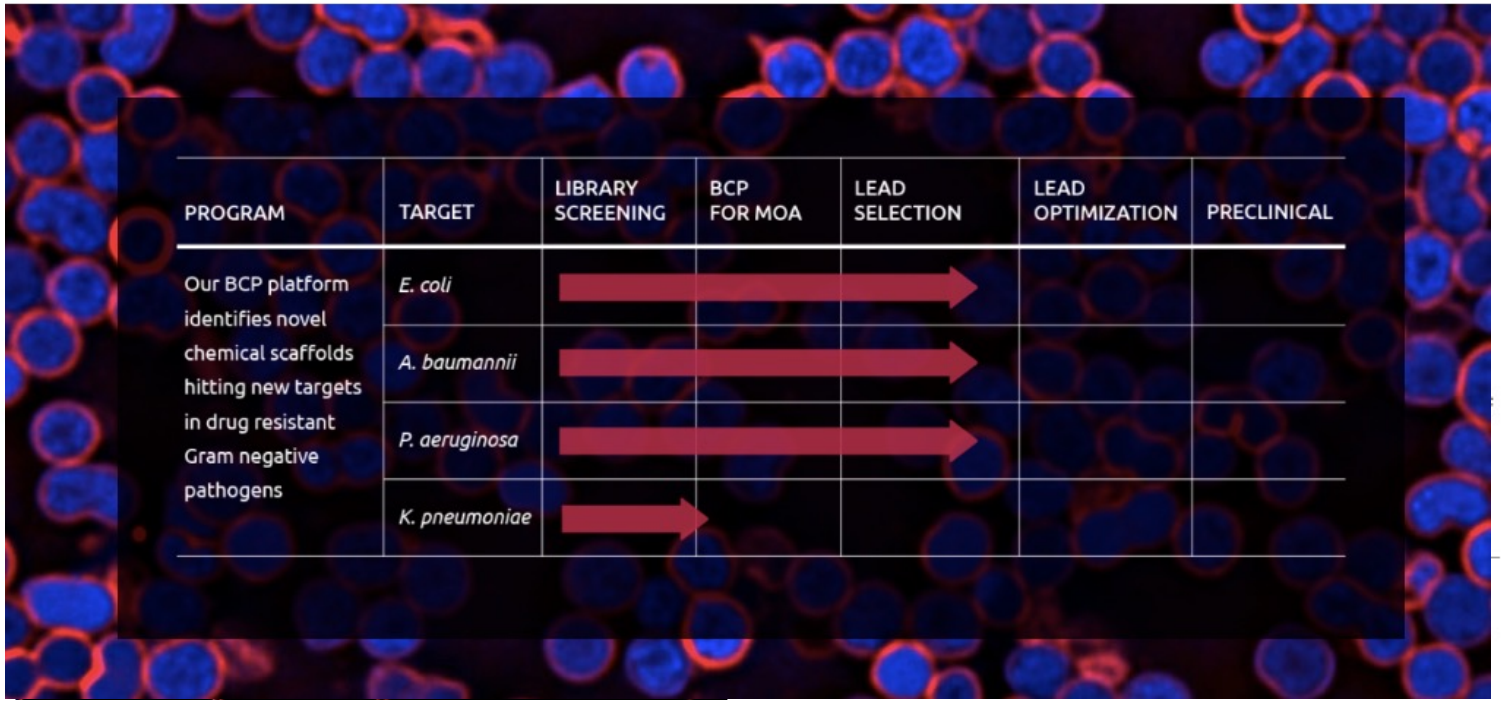


Floresans Mikroskobik Yöntemler

LINNAEUS BIOSCIENCE

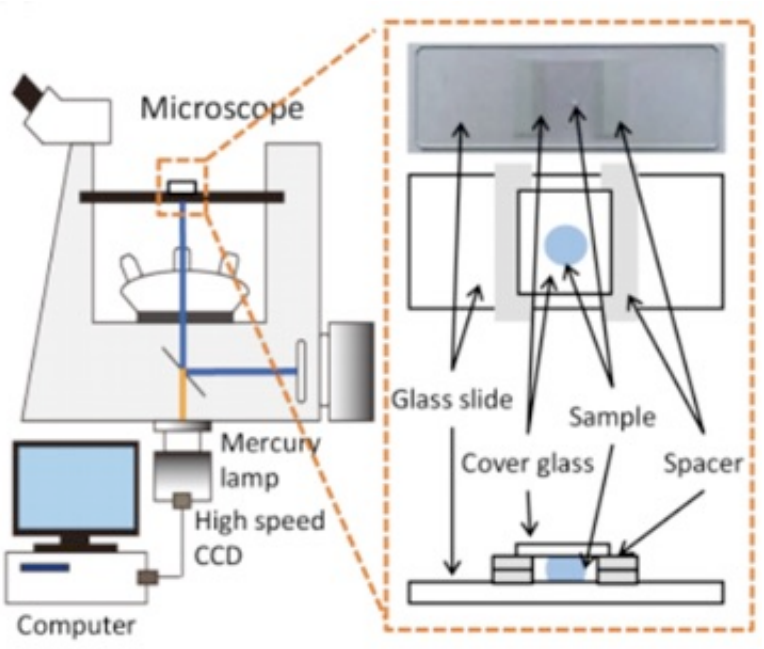
ABOUT US TECHNOLOGY SERVICES PIPELINE CONTACT

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.



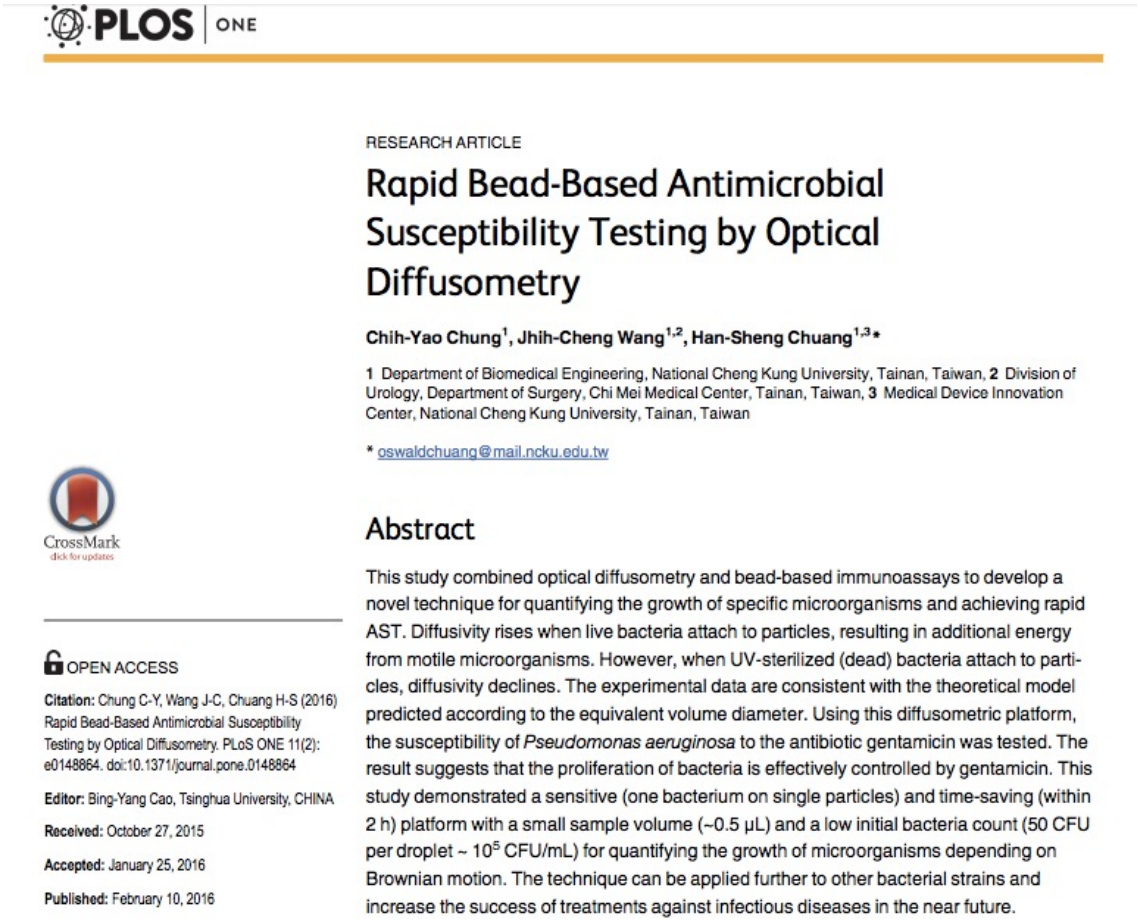
Optikal Difüzometri

- 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

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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

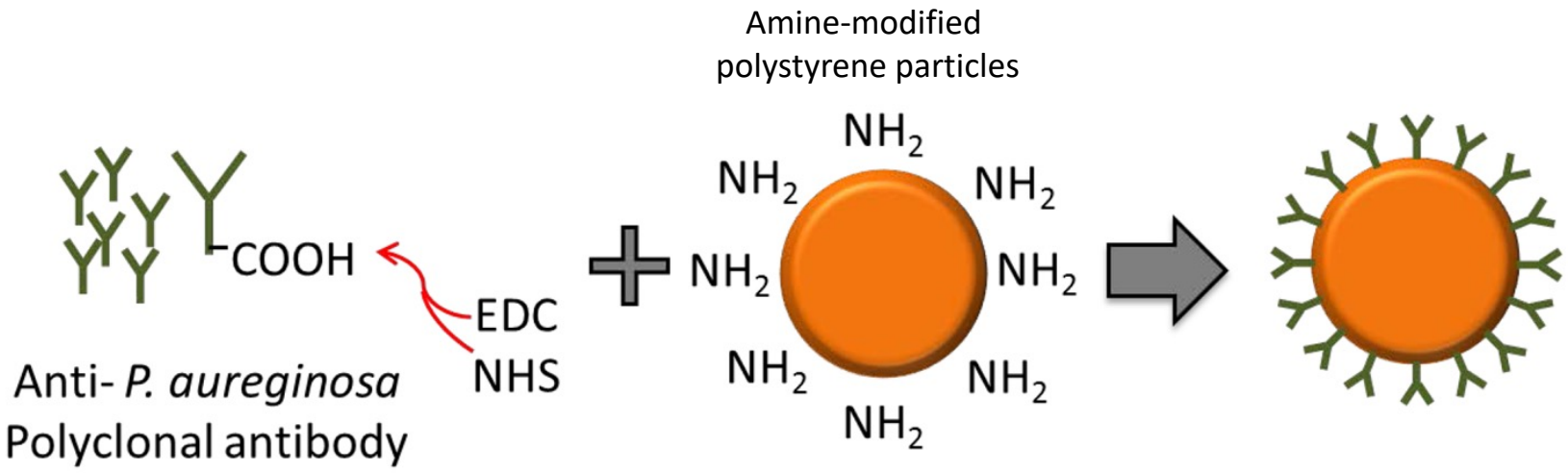
Abstract

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

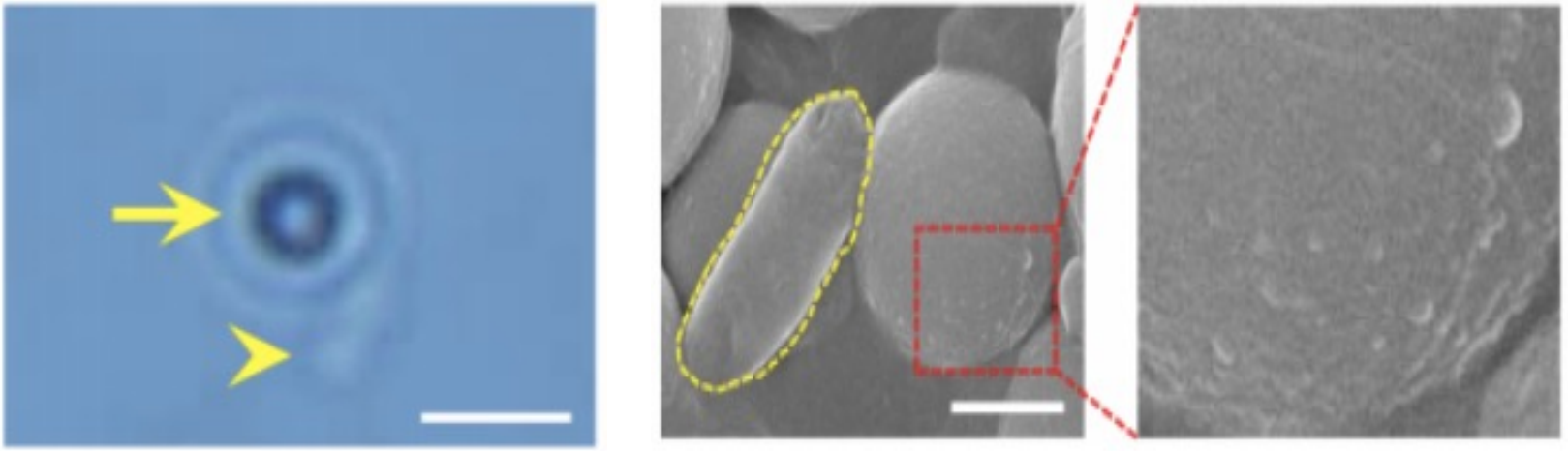
- Antikorun aktive edilmesinde EDC-NHS kimyasalları kullanılarak antikorun polystyrene matrikse bağlanması.

1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)
N-hydroxysuccinimide (NHS)



Optikal Difüzometri

- Boncuklar üzerinde yakalanmış *P. aeruginosa*.



Optikal Difüzometri

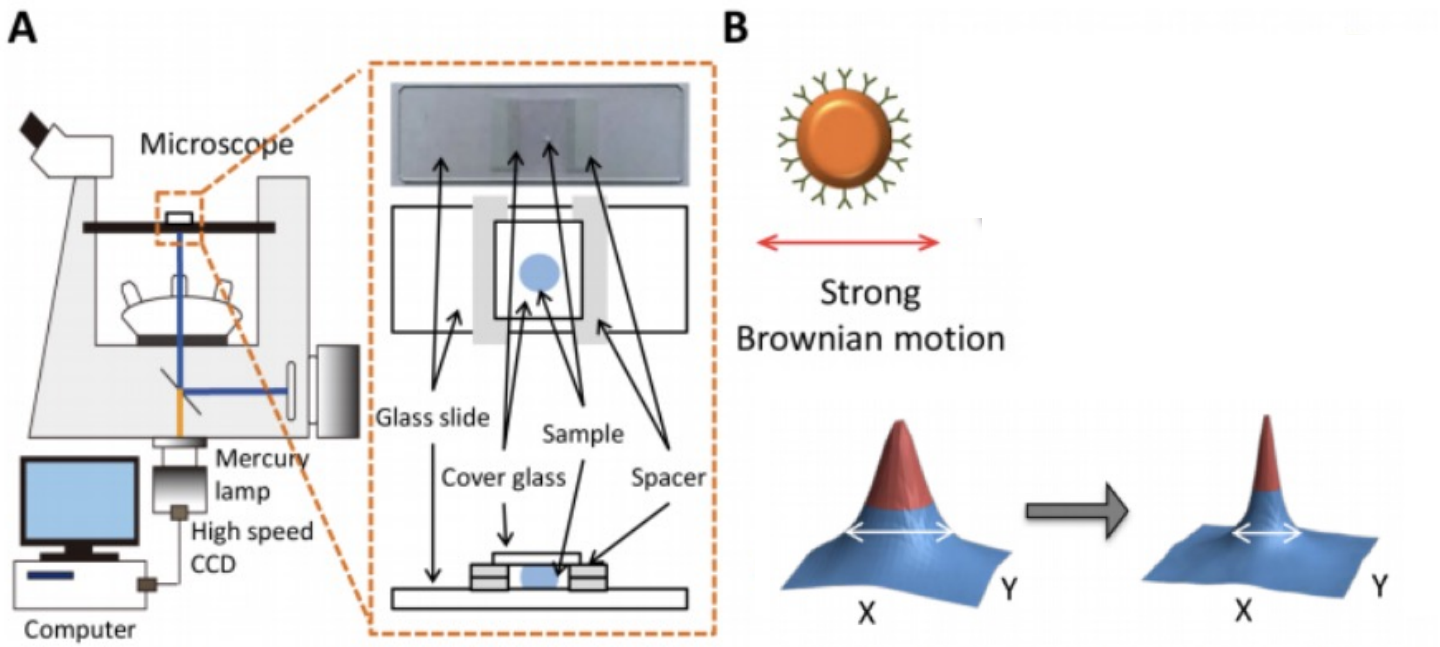
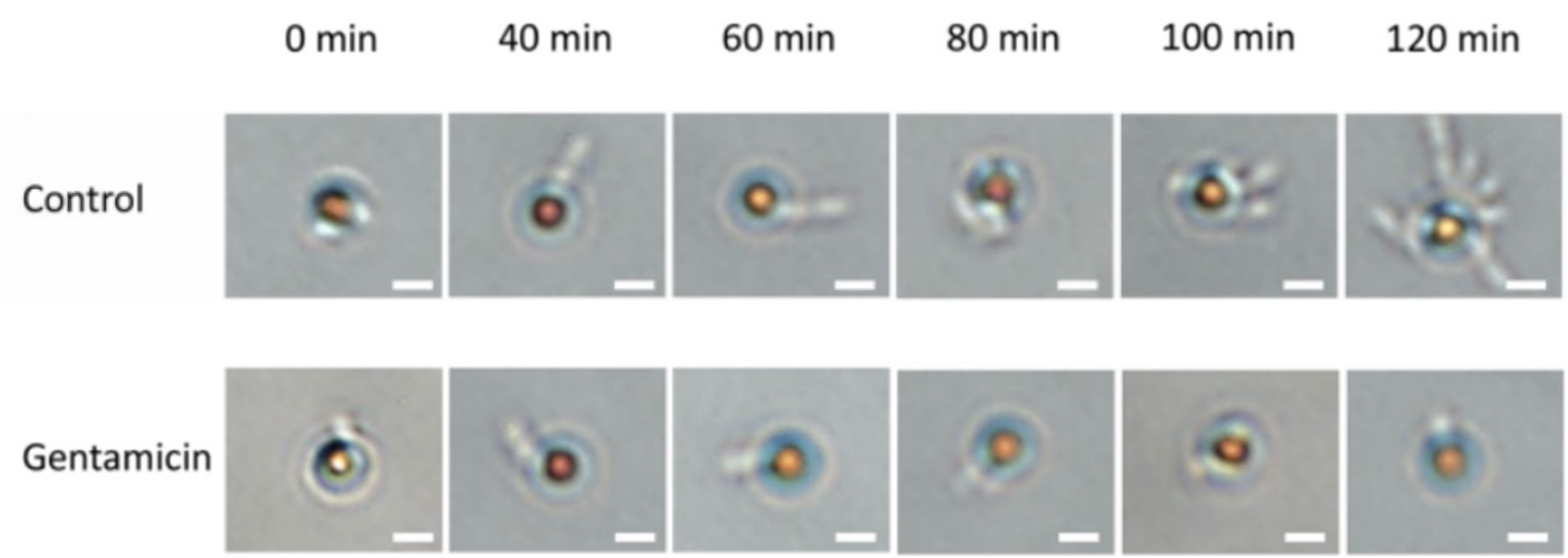


Fig 1. The optical diffusometric platform. (A) Schematic of the optical diffusometry. (B) The relationship of Brownian motion and the particle size change due to the bacterium-particle binding. The corresponding diffusivity values are derived from the cross-correlation algorithm. A large particle diameter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

doi:10.1371/journal.pone.0148864.g001

Optikal Difüzometri



İnavasyon ve Mikrobiyoloji

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

ACCELERATE
DIAGNOSTICS

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.

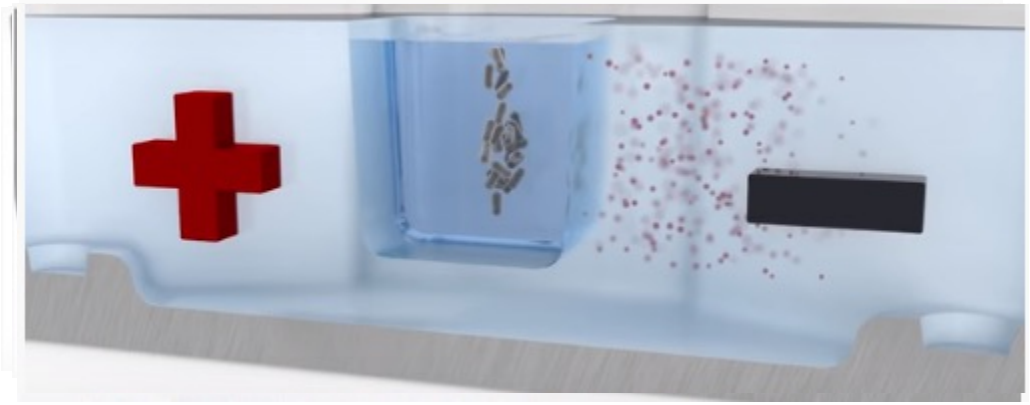
This earlier infection intelligence equips hospital teams to reduce adverse effects of antibiotic overuse — and gain ground against sepsis morbidity and mortality — by ensuring patients receive optimal, individualized sepsis treatment as quickly as possible.

İnavasyon ve Mikrobiyoloji

- Accelerate PhenoTest™ BC

  Örnek muayene maddesinden mikroorganizmanın saflaştırılması için

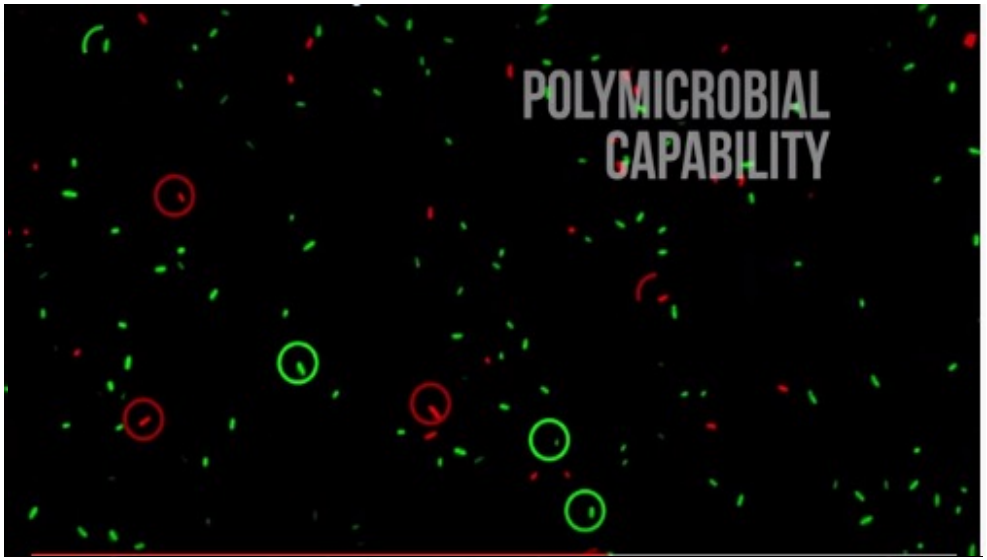
  elektroforez ve filtreleme sistemi (elektrofiltrasyon).



İnavasyon ve Mikrobiyoloji

- Accelerate PhenoTest™ BC

  Multiplexed Fluorescence in situ Hybridization (FISH)



İnavasyon ve Mikrobiyoloji

- Accelerate PhenoTest™ BC

  Antimikrobial duyarlılık testi;

  zaman-atlamalı mikroskop ile üreme/inhibisyonun tespiti.

İnavasyon ve Mikrobiyoloji

- 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

İnavasyon ve Mikrobiyoloji

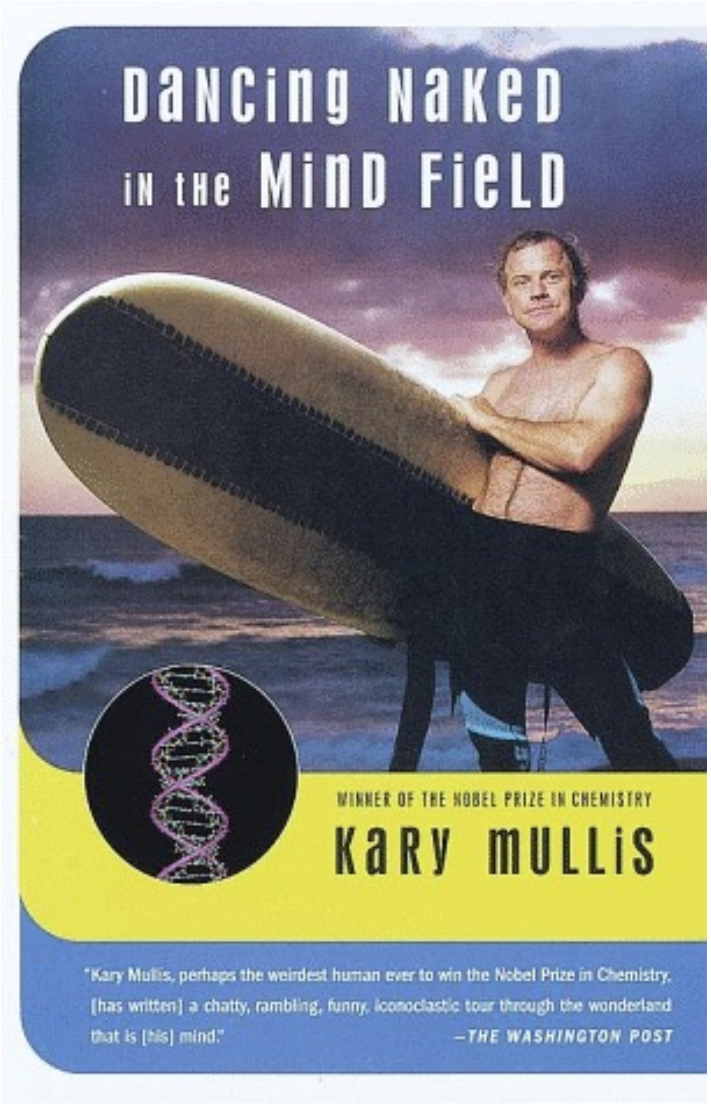
- Accelerate PhenoTest™ BC

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



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 isolat 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 serop men. This method of DNA amplification days, whereas virus isolation takes up to used to complement or replace virus isolat 1 infection.

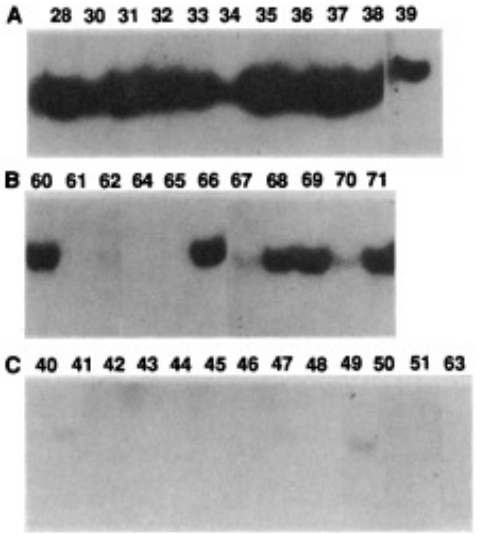


Fig. 1. (**A** to **C**) Representative DNA amplification analysis of peripheral blood lymphocyte DNA from HIV-1–seropositive and seronegative persons (see Table 1). DNA samples were amplified for 35 rounds with the primer pair SK68/69 (Table 2) representing a conserved gp41 region, restricted with BstN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

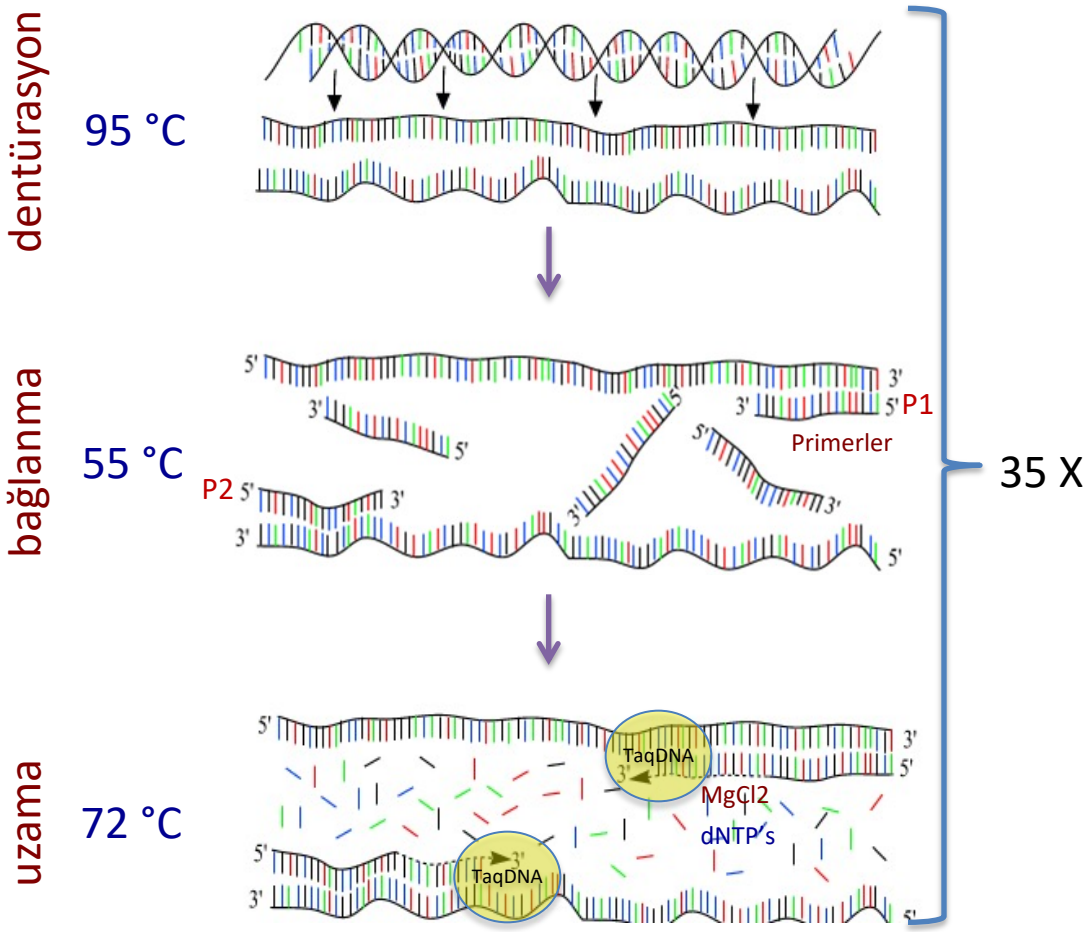
Polymerase Chain Reaction (PCR) Keşfi

PCR

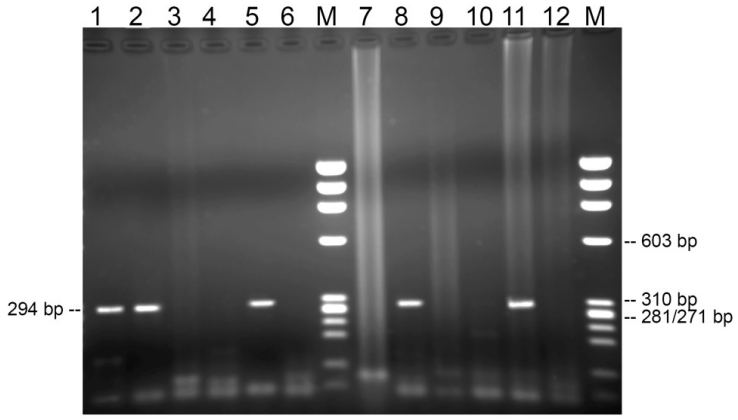
- TMA
- LAMP
- NASBA
- *bDNA*
- *Hybrid Capture*

Turgut Özal Tıp Merkezi

Polymerase Chain Reaction (PCR)

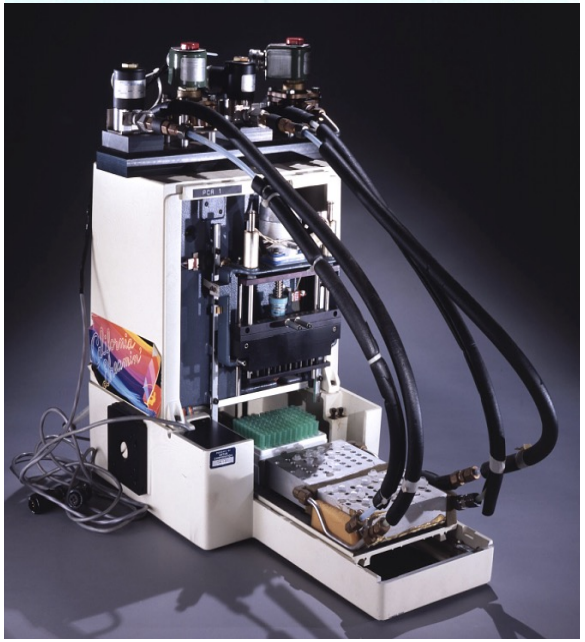


EtBr



Isı döngü cihazlarındaki gelişmeler

- Isı döngü cihazlarındaki gelişmeler



40 cycle PCR in 20 minutes

Isı döngü cihazlarındaki gelişmeler

- Isı döngü cihazlarındaki gelişmeler

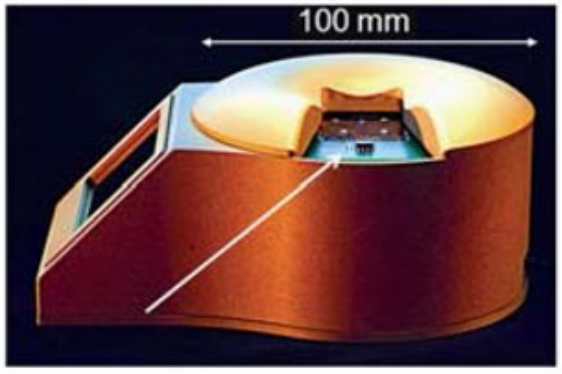
Palm PCR™
G1-12 Portable PCR System

- **Battery-powered stand-alone device**
  More than 4 hours of operation on a single charge
- **Unprecedented high-speed**
  30-cycles within 30 min down to 18 min (≤ 1 kbp)
- **Extreme high-efficiency**
  Near single-copy amplification sensitivity
- **Wide dynamic range**
  Up to 2 kbp with the high-speed and high-efficiency
- **Unmatched well-to-well uniformity**
  Better than ± 0.1°C
- **Conforms to the standard 9 mm well spacing**
  To use disposable plastic Palm PCR sample tubes
- **Simple and adaptable**
  Easy to program and compatible with standard tools

"It is now possible to do PCR anywhere, anytime."

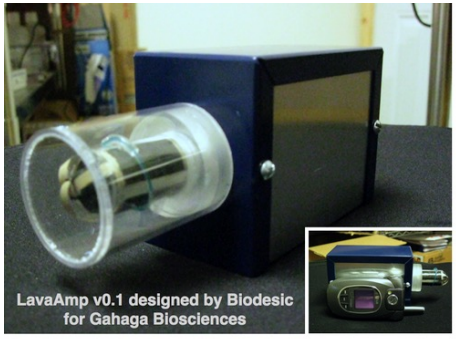
Pocket PCR: The whole chain reaction in his hand
10/26/2010
Andrew S. Wiecek

To advance point-of-care diagnostics in developing countries, a team from the Korean Institute of Science and Technology has developed an affordable, low-powered PCR system that fits in the palm of your hand. Andrew Wiecek reports.



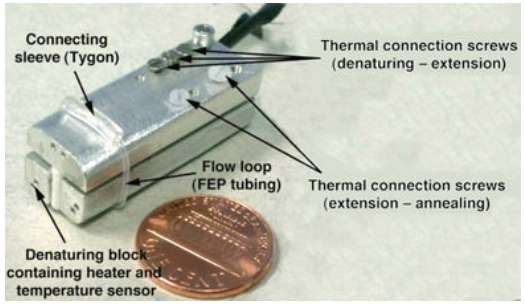
LavaAmp: cheapest pocket PCR thermocycler dreamed for DIY biologists

Posted by attilachordash on October 31, 2009


LavaAmp v0.1 designed by Biodesic for Gahaga Biosciences

Pocket-sized PCR machine

Scientists in the US report being one step closer to designing a miniaturised, portable PCR machine that could be used for applications such as point-of-care diagnostics.



Isı döngü cihazlarındaki gelişmeler

Laboratory Products — Oct 01 2015 12:00 AM

New PCR Thermal Cycler Fits in the Palm of your Hand

Request more information

Reader comments

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Printer friendly version

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Share 0

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Share 0

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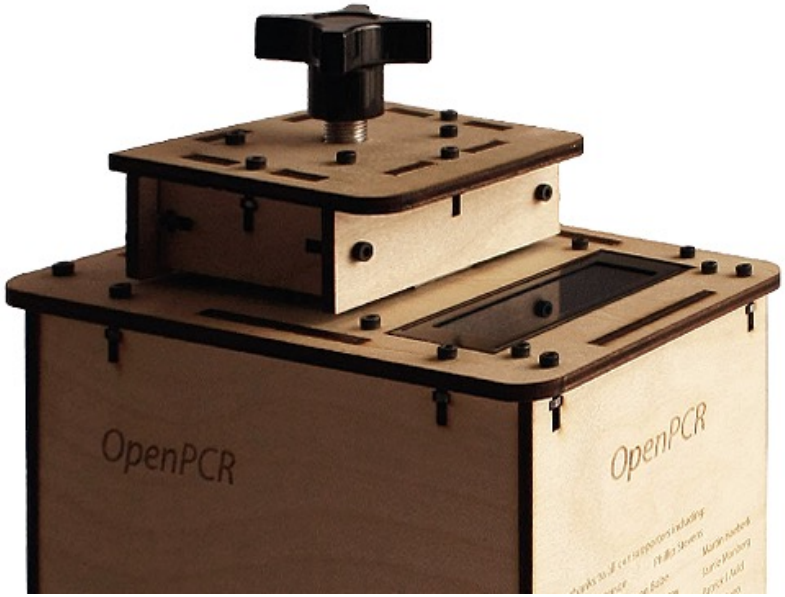
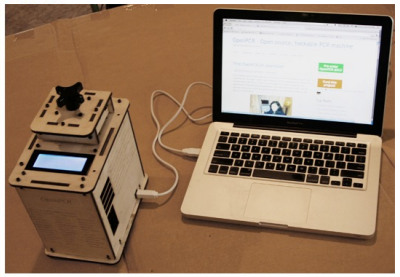
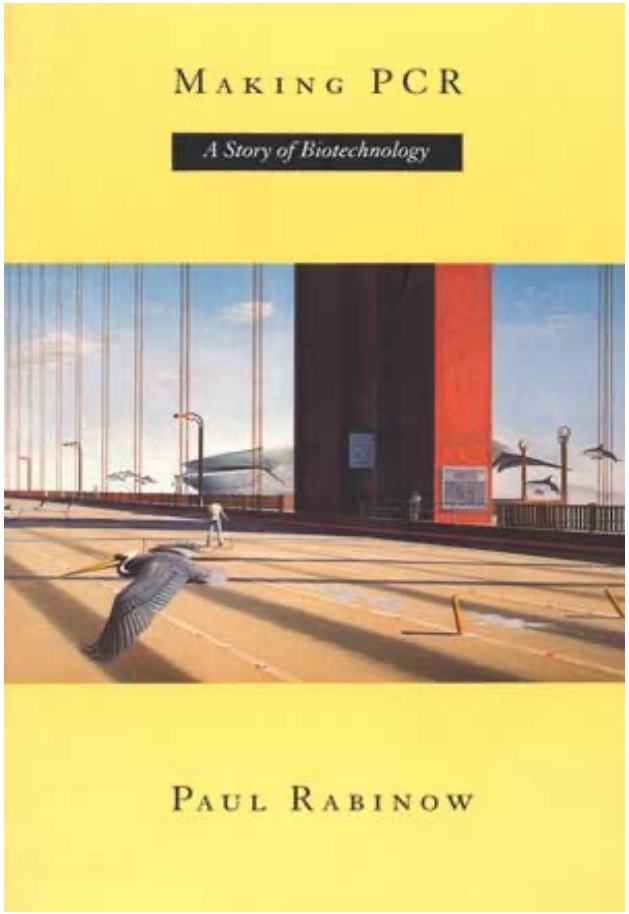
Isı döngü cihazlarındaki gelişmeler





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

- PCR'ın keşfinden sonraki en önemli gelişme.

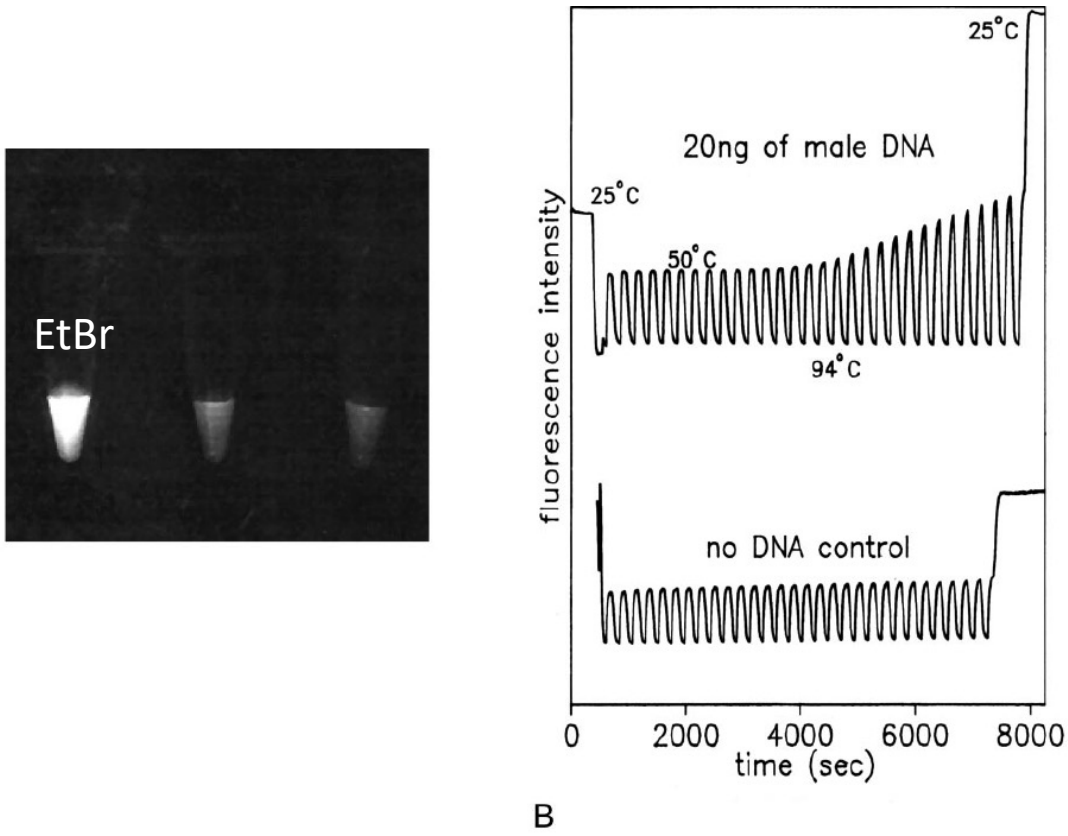
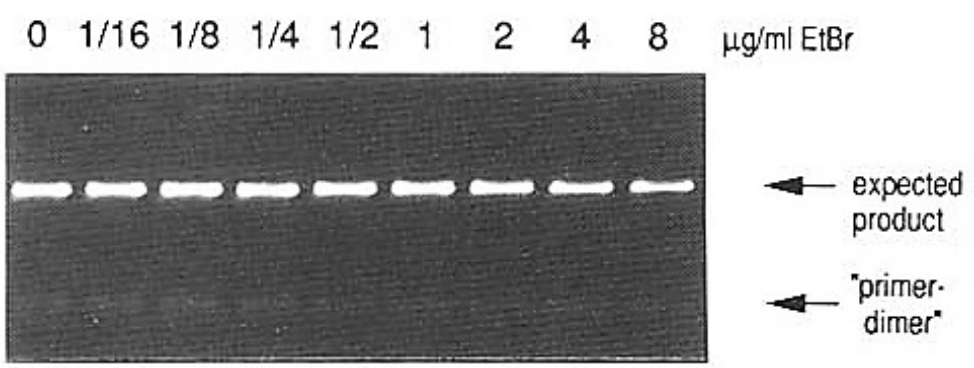


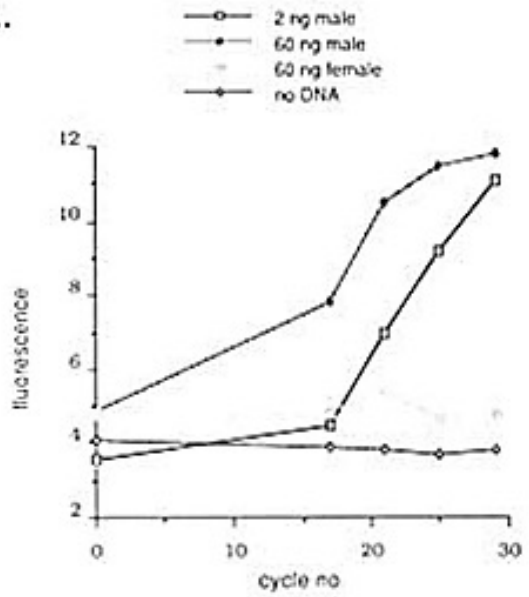
Fig. 1. Real-time visualization of PCR.

(*A*), three completed PCRs in microfuge tubes illuminated by UV light. All tubes contained ethidium bromide. The first tube on the *left* had target sequence cognate to the primers; the two others did not. (*B*), fluorescence traces from ethidium-bromide-containing PCRs taken with a fiber optic cable and a spectrophotometer. The *upper trace* is from a PCR begun with 20 ng of human male DNA and primers specific to a Y-chromosome sequence. The *lower trace* is from a control PCR with the primers but without the target DNA added. Fig. 1B was reprinted with permission from *Biotechnology* 1992;10:413–7.

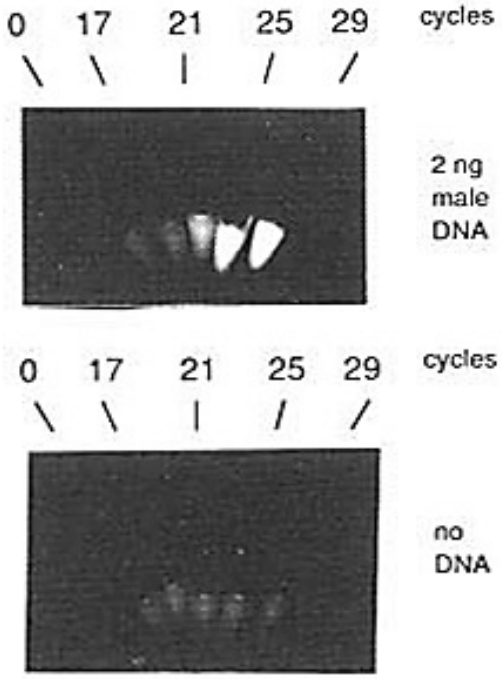
Real-time PCR'ın Keşfi-1992



A.



B.



Real-time PCR'ın Keşfi-1992

nature biotechnology

Research Papers

Nature Biotechnology **10**, 413 - 417 (1992)
doi:10.1038/nbt0492-413

Simultaneous Amplification and Detection of Specific DNA Sequences

Russell Higuchi[1], [*], Gavin Dollinger[2], P. Sean Walsh[1] & Robert Griffith[1]

We have enhanced the polymerase chain reaction (PCR) such that specific DNA sequences can be detected without opening the reaction tube. This enhancement requires the addition of ethidium bromide (EtBr) to a PCR. Since the fluorescence of EtBr increases in the presence of double-stranded (ds) DNA an increase in fluorescence in such a PCR indicates a positive amplification, which can be easily monitored externally. In fact, amplification can be continuously monitored in order to follow its progress. The ability to simultaneously amplify specific DNA sequences and detect the product of the amplification both simplifies and improves PCR and may facilitate its automation and more widespread use in the clinic or in other situations requiring high sample through-put.

- Bunu PCR reaksiyonuna EtBr ekleyerek başarmışlar.

Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences. Biotechnology 1992;10:413-417.

Real-time PCR'ın Keşfi-1992

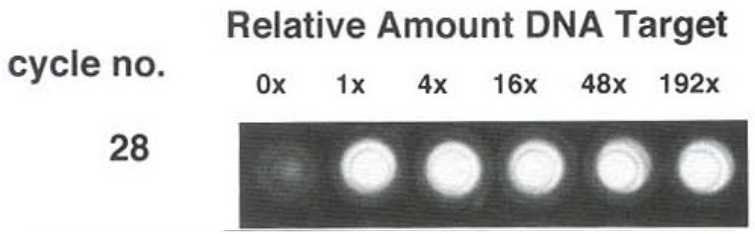


FIGURE 2. Composite of portions of video images taken using the set-up shown in Figure 1. These images are of EtBr-containing PCRs in the thermocycler block (Perkin-Elmer Model 480) and are taken looking down through the tube caps under UV (302 nm) illumination. The three images were of the same six PCRs held at the annealing/extension temperature before beginning thermocycling (cycle 0), and during the annealing/extension phase of cycles 14 and 20. Only one row of samples in the image is shown; a full block of 48 samples in a TC 480 or 96 samples in a TC 9600 can be imaged. The PCRs were initiated using a dilution series of target DNA (a 242 bp segment of HLA DQα gene[18]) at the indicated relative levels (192× = 3×10^{10} target molecules <8 ng DNA). These images demonstrate the general principle that the higher the starting amount of target DNA, the earlier the cycle at which increased fluorescence is detectable.

Real-time PCR'ın Keşfi-1992

Clinical Chemistry 51:3
661–671 (2005)

History

1955 – 2004 50 FIFTY YEARS OF PUBLISHING

Fifty Years of Molecular (DNA/RNA) Diagnostics

THOMAS R. GINGERAS,[1] RUSSELL HIGUCHI,[2] LARRY J. KRICKA,[3] Y.M. DENNIS LO,[4] and CARL T. WITTWER[5,6*]

Real-time PCR was essentially based on a scientific mistake; preferably thought of as an "unplanned experiment."

Real-time PCR: History and Fluorogenic Chemistries.

Real-Time PCR

—Russ Higuchi

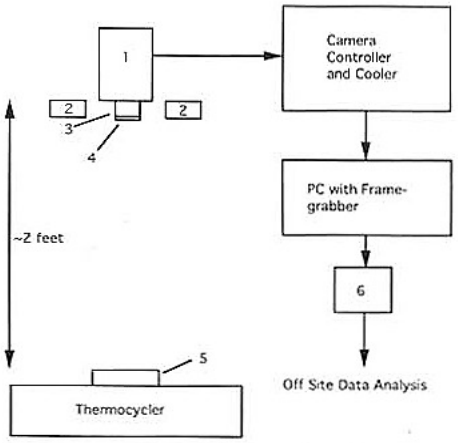
When late in 1986 I joined Cetus, the birthplace of PCR, "closed-tube" PCR using a thermostable DNA polymerase had just been achieved *(1, 2)*. Before this, the thermolabile polymerase had to be added at each cycle. Tom White, our vice-president in charge of Research and Development, told me that what was needed now was some way to visualize the amplification product, also without opening the tube. Such closed-tube detection was termed "homogenous".

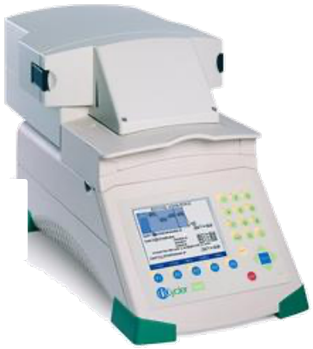
A few years later I was working with Gavin Dollinger on his idea of "tagging" things that needed to be traced (e.g., explosives, money, and pharmaceuticals) with specific, amplifiable DNA sequences *(3)*. We noticed that very high molecular weight DNA was occasionally generated in PCRs from such tagged substances. If we could reproducibly promote such large DNA, Gavin reasoned, it might be detected directly in the tube by light scatter. We set out to use biotinylated primers and streptavidin in the PCR to try to catalyze the formation of long branched chains of amplicons. We succeeded only in forming precipitates. To determine whether DNA was in the precipitates, we added ethidium bromide to the completed PCRs and held the tubes up to ultraviolet (UV) light. The precipitates were fluorescent, but the fluorescence was not specific to the presence of double-stranded DNA (dsDNA). Bob Griffith, who was helping us with these experiments, was directed to try some different conditions. He showed me the gel with the expected DNA band from the control without streptavidin and mentioned that, without thinking, he had added the ethidium bromide at the beginning of the PCR.

I had two reactions. One was surprise that the PCR had worked at all in the presence of ethidium bromide, a known DNA polymerase inhibitor. Indeed, PCR would have been inhibited if the concentration of ethidium bromide had been somewhat higher. The second was to realize that because PCR creates much more dsDNA than is put into it, there should be more ethidium bromide fluorescence created as well. Such fluorescence would be detectable without opening the tube.

The PCRs were repeated without streptavidin. When held up to a UV light, the tube containing the amplification target glowed brightly compared with negative controls (Fig. 1A). Although this "endpoint" reading of PCR was of itself very useful, we thought that taking fluorescence readings on a cycle-by-cycle basis might provide a

Real-time PCR'ın Keşfi-1992

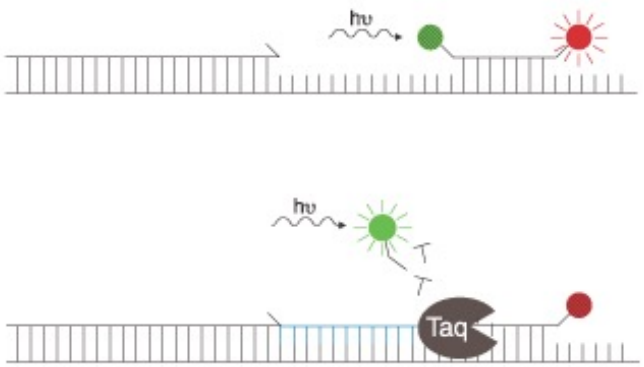




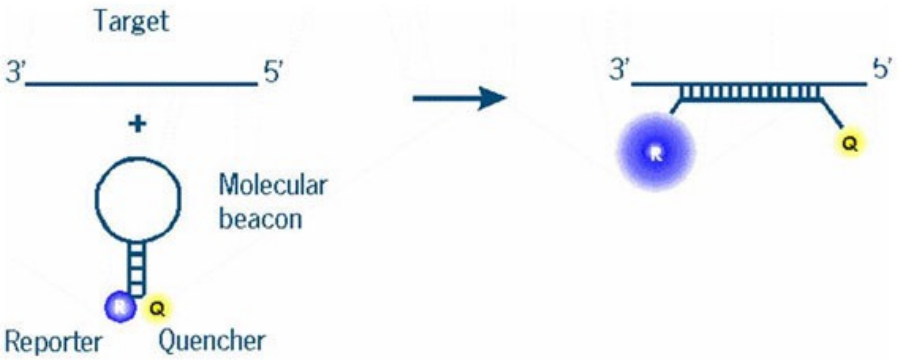
Real-time PCR'ın Keşfi-1992

- Real-time PCR yönteminde kullanılan problar

TaqMan Prob



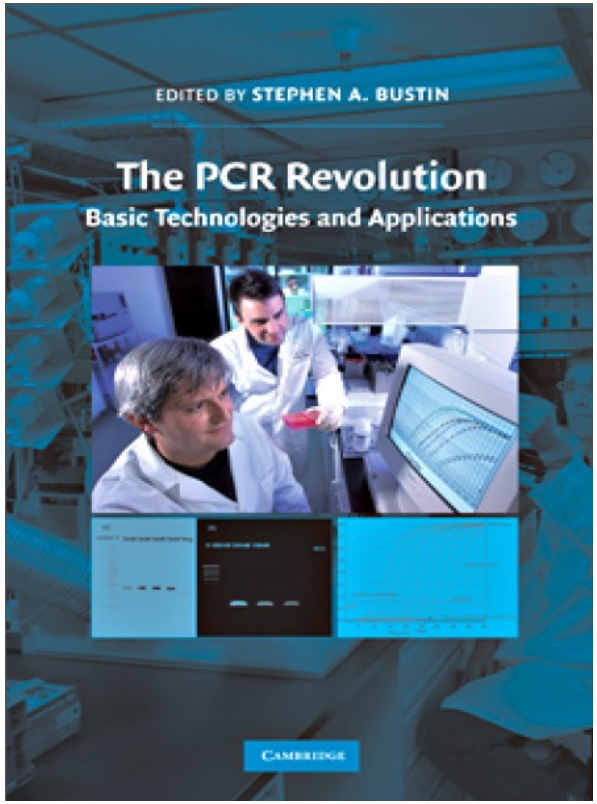
Molecular Beacons



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 car
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 so
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

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

Amplifikasyon Temelli Yöntemler

- Yeni yaklaşımlar
  - *İzolasyondan itibaren otomatize sistemler*

Amplifikasyon Temelli Yöntemler

- Yeni yaklaşımlar
  - *İzolasyondan itibaren otomatize sistemler*

Real-time PCR Cihazları

- Yeni yaklaşımlar
  - Nükleik asit izolasyonu ve gerçek zamanlı PCR

Healthcare Associated Infections

- Xpert MRSA
- Xpert SA Nasal Complete
- Xpert MRSA/SA SSTI
- Xpert MRSA/SA BC
- Xpert C. difficile
- Xpert C. difficile/Epi
- Xpert vanA
- Xpert Norovirus
- Xpert Carba-R

Critical Infectious Diseases

- Xpert MTB/RIF
- Xpert Flu
- Xpert Flu/RSV XC
- Xpert EV

Sexual Health

- Xpert CT/NG
- Xpert GBS
- Xpert GBS LB

Real-time PCR Cihazları

Healthcare Associated Infections

- Xpert MRSA
- Xpert SA Nasal Complete
- Xpert MRSA/SA SSTI
- Xpert MRSA/SA BC
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- Xpert C. difficile/Epi
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Critical Infectious Diseases

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- Xpert EV

Sexual Health

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- Xpert GBS
- Xpert GBS LB

Amplifikasyon Temelli Yöntemler

- Yeni yaklaşımlar
  - İzolasyondan itibaren otomatize sistemler

Cepheid.

« Previous Release | Next Release »

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**

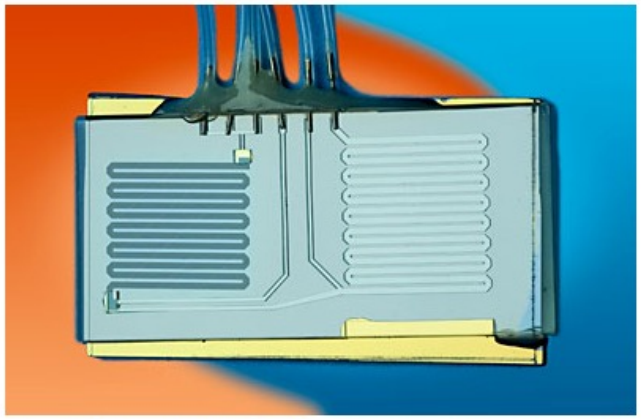
- **Ubiquitome Quickens Pace of POC Apps for Its Freedom4**
- **Cepheid Unveils its POC Diagnostics System**
- **Hopkins Crew Brews "Coffee Mug-Sized" Gizmo for Fully Automated Chlamydia Testing**

Amplifikasyon Temelli Yöntemler

- Yeni yaklaşımlar
  - *Microfludic* cihazlar

Portable device detects anthrax in under an hour

August 1, 2011 By Krishna Ramanujan

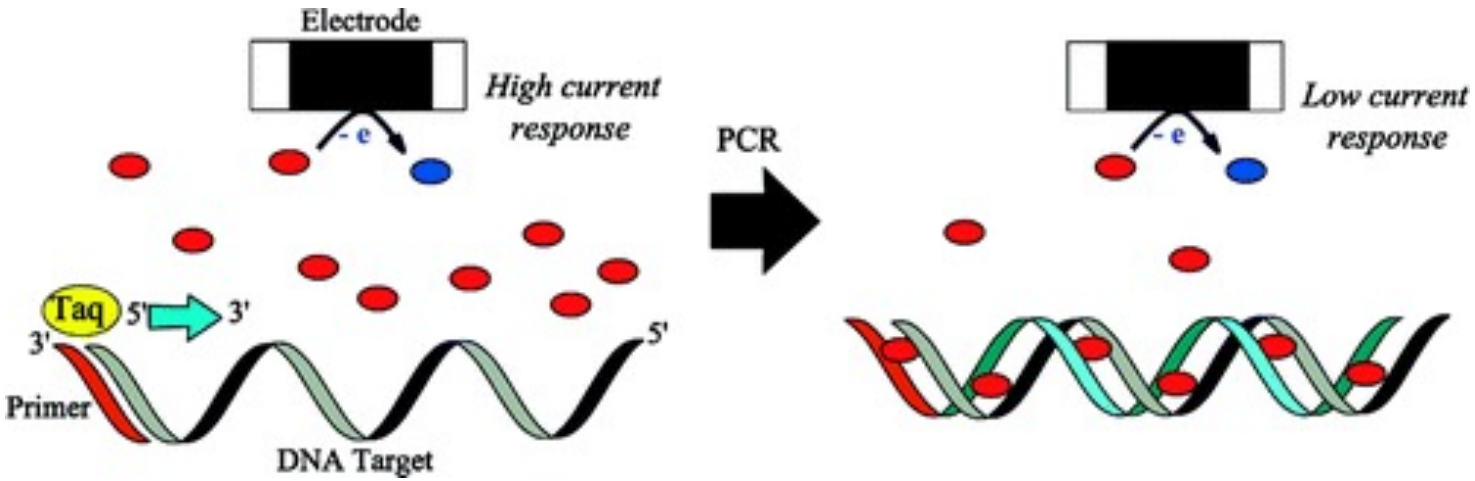


This photograph shows the device's microfluidic chip, which measures approximately one centimeter by 3 centimeters and integrates sample purification and real-time PCR analysis chambers. Credit: Kent Loeffler, Cornell University

A portable device can detect the presence of the anthrax bacterium in about one hour from a sample containing as few as 40 microscopic spores, report Cornell and University of Albany researchers who invented it. The device could provide early detection in the case of an anthrax attack, saving many lives.

Amplifikasyon Temelli Yöntemler

- Yeni yaklaşımlar
  - *Elektrokimyasal Real-Time PCR*
    - PCR ile çoğaltılan hedef nükleik asitin optik olmayan yöntemlerle monitörize edilir.
    - Reaksiyon karışımında yer alan *redox prop*, çoğalan dsDNA'ya bağlanır (interkale olur) ve ortamın elektrokimyasal sinyal oranını azaltır.
    - Hassas voltmetre cihazları ile ölçülen elektrokimyasal potansiyel fark, hedef nükleik asitin çoğaldığını gösterir



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

Amplifikasyon Temelli Yöntemler

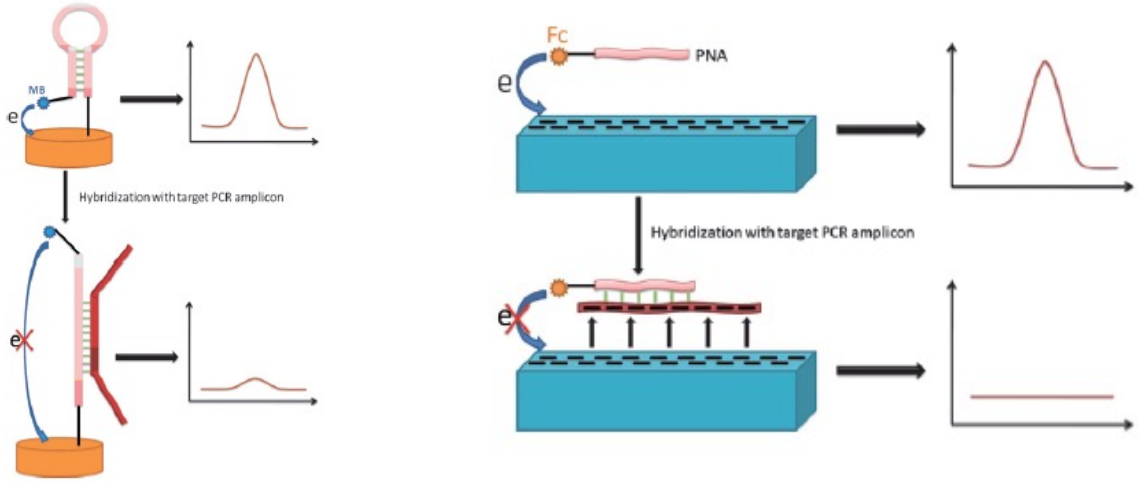
- Yeni yaklaşımlar
  - *Elektrokimyasal Real-Time PCR*

MINIREVIEW www.rsc.org/analyst | Analyst

Electrochemical techniques on sequence-specific PCR amplicon detection for point-of-care applications

Xiaoteng Luo[a] and I-Ming Hsing*[ab]

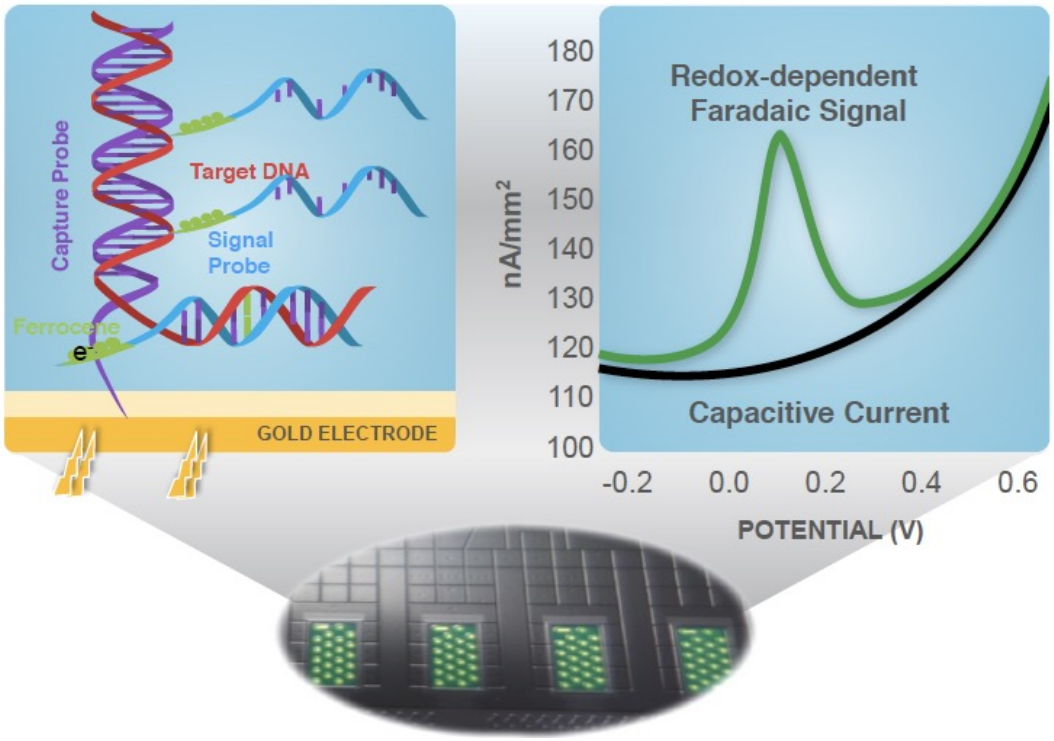
First published as an Advance Article on the web 18th August 2009
DOI: 10.1039/b912653h



Amplifikasyon Temelli Yöntemler

- Yeni yaklaşımlar
  - *Elektrokimyasal Real-Time PCR*

Electrochemical detection enabling high degree multiplexing



Amplifikasyon Temelli Yöntemler

Curiosity Diagnostics

Help when you need it most

PCR|ONE For Investors About Us

(I)
COLLECT SAMPLE

SEPSIS GOLDEN HOUR

Saving patients with systemic infections requires rapid administration of drugs. PCR|ONE system allows drugs to be specifically targeted for optimal treatment.

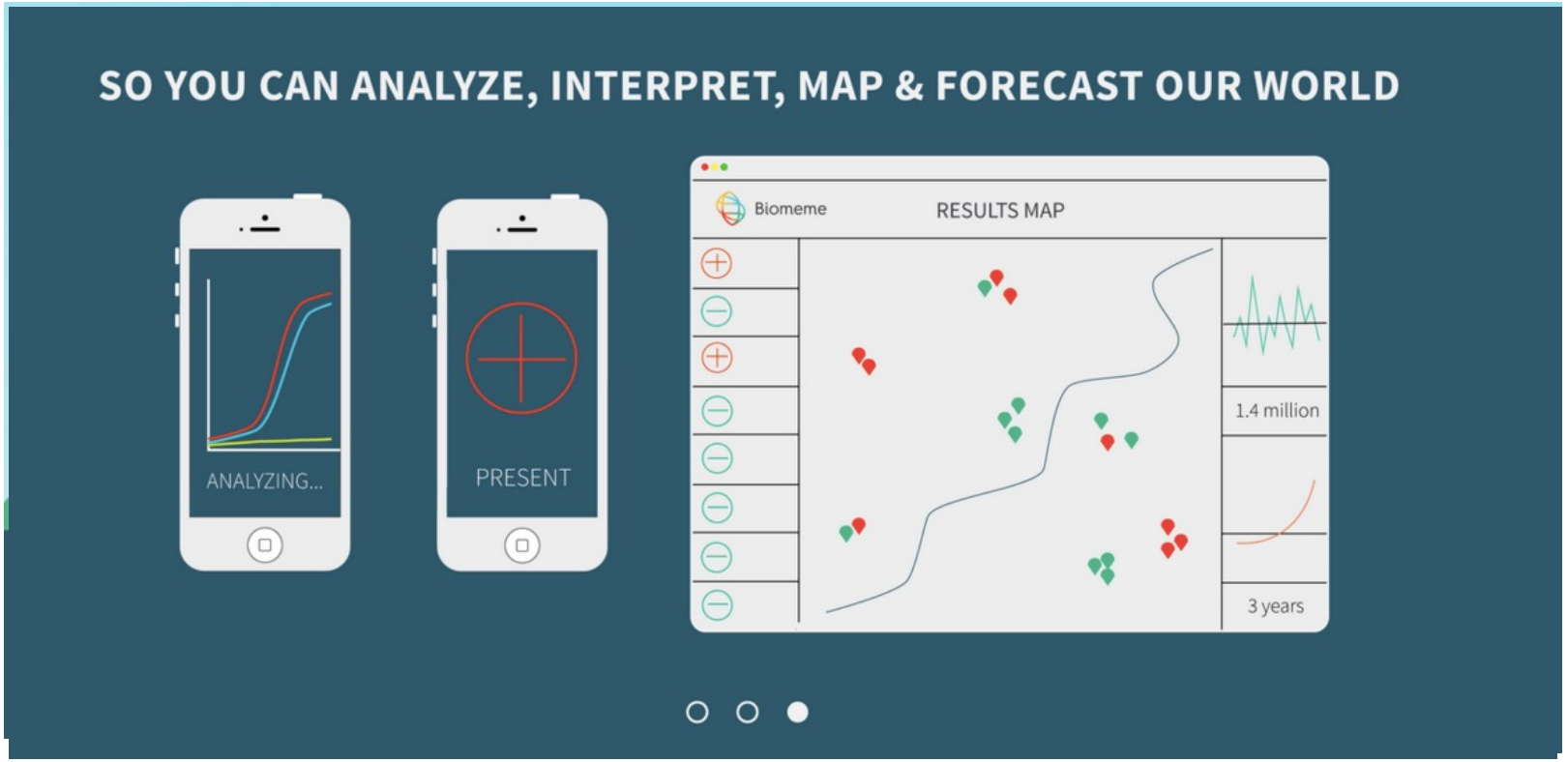
Amplifikasyon Temelli Yöntemler

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

Amplifikasyon Temelli Yöntemler

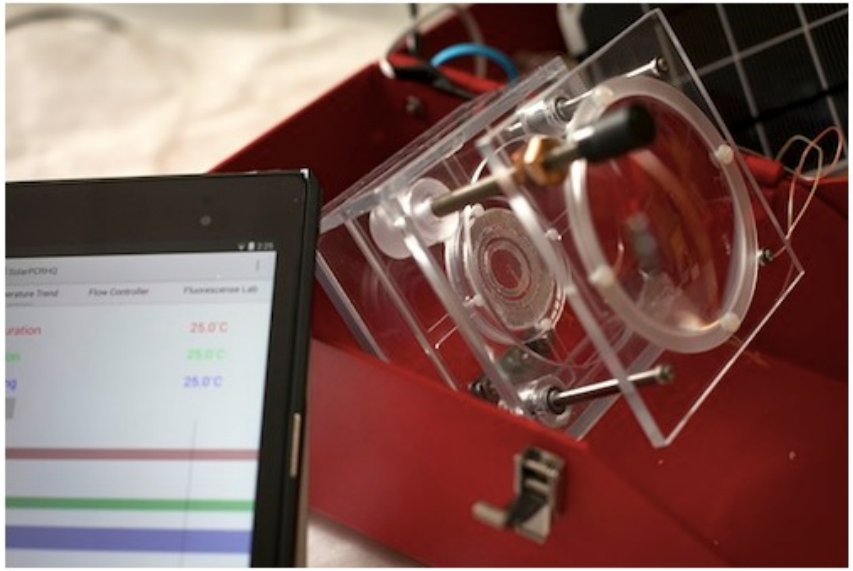
Biomeme Sample Prep for Children

Amplifikasyon Temelli Yöntemler



Amplifikasyon Temelli Yöntemler

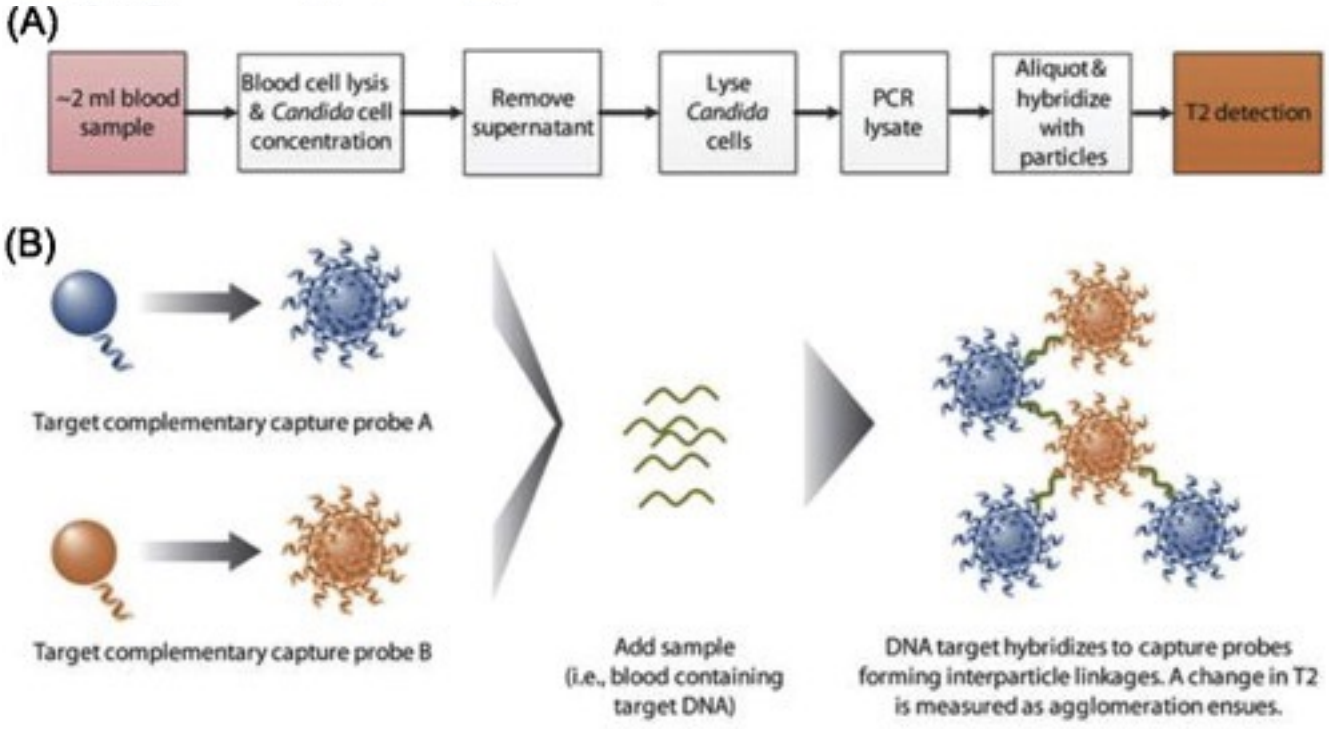
Sun And Smartphone Power



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.

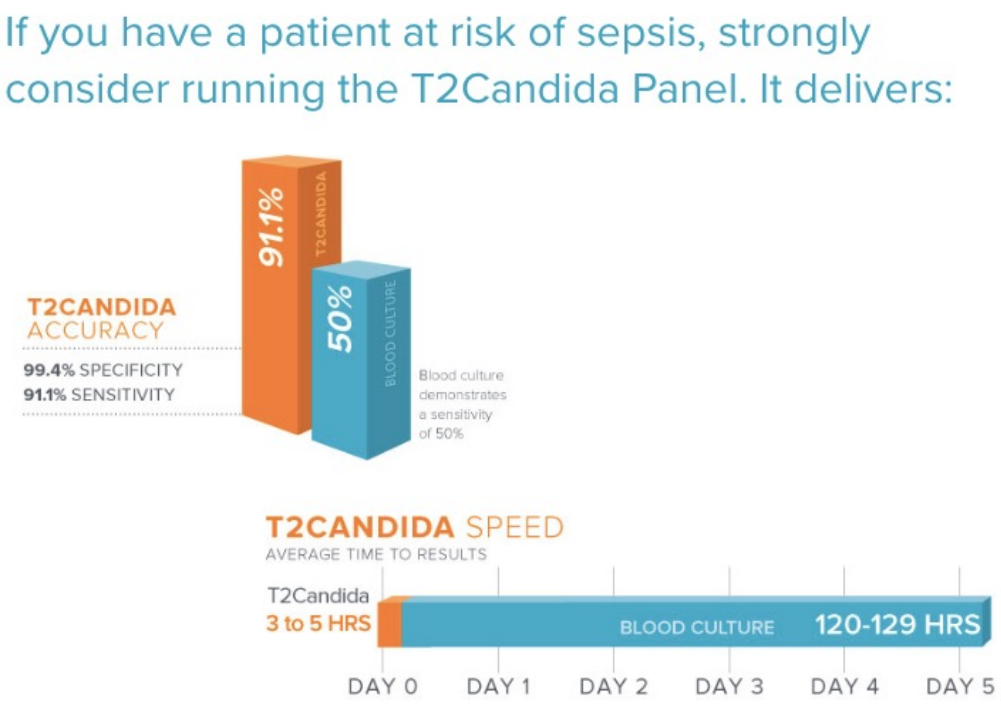
Amplifikasyon Temelli Yöntemler

- *T2 Candida Panel- magnetic resonance*



Amplifikasyon Temelli Yöntemler

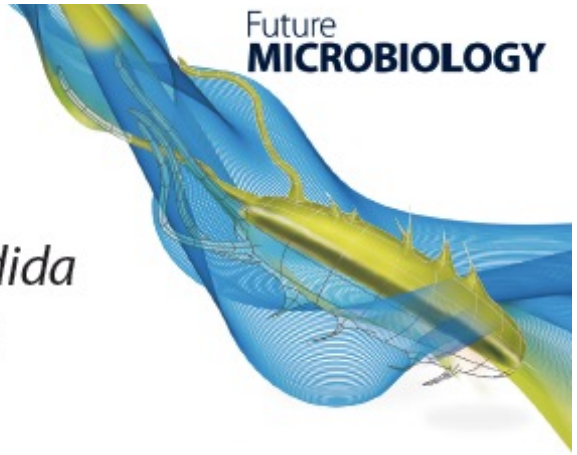
- *T2 Candida Panel- magnetic resonance*



Amplifikasyon Temelli Yöntemler

- *T2 Candida Panel- magnetic resonance*

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



RESEARCH ARTICLE

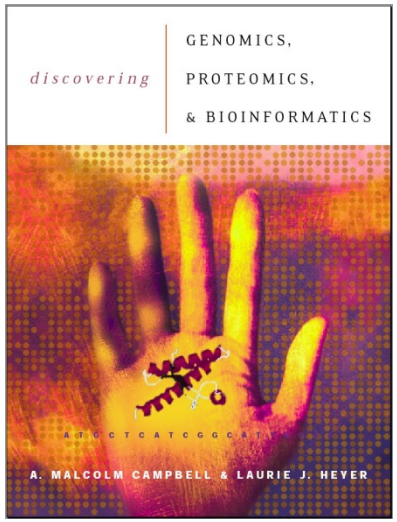
For reprint orders, please contact: reprints@futuremedicine.com

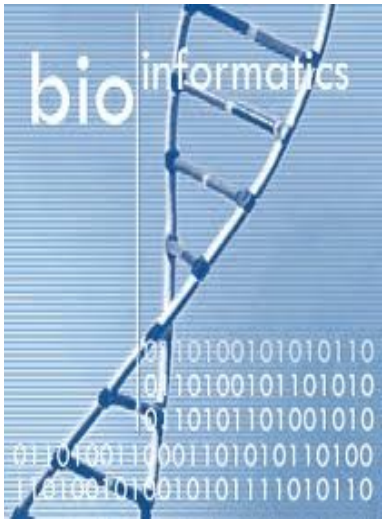
The economic impact of rapid *Candida* species identification by T2Candida among high-risk patients

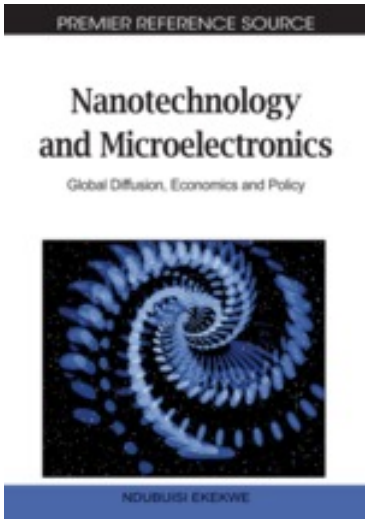
Sara Pinar Bilir*[1], Cheryl P Ferrufino[1], Michael A Pfaller[2,3] & Julie Munakata[1]

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

- Genomik, biyoinformatik ve mikroelektronik alanında yaşanan hızlı gelişmelerin en göze çarpan sonuçları,
  - Biyosensörlerdir 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.

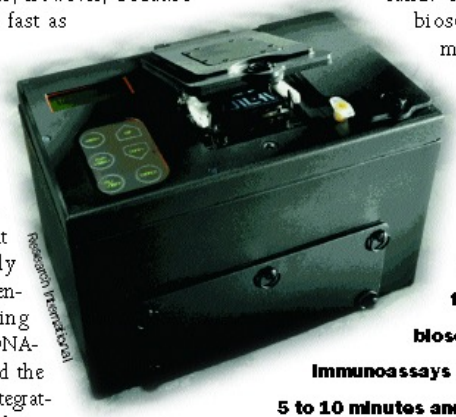


Figure 2. Fiber-optic, fully automated biosensor performs four immunoassays simultaneously in 5 to 10 minutes and shows the results on an LCD screen in words.

nostics, environmental monitoring, and food processing," says S. J. Alcock, 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 Ligler 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-

cose, pion
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sports-phys
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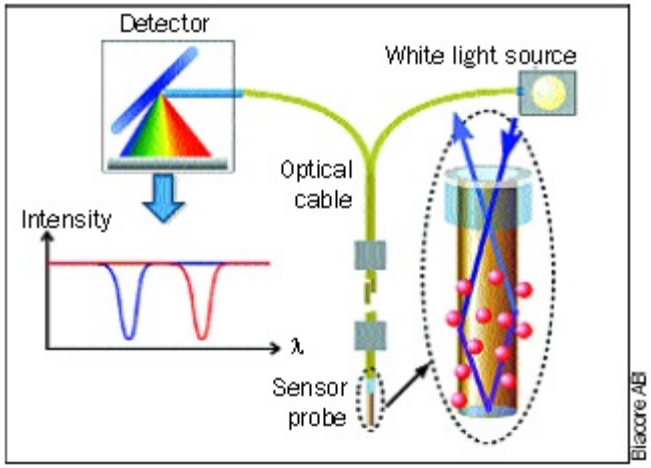
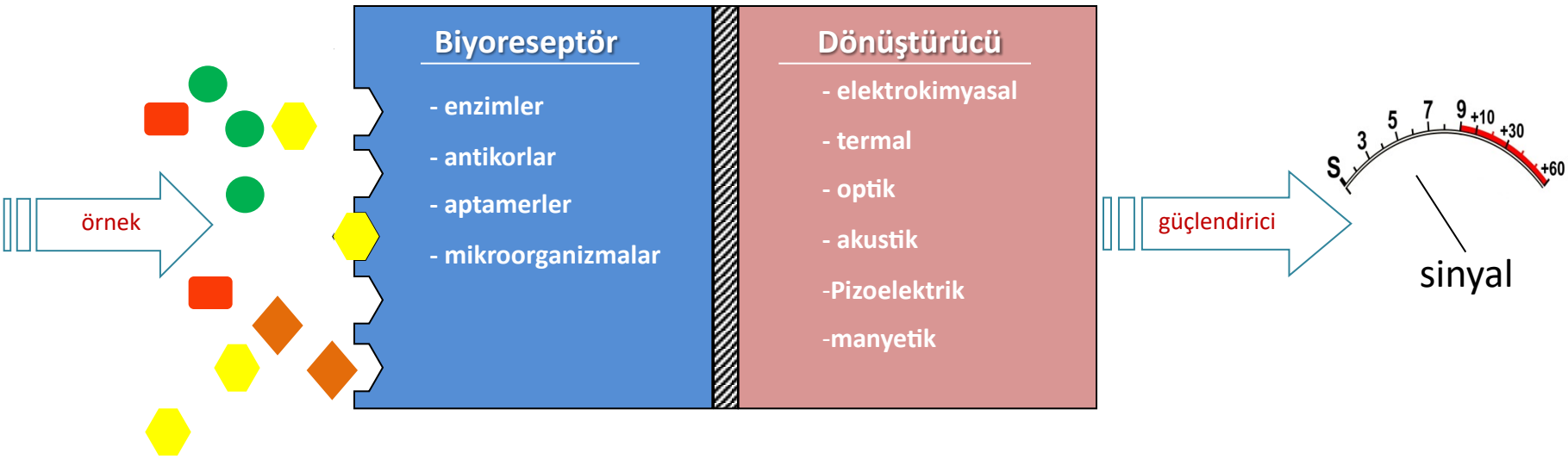
Prompte



Biyosensör Temeli Yöntemler



Biyosensör Temeli Yöntemler

Biyosensör Temeli Yöntemler

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LIFE SCIENCE PATHFINDER

SEARCH

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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 decentralized 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 in-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

- Mantarların tanısında biyosensör mantarlar

SCIENCE ADVANCES | RESEARCH ARTICLE

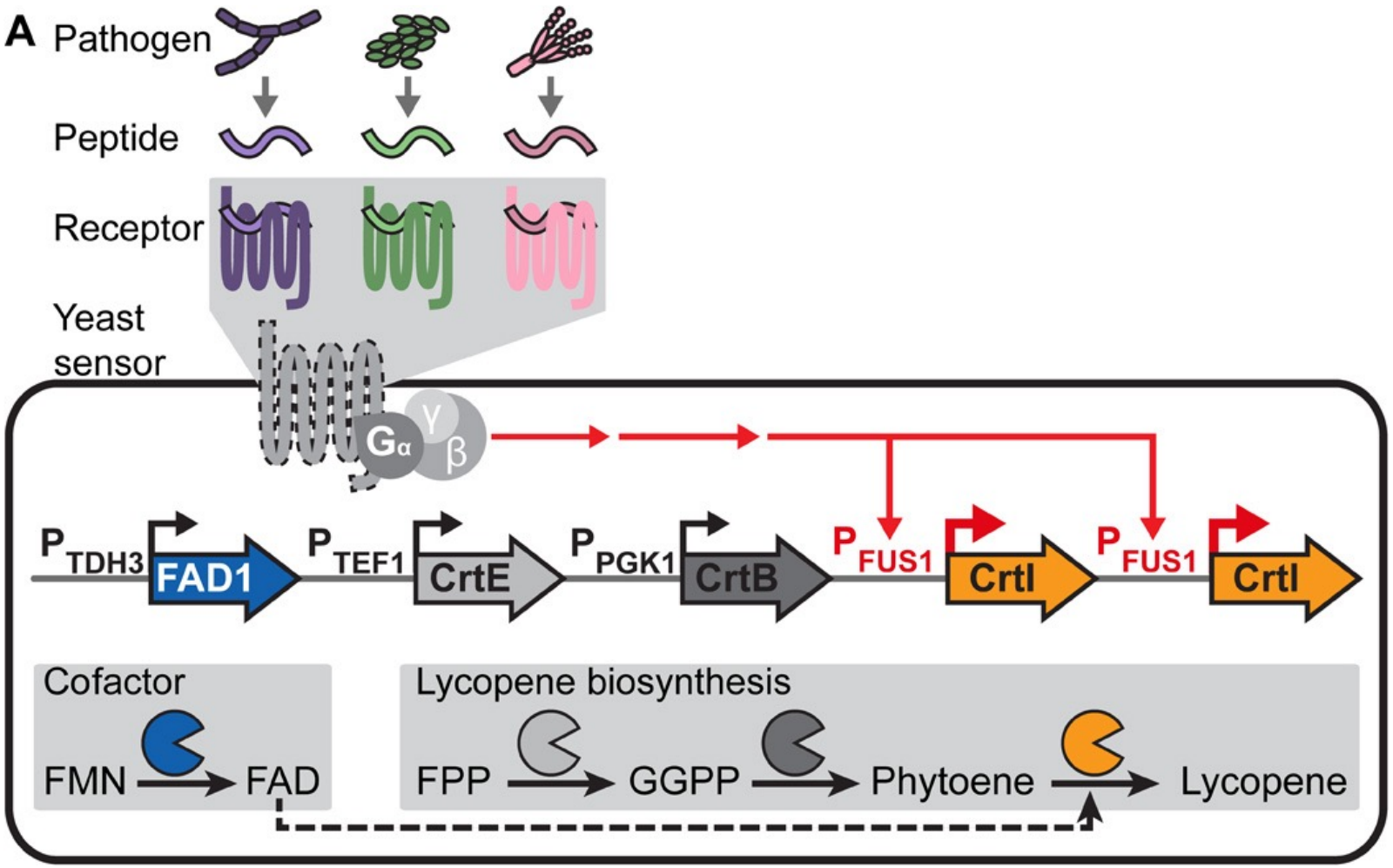
HEALTH AND MEDICINE

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

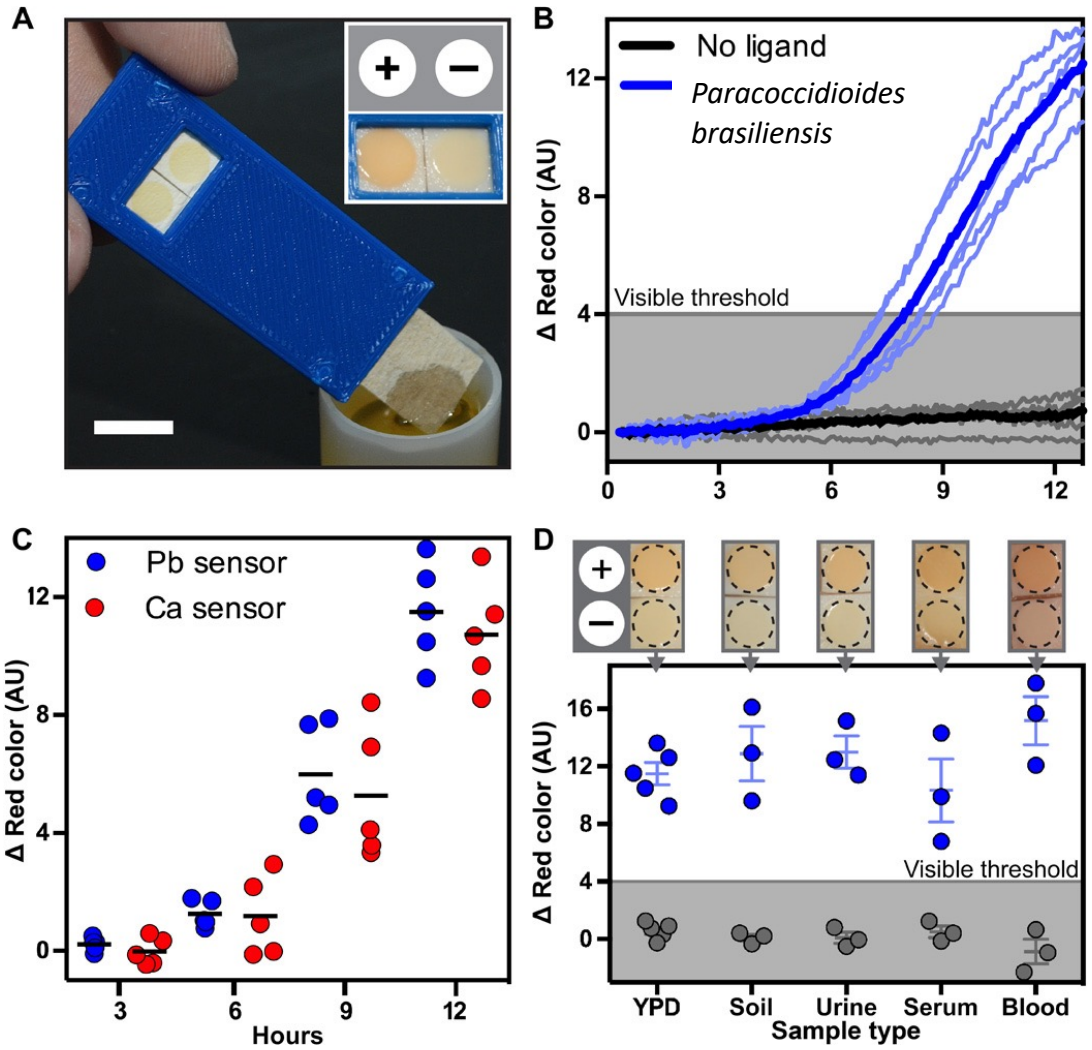
Nili Ostrov,[1*†] Miguel Jimenez,[1*‡] Sonja Billerbeck,[1*] James Brisbois,[1] Joseph Matragrano,[1] Alastair Ager,[2,3] Virginia W. Cornish[1,4§]

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

Biyosensör Temeli Yöntemler



Biyosensör Temeli Yöntemler



Biyosensör Temeli Yöntemler

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

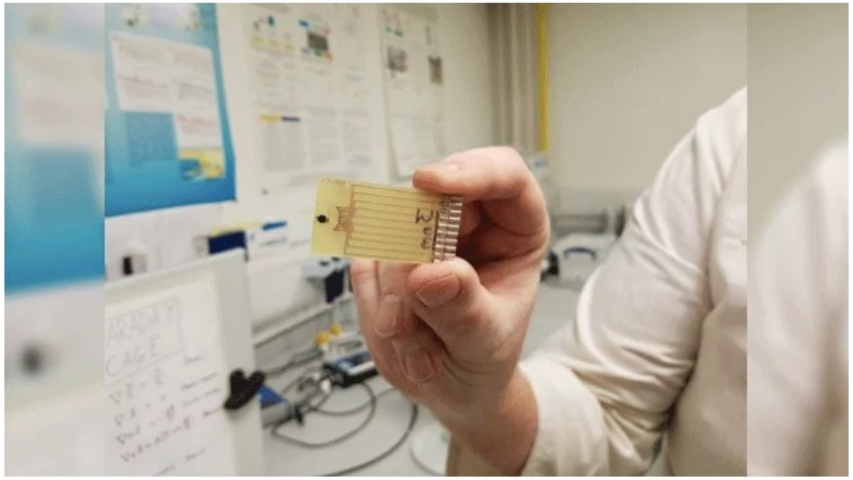
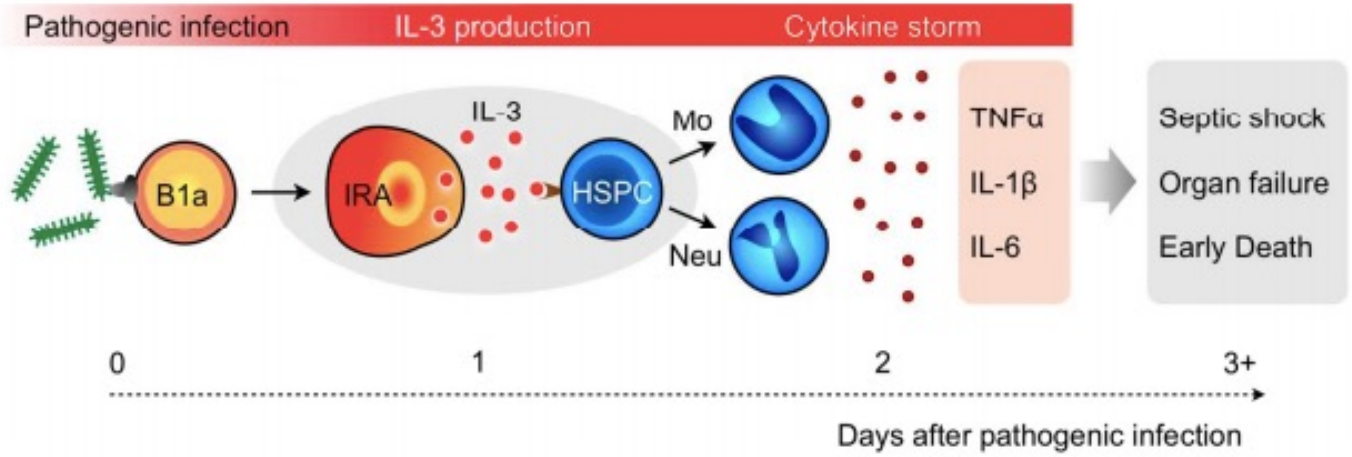


Image credit: the University of Strathclyde Glasgow

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



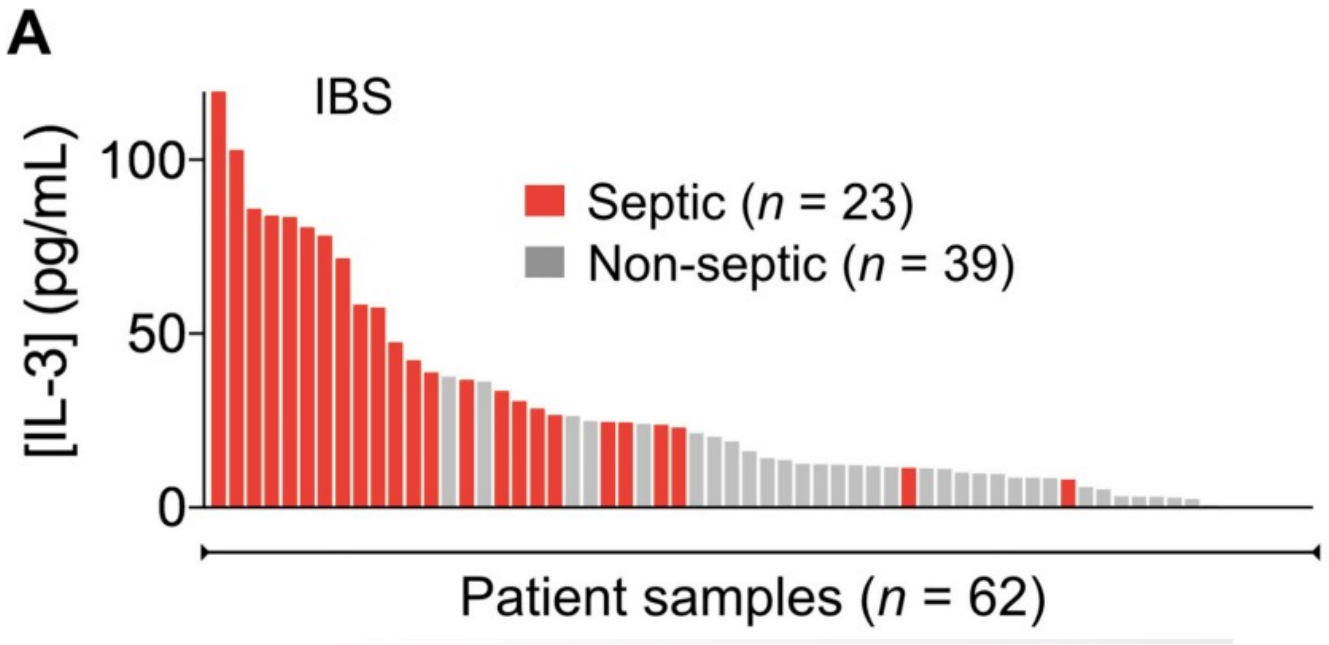
Scheme 1. IL-3 mediated mechanism of sepsis. Peritoneal B1a cells are activated by pathogens and give rise to IL-3+ B cells (IRA, innate response activator). IL-3 acts on hematopoietic stem progenitor cells (HSPC) to promote the emergency generation of inflammatory leukocytes that are released into the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

Biyosensör Temeli Yöntemler

Published in final edited form as:
ACS Nano. 2018 April 24; 12(4): 3378–3384. doi:10.1021/acsnano.7b08965.

Integrated Biosensor for Rapid and Point-Of-Care Sepsis Diagnosis

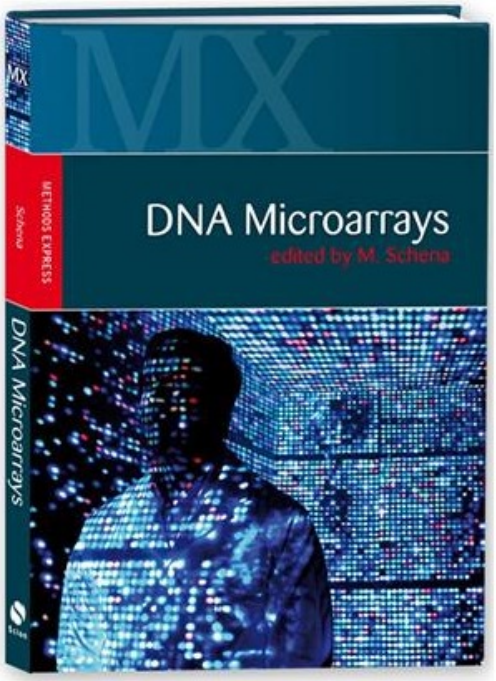
Jouha Min[1,2], **Maria Nothing**[3], **Ben Coble**[1], **Hui Zheng**[4], **Jongmin Park**[1,2], **Hyungsoon Im**[1,2], **Georg F. Weber**[3], **Cesar M. Castro**[1,5], **Filip K. Swirski**[1], **Ralph Weissleder**[1,2,6,*], and **Hakho Lee**[1,2,*]



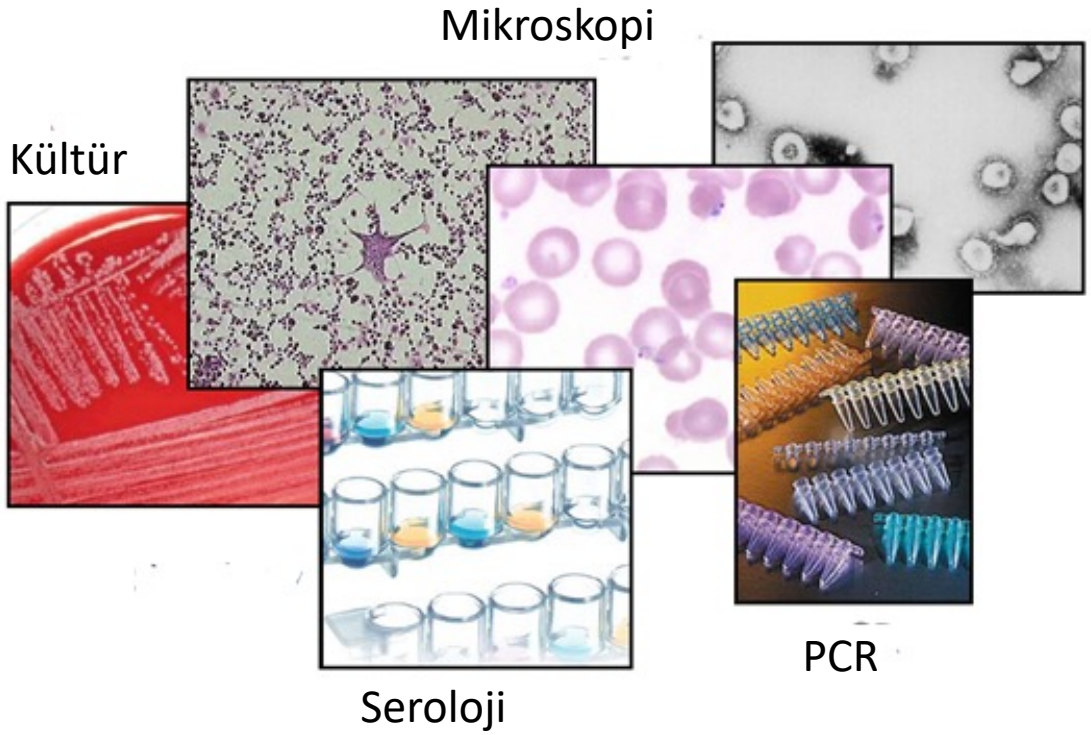
DNA Mikroçip Teknolojileri

- DNA mikroçip teknolojileri, çevresel ve

  klinik örneklerden mikroorganizmaların tanısı için giderek artan oranda kullanılmaktadır.



Geleneksel Mikrobiyolojik Tanı Yöntemleri

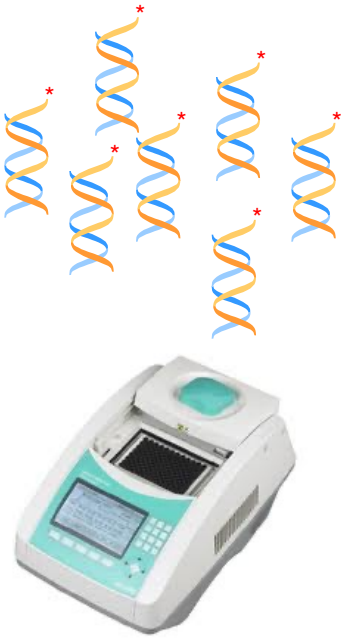


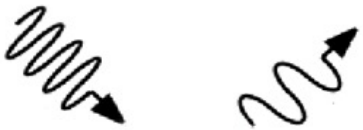
DNA Mikroçip Teknolojileri

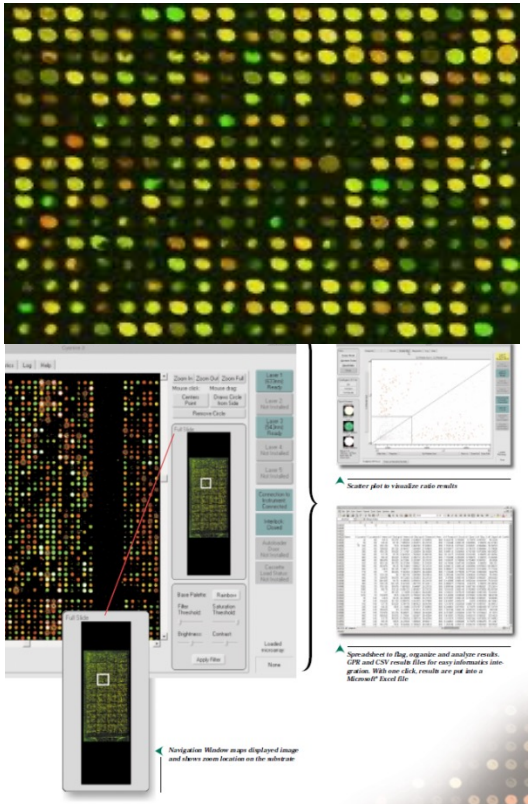
- PCR ile elde edilen floresanla işaretli amplikonların

  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

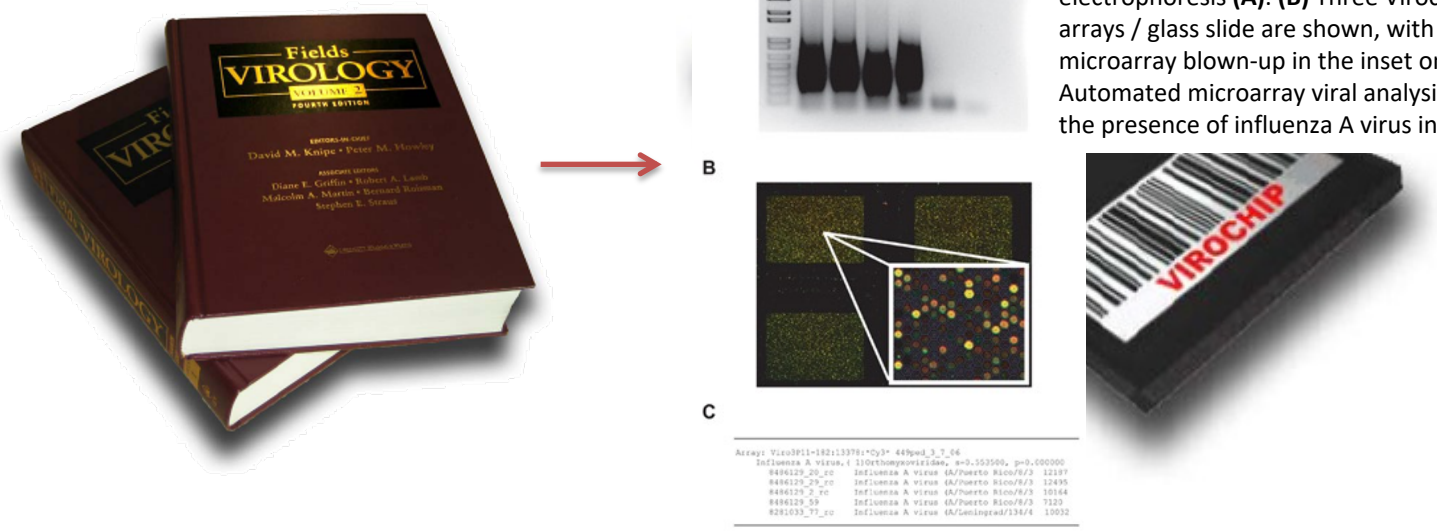
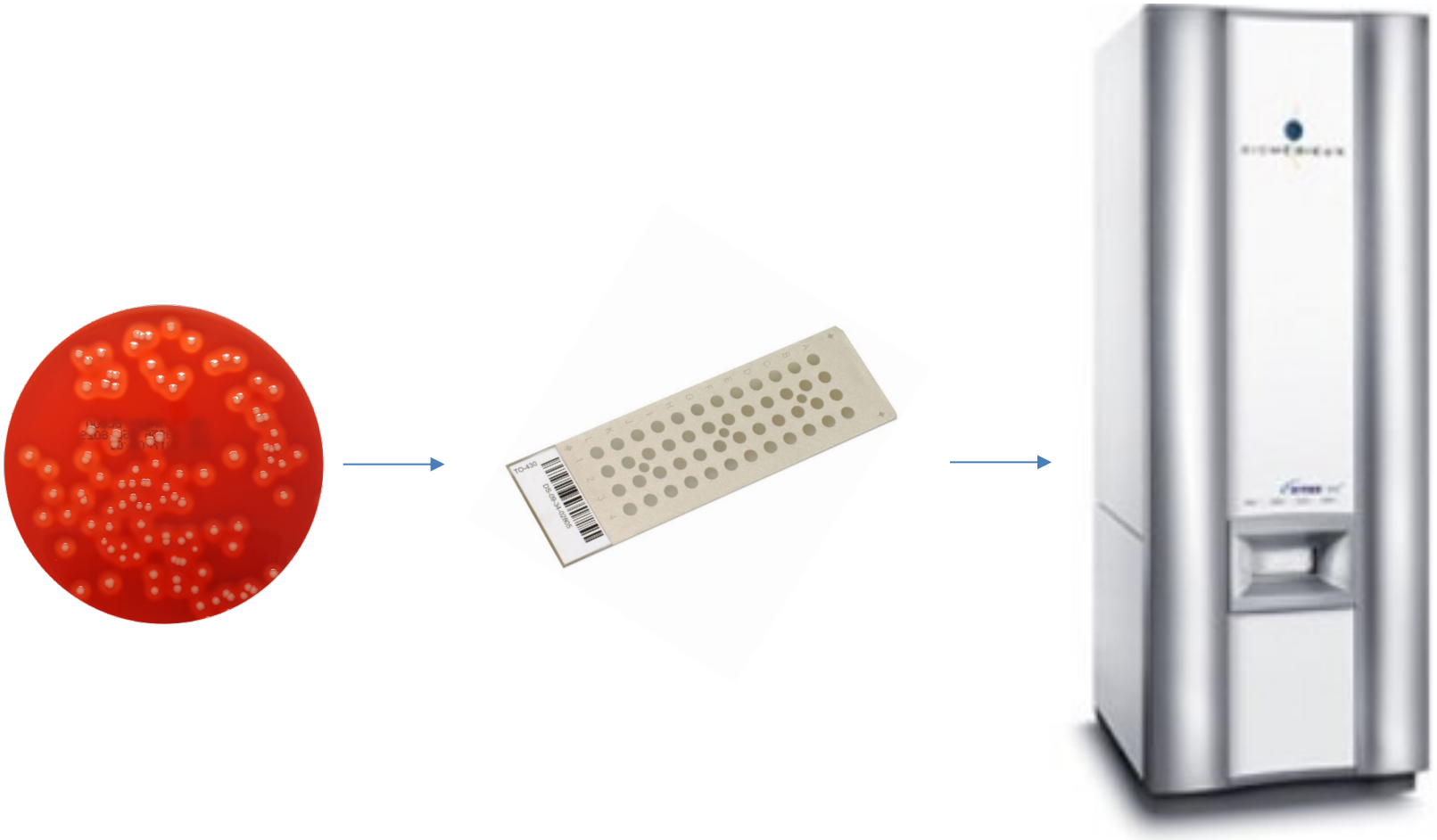


Figure 2. Steps in the Virochip assay. After amplification by random PCR, a smear of 200 - 1000 bp can be visualized by gel electrophoresis **(A)**. **(B)** Three Virochip microarrays out of the 8 arrays / glass slide are shown, with a small region of one microarray blown-up in the inset on the bottom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

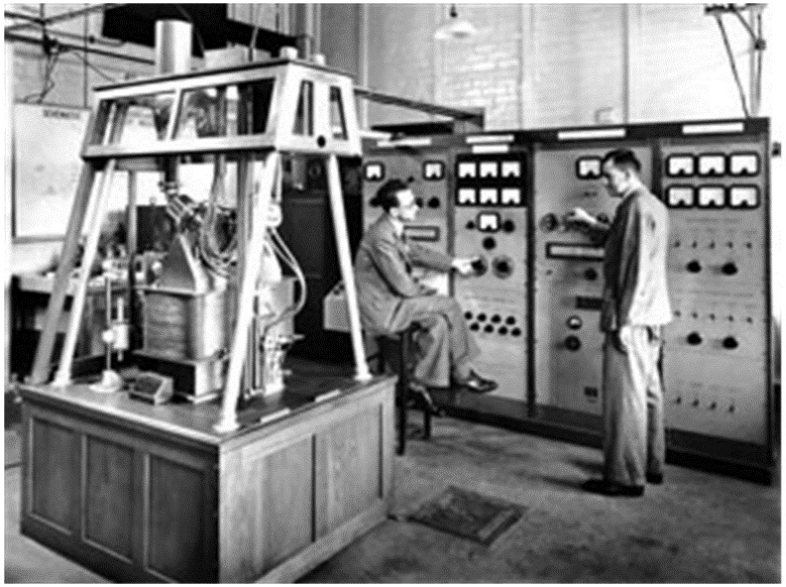
Kütle Spektrometrik Yöntemler

- 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



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 carboxypeptidase-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.

2009

FDA onayı ile klinik mikrobiyolojik tanıya girdi

Günümüzde 2000'e yakın bakteri ve mantar tanımlanabiliyor

Kütle Spektrometrik Yöntemler- Hangi Amaçlarla Kullanılıyor?

Biyokimya: Proteomik çalışmaları, proteinlerin hızlı tanısı ve ölçümü

Organik kimya: Aşırı büyük kütleli sentetik moleküllerin (katenan, dendrimer vb) tanımlanması.

Polimer kimyası: Büyük polimerlerin moleküler büyüklüğünün saptanması

Tıp-İnsan Doku ve Hücreleri: Genetik anormallikler sonucu oluşan sorunlu proteinlerin saptanması

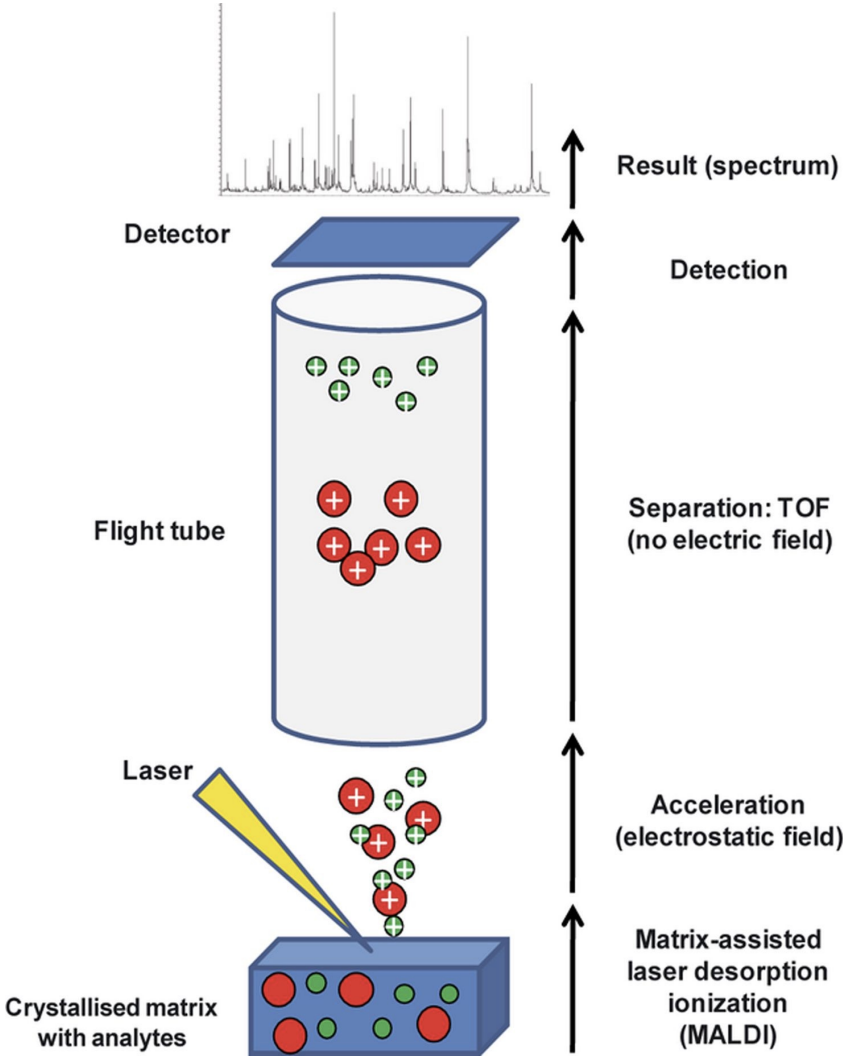
Genetik: çoğaltılmış nükleik asitlerin gösterilmesi

Mikrobiyoloji: bakteri ve mantar tanımlanması

Diğer: çevrede ve gıdalara bulaşmış toksinlerin saptanması

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.

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

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, inkübasyon 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.

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- Direkt Örnekten

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

JCM
Journals.ASM.org

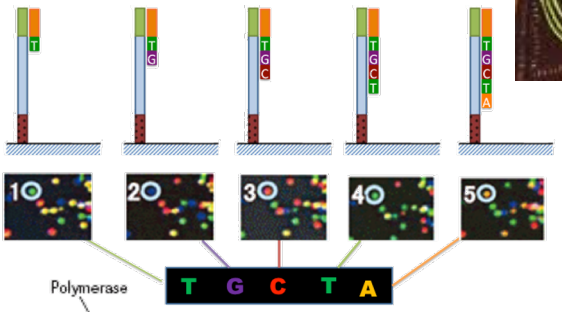
Direct MALDI-TOF Mass Spectrometry Assay of Blood Culture Broths for Rapid Identification of *Candida* Species Causing Bloodstream Infections: an Observational Study in Two Large Microbiology Laboratories

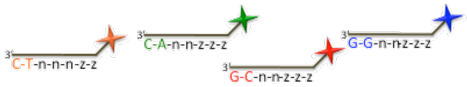
TABLE 1 Performances of Bruker Biotyper for direct identification of yeasts from blood culture bottles with culture-based identification as reference

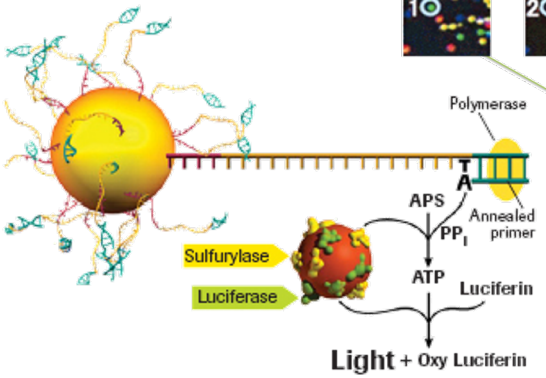
Comparison method ID	No. of isolates		
	Total tested	Concordant ID[a]	% Sensitivity (95% CI)[b]
Candida albicans	195	187	95.9 (91.8–98.1)
Candida famata	1	0	NT
Candida glabrata	26	22	84.6 (64.3–94.9)
Candida guilliermondii	10	6	60.0 (27.4–86.3)
Candida krusei	8	6	75.0 (35.6–95.5)
Candida lusitaniae	2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

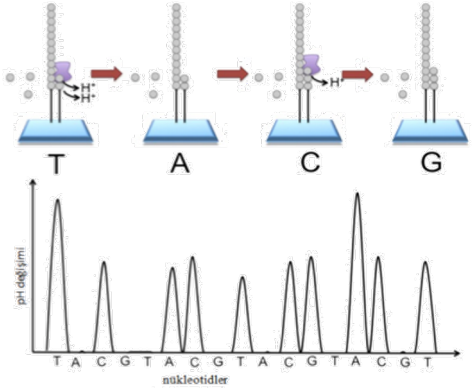
[a] Species identification furnished by the Bruker Biotyper was concordant with that of the comparison method.
[b] NT, not tested. Sensitivity was not calculated when <5 isolates were found.

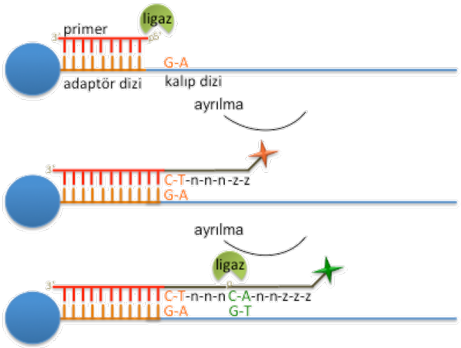
Yeni Nesil Dizileme Sistemleri







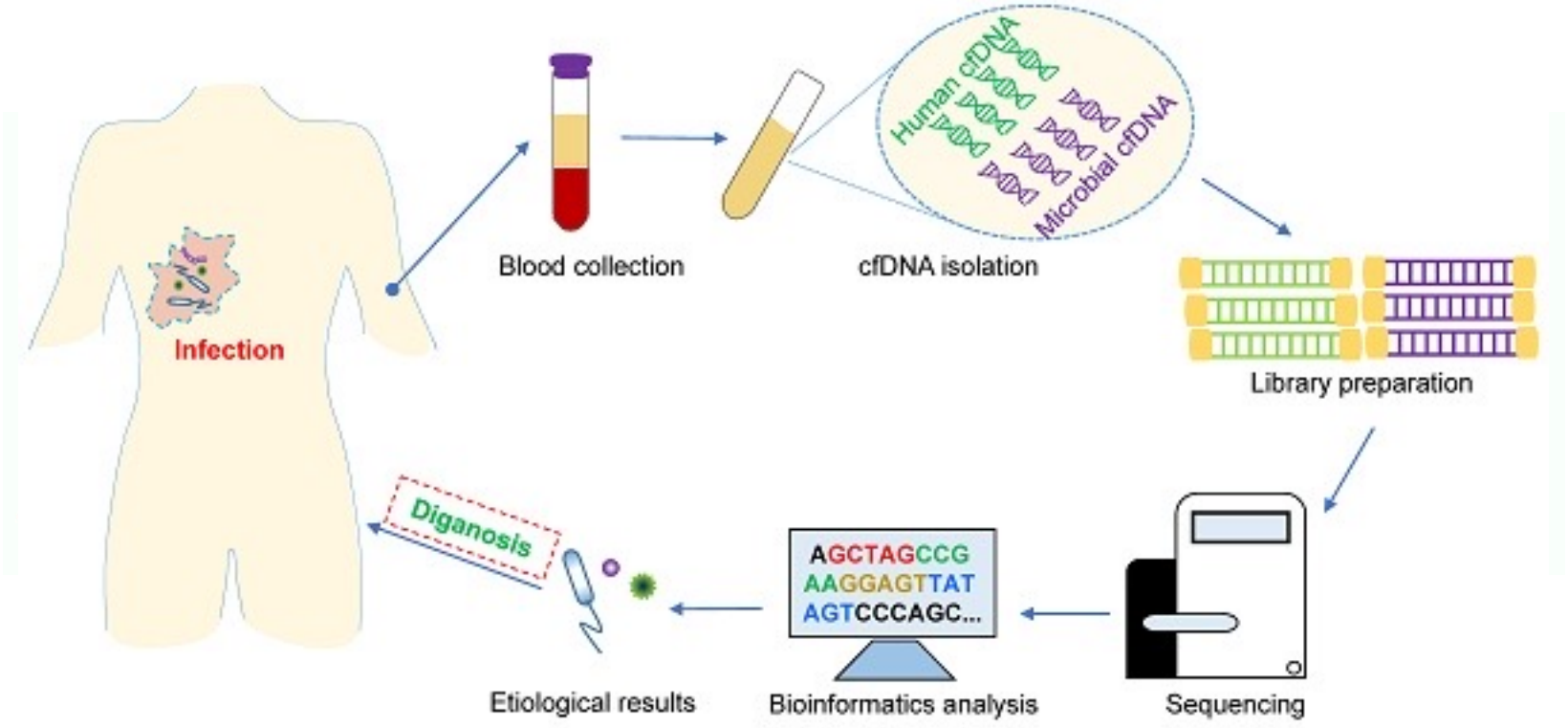




Yeni Nesil Dizileme Sistemleri

- Metageonomik Yaklaşımlar

  metagenomik dizileme, mikrobiyal türlerin tespiti ve karakterizasyonunda devrim yaratmıştır.



Yeni Nesil Dizileme Sistemleri

- Metageonomik Yaklaşımlar

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*

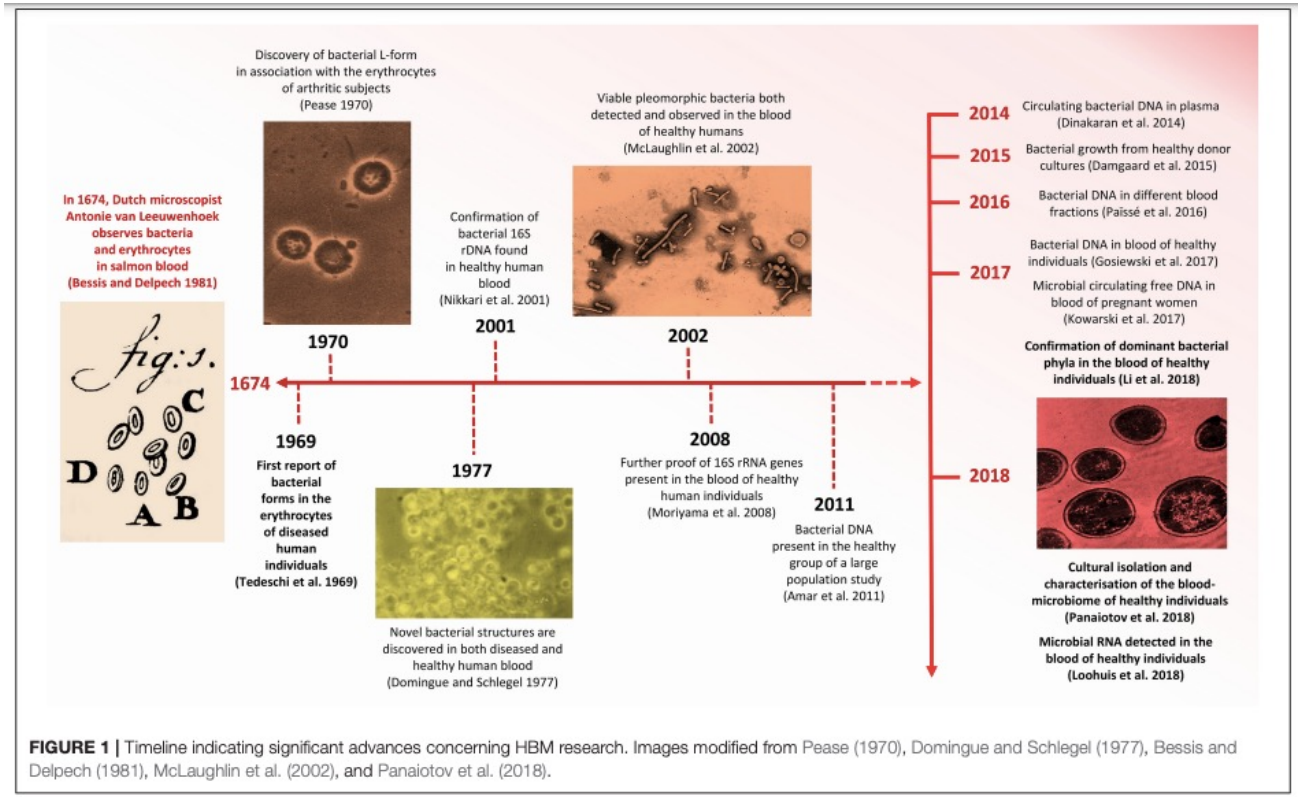
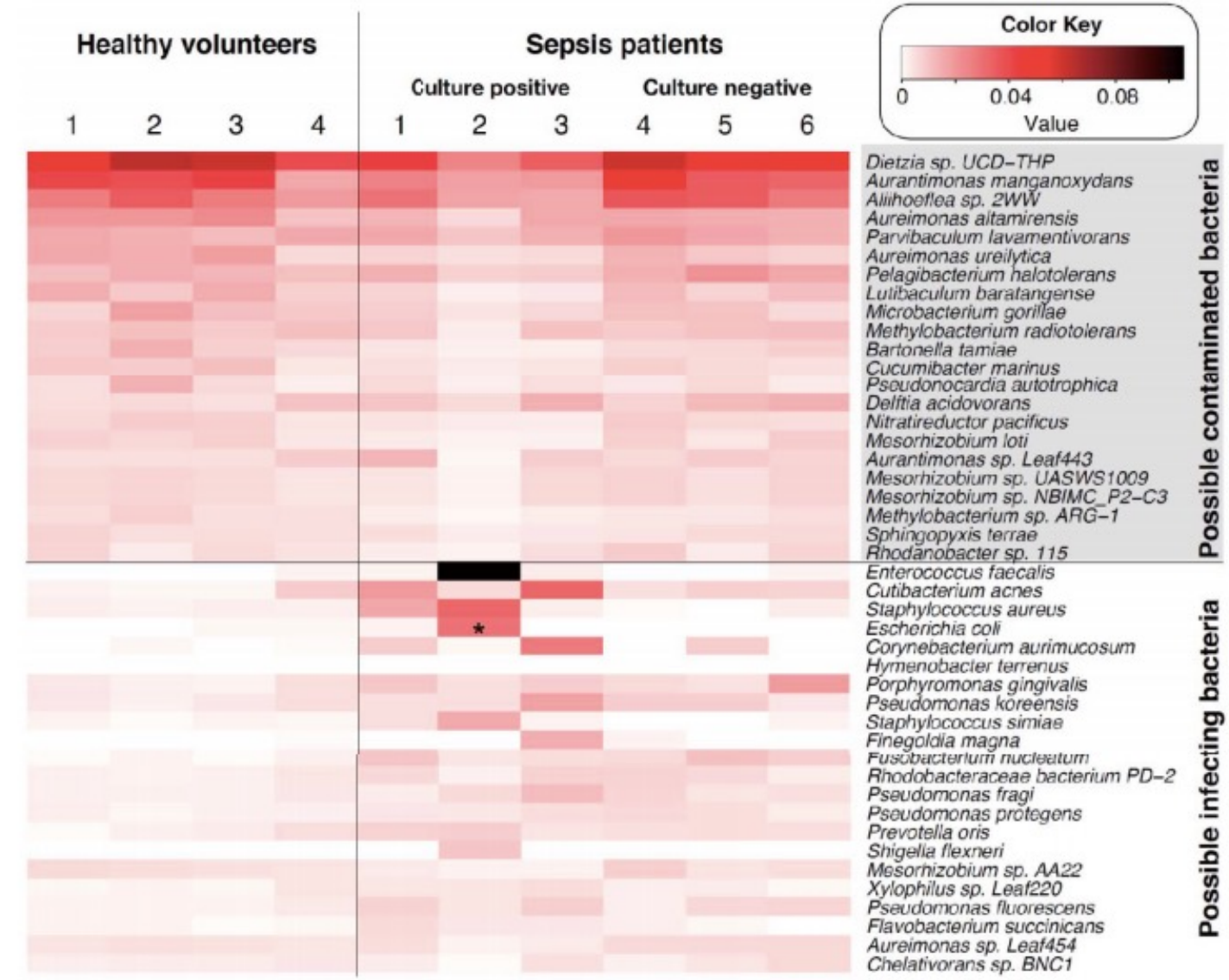
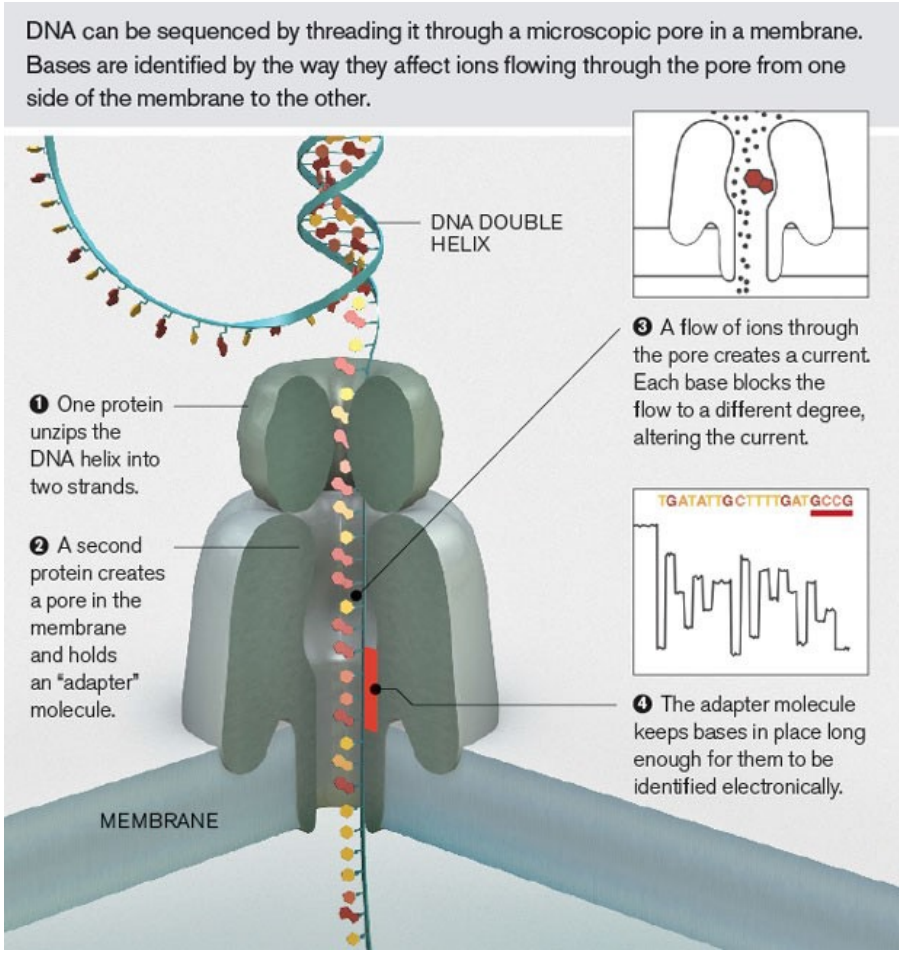


FIGURE 1 | Timeline indicating significant advances concerning HBM research. Images modified from Pease (1970), Domingue and Schlegel (1977), Bessis and Delpech (1981), McLaughlin et al. (2002), and Panaiotov et al. (2018).

Sepsisin Tanısında Metagenomik Yaklaşım

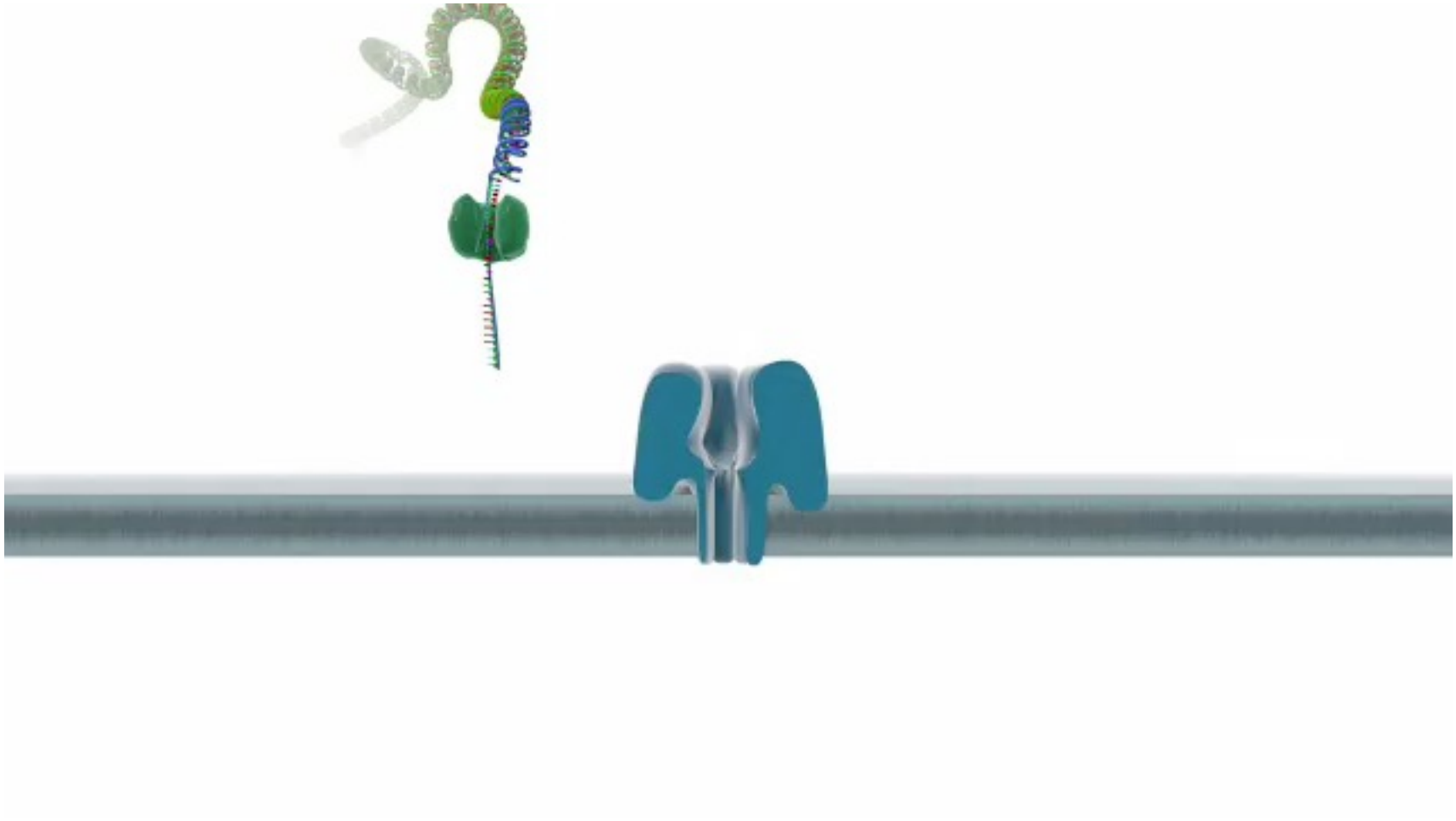


Oxford Nanopore Technology



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

Nick Loman
@pathogenomenick

Follow

A genuine >8kb Oxford @nanopore read off our instrument THIS MORNING. And happily it's actually useful for diagnosis! figshare.com/articles/A_P_a…

9:40 AM - 11 Jun 2014

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Pseudomonas aeruginosa LESB65

Sequence ID: ref|NZ_CP006983.1| **Length:** 6527005 **Number of Matches:** 7

Range 1: 2065558 to 2068554 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
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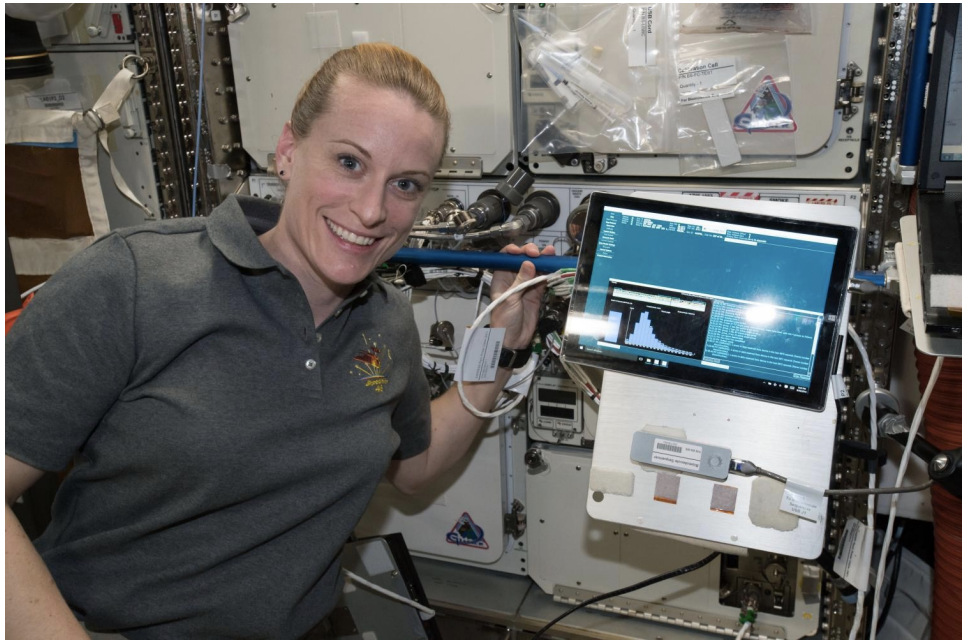
Oxford Nanopore
@nanopore

Takip ediliyor

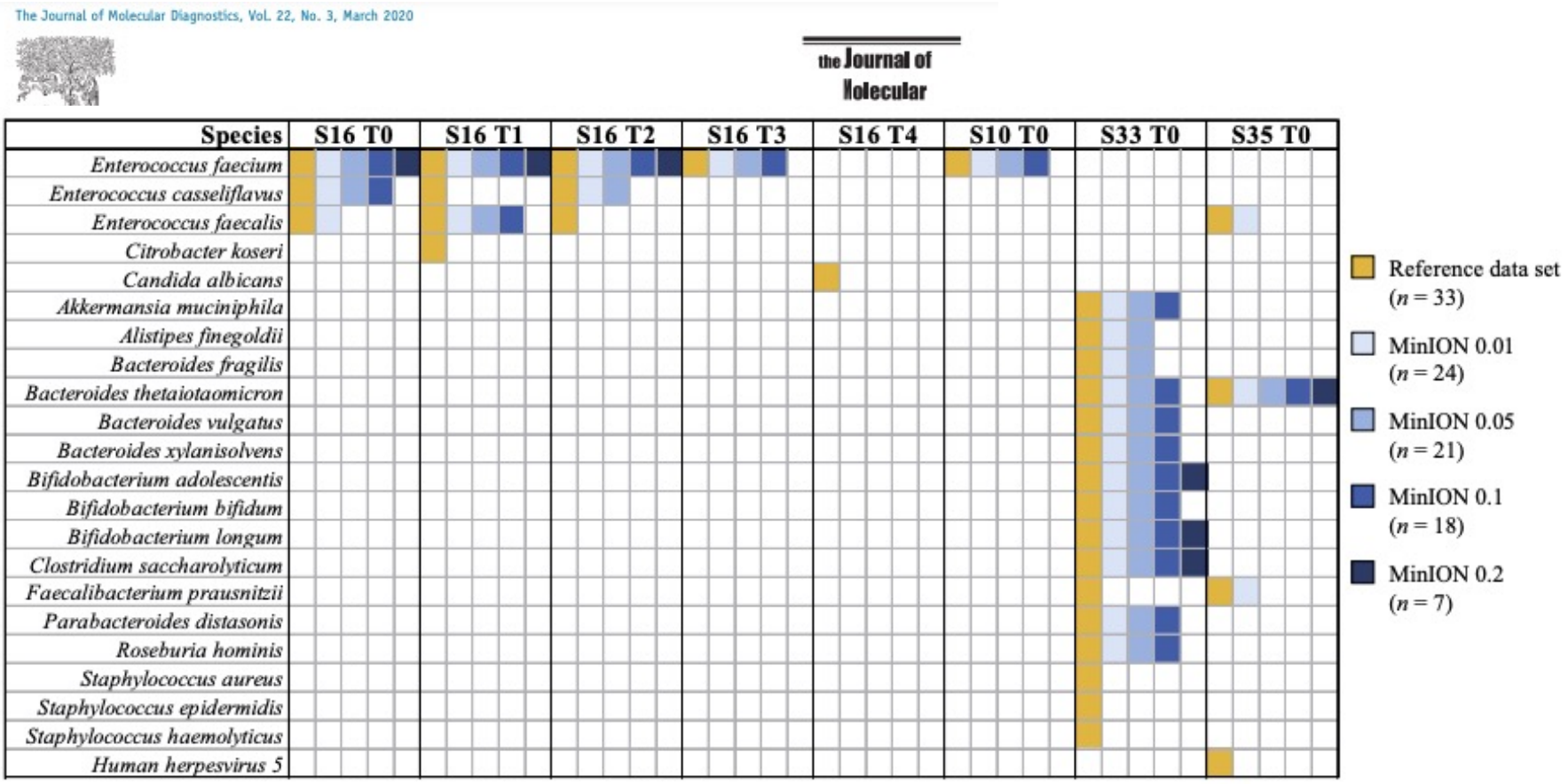
New @nanopore supplies have arrived @space_station to sequence and ID on-board organisms. NASA blog go.nasa.gov/2oL14sL

İngilizce dilinden çevir



Oxford Nanopore Technology

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



Moleküler Mikrobiyolojik Teknikler

- Moleküler hızlı tanı yöntemlerine ve moleküler epidemiyolojiye harcanan

  her 1 USD harcama 5 USD olarak geri dönüyor.

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

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