ENFEKSİYON HASTALIKLARINDA

İNOVASYON OLANAKLARI VE FIRSATLARI

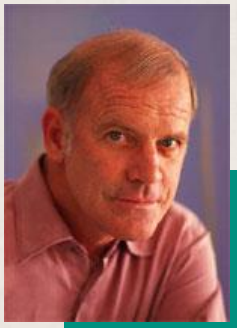
Prof. Dr. Tanıl Kocagöz

ACIBADEM

MEHMET ALİ AYDINLAR
ÜNİVERSİTESİ

Enfeksiyon Hastalıkları ile İlgili Araştırmalar

* Hızlı, duyarlı, özgül, ekonomik, uygulaması kolay tanı araçları geliştirilmesi
* Yeni etkin, yan etkisi az, uygulaması kolay, ekonomik tedavi yöntemlerinin geliştirilmesi



Polimeraz Zincirleme Tepkimesi (PZT)

(Polymerase Chain Reaction –PCR-)

Karry Mullis

Kalıp DNA

↓

95° C zincirlerin ayrılması

↓

50-70° C

primerlerin birleşmesi

↓

72° C DNA yapımı

↓

Ürünler

Karikatürist
Eren Özdemir
8 Yaşında

Dekontaminasyon ve Konsantrasyon

Petroff Yöntemi: 1915

- Balgam 1N NaOH ile dekontamine edilir
- pH nötralizasyonu 1N HCl ile yapılır
- pH indikatörü olarak brom timol mavisi kullanılır.
- Çözeltileri hazırlaması kolay
- pH ayarlaması zor
- Santrifüj gerektiriyor

Kubica Yöntemi: 1963

* Decontaminasyon için NaOH-NALC kullanılır
* Nötralizasyon için fosfat tamponu kullanılır
* pH ayarlaması daha iyi ama deneyim ve dikkat gerektiriyor
* Çözeltilerin hazırlanması zor, yarı ömrü kısa
* Kontaminasyon olasılığı yüksek
* Santrifüj gerektiriyor
* 45 dakika sürüyor

MYCOPROSAFE (2001)

- Kubica yöntemini kolay uygulamak için hazırlanmış kit
- Her hasta için ayrı steril çözeltiler içeriyor
- Kontaminasyon oranını çok düşürüyor
- Çözelti hazırlama zahmetini ortadan kaldırıyor
- Her örnek için taze çözelti hazırlanmasını sağlıyor

Bugüne dek yurt içi ve yurt dışında 3 milyondan fazla örneğin işlenmesinde kullanılmış durumda

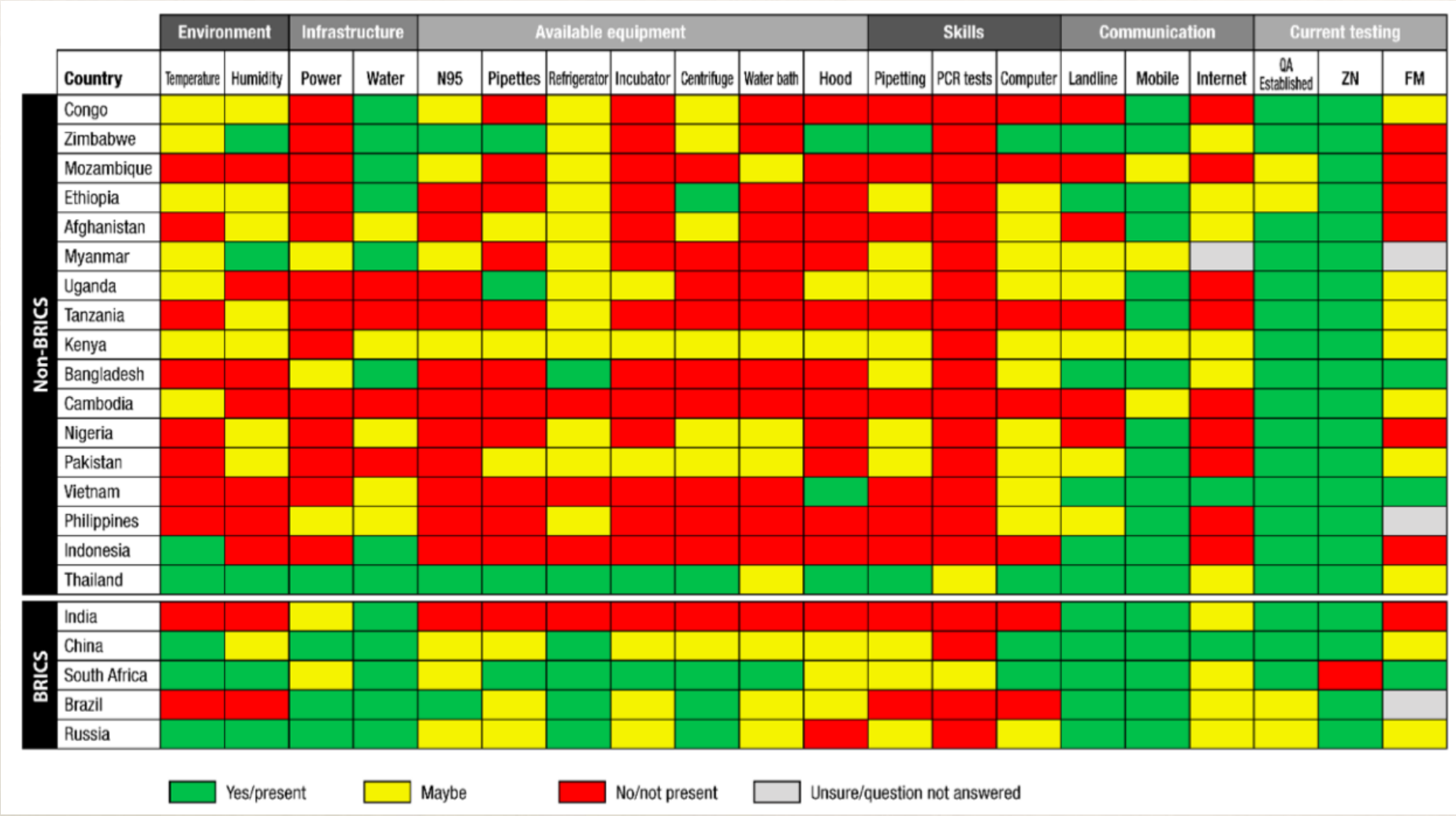
Decocent (2014)

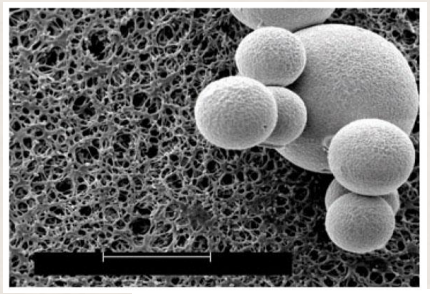
* Dekontaminasyon ve nötralizasyon sıvıları pH tam ayarlanacak derişim ve miktarda hazırlanmıştır
* Tüplerdeki çözeltilerin tamamı kullanılır, ölçüm yapmaya gereksinim yoktur
* pH kolayca ve tam doğru olarak ayarlanır.
* 30 veya 50ml'lik tüplerde örnek işleme yapılabilir.
* Ekonomiktir

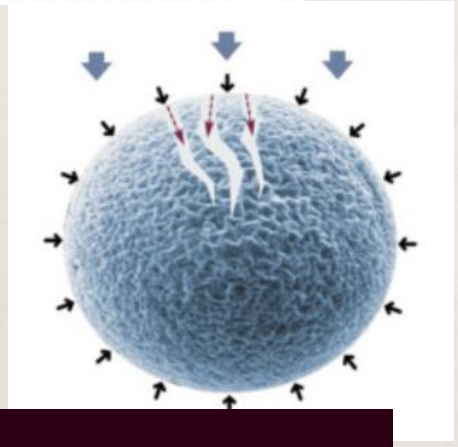
Uganda’da
bir laboratuvar

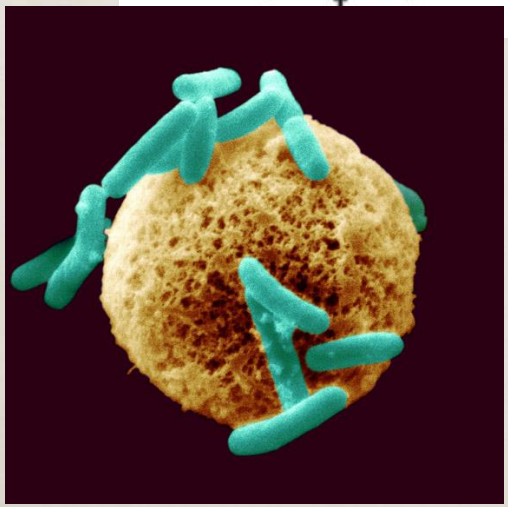
Hindistan’da
bir laboratuvar

Kaynakları Sınırlı Ülkelerde Laboratuvar Koşulları









Decomics
(2012)

- Dekontaminasyon işlemi için örneğe çok fazla sıvı eklenmesi gerektiğinden, seyrelmiş örnekteki mikobakterileri yoğunlaştırma için santrifüj gerekmektedir.
- Decomics yönteminde dekontaminasyon ve nötralizasyon için eklenen sıvılar emici boncuklar tarafından emildiği için, santrifüj gereksinimi ortadan kalkmıştır.

Hızlı Mikobakteri Kültür Sistemleri

- BACTEC (Becton Dickinson, USA)
- MGIT (Becton Dickinson, USA)
- Bac-T Alert (Biomerieux, France)
- Versatrek (Thermoscientific, USA)
- TK Kültür Sistemi (Salubris)



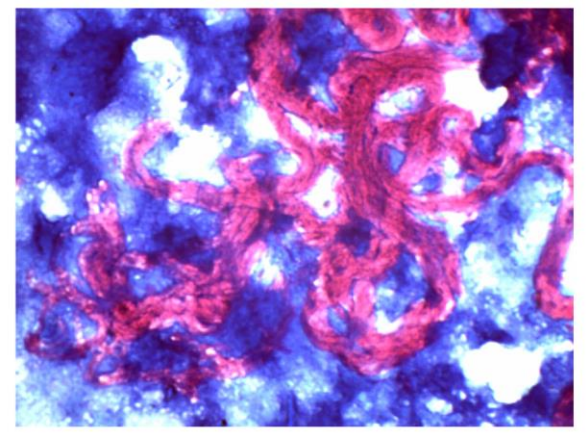






TK Besiyeri

* Kullanıma hazır: OADC ve seçici antimikrobiyal eklenmesini gerektirmiyor.
* Koloniler belirmeden önce besiyerinin rengi kırmızıdan sarıya değişerek üremeyi gösteriyor. Üreme saptama süresi ortalama yarıya iniyor.
* Mikobakteri dışında bir tür üreyecek olursa renk kırmızıdan yeşile dönerek kontaminasyonun ayırt edilmesini sağlıyor.
* Üreme hem gözle hem de otomatik aygıt ile izlenebiliyor.

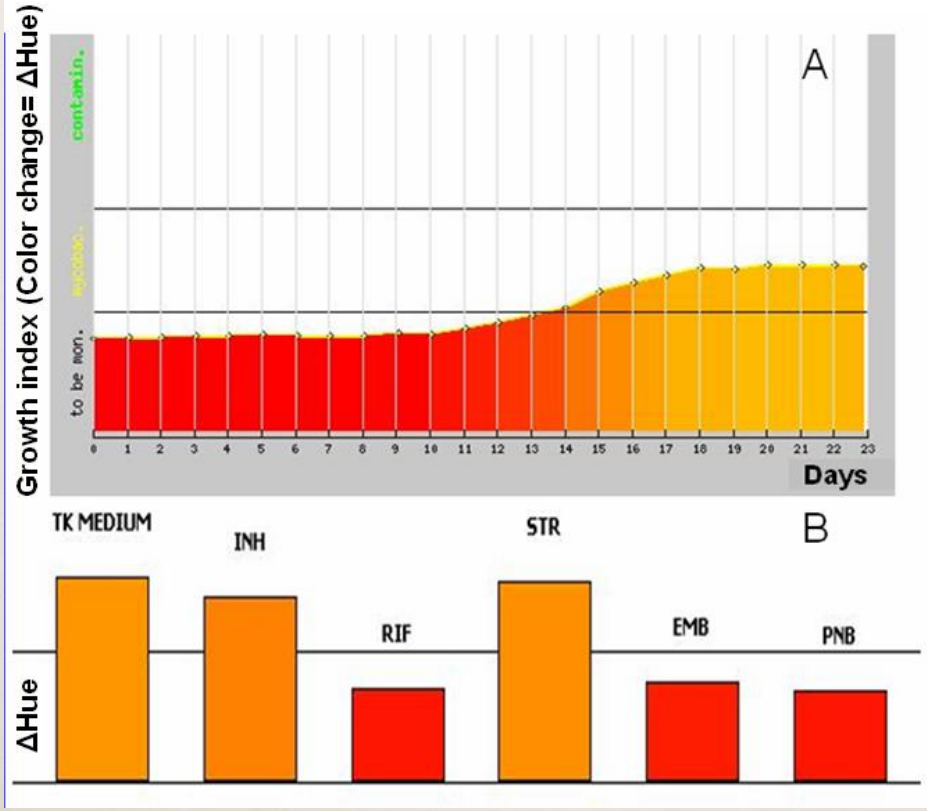


TK Mikobakteri Kontaminasyo

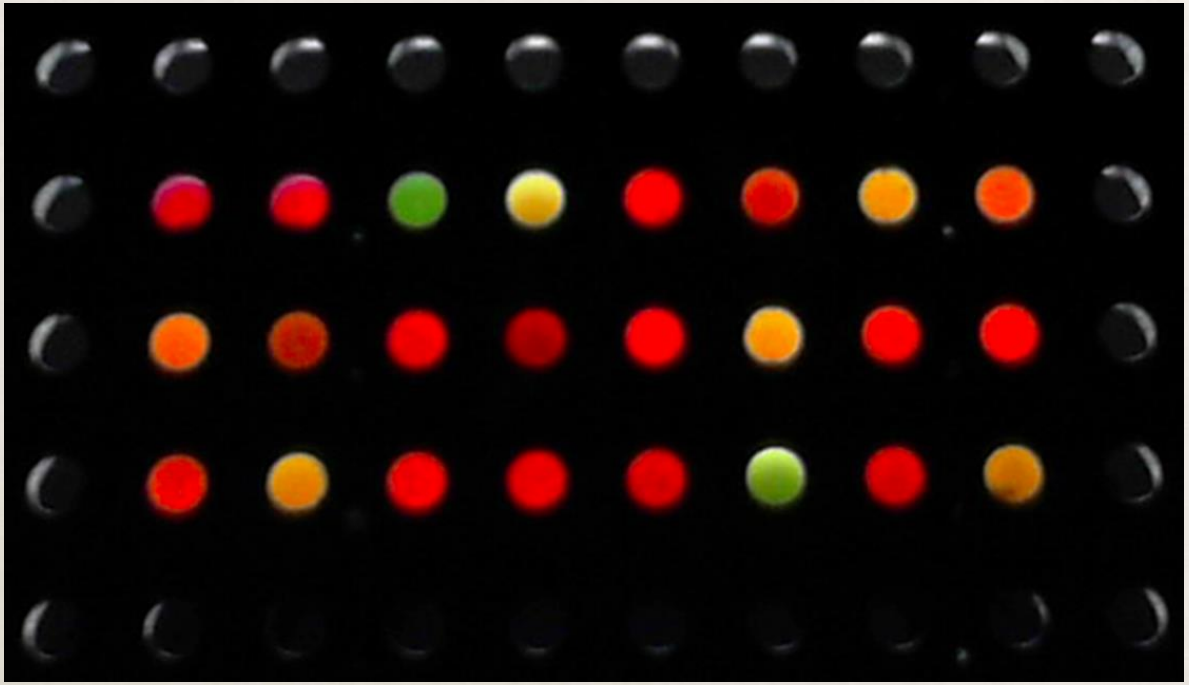
Mycolor TK

- TK Medium için bilgisayarlı otomatik inkübatör ve okuyucu
- Her örnek için üreme eğrileri çiziyor
- Uzman sistemi ile M. tuberculosis, atipik mikobakteri, kontaminant organizma ayrımı yapabiliyor
- Otomatik ilaç duyarlılığı belirleme ve tiplendirme yapabiliyor

Modular 336

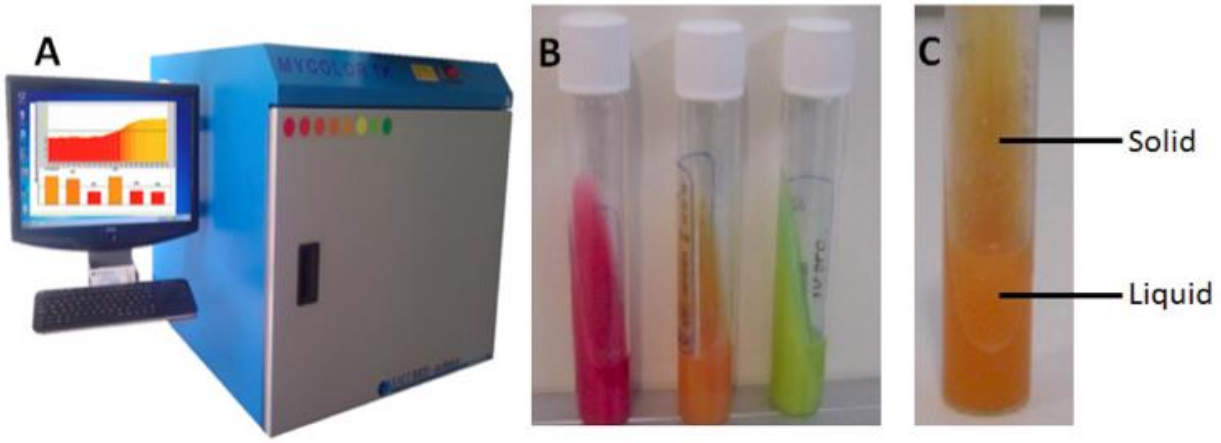


Sahne Arkası



Salubris Inc. offers a series of products that also perform with automated TB culture (Figure 14). They manufacture: TK MEDIUM® SLC; a biphasic, culture medium that can be incubated and automatically monitored in the MYCOLOR TK® instrument (Figure 14); and TK MEDIUM SLC-L, a liquid formulation.

Figure 14. A: MYCOLOR TK automated incubator; B: TK colorimetric media with changes from red to orange (mycobacterial positive culture) red to green (contaminated culture); C: biphasic format of TK media with both solid and liquid media



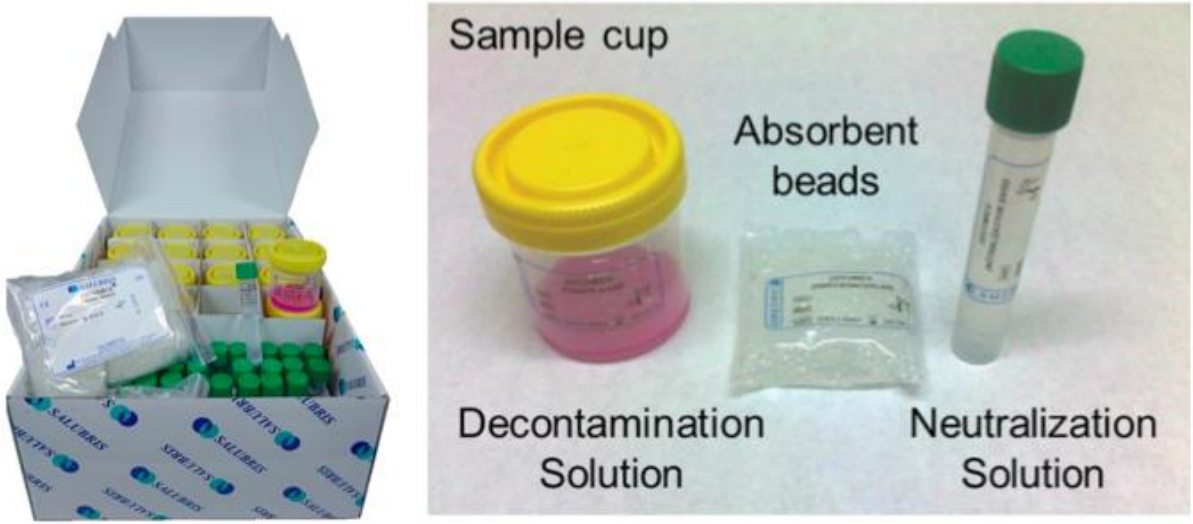
Source: Images reproduced with permission from Salubris Inc.

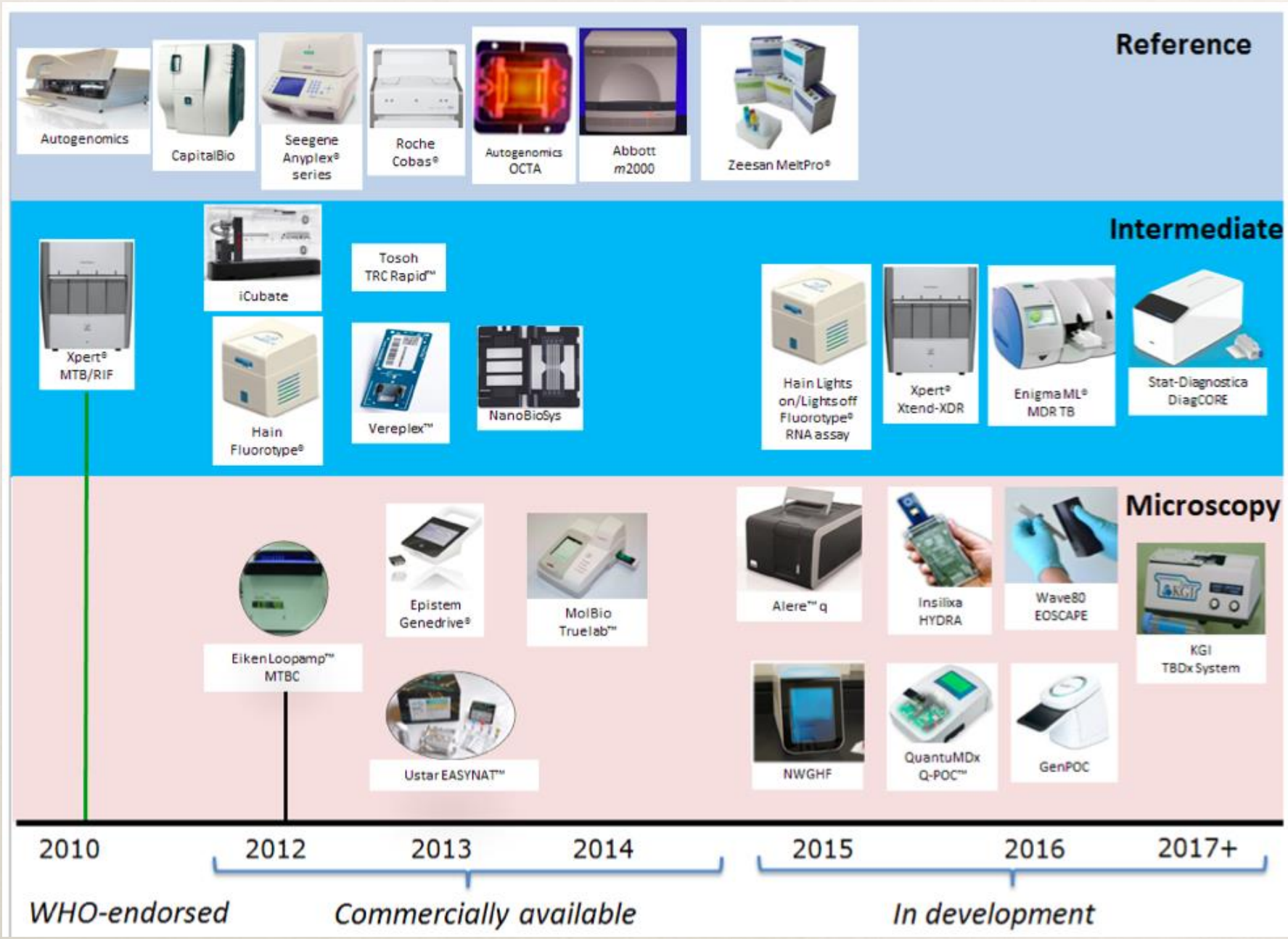
TK MEDIUM® is an egg-based solid medium similar to L-J.[92] Unlike the liquid media used in other automated platforms, the TK media do not require any additives, creating a simplified workflow and reducing risk of contamination.[92] The media contain dyes that react upon the growth of microorganisms (Figure 14).[93] Upon mycobacterial growth, the original red colour turns orange and then yellow. The colour change is indicated before colonies are visible on the agar, improving the time to detection. Contamination by fungi or Gram-negative bacteria produces a green pigment; some Gram-negative bacteria can produce orange/yellow. The TK SLC medium is biphasic having both solid and liquid media (Figure 14). The TK

4.2.4. *Culture-based tools for the diagnosis of TB and DST*

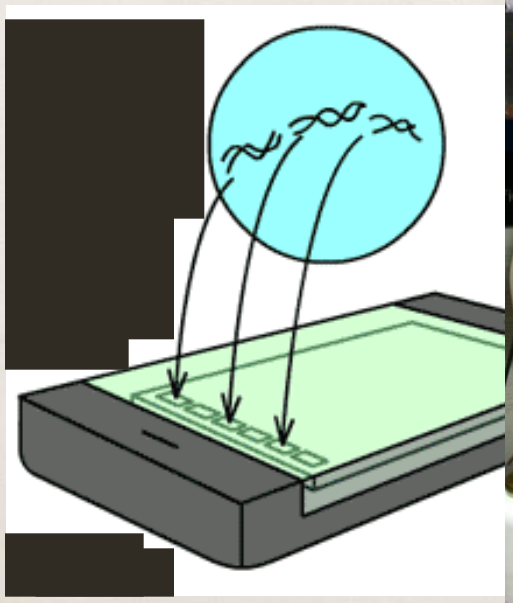
There is little to report in terms of new product information from developers regarding culture-based diagnosis of TB. Salubris Inc. (USA) no longer supplies the biphasic TK MEDIUM® SLC for use in its MYCOLOR TK® automated culture system. The company now offers the TK MEDIUM® SLC-L, a liquid media that is now housed in a plastic tube to limit risks of mishandling. A consistent issue with the conventional decontamination method of sodium hydroxide and N-acetyl cysteine (NaOH-NALC) is the risk of incomplete decontamination or overexposure to reagents killing the MTB cells. The NaOH-NALC method also requires centrifugation that can limit processivity rate and incorrect use of this can result in infection risks via aerolisation. The Decomics® kit (Figure 9) uses absorbent beads and reagents to liquefy, decontaminate and neutralize samples in less than 25 minutes without requiring a centrifuge or NaOH-NALC.

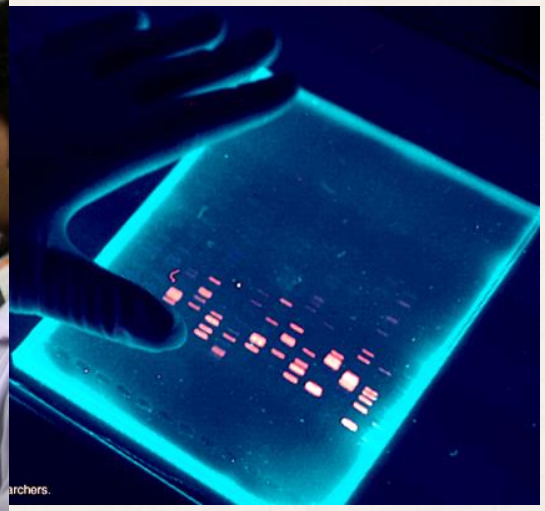
Figure 9. Decomics® kit from Salubris Inc.: individual components including sample cup, decontamination solution, beads and neutralization solution

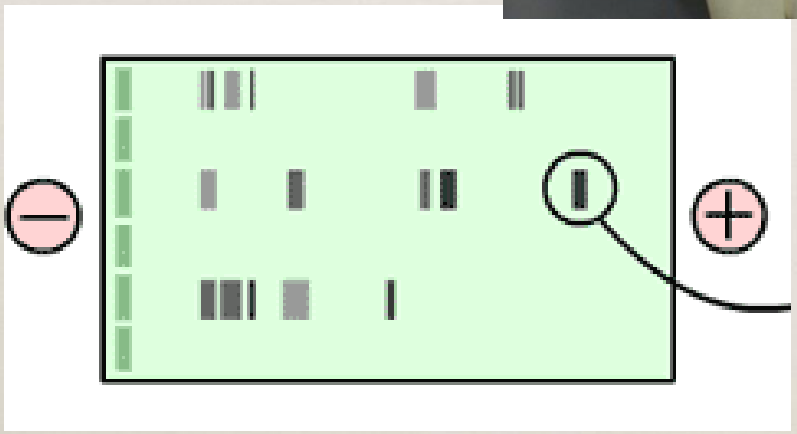


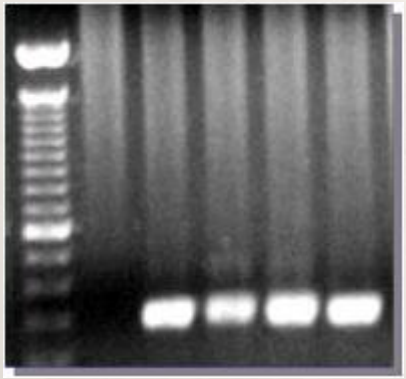

Reference
Autogenomics
CapitalBio
Seegene Anyplex® series
Roche Cobas®
Autogenomics OCTA
Abbott m2000
Zeesan MeltPro®
Intermediate
Xpert® MTB/RIF
iCubate
Tosoh TRC Rapid™
Hain Fluorotype®
Vereplex™
NanoBioSys
Hain Lights on/Lights off Fluorotype® RNA assay
Xpert® Xtend-XDR
Enigma ML® MDR TB
Stat-Diagnostica DiagCORE
Microscopy
Eiken Loopamp™ MTBC
Epistem Genedrive®
MolBio Truelab™
Alere™ q
Insilixa HYDRA
Wave80 EOSCAPE
KGI TBDx System
Ustar EASYNAT™
NWGHF
QuantuMDx Q-POC™
GenPOC
2010
2012
2013
2014
2015
2016
2017+
WHO-endorsed
Commercially available
In development

PZT çoğaltma ürünleri elektroforez ile incelenir



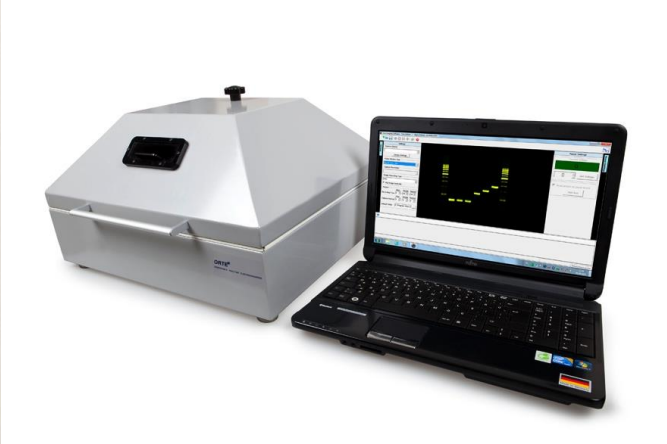


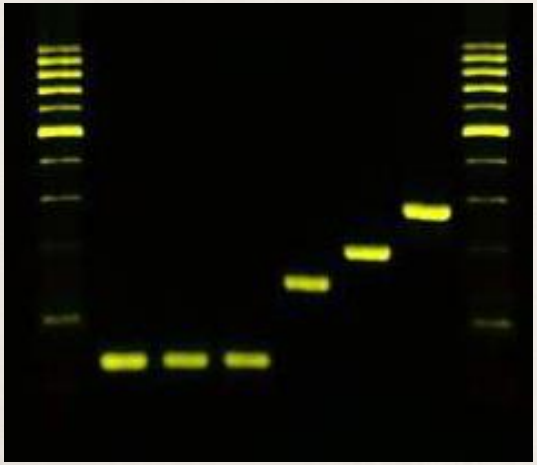


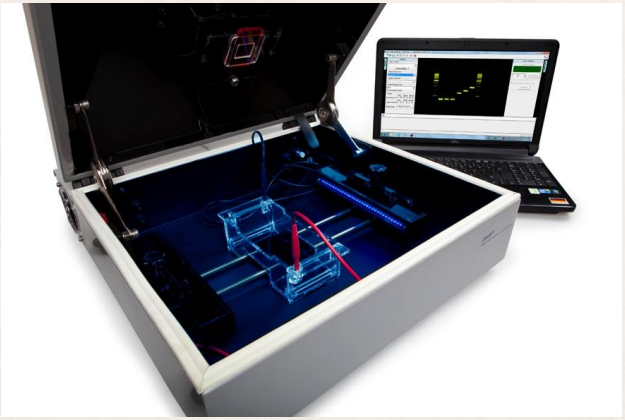


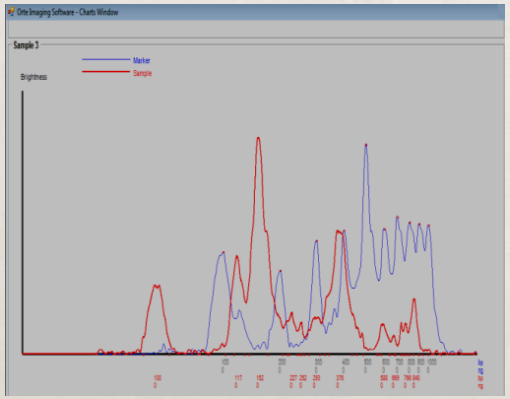
ORTE
İzlenebilir
Elektroforez

Observable Real Time Electrophoresis







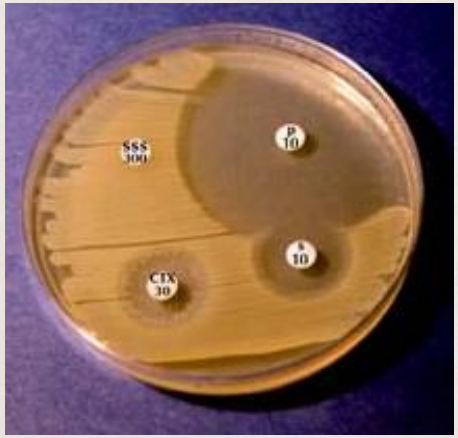


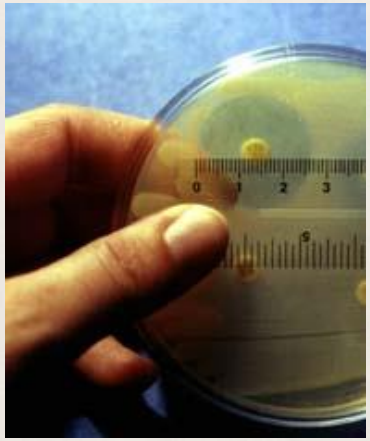
Kullanımdaki Antibiyotik Duyarlılık Testleri

No	Teknoloji	Yöntem	Süre (saat)	MIC	FDA onayı	Emek	Fiyat
1	Disk difüzyon MHA	Agarda üreme	16-24		✔	2	1
2	Disk difüzyon Quicolor	Agarda üreme	4-6		*	2	1
3	Agar dilüsyon	Agarda üreme	16-24	✔	✔	2	2
4	E-test MHA	Agarda üreme	16-24	✔	✔	2	3
5	E-test Quicolor	Agarda üreme	4-6	✔	*	2	3
6	Sıvı dilüsyon MHB	Sıvıda üreme	16-24	✔	✔	2	2
7	MicroScanWalkAway	Sıvıda üreme	4.5-18	✔	✔	1	5
8	Vitek1/Vitek2	Sıvıda üreme	6-11	✔	✔	1	5
9	Phoenix	Sıvıda üreme	9-15	✔	✔	1	5
10	Sensititre	Sıvıda üreme	9-15	✔	✔	1	5

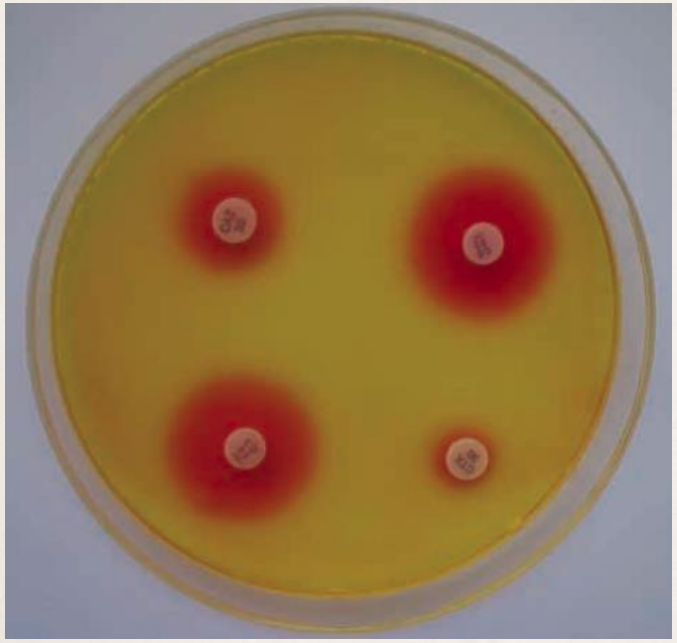
* Güvenilirliği bilimsel çalışma ve yayınlarla kanıtlanmış

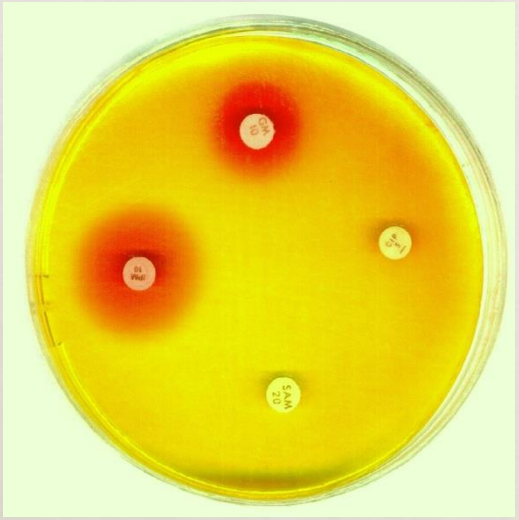
Quicolor

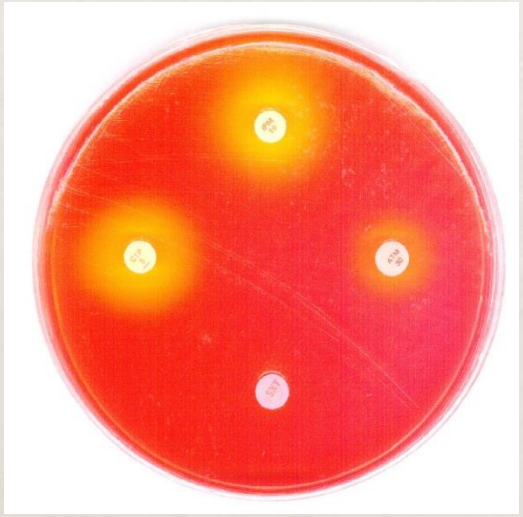




Mueller Hinton 16-24 saat







QUICOLOR
4-6 saat

US006265182B1

(12) **United States Patent**
Kocagoz

(10) **Patent No.: US 6,265,182 B1**
(45) **Date of Patent: Jul. 24, 2001**

(54) **ANTIBACTERIAL SUSCEPTIBILITY TEST**

(76) Inventor: **Zuhtu T. Kocagoz**, Koza Sokak, 88/6 Gazi Osman Pasa, Ankara (TR)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/423,087**

(22) PCT Filed: **May 4, 1998**

(86) PCT No.: **PCT/TR98/00005**

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Cruz, F.O., Database WPI on EPOQUE, week 9109, London:Derwent Publications, Ltd., AN 91–058363, Class D16, BR 8903571 A, abstract.

* cited by examiner

Annals of Microbiology, **57 (1)** 131-135 (2007)

Quicolor: A novel system for rapid antibacterial susceptibility testing

Tanıl KOCAGÖZ[1,2,3]*, Serpil ERCIS[4], Özge DARKA[5], Siavosh SALMANZADEH-AHRABI[6], Sesin KOCAGÖZ[1], Gülşen HASÇELIK[4]

TABLE 1 - The percentage of agreement between Quicolor and CLSI disk diffusion susceptibility results for Enterobacteriaceae (n = 66)

Antibacterial	Minor discrepancy (%)	Major discrepancy (%)	Total discrepancy (%)	Total agreement (%)
Amikacin	0	1.5	1.5	98.5
Ampicillin	4.5	0	4.5	95.5
Cefazolin	4.5	0	4.5	95.5
Cefoperazone	4.5	0	4.5	95.5
Cefotaxime	4.5	0	4.5	95.5
Ceftazidime	3.0	0	3.0	97.0
Cefuroxime	0	0	0	100
Ciprofloxacin	1.5	1.5	3.0	97.0
Gentamicin	1.5	2.0	4.5	95.5
Imipenem	0	0	0	100
Meropenem	0	0	0	100
Piperacillin	1.5	0	1.5	98.5
Ampicilllin/sulbactam	7.5	0	7.5	92.5
Trimethoprim/sulfamethoxazole	4.5	3.0	7.5	92.5
Total	2.7	0.6	3.3	96.7

TABLE 2 - The percentage of agreement between Quicolor and CLSI disk diffusion susceptibility results for staphylococci (n = 41)

Antibacterial	Minor discrepancy (%)	Major discrepancy (%)	Total discrepancy (%)	Total agreement (%)
Cefazolin	0	4.8	4.8	95.2
Ciprofloxacin	4.8	0	4.8	95.2
Clindamycin	4.8	0	4.8	95.2
Erythromycin	7.1	2.4	9.5	90.5
Fusidic acid	0	0	0	100
Oxacillin	0	0	0	100
Penicillin	0	0	0	100
Rifampicin	0	0	0	100
Ampicillin/sulbactam	0	4.8	4.8	95.2
Cefotaxime	2.4	4.8	7.2	92.8
Teicoplanin	0	0	0	100
Trimethoprim/sulfamethoxazole	0	4.8	4.8	95.2
Vancomycin	0	0	0	100
Total	1.5	1.7	3.2	96.8

Table 3 - The percentage of agreement between Quicolor and CLSI disk diffusion susceptibility results for non-fermentative Gram-negative bacteria (n = 70; *Pseudomonas, Acinetobacter, Stenotrophomonas* species)

Antibacterial	Minor discrepancy (%)	Major discrepancy (%)	Total discrepancy (%)	Total agreement (%)
Amikacin	7.0	1.4	8.4	91.6
Aztreonam	7.0	0	7.0	93.0
Ceftazidime	2.8	1.4	4.2	95.8
Ciprofloxacin	2.8	0	2.8	97.2
Gentamicin	2.8	0	2.8	97.2
Imipenem	9.9	0	9.9	90.1
Meropenem	7.0	1.4	8.4	91.6
Piperacillin	0	2.8	2.8	97.2
Total	4.9	0.9	5.8	94.2

Is rapid antibacterial susceptibility testing medium reliable for routine laboratory practices?

Yagmur G, Ercal BD, Mengeloglu Z, Sariguzel FM, Berk E, Saglam D - Pak J Med Sci (2015 Mar-Apr)

Bottom Line: In the present study, it was aimed to compare the antibiotic susceptibility results of Quicolor (Salubris Inc., Massachusetts, USA) and standard disk diffusion method.Antibiotic susceptibility in clinical isolates was evaluated using Mueller-Hinton (MH) agar and Quicolor (ES and NF) agar plates.For Enterobacteriaceae, frequency of total concordance, major error, and minor error between the tests were found as 96.8%, 0.8%, and 2.4%, respectively.

View Article: PubMed Central - PubMed

Affiliation: Gulhan Yagmur, Department of Postmortem Microbiology, Council of Forensic Medicine, Istanbul, Turkey.

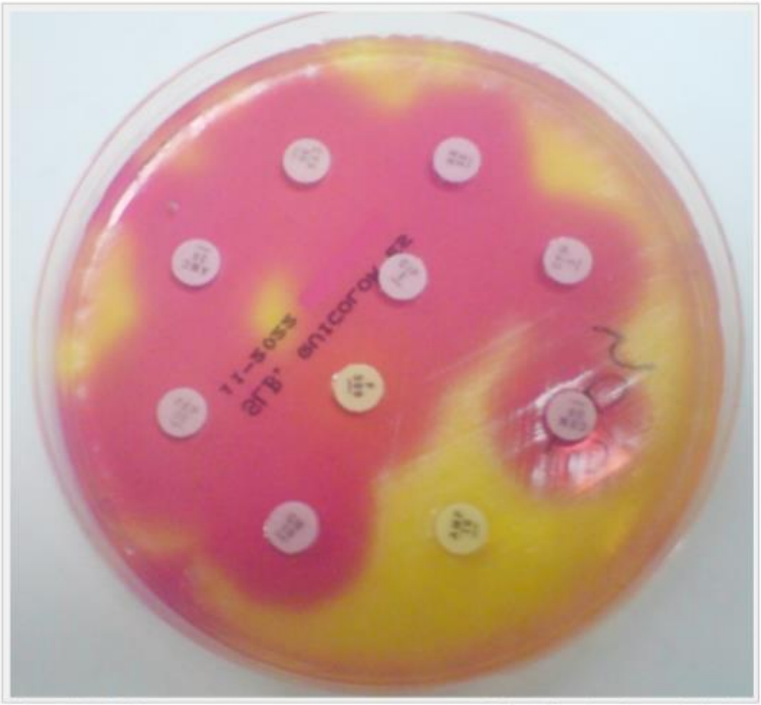
ABSTRACT

Objective: Early detection of antibiotic susceptibility profile of the isolates has critical importance in terms of immediate beginning of the appropriate treatment and increasing of treatment success, such as meningitis, bacteriemia and sepsis. In the present study, it was aimed to compare the antibiotic susceptibility results of Quicolor (Salubris Inc., Massachusetts, USA) and standard disk diffusion method.

Methods: One hundred twenty three isolates were included in this study (80 Enterobacteriaceae, 15 Staphylococci and 28 nonfermentative Gram-negative bacteria). Antibiotic susceptibility in clinical isolates was evaluated using Mueller-Hinton (MH) agar and Quicolor (ES and NF) agar plates.

Results: For Enterobacteriaceae, frequency of total concordance, major error, and minor error between the tests were found as 96.8%, 0.8%, and 2.4%, respectively. For Staphylococci, frequency of total concordance, major error, and minor error among the tests were found as 95.7%, 3.5%, and 0.8%, respectively. For non fermentative bacteria, frequency of total concordance, major error, and minor error among the tests were found as 83.9%, 9.6%, and 6.4%, respectively.

Conclusions: Quicolor media provided reliable susceptibility results in enteric bacteria and Staphylococci. However, further studies including higher number of nonfermentative bacteria are required to determine whether the chromogenic media are appropriate for this group of bacteria.

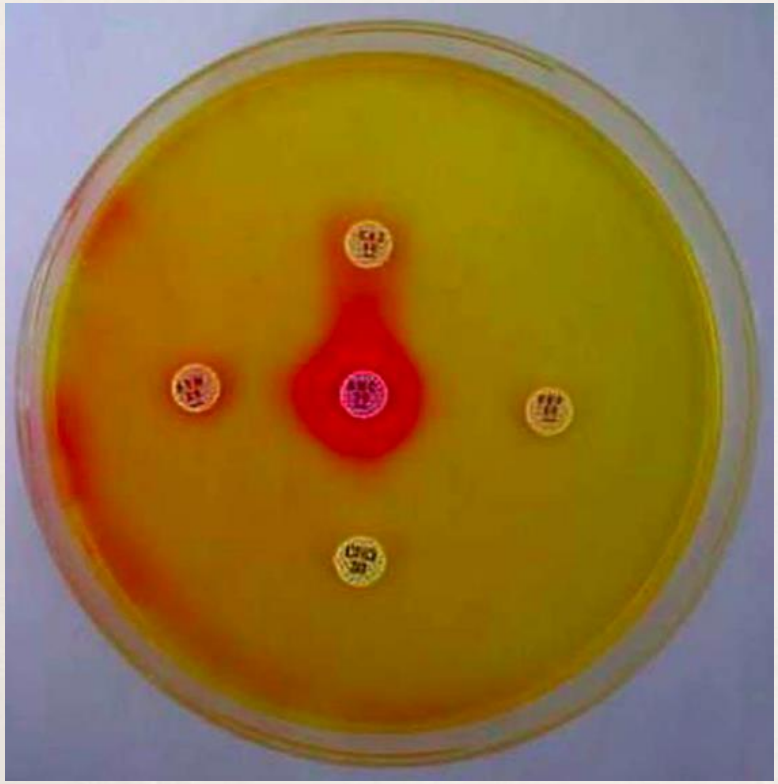


Related In: Results - Collection
Show All Figures

Figure 1: A sample for Quicolor media.

BMC Infectious Diseases 2003, **3**:22

Atahan Çağatay,
Tanıl Kocagöz,
Haluk Eraksoy

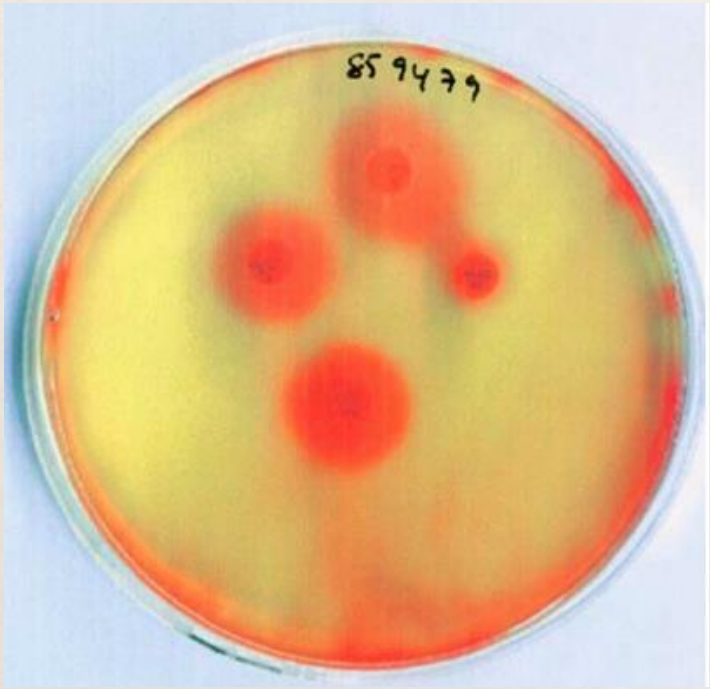


Quicolor ile 4 saatte GSBL saptama

Evaluation of a chromogenic medium for rapid detection of extended spectrum β-lactamase producing *Salmonella* spp.

Indian J Med Res 124, October 2006, pp 443-446

Sesin Kocagöz, Fatma Budak* & Deniz Gür**



Quicolor ile Salmonella'larda 4 saatte GSBL saptama

Scandinavian Journal of Infectious Diseases, 2007; 39: 781–785

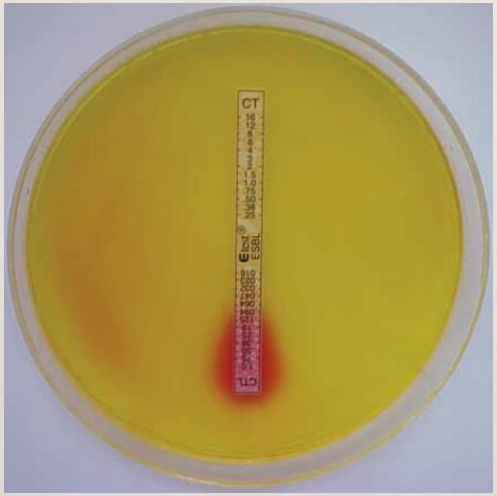
informa
healthcare

ORIGINAL ARTICLE

Rapid 4 to 6 hour detection of extended-spectrum beta-lactamases in a routine laboratory

SERPIL ERCIS[1], BANU SANCAK[1], TANIL KOCAGÖZ[2–4], SESIN KOCAGÖZ[2], GÜLŞEN HASÇELIK[1] & ANNE BOLMSTRÖM[5]

From the [1]Hacettepe University Faculty of Medicine, Ankara, [2]Yeditepe University Faculty of Medicine, [3]Turkish Innovative Biotechnology Organization, [4]Salubris A.S., Istanbul, Turkey, and [5]AB BIODISK, Solna, Sweden

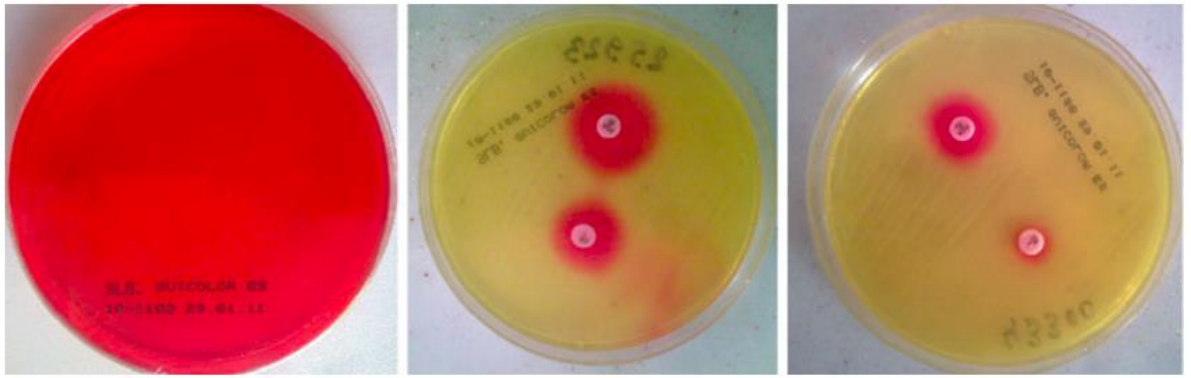


World J Microbiol Biotechnol (2011) 27:1901–1904
DOI 10.1007/s11274-011-0649-y

ORIGINAL PAPER

Rapid detection of methicillin resistance in *Staphylococcus aureus* isolates; evaluation of colorimetric Quicolor ES agar and determination of breakpoint inhibition zone diameters of cefoxitin

Ahmet Yilmaz Coban · Ugur Demırpek · Tuba Yıldırım · Yeliz Tanrıverdı Caycı · Tanil Kocagoz · Belma Durupınar



A QC ES agar (There is no growth) **B** *S. aureus* ATCC 25923 **C** *S. aureus* ATCC 43300

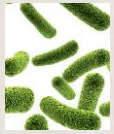
Quicolor ile MRSA saptama 4-7 saat

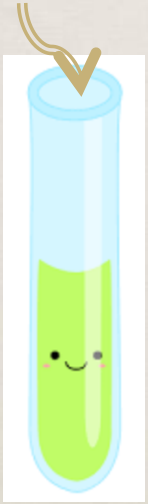
Geliştirilmekte olan Antibiyotik Duyarlılık Testleri

No	Teknoloji	Yöntem	Süre (saat)	MIC	FDA onayı
1	Multiplex Automated Digital Imaging Microscopy (MADM)	Mikroskopla izleme	3-5	✔	✔
2	Single cell morphological analysis (SCMA)	Mikroskopla izleme	3-4	✔	
3	oCelloscope	Optik sistem izleme	1-4	✔	
4	BacterioScan FLLC	Bakteri boy ve sayı	3-10	✔	
5	Lifescale Microchannel Resonator	Bakteri boy ve sayı	3-10	✔	
6	Genefluidics	16s RNA artışı	4	✔	
7	AFM cantilerver	Atomik Güç Mikros.	2	✔	
8	PIT	Bakteri hareketi	2	✔	
9	Akım Sitometrisi	Akım sitometrisi	2-3	✔	
10	IMC (Infrared Measurment)	Isı biçemi	3-14	✔	
11	Lumisen	Biyoışıma	1	✔	

LUMISEN

Lusiferin —(ATP / Lusiferaz)→ Okside Lusiferin+



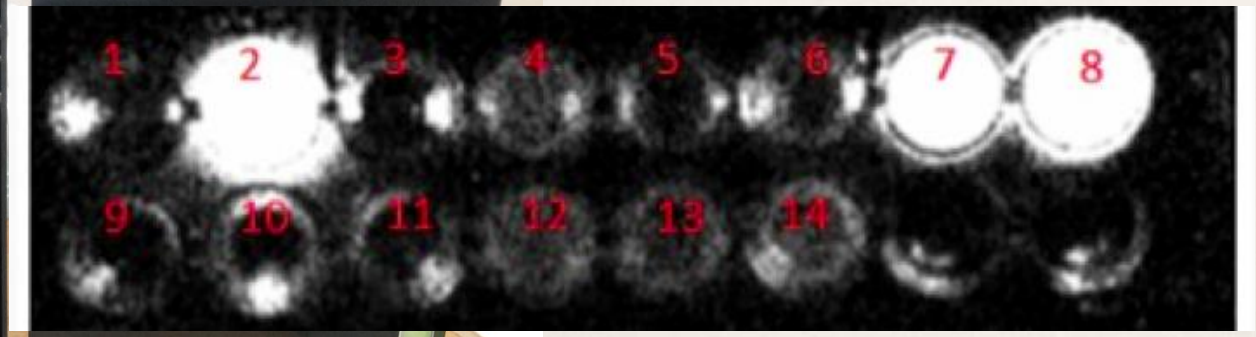


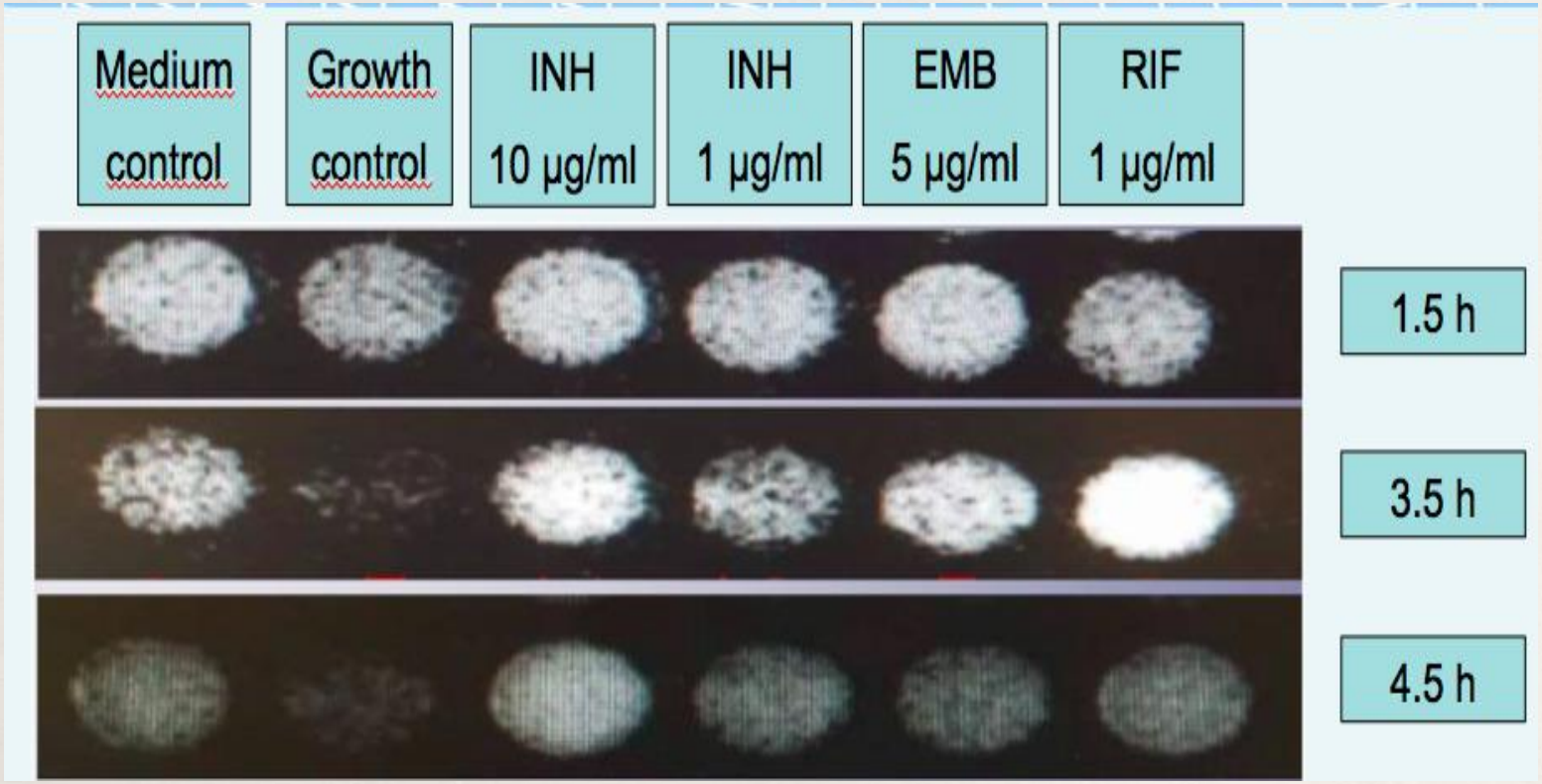
ATP'li besiyeri

1 saat

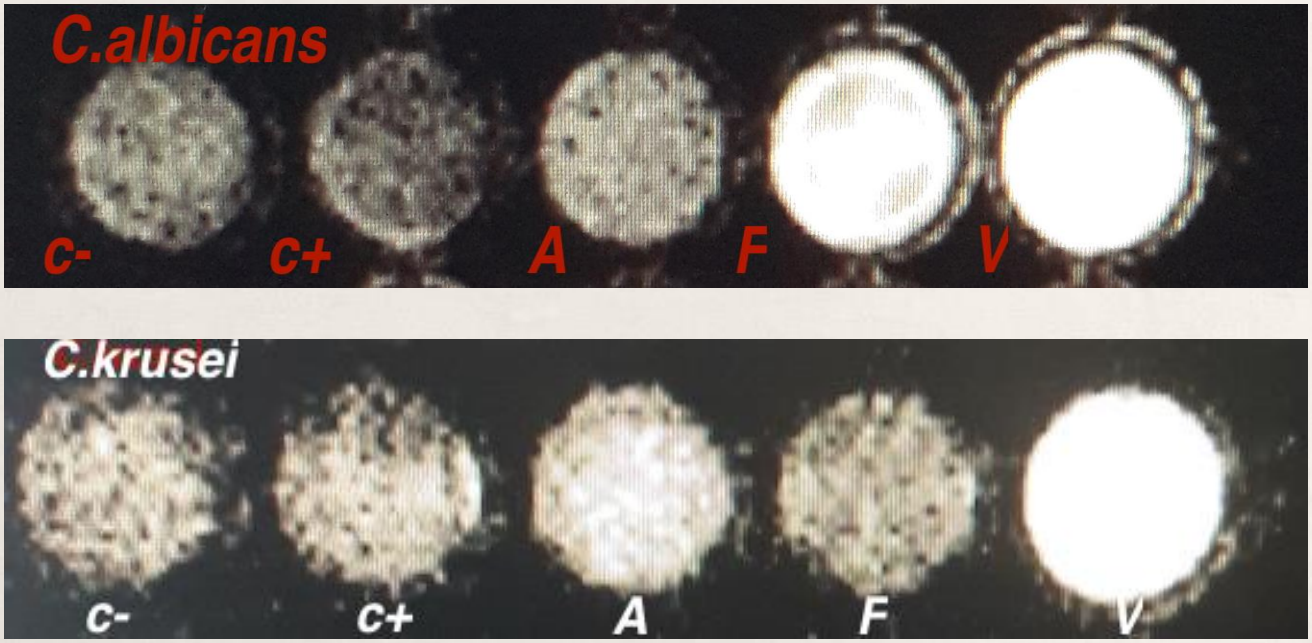
Biyoışıma Temelli Hızlı Antibiyotik Duyarlılık Testi

LUMISEN





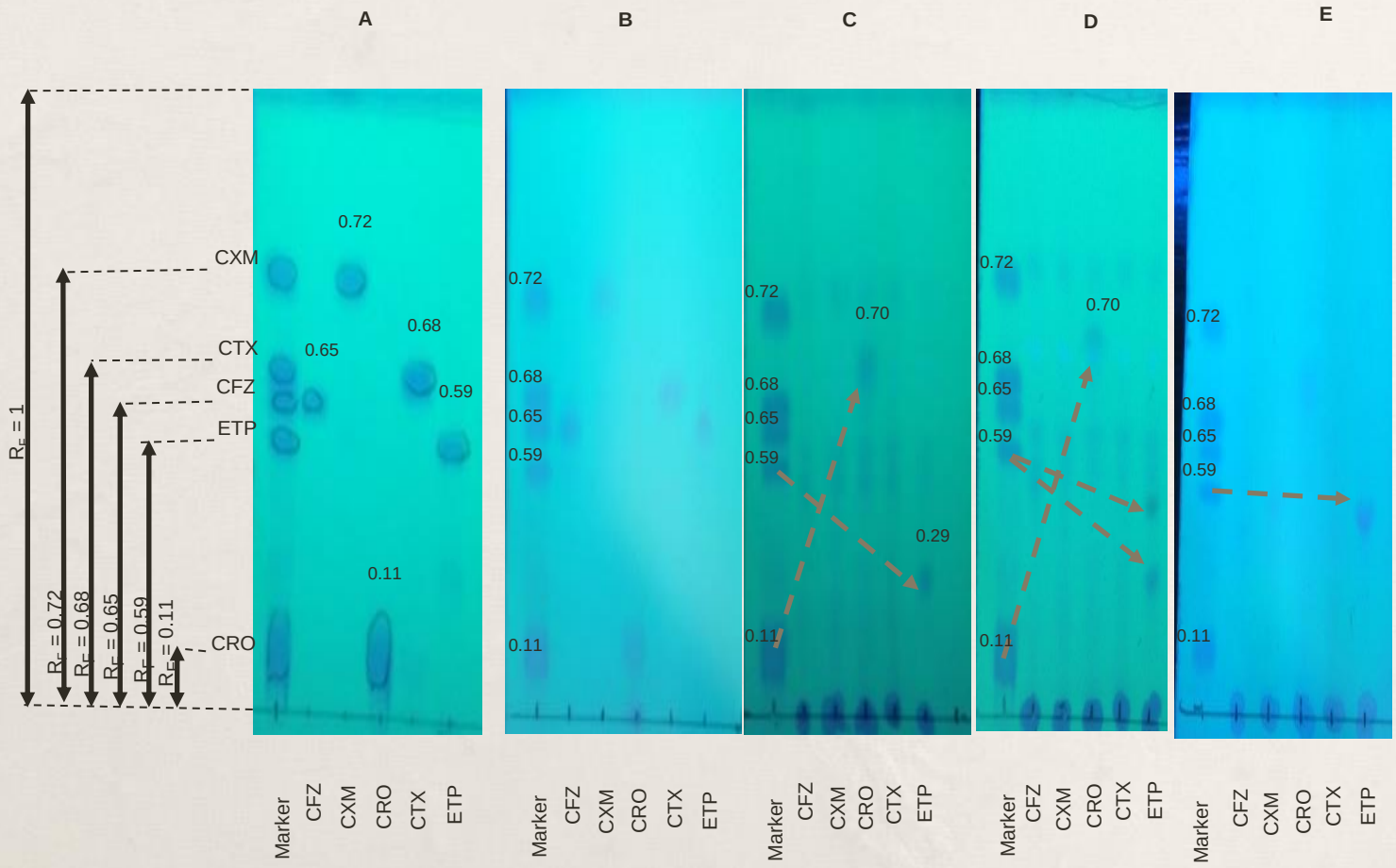
Mycobacterium tuberculosis

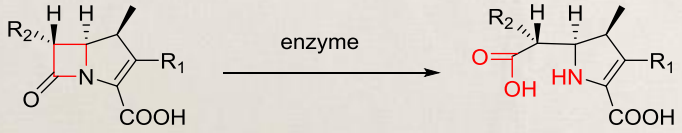


2-6 saat

Mayalar

Lactamaster

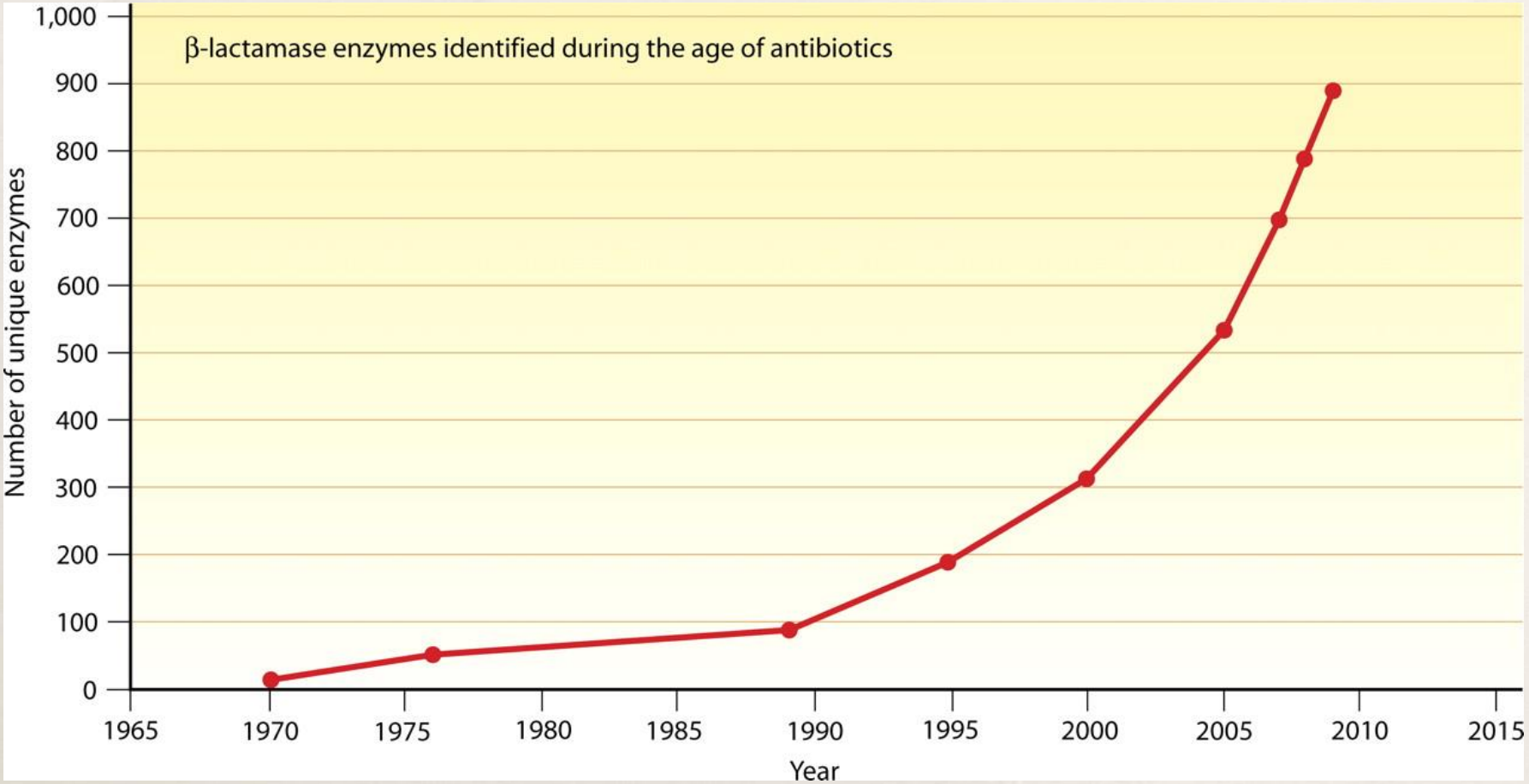




β-Lactam Antibiotic

Degraded Product

Beta-laktamazların önlenemez yükselişi

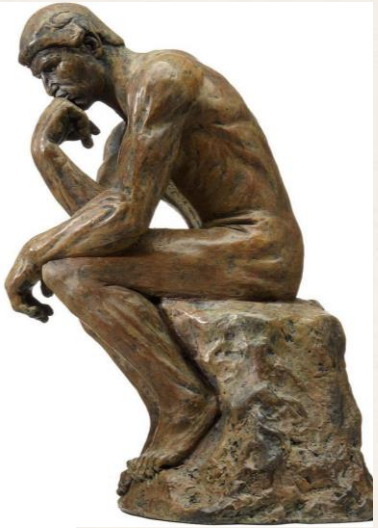


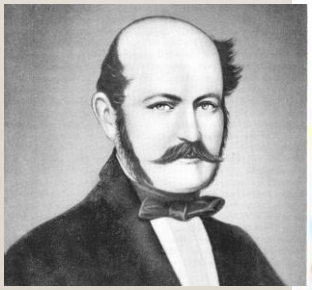
History of antibiotic discovery and concomitant development of antibiotic resistance.

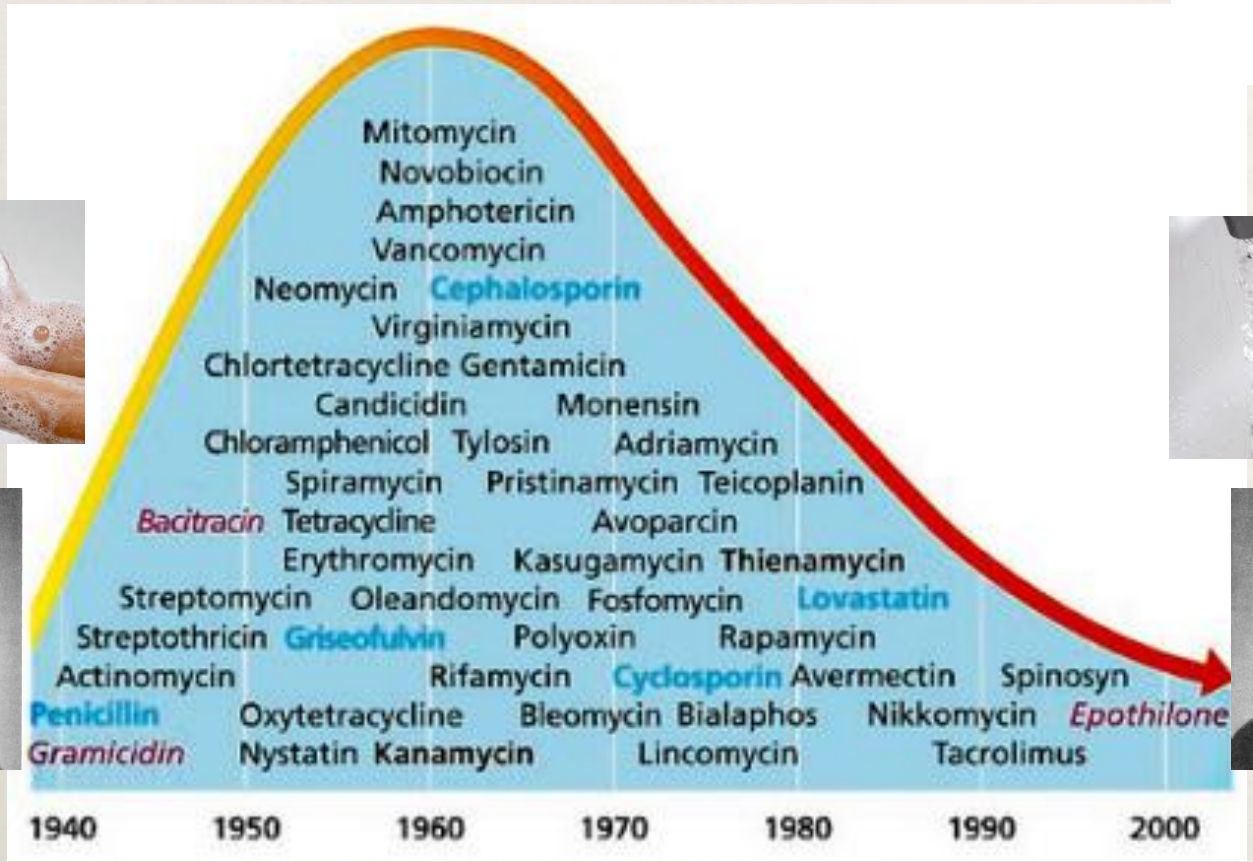
Davies J , and Davies D Microbiol. Mol. Biol. Rev. 2010;74:417-433

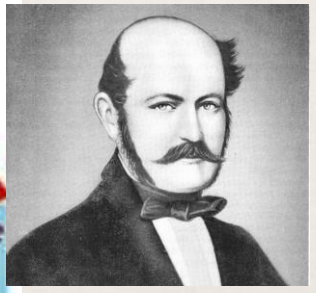
Microbiology and Molecular Biology Reviews

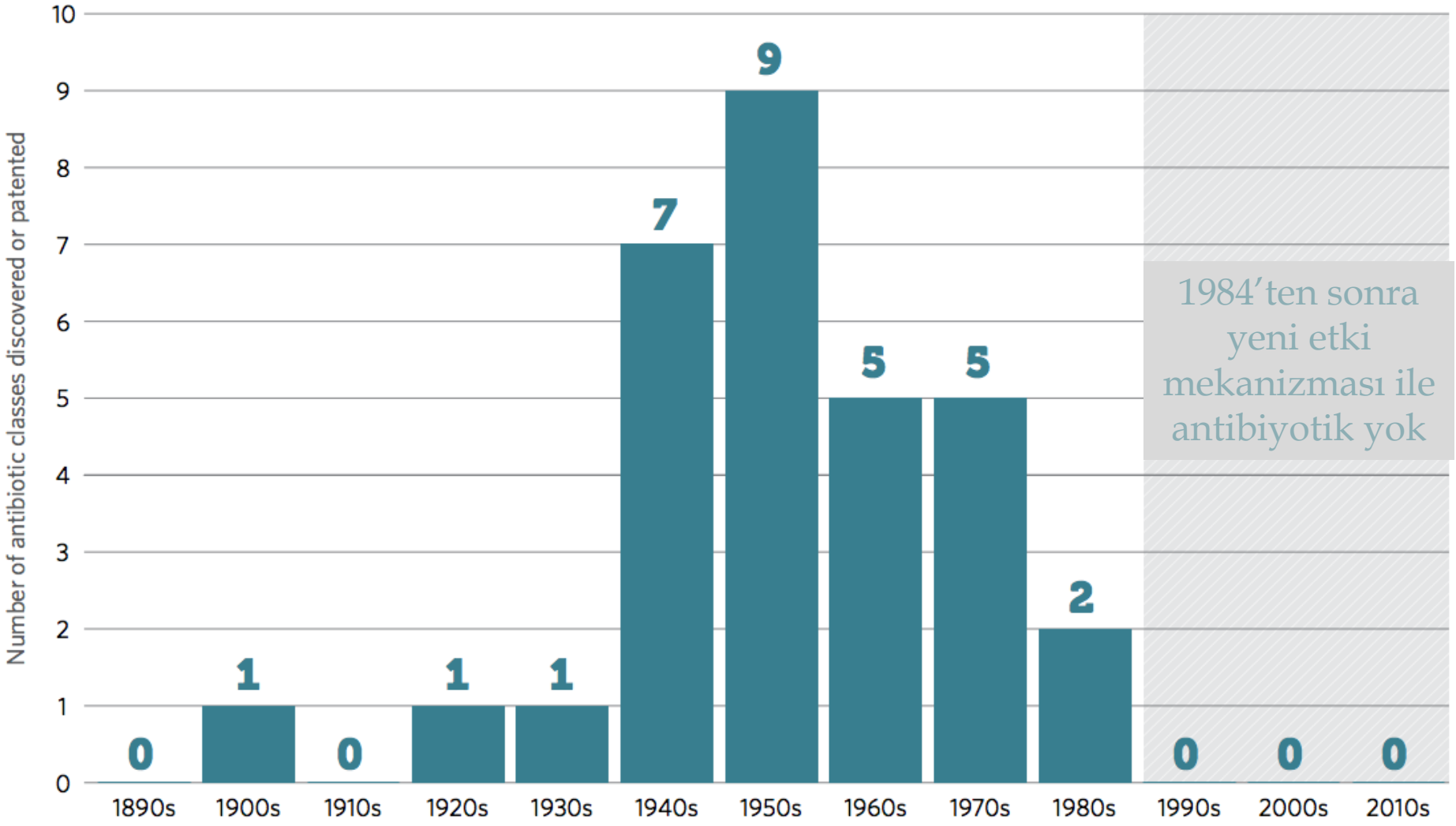
Semmelweis'dan Semmelweis'a





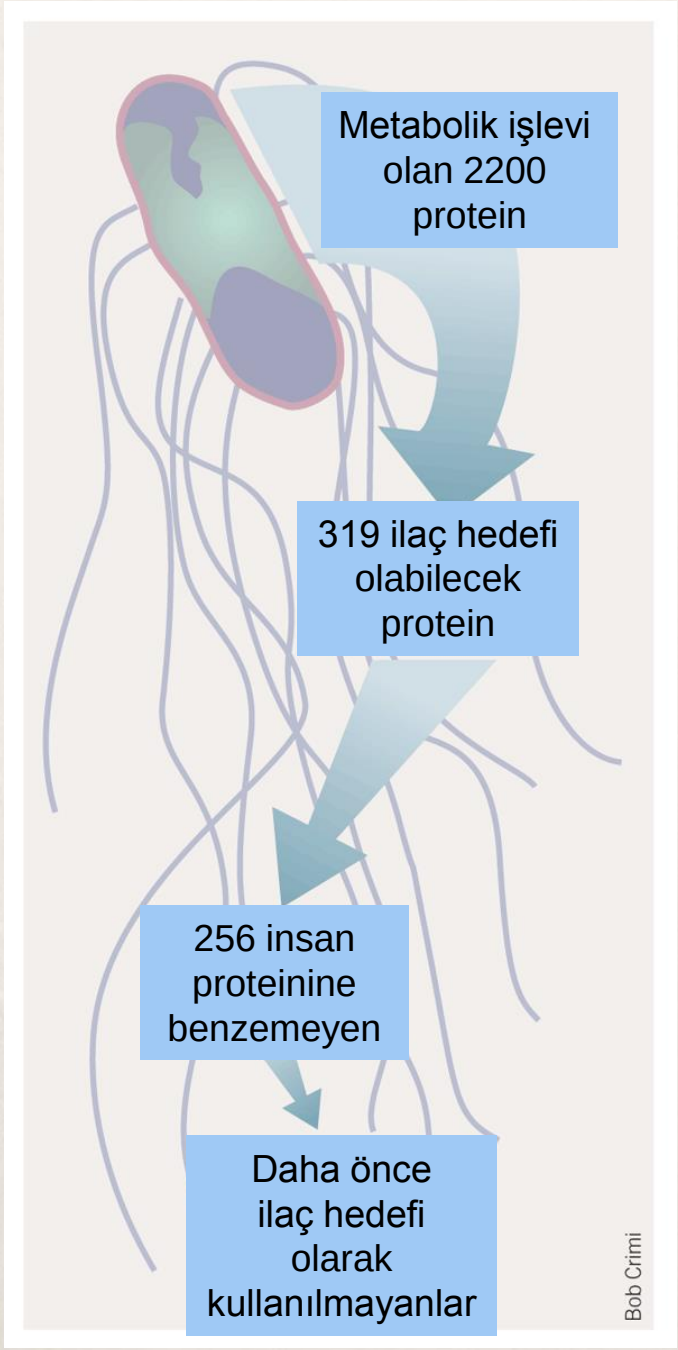





Number of antibiotic classes discovered or patented
10
9
8
7
6
5
4
3
2
1
0
0
1
0
1
1
7
9
5
5
2
0
0
0
1890s
1900s
1910s
1920s
1930s
1940s
1950s
1960s
1970s
1980s
1990s
2000s
2010s
1984'ten sonra yeni etki mekanizması ile antibiyotik yok

Biyoinformatik Yaklaşım

* Bakterilerde ilaç hedefi olabilecek proteinler saptanmakta, insan hücrelerindeki benzerlikleri ile karşılaştırılmakta.
* *E. coli* proteinlerinin 319 tanesinin (%7'sinin) ilaç hedefi olabileceği belirlenmiş, bunlardan 63 tanesinin insan proteinlerine belirgin oranda benzerlik gösterdiği saptanmıştır.
* Geriye kalan 256 proteinin insan hücrelerinde bulunmadığı ve ilaç hedefi olabileceği sonucuna varılmıştır. Bu tür yaklaşımlar çok sayıda yeni antibakteriyel ilacın akılcı bir şekilde geliştirilmesini sağlayabilir.



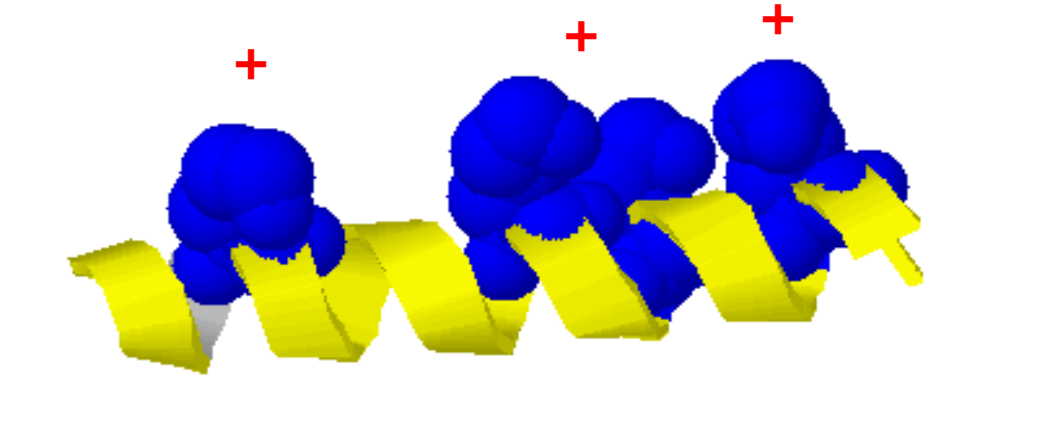
Ortak yanımız nedir?

Peptit antibiyotikler

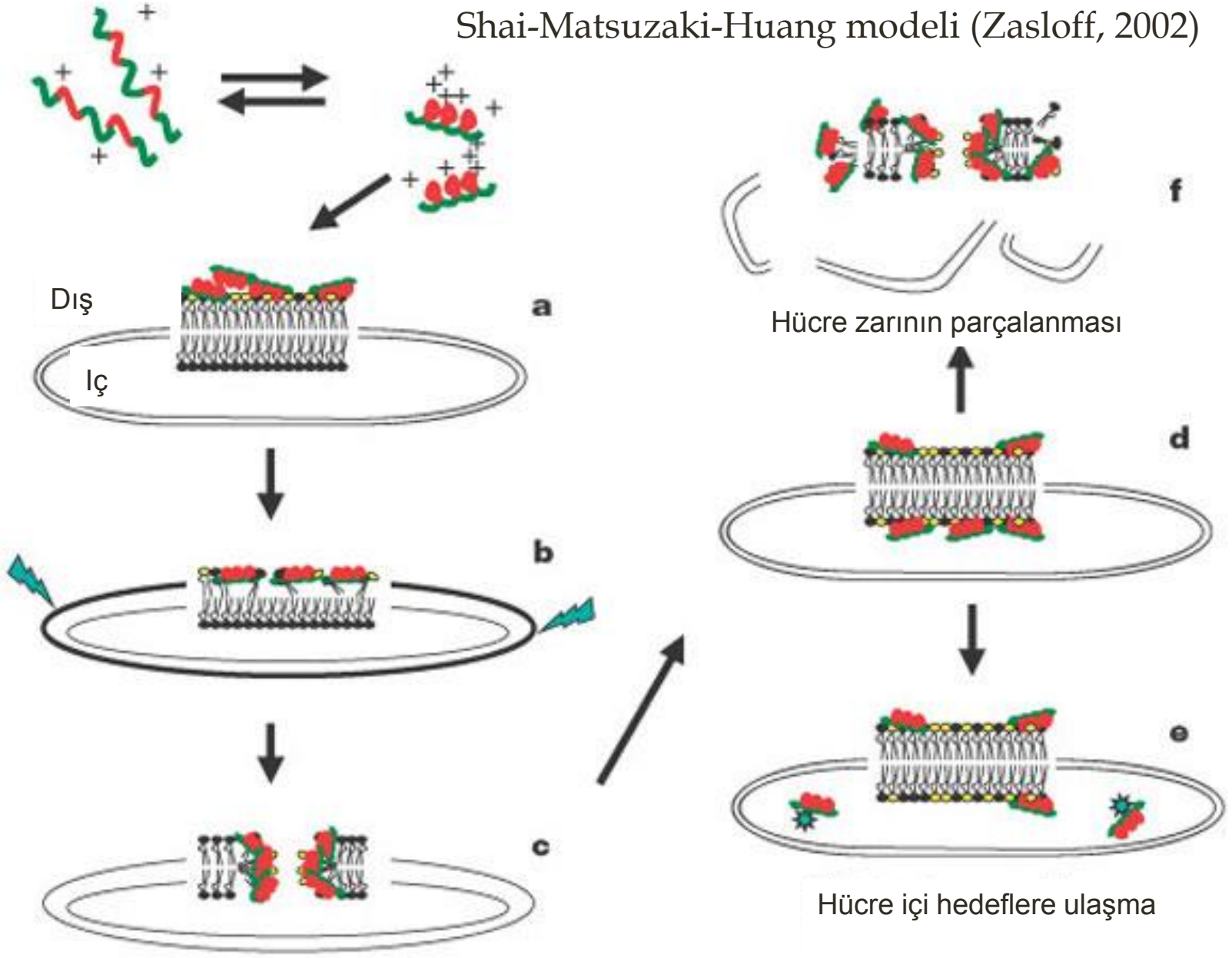
Peptit Antibiyotik Genlerinde Türler Arasında Korunmuş Bölgeler

```
057 ----------mrlhhllltllflvlsag----------------sgf-----------
058 ----------mkifffifaalillaqif----------------q-------------
059 ------malirktfyflfavffilvqlp----------------sgc-qagl--dfsqp
060 ----------mrtsylllftlclllsema----------------sgd-----------
061 ----------mrtflflfvvlffltpak----------------n-------------
062 ---------mr-rlvllltisvllyqdl----------------p-------------
063 ----------mrtsylllftlcllmsema----------------sgd----------
064 ----------mrlhrlllvfllmvllpv----------------pgl----------
065 ------mpgamkifffifaalillaqif----------------q-------------
066 ----------mkifffifaalillaqif----------------q-------------
067 ----------mrtflflfavlffltpak----------------n-------------
068 ------malirktfyfvfavffilvqqp----------------sgc-qagl--efsep
069 ---------mr-rlvlllaislllyqdl----------------p-------------
070 ----------mrtsylllftlclllsema----------------sgg----------
071 ----------mklllltlaalllv-----------------------------------
072 ----------mrvlffvfgvlslmftvp----------------p-------------
073 ------malikktffflfamffilvqls----------------sgc-qagl--dfsqp
074 ---------mpqtffvfcflffvflqlf----------------p-------------
075 ----------mktflflfavlffldpak----------------n-------------
```

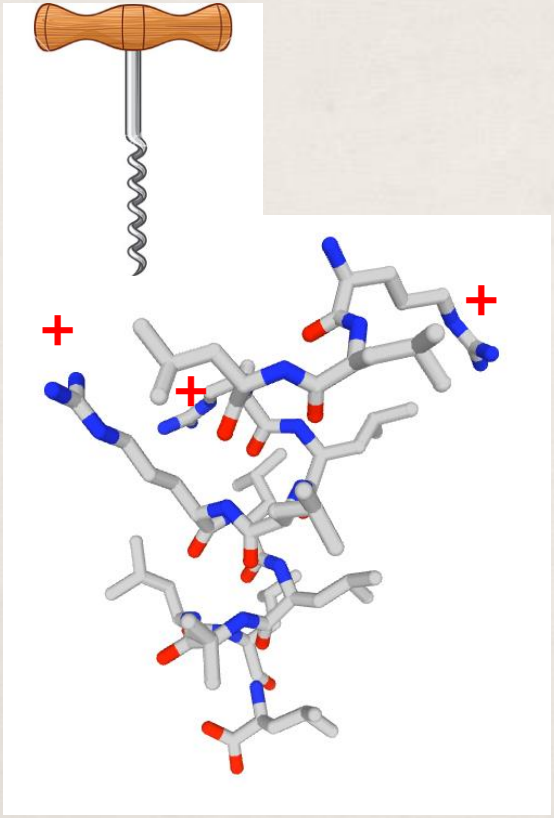
Katelisidinler

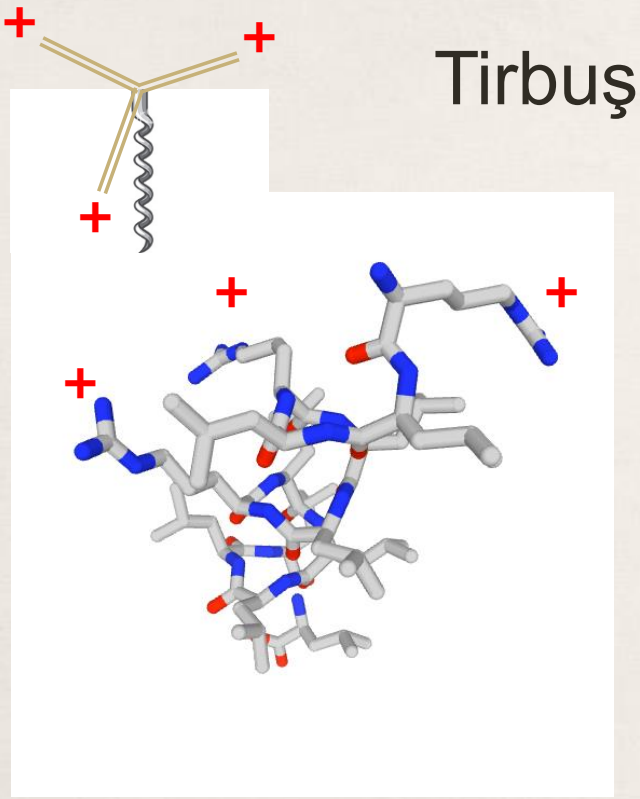


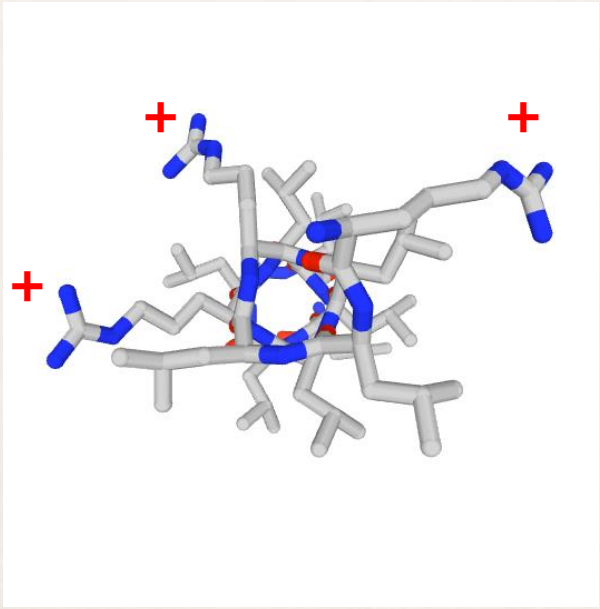
Shai-Matsuzaki-Huang modeli (Zasloff, 2002)

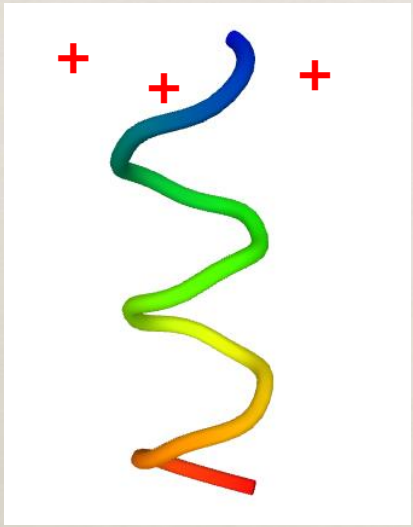


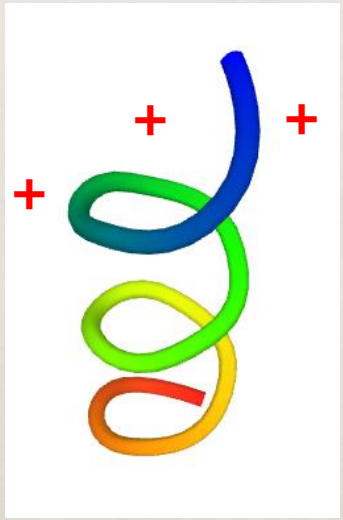
Tirbuşon antibiyotik

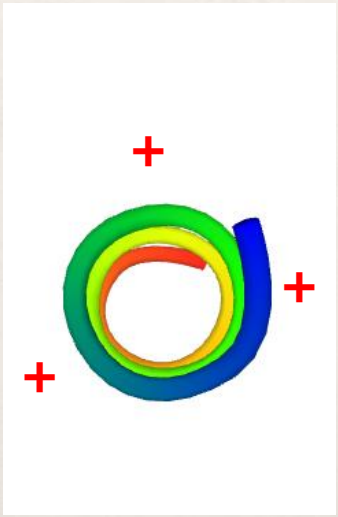




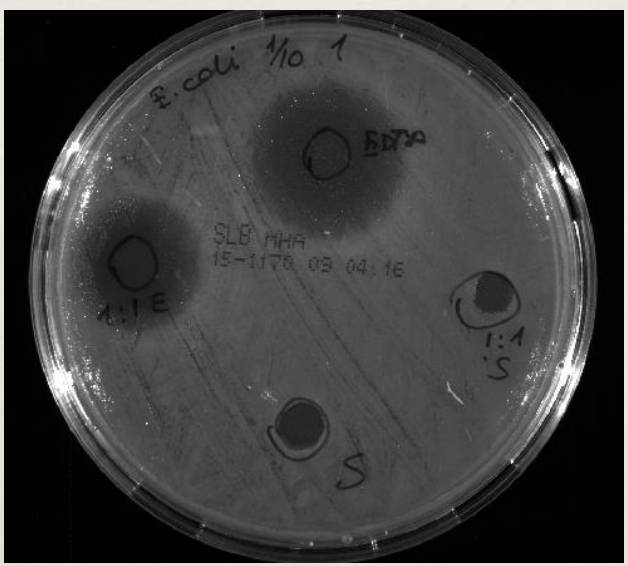


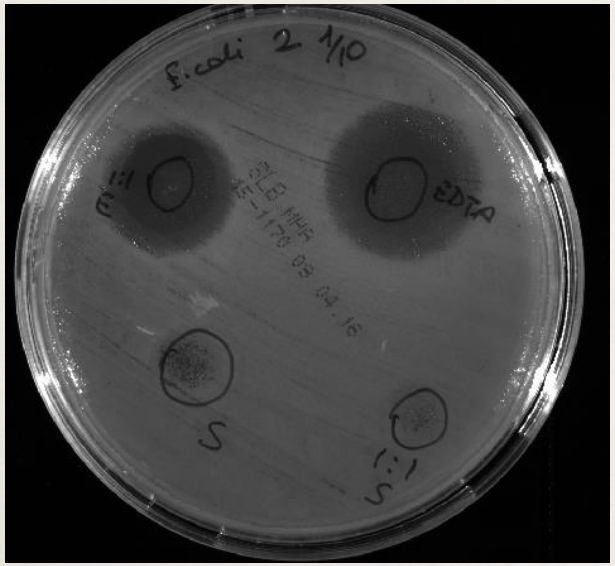


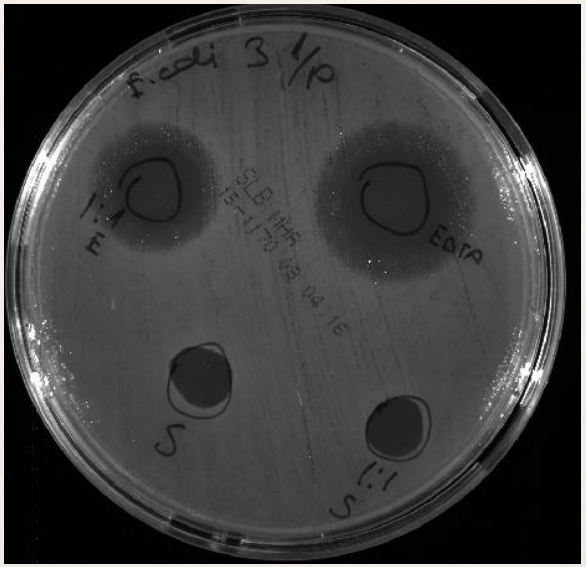


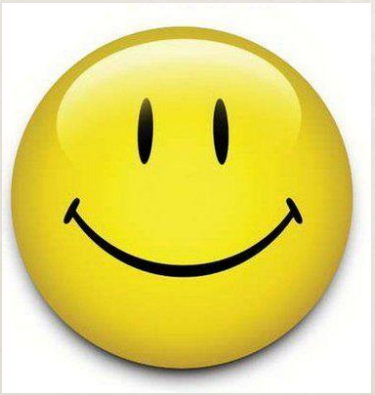


E. coli’ye etkinlik

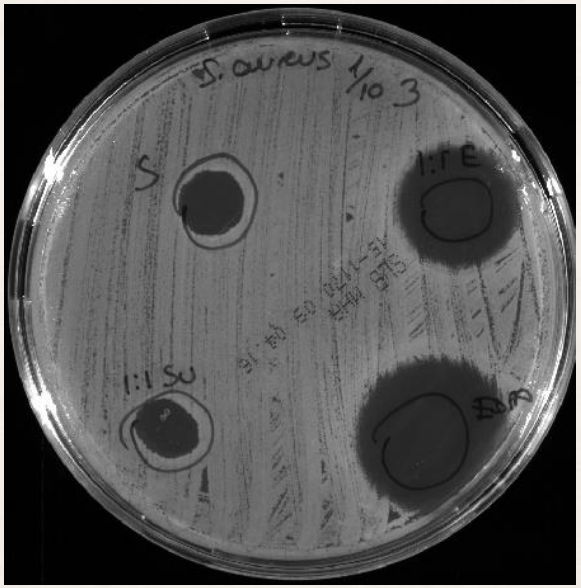




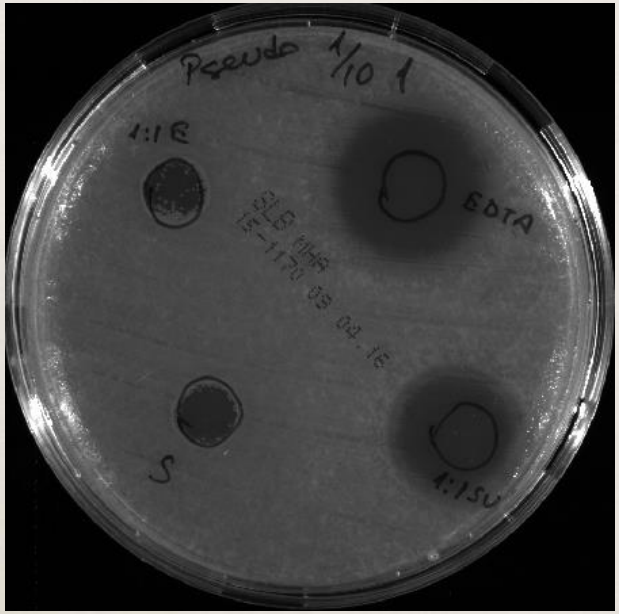


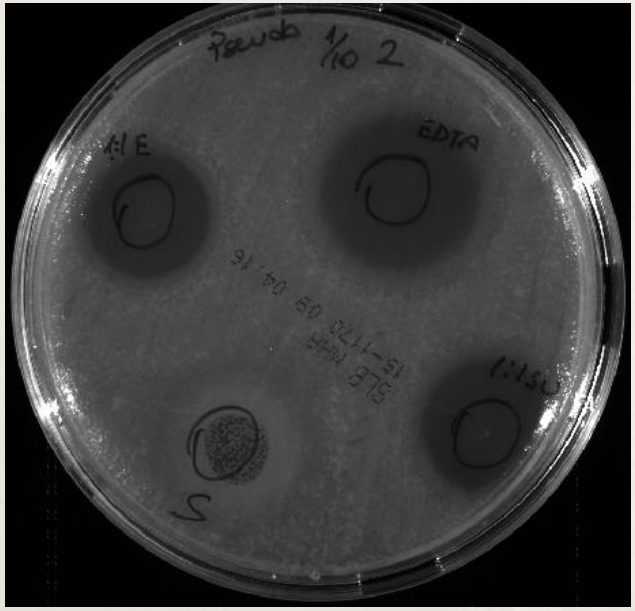


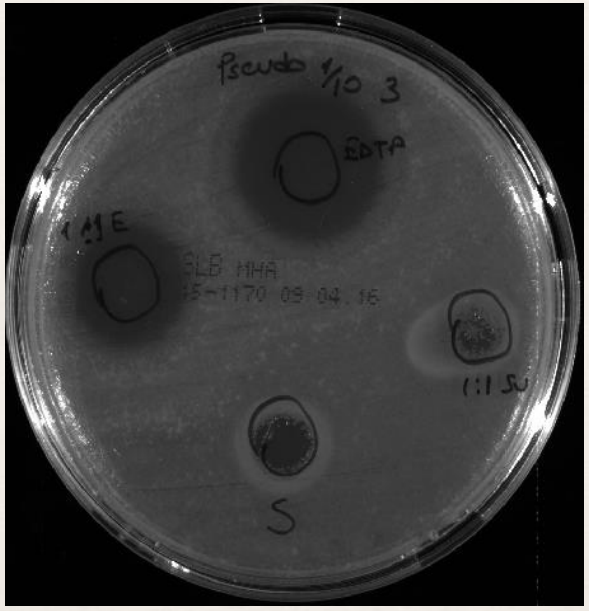
Staphylococcus aureus'a etkinlik



Pseudomonas aeruginosa'ya etkinlik

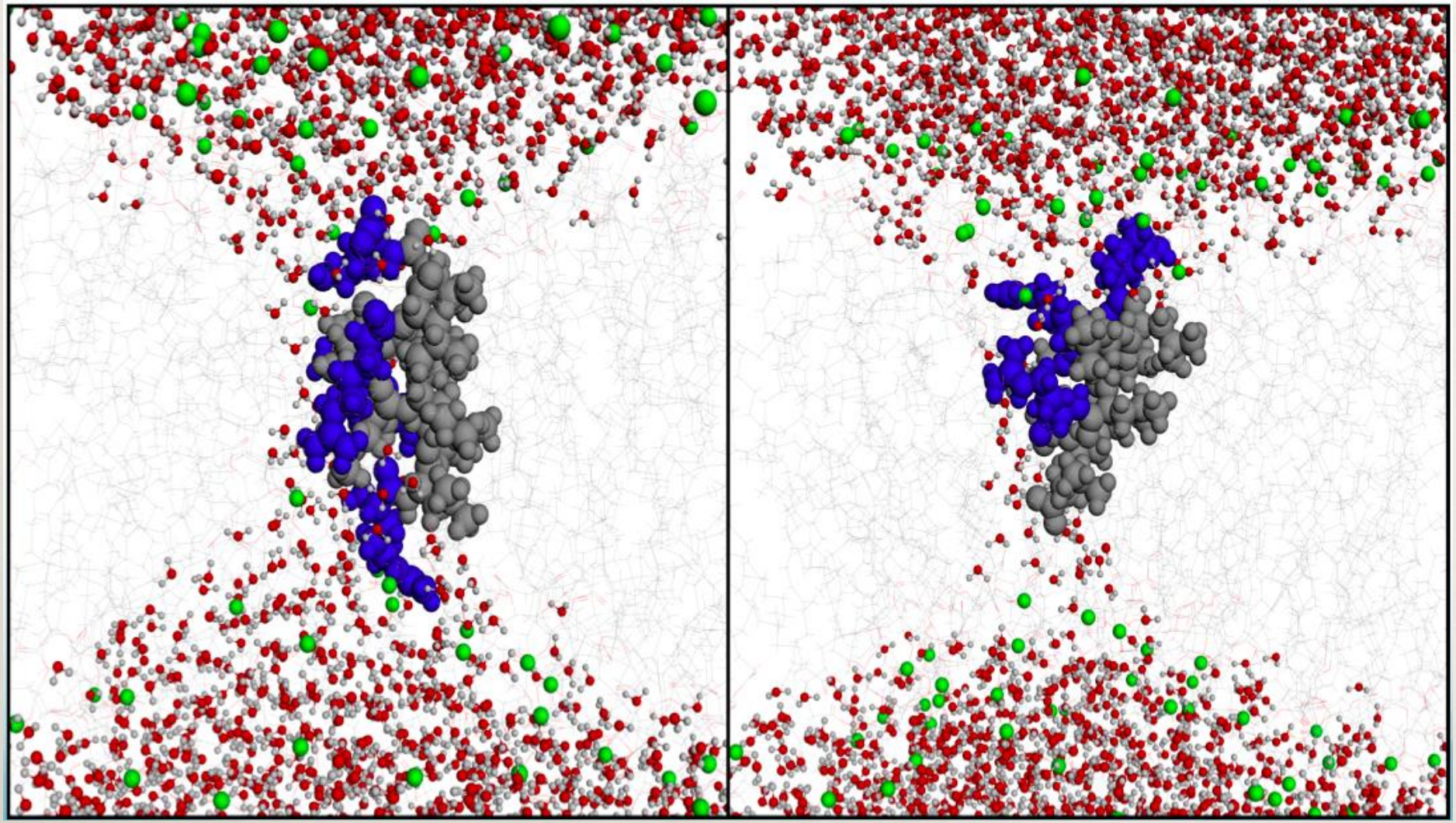






MIC (µg/ml)	*S. aureus* ATCC 29213	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 10031	*P. aeruginosa* ATCC 27853
TN1	16	2	16	16
TN2	64	16	512	> 512
TN3	8	4	16	16
TN4	> 512	> 512	512	256
TN5	>512	> 512	> 512	> 512
TN6	4	4	8	32
TN7	256	64	256	64

Hesaplamalı moleküler modelleme ile TN1 ve TN3'ün hücre zarında oluşturduğu kanal



Yeni Enfeksiyon Tedavisi Yaklaşımları

* Faj tedavisi
* Monoklonal antikorlar
* siRNA
* Probiyotik, mikrobiyom

Kaynaklar

* Tübitak projeleri: Kurum hissesi %40'a çıktı
* Birçok araştırma kuruluşunda alt yapı gelişti. Bunlardan herkesin yararlanabileceği bir sistem kurmak olanaklı
* Endüstri ve finans Türkiye'de biyoteknolojiye yatırım yapmaya başladı. Üniversite-Endüstri işbirliğini geliştirmenin tam zamanı

Teşekkürler