Gülhane Mikrobiyoloji Günleri

20 - 22 Nisan 2010

Antimikrobik Kemoterapi Laboratuvar Uygulamaları ve Yenilikler

KLİMUD

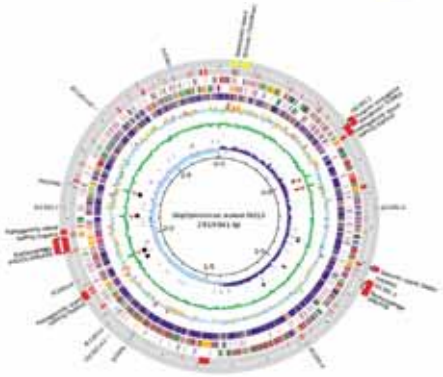
GÜLHANE ASKERİ TIP AKADEMİSİ 1898

TÜRK MİKROBİYOLOJİ CEMİYETİ 1931

Kan Akımı Enfeksiyonlarında MRSA Moleküler Tanısı

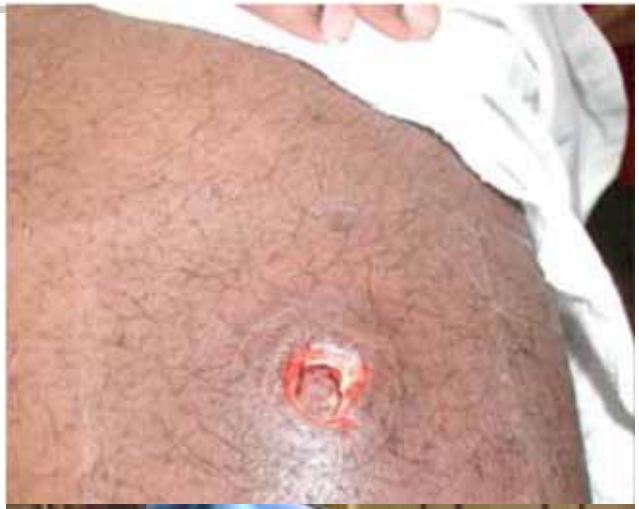
Dr. Abdullah KILIÇ

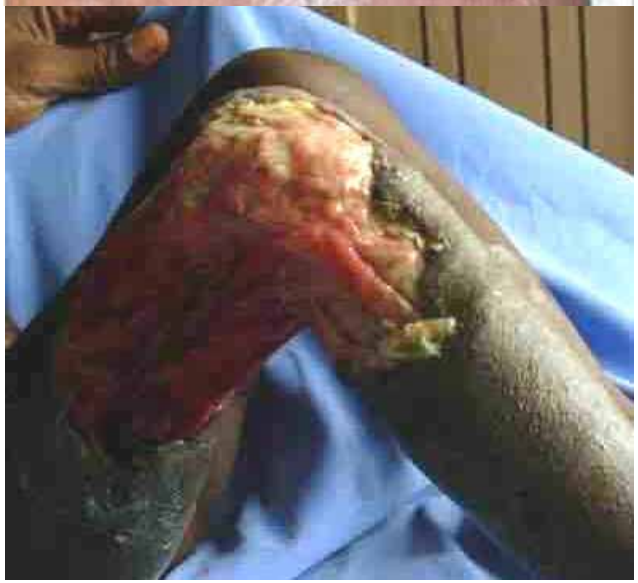
GATA Tıbbi Mikrobiyoloji AD



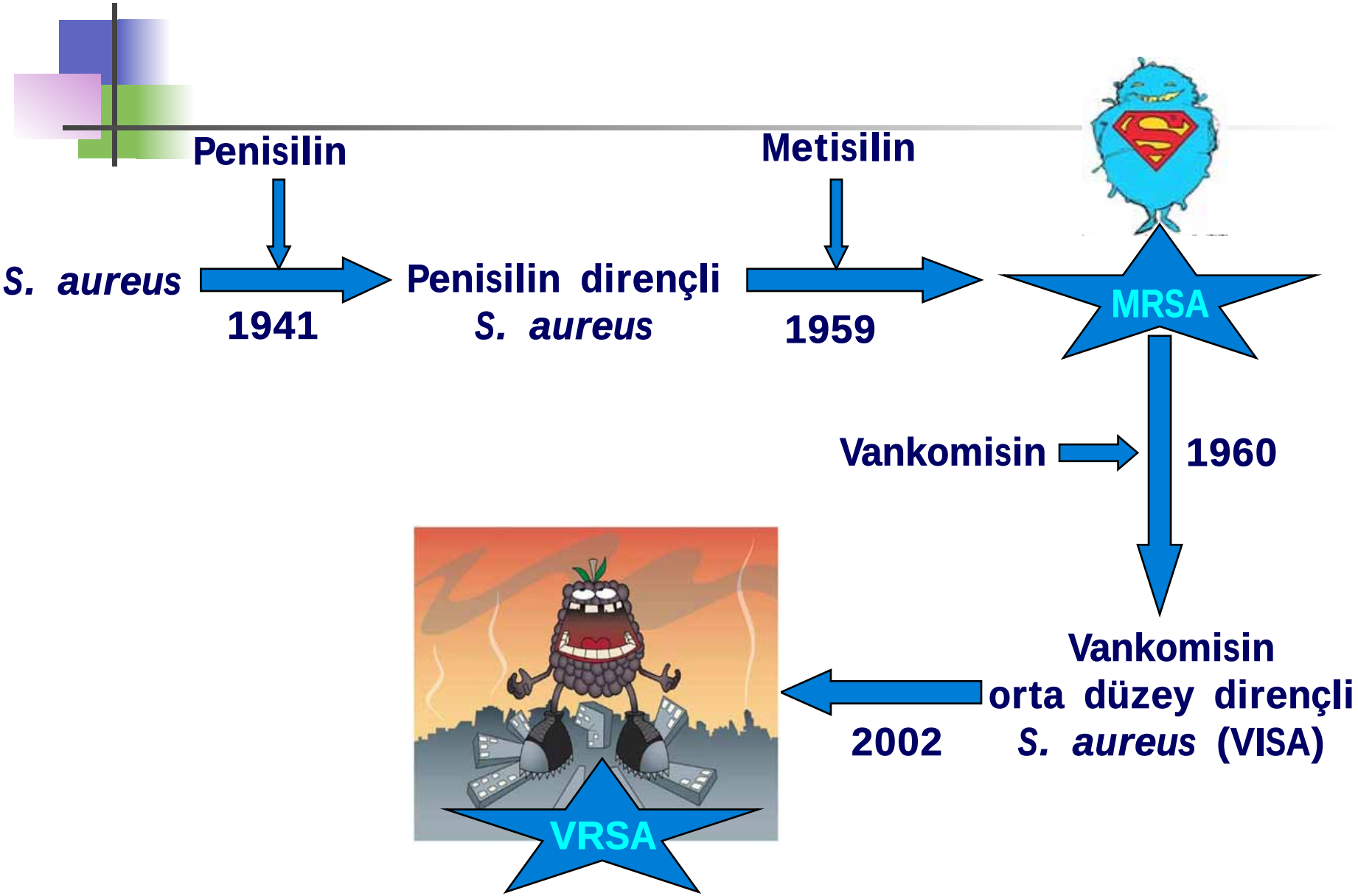
Staphylococcus aureus

- Hastane ve toplum kaynaklı enfeksiyonlara neden olmakta
- Enfeksiyonlar
  - Alt solunum sistemi
  - Cerrahi yara
  - Kan akımı
  - Kardiyovasküler sistem (endokardit)
  - Deri ve yumuşak doku enfeksiyonları
  - Osteomiyelit, septik artrit
- ABD'de yıllık 300.000 vaka, 12.000 ölüm, 2,7 milyon gün yatış süresi, 9.5 milyar $ ilave maliyet

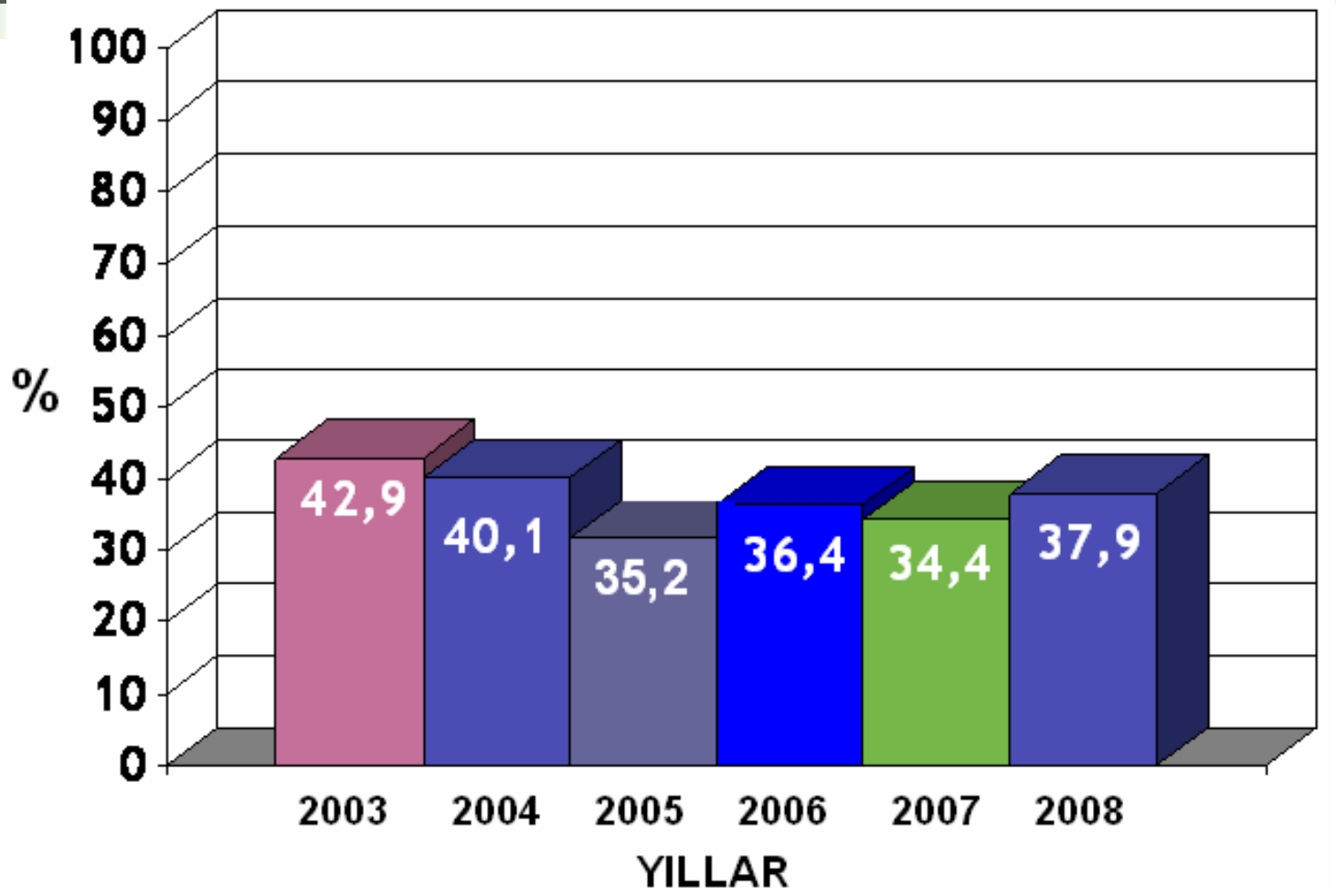




Noskin et al. Arch Intern Med (2005) 1756-59.

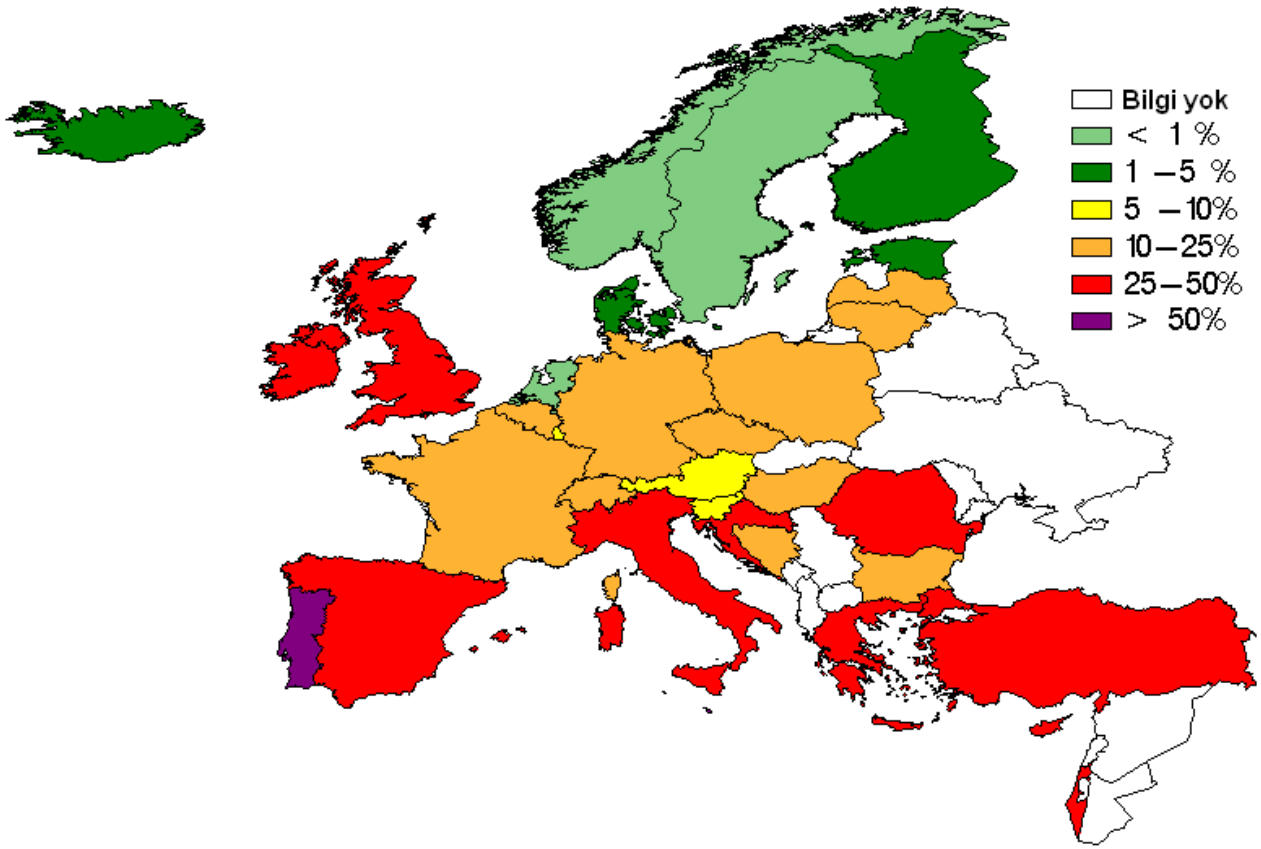

Penisilin
Metisilin
S. aureus
1941
Penisilin dirençli
S. aureus
1959
MRSA
Vankomisin
1960
Vankomisin
orta düzey dirençli
S. aureus (VISA)
2002
VRSA

European Antimicrobial Resistance Surveillance System Türkiye MRSA Oranları



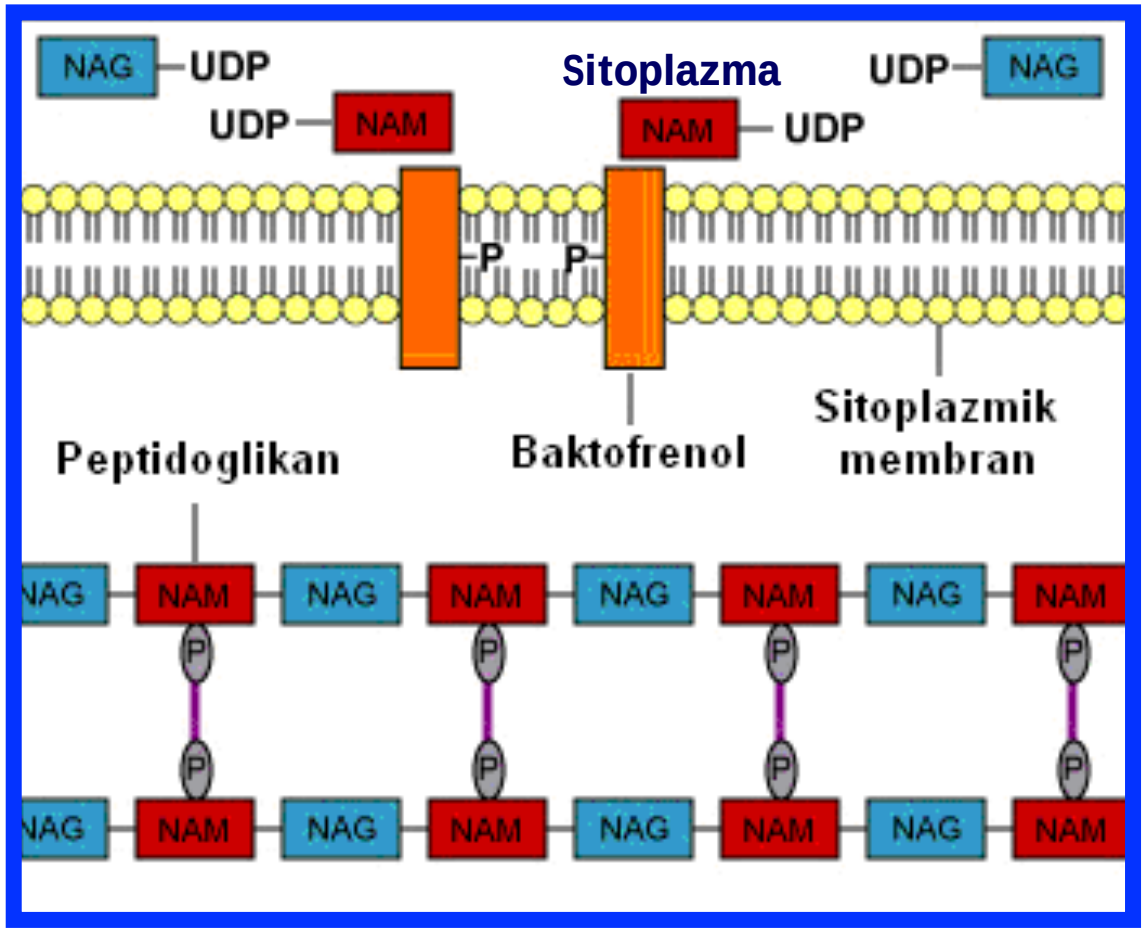
http://www.rivm.nl/earss/database/

European Antimicrobial Resistance Surveillance System Avrupa MRSA Oranları



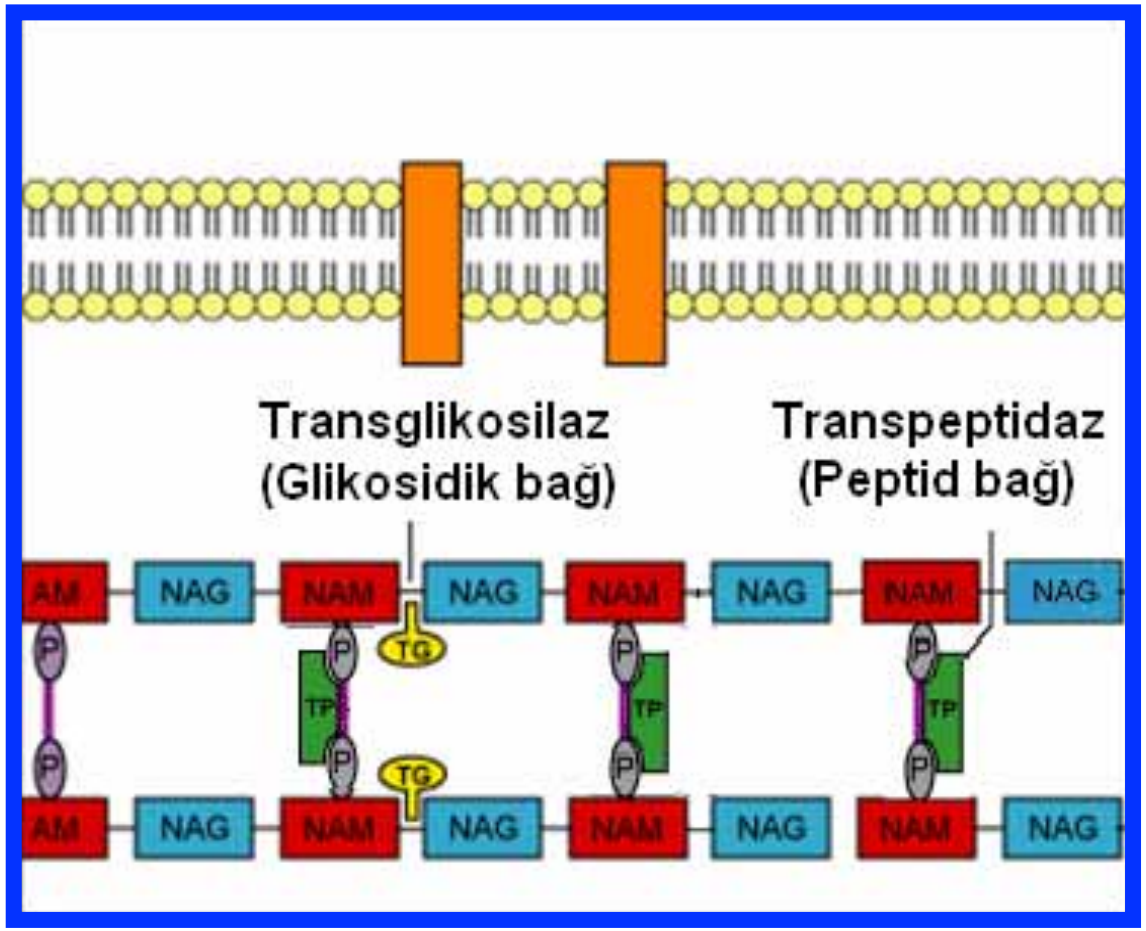
http://www.rivm.nl/earss/database/

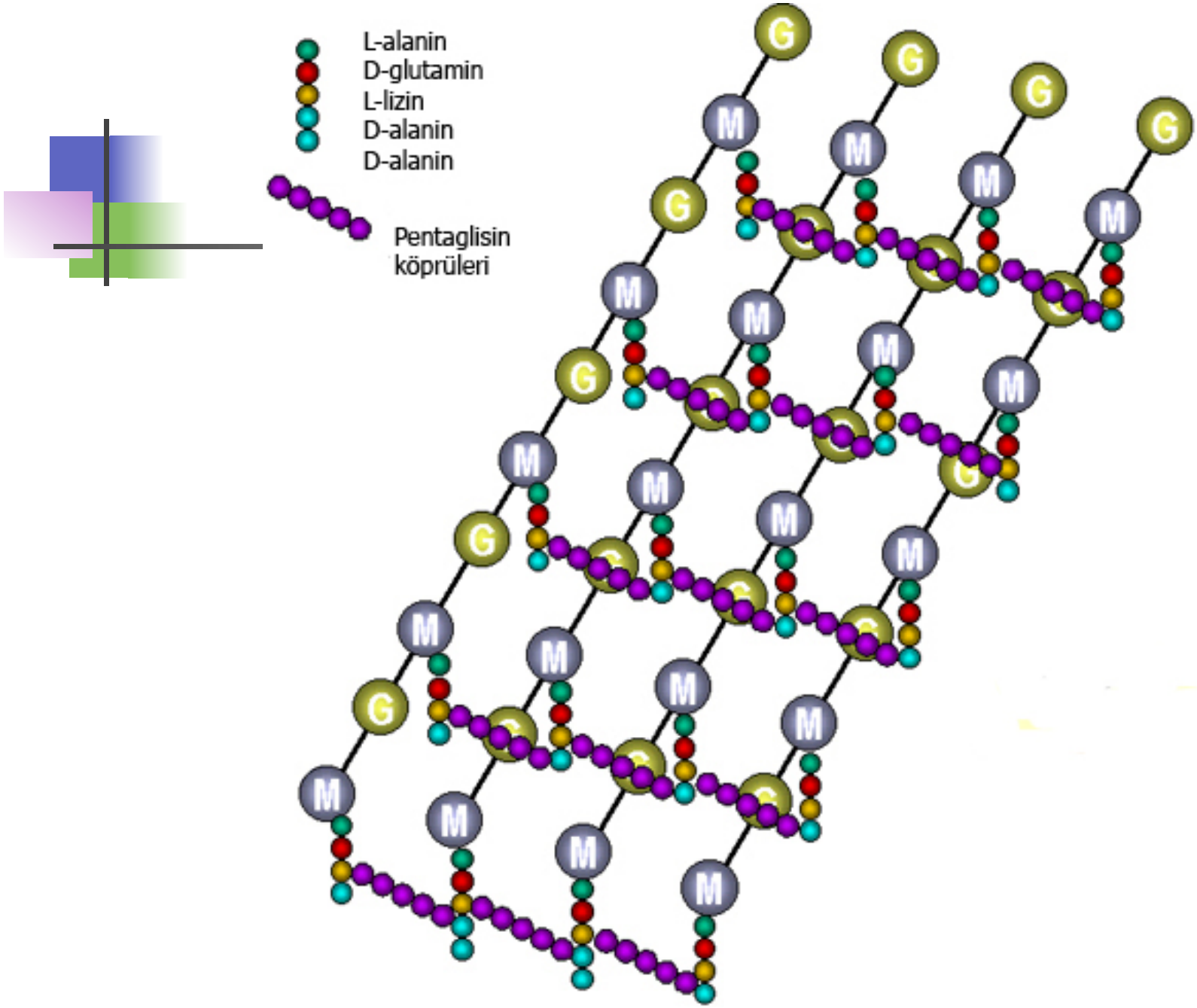
Hücre Duvar Sentezi



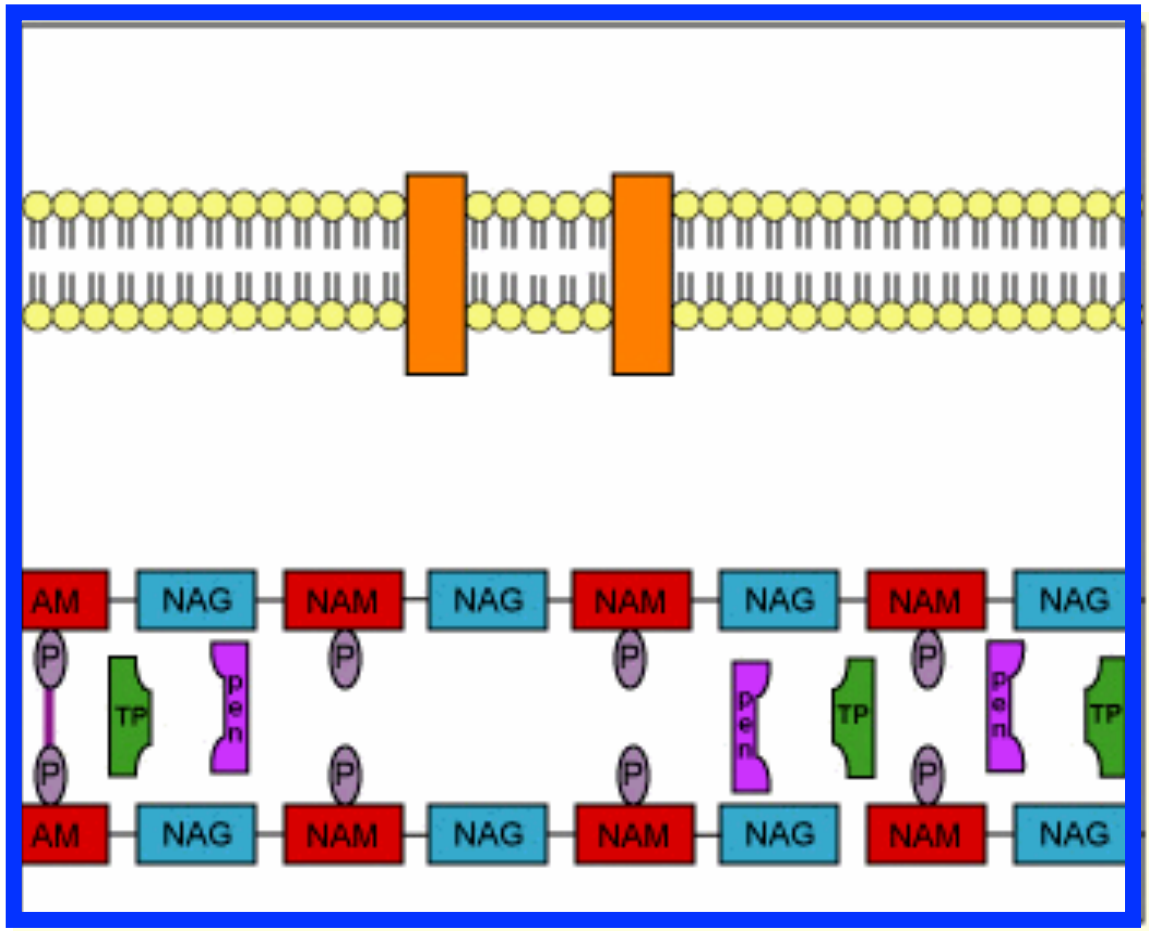
NAG: N-Asetil Glukoz Amin
NAM: N-Asetil Muramik Asit

Hücre Duvar Sentezi

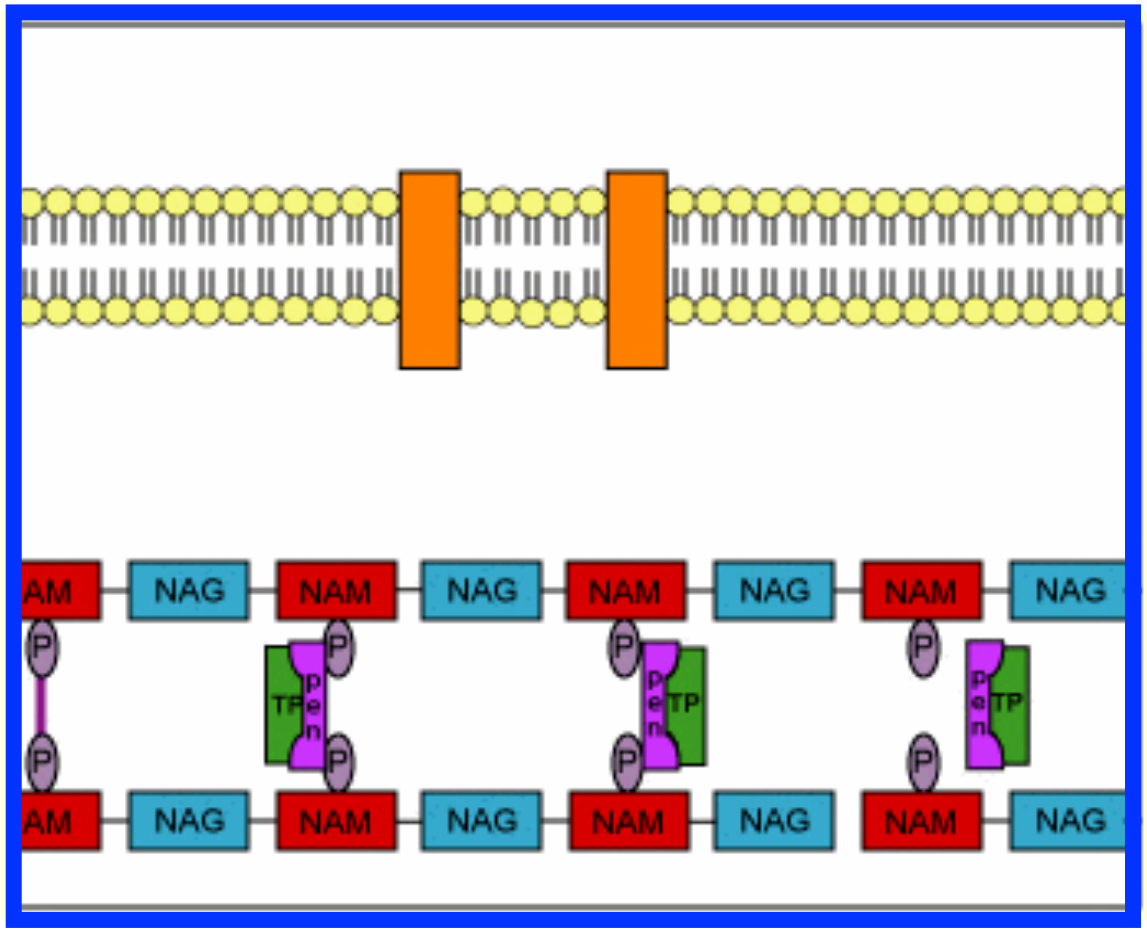


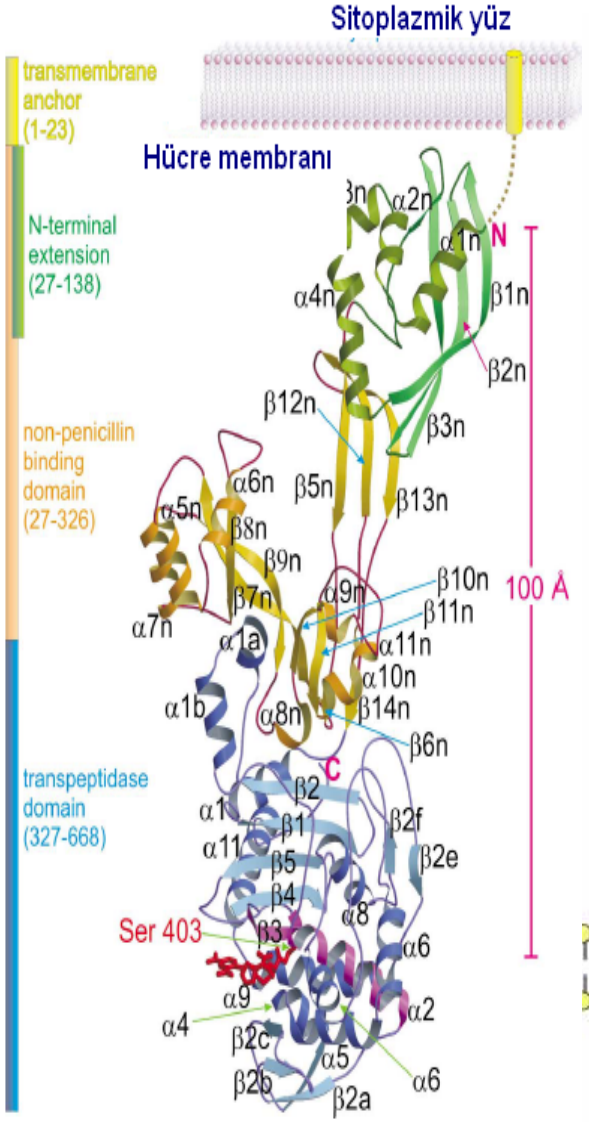

L-alanin
D-glutamin
L-lizin
D-alanin
D-alanin
Pentaglisin
köprüleri
G
M

Beta-laktam AB Etkisi

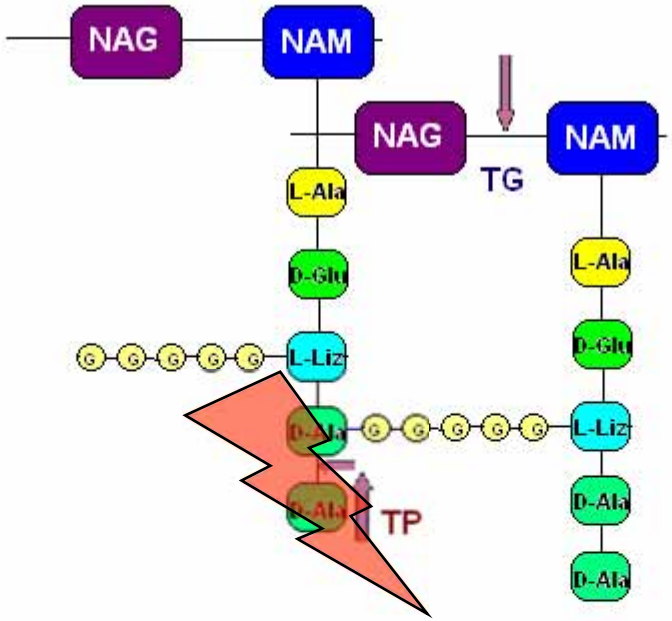


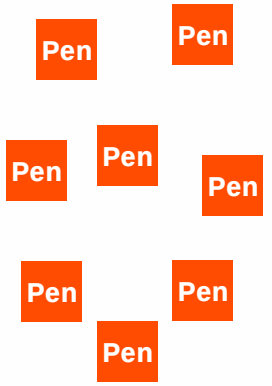
Beta-laktam AB Etkisi

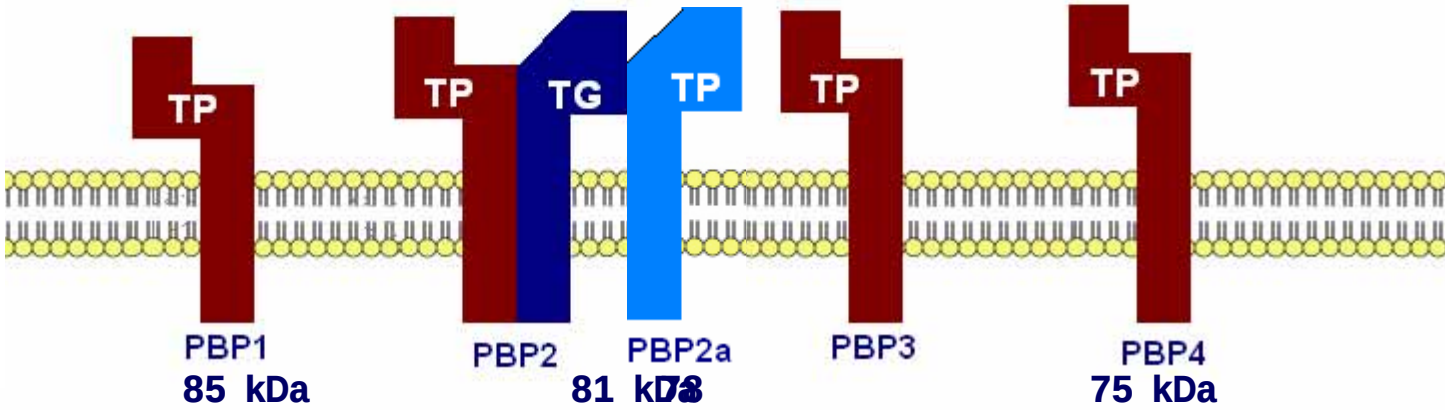




PBP yapısı

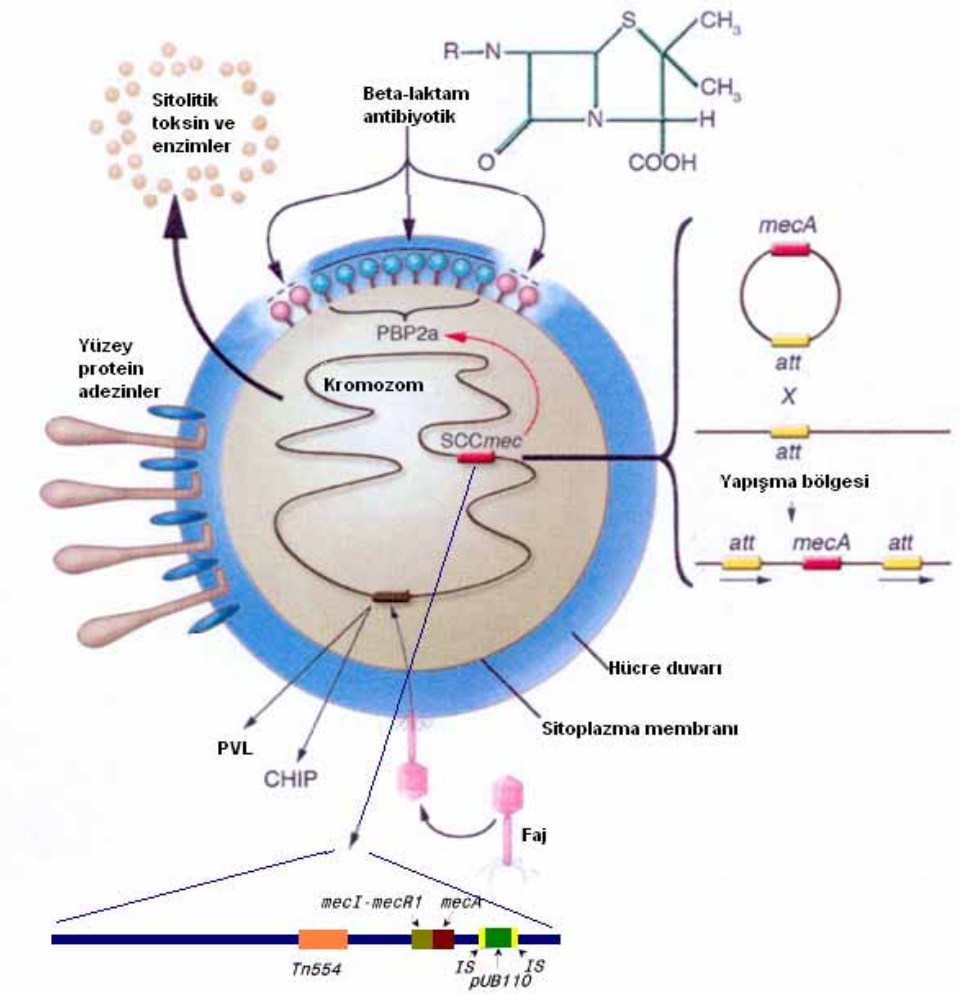






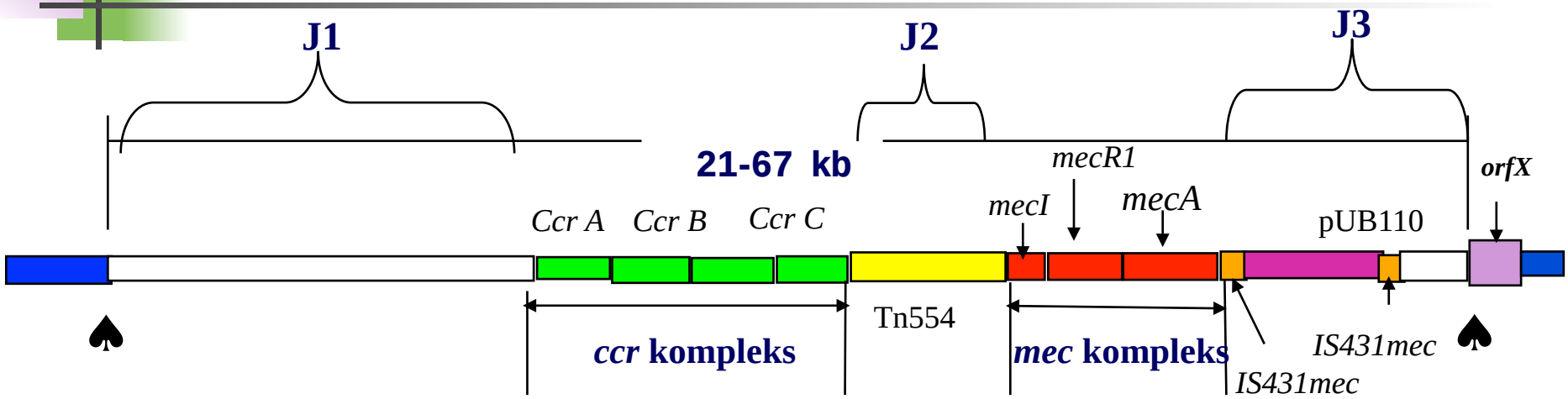
Georgopapadakou et al Antimicrob Agents Chemother (1980) 894-6

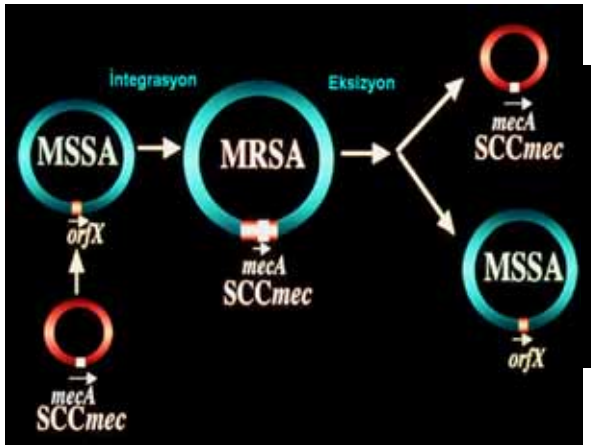
PBP2a Sentezinin Düzenlenmesi



Katayama et al Antimicrob Agents Chemother (2000) 549-55

SCC*mec* Gen Kaset Yapısı

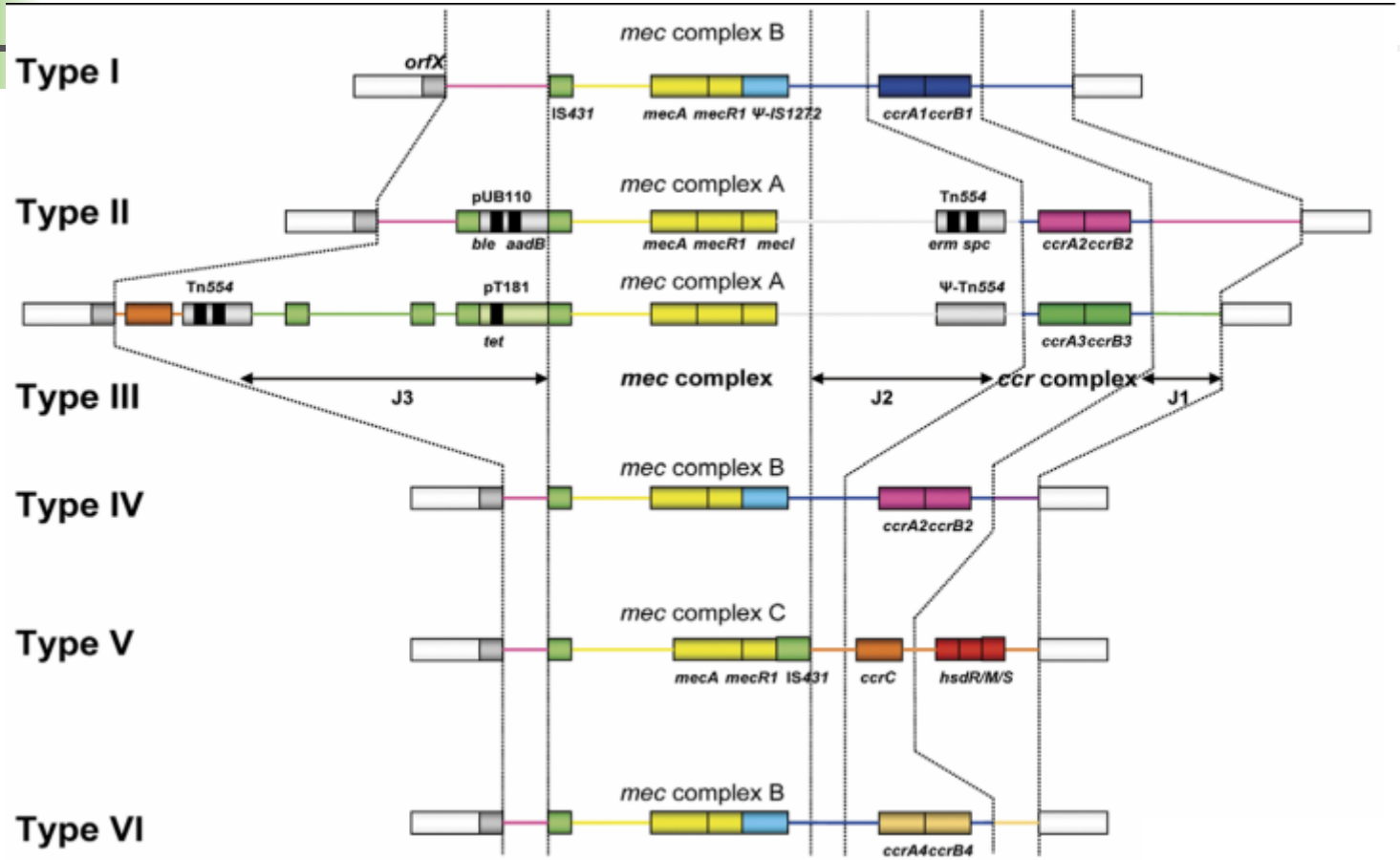




Beta-laktam direnci ve bu direncin düzenlenmesi

Katayama et al Antimicrob Agents Chemother (2001) 1955-63.

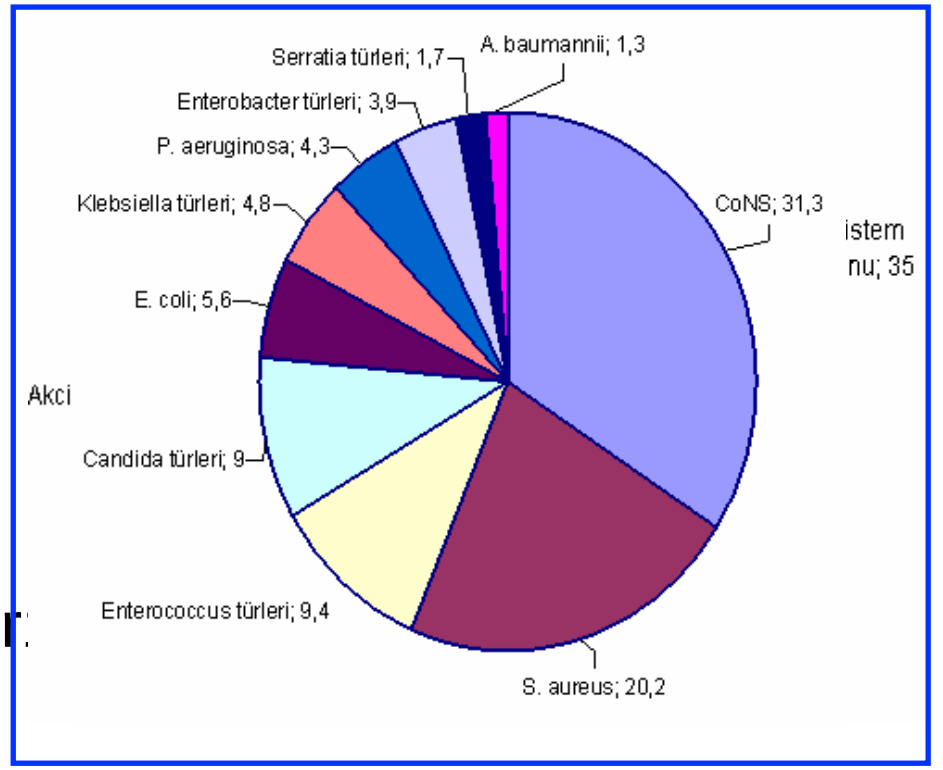
SCC*mec* Yapısı (I-VI)



Ito et al Drug Resist Updat (2003) 41-52

Kan Akımı Enfeksiyonları (KAE)

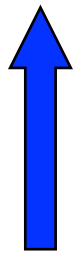
- ABD'de yıllık 250.000 vaka (Hastane enfeksiyonlarının %10'u)
- Mortalite oranı %25-30 (ilk 25 saat içinde uygun tedavi almayanlarda 2 kat fazla)
- %20'sinde *S. aureus*, %50 civarı MR



Wenzel RP, Edmond MB. N Engl J Med (2001) 345:1384-92
Weber DJ, Rutala WA. Infect Dis Clin North Am (2004) 18:309-17

MRSA KAE

- Metisilin direnci hastanede yatış süresini 1.29-2 kat arttırır
- Metisilin direnci hasta maliyetini 1.44-3 kat arttırır
- Metisilin direnci mortaliteyi yaklaşık 1.5-2.6 kat arttırır
- Gecikmiş tedavi mortalite oranını 4 kat hasta yatış süresini 1.5 kat arttırır



Cosgrove et al Infect Control Hosp Epidemiol (2005) 166-74
Ben-David et al Infect Control Hosp Epidemiol (2009) 453-60
Rieg et al J Infect (2009) 232-9
Lodise et al Clin Infect Dis (2003) 1418-23

MAJOR ARTICLE

Clinical Infectious Diseases 2003; 36:1418–23

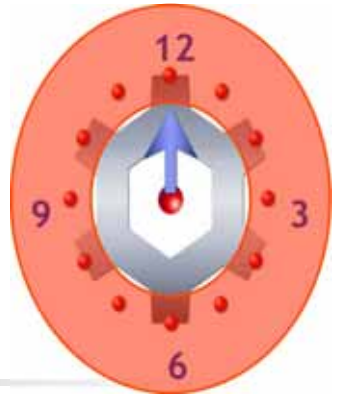
Outcomes Analysis of Delayed Antibiotic Treatment for Hospital-Acquired *Staphylococcus aureus* Bacteremia

Thomas P. Lodise,[a] Peggy S. McKinnon, Linda Swiderski, and Michael J. Rybak

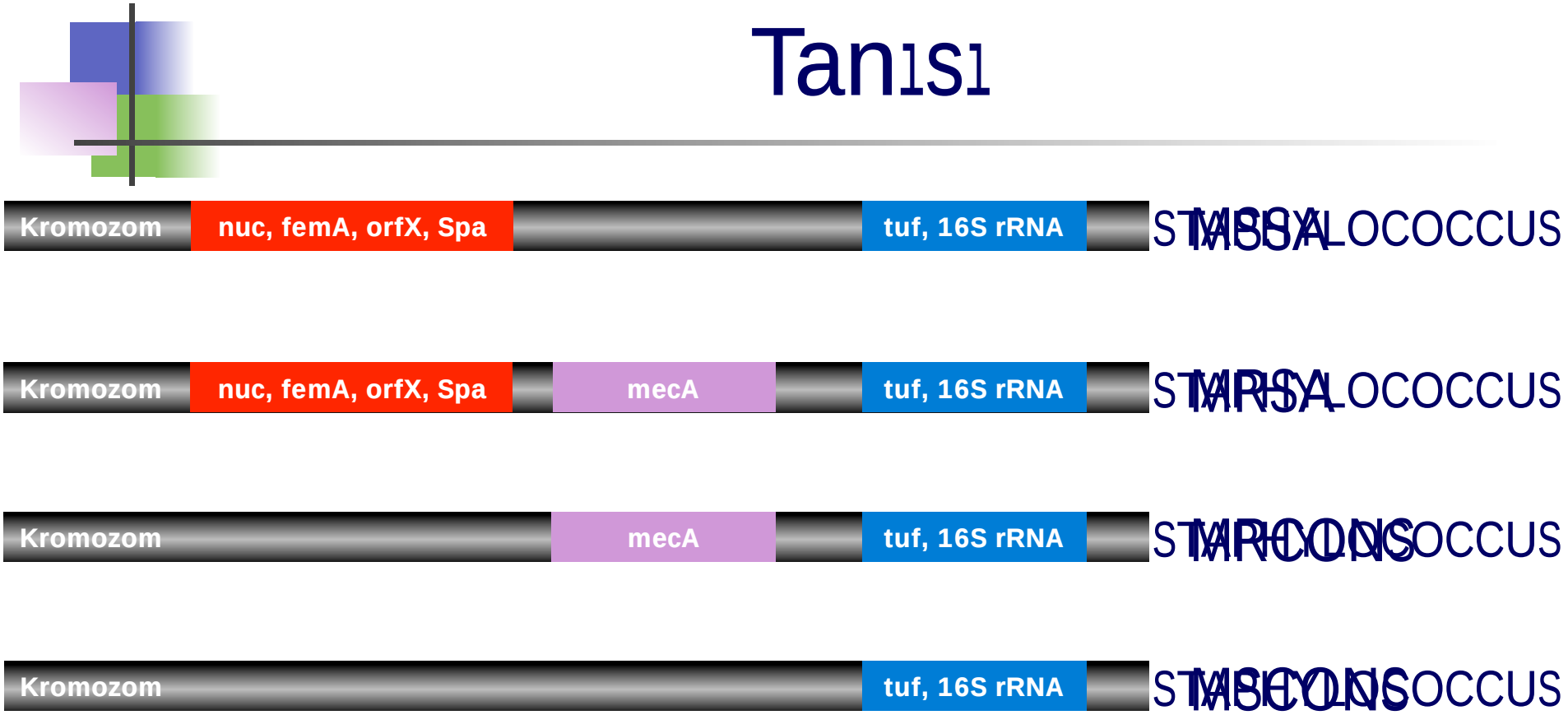
Anti-Infective Research Laboratory, Detroit Receiving Hospital and University Health Center, and Eugene Applebaum College of Pharmacy and Health Sciences, Wayne State University, Detroit, Michigan

The objective of this study was to determine the effect of delayed therapy on morbidity and mortality associated with nosocomial *Staphylococcus aureus* bacteremia. The study included all episodes of *S. aureus* bacteremia that developed >2 days after hospital admission during 1999 to 2001. Classification and regression tree analysis (CART) was used to select the mortality breakpoint between early and delayed treatment. During the 25-month study period, 167 patients met the inclusion criteria. The breakpoint between delayed and early treatment derived using CART was 44.75 hours. On multivariate analysis, delayed treatment was found to be an independent predictor of infection-related mortality (odds ratio, 3.8; 95% confidence interval, 1.3–11.0; $P = .01$) and was associated with a longer hospital stay than was early treatment (20.2 days versus 14.3 days; $P = .05$). These findings support the notion that delay of therapy has deleterious effects on clinical outcomes, and efforts should be made to ensure that appropriate therapy is initiated promptly.

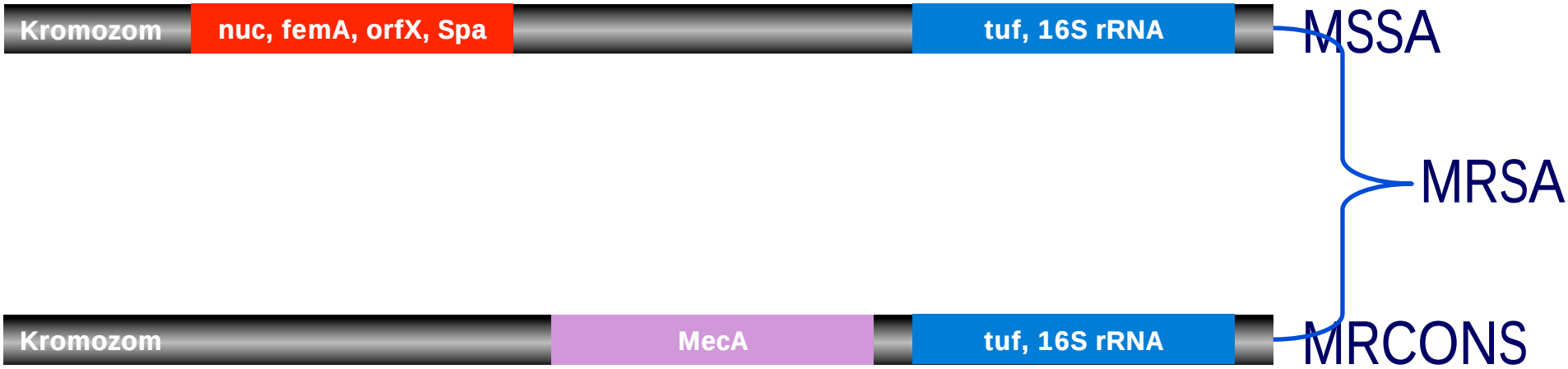
MRSA KAE'u Laboratuvar Tanısı



MRSA KAE'u Moleküler Tanısı

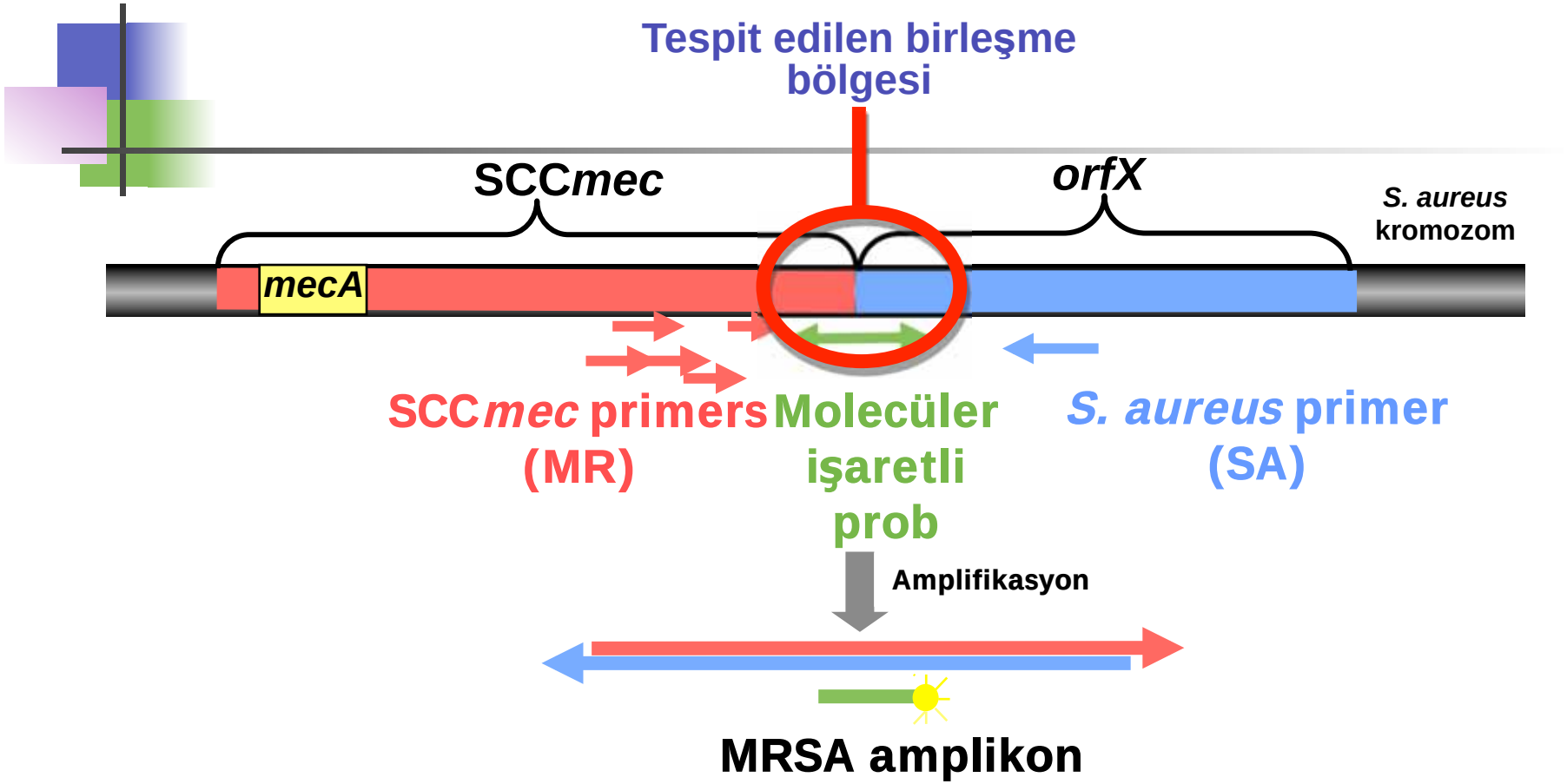


MRSA KAE'u Moleküler Tanısı



- Burun örneklerinde %3.4-4.6
- Steril örneklerde %0-2.5

Becker et al J Clin Microbiol (2006) 229-31
Wolk et al J Clin Microbiol (2009) 823-6


Tespit edilen birleşme bölgesi
SCCmec
orfX
S. aureus kromozom
mecA
SCCmec primers (MR)
Molecüler işaretli prob
S. aureus primer (SA)
Amplifikasyon
MRSA amplikon

FDA Onayı Almış MRSA Testleri

- **BD GeneOhmTM MRSA**
- **Xpert MRSA/SA BC**

FDA Onayı Almış MRSA Testleri

BD GeneOhm™ MRSA

Pozitif kan kültürü

Örnek Hazırlama

Lizis-DNA ekstraksiyonu

Rekonstrüksiyon

Real-time PCR SmartCycler

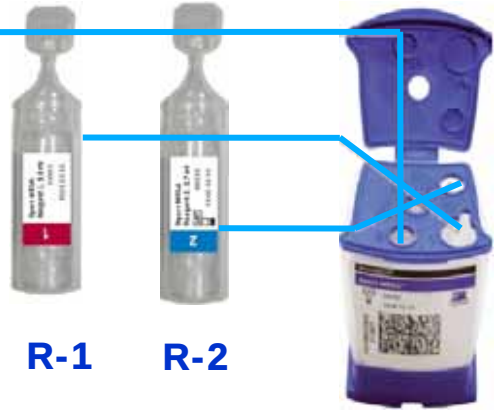
Ekranda kesin sonuçlar

15 örnek için 20 dk ~ 70 dk < 2 saat

Xpert MRSA / SA BC

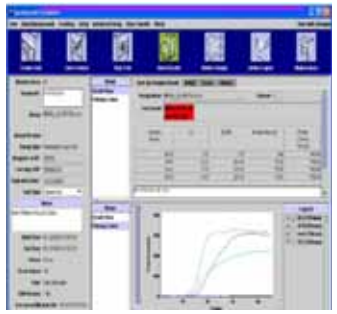
Pozitif kan kültürü

Lizis solüsyon



R-1 R-2

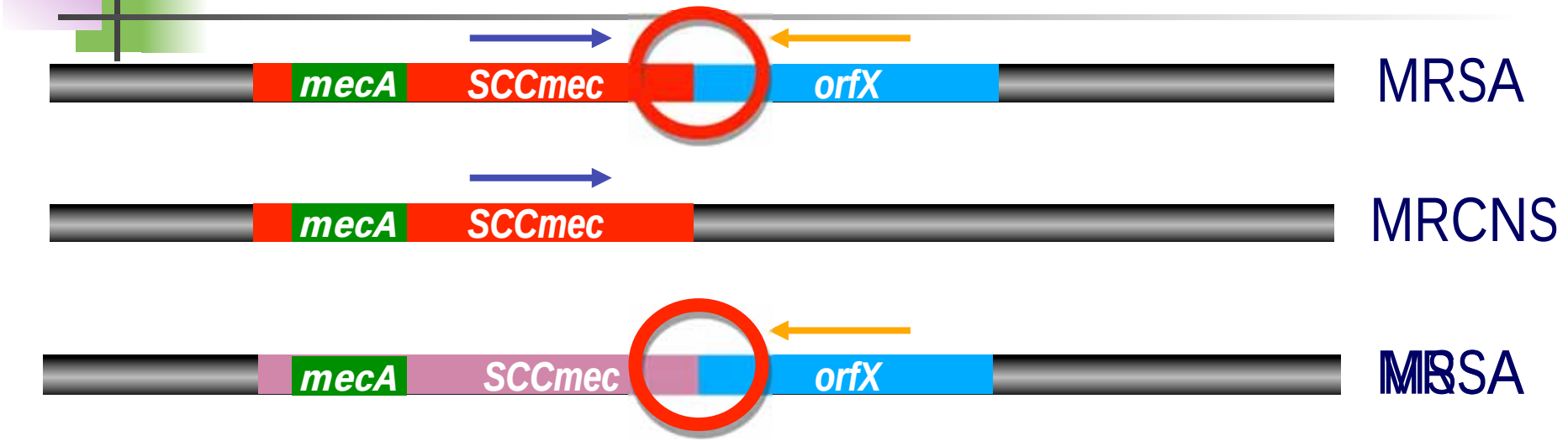
Real-time PCR GenExpert Cihazı



Ekranda Kesin sonuçlar

2 dk ~ 70 dk < 2 saat

BD GeneOhmTM MRSA



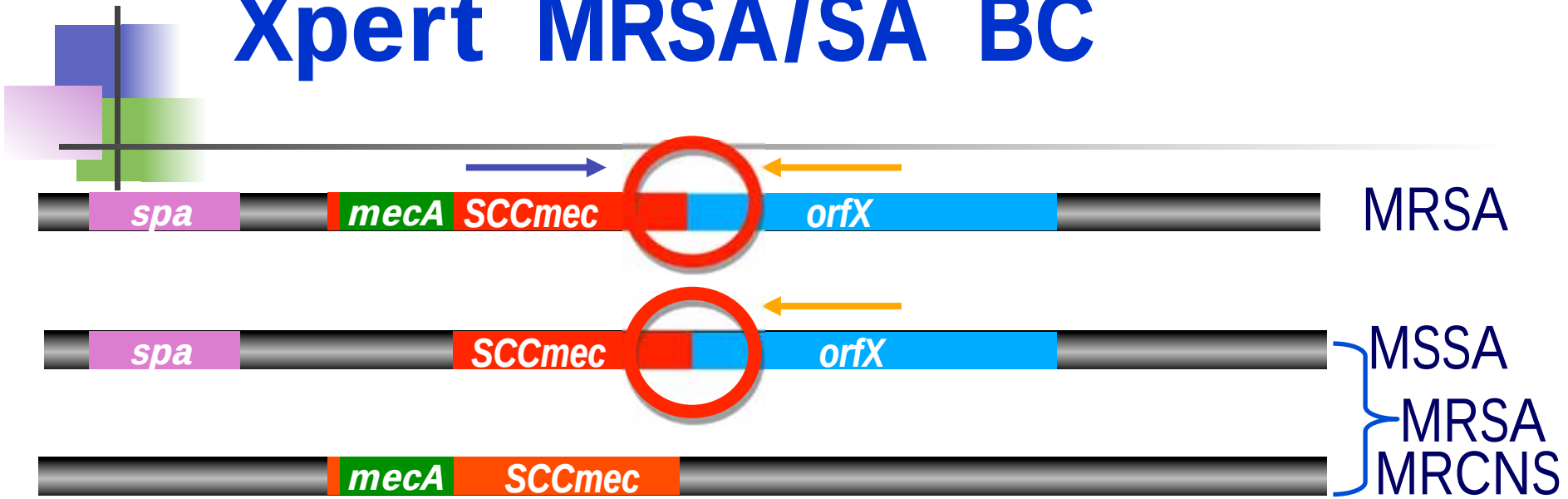
- *orfX* (*S. aureus*) and SCC*mec* gen kaset (direnç geni bölgesi) birleşim yeri
- SCC*mec* tip I - IV tespiti: Hastane ve toplum kaynaklı
  - Yalancı pozitiflik: "empty mecA" fenomene mec varyant izolatlar
  - Yalancı negatiflik: Varyant SCC*mec* birleşme bölgesi

Huletsky et al J Clin Microbiol (2004) 1875-84
Bartels et al J Clin Microbiol (2009) 1524-7
Thomas et al J Clin Microbiol (2008) 4116-7

BD GeneOhmTM MRSA

Kaynak	Duyarlılık	Özgüllük
Gröbner et al JCM 2009	%95.6 (43/45)	%95.3 (61/64)
Kimura et al RB 2009	%100 (67/67)	% 97.4 (37/38)
Snyder et al JCM 2009	%95.0 (56/59)	%97.5 (40/41)

Xpert MRSA/SA BC



- *orfX* (*S. aureus*) and SCC*mec* gen kaset (direnç geni bölgesi) birleşim yeri, *spa*, *mecA*
- SCC*mec* tip I - V tespiti: Hastane ve toplum kaynaklı
  - Yalancı pozitiflik: "empty mecA" fenomene (spa ve SCCmec)+ MRCNS bir arada olması (mecA)
  - Yalancı negatiflik: Yetersiz bakteri miktarı

Wolk et al J Clin Microbiol (2009) 823-6

Xpert MRSA/SA BC

Kaynak	Duyarlılık	Özgüllük
Parta et al JCM 2009	% 97.9 (46/47)	% 100 (21/21)
Wolk et al JCM 2009	% 98.3 (57/58)	% 99.4 (346/348)

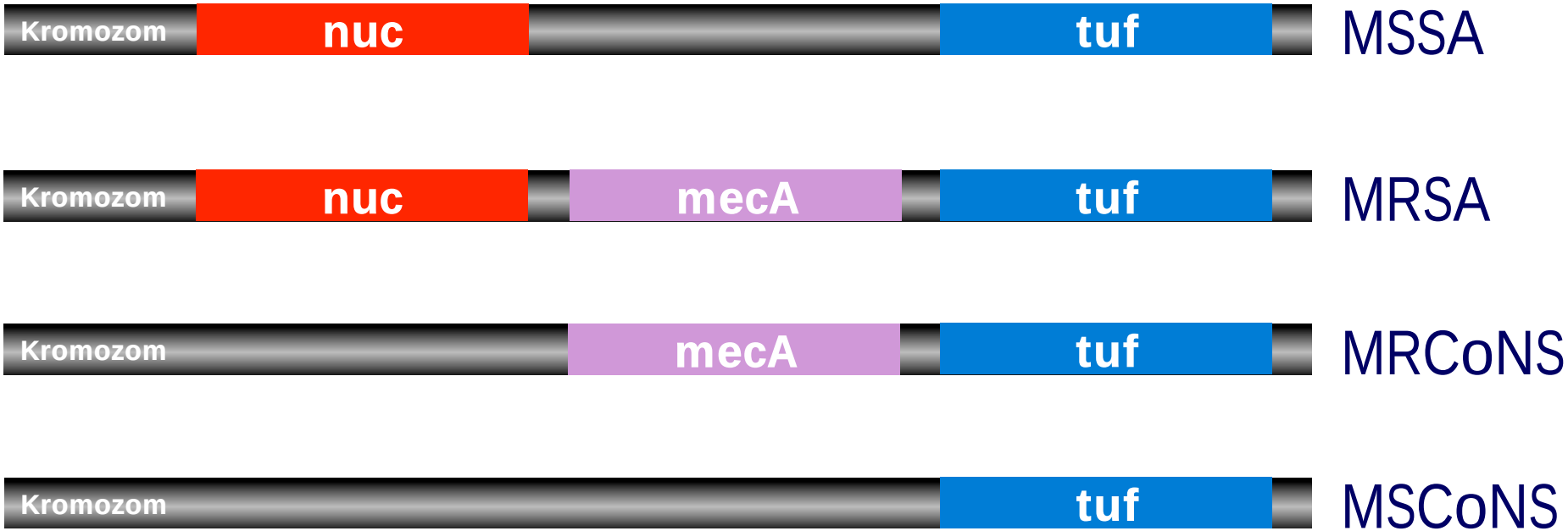
Tripleks Real-Time PCR Yöntemi ile Kan Kültürü Örneklerinden Stafilokokların ve Metisilin Direncinin Tespit Edilmesi

Amaç: KAE'una neden olan stafilokokların ve metisilin direncinin real-time PCR yöntemi ile tanımlanması

Fenotipik Yöntem

- 12 Kasım 2008-11 Ağustos 2009 arasında 341 gram pozitif kok kümesi (GPKK) görülen kan kültür örneği çalışmaya alındı
- Konvansiyonel yöntem ile
  - 22 MRSA
  - 22 MSSA
  - 230 MRCoNS
  - 54 MSCoNS
  - 13 stafilokok dışı GPKK yapmış organizma

Genotipik yöntem

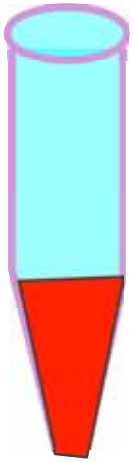



ANA SAYFA
DİZİ
Staphylococcus aureus nuc gene
sirküler
lineer
Preset Oligo
Tm
PRİMER UZUNLUĞU 20
BAŞLANGIÇ YERİ 1
BİTİŞ YERİ 648
ARA
Referans Dizi No: 4
Toplam Dizi Sayısı: 12
Dizi Uzunluğu: 648
Amplikon Uzunluğu: 192
GTATGGCAATTGTTTCAATATTACTTATAGGGATGGCTATCAGTAATGTTTCGAAAGGGCAATACGCAAAGAGGTTTTT
CTATTTCGCTACTAGTTGTTTAGTGTTAACTTTAGTTGTAGTTTCAAGTCTAAGTAGCTCAGCAAATGCATCACAAACA
GATAATGGCGTAAATAGAAGTGGTTCTGAAGATCCAACAGTATATAGTGCAACTTCAACTAAAAAATTACATAAAGAAC
CTGCGACATTAATTAAAGCGATTGATGGTGATACGGTTAAATTAATgtacaaaggtcaaccaatgacattcaGACTATT
ATtggttgatacacctgaaacaaagcatccTAAAAAAGGTGTAGAGAAATATGGTCCTGAAGCAAGTGCATTTACGAAA
AAAATGGTAGAAAATGCAAAGAAAATTGAAGTCGAGTTTGACAAAGGTCAAAGAactgataaatatggacgtggcttag
cGTATATTTATGCTGATGGAAAAATGGTAAACGAAGCTTTAGTTCGTCAAGGCTTGGCTAAAGTTGCTTATGTTTATAA
ACCTAACAATACACATGAACAACTTTTAAGAAAAAGTGAAGCACAAGCAAAAAAAGAGAAATTAAATATTTGGAGCGAA
GACAACGCTGATTCAG
1.Primer
revers yap
3' -> 5' YAP
5'
gtacaaaggtcaaccaatgacattca
3'
Başlangıç Nükleotidi : 284
2.Primer
revers yap
3' -> 5' YAP
5'
actgataaatatggacgtggcttagc
3'
Başlangıç Nükleotidi : 450
3.Primer
revers yap
3' -> 5' YAP
5'
tggttgatacacctgaaacaaagcatcc
3'
Başlangıç Nükleotidi : 319
4.Primer
revers yap
3' -> 5' YAP
5'
3'
Başlangıç Nükleotidi :
5.Primer
revers yap
3' -> 5' YAP
5'
3'
Başlangıç Nükleotidi :
6.Primer
revers yap
3' -> 5' YAP
5'
3'
Başlangıç Nükleotidi :
DİZİ ARA
DİZİ GÖSTER
PRİMER ANALİZ
HESAPLAMALAR
YENİ DİZİ GİRİŞİ
PRİMERLER
ENZİM KESİMİ
ÇIKIŞ

Hedef gen	Primerler	Proplar	Genbank No
nuc	5’-gttgcttagtgttaactttagttgta-3’, 5’-aatgtcgcaggttctttatgtaattt-3’	5’-VIC-aagtctaagtagctcagcaaatgca-BHQ1-3’	EF529599 EF529596 EF529595 EF529593
mecA	5’-aaatattattagctgattcaggttac-3’ 5’-cgttaatattgccattattttctaat-3	5’-FAM-caaggtgaaatactgattaacccagta-BHQ1-3’	AB221124 AB221123 AB221122 AB221121 AB221120
tuf	5’-aaacaactgttactggtgtagaaatg-3’ 5’-agtacggaaatagaattgtg-3’	5’-TR-tccgtaaattattagactacgctgaagc BHQ2-3’	GQ141079 EU652826 EU652822 EU652816 EU652802 EU652800 EU652794 EU652785

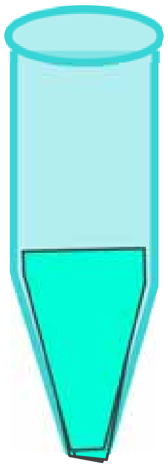
20 µl

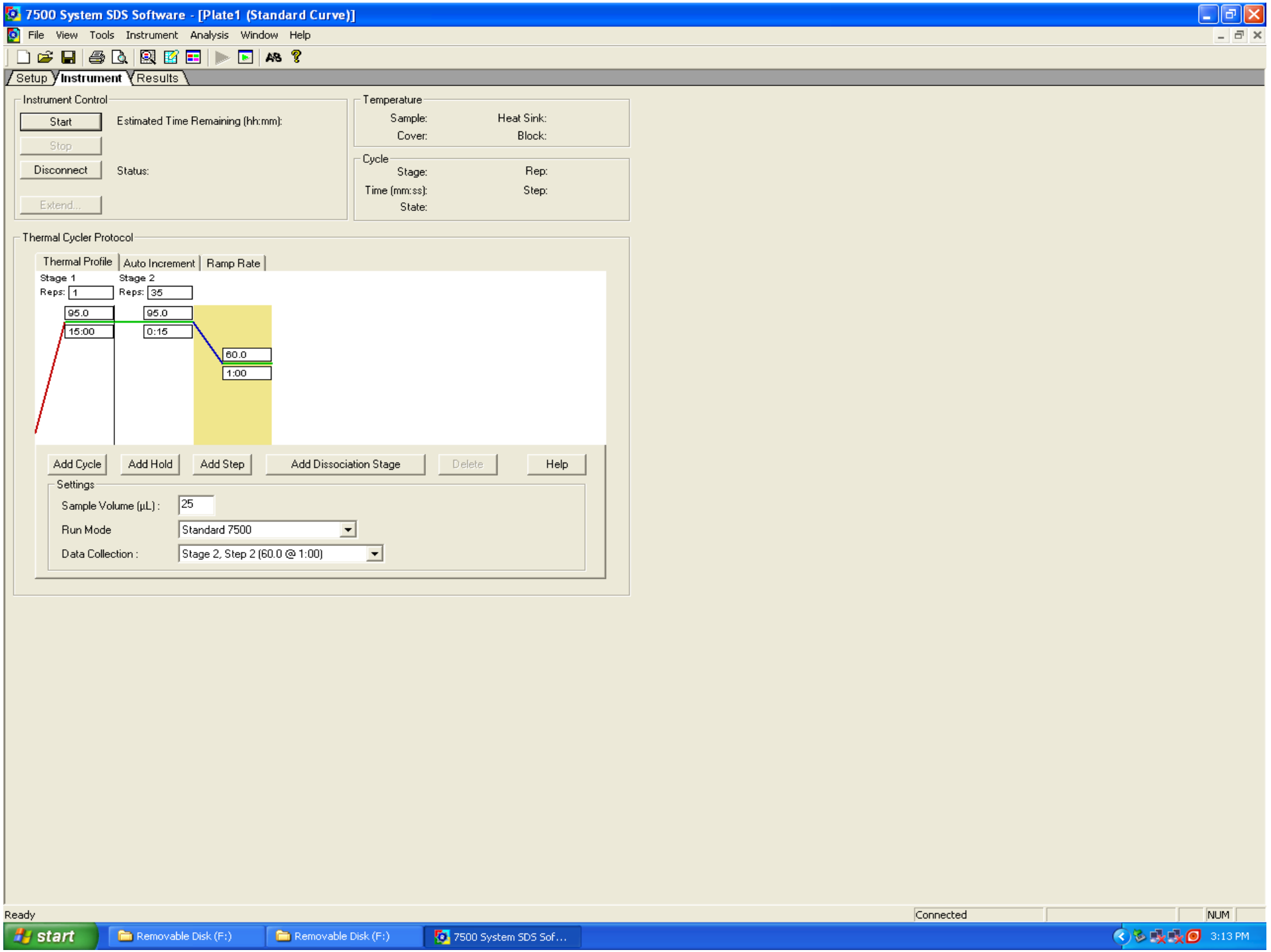
Sodyum polyanetolsulfonat (SPS)

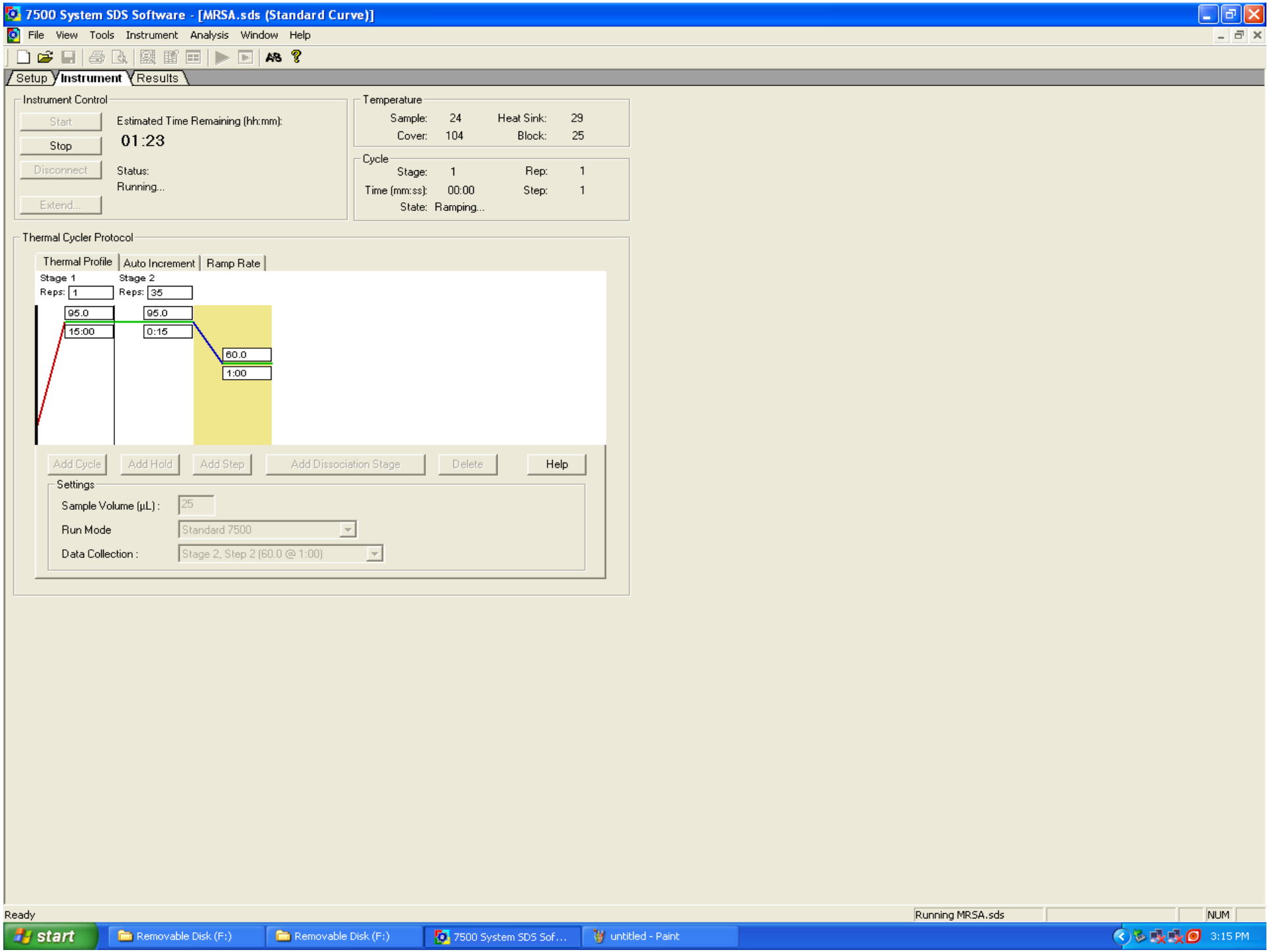


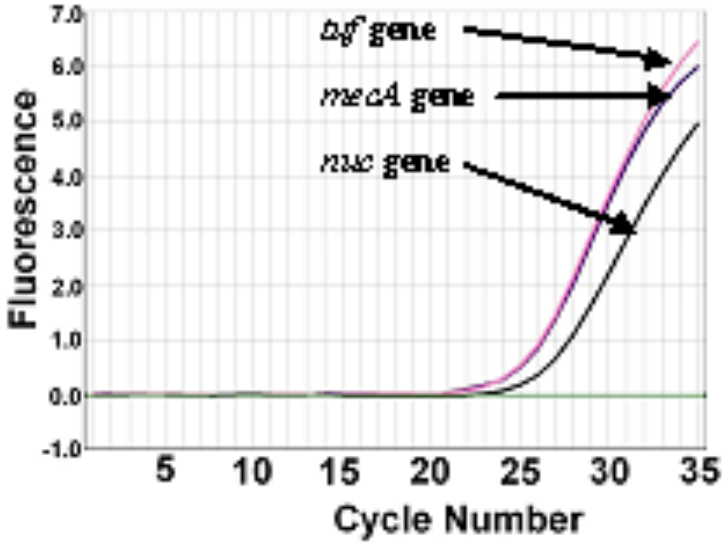
1980 µl

Örnek (1)



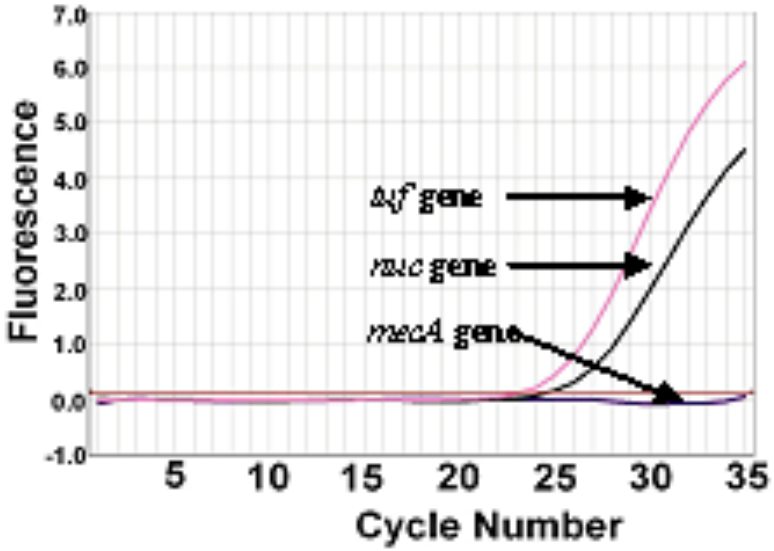

7500 System SDS Software - [Plate1 (Standard Curve)]
File View Tools Instrument Analysis Window Help
Setup Instrument Results
Instrument Control
Start
Estimated Time Remaining (hh:mm):
Stop
Disconnect
Status:
Extend...
Temperature
Sample:
Heat Sink:
Cover:
Block:
Cycle
Stage:
Rep:
Time (mm:ss):
Step:
State:
Thermal Cycler Protocol
Thermal Profile
Auto Increment
Ramp Rate
Stage 1
Reps: 1
Stage 2
Reps: 35
95.0
15:00
95.0
0:15
60.0
1:00
Add Cycle
Add Hold
Add Step
Add Dissociation Stage
Delete
Help
Settings
Sample Volume (µL) : 25
Run Mode
Standard 7500
Data Collection :
Stage 2, Step 2 (60.0 @ 1:00)
Ready
Connected
NUM
start
Removable Disk (F:)
Removable Disk (F:)
7500 System SDS Sof...
3:13 PM


7500 System SDS Software - [MRSA.sds (Standard Curve)]
File View Tools Instrument Analysis Window Help
Setup Instrument Results
Instrument Control
Start
Stop
Disconnect
Extend...
Estimated Time Remaining (hh:mm):
01:23
Status:
Running...
Temperature
Sample: 24
Heat Sink: 29
Cover: 104
Block: 25
Cycle
Stage: 1
Rep: 1
Time (mm:ss): 00:00
Step: 1
State: Ramping...
Thermal Cycler Protocol
Thermal Profile
Auto Increment
Ramp Rate
Stage 1
Reps: 1
Stage 2
Reps: 35
95.0
15:00
95.0
0:15
60.0
1:00
Add Cycle
Add Hold
Add Step
Add Dissociation Stage
Delete
Help
Settings
Sample Volume (µL) : 25
Run Mode Standard 7500
Data Collection : Stage 2, Step 2 (60.0 @ 1:00)
Ready
Running MRSA.sds
NUM
start
Removable Disk (F:)
Removable Disk (F:)
7500 System SDS Sof...
untitled - Paint
3:15 PM



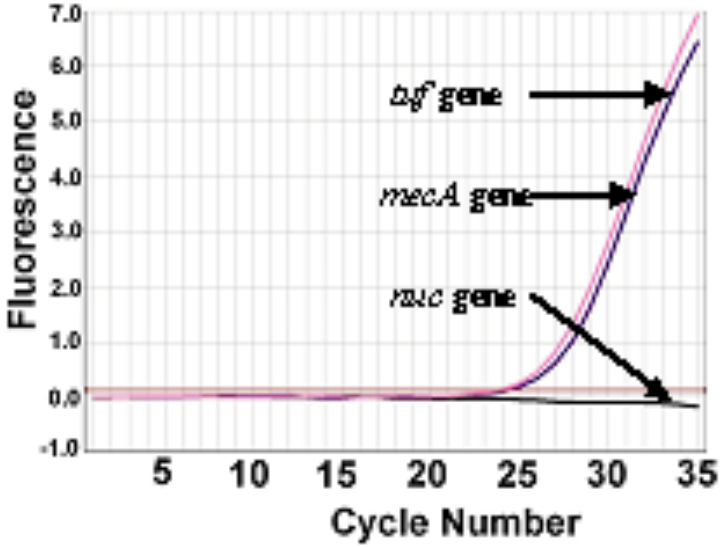
A

MRSA



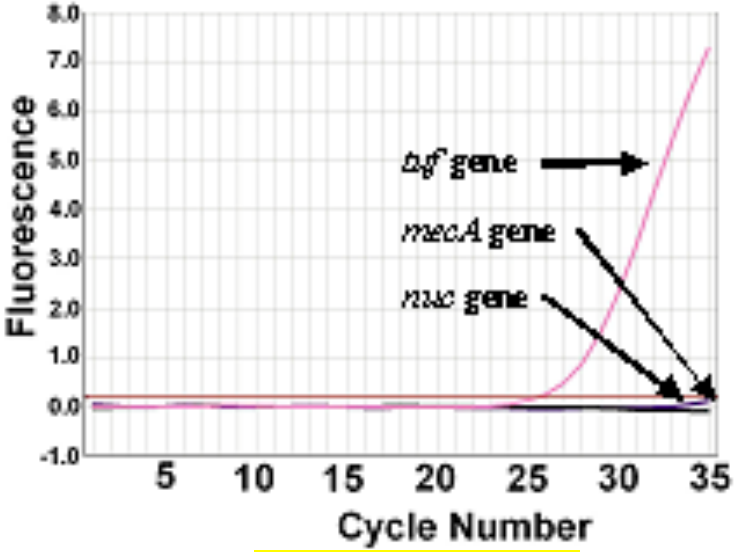
B

MSSA



C

MRCoNS

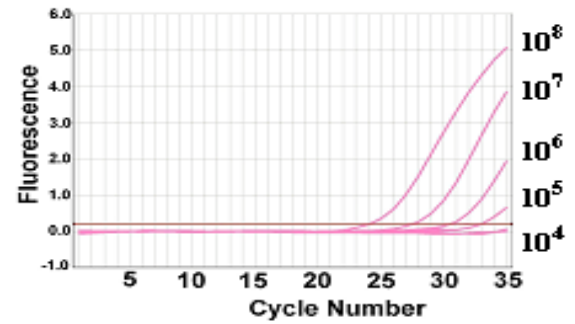


D

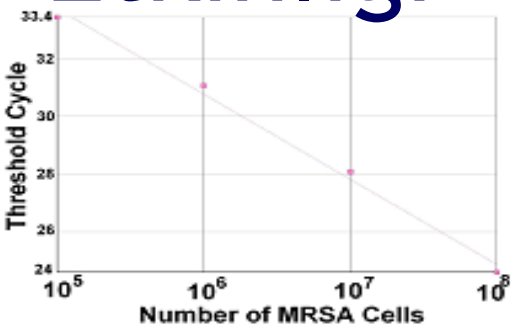
MSCoNS

Mikroorganizmalar	Tripleks PCR sonucu		
	tuf	*nuc*	*mecA*
MRSA NCTC10442, N315, *85/2082,* JCSC4744, WIS (WBG8318)	+	+	+
S. aureus ATCC25923, ATCC29213	+	+	0
S. hominis ATCC700236; *S. epidermidis* ATCC35984	+	0	+
S. warneri ATCC25614; *S. simulans* ATCC27848; *S. haemolyticus* ATCC29970; *S. capitis* ATCC27840; *S. lugdunensis* ATCC43809; *S. saprophyticus* ATCC15305; *S. sciuri* ATCC29061	+	0	0
Enterococcus casseliflavus ATCC25788; *Enterococcus gallinarum* GS; *Enterococcus faecalis* ATCC27270; *Enterococcus faecium* B7641; *Streptococcus pneumoniae* NCTC12695; *Streptococcus pyogenes* NCTC12696; *Acinetobacter haemolyticus* ATCC19002; *Acinetobacter septicus* DSM19415; *Pseudomonas aeruginosa* ATCC27853; *Enterobacter aerogenes* ATCC13048; *Escherichia coli* ATCC35218; *Klebsiella pneumoniae* ATCC13883; *Salmonella typhimurium* NCTC12023	0	0	0

Analitik Duyarlılık ve Reaksiyon Etkinliği



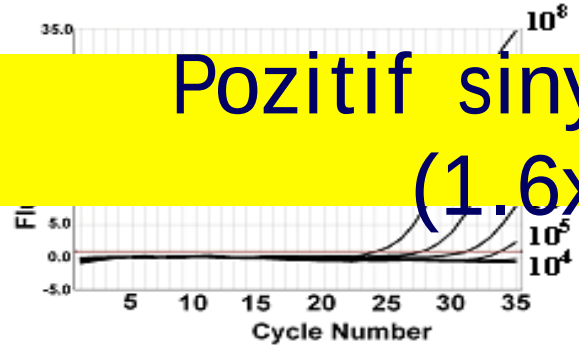
A1



A2

RE (*tuf*)= %120

Pozitif sinyal=$1.2x10^8$ cfu/ml ($1.6x10^7$-$1.1x10^9$)



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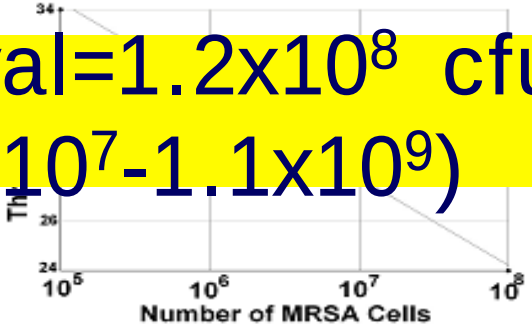

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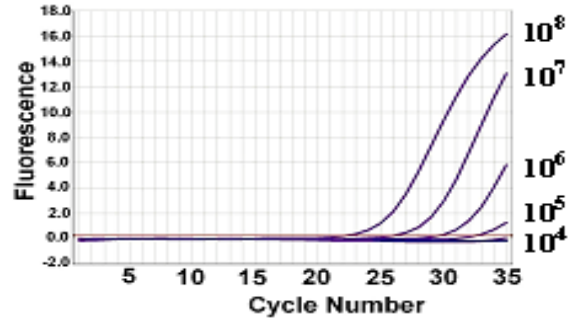
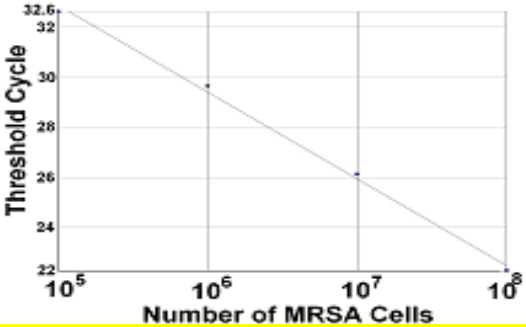


C1



RE (*mecA*)= %90

Reaksiyon etkinliği=$10^{(-1/slope)}-1$

Değişken	Tespit edilen örnek sayısı[a]		Toplam örnek sayısı
	Real-time PCR+	Real-time PCR-	
nuc gen[b]			
S. aureus	44	0	44
CoNS	0	284	284
Non-staphylococcal izolat[c]	0	13	13
tuf gen[d]			
S. aureus	44	0	44
CoNS	283	1	284
Non-staphylococcal izolat[c]	0	13	13

b Duyarlılık = %100; özgüllük = % 100

c **10** *Enterococcus faecalis*, **3** *Enterococcus faecium*.

d Duyarlılık = %99.7; özgüllük = % 100

Mikroorganizma	Tespit edilen örnek sayısı[a]		Toplam örnek sayısı
	Real-time PCR+	Real-time PCR-	
Tüm staphylococci (n = 328)[b]			
methicillin-resistant	250	2	252
methicillin-susceptible	1	75	76
S. aureus (n = 44)[c]			
methicillin-resistant	22	0	22
methicillin-susceptible	0	22	22
CoNS (n = 284)[d]			
methicillin-resistant	228	2	230
methicillin-susceptible	1	53	54

b Duyarlılık = %99.2; özgüllük = % 98.7

c Duyarlılık = 100%; özgüllük = % 100

d Duyarlılık = %99.1; özgüllük = % 98.1

- Avantajları
  - En önemli avantajı PCR amplifikasyon öncesi zaman kaybı ve kontaminasyon riski olan DNA elde etme yöntemi olmaması
  - İkincisi her üç gen bölgesini aynı reaksiyonda tespit etmesi
- Dezavantajları
  - MSSA ve MRCoNS karışık örneklerde (kokolonizasyon) yalancı pozitiflik
  - İki CoNS izolat (*S. haemolyticus* ve *S. hominis*) örneği real-time PCR ile *mecA* negatif bulundu ve başka bir direnç mekanizmasının sorumlu olduğu düşünüldü
  - Bir vakada (*S. hominis*) *tuf* geni 1:100 dilüsyonda tespit edilemedi ve sonrasında 1:10 dilüsyonda tespit edildi

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Diagnostic Microbiology and Infectious Disease 66 (2010) 349–355
DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE
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Triplex real-time polymerase chain reaction assay for simultaneous detection of *Staphylococcus aureus* and coagulase-negative staphylococci and determination of methicillin resistance directly from positive blood culture bottles

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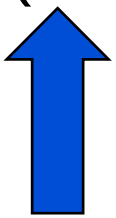
Received 13 October 2009; accepted 23 November 2009

Abstract

We describe here a 1-step, triplex real-time polymerase chain reaction (PCR) assay for the detection and identification of staphylococci directly from signal-positive blood culture bottles containing Gram-positive cocci in clusters (GPCC). The triplex assay targeted and detected *tuf*, *nuc*, and *mecA* genes in a single tube and had a detection limit of 10^5 CFU/mL for each gene target. A total of 341 GPCC-positive blood culture bottles were collected between November 12, 2008, and August 11, 2009. Among them, 230 methicillin-resistant coagulase-negative staphylococci (CoNS), 54 methicillin-susceptible CoNS, 22 methicillin-resistant *Staphylococcus aureus*, 22 methicillin-susceptible *S. aureus*, and 13 nonstaphylococci species were identified by conventional methods. The results obtained by triplex assay were in agreement with those of conventional methods for *tuf* (99.7%), *nuc* (100.0%), and *mecA* (99.1%), respectively. The triplex assay was found to have sensitivities of 99.7%, 100%, and 99.2% and specificities of 100%, 100%, and 98.7%, respectively, for the *tuf*, *nuc*, and *mecA* gene targets. The triplex real-time PCR assay accurately detects and identifies staphylococci directly from positive blood cultures without nucleic acid extraction prior to amplification.

KAE'unda Kontaminasyon

- CoNS tüm KAE'ların %30'undan izole edilmektedir
- İzole edilen CoNS'lerin %70-94'ü kontaminanttır
- Hastanemizde 249 epizotun 45'inde (%18.1)'inde gerçek CoNS KAE tespit edildi (GMG,2010)
- Uygunsuz AB kullanımına bağlı (%34'ünde vankomisin)
  - Maliyet
  - Hastaların hastanede kalış süresi
  - Direnç oranları



- Gerçek CoNS KAE'larının tanımlanması etkili ve uygun antibiyotik (glikopeptid) kullanılması için önemlidir

Ben-David et al Infect Control Hosp Epidemiol (2009) 453-60
Souvenir et al J Clin Microbiol (1998) 1923-6

Surveillance Definitions for Primary BSIs, National Nosocomial Infections Surveillance System

Laboratory-Confirmed BSI

Should meet at least one of the following criteria:

Criterion 1: Patient has a recognized pathogen cultured from one or more blood cultures, and the pathogen cultured from the blood is not related to an infection at another site.

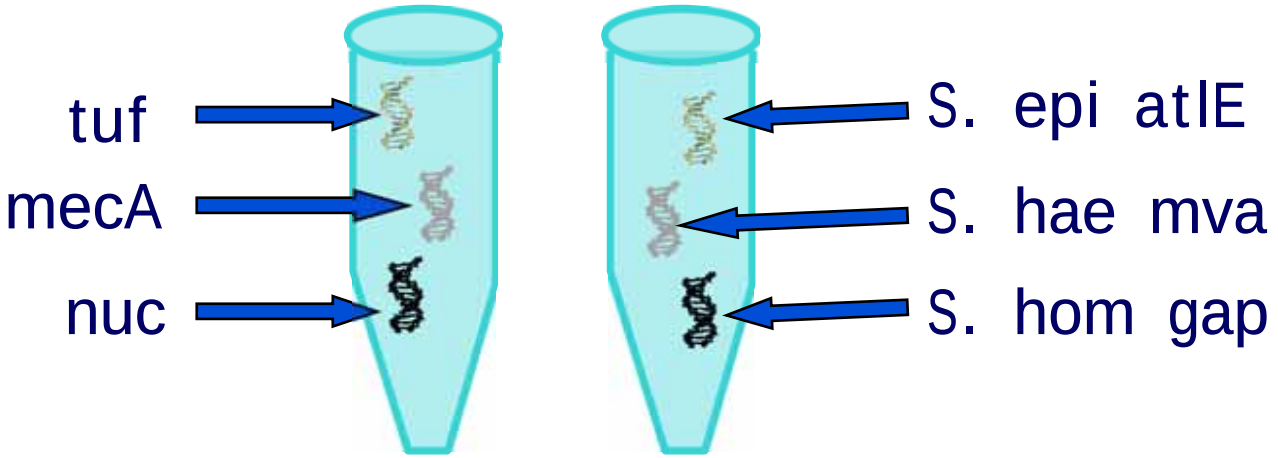
Criterion 2: Patient has at least one of the following signs or symptoms: fever (>100.4° F [>38° C]), chills, or hypotension, and at least one of the following:

1. Common skin contaminant (e.g., diphtheroids, *Bacillus* spp., *Propionibacterium* spp., coagulase-negative staphylococci, or micrococci) cultured from two or more blood cultures drawn on separate occasions.
2. Common skin contaminant (e.g., diphtheroids, *Bacillus* spp., *Propionibacterium* spp., coagulase-negative staphylococci, or micrococci) cultured from at least one blood culture from a patient with an intravenous line, and the physician institutes appropriate antimicrobial therapy.
3. Positive antigen test on blood (e.g., *Hemophilus influenzae, Streptococcus pneumoniae, Neisseria meningitides,* or group B streptococcus).

and signs and symptoms with positive laboratory results are not related to an infection at another site.

MMWR Aug, 2000,51, No. RR-10

CoNS Çalışması

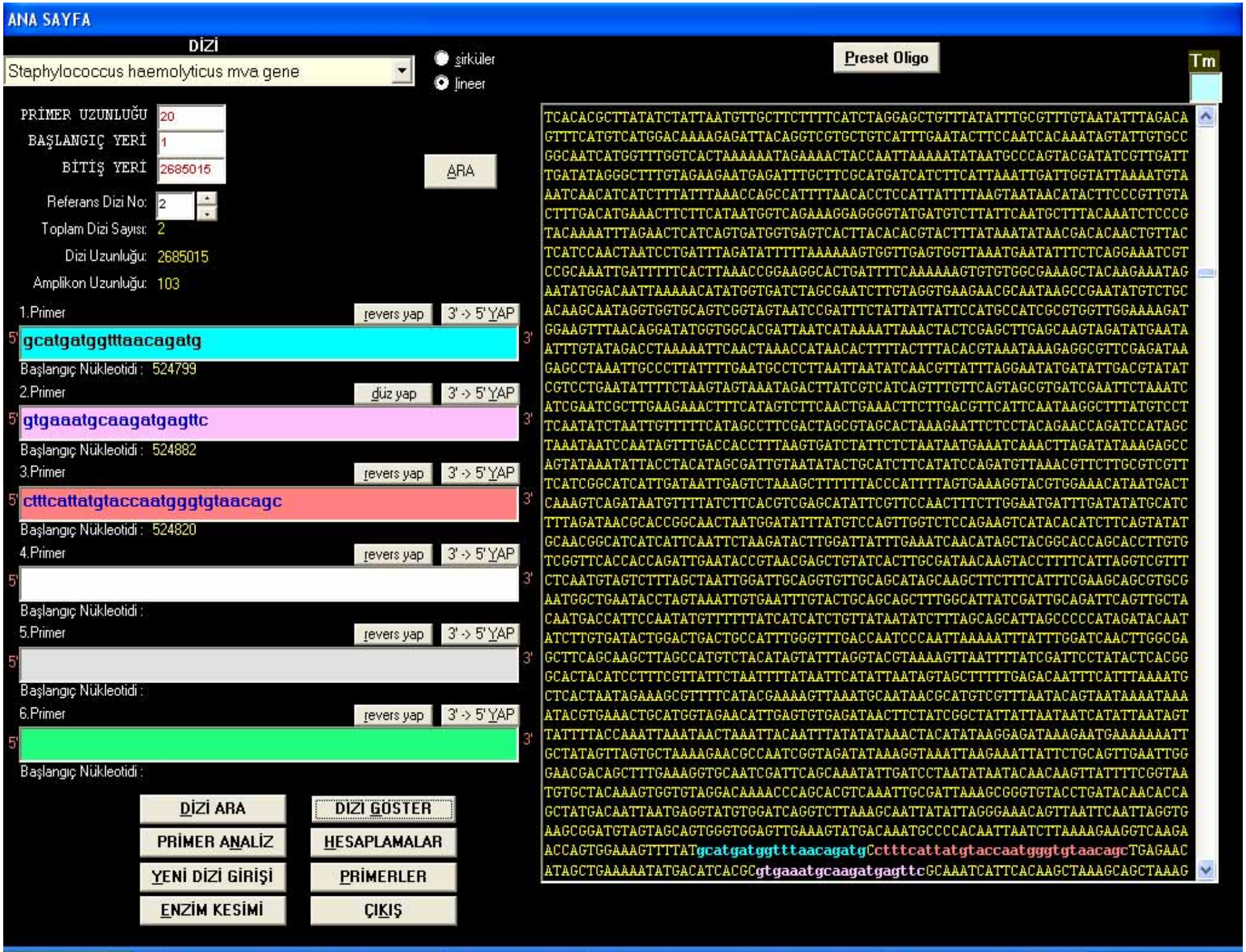


MRSA
MSSA
MRCoNS
MSCoNS

S. epidermidis
S. hominis
S. haemolyticus

Fenotipik Yöntem

- 15 Ağustos 2009-11 Şubat 2010 tarihleri arasında 238 gram pozitif kok kümesi (GPKK) görülen kan kültür örneği çalışmaya alındı
- Konvansiyonel yöntem ile
  - 11 MRSA
  - 28 MSSA
  - 176 MRCoNS
  - 21 MSCoNS
  - 2 stafilokok dışı GPKK yapmış organizma


ANA SAYFA
DİZİ
Staphylococcus haemolyticus mva gene
sirküler
lineer
Preset Oligo
Tm
PRİMER UZUNLUĞU 20
BAŞLANGIÇ YERİ 1
BİTİŞ YERİ 2685015
ARA
Referans Dizi No: 2
Toplam Dizi Sayısı: 2
Dizi Uzunluğu: 2685015
Amplikon Uzunluğu: 103
1.Primer
revers yap
3' -> 5' YAP
5' gcatgatggtttaacagatg 3'
Başlangıç Nükleotidi : 524799
2.Primer
düz yap
3' -> 5' YAP
5' gtgaaatgcaagatgagttc 3'
Başlangıç Nükleotidi : 524882
3.Primer
revers yap
3' -> 5' YAP
5' ctttcattatgtaccaatgggtgtaacagc 3'
Başlangıç Nükleotidi : 524820
4.Primer
revers yap
3' -> 5' YAP
5' 3'
Başlangıç Nükleotidi :
5.Primer
revers yap
3' -> 5' YAP
5' 3'
Başlangıç Nükleotidi :
6.Primer
revers yap
3' -> 5' YAP
5' 3'
Başlangıç Nükleotidi :
DİZİ ARA
DİZİ GÖSTER
PRİMER ANALİZ
HESAPLAMALAR
YENİ DİZİ GİRİŞİ
PRİMERLER
ENZİM KESİMİ
ÇIKIŞ
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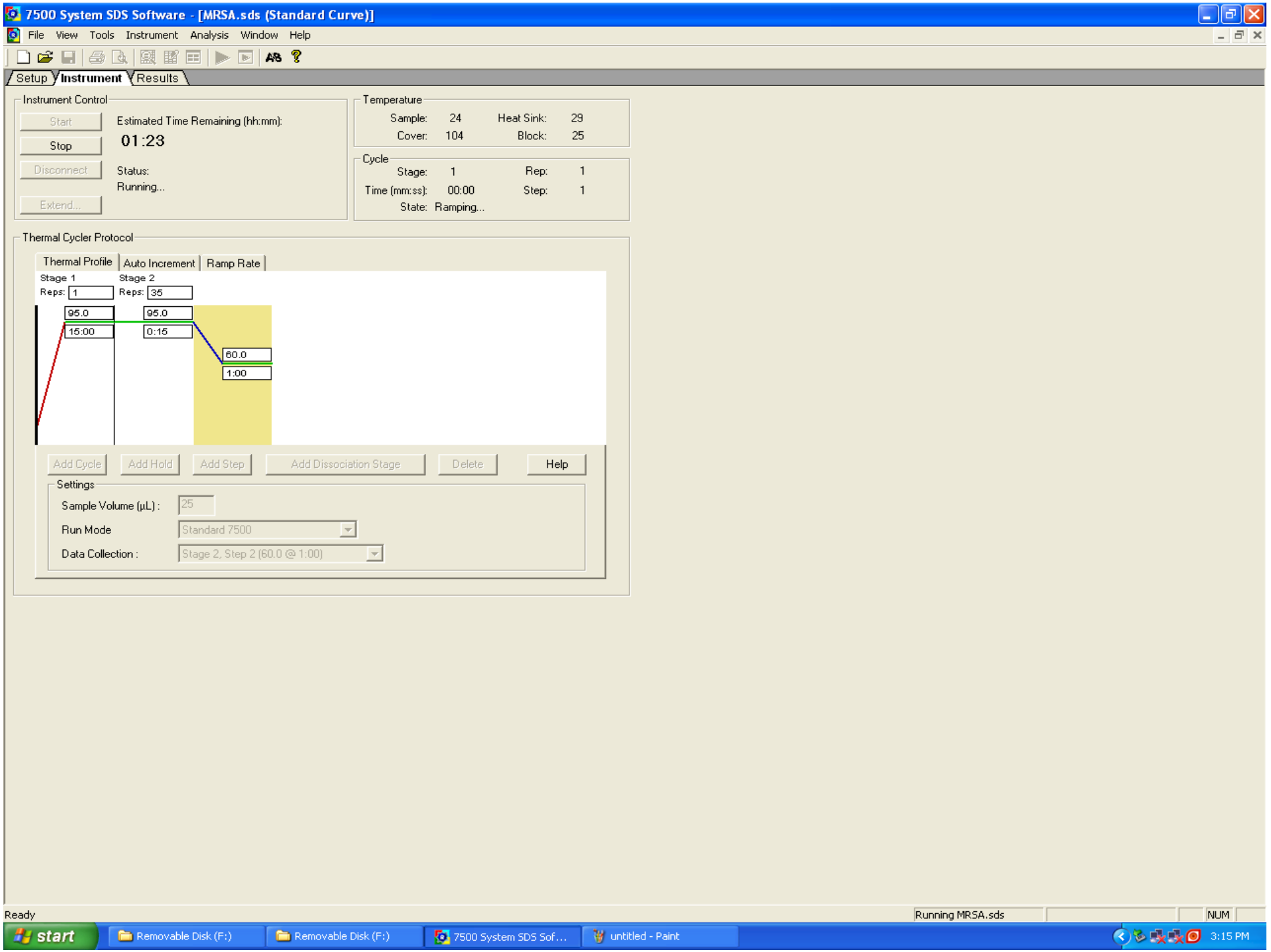
ACCAGTGGAAAGTTTTATgcatgatggtttaacagatgCctttcattatgtaccaatgggtgtaacagcTGAGAAC
ATAGCTGAAAAATATGACATCACGCgtgaaatgcaagatgagttcGCAAATCATTCACAAGCTAAAGCAGCTAAAG
Başlat
Gelen Kutus...
KNS hemokül...
Microsoft Po...
Microsoft Ex...
ÖSYM:::Yüks...
ANA SAYFA
TR
15:46

Hedef gen	Primerler	Proplar	Genbank No
atlE	5’-ggaggaactaataataagttaactg -3’, 5’-gtcataaacagttgtatataagcc -3’	5’-fam-ctgctaatcgtggtgttgctcaaattaaa a-bhq1-3’	AJ887986 AJ887985 AJ887984 AJ887983
gap	5’-tagatggatctgaaacagtagtat -3’ 5’-ccttcaacaataccaaattcgtc -3	5’-tr-aggtgcttcatgtactacaaactcattg -bhq1-3’	DQ321688
mva	5’-tatgcatgatggtttaacagatg -3’ 5’-gaactcatcttgcatttcac -3’	5’-vıc-ctttcattatgtaccaatgggtgtaacagc bhq2-3’	AP006716 AF290088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Cycle
Stage: 1
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Auto Increment
Ramp Rate
Stage 1
Reps: 1
Stage 2
Reps: 35
95.0
15:00
95.0
0:15
60.0
1:00
Add Cycle
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Removable Disk (F:)
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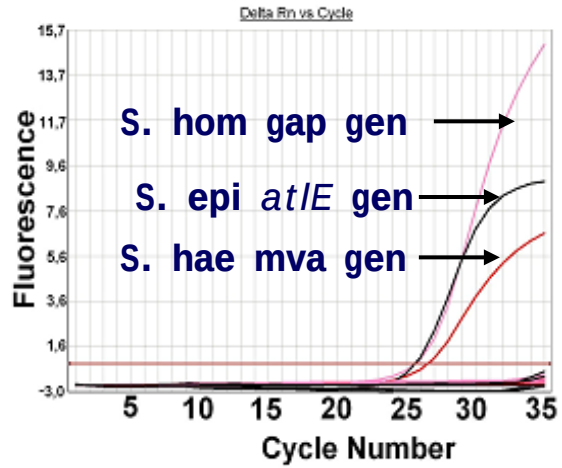
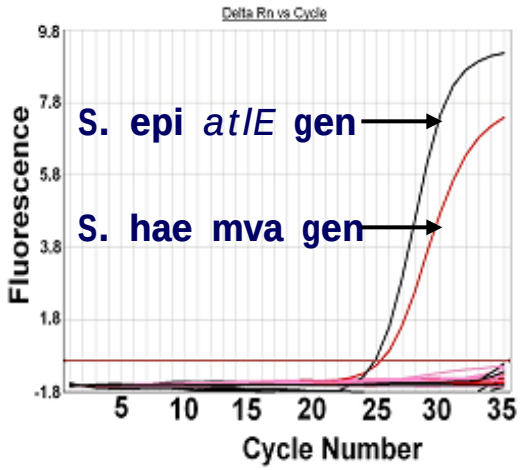
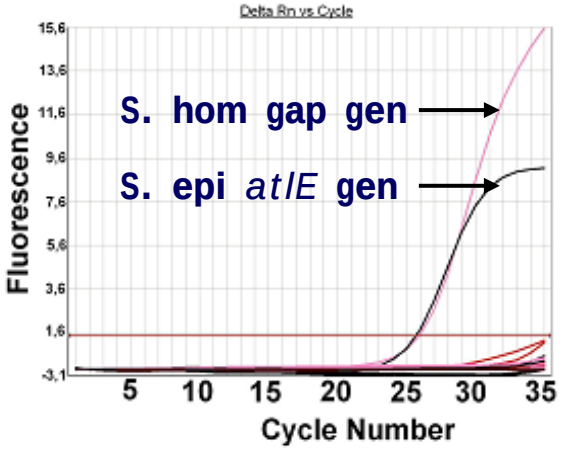
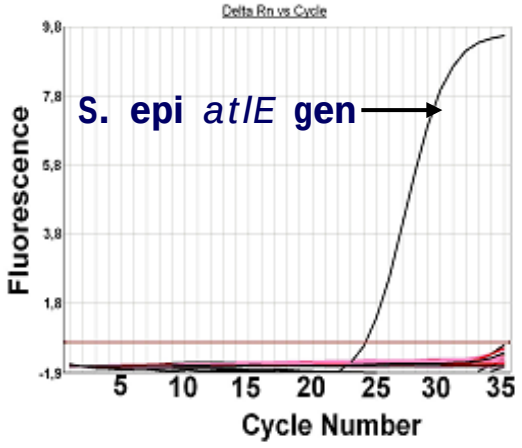
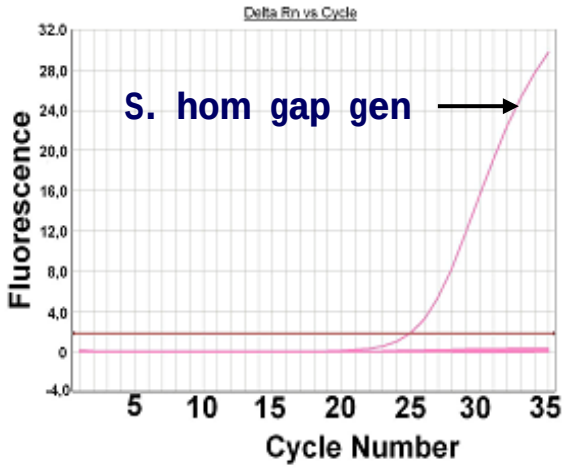
3:15 PM

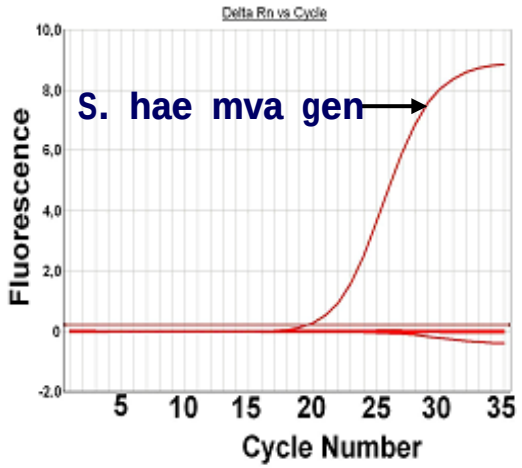

Delta Rn vs Cycle
S. hom gap gen
S. epi atlE gen
S. hae mva gen
Fluorescence
Cycle Number


Delta Rn vs Cycle
S. epi atlE gen
S. hae mva gen
Fluorescence
Cycle Number

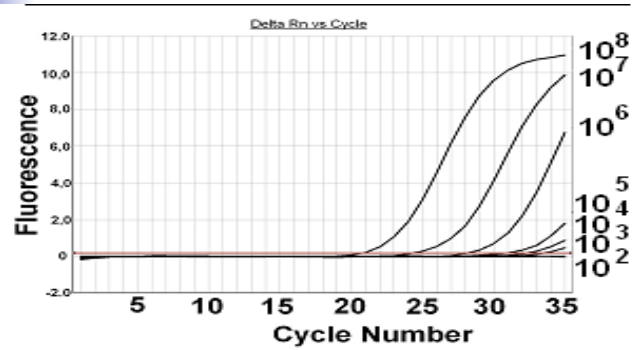

Delta Rn vs Cycle
S. hom gap gen
S. epi atlE gen
Fluorescence
Cycle Number

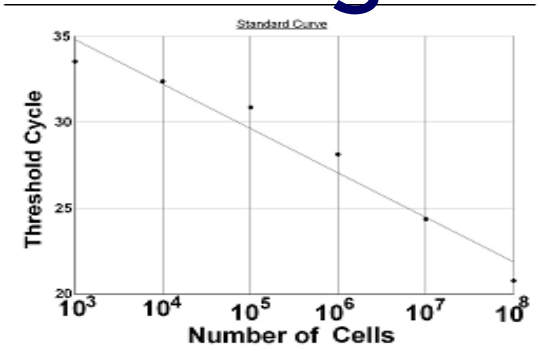

Delta Rn vs Cycle
S. epi atlE gen
Fluorescence
Cycle Number


Delta Rn vs Cycle
S. hom gap gen
Fluorescence
Cycle Number

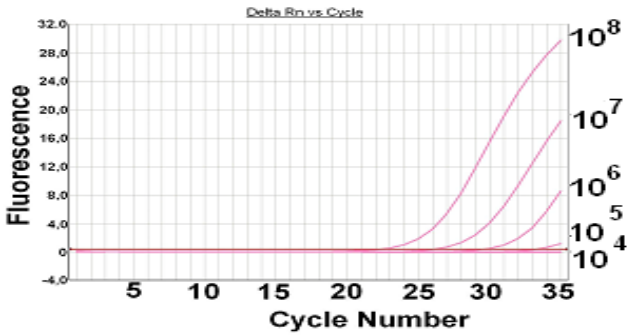

Delta Rn vs Cycle
S. hae mva gen
Fluorescence
Cycle Number

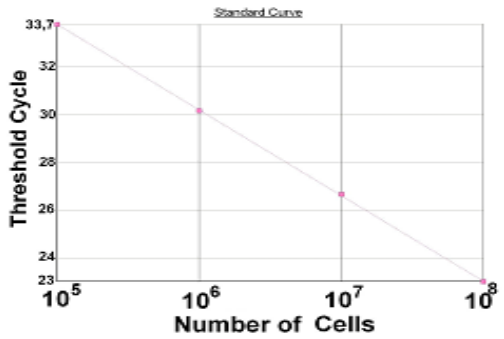
Analitik Duyarlılık ve Reaksiyon Etkinliği



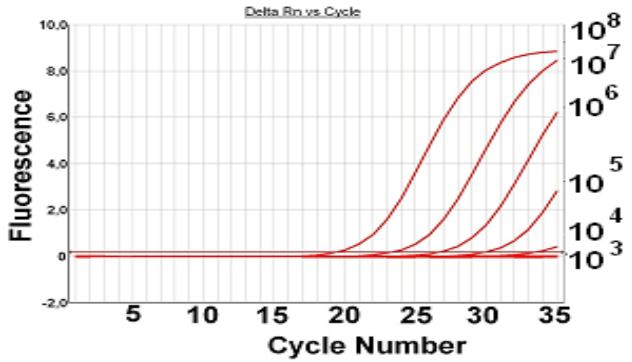


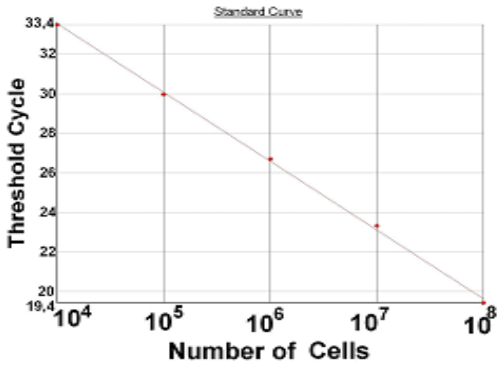
RE (*etlA*)= %143





RE (*gap*)= %93





RE (*mva*)= %90

Reaksiyon etkinliği=$10^{(-1/slope)}$-1

Mikroorganizmalar	Tripleks PCR sonucu		
	atlE	*mva*	*gap*
S. epidermidis ATCC35984	+	0	0
S. hominis ATCC700236	0	0	+
S. haemolyticus ATCC29970	0	+	0
MRSA NCTC10442, N315	0	0	0
S. aureus ATCC25923, ATCC29213	0	0	0
S. warneri ATCC25614; *S. simulans* ATCC27848; *S. capitis* ATCC27840; *S. lugdunensis* ATCC43809; *S. saprophyticus* ATCC15305; *S. sciuri* ATCC29061	0	0	0
Enterococcus casseliflavus ATCC25788; *Enterococcus gallinarum* GS; *Enterococcus faecalis* ATCC27270; *Enterococcus faecium* B7641; *Streptococcus pneumoniae* NCTC12695; *Streptococcus pyogenes* NCTC12696; *Acinetobacter haemolyticus* ATCC19002; *Acinetobacter septicus* DSM19415; *Pseudomonas aeruginosa* ATCC27853; *Enterobacter aerogenes* ATCC13048; *Escherichia coli* ATCC35218; *Klebsiella pneumoniae* ATCC13883	0	0	0

Mikroorganizmalar (Fenotipik Yöntem) Phenix 0.5 McFarland	*S. epidermidis atlE gen*		*S. hominis gap gen*		*S. haemolyticus mva gen*		Mikroroganizmalar (moleküler yöntem)
	PCR+	PCR—	PCR+	PCR—	PCR+	PCR—	
S. epidermidis (n=120)	107	13	21	99	2	118	*S.epidermidis* (n=107) *S.hominis* (n=11) *S.epidermidis+S.hominis* (n=9) *S.epidermidis+S.haemolyticus* (n=1) *S. hominis +S.haemolyticus* (n=1) Negatif (n=2)
S. hominis (n=64)	4	60	64	0	0	64	*S.hominis* (n=60) *S.hominis+S.epidermidis* (n=4)
S. haemolyticus (n=17)	1	16	1	16	17	0	*S.haemolyticus* (n=15) *S.haemolyticus+S.epidermidis* (n=1) *S.haemolyticus+S.hominis* (n=1)
*Diğer (n=6)	0	6	0	6	0	6	Negatif (n=6)

JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2007, p. 4088–4089 Vol. 45, No. 12
0095-1137/07/$08.00+0 doi:10.1128/JCM.01847-07

Letters to the Editor

Evaluation of the Inoculation Procedure Using a 0.25 McFarland Standard for the BD Phoenix Automated Microbiology System[▽]

[a] The 132 clinical strains were 55 *Enterobacteriaceae*, 31 *Pseudomonaceae*, 25 staphylococci, and 21 enterococci. Discordant IDs were tested by API strips (API-20E, 32-STREP, and 32-STAPH; bioMerieux, Marcy l'Etoile, France), and the API result was used to select or adjudicate the correct ID result.

The performance of the low-inoculum-density method based on presented results has allowed us to start to use this approach routinely in our laboratory.

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1 Avenue Claude Vellefaux
Paris, France

Mikroorganizmalar (Fenotipik Yöntem) Phoenix 0.25 McFarland	*S. epidermidis atlE gen*		*S. hominis sodA gen*		*S. haemolyticus mva gen*		Mikroroganizmalar (moleküler yöntem)
	PCR+	PCR—	PCR+	PCR—	PCR+	PCR—	
S. epidermidis (n=109)	107	2	10	99	2	107	*S.epidermidis* (n=107) *S.hominis* (n=0) *S.epidermidis+S.hominis* (n=9) *S.epidermidis+S.haemolyticus* (n=1) *S. hominis +S.haemolyticus* (n=1) Negatif (n=2)
S. hominis (n=75)	4	71	75	0	0	75	*S.hominis* (n=71) *S.epidermidis+S.hominis* (n=4)
S. haemolyticus (n=17)	1	16	1	16	17	0	*S.haemolyticus* (n=15) *S.haemolyticus+S.epidermidis* (n=1) *S.haemolyticus+S.hominis* (n=1)
*Diğer (n=6)	0	6	0	6	0	6	Negatif (n=6)

*S.simulans, S.capitis (n=2), S.saprophyticus (n=2), S.equorum

Sonuç

- İkili multipleks real-time PCR ile kan kültürü örneklerinden 83 dk'da
  - MRSA ve MSSA
  - MR *S. epidermidis* ve MS *S. epidermidis*
  - MR *S. hominis* ve MS *S. hominis*
  - MR *S. haemolyticus* ve MS *S. haemolyticus*
- Karışık örneklerin tanımlayabilmektedir
- Phoenix sistemi ile CoNS tanımlanmasında düşük inokulum daha iyi sonuç vermektedir

Gülhane Mikrobiyoloji Günleri

20 - 22 Nisan 2010

Antimikrobik Kemoterapi Laboratuvar Uygulamaları ve Yenilikler

KLİMUD

GÜLHANE ASKERİ TIP AKADEMİSİ 1898

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