Kan Dolaşımı Enfeksiyonlarının Tanısında Sendromik Yaklaşım

Dr. Banu Sancak

- Kan akımı enfeksiyonları morbidite ve morbidite⬆
- Uygun olmayan AB tedavisi: En önemli prognostik faktör
  - Uygun ampirik tedavi 1 saat içinde başlanmalı
  - >%30 hasta: Uygun olmayan tedavi almakta

Etken spektrumu dinamik.....

1990lar:

- Enterobacteriaceae ⬇
- Gram (+) koklar ⬆

2000 sonrası:

- ESKAPE
- CRE (karbapenem dirençli Enterobacteriaceae)
- Karba dirençli *Acinetobacter*

2015 sonrası:

- ESCAPE
- CRE (karbapenem dirençli Enterobacteriaceae)
- Karba dirençli *Acinetobacter*

Antibiotic Resistance: From the Bench to Patients

Mário Gajdács [1,*] and Fernando Albericio [2,3]

From ESKAPE to ESCAPE,
From KPC to CCC

Table 2. Current list of ESKAPE pathogens.

Pathogens
Enterococcus faecium
Staphylococcus aureus (*Stenotrophomonas maltophilia*)
Klebsiella pneumoniae (*Clostridioides difficile*)
Acinetobacter spp.
Pseudomonas aeruginosa
Enterobacter spp. (members of *Enterobacterales*)

on a "CCC" strategy, aiming at carbapenemase-producing Enterobacteriaceae, *C. difficile*, and *Candida* species.

Çok farklı etkenler görmeye başladık…

- Enfeksiyona yatkın hasta grupları ⬆⬆
- Mikrobiyota üyeleri göz ardı edemiyoruz…

➡ Etken olabiliyorlar

1

Etkenlerin hızlı ve doğru tanımlanması...

Timeliness of antibiotics for patients with sepsis and septic shock

Michiel Schinkel[1,2], Rishi S. Nannan Panday[1], W. Joost [illegible]inga[2], Prabath W. B. Nanayakkara[1]

The Journal of Infectious Diseases® 2020;222(S2):S110–8

A Critical Analysis of the Literature on Time-to-Antibiotics in Suspected Sepsis

[illegible]einberger,[1,2] Chanu Rhee,[1,2] and Michael Klompas[1,2]

Every hour of delay
in the administration of ABs
decreased the chances of survival by

7.6%

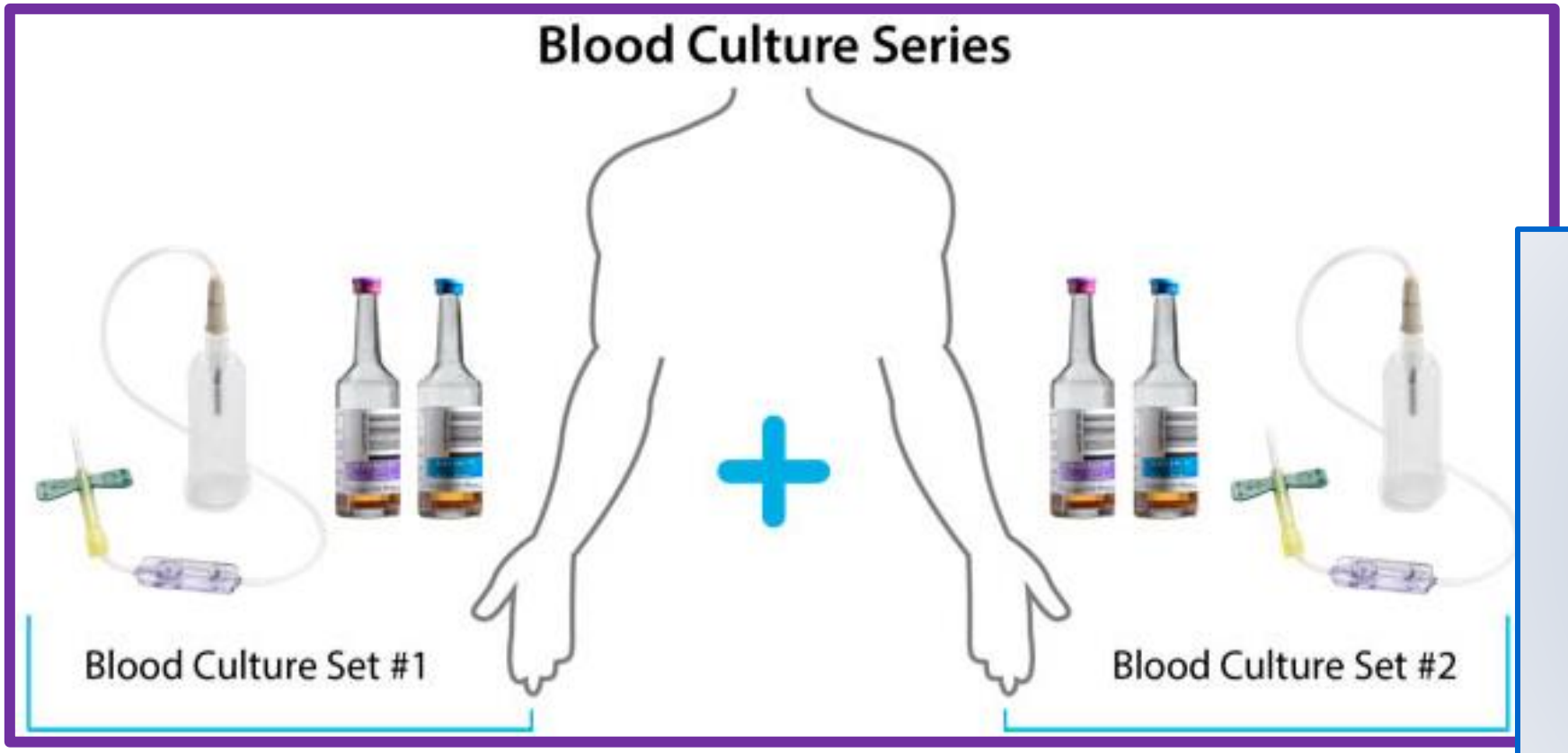
Time to administration of antibiotics and mortality in sepsis

Karina Siewers BSc[1,2] | S M Osama Bin Abdullah MD[3] |

JACEP Open 2021;2:e12435.

Kan kültürü
Hala Altın Standart

Kan kültüründen tanımlama ve duyarlılık için kullanılan standart yöntemler nedir?



Kan kültürü **sonuçlarını etkileyen en önemli faktör** alınan kan miktarıdır:

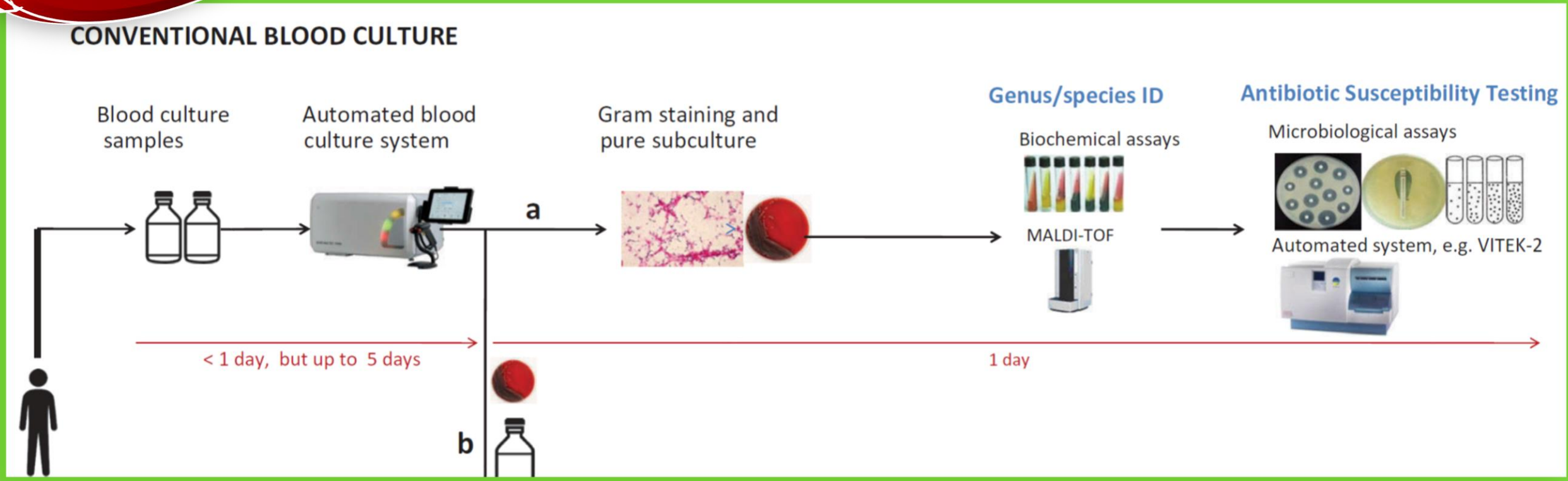
40-80 ml

2-4 set kan kültürü

Kan kültürü SİNYAL verdikten sonra

GRAM BOYAMA

When a blood culture is (+),
the morphology and staining characteristics of the pathogen can be determined through Gram staining.



- Kan kültürü SİNYAL verdikten sonra

↓

identifikasyon ve duyarlılık için

↓

24-72 saat gerekli!!!!

Kan kültürü: Hala Altın Standart

AMA

- Etkenin saptanabilmesi için en az 24-72 saat gerekli
- Nazlı bakteriler KK ile saptanamıyor

- TAT: Uzun
- Duyarlılık: Düşük
- **AB kullanımı** varsa: Duyarlılık daha da azalır

↓

- Yeni yöntemler gerekli

↓

- Ama bunların klinik ve ekonomik etkileri neler?

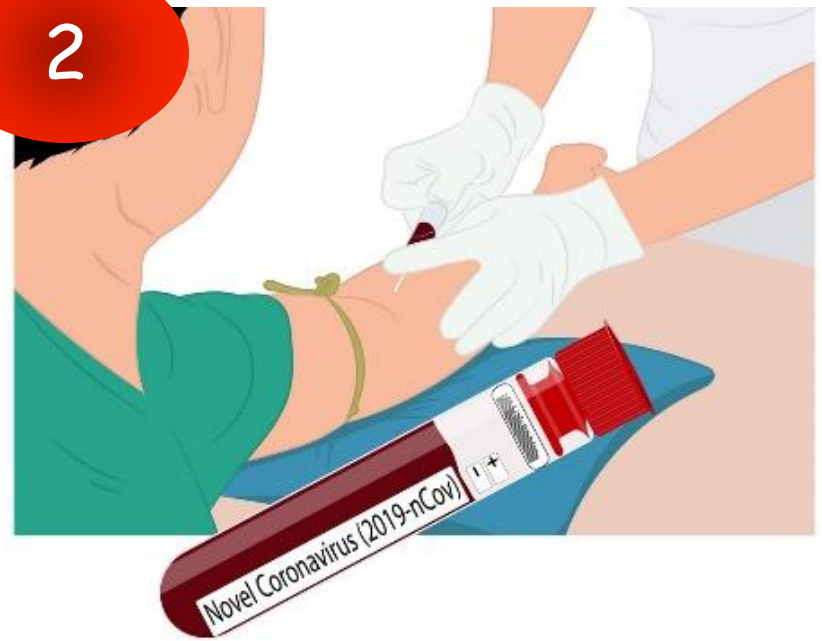

Yeni
Yaklaşımlar
neler?

Yeni yaklaşımlar neler?

1

(+) sinyal alınan
kan kültürü şişesinden

2



Direkt kandan

A

Pozitif kan kültüründen direkt tanımlama

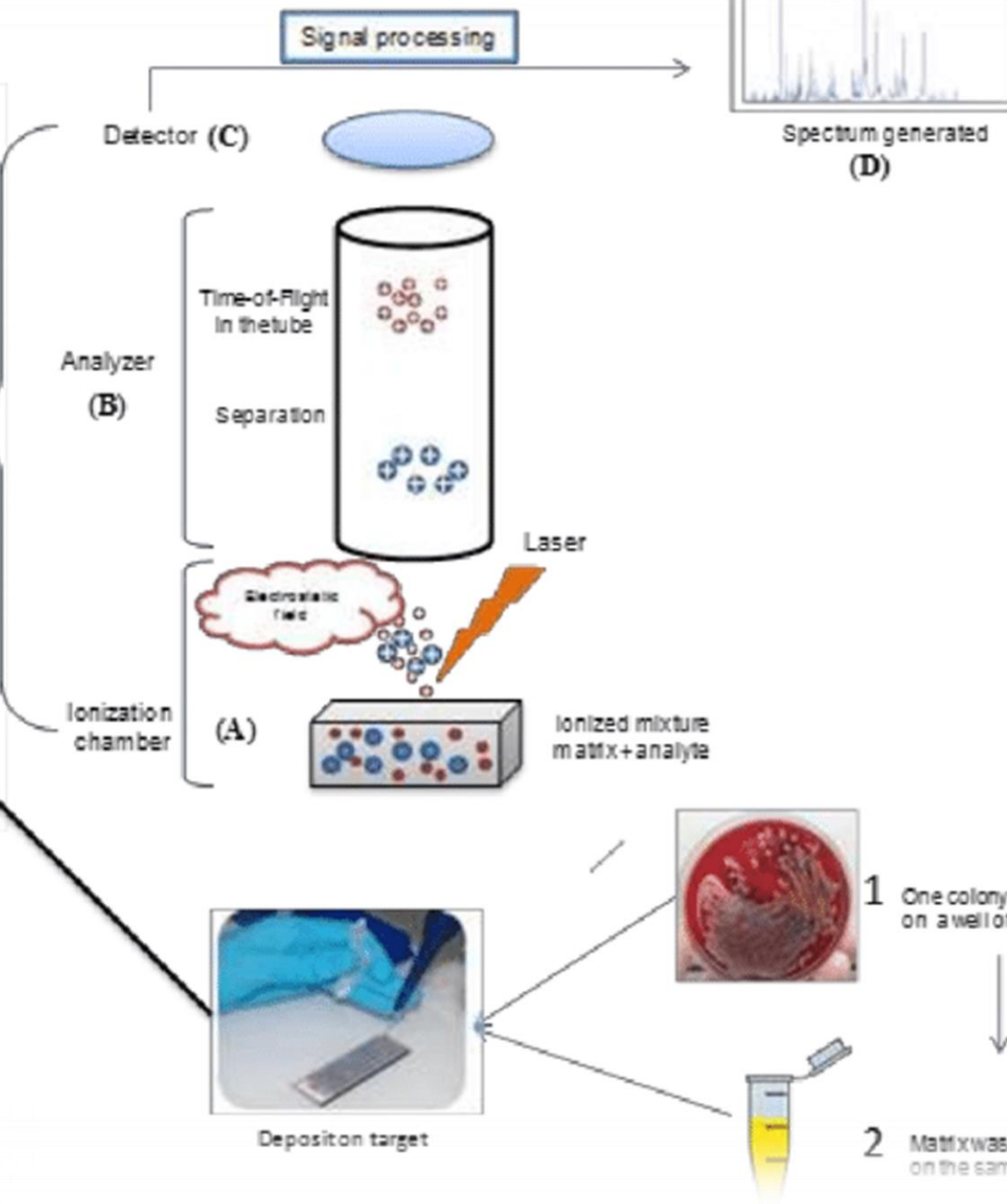
Pozitif sinyal veren
Kan kültürü şişelerinden tanımlama

1. MALDI-TOF MS
2. Floresan in situ hibridizasyon
3. Mikroarray
4. NA amplifikasyon

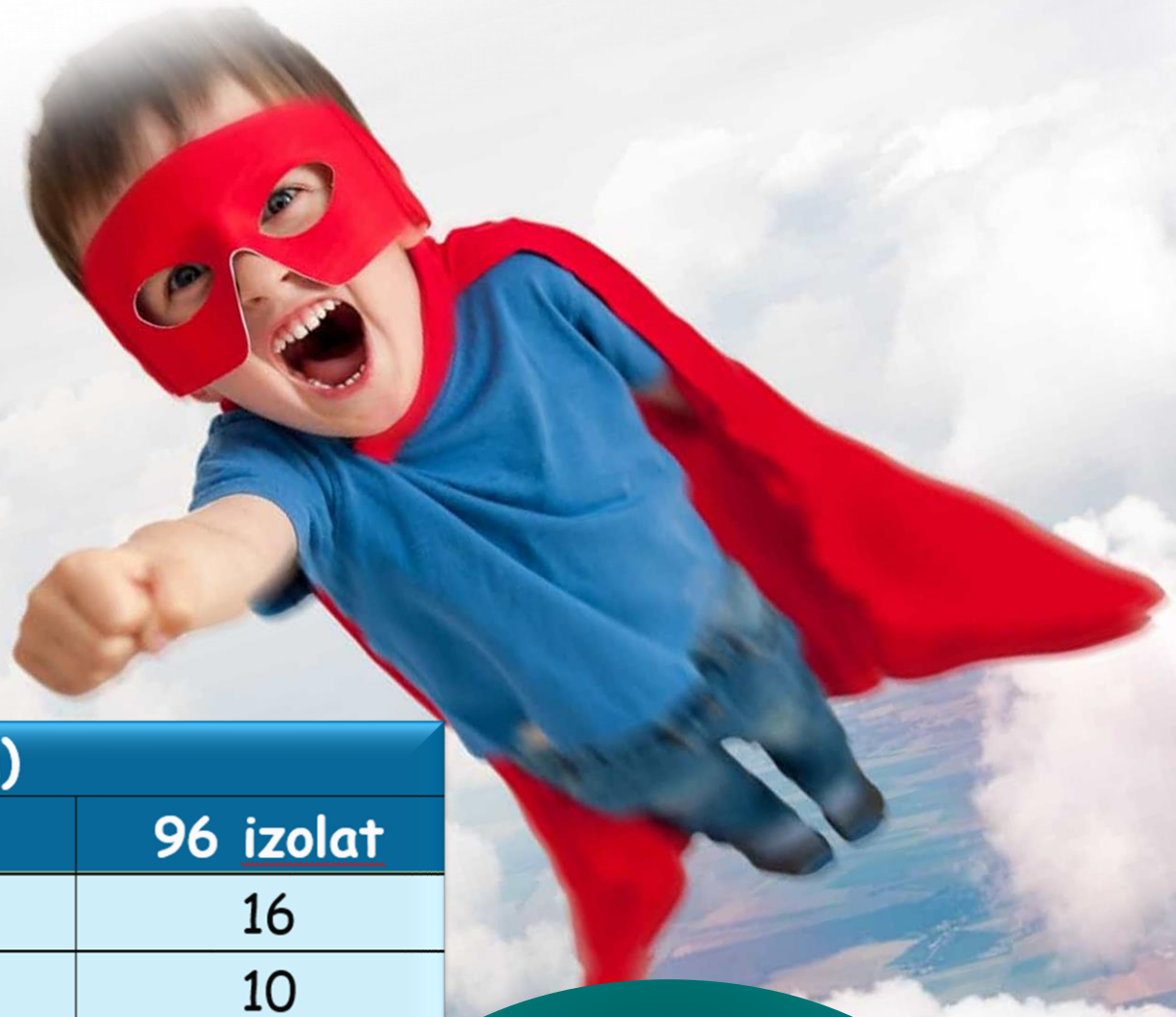
Pozitif KK şişesinden tanımlama

1 MALDI-TOF MS

MALDI-TOF VITEK® MS



HIZLI

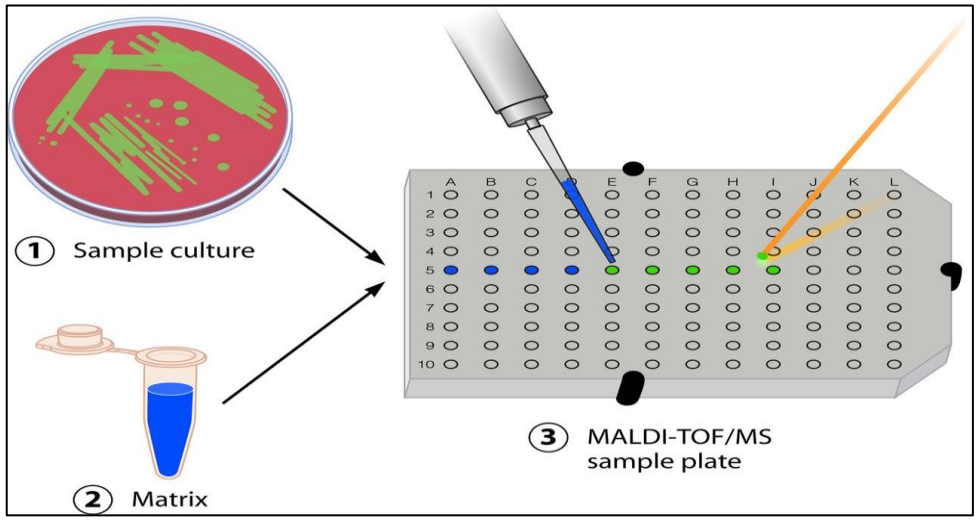


Workflow step	TIME (min)		
	5 izolat	24 izolat	96 izolat
Application on plate	1	5	16
Application matrix	1	3	10
Drying	2	2	7
Read in system	5	12	43
Time to result			
TOTAL	9	22	76

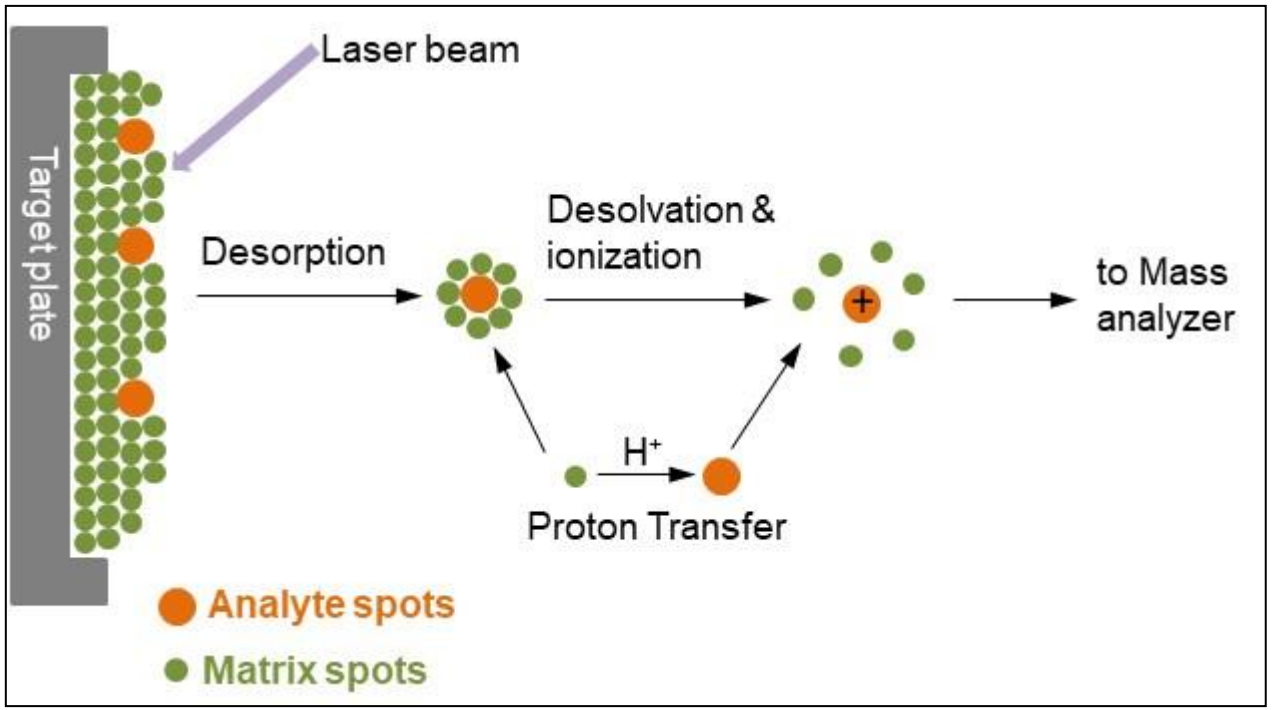
Dakikalar…

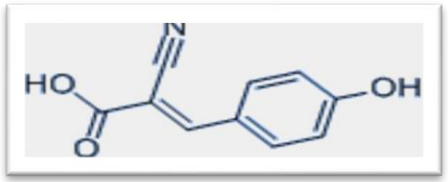
Çalışma Prensipi nedir?

Matrix Assisted Laser Desorption Ionization-Time of Flight-Mass Spectrometry



Peptit parmak izi

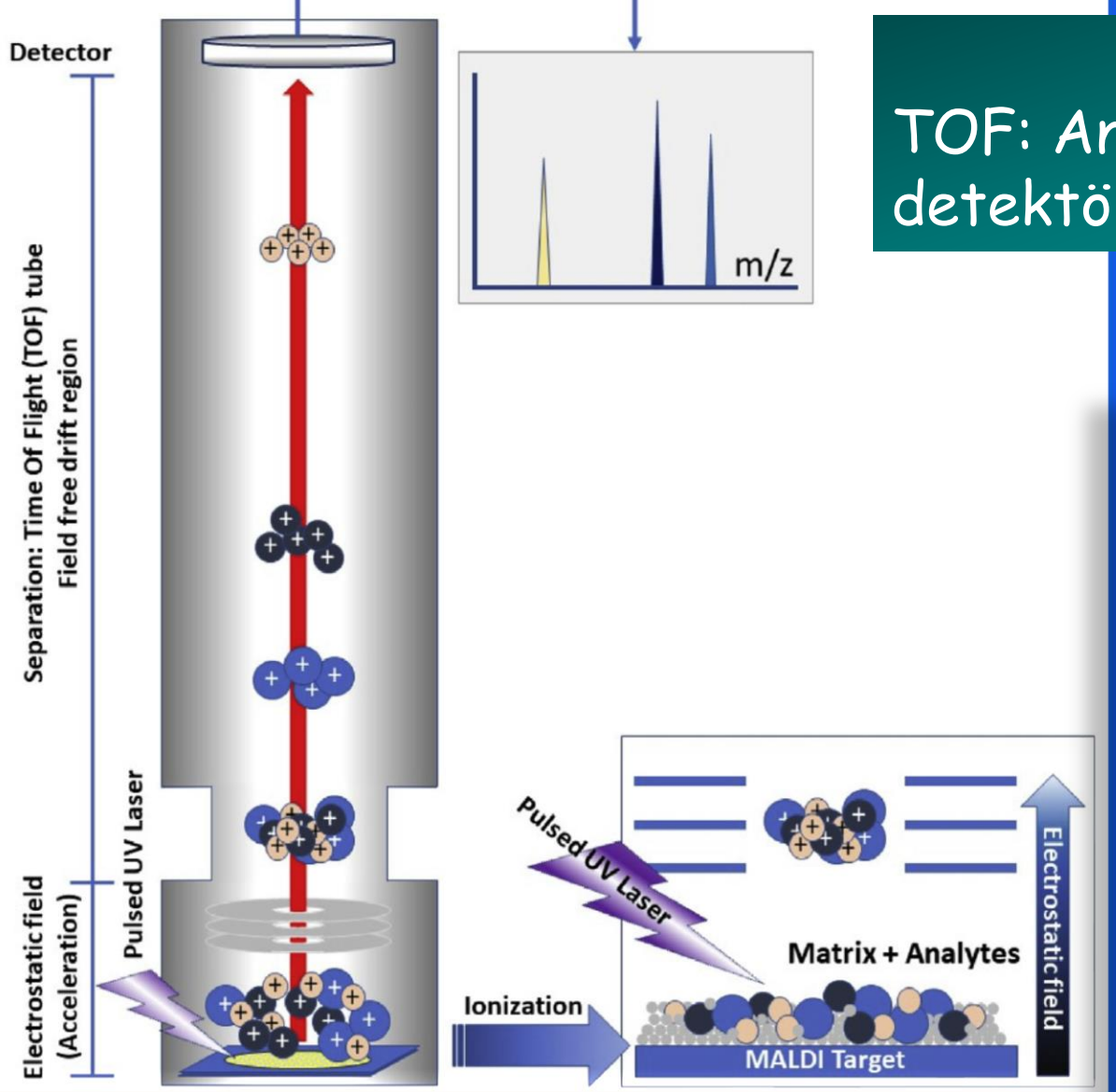


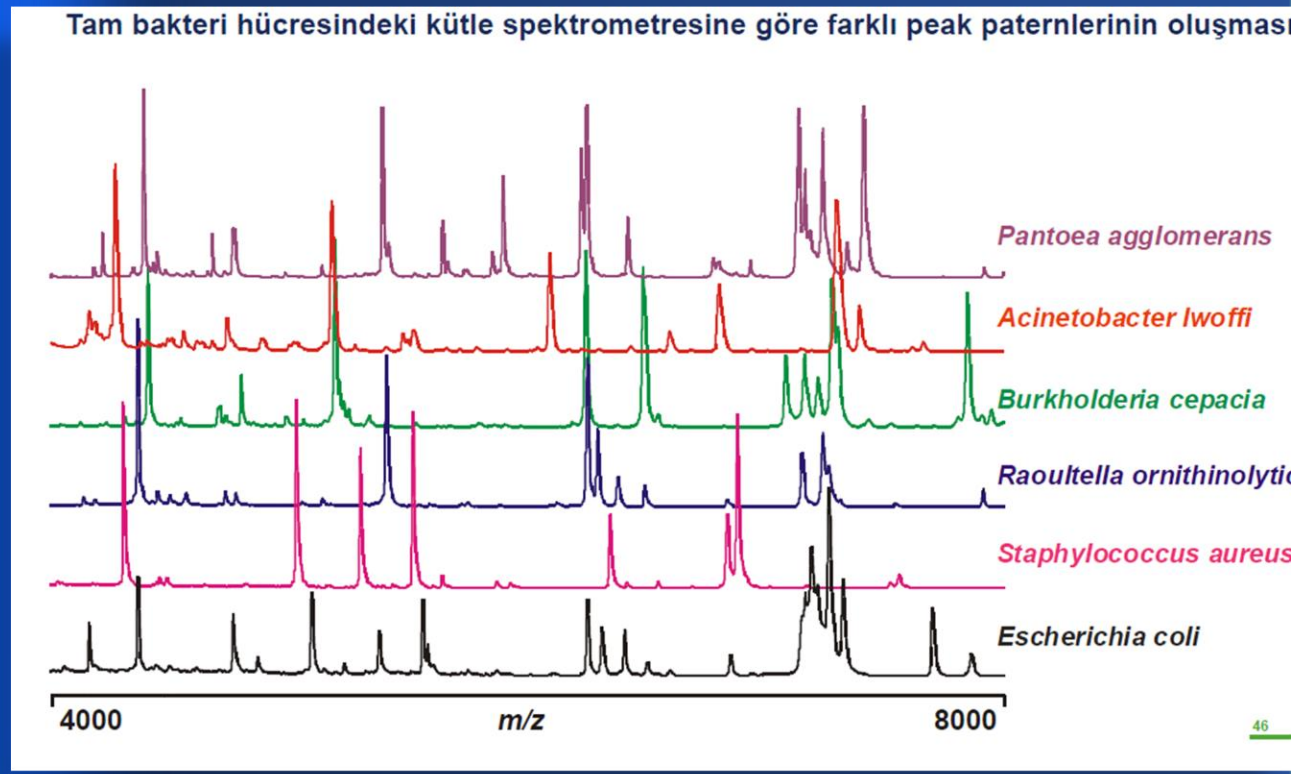


α-cyano-4-hydroxycinnamic acid

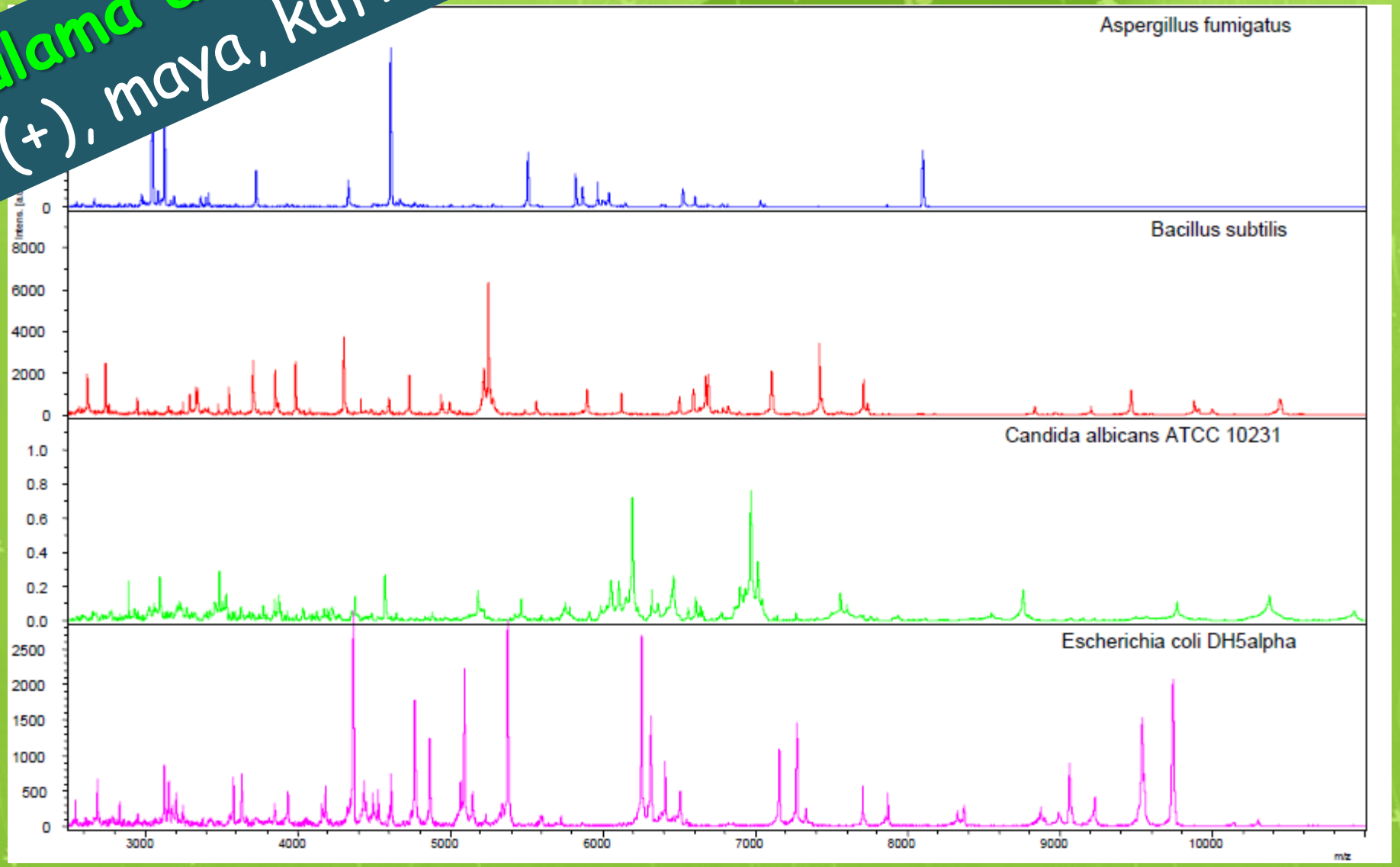
Uçuş tüpü

TOF: Analitlerin detektöre ulaşma zamanı





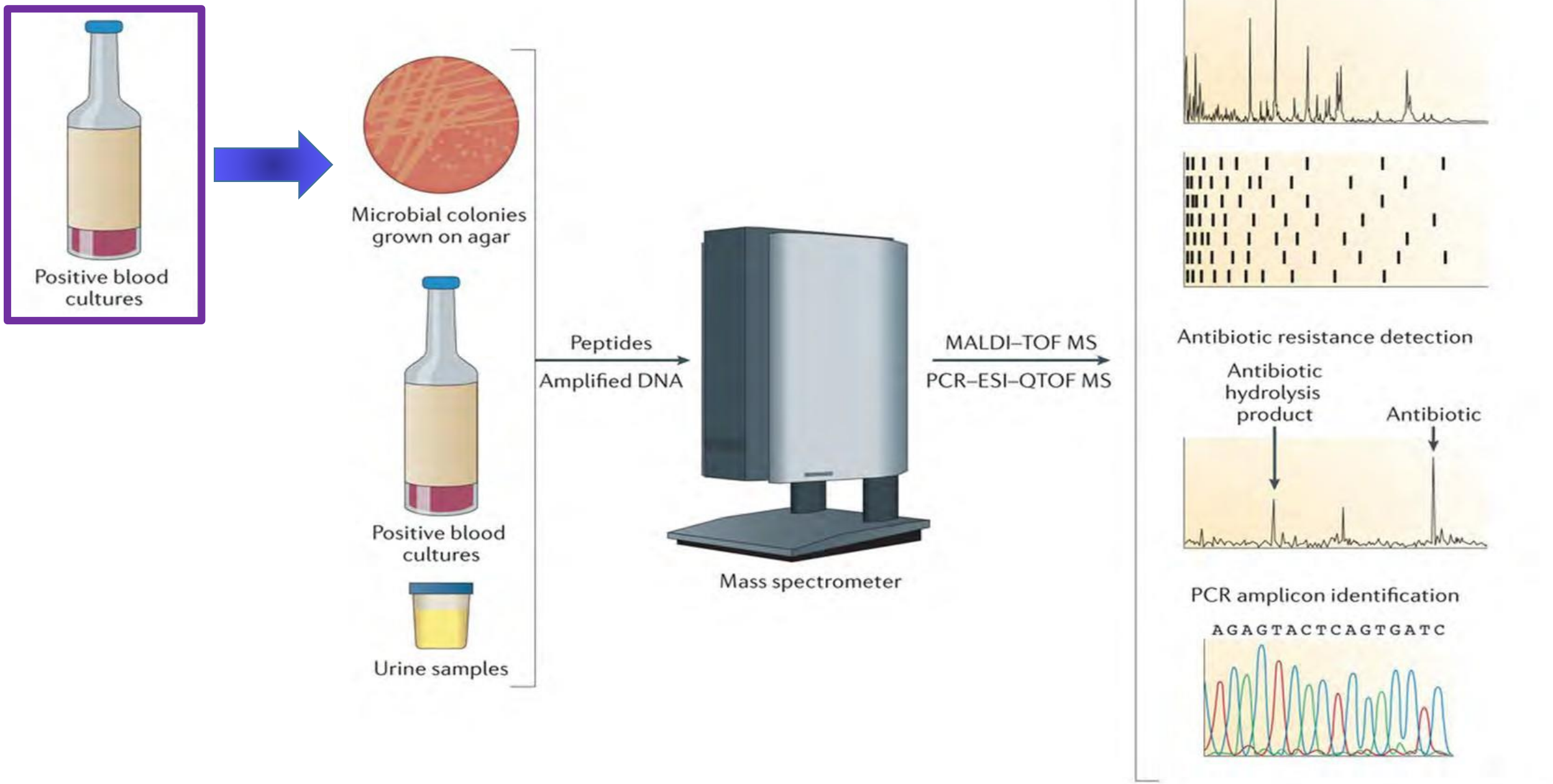
Yaygın uygulama alanı var:
Gr(-), Gr(+), maya, küf …



Mikroorganizma tanımlamalarında gelişme

- Konvansiyonel yöntemlerle tanımlanması zor olan m.o.lar tanımlanır
- Yeni veri tabanı oluşturmak için:

  Yeterli sayıya ulaşmak gerek


Positive blood cultures
Microbial colonies grown on agar
Positive blood cultures
Urine samples
Peptides
Amplified DNA
Mass spectrometer
MALDI–TOF MS
PCR–ESI–QTOF MS
Isolate identification
Antibiotic resistance detection
Antibiotic hydrolysis product
Antibiotic
PCR amplicon identification
AGAGTACTCAGTGATC

Direkt kan kültüründen

1) *Katı b.y.de kısa inkübasyon* m.g. üreme

Ucuz ve basit

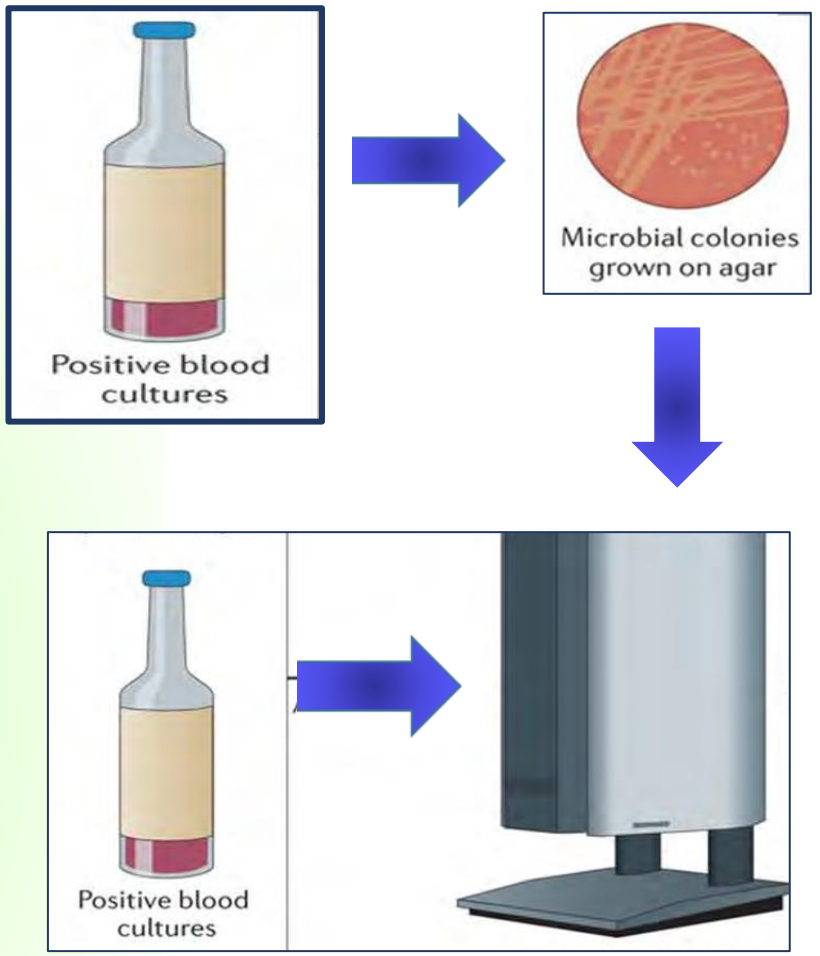
Gr (–) için: 2 saat

Gr (+) için: 6 saat

Anaerop başarısız: 4-6 sa %50 tanımlama

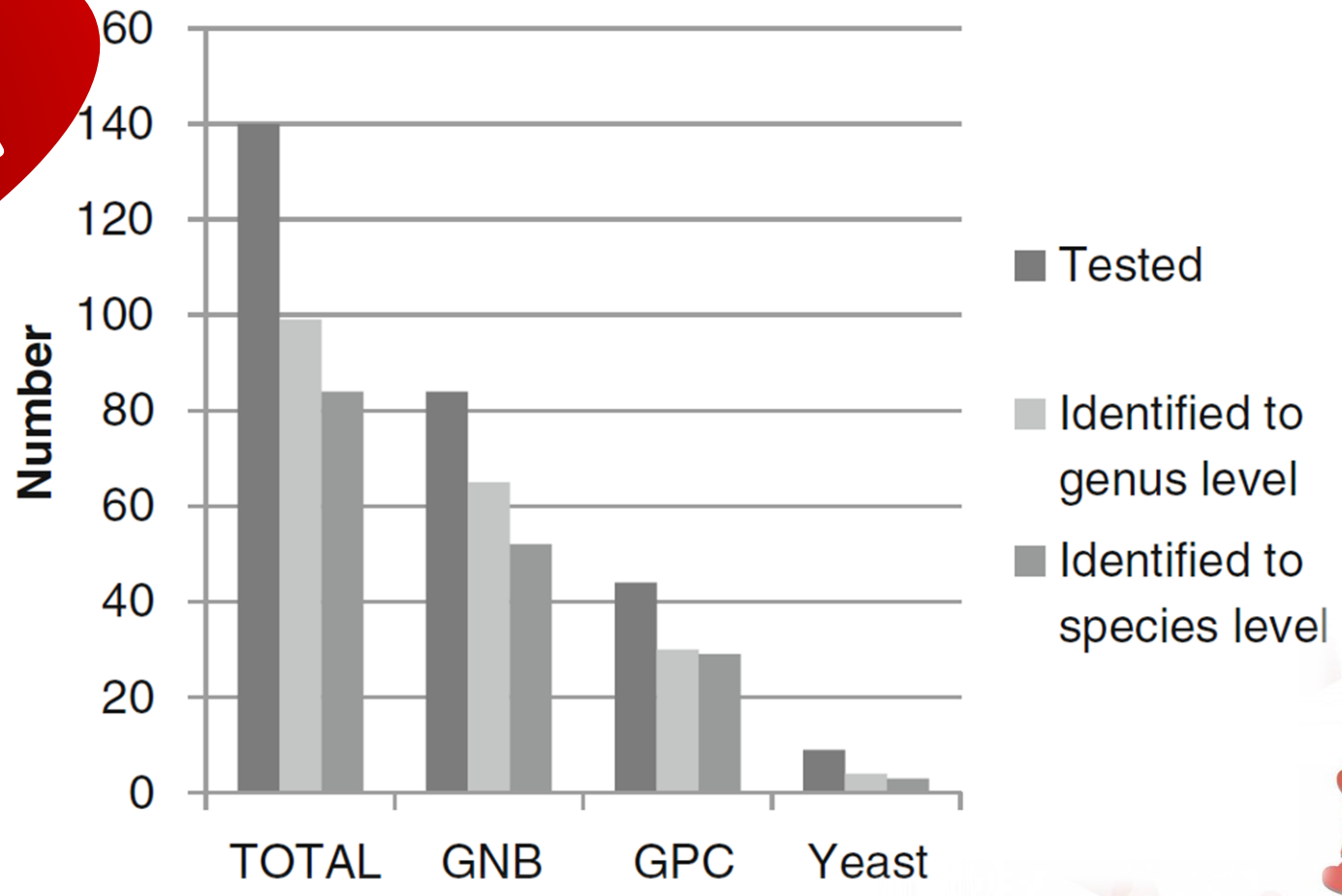
2) *Direkt kan kültürü sıvı b.y.den* tanımlama

Lizis, santrifügasyon, fitrasyon, protein ekstraksiyonu



Bruker SepsiTyper®
bioMerieux Vitek®MS BC kits

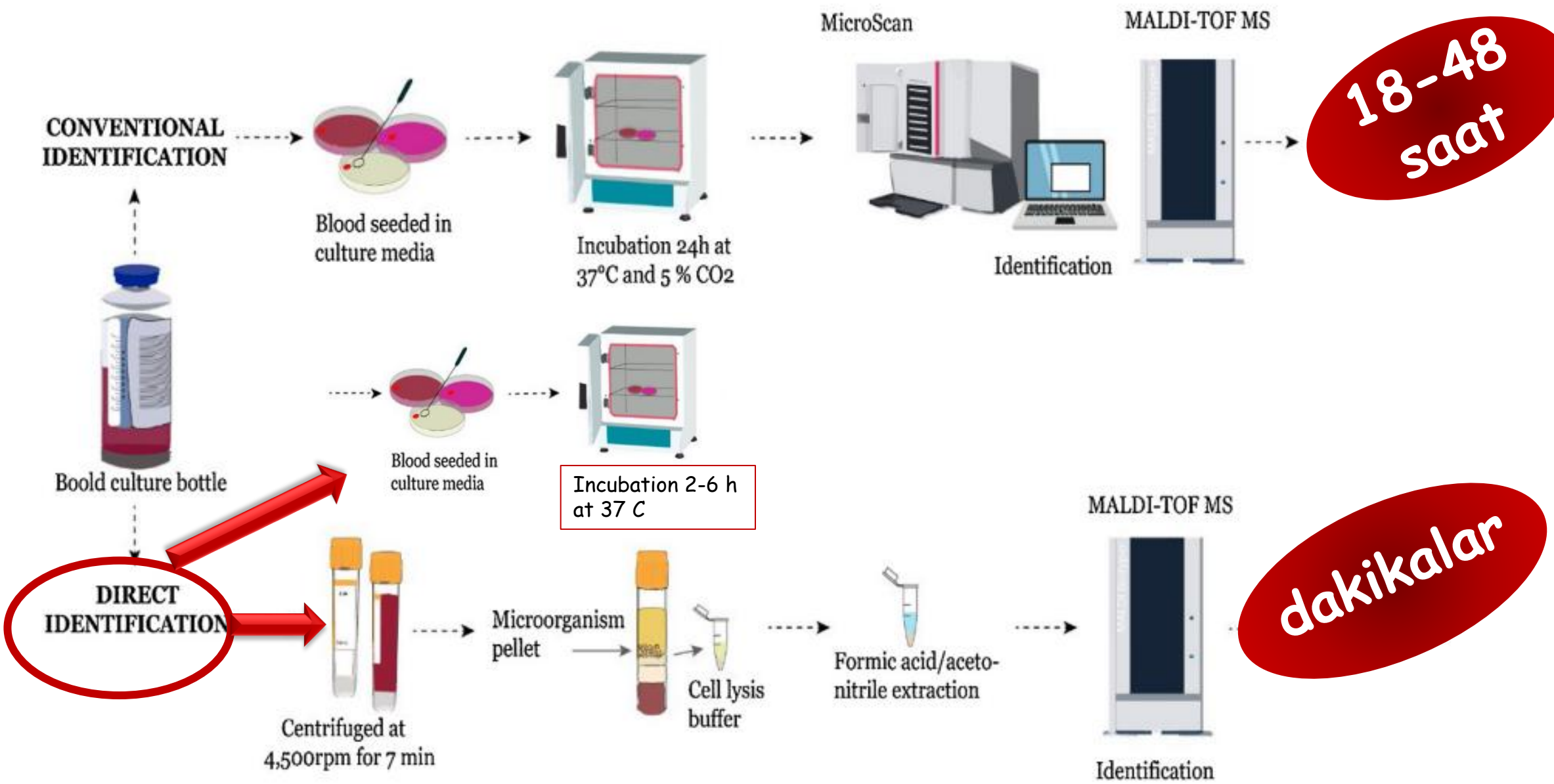
Direkt kan kültüründen



- Duyarlılık ve özgüllük yüksek
  - Gram (-)'lerde en iyi
  - Mayalarda ve anaeroplarda başarı düşük
- Polimikrobiyal: ⬇
- Nazlı bakteriler:⬇

Pozitif KK şişesinden tanımlama

1 MALDI-TOF MS



Özetle……

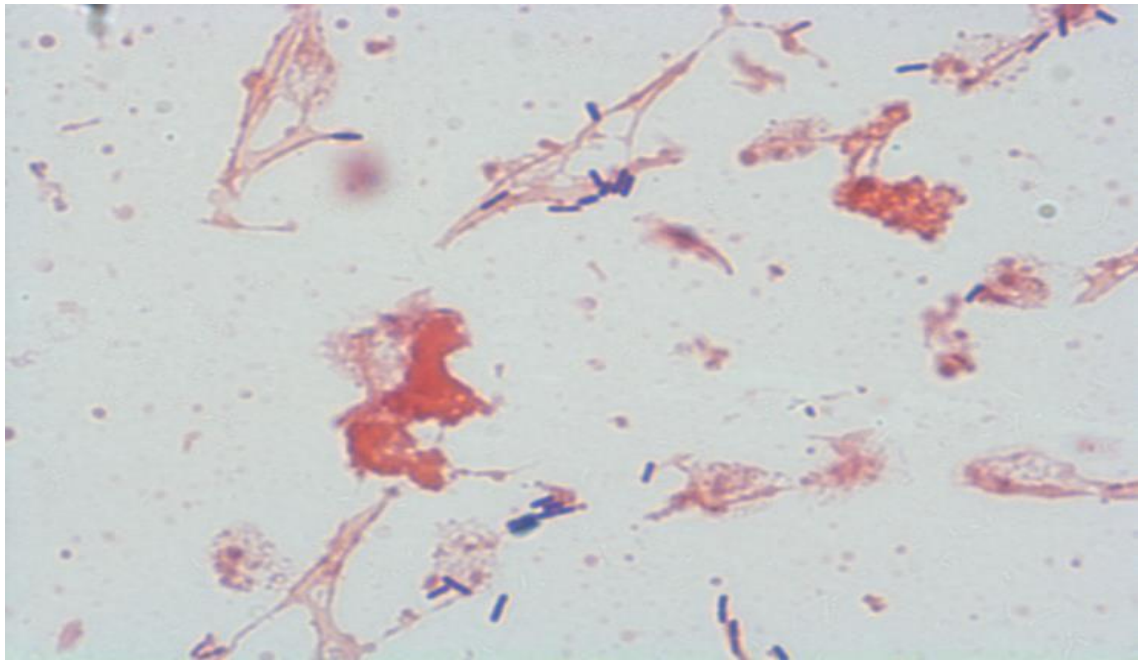
Blood culture
bacterial pellet

Gram staining ➡ **≤1h**
> 100% accurate

MALDI-TOF MS ➡ **≤1h**
> 99% accurate

Olgu

- Kan kültürü
- 28 saat sonra pozitif sinyal

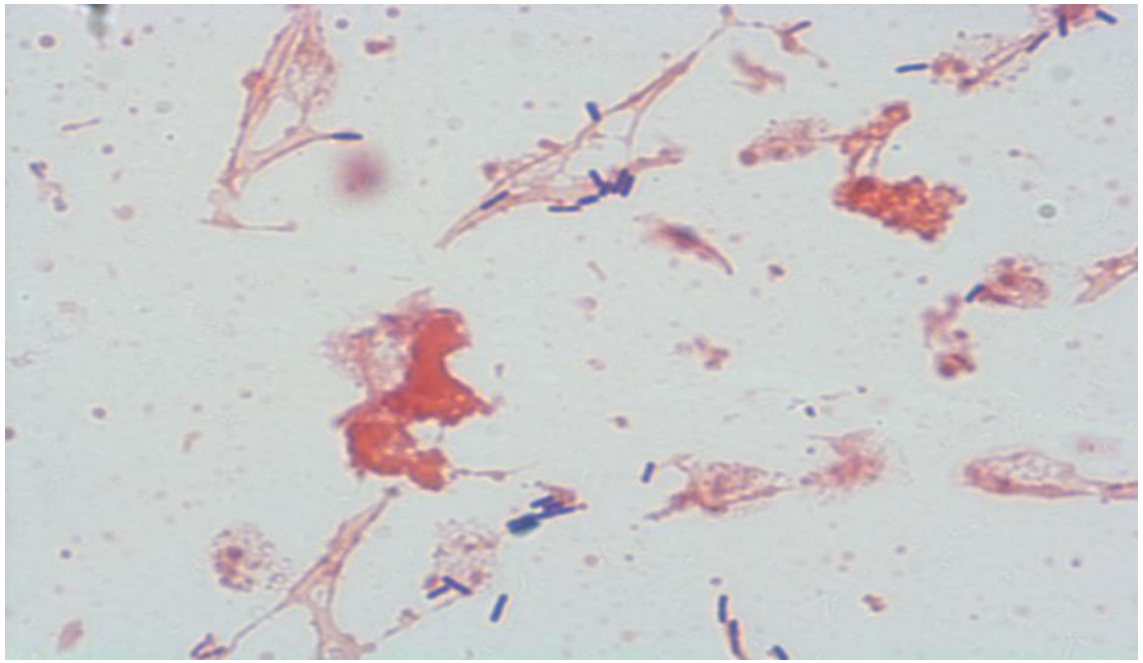


- Gram inceleme: Gram pozitif basil
- Difteroid mi?
- Kontaminant

Olgu

- Kan kültürü
- 28 saat sonra pozitif sinyal

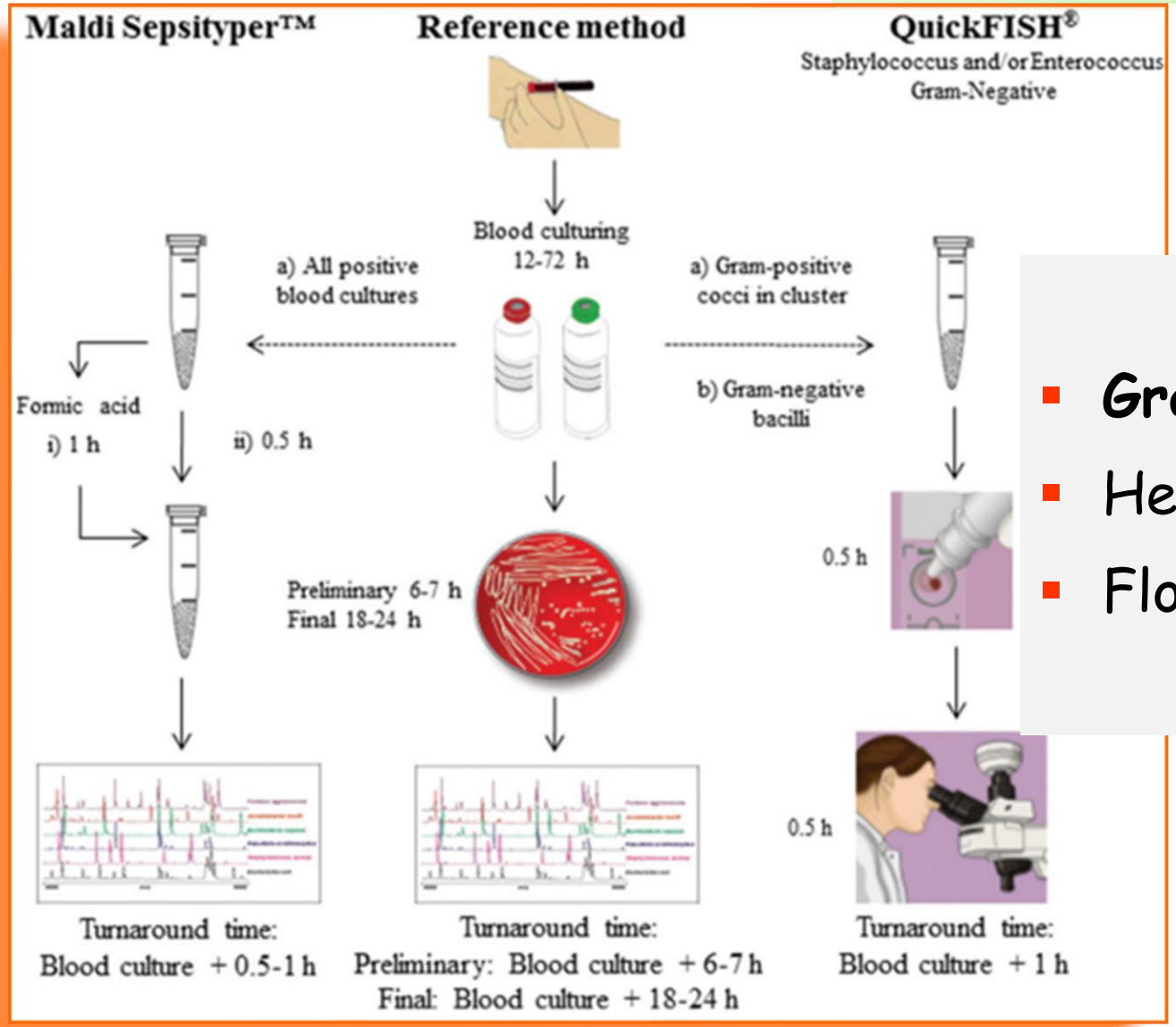
- Gram inceleme: Gram pozitif basil
- Difteroid mi?
- Kontaminant



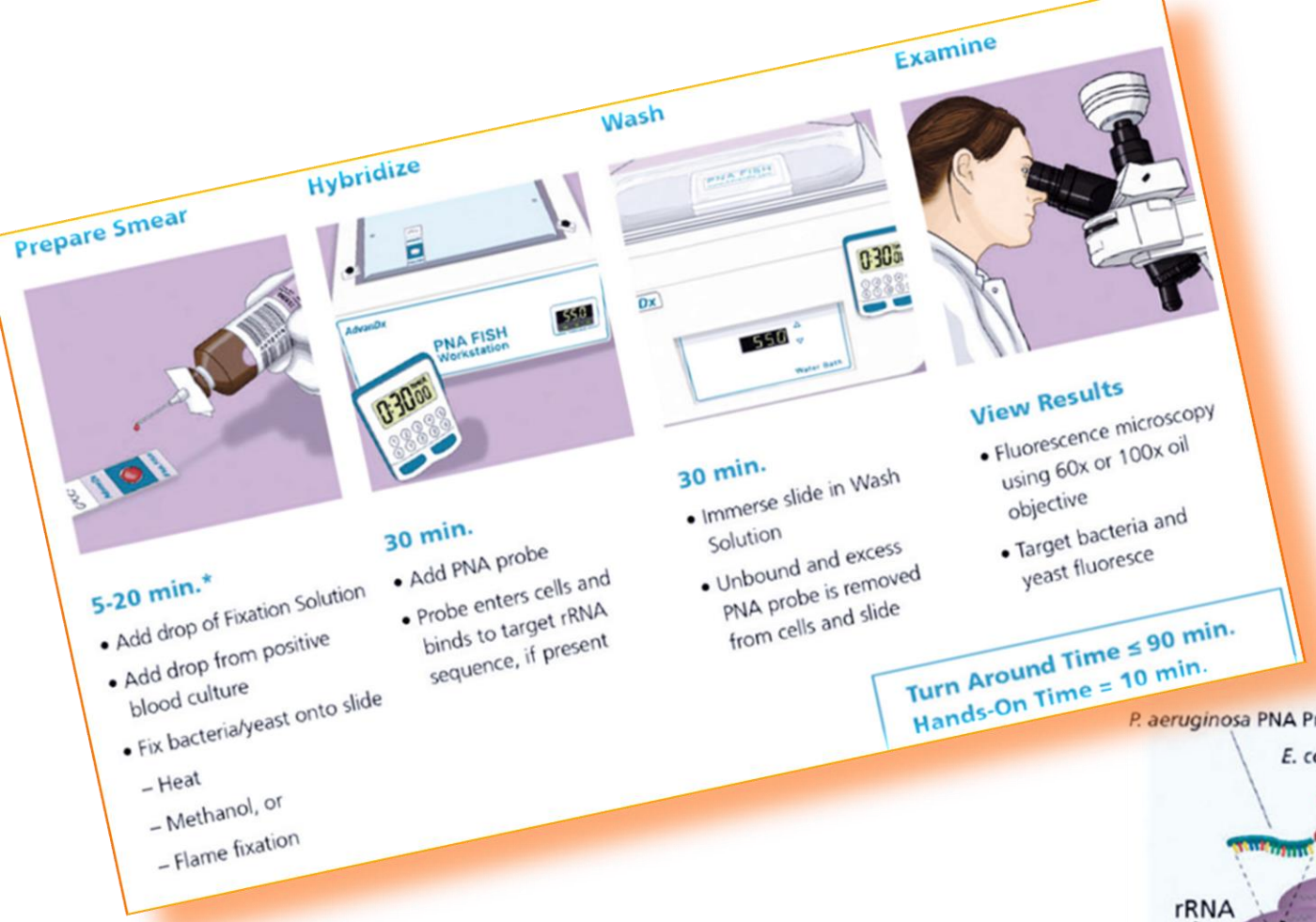
- MALDI-TOF MS: *Listeria monocytogenes*

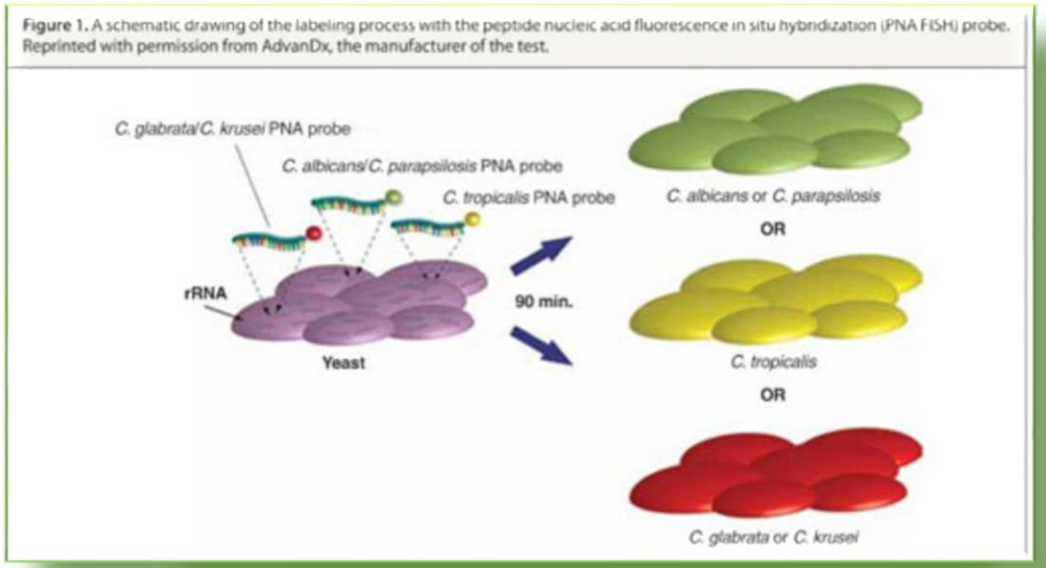
Pozitif KK şişesinden tanımlama

2 Floresan in situ hibridizasyon (FISH)



- **Gram boyama şart**
- Hedefe özgü floresan işaretli prob
- Floresan mikroskop ile değerlendirme





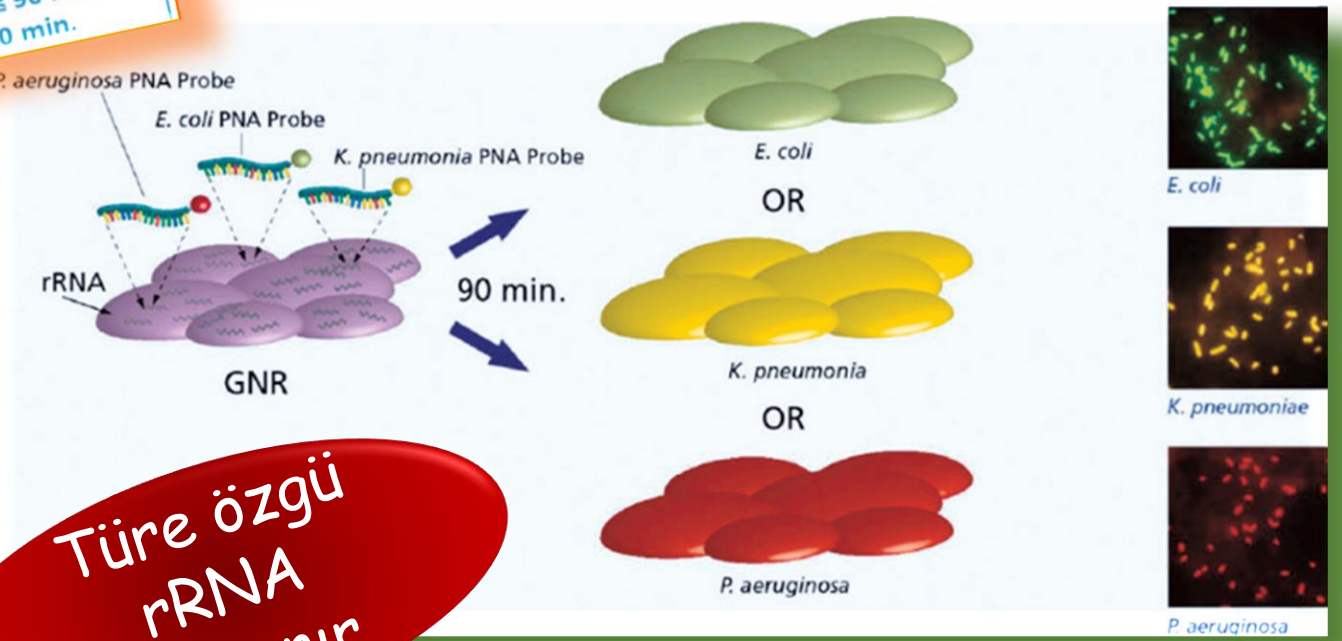
Once a blood culture turns (+)

⬇

Gram stain is performed

⬇

based on the results
the appropriate PNA FISH test is selected



Türe özgü rRNA saptanır

Manufacturer, assay	Methodology	Organisms panel	Direct genotypic or phenotypic resistance information	Turn around time hands-on time throughput limit of detection (LOD)[a]	FDA sensitivity and specificity information (in comparison with culture)[a]	Advantages	Drawbacks
OpGen, AdvanDx, *QuickFISH* BC Assays for *Staphylococcus*, *Enterococcus*, Gram-negative	FISH using PNA probes to organism-specific ribosomal RNA sequences followed by examination by fluorescence microscopy	Bacteria *Staphylococcus aureus*, CoNS *Enterococcus faecalis* other Enterococci *E. coli*, *P. aeruginosa*, *K. pneumoniae*	None	20–25 min 5 min hands on time 1 sample processed at a time LOD: 1–4.5 × 10^5 CFU/mL			panel rmation iate MSSA from isidentified as n occur with mixed th prolonged times men collection and
OpenGen, AdvanDx, *Yeast Traffic Light PNA FISH* Assay for *Candida*	FISH using PNA probes to organism specific ribosomal RNA sequences followed by examination by fluorescence microscopy	Fungi *C . tropicalis*, *C. albicans and C. parapsilosis*, *C. glabrata and C. krusei*	None	30 min 5 min hands on time 1 sample processed at a time LOD: 1 × 10^5 CFU/mL			panel rmation distinguish between *C. parapsilosis* or between *C. glabrata* and *C. krusei*
OpenGen, AdvanDx, PNA FISH BC Assays for *S. aureus*/CoNS, *E. feacalis*/*OE*, Gram-Negative and *Candida*	FISH using PNA probes to organism specific ribosomal RNA sequences followed by examination by fluorescence microscopy	Bacteria *S. aureus*, CoNS *E. feacalis*, other Enterococci *E. coli*, *P. aeruginosa* Fungi *C. albicans* and/or *C. parapsilosis*, *C. glabrata* and/or *C. krusei*, and *C. tropicalis*	None			one	Limited organism panel No resistance information Low throughput Does not differentiate MSSA from MRSA False negatives can occur with mixed cultures *Micrococcus* may be misidentified as *Staphylococcus* *S. anginosus* may be misidentified as *Enterococcus* The test does not distinguish between *C. albicans* and *C. parapsilosis* or between *C. glabrata* and *C. krusei*

(*C. tropicalis*); Sp: 100%

QuickFISH

- 20-30 dak
- **Panel: sınırlı** mo
- **Direnç saptamaz**
- MRSA/MSSA ayrımı yok
- C.glabrata/krusei ayrımı yok

PNA FISH

- 90 dak
- **Panel: sınırlı** sayıda mo
- **Direnç saptamaz**
- Mikrokok- Stafilokok ?
- S.anginosus-Enterokok?

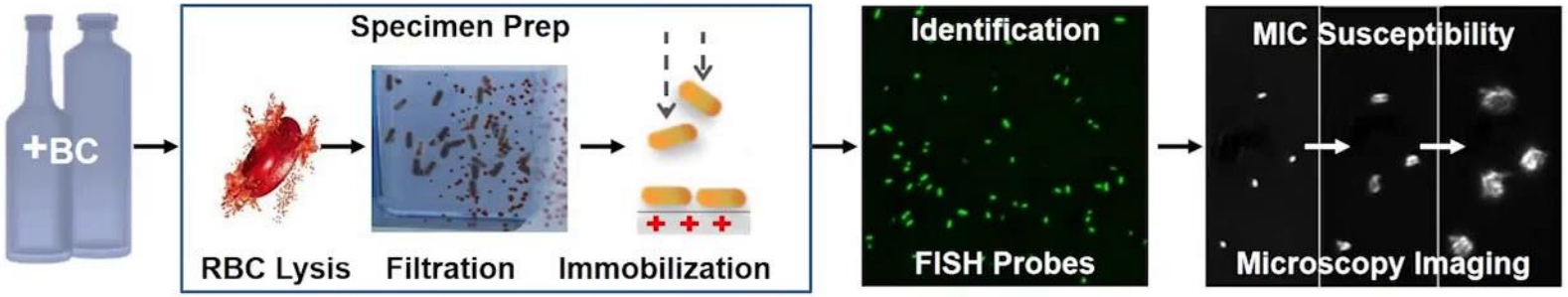
Accelerate Pheno™ System

AST : ~ 7 h
Identification:~ 2 h

System
- 1-4 module(s)
- Control & Analysis PCs
- Touchscreen monitor

Module
- Automated pipetting robot
- Digital camera
- Custom microscope

Kit
- 48 flow-channel cassette
- Reagent cartridge
- Sample vial



morphokinetic cellular analysis

- **Otomatize**
- Tanımlama (FISH) + antibiyogram (otomatize dijital mikroskop)
- Kan kültürü pozitif sinyal verdikten sonra 7 saat içinde sonuç

Gr (+)

	Identification	Ampicilin	Ceftaroline	Daptomycin	Linezolid	Vancomycin	Methicillin resistance (Cefoxitin)
S. aureus	●		●	●	●	●	●
S. lugdunensis	●					●	●
CNS spp.	●			●		●	●
E. faecalis	●	●		●	●	●	
E. faecium	●	●		●	●	●	
Streptococcus spp.	●						

Gr (-)

	Identificatio	Ampicillin-Sulbactam	Piperacillin-Tazobactam	Cefepime	Ceftazidime	Ceftriaxone	Ertapenem	Meropenem	Amikacin	Gentamicin	Tobramycin	Ciprofloxaci	Aztreonam
	●	●	●	●	●	●	●	●	●	●	●	●	●
Klebsiella spp.	●	●	●	●	●	●	●	●	●	●	●	●	●
Enterobacter spp.	●		●	●	●	●	●	●	●	●	●	●	●
Proteus spp.	●	●	●	●	●	●	●	●	●	●	●	●	●
Citrobacter spp.	●		●	●	●	●	●	●	●	●	●	●	●
S. marcescens	●		●	●	●	●	●	●	●	●	●	●	●
P. aeruginosa	●		●	●	●							●	●
A. baumannii	●		●										

Maya

Candida albicans	●
Candida glabrata	●

***Klebsiella* spp.**
K. oxytoca
K. pneumoniae

***Enterobacter* spp.**
E. cloacae
E. aerogenes

***Proteus* spp.**
P. mirabilis
P. vulgaris

***Citrobacter* spp.**
C. freundii
C. koseri

CNS spp.
S. capitis
S. epidermidis
S. haemolyticus
S. hominis
S. lugdunensis
S. warneri

***Streptococcus* spp.**
S. agalactiae
S. gallolyticus
S. mitis
S. oralis
S. pneumoniae

16 organizma

- VME: Bazı BL'larda (pip-taz, 3/4. sefalosporin)
- *P.aeruginosa*: sonuçları iyi değil (program yenilendi!!!)

Manufacturer, assay	Methodology	Organisms panel	Direct genotypic or phenotypic resistance information	Turn around time hands-on time throughput limit of detection (LOD)[a]	FDA sensitivity and specificity information (in comparison with culture)[a]	Advantages	Drawbacks
Accelerate Diagnostics, Accelerate PhenoTest BC kit	FISH using PNA probes to organism specific ribosomal RNA followed by antimicrobial susce testin lapse bacte	*Bacteria* *S. aureus*, *S. lugdunensis*, CoNS, *E. faecalis*, *E. faecium*, *Streptococcus*	*Phenotypic* Provides MIC-based resistance information	90 min (identification) 6.5 h (AST) 2 min hands on time 1 sample processed at a time LOD: 1×10^4 CFU/mL	*Bacteria* Se (*S. aureus*): 97.9%; Se (*S. lugdunensis*): 97.5%; Se (CoNS): 95.3%; Se (*E. faecalis*): 97%; Se (*E. faecium*): 98.3%; Se	Only platform to provide MIC-based resistance information (rather than resistance genes nformation that may not correlate with phenotypic esistance)	Limited organism panel Does not provide genotypic resistance information Low throughput Time lag (5 h) between provision of organism identification and AST *S. pneumoniae* not differentiated from other *Streptococcus* spp No AST for *Candida* or *Streptococcus* spp Overcalls resistance to Ceftazidime and Cefepime Too little data to determine sensitivity for Daptomycin resistance False negatives can occur with mixed cultures

Accelerate PhenoTest

- **MIK belirleyen tek sistem** (fenotipik direnç)
- ***Candida, Streptococcus* spp: AST yok**
- Tanımlama: 90 dak
- AST: 6.5-7 saat
- Panel kısıtlı
- Pnömokok-diğer Strep ???

Pozitif KK şişesinden tanımlama

3

Mikroarray

- Verigene™ system (Luminex, USA)

Gram-Positive Blood Culture Test (BC-GP)

Species

Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus lugdunensis
Streptococcus agalactiae
Streptococcus pneumoniae
Streptococcus pyogenes
Enterococcus faecalis
Enterococcus faecium

Group

Streptococcus anginosus

Genus

Staphylococcus spp.
Streptococcus spp.
Micrococcus spp.+
Listeria spp.

Resistance

mecA (methicillin)
vanA (vancomycin)
vanB (vancomycin)

Gram-Negative Blood Culture Test (BC-GN)

Species

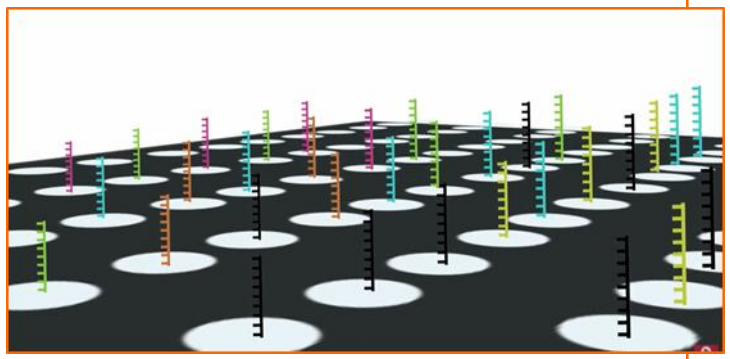
*Escherichia coli**
Klebsiella pneumoniae
Klebsiella oxytoca
Pseudomonas aeruginosa
Serratia marcescens++

Genus

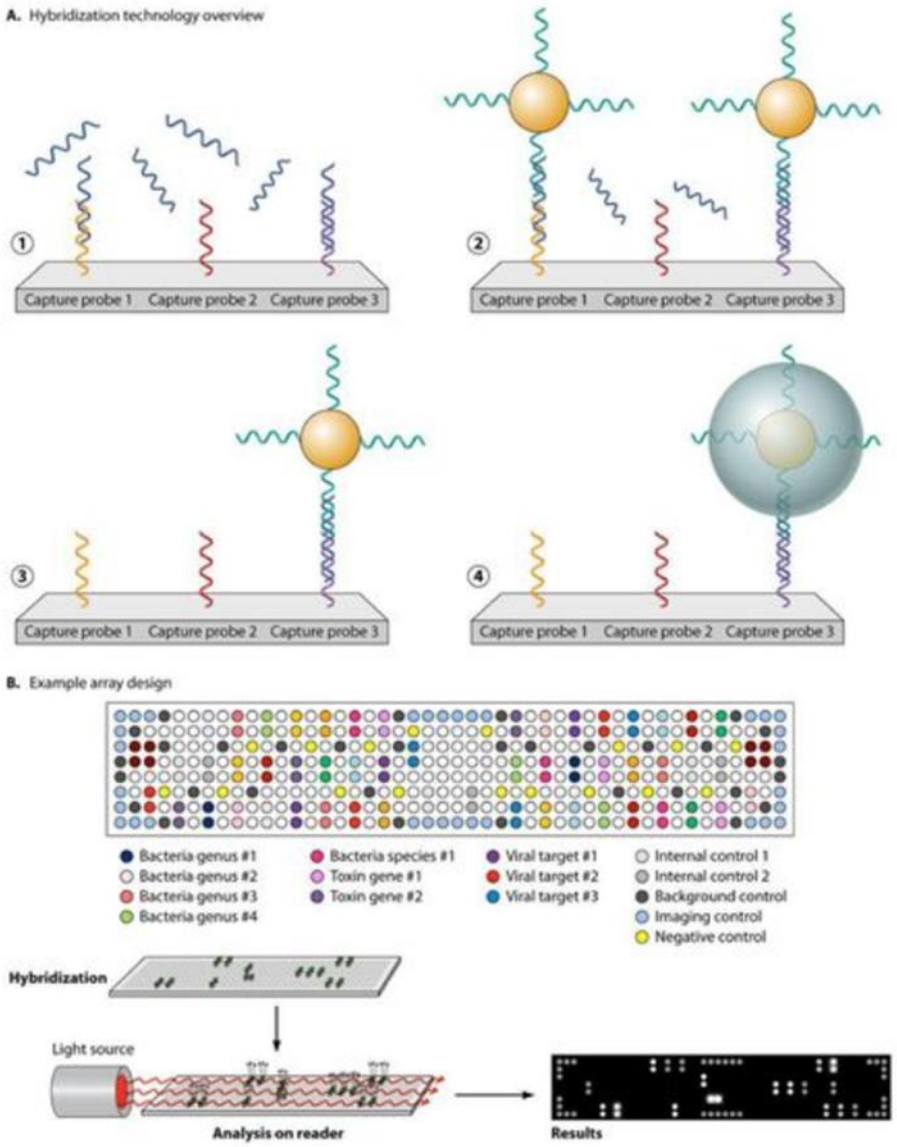
Acinetobacter spp.
Citrobacter spp.
Enterobacter spp.
Proteus spp.

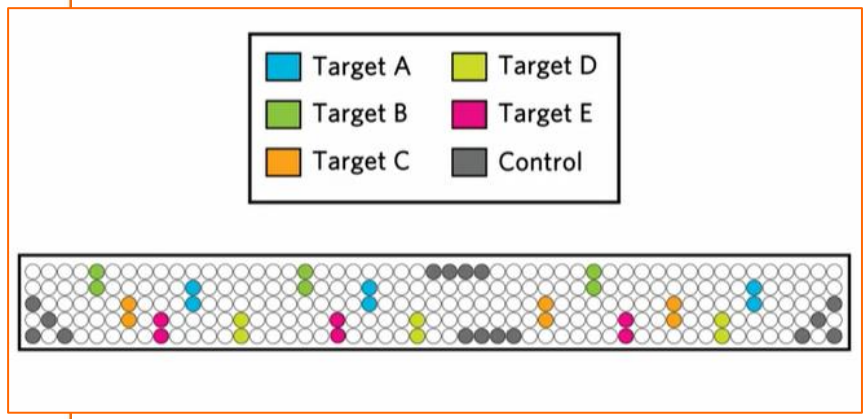
Resistance

CTX-M (ESBL)
IMP (carbapenemase)
KPC (carbapenemase)
NDM (carbapenemase)
OXA (carbapenemase)
VIM (carbapenemase)



Verigene solid-phase microarray.





frontiers in Cellular and Infection Microbiology

REVIEW
published: 16 March 2022
doi: 10.3389/fcimb.2022.859935

Diagnosis and Management of Bloodstream Infections With Rapid, Multiplexed Molecular Assays

Sherry A. Dunbar, Christopher Gardner and Shubhagata Das*

43 çalışma

2013-2020

The biggest discrepancy observed was a false-positive rate of 43.1% (25/58) for *Streptococcus pneumoniae*. Most of these were identified as *Streptococcus mitis/oralis* (21/25) by conventional testing and this finding was not unexpected since these species share 99% homology at the sequence level. The authors recommended that identification of *S. pneumoniae* by the BC-GP panel be confirmed by Gram stain plus bile solubility or other

As described in the study above, BC-GN identified at least one pathogen in the majority of polymicrobial cultures but could only identify all pathogens present in about half of these cases.

(Lamy et al., 2020). In addition, detection of genetic resistance markers does not preclude phenotypic resistance due to other mechanisms. Identification of the pathogens present in a polymicrobial culture is also difficult and could impact the clinical use of tests such as BC-GN, as a lower sensitivity when multiple pathogens are present could limit the ability to modify antibiotic treatment. And, as reported for BC-GP, differentiation of *S. pneumoniae* from *S. mitis/oralis* is particularly challenging for a molecular test, since the organisms share a 99% sequence homology. Therefore, it would be prudent to confirm *S. pneumoniae* results with additional tests and to refrain from reporting it to the species level.

frontiers in Cellular and Infection Microbiology

REVIEW
published: 16 March 2022
doi: 10.3389/fcimb.2022.859935

Diagnosis and Management of Bloodstream Infections With Rapid, Multiplexed Molecular Assays

Panel kısıtlı

13 Gr(+)
9 Gr(-)
9 Direnç geni

TABLE 1 | Targets included in the VERIGENE Gram-Positive Blood Culture test.

Species	Genus	Group	Resistance
Staphylococcus aureus	*Staphylococcus* spp.	*Streptococcus anginosus*	*mecA* (methicillin)
Staphylococcus epidermidis	*Streptococcus* spp.		*vanA* (vancomycin)
Staphylococcus lugdunensis	*Micrococcus* spp.[a]		*vanB* (vancomycin)
Streptococcus agalactiae	*Listeria* spp.		
Streptococcus pneumoniae			
Streptococcus pyogenes			
Enterococcus faecalis			
Enterococcus faecium			

[a]*Micrococcus spp. is not IVD-cleared in the United States.*

TABLE 2 | Targets included in the VERIGENE Gram-Negative Blood Culture test.

Species	Genus	Resistance
Escherichia coli[a]	*Acinetobacter* spp.	CTX-M (ESBL)
Klebsiella pneumoniae	*Citrobacter* spp.	IMP (carbapenemase)
Klebsiella oxytoca	*Enterobacter* spp.	KPC (carbapenemase)
Pseudomonas aeruginosa	*Proteus* spp.	NDM (carbapenemase)
Serratia marcescens[b]		OXA (carbapenemase)
		VIM (carbapenemase)

[a]*Test does not distinguish Escherichia coli from Shigella spp. (S. dysenteriae, S. flexneri, S. boydii, and S. sonnei).*
[b]*Serratia marcescens is not IVD-cleared in the United States.*

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challenging
% sequence
homology. Therefore, it would be prudent to confirm *S. pneumoniae* results with additional tests and to refrain from reporting it to the species level.

Manufacturer, assay	Methodology	Organisms panel	Direct genotypic or phenotypic resistance information	Turn around time hands-on time throughput limit of detection (LOD)[a]	FDA sensitivity and specificity information (in comparison with culture)[a]	Advantages	Drawbacks
Luminex, Verigene GP Blood Culture Assay	Rapid microarray-based detection of specific nucleic acid targets	*Bacteria* *S. aureus*, *S. lugdunensis*, CoNS, *Streptococcus* spp., *S. pneumoniae*, *S. pyogenes*, *S. agalactiae*, *S. anginosus* group *E. faecalis*, *E. faecium*, *Listeria* spp.	*Genotypic* Methicillin (vancomy (*vanA* an *vanB*)	2.5 h	*Bacteria*	Provides genotypic	Limited organism panel (includes no rgets) tect rare *mecC* variants around time than g assays ay be misidentified as *m* ives can occur with mixed
Luminex, Verigene GN Blood Culture Assay	Rapid microarray-based detection of specific nucleic acid targets	*Bacteria* *E. coli*, *K. oxytoca*, *K. pneumoniae*, *Proteus* spp., *Enterobacter* spp., *Citrobacter* spp., *Acinetobacter* spp., *P. aeruginosa*	*Genotypic:* ES (CTX-M), Carbapene (KPC, IMP, NDM, OXA VIM)		Se (*Acinetobacter* spp.): 98.2%; Se (*P. aeruginosa*): 97.6%; Se (CTX-M): 98.7%; e (OXA): 95.3%; Se (KPC): 100%; Se (NDM): 100%; Se (IMP): 100%; Se (VIM): 100%; Sp: 99.4%–100%	per reader (though at increased cost)	sm panel (includes no ets) round time than assays istinguished from p. s can occur with mixed cultures

VERIGENE

- TAT: 2-2.5 saat
- QuickFISH, Accelerate Phone kıyasla biraz daha geniş panel
- **Mantar paneli yok**
- *S.pneumoniae-S.mitis/oralis*
- Genotipik direnç (+)
- Gr(-): KPC, IMP,NDM, OXA, VIM, ESBL (CTX-M)
- Gr(+): **mecC yok**

Pozitif KK şişesinden tanımlama

GRAM-NEGATIVE BACTERIA:

- *Acinetobacter calcoaceticus-baumannii complex*
- *Bacteroides fragilis**
- *Enterobacterales*
  - *Enterobacter cloacae complex*
  - *Escherichia coli*
  - *Klebsiella aerogenes**
  - *Klebsiella oxytoca*
  - *Klebsiella pneumoniae group*
  - *Proteus*
  - *Salmonella**
  - *Serratia marcescens*
- *Haemophilus influenzae*
- *Neisseria meningitidis*
- *Pseudomonas aeruginosa*
- *Stenotrophomonas maltophilia**

GRAM-POSITIVE BACTERIA:

- *Enterococcus faecalis**
- *Enterococcus faecium**
- *Listeria monocytogenes*
- *Staphylococcus*
  - *Staphylococcus aureus*
  - *Staphylococcus epidermidis**
  - *Staphylococcus lugdunensis**
- *Streptococcus*
  - *Streptococcus agalactiae*
  - *Streptococcus pneumoniae*
  - *Streptococcus pyogenes*

YEAST:

- *Candida albicans*
- *Candida auris**
- *Candida glabrata*
- *Candida krusei*
- *Candida parapsilosis*
- *Candida tropicalis*
- *Cryptococcus neoformans/gattii**

ANTIMICROBIAL RESISTANCE GENES:

- Carbapenemases
  - IMP*
  - KPC
  - OXA-48-like*
  - NDM*
  - VIM*
- Colistin Resistance
  - *mcr-1**
- ESBL
  - CTX-M*
- Methicillin Resistance
  - *mecA/C*
  - *mecA/C and MREJ (MRSA)**
- Vancomycin Resistance
  - *vanA/B*

*Indicates a new target on the BioFire BCID2 Panel

4 NA amplifikasyon

FilmArray®

(BioFire Diagnostics, Salt Lake City, USA)

- «Nested multiplex PCR»
- DNA is analyzed through melting curves

43 hedef:
15 Gr (-),
11 Gr (+),
7 maya
10 Direnç geni

1 saat

TEK PANEL

Usefulness of BioFire FilmArray BCID2 for Blood Culture Processing in Clinical Practice

Benjamin Berinson,[a] Anna Both,[a] Laura Berneking,[a] Martin Christner,[a] Marc Lütgehetmann,[a] Martin Aepfelbacher,[a]

Study no.	SOC identification	BCID2 identification
Monomicrobial Gram positive		
6	*E. faecalis*	*E. faecalis*, *Staphylococcus* spp.
47	*S. haemolyticus*	*S. epidermidis*
54	*E. faecalis*	*E. faecalis*, *S. epidermidis*
62	*S. haemolyticus*	*S. epidermidis*
97	*S. haemolyticus*	*S. epidermidis*
118	*S. haemolyticus*	*S. epidermidis*
Monomicrobial Gram negative		
17	*K. pneumoniae*	None
28	*E. coli*	*E. coli*, *S. epidermidis*
70	*E. coli*	*E. coli*, *S. epidermidis*
Polymicrobial culture		
5	*K. pneumoniae*, *S. capitis*	*K. pneumoniae* group
14	*P. aeruginosa*, *S. maltophilia*	*P. aeruginosa*
20	*E. faecium*, *S. haemolyticus*	*E. faecium*, *S. epidermidis*
51	*E. faecium*, *S. epidermidis*	*E. faecium*
58	*E. coli*, *A. veronii*	*E. coli*, *K. pneumoniae* group
73	*E. coli*, *S. epidermidis*	*E. coli*, *Staphylococcus* spp.
75	*S. haemolyticus*, *C. krusei*	*S. epidermidis*, *C. krusei*
82	*E. coli*, *S. anginosus* group	*E. coli*, *B. fragilis*, *Streptococcus* spp.
123	*C. perfringens*, *S. epidermidis*	None
127	*E. faecalis*, *E. faecium*, *Candida albicans*	*E. faecalis*, *E. faecium*
129	*K. oxytoca*, *E. faecium*	
178	*P. agglomerans*, *S. h*	

KNS ?

Polimikrobiyal

Ped kan kültürü ??

TABLE 2 Distribution of resistance markers detected by BCID2

Isolate	Resistance marker detected by BCID2 (*n*)			
	bla_{CTX-M}	bla_{OXA-48}-like	bla_{VIM}	None detected
Phenotypic third-generation cephalosporin resistance				
E. coli (*n* = 12)	11	0	0	1[a]
K. pneumoniae group (*n* = 3)	1	0	0	2[b]
K. oxytoca (*n* = 1)	0	0	0	1[c]
Carbapenem-resistant isolates				
P. aeruginosa (*n* = 1)	0	0	1	0
K. pneumoniae group (*n* = 1)	1	1	0	0

[a]Molecular analysis revealed the presence of bla_{TEM}.
[b]Molecular analysis revealed the presence of bla_{SHV} or a combination of bla_{SHV} and bla_{TEM}.
[c]Molecular analysis did not reveal the presence of a bla_{TEM}, bla_{SHV}, or bla_{CTX-M}.

Direnç: diğer mek..

Comparing BioFire FilmArray BCID2 and BCID Panels for Direct Detection of Bacterial Pathogens and Antimicrobial Resistance Genes from Positive Blood Cultures

Venere Cortazzo,[a] Tiziana D'Inzeo,[a,b] Liliana Giordano,[a] Giulia Menchinelli,[a,b] Flora Marzia Liotti,[a,b] Barbara Fiori,[b,c] Flavio De Maio,[a,b]

Berinson B; J Clin Microbiol 2021; 59:e00543- 21.

Cortazzo; J Clin Microbiol 2021; 59:e03163-20.

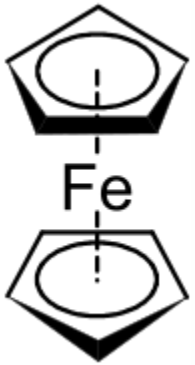
Manufacturer, assay	Methodology	Organisms panel	Direct genotypic or phenotypic resistance information	Turn around time hands-on time throughput limit of detection (LOD)[a]	FDA sensitivity and specificity information (in comparison with culture)[a]	Advantages	Drawbacks
BioFire, FilmArray Blood Culture Identification Panel	Multiplex real-time PCR followed by high resolution melting analysis to identify multiple bacterial and yeast nucleic acids and	*Bacteria* *S. aureus*, *Streptococcus* spp. *(S. agalactiae, S. pneumoniae, and S. pyogenes)*, *Enterococcus*, *L. monocytogenes*,	*Genotypic* Methicillin (*mecA*), vancomycin (*vanA/vanB*), and carbapenems (KPC)	1 h 2 min hands-on time May process up to 12 samples at a time depending on number of bays per tower LOD: $6.12 \times 10^7 - 9.5 \times 10^8$ CFU/mL	*Bacteria* Se (*S. aureus):* 98.4%; Se (Staphylococci): 96.5%; *Se* (Streptococci): 97.5%; Se (*Enterococcus*): 97.7%; Se (*L. monocytogenes*): 100%; Se (*A. baumannii*): 100%; Se (*E. cloacae complex*): 97.4%; Se (*E. coli*): 98%; Se (*K. oxytoca):* 92.2% Se (*K. pneumoniae):* 97.1%; Se (*Proteus*): 100%; Se (*S. marcescens):* 98.7%; Se (*H. influenzae*): 100%; Se (*N. meningitidis*): 100%; Se (*P. aeruginosa)* 98.1% *Fungi* Se (*C. albicans*): 100%; Se (*C. glabrata*): 100%; Se (*C. krusei*): 100%; Se (*C. parapsilosis*):100%; Se (*C. tropicalis*): 100%; Se (*mecA*): 98.4%; Se (*vanA/vanB*): 100%; Se (KPC): 100%; Sp: 99.1%–100%	Expanded organism panel Provides genotypic resistance information (but limited) Throughput can be enhanced by using an instrument with multiple bays and towers (though at increased cost)	Carbapenem genetic resistance information limited to KPC Does not detect rare *mecC* variants Does not provide *Enterococcus* species identification *Enterococcus* may be misidentified as *Staphylococcus* *E. aerogenes*, *S. marcescens* and *Raoultella ornithinolytica* may be misidentified as *K. pneumoniae* False negatives can occur with mixed cultures

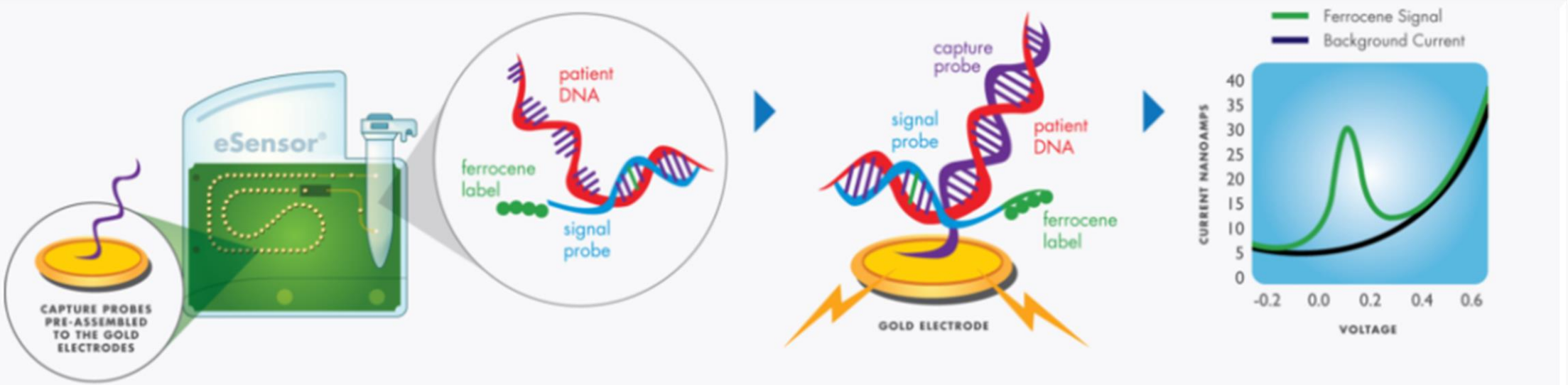
FilmArray

- TAT: 1 saat
- Geniş Panel: **33 organizma**; 10 R geni
  - Diğerlerinde yer almayan
- *Candida auris* dahil
- Genotipik direnç (+)
  - Gr(-): KPC, IMP,NDM, OXA, VIM, **mcr1**
  - Gr(+): **vanA/B**, **mecA/C**

ePlex® BCID Panel:

- NA extraction, PCR amplification
- Detection via GenMark's proprietary eSensor® technology.
- Sample loaded by the operator into the cartridge, which is then inserted into the instrument for processing.
- *GenMark's proprietary eSensor technology* is based on competitive DNA hybridization and electrochemical detection (highly specific)





1 The target DNA is mixed with the signal probe solution. If the applicable target DNA is present, hybridization to the signal probes occurs immediately.

2 The solution is pumped through the cartridge's microfluidic chamber and the target DNA/signal probe complex completes the reaction with the pre-assembled capture probe.

3 Voltage is swept across each electrode and target DNA is analyzed by electrochemical detection.

ePlex® BCID-GP Panel

Gram-Positive Organisms
- *Bacillus cereus group*
- *Bacillus subtilis group*
- *Corynebacterium*
- *Cutibacterium acnes (Propionibacterium acnes)*
- *Enterococcus*
- *Enterococcus faecalis*
- *Enterococcus faecium*
- *Lactobacillus*
- *Listeria*
- *Listeria monocytogenes*
- *Micrococcus*
- *Staphylococcus*
- *Staphylococcus aureus*
- *Staphylococcus epidermidis*
- *Staphylococcus lugdunensis*
- *Streptococcus*
- *Streptococcus agalactiae (GBS)*
- *Streptococcus anginosus group*
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes (GAS)*

Resistance Genes
- *mecA*
- *mecC*
- *vanA*
- *vanB*

Pan Targets
- Pan Gram-Negative
- Pan *Candida*

ePlex® BCID-GN Panel

Gram-Negative Organisms
- *Acinetobacter baumannii*
- *Bacteroides fragilis*
- *Citrobacter*
- *Cronobacter sakazakii*
- *Enterobacter (non-cloacae complex)*
- *Enterobacter cloacae complex*
- *Escherichia coli*
- *Fusobacterium nucleatum*
- *Fusobacterium necrophorum*
- *Haemophilus influenzae*
- *Klebsiella oxytoca*
- *Klebsiella pneumoniae group*
- *Morganella morganii*
- *Neisseria meningitidis*
- *Proteus*
- *Proteus mirabilis*
- *Pseudomonas aeruginosa*
- *Salmonella*
- *Serratia*
- *Serratia marcescens*
- *Stenotrophomonas maltophilia*

Resistance Genes
- *CTX-M*
- *IMP*
- *KPC*
- *NDM*
- *OXA (OXA-23 and OXA-48)*
- *VIM*

Pan Targets
- Pan Gram-Positive
- Pan *Candida*

ePlex® BCID-FP

Fungal Organisms
- *Candida albicans*
- *Candida auris*
- *Candida dubliniensis*
- *Candida famata*
- *Candida glabrata*
- *Candida guilliermondii*
- *Candida kefyr*
- *Candida krusei*
- *Candida lusitaniae*
- *Candida parapsilosis*
- *Candida tropicalis*
- *Cryptococcus gattii*
- *Cryptococcus neoformans*
- *Fusarium*
- *Rhodotorula*

identification of bacteria and fungi
as well as AB resistance genes

⬇

within ~ 1.5 h
of BC bottle positivity,

As much as 15-30% of (+) BC may be due to contaminants which can result in continuation of unnecessary ABs

rapidly differentiation:contaminant/true inf, enabling rapid de-escalation and discharge of patients with a BSI 2-3 days earlier than conv. methods.
Common contaminants included on the ePlex® BCID-GP panel but not on most competitor's panels include:

- *Bacillus subtilis*
- *Corynebacterium*
- *Cutibacterium acnes*
- *Micrococcus*
- *Lactobacillus*

A Multicenter Clinical Study To Demonstrate the Diagnostic Accuracy of the GenMark Dx ePlex Blood Culture Identification Gram-Negative Panel

Donna M. Wolk,[a] Stephen Young,[b] Natalie N. Whitfield,[c] Jennifer L. Reid,[c] Adam Thornberg,[c] Karen C. Carroll,[d]

TABLE 1 ePlex blood culture identification Gram-negative panel

Target type	Target
Bacterial targets	*Acinetobacter baumannii*
	Bacteroides fragilis
	Citrobacter spp.
	Cronobacter sakazakii
	Enterobacter cloacae complex
	Enterobacter (non-*cloacae* complex)
	Escherichia coli
	Fusobacterium necrophorum
	Fusobacterium nucleatum
	Haemophilus influenzae
	Klebsiella oxytoca
	Klebsiella pneumoniae group[a]
	Morganella morganii
	Neisseria meningitidis
	Proteus spp.
	Proteus mirabilis
	Pseudomonas aeruginosa
	Salmonella spp.
	Serratia spp.
	Serratia marcescens
	Stenotrophomonas maltophilia
Antimicrobial resistance markers	CTX-M (bla_{CTX-M})
	IMP (bla_{IMP})
	KPC (bla_{KPC})
	NDM (bla_{NDM})
	OXA (bla_{OXA})[b]
	VIM (bla_{VIM})
Pan targets	Pan-Gram-positive[c]
	Pan-*Candida*[d]

Wolk DM; J Clin Microbiol 2021; 59:e02484-20.

Clinical Performance of the Novel GenMark Dx ePlex Blood Culture ID Gram-Positive Panel

Karen C. Carroll,[a] Jennifer L. Reid,[b] Adam Thornberg,[b] Natalie N. Whitfield,[b] Deirdre Trainor,[b] Shawna Lewis,[a]

TABLE 1 Targets detected by the ePlex BCID-GP Panel

Type of target	Organism(s) or gene
Bacterium	*Bacillus cereus* group[a]
	Bacillus subtilis group[b]
	Corynebacterium
	Enterococcus[c]
	Enterococcus faecalis
	Enterococcus faecium
	Lactobacillus[d]
	Listeria[c]
	Listeria monocytogenes
	Micrococcus
	Cutibacterium (*Propionibacterium*) *acnes*
	Staphylococcus[c]
	Staphylococcus aureus
	Staphylococcus epidermidis
	Staphylococcus lugdunensis
	Streptococcus[c]
	Streptococcus agalactiae
	Streptococcus anginosus group[e]
	Streptococcus pneumoniae
Antimicrobial resistance gene[f]	*mecA* (methicillin resistance)
	mecC (methicillin resistance)
	vanA (vancomycin resistance)
	vanB (vancomycin resistance)
Pan target	Pan Gram-Negative[g]
	Pan *Candida*[h]

Carroll KC, J Clin Microbiol 2020;58:e01730-19.

Yavaş üreyenler
Gram değişken boyananlar
van A/van B ayrımı

frontiers in Cellular and Infection Microbiology

ORIGINAL RESEARCH
published: 26 November 2020
doi: 10.3389/fcimb.2020.594951

Evaluation of Microbiological Performance and the Potential Clinical Impact of the ePlex® Blood Culture Identification Panels for the Rapid Diagnosis of Bacteremia and Fungemia

Sabrina Bryant[1], Iyad Almahmoud[2], Isabelle Pierre[3], Julie Bardet[3], Saber Touati[3],

TABLE 3 | Results and performance of fungal identification using ePlex BCID-FP panel compared to standard of care results.

BCID-FP Panel Targets (n = 15)	Identification and resistance results by SOC testing	Se (%)	Sp (%)
Candida albicans	*C. albicans*	5/5 (100)	10/10 (100)
Candida glabrata	*C. glabrata*	3/3 (100)	12/12 (100)
Candida parapsilosis	*C. parapsilosis*	2/2 (100)	13/13 (100)
Candida guilliermondii	*C. guilliermondii*	2/2 (100)	13/13 (100)
Candida kefy	*C. kefyr*	1/1 (100)	14/14 (100)
Candida krusei	*C. krusei*	1/1 (100)	14/14 (100)
Other targets (*Candida auris*, *Candida dubliniensis*, *Candida famata*, *Candida lusitaniae*, *Candida tropicalis*, *Cryptococcus gattii*, *Cryptococcus neoformans*, *Fusarium*, *Rhodotorula*)	None		15/15 (100)
No Target Detected	*C. orthopsilosis* (1); *C. inconspicua* (1)		

Accuracy of Broad-Panel PCR-Based Bacterial Identification for Blood Cultures in a Pediatric Oncology Population

C. D. Garner,[a]* J. Brazelton de Cardenas,[a] S. Suganda,[a] R. T. Hayden[a]

BCID Gram-negative (GN) and Gram-positive (GP) panels were evaluated in a predominantly pediatric oncology population. A total of 112 blood cultures were tested by the ePlex BCID GN and GP panels and results were compared to those from standard-of-care testing. Accuracy for on-panel organisms was 89% (CI, 76% to 95%) for the Gram-positive panel, with four misidentifications and one not detected, and 93% (CI, 82% to 98%) for the Gram-negative panel, with two misidentifications and one not detected.

TABLE 2 Organisms detected correctly in monomicrobial samples

Gram-negative	No.	Gram-positive	No.
Citrobacter	1	*Enterococcus faecalis*	1
Escherichia coli	27	Micrococcus	2
Enterobacter cloacae complex	3	*Staphylococcus aureus*	3
Klebsiella pneumoniae	4	*Staphylococcus epidermidis*	22
Pseudomonas aeruginosa	3	*Streptococcus pneumoniae*	1
Serratia marcescens	1	*Staphylococcus*	4
Stenotrophomonas maltophilia	2	*Streptococcus*	6

TABLE 3 Discrepant samples for monomicrobial samples

Sample ID	SOC result[a]	ePlex BCID panel result[a]	WGS BLAST/WGS KMER result[a,b]
BCID-GP panel			
BCID 8	VGS	*Streptococcus pneumoniae*	*Streptococcus mitis*
BCID 30	VGS	ND	*Streptococcus salivarius*
BCID 35	VGS	*Streptococcus pneumoniae*	*Streptococcus mitis*
BCID 57	CoNS	*Staphylococcus epidermidis*	*Staphylococcus hominis*
BCID 69	CoNS	*Staphylococcus epidermidis*	*Staphylococcus pasteuri*
BCID 50	*Rothia dentocariosa*	ND	*Rothia mucilaginosa*[b]
BCID-GN panel			
BCID 12	*Escherichia coli*	ND	*Escherichia coli*
BCID 31	*Klebsiella oxytoca*	*Klebsiella oxytoca*; *Enterobacter cloacae* complex	NP
BCID 43	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*; *Escherichia coli*	NP
BCID 24	*Pantoea agglomerans*	ND	*Pantoea vagans*[b]
BCID 28	*Rhizobium radiobacter*	ND	*Rhizobium* sp.[b]
BCID 70	*Rahnella aquatilis*	ND	*Rahnella aquatilis*[b]
BCID 83	*Pantoea sp.*	ND	*Pantoea vagans* b
BCID 98	*Pseudomonas putida*	ND	*Pseudomonas monteilii*[b]
BCID 104	*Pseudomonas oryzihabitans*	ND	*Pseudomonas oryzihabitans*[b]

Garner CD, Microbiol Spectr 2021; 9:e00221-21..

Manufacturer, assay	Methodology	Organisms panel	Direct genotypic or phenotypic resistance information	Turn around time hands-on time throughput limit of detection (LOD)[a]	FDA sensitivity and specificity information (in comparison with culture)[a]	Advantages	Drawbacks
GenMark Diagnostics, ePlex BCID-GP	Multiplexed PCR identification of multiple bacterial nucleic acids and select genetic determinants of antimicrobial resistance via	*Bacteria* *Bacillus cereus* group, *B. subtilis* group, *Corynebacterium*, *C. acnes*, *Enterococcus* spp., *E. feacalis*, *E. feacium*,	*Genotypic* Methicillin (*mecA*, *m* Vancomy (*vanA*, *va*	90 min	*Bacteria*	Very expanded	Relatively low sensitivity for *Corynebacterium* spp. ver sensitivity for *vanA* detection in *E. feacalis* (88%) se negatives can occur with mixed cultures
GenMark Diagnostics, ePlex BCID-GN	Multiplexed PCR identification of multiple bacterial nucleic acids and select genetic determinants of antimicrobial resistance via competitive nucleic acid hybridization using a sandwich assay format	*Bacteria* *A. baumannii*, *B. fragilis*, *Citrobacter* spp., *C. sakazakii*, *Enterobacter* (non-cloacae complex), *E. cloacae*, *E. coli*, *F. necrophorum*, *H. influenza*, *K. oxytoca*, *K. pneumonia*, *M. morganii*, *N. meningitidis*, *Proteus* spp., *P. mirabilis*, *P. aeruginosa*, *Salmonella* spp., *Serratia* spp., *S. marcescens*, *S. maltophilia*, Pan Gram-positive Pan *Candida*	*Genotypic* ESBL (CTX-M) Carbapener (KPC, IMP, NDM, OXA, VIM)				an occur with mixed vity for
GenMark	Multiplexed PCR	*Fungi*	None	90 min	*Fungi*	Very expanded	No resistance information

ePlex

- Tanımlama: 90 dak
- Çok geniş Panel
- Anaerop (*Bacteroides, Fusobacterium*)
- Kontaminantlar dahil:
  - *Corynebacterium*
  - *Bacillus subtilis*
  - *Cutibacterium acnes*
  - *Micrococcus*
  - *Lactobacillus*
- *C. auris* , ***Fusarium***, ***Rhodotorula***, *Cryptococcus*
- Genotipik direnç (+)
- Gr(-): KPC, IMP,NDM, OXA (23, 48), VIM,
- Gr(+): vanA, van B, mecA, mecC

FDA-cleared multiplex blood culture panels include

- BioFire FilmArray BCID Panel
- GenMark ePlex BCID-GP, FP, and GN panels,
- Luminex Verigene GP,GN Blood Culture tests
- Accelerate Pheno

Farklılıklar:

- D: %81-100; Ö: %100
- TAT: 30 dak-3 saat
- Kan miktarı değişken

Önemli noktalar:

- Kültür (-) sendromik test (+)
  - AB kullanımı
  - «Short-lived nucleic acid»
- Kültür (+), ST (-): Panelde yok
- Polimikrobiyal enf

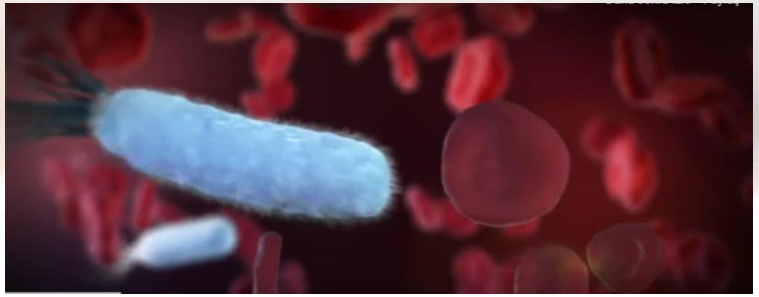
Direnç genleri

- Genotipik= Fenotipik değil
- Karbapenem ve Sefalosporin direnci:

  Farklı mekanizmalar mevcut…

B

Tam kandan tanımlama



Direkt kandan Moleküler yöntemlerin kısıtlılıkları neler?

- Örnekteki **bakteri yükü** çok önemli
- Kanda PCR inhibitörleri (+)
- Patojen DNA'sı düşük miktarda ama tam kanda insan DNA'sı fazla. «High Background»

  ⬇

  Duyarlılık; özgüllük düşük

- Çoğu kitin **FDA onayı yok**

Tam kandan tanımlama

1. NA amplifikasyon
2. T2 magnetik rezonans
3. Metagenomik

1

NA amplifikasyon

Magicplex™ system (Seegene, G.Kore)

- Multipleks Real-time PCR
- 1 ml kan
- TAT: 3-6 h
- Duy: ~(%29-47); Özg:~ (%95)

Magicplex™ system

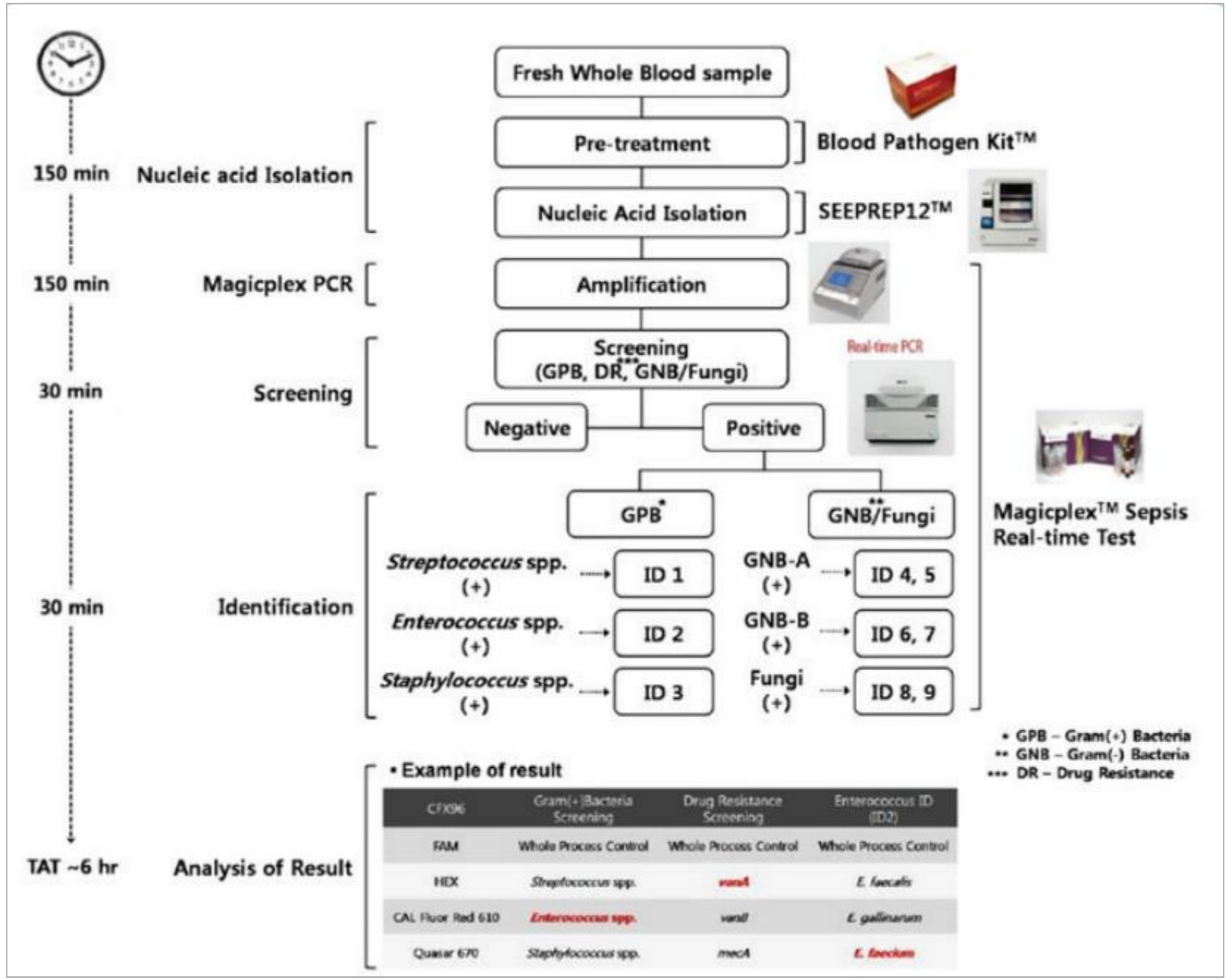
Gr(-) direnç ??

Amplification	Screeening	Identification
Amplicon Bank 1. Gram (+) bacteria / DR 73 Gram (+) bacteria 3 Drug resistance markers	Gram (+) bacteria Screening *Streptococcus* spp. *Enterococcus* spp. *Staphylococcus* spp. Drug Resistance (DR) Screening *vanA* *vanB* *mecA*	ID1. *Streptococcus* spp. *S. agalactiae* *S. pyogenes* *S. pneumoniae* ID2. *Enterococcus* spp. *E. faecalis* *E. gallinarum* *E. faecium* ID3. *Staphylococcus* spp. *S. epidermidis* *S. haemolyticus* *S. aureus*
Amplicon Bank 2. Gram (-) bacteria / Fungi 12 Gram(-)bacreria 6 fungi	Gram (-) bacteria / Fungi Screening Gram (-) bacteria-A Gram (-) bacteria-B Fungi	ID4. Gram (-) bacteria-A *P. aeruginosa* *A. baumannii* *S. maltophilia* ID5. Gram (-) bacteria-A *S. marcescens* *B. fragilis* *S. typhi* ID6. Gram (-) bacteria- B *K. pneumoniae* *K. oxytoca* *P. mirabilis* ID7. Gram (-) bacteria- B *E. coli* *E. cloacae* *E. aerogenes* ID8. Fungi *C. albicans* *C. tropicalis* *C. parapsilosis* ID9. Fungi *C. glabrata* *C. krusei* *A. fumigatus*

Screening:

- > 90 pathogens +
- only 3 res gene
  - vanA vanB
  - mecA

Further identification of 27 pathogens
detected within 30 min
with no additional amplification


Fresh Whole Blood sample
Pre-treatment
Blood Pathogen Kit™
Nucleic Acid Isolation
SEEPREP12™
Amplification
Real-time PCR
Screening (GPB, DR***, GNB/Fungi)
Negative
Positive
GPB*
GNB/Fungi**
Magicplex™ Sepsis Real-time Test
150 min
Nucleic acid Isolation
150 min
Magicplex PCR
30 min
Screening
30 min
Identification
Streptococcus spp. (+)
ID 1
Enterococcus spp. (+)
ID 2
Staphylococcus spp. (+)
ID 3
GNB-A (+)
ID 4, 5
GNB-B (+)
ID 6, 7
Fungi (+)
ID 8, 9
TAT ~6 hr
Analysis of Result
• Example of result
CFX96
Gram(+)Bacteria Screening
Drug Resistance Screening
Enterococcus ID (ID2)
FAM
Whole Process Control
Whole Process Control
Whole Process Control
HEX
Streptococcus spp.
vanA
E. faecalis
CAL Fluor Red 610
Enterococcus spp.
vanB
E. gallinarum
Quasar 670
Staphylococcus spp.
mecA
E. faecium
* GPB – Gram(+) Bacteria
** GNB – Gram(-) Bacteria
*** DR – Drug Resistance

frontiers in Cellular and Infection Microbiology

ORIGINAL RESEARCH
published: 12 March 2019
doi: 10.3389/fcimb.2019.00056

Evaluation of the Magicplex™ Sepsis Real-Time Test for the Rapid Diagnosis of Bloodstream Infections in Adults

Yuliya Zboromyrska[1†], Catia Cillóniz[2†], Nazaret Cobos-Trigueros[3], Manel Almela[1], Juan Carlos Hurtado[4], Andrea Vergara[4], Caterina Mata[5], Alex Soriano[3], Josep Mensa[3], Francesc Marco[4] and Jordi Vila[4]*

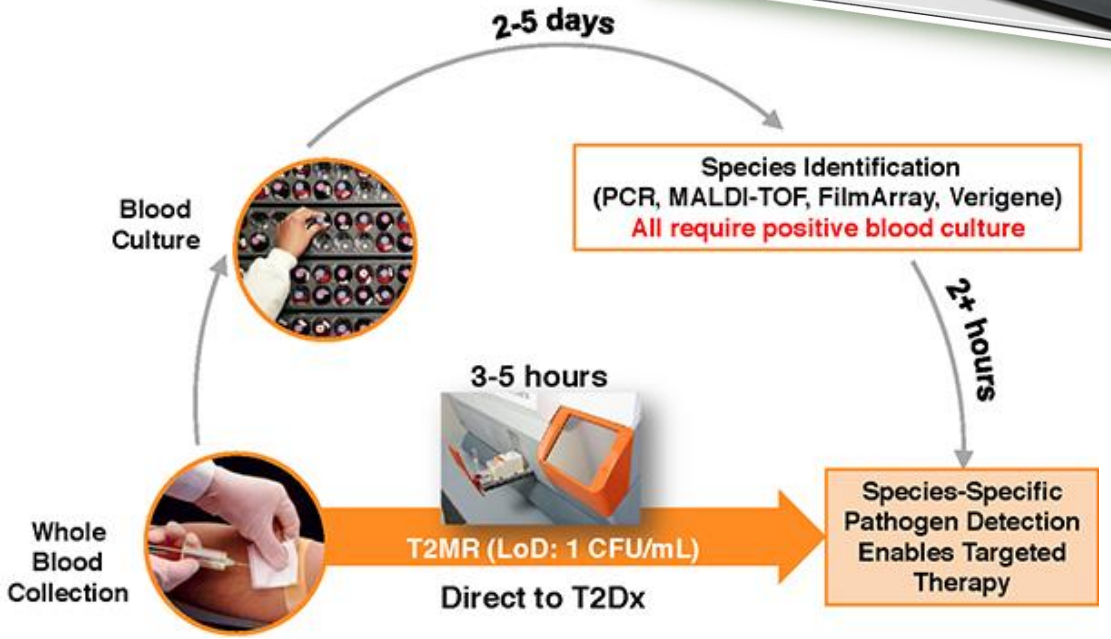
delays the start of appropriate antimicrobial therapy. This prospective study evaluated a multiplex real-time polymerase chain reaction, the Magicplex™ Sepsis test (MP), for the detection of pathogens from whole blood, comparing it to routine BC. We analyzed 809 blood samples from 636 adult patients, with 132/809 (16.3%) of the samples positive for one or more relevant microorganism according to BC and/or MP. The sensitivity and specificity of MP were 29 and 95%, respectively, while the level of agreement between BC and MP was 87%. The rate of contaminated samples was higher for BC (10%) than MP (4.8%) ($P < 0.001$). Patients with only MP-positive samples were more likely to be on antimicrobial treatment (47%) than those with only BC-positive samples (18%) ($P = 0.002$). In summary, the MP test could be useful in some clinical setting, such as among patients on antibiotic therapy. Nevertheless, a low sensitivity demonstrated impairs its use as a part of a routine diagnostic algorithm.

Tam kandan tanımlama

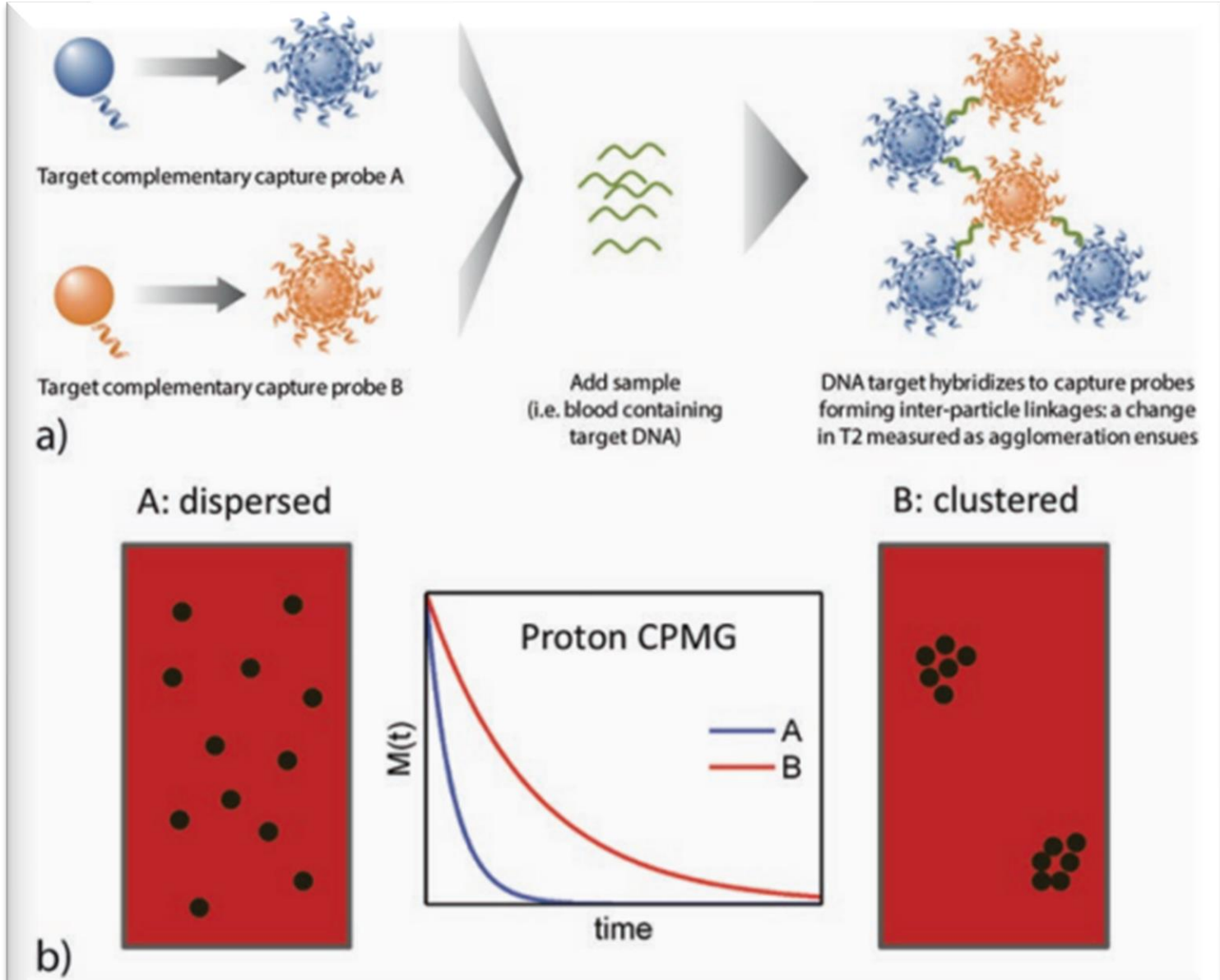
2 T2 magnetik rezonans

T2 magnetic resonance
(T2MR; T2Biosystems, Lexington,MA, USA)

- Direct from whole blood
  - BC (-)
  - NA purification (-)
  - NA extraction (-)
- Detection as low as 1 CFU/mL
- No interference from ABs
- 4-7 saat



DNA amplified by PCR binds by hybridization to probes enriched with superparamagnetic nanoparticles, which allow the detection and identification of the amplicons by changes in the magnetic signal



Tam kandan tanımlama

2 T2 magnetik rezonans

T2BACTERIA PANEL

Sensitivity: 90%[1,*]
Specificity: 98%[1,*]

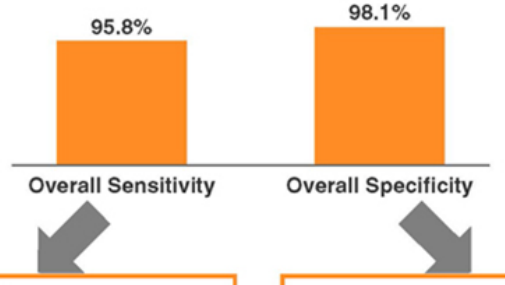
E. faecium
S. aureus
K. pneumoniae
A. baumannii
P. aeruginosa
E. coli

ESKAPE bacteria

T2Candida reported in 3 groups:

- *C. albicans* and *C. tropicalis*;
- *C. glabrata*, *C. krusei*, *S. cerevisiae*,

  C. bracarensis, reported as *C. glabrata*/*C. krusei*;
- *C. parapsilosis*,*C. orthopsilosis*, *C. metapsilosis*, reported as *C. parapsilosis*.



Sensitivity by Test

		95% Confidence Interval
A. baumannii	97.5% (39/40)	87.1% - 99.6%
E. coli	90.9% (30/33)	76.4% - 96.9%
E. faecium	100.0% (41/41)	91.4% - 100.0%
K. pneumoniae	100.0% (46/46)	92.3% - 100.0%
P. aeruginosa	97.7% (43/44)	88.2% - 99.6%
S. aureus	89.1% (49/55)	78.2% - 94.9%
Total	95.8% (248/259)	92.6% - 97.6%

Specificity by Test

		95% Confidence Interval
A. baumannii	99.2% (1713/1727)	98.6% - 99.5%
E. coli	95.4% (1637/1716)	94.3% - 96.3%
E. faecium	99.5% (1717/1726)	99.0% - 99.7%
K. pneumoniae	98.6% (1697/1721)	97.9% - 99.1%
P. aeruginosa	97.7% (1682/1722)	96.9% - 98.3%
S. aureus	98.4% (1683/1711)	97.6% - 98.9%
Total	98.1% (10,129/10,323)	97.8% - 98.4%

T2RESISTANCE PANEL

Gram-negative marker
KPC
OXA-48
NDM/VIM/IMP
CTX-M 14/15
AmpC(CMY/DHA)

Gram-positive marker
vanA/B
mecA/C

Received: 24 January 2021 | Revised: 10 May 2021 | Accepted: 17 May 2021

DOI: 10.1002/mbo3.1210

ORIGINAL ARTICLE

MicrobiologyOpen Open Access WILEY

Direct detection of ESKAPEc pathogens from whole blood using the T2Bacteria Panel allows early antimicrobial stewardship intervention in patients with sepsis

Pavel Drevinek[1] | Jakub Hurych[1] | Milena Antuskova[1] | Jan Tkadlec[1] |

treating physicians. A total of 55 samples from 53 patients were evaluated. The sensitivity and specificity of the T2Bacteria panel was 94% (16 out of 17 detections of T2Bacteria-targeted organisms) and 100%, respectively, with 36.4% (8 of 22) causes of BSI detected only by this method. The T2Bacteria Panel detected pathogens on

Clinical Infectious Diseases

MAJOR ARTICLE

Multicenter Prospective Study of Biomarkers for Diagnosis of Invasive Candidiasis in Children and Adolescents

Brian T. Fisher,[1,2] Craig L. K. Boge,[1] Rui Xiao,[2] Sydney Shuster,[1] Dawn Chin-Quee,[3] John Allen IV,[3] Shareef Shaheen,[3] Randall Hayden,[4] Sri Suganda,[4]

Background. Diagnosis of invasive candidiasis (IC) relies on insensitive cultures; the relative utility of fungal biomarkers in children is unclear.

Methods. This multinational observational cohort study enrolled patients aged >120 days and <18 years with concern for IC from 1 January 2015 to 26 September 2019 at 25 centers. Blood collected at onset of symptoms was tested using T2Candida, Fungitell (1→3)-β-D-glucan, Platelia *Candida* Antigen (Ag) Plus, and Platelia *Candida* Antibody (Ab) Plus assays. Operating characteristics were determined for each biomarker, and assays meeting a defined threshold considered in combination. Sterile site cultures were the reference standard.

Results. Five hundred participants were enrolled at 22 centers in 3 countries, and IC was diagnosed in 13 (2.6%). Thirteen additional blood specimens were collected and successfully spiked with *Candida* species, to achieve a 5.0% event rate. Valid T2Candida, Fungitell, Platelia *Candida* Ag Plus, and Platelia *Candida* Ab Plus assay results were available for 438, 467, 473, and 473 specimens, respectively. Operating characteristics for T2Candida were most optimal for detecting IC due to any *Candida* species, with results as follows: sensitivity, 80.0% (95% confidence interval, 59.3%–93.2%), specificity 97.1% (95.0%–98.5%), positive predictive value, 62.5% (43.7%–78.9%), and negative predictive value, 98.8% (97.2%–99.6%). Only T2Candida and Platelia *Candida* Ag Plus assays met the threshold for combination testing. Positive result for either yielded the following results: sensitivity, 86.4% (95% confidence interval, 65.1%– 97.1%); specificity, 94.7% (92.0%–96.7%); positive predictive value, 47.5% (31.5%–63.9%); and negative predictive value, 99.2% (97.7%–99.8%).

Conclusions. T2Candida alone or in combination with Platelia *Candida* Ag Plus may be beneficial for rapid detection of *Candida* species in children with concern for IC.

Clinical Trials Registration. NCT02220790.

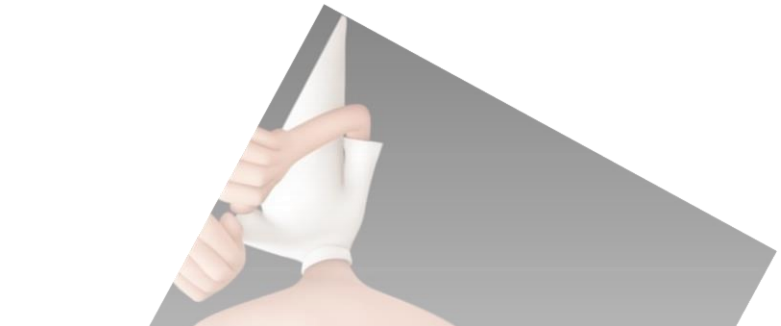
Table 1
Nucleic acid amplification-based technologies for the diagnosis of bloodstream infections from whole blood

Technology	Assay (manufacturer)	TAT (h)	Organisms detected	Resistance genes detected	Complexity—Personnel experience level	Sensitivity/ specificity	FDA clearance/ CE marked
Multiplex real-time PCR	Magicplex™ Sepsis Real-Time test (Seegene)	3–5	73 Gram positives, 12 Gram negatives, 6 fungi	*mecA*, *van A/B*	Multi-step automated Specially trained personnel	29%–65%/66%–95%	CE marked
Multiplex real-time PCR	Fungiplex® Candida (Bruker Daltonik)	3	*Candida* spp. (*C. albicans*, *C. parapsilosis*, *C. dubliniensis*, *C. tropicalis*), *Candida glabrata*, *Candida krusei*	—	Partially automated Specially trained personnel	100%/94.1%	CE marked
PCR + miniaturized magnetic resonance	T2Candida® panel (T2Biosystems)	3–5	5 *Candida* species *C. albicans/C. tropicalis*, *C. glabrata/C. krusei and C. parapsilosis*	—	Fully automated Trained personnel	91%/99%	FDA approved CE marked
	T2Bacteria® panel (T2Biosystems)	4–7	*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*[a], *Pseudomonas aeruginosa*, *Escherichia coli*	—	Fully automated Trained personnel	90%/96%–98%	FDA approved CE marked
	T2Resistance® panel (T2Biosystems)	3–5	—	*CTX-M 14*, *CTX-M 15*, *CMY*, *DHA*, *KPC*, *OXA-48*, *NDM*, *VIM*, *IMP*, *vanA|B*, *mecA|C*	Fully automated Trained personnel	NA	CE marked FDA 'Breakthrou Device' designat
	T2Cauris™ (T2Biosystems)	5	*Candida auris*, *Candida duobushaemulonii*, *Candida haemulonii*	—	Fully automated Trained personnel	89%/98%	None yet

Abbreviations: CE, European Conformity; FDA, US Food and Drug Administration; TAT, turnaround time; NA, Not available.
[a] *Acinetobacter baumanni* is CE marked only and not FDA approved, so is not in the panel in the USA.

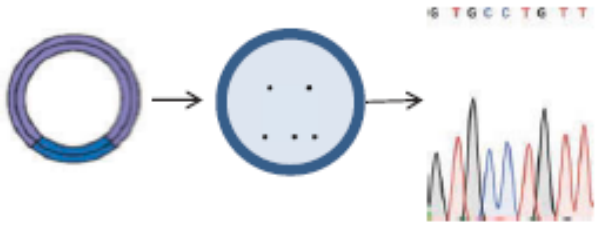
3

Metagenomik



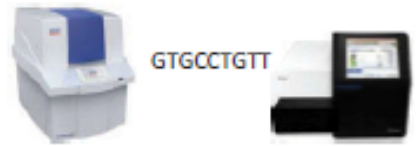
- 16 s metagenomik
- Shotgun metagenomik

First generation sequencing
Sanger sequencing



Population coverage: low
False positivity rate: low

Second generation sequencing
• Pyrosequencing
• Illumina sequencing (e.g. Miseq)



Population coverage: high
False positivity rate: high

Third generation sequencing
• PacBio SMRT Technology
• Oxford Nanopore Technologies
(e.g. Minion™)

Population coverage: high
False positivity rate: high

1977 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016

46 59 38 45 44 43 25 42 24 26 41 22 12 36 40 39 27 21 23

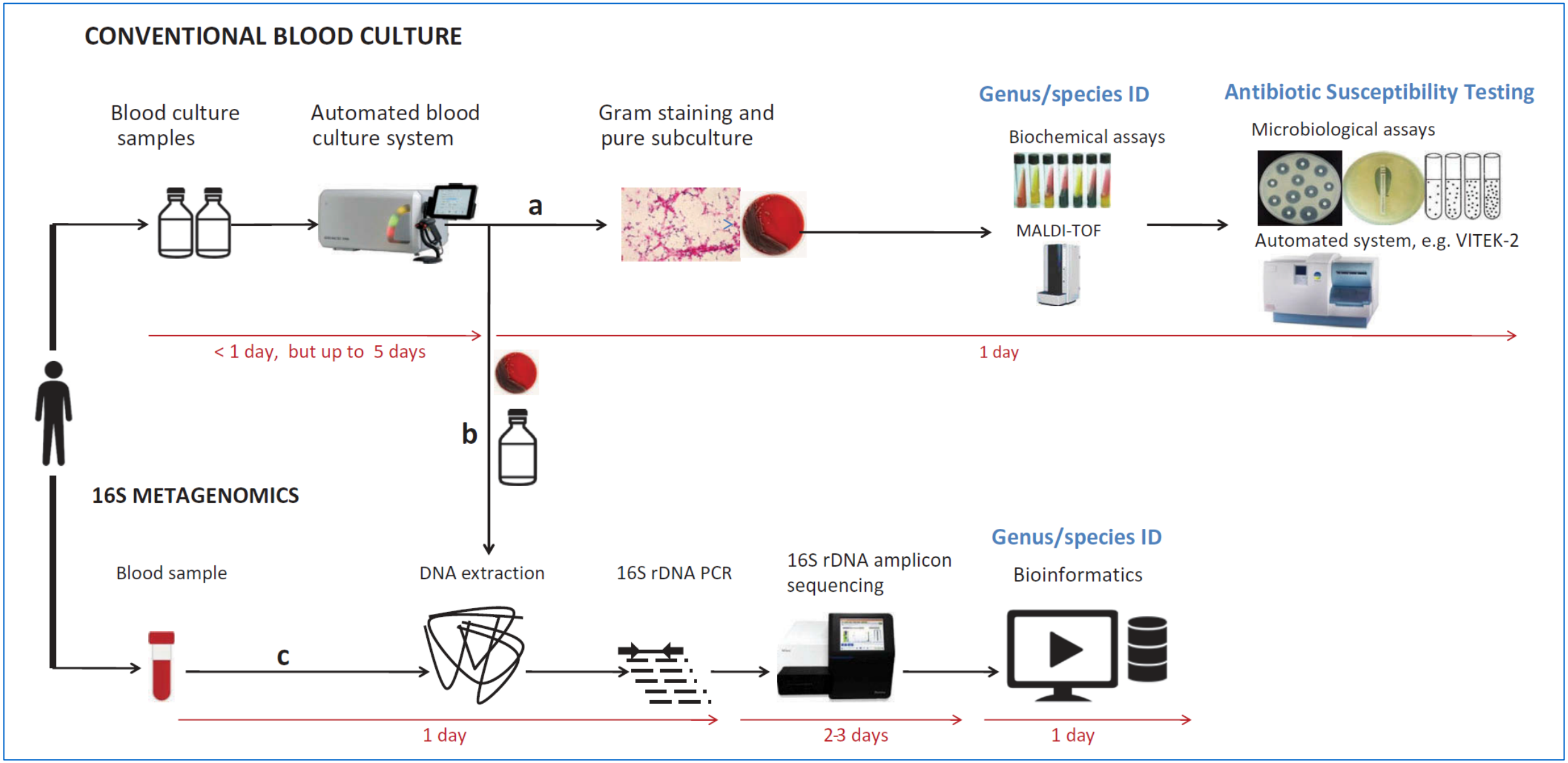
: Studies which used Sanger sequencing

: Studies which used pyrosequencing

: Studies which used Illumina sequencing (Miseq)

* : Studies conducted on whole blood

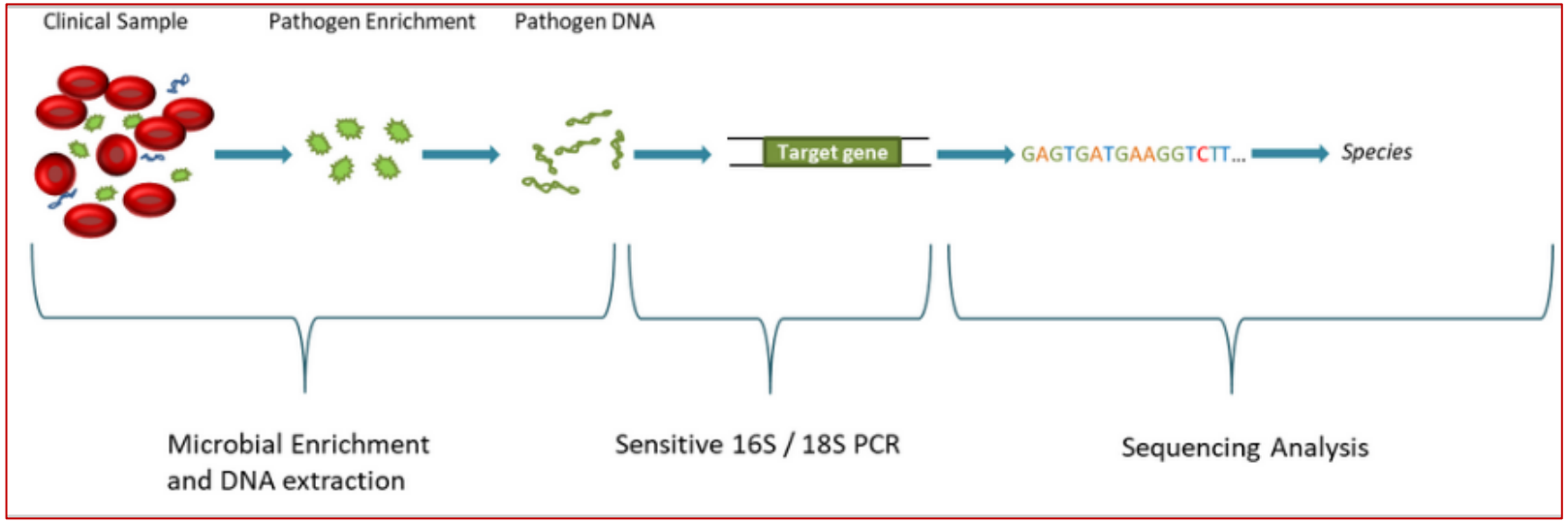
▪ 16 s metagenomik



Tam kandan tanımlama

SepsiTest™
(Molzym, Bremen, Germany)

345 bakteri
+
8 mantar



- Universal **PCR amplification (**bacterial 16S rRNA and fungal 18S rRNA)
- **Nucleic acid sequencing**

- (+) veya (-) sonuç: 4 saat
- TAT: 8-12 saat
- Sonuç (+) ise, var olan etkeni belirlemek için sekans analizi
- Direnç geni saptamaz

3

Metagenomik

Shotgun metagenomik:

Fark:

- Hedef yok. Örnekte bulunan tüm NA amplifiye edilir
- Etken tanımlama + tüm direnç determinantları

Kısıtlılıkları:

- TAT uzun
- Standartlar iyi tanımlanmamış
- Kolonizasyon mu/enfeksiyon etkeni mi?
- Maliyet yüksek

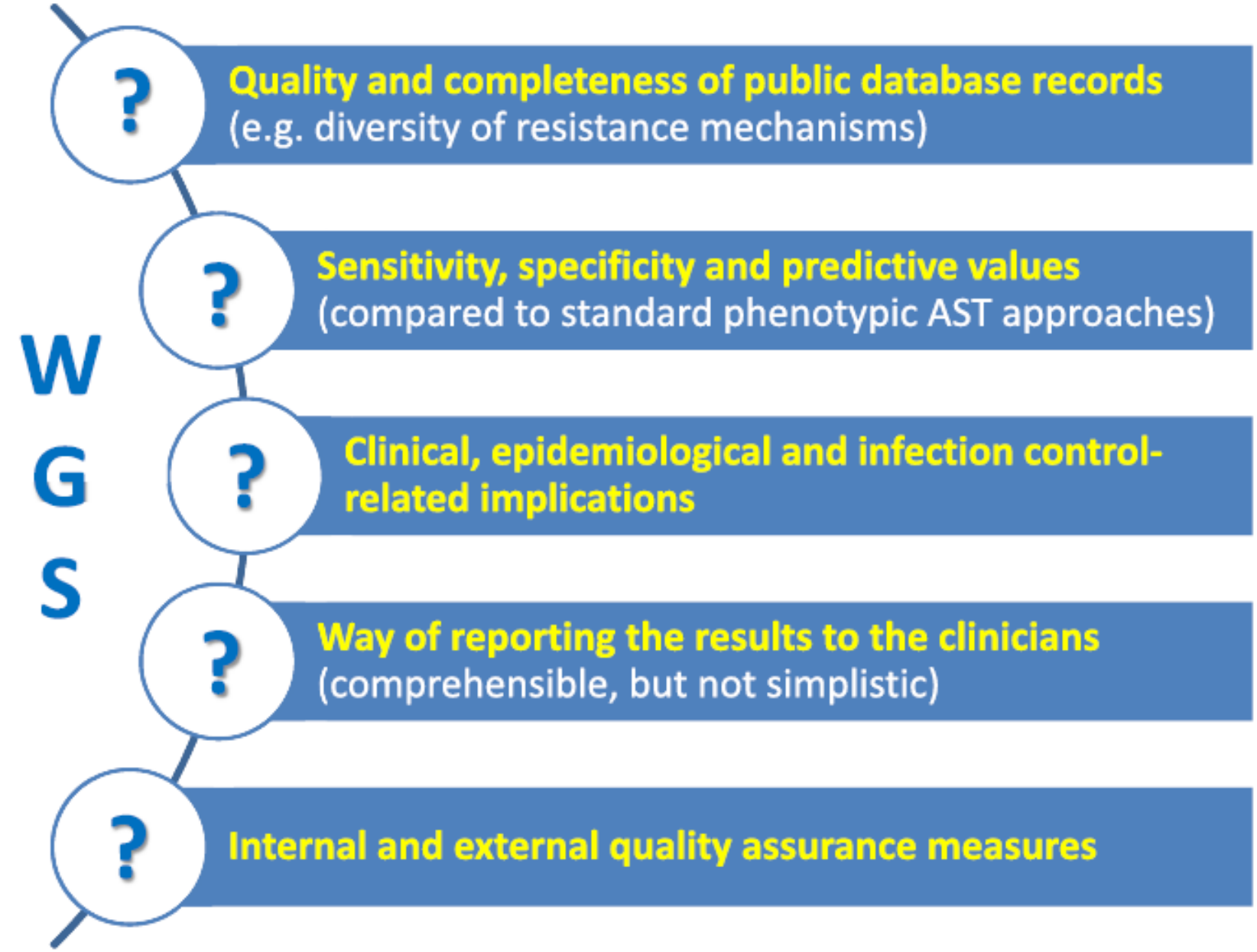


Fig. 2. Unsolved questions standing in the way of establishing whole genome sequencing (WGS) as approach for routine antimicrobial susceptibility testing (AST).

Tam kandan tanımlama

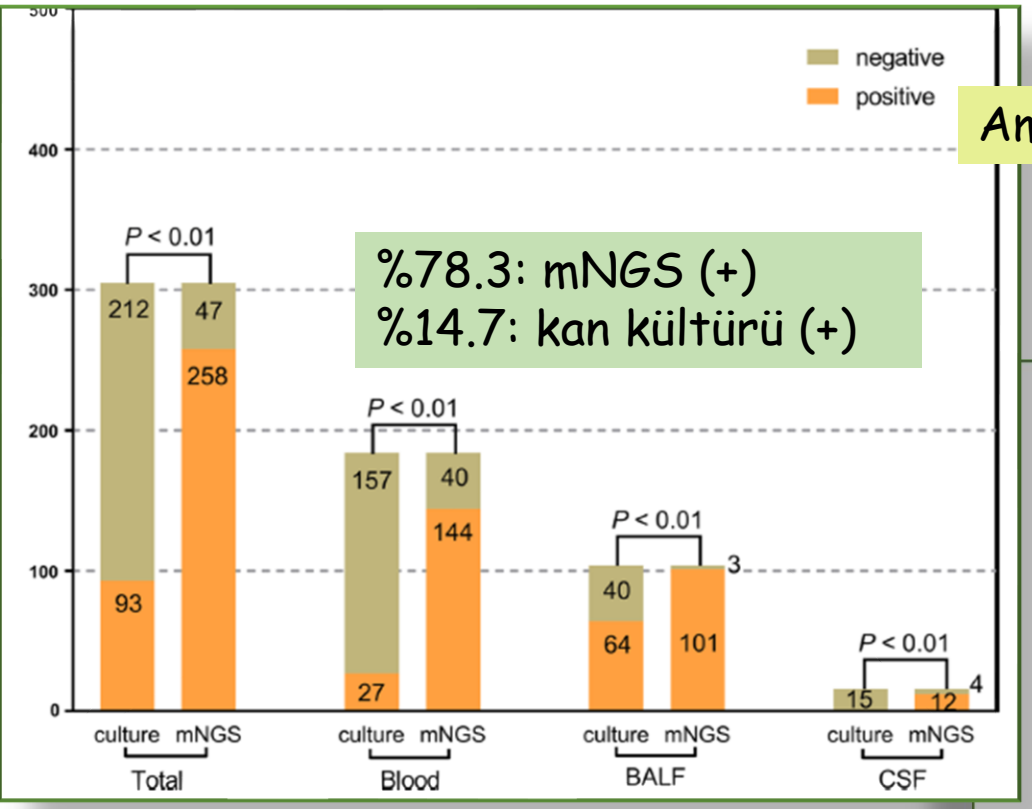
3

Metagenomik

Shotgun metagenomics:

- **iDTECT® Dx Blood test** (PathoQuest, Paris, France) is a CE-marked
- **Karius NGS Plasma Test™**(Karius, Redwood City, CA, USA)

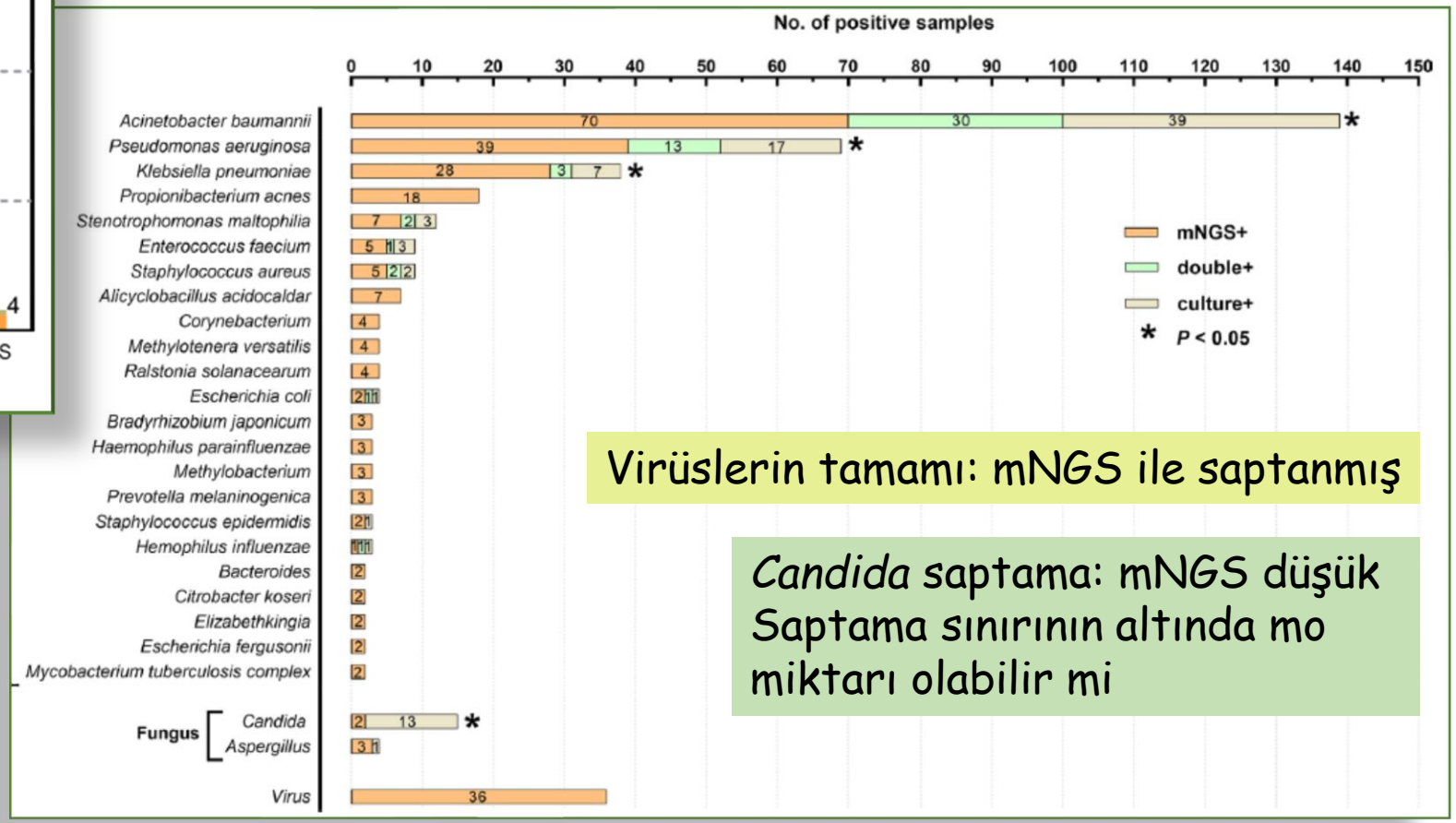
Technology	Assay (manufacturer)	TAT (h)	Organisms detected	Resistance genes detected	Sensitivity/ specificity (%)
Broad range PCR + sequencing	SepsiTest (Molzym)	8–18	Over 345 bacteria and 8 fungi	-	48/86
Untargeted NGS	iDTECT Dx Blood (PathoQuest)	60[a]	Over 1200 pathogens (bacteria and viruses)	-	(Negative predictive value: 98.4%)
Untargeted NGS	Karius NGS plasma Test (Karius)	53[a]	Over 1200 pathogens (bacteria, fungi, viruses and parasites)	-	93/63



%78.3: mNGS (+)
%14.7: kan kültürü (+)

Ampirik AB tedavisi .....

viremiler

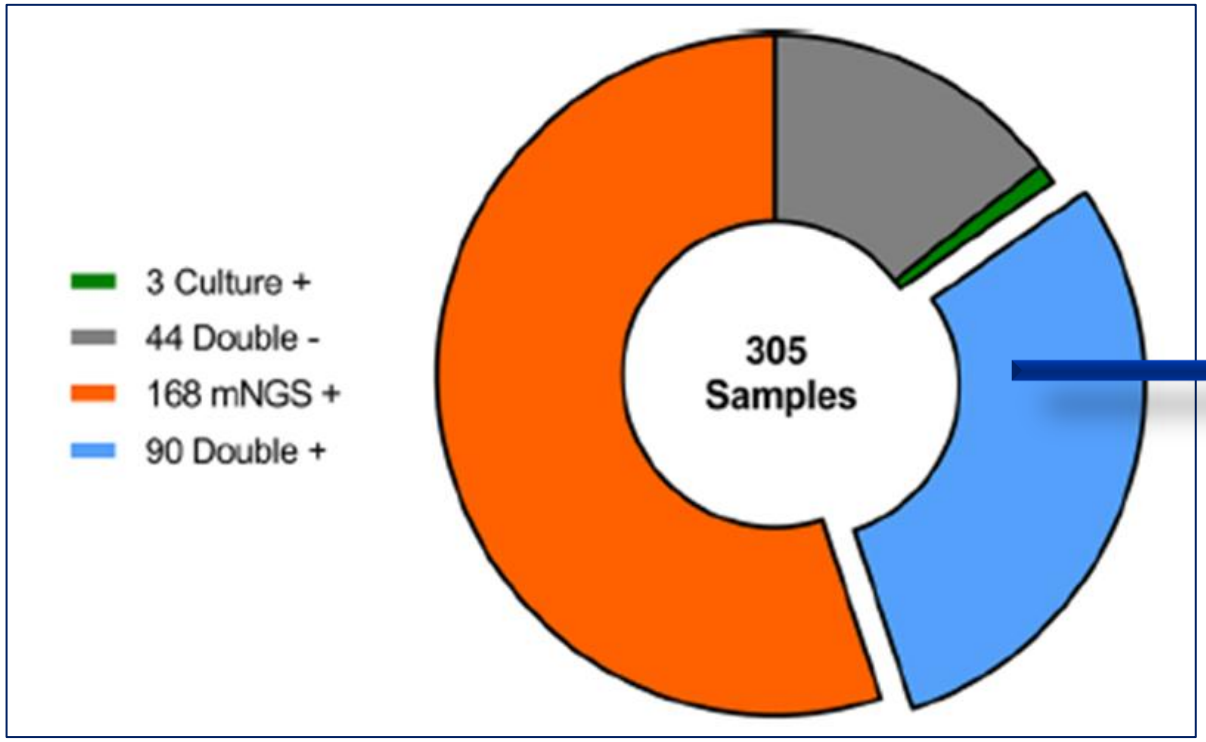


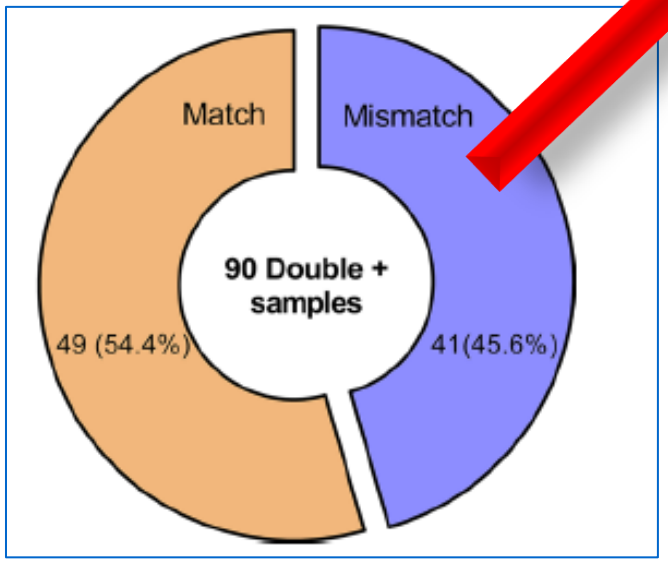
Virüslerin tamamı: mNGS ile saptanmış

Candida saptama: mNGS düşük
Saptama sınırının altında mo miktarı olabilir mi

Ren et al. BMC Infectious Diseases (2021) 21:1257

Uyumsuz sonuçlar için
3. yönteme ihtiyaç var





Ren et al. BMC Infectious Diseases (2021) 21:1257

Diagnosis and Surveillance of Neonatal Infections by Metagenomic Next-Generation Sequencing

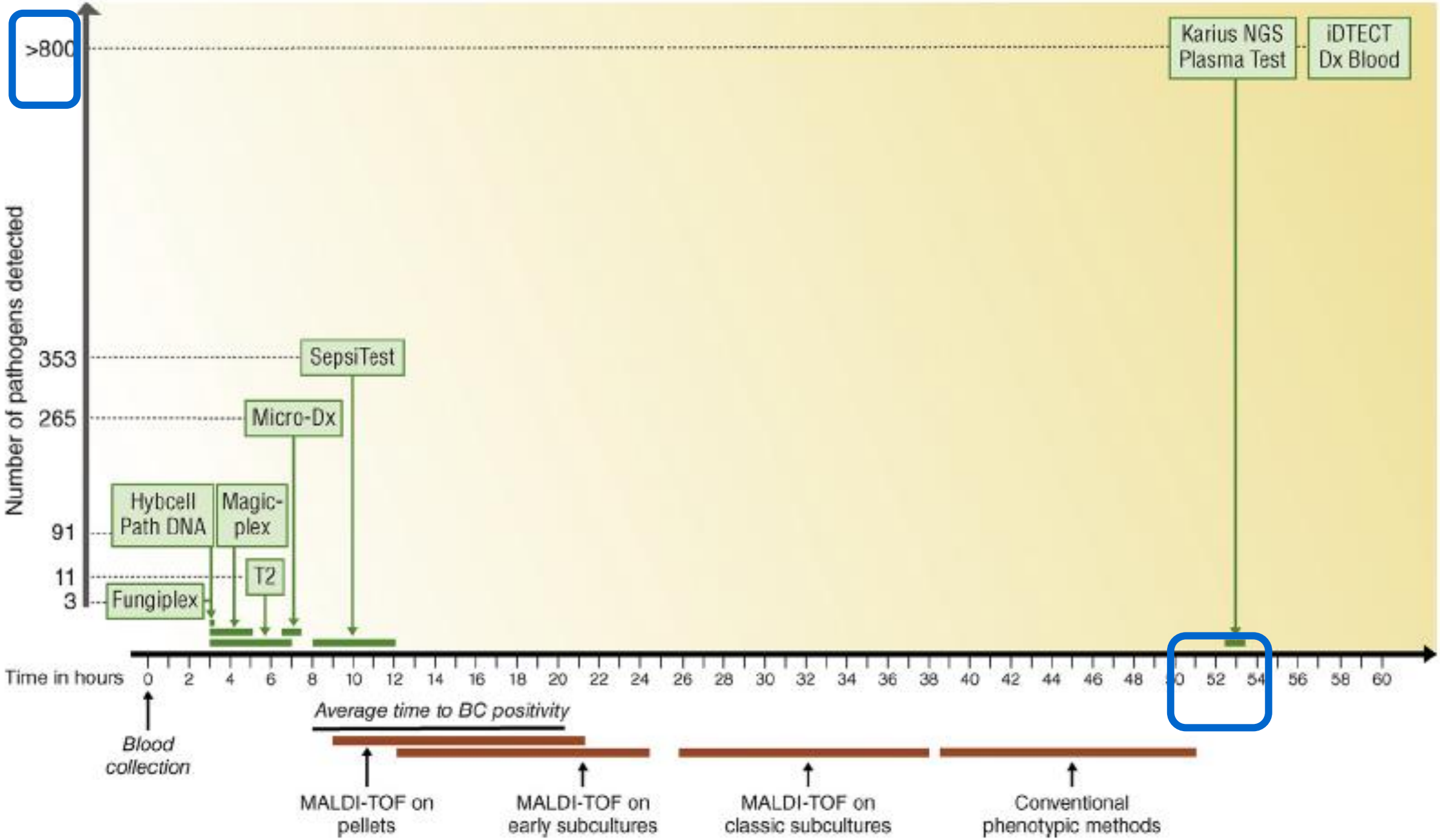
Rong Zhang[1], Yan Zhuang[1], Zheng-hui Xiao[2], Cai-yun Li[3], Fan Zhang[1], Wei-qing Huang[1], Min Zhang[1], Xiao-ming Peng[1] and Chao Liu[3]**

TABLE 1 | Clinical characteristics of the 10 patients.

Patient no./ Sex/Age, d	Infection data			Initial signs	RF	CF	NA	LOS in NICU, d	Final diagnosis
	CRP (mg/dl)	PCT (ng/ml)	IL-6 (pg/ml)						
1/M/19	205.08	16.44	1,387	fever, tachypnea	+	+	–	19	*Mycobacterium tuberculosis*
2/F/4	>320	1.07	181.5	fever, cough, dyspnea	+	+	–	33	*Legionella pneumophila*
3/F/2	206.68	>100	>5,000	cough, fever	+	+	–	26	*Moraxella catarrhalis* *Staphylococcus aureus*
4/F/38	3.74	0.16	13.46	spasmodic cough	+	–	–	11	*Chlamydia trachomatis*
5/M/52	41.84	0.17	76.92	fever, abdominal distention	+	–	–	83	*Ureaplasma parvum*
6/M/5	98.46	2.32	165.8	fever, cyanosis	+	+	+	47	*Streptococcus mitis*
7/M/23	33.06	0.31	101.0	cough, fever, tachypnea	+	–	+	43	*Streptococcus pasteuri* *Human betaherpesvirus 5*
8/M/7	122.59	>100	>5,000	fever, convulsion	+	+	+	40	*Escherichia coli*
9/M/3	192.9	12.18	324.1	fever, convulsion	–	–	+	26	*Streptococcus agalactiae*
10/F/15	168.1	18.12	23.06	fever, dyspnea, convulsion	+	+	+	13	*Bacillus cereus*

F, female; M, male; CRP, C-reactive protein; PCT, Procalcitonin; IL-6, cinterleukin 6; RF, Respiratory failure; CF, Circulatory failure; NA, Neurological abnormality; LOS, length of stay; and NICU, neonatal intensive care unit.

Zhang R, (2022) Front. Microbiol.


>800
Karius NGS Plasma Test
iDTECT Dx Blood
Number of pathogens detected
353
SepsiTest
265
Micro-Dx
Hybcell Path DNA
Magic-plex
91
11
T2
3
Fungiplex
Time in hours
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60
Blood collection
Average time to BC positivity
MALDI-TOF on pellets
MALDI-TOF on early subcultures
MALDI-TOF on classic subcultures
Conventional phenotypic methods

The Effect of Molecular Rapid Diagnostic Testing on Clinical Outcomes in Bloodstream Infections: A Systematic Review and Meta-analysis

Tristan T. Timbrook,[1,4] Jacob B. Morton,[1,4] Kevin W. McConeghy,[2] Aisling R. Caffrey,[1,2,4] Eleftherios Mylonakis,[3] and Kerry L. LaPlante[1,2,4]

[1]Rhode Island Infectious Diseases Research Program, Providence Veterans Affairs Medical Center, [2]Center of Innovation in Long Term Services and Supports, Providence Veterans Affairs Medical Center, [3]Infectious Diseases Division, Warren Alpert Medical School of Brown University, Providence, and [4]College of Pharmacy, University of Rhode Island, Kingston

Background. Previous reports on molecular rapid diagnostic testing (mRDT) do not consistently demonstrate improved clinical outcomes in bloodstream infections (BSIs). This meta-analysis seeks to evaluate the impact of mRDT in improving clinical outcomes in BSIs.

Methods. We searched PubMed, CINAHL, Web of Science, and EMBASE through May 2016 for BSI studies comparing clinical outcomes between mRDT and conventional microbiology methods.

Results. Thirty-one studies were included with 5920 patients. The mortality risk was significantly lower with mRDT than with conventional microbiology methods (odds ratio [OR], 0.66; 95% confidence interval [CI], .54–.80), yielding a number needed to treat of 20. The mortality risk was slightly lower with mRDT in studies with antimicrobial stewardship programs (ASPs) (OR, 0.64; 95% CI, .51–.79), and non-ASP studies failed to demonstrate a significant decrease in mortality risk (0.72; .46–1.12). Significant decreases in mortality risk were observed with both gram-positive (OR, 0.73; 95% CI, .55–.97) and gram-negative organisms (0.51; .33–.78) but not yeast (0.90; .49–1.67). Time to effective therapy decreased by a weighted mean difference of −5.03 hours (95% CI, −8.60 to −1.45 hours), and length of stay decreased by −2.48 days (−3.90 to −1.06 days).

Conclusions. For BSIs, mRDT was associated with significant decreases in mortality risk in the presence of a ASP, but not in its absence. mRDT also decreased the time to effective therapy and the length of stay. mRDT should be considered as part of the standard of care in patients with BSIs.

2

Tek başına erken tanımlama

etkisi düşük

Aynı zamanda hızlı duyarlılık şart

Hızlı Antimikrobiyal Duyarlılık Testleri

- Bir gece inkübasyon yerine aynı gün ABgram çok kıymetli
  - ✓ Polimikrobiyal/ yavaş üreyen bakteriler: kısıtlılık
  - ✓ Gr(-) sonuçlar iyi; Gr(+), maya: ⬇
- (+) KK şişesinden:
    - ✓ santrifüj ➡ pellet ➡ AB
    - ✓ kısa süreli subkültür ➡ AB ➡ daha iyi sonuçlar

J Antimicrob Chemother 2020; **75**: 3230–3238
doi:10.1093/jac/dkaa333 Advance Access publication 13 August 2020

Journal of Antimicrobial Chemotherapy

EUCAST rapid antimicrobial susceptibility testing (RAST) in blood cultures: validation in 55 European laboratories

Anna Åkerlund[1,2,3]*, Emma Jonasson[4,5], Erika Matuschek[5], Lena Serrander[2,3], Martin Sundqvist[6] and Gunnar Kahlmeter[4,5] on behalf of the RAST Study Group†

J Antimicrob Chemother 2020; **75**: 968–978
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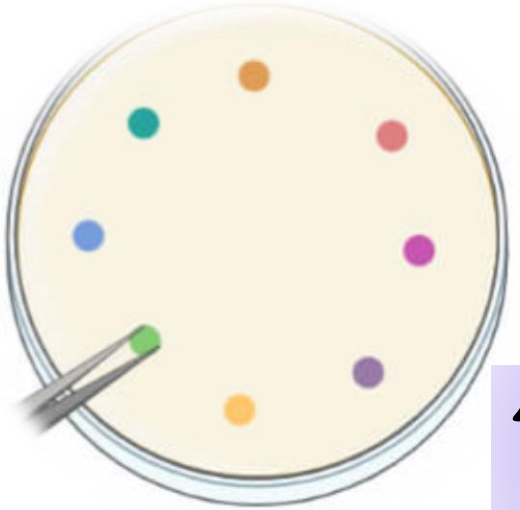
Journal of Antimicrobial Chemotherapy

The EUCAST rapid disc diffusion method for antimicrobial susceptibility testing directly from positive blood culture bottles

Emma Jonasson[1]*, Erika Matuschek[2] and Gunnar Kahlmeter[1,2]

EUCAST rapid antimicrobial susceptibility testing (RAST) directly from positive blood culture bottles

125±25µl
KAN



4/6/8 saat

EUCAST rapid AST (RAST) directly from (+) blood culture bottles

Table 1. Incubation conditions for antimicrobial susceptibility test plates.

Organism	Incubation time	Medium	Incubation
Escherichia coli *Klebsiella pneumoniae* *Acinetobacter baumannii* *Staphylococcus aureus* *Enterococcus faecalis* *Enterococcus faecium*	4, 6 and 8 hours	MH	35±1°C in air
Pseudomonas aeruginosa	6 and 8 hours	MH	35±1°C in air
Streptococcus pneumoniae	4, 6 and 8 hours	MH-F	35±1°C in 4-6% CO_2 in air

European Committee on Antimicrobial Susceptibility Testing

Zone diameter breakpoints for rapid antimicrobial susceptibility testing (RAST) directly from blood culture bottles

Version 3.0, valid from 2021-01-01

Escherichia coli

EUCAST RAST Breakpoint Tables v. 3.0, valid from 2021-01-01

Zone diameter breakpoints for RAST directly from blood culture bottles

EUCAST rapid disk diffusion method directly from positive blood culture bottles
Medium: Mueller-Hinton (MH) agar
Inoculum: 125±25 µL directly from a positive blood culture bottle
Incubation: Air, 35±1°C
Incubation time: 4, 6 and 8 hours
Reading: Remove lid and read zone edges from the front against a dark background illuminated with reflected light.
QC for implementation of RAST

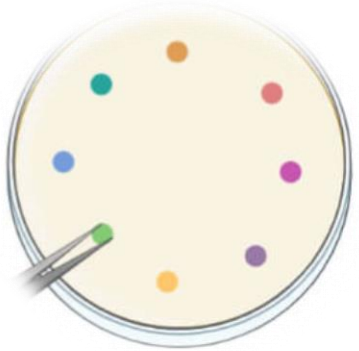
Antimicrobial agent	Disk content (µg)	4 hours			6 hours			8 hours		
		S ≥	ATU	R <	S ≥	ATU	R <	S ≥	ATU	R <
Piperacillin-tazobactam	30-6	17	14-16	14	18	15-17	15	18	15-17	15
Cefotaxime[1]	5	15	13-14	13	16	14-15	14	17	15-16	15
Ceftazidime[1]	10	15	12-14	12	16	14-15	14	17	15-16	15
Ceftazidime-avibactam[1]	10-4	12	10-11	10	12	10-11	10	12	10-11	10
Ceftolozane-tazobactam[1]	30-10	16	14-15	14	18	16-17	16	18	16-17	16
Imipenem[2]	10	16	14-15	14	17	15-16	15	17	15-16	15
Meropenem[2]	10	18	15-17	15	17	15-16	15	17	15-16	15
Ciprofloxacin	5	17	14-16	14	20	17-19	17	20	17-19	17
Levofloxacin	5	16	14-15	14	18	15-17	15	17	15-16	15
Amikacin[3]	30	15	13-14	13	15	13-14	13	15	13-14	13
Gentamicin[3]	10	14	12-13	12	14	12-13	12	14	12-13	12
Tobramycin[3]	10	14	12-13	12	15	13-14	13	15	13-14	13
Trimethoprim-sulfamethoxazole	1.25-23.75	12	10-11	10	14	12-13	12	14	12-13	12

Notes

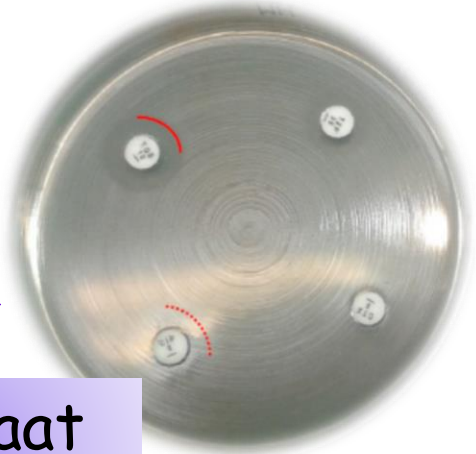
1. Cephalosporin breakpoints for *E. coli* will detect all clinically important resistance mechanisms. The presence or absence of an ESBL does not in itself influence the categorisation of susceptibility. However, ESBL detection and characterisation are recommended for public health and infection control purposes.

EUCAST rapid AST (RAST)
directly from (+) blood culture bottles

125±25µl
KAN



4/6/8 saat



K. pneumoniae

AYNI GÜN içinde
tanımlama
+
duyarlılık sonucu

Klinik Örnek: Kan
Escherichia coli

MİK (µg/ml)

amikacin	R
cefepime	R
ceftriaxone	R
ciprofloxacin	R
ertapenem	R
gentamicin	R
meropenem	R
pip-taz	R
tobramycin	R
trimeth-sulfa	R

- Bu izolat karbapenemaz oluşturuyor mu?
- Cevap evet ise hangisi?

- Hangi tedavi uygulanacak?
- Enfeksiyon kontrol önlemleri gerekli mi?
- Surveyans gerekli mi?

3

Hangi
karbapenemaz enzimi var?

Yeni Blaz-Blaz inhibitörleri kombinasyonlarının aktiviteleri

TABLE 1 | Activity of recent beta-lactam/beta-lactamase inhibitor combinations against microorganisms containing carbapenemases[a].

Antimicrobial Agent	FDA status[a]	EMA status[b]	Carbapenemase (Class)				
			KPC (A)	NDM (B)	IMP (B)	VIM (B)	OXA-48 (D)
Ceftzidime-avibactam	Approved	Authorized	Yes	No	No	No	Limited
Meropenem-vaborbactam	Approved	Authorized	Yes	No	No	No	No
Ceftolozane-tazobactam	Approved	Authorized	No	No	No	No	No
Imipenem-cilastatin-relebactam	Approved	Authorized	Yes	No	No	No	No
Cefiderocol	Approved	Authorized	Yes	Yes	Yes	Yes	Yes
Aztreonam-avibactam	Phase III clinical trial	Authorized	Yes	Yes	Yes	Yes	Yes

[a]*Adapted from https://www.centerwatch.com/directories/1067-fda-approved-drugs/topic/116-infections-and-infectious-diseases accessed 4-8-2021.*

[b]*European Medicines Agency; https://www.ema.europa.eu/en/medicines/human accessed 6-19-2021.*

Panellerde neler saptanabiliyor??

Test name; Manufacturer	Technology; Specimen types; availability[a]	Carbapenem resistance genes detected
Xpert® Carba-R; Cepheid, Sunnyvale, CA	NAAT; Pure cultures of carbapenem-resistant organisms, rectal swabs, peri-rectal swabs; EU and US	bla_{IMP}, bla_{KPC}, bla_{NDM}, $bla_{OXA-48-like}$, and bla_{VIM}
CARBA-5; NG Biotech, Guipry, France	Immunochromatographic; Pure cultures of carbapenem-resistant organisms; EU and US	bla_{IMP}, bla_{KPC}, bla_{NDM}, $bla_{OXA-48-like}$, and bla_{VIM}
BioFire BCID2; BioFire, Salt Lake City, UT, USA	Film array; Blood culture bottles; EU and US	bla_{KPC}, bla_{IMP}, bla_{NDM}, $bla_{OXA-48-like}$, and bla_{VIM}
Luminex Verigene BC-GN; Luminex, Toronto, CA	NAAT; Blood culture bottles; EU and US	bla_{KPC}, bla_{IMP}, bla_{NDM}, bla_{OXA-48}, and bla_{VIM}
GenMark ePlex BCID-GN; Carlsbad, CA, USA	NAAT; Blood culture bottles; EU and US	bla_{KPC}, bla_{IMP}, bla_{NDM} bla_{OXA-23}, bla_{OXA-48}, and bla_{VIM}

Central Asian and European Surveillance of Antimicrobial Resistance

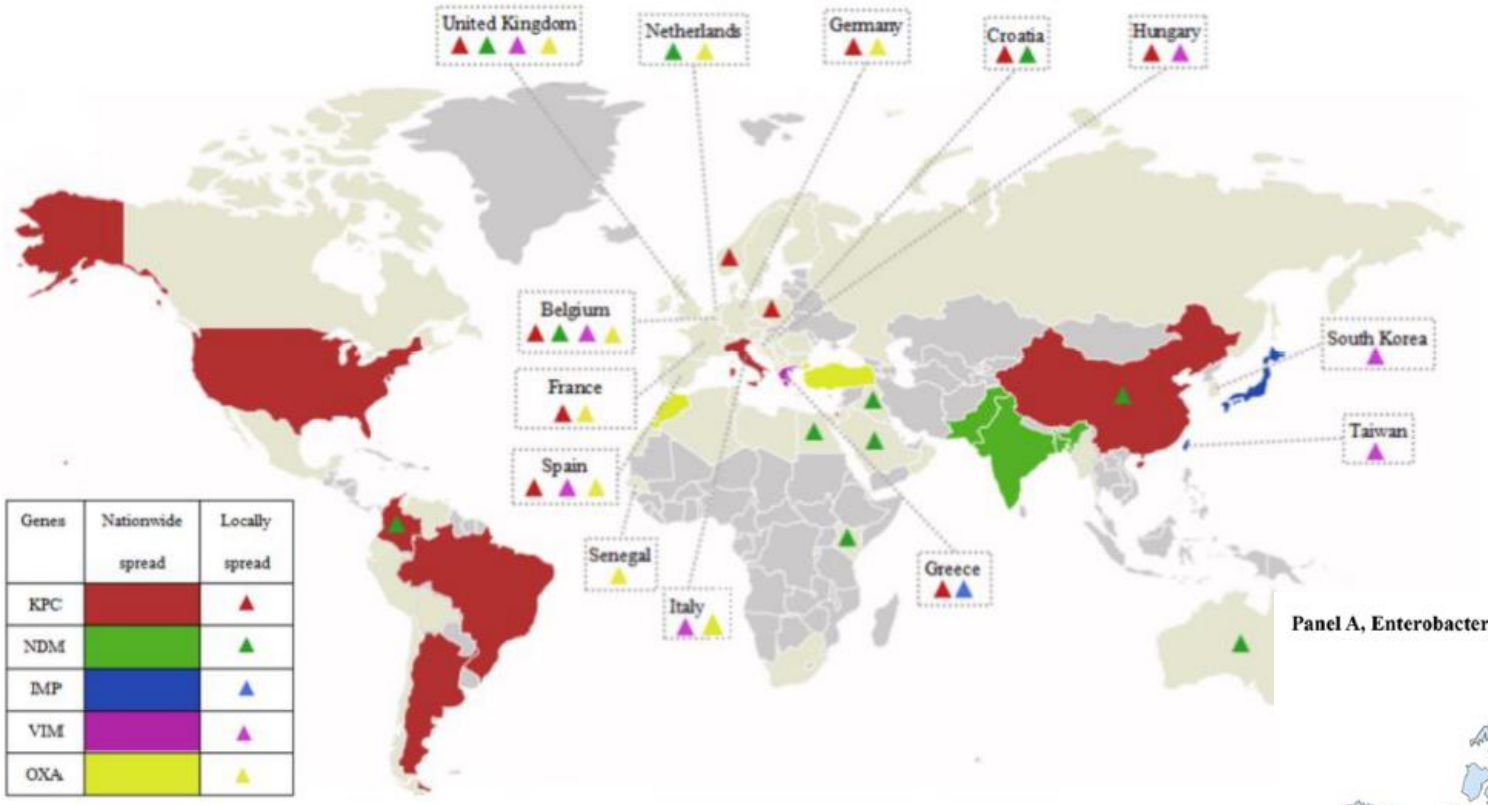
Annual report 2020

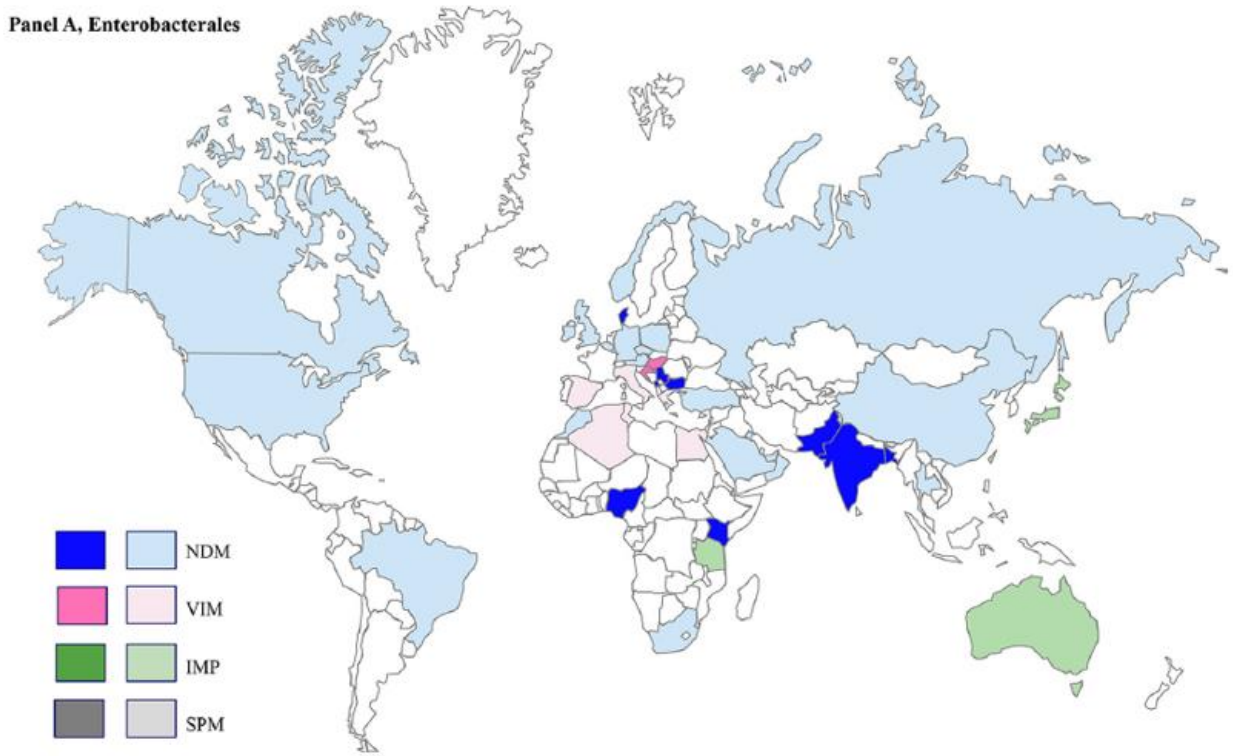
Table 6.70 Resistance levels for *E. coli* and *K. pneumoniae* among blood and CSF isolates in Turkey in 2019

	E.coli			*K.pneumoniae*		
Antibiotic (group)	**N**	**%R**	**%I**	**N**	**%R**	**%I**
Ampicillin/amoxicillin	4289	79	0	NA	NA	NA
Amoxicillin-clavulanic acid	3487	61**	0**	2772	75**	0**
Piperacillin-tazobactam	4369	22	4	3565	60	7
Cefotaxime/ceftriaxone	4598	53	1	3602	73	1
Ceftazidime	4537	47	6	3742	70	3
Ertapenem	4559	9	0	3647	51	0
Imipenem/meropenem	4965	3	1	4028	39	6
Gentamicin/tobramycin	4616	26	1	3925	45	2
Amikacin	4552	2	4	3760	27	5
Ciprofloxacin/levofloxacin/ofloxacin	4852	52	5	3933	65	5
Multidrug resistance[a]	4495	18	NA	3689	40	NA

Table 6.72 Resistance levels for *P. aeruginosa* and *Acinetobacter* spp. among blood and CSF isolates in Turkey in 2019

	P.a.			*Acinetobacter*		
Antibiotic (group)	**N**	**%R**	**%I**	**N**	**%R**	**%I**
Piperacillin-tazobactam	1533	34	0	NA	NA	NA
Ceftazidime	1645	28	0	NA	NA	NA
Cefepime	1630	31	0	NA	NA	NA
Imipenem/meropenem	1712	38	3	2390	90	1
Gentamicin/tobramycin	1681	21	0	2404	80	0
Amikacin	1579	14	4	2179	70	5
Ciprofloxacin/levofloxacin	1637	35	0	2391	91	6
Multidrug resistance[a]	1424	30	NA	2362	80	NA





Boyd SE, Livermore DM, Hooper DC, Hope WW. 2020. Metallo-β-lactamases: structure, function, epidemiology, treatment options, and the development pipeline. Antimicrob Agents Chemother 64:e00397-20.

Türkiye'de 2019 Yılı İçinde İzole Edilen *Escherichia coli* ve *Klebsiella pneumoniae* İzolatlarında Karbapenemaz Epidemiyolojisi

daha net anlaşılmasına olanak sağlamaktadır. Bu çalışmada *Escherichia coli* ve *Klebsiella pneumoniae* izolatlarında moleküler tabanlı pilot karbapenem direnci sürveyans sisteminden elde edilen verilere göre ülke genelinde karbapenemaz epidemiyolojisinin belirlenmesi amaçlanmıştır. Türkiye'nin 26 istatistiki düzey-II bölgesinden 28 hastane çalışmaya dahil edilmiştir. Çalışmaya dahil edilen hastaneler 1 Mart-31 Ağustos 2019 ya da 1 Nisan-30 Eylül 2019 tarihleri arasında altı aylık dönemde klinik örneklerden izole edilen 10 adet karbapenem duyarlı, 10 adet karbapenem dirençli *E.coli* ve *K.pneumoniae* izolatını laboratuvarımıza göndermiştir. Çalışmaya katılan 28 hastanenin 26 tanesinden toplam 509 izolat gönderilmiştir. İzolatlar matriks aracılı lazer desorpsiyon iyonizasyon uçuş süresi kütle spektrometrisi ["matrix assisted laser desorptionization-time of flight mass spectrophotometry" (MALDI-TOF MS)] (Bruker Daltonics, Almanya) yöntemi ile tanımlanmış ve imipenem, meropenem ve kolistin duyarlılıkları sıvı mikrodilüsyon ile amikasin, amoksisilin klavulonik asit, ampisilin, aztreonam, sefepim, sefotaksim, seftazidim, siprofloksasin, ertapenem, gentamisin, pipera lanan 509 izolatın 493'ü tür düzeyinde *E.coli* (%25.7, n= 127) ve *K.pneumoniae* (%74.3, n= 366) olarak tanımlanmış ve çalışmaya dahil edilmiştir. Değerlendirilen izolatların %31'inin toplum kökenli enfeksiyon etkeni, %69'unun ise sağlık hizmetleri ile ilişkili enfeksiyon etkeni ya da kolonize olan bakteri olduğu tespit edilmiştir. İzolatların 248 (%50.3)'i karbapenemlere duyarlı, 245 (%49.7)'i karbapenemlere dirençli olarak belirlenmiştir. Karbapenemlerden en az birine dirençli olan izolatlarda tespit edilen karbapenemaz türleri OXA-48 (%52.2), KPC (%16.1), NDM-1 (%15), OXA-48 + NDM-1 (%12.6), KPC + NDM-1 (%2.8) ve birer izolatta VIM (%0.5) ve OXA-48 + VIM (0.5) belirlenmiştir. İzolatların %23.3'ünde kolistin direnci tespit edilmiş olup *mcr* 1-8 genleri tespit edilememiştir. Kolistine dirençli izolatların tümünün en az bir karbapeneme dirençli olduğu görülmüştür. Ülkemizde moleküler tabanlı antibiyotik direnç sürveyans sis-

Sadece karbapenemaz enzimlerini saptamak yeterli mi?

Sadece van A/B ya da mecA/mecC saptamak yeterli mi?

ne yapılabilir?

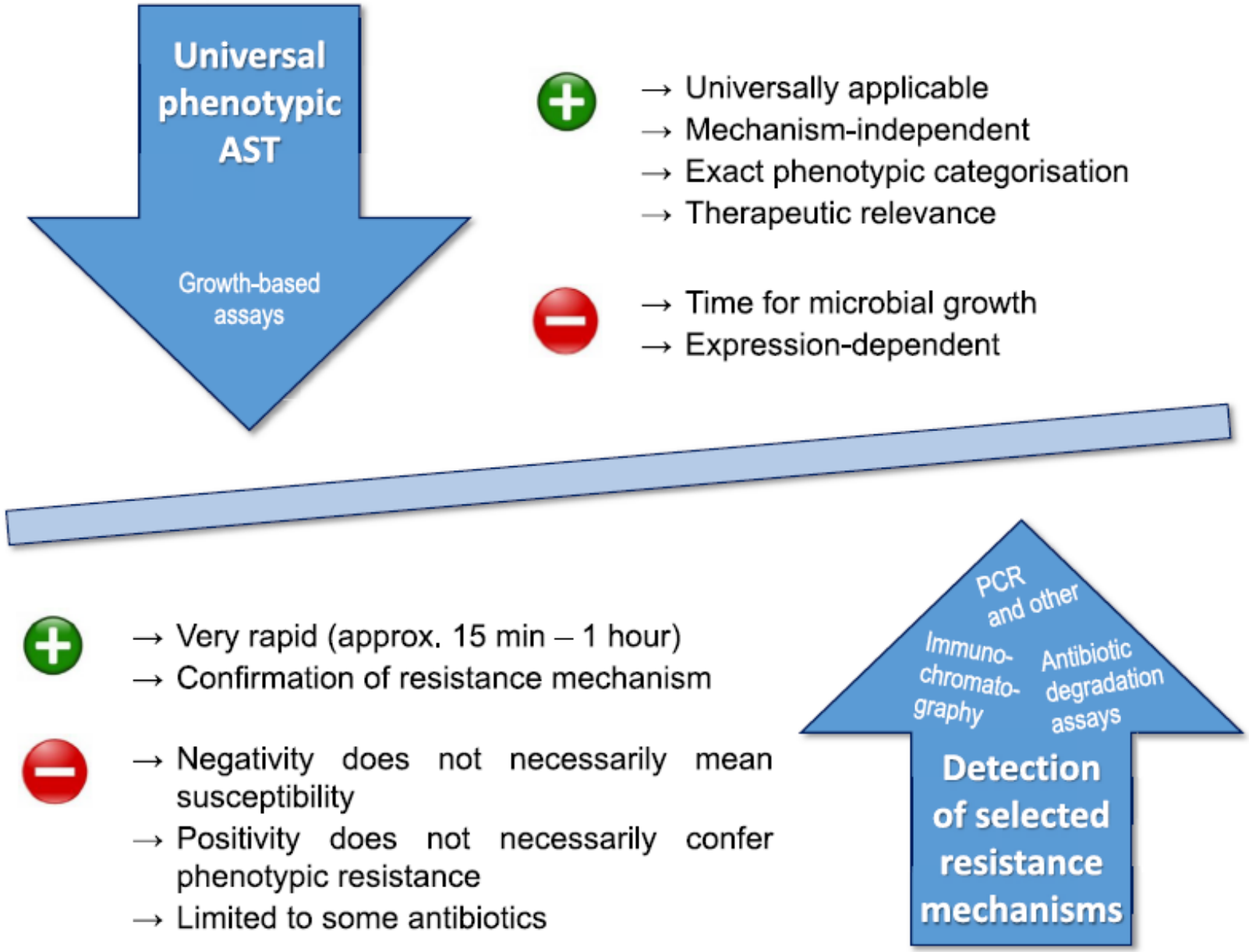


Fig. 1. Detection of particular resistance mechanisms vs. universal phenotypic susceptibility testing. AST, antimicrobial susceptibility testing.

Technology	Assay (manufacturer)	TAT (h)	Organisms detected	Resistance genes detected	Sensitivity/ specificity (%)
From positive blood cultures					
Multiplex PCR	The BioFire FilmArray blood culture identification panel 2 (BCID2) (bioMérieux)	1	11 Gram positives *Staphylococcus* spp., *Staphylococcus aureus*, *S. epidermidis*, *S. lugdunensis*, *Streptococcus* spp., *S. agalactiae*, *S. pyogenes*, *S. pneumoniae*, *E. faecalis*, *E. faecium*, *L. monocytogenes* 15 Gram negatives *A. calcoaceticus-baumannii complex*, *B. fragilis*, *H. influenzae*, *N. meningitidis*, *P. aeruginosa*, *S. maltophila*, *Enterobacterales*: *E. coli*, *E. cloacae complex*, *K. aerogenes*, *K. oxytoca*, *K. pneumoniae* group, *Proteus* spp., *Salmonella*, *S. marcescens* 7 fungal species *C. albicans*, *C. auris*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. neoformans/gattii*	*mecA/C*, *mecA/C and MREJ* (MRSA), *van A/B*, *blaKPC*, *blaIMP*, *blaOXA-48*, *blaNDM*, *blaVIM*, *mcr-1*, *CTX-M*	91–96/98–100
Real-time multiplex PCR	Xpert MRSA/SA Blood Culture Assay (Cepheid)	1–2	*Staphylococcus aureus*, MRSA	*mecA*	98–100/99.5
DNA microarray	Verigene Gram Positive Blood Culture Test (Luminex)	2.5	13 Gram positives *Staphylococcus* spp., *Staphylococcus aureus*, *S. epidermidis*, *S. lugdunensis*, *Streptococcus* spp., *S. agalactiae*, *S. pneumoniae*, *S. pyogenes*, *S. anginosus*, *E. faecalis*, *E. faecium*, *Micrococcus* spp., *Listeria* spp.	*mecA*, *van A/B*	93–100/94.5–100
	Verigene Gram Negative Blood Culture Test (Luminex)	2.5	9 Gram negatives *E. coli*, *K. pneumoniae*, *K. oxytoca*, *S. marcescens*, *Citrobacter* spp., *Enterobacter* spp., *Proteus* spp., *Acinetobacter* spp., *P. aeruginosa*	*mecA*, *van A/B*, bla_{CTX-M}, bla_{KPC}, bla_{OXA-48}, bla_{IMP}, bla_{VIM}, bla_{NDM}	98/100
In situ hybridization	-*Staphylococcus aureus*/CNS PNA FISH (AdvanDx)	1.5–3	*S. aureus*, *CoNS*	-	88–98/>98
	-*E. faecalis*/OE PNA FISH (AdvanDx)		*E. faecalis*, *E. faecium*, *Enterococcus* spp.	-	97/100
	-Gram-Negative PNA FISH (AdvanDx)		*E. coli*, *K. pneumoniae*, *P. aeruginosa*	-	99/98
	-*Candida* PNA FISH (AdvanDx)		*C. albicans* / *C. parapsilosis*, *C. tropicalis*, *C. glabrata* / *C. krusei*	-	99/100
	Quick-FISH	0.5	(same 4 panels of PNA-FISH)	-	98–100/98–100
In situ hybridization + morphokinetic cellular analysis for AST	Accelerate PhenoTest BC (Accelerate Diagnostics)	1 (7 for A-S-T)	6 Gram positives *CoNS* spp., *E. faecalis*, *E. faecium*, *S. aureus*, *S. lugdunensis*, *Streptococcus* spp. 8 Gram negatives *A. baumannii*, *Citrobacter* spp., *Enterobacter* spp., *E. coli*, *Klebsiella* spp., *Proteus* spp., *P. aeruginosa*, *S. marcescens* 2 *Candida* species *C. albicans*, *C. glabrata*	AST results as MIC	95–97.5/99–99.5 (for ID)
From whole blood					
Multiplex real-time PCR	Magicplex Sepsis Real-Time test (Seegene)	3–5	73 Gram positives (40 *Streptococcus* spp., *30 Staphylococcus* spp., *3 Enterococcus* spp.)	*mecA*, *van A/B*	29–65/66–95

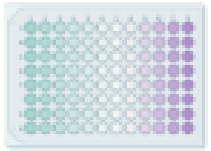
MIK

Methods of antimicrobial susceptibility testing

Phenotypic methods

Constrained by bacterial growth time

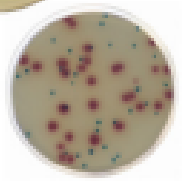
Dilution — 48 h



Diffusion — 48 h

Gradient test — 48 h

Chromogenic media — 24 h

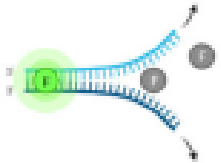


Automated devices — > 20 h

Molecular-based methods

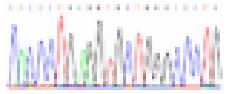
PCR

qPCR



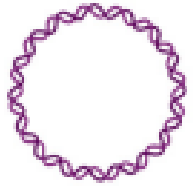
several h

Cycle sequencing



24 - 48 h

NGS



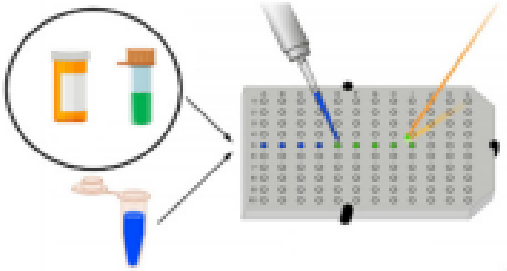
several days

** estimated time depend on a sample analysed (clinical specimen vs. isolated bacterial culture)*

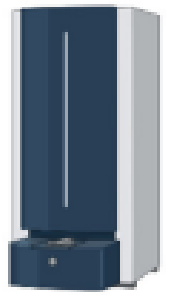
Mass spectrometry

MALDI–TOF MS

Incubation (antibiotic + sample)



Matrix



several h

** estimated time depend on a sample analysed (clinical specimen vs. isolated bacterial culture)*

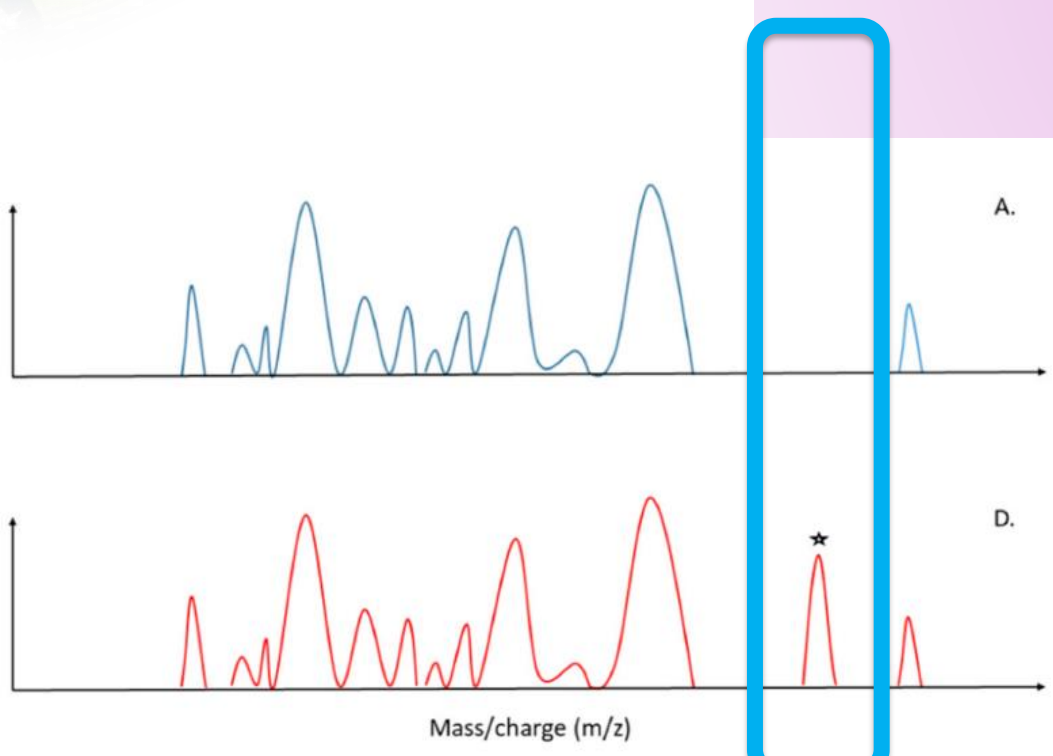
MALDI-TOF MS
AST

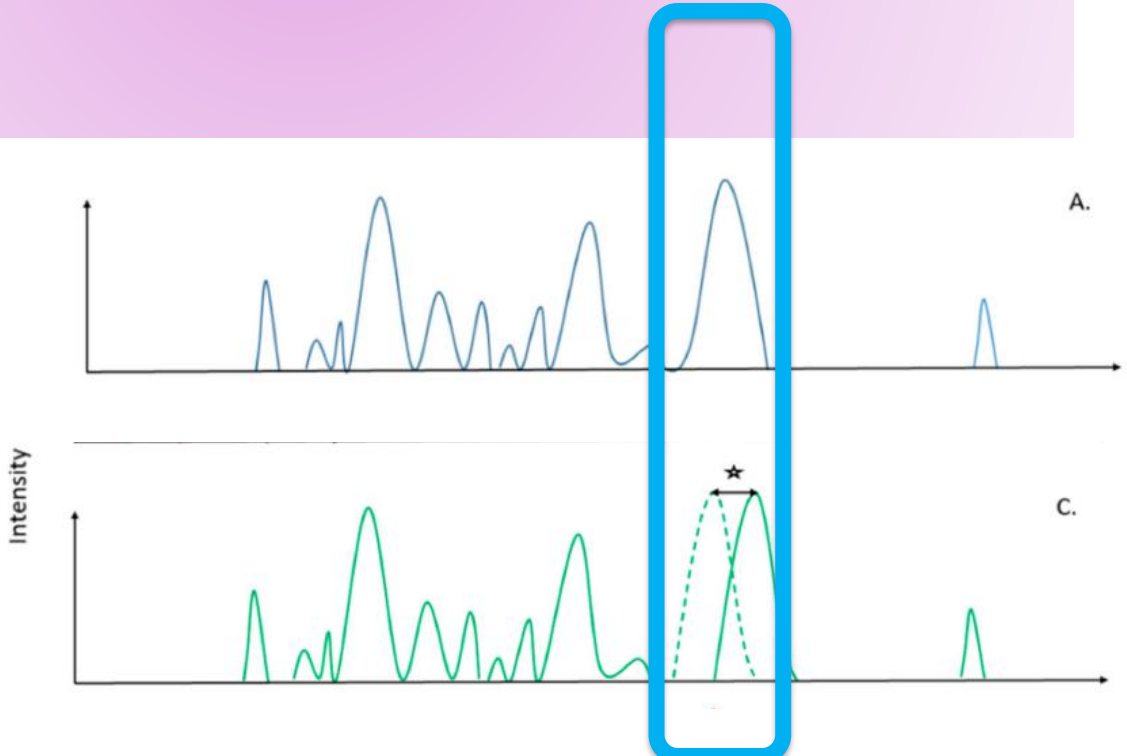
Farklı yaklaşımlar var:

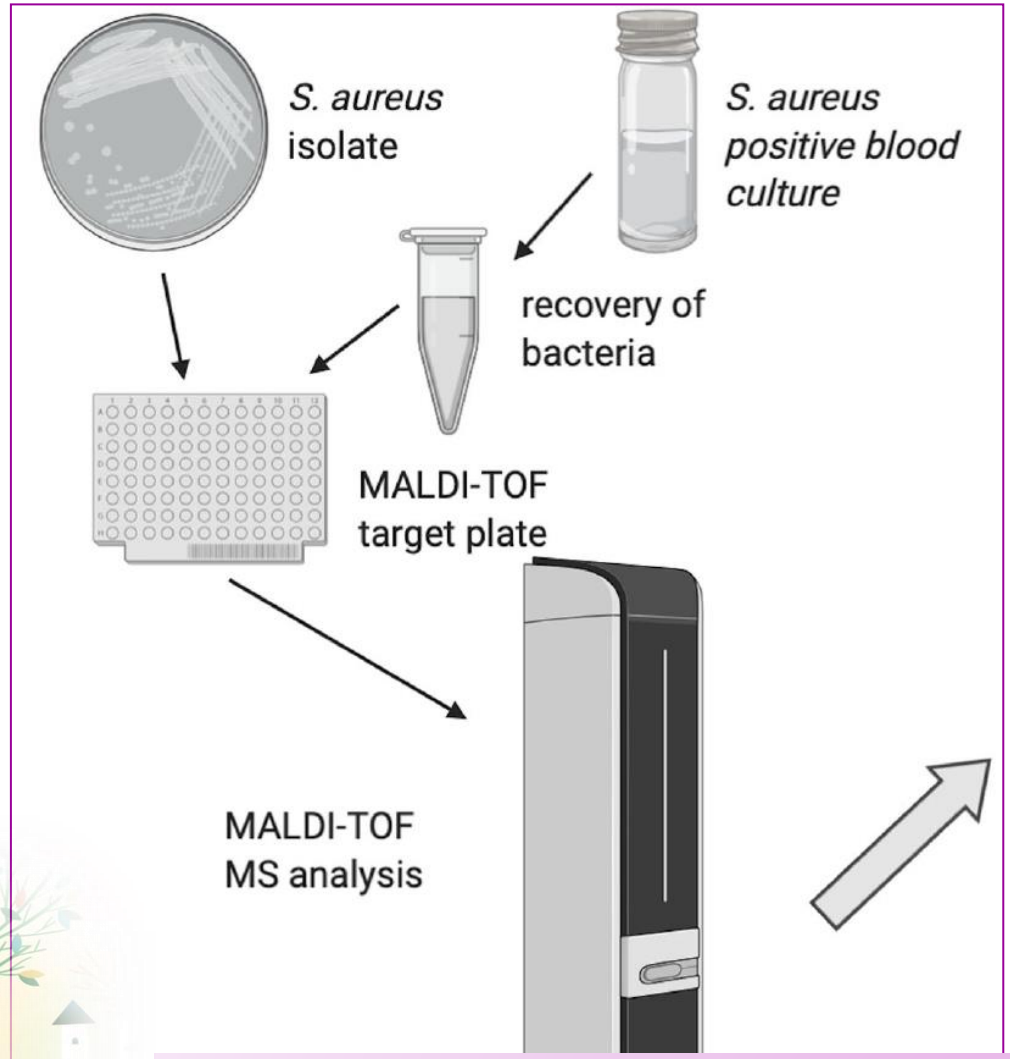
1) Biomarker
2) Antibiyotikte değişikliklerin saptanması
3) MBT-ASTRA
4) DOT

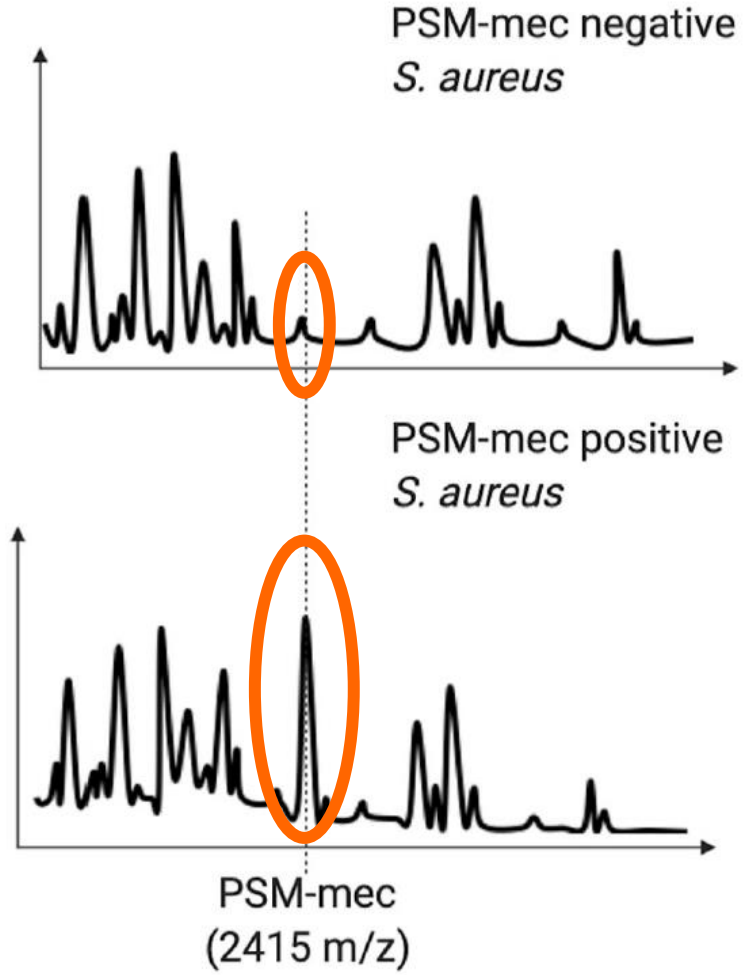
I. Biomarker:

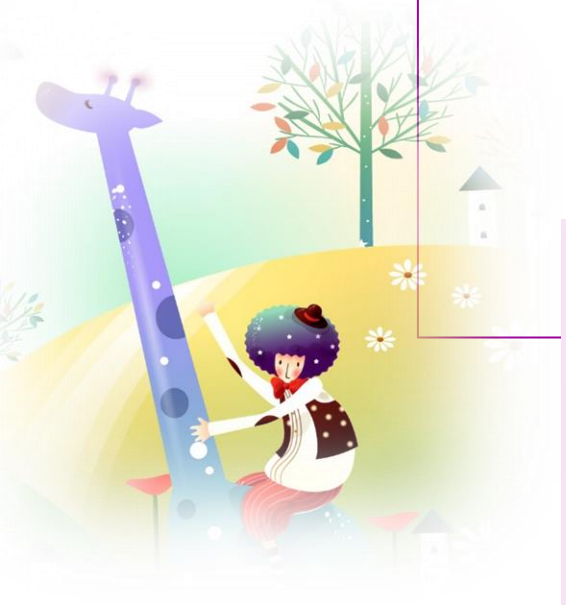
Özgül tek pik elde edilmesi ya da kütle/yük değerinde kayma görülmesi olarak tanımlanır











Stafilokok-metisilin direnci:

Metisilin direnci: mecA ile kodlanıyor.

2415 m/z pik: PSM-mec (phenol soluable modulin molecule)

SCCmec tipleri II,III ve VIII içinde yer alıyor

II. Antibiyotikteki değişikliklerin saptanması
Hidroliz, dekarboksilasyon, asetilasyon

- AB hidrolizine bağlı kütle değişmesi mg.
  AB'ler yıkıldığı zaman ortaya çıkan metabolitler özgül pikler oluşturur.
  AB'ye ait pikler azalır; hidroliz ürünlerine ait pikler oç

- Duyarlılık testi olarak MALDI ile bu yıkım ürünü saptanır

II. ANTİBİYOTİKteki değişikliklerin saptanması

Hidroliz, dekarboksilasyon, asetilasyon

B-laktamaz

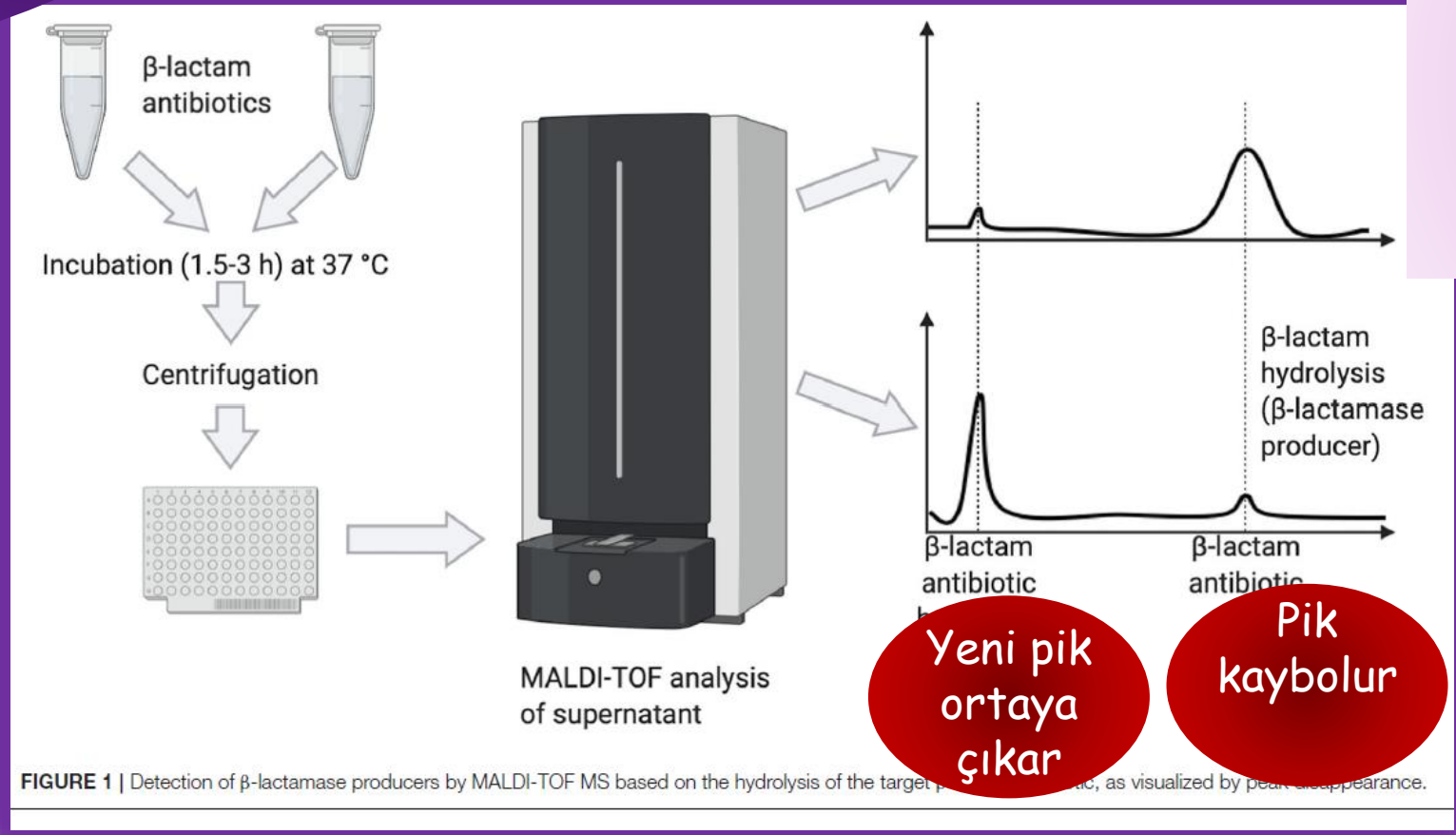
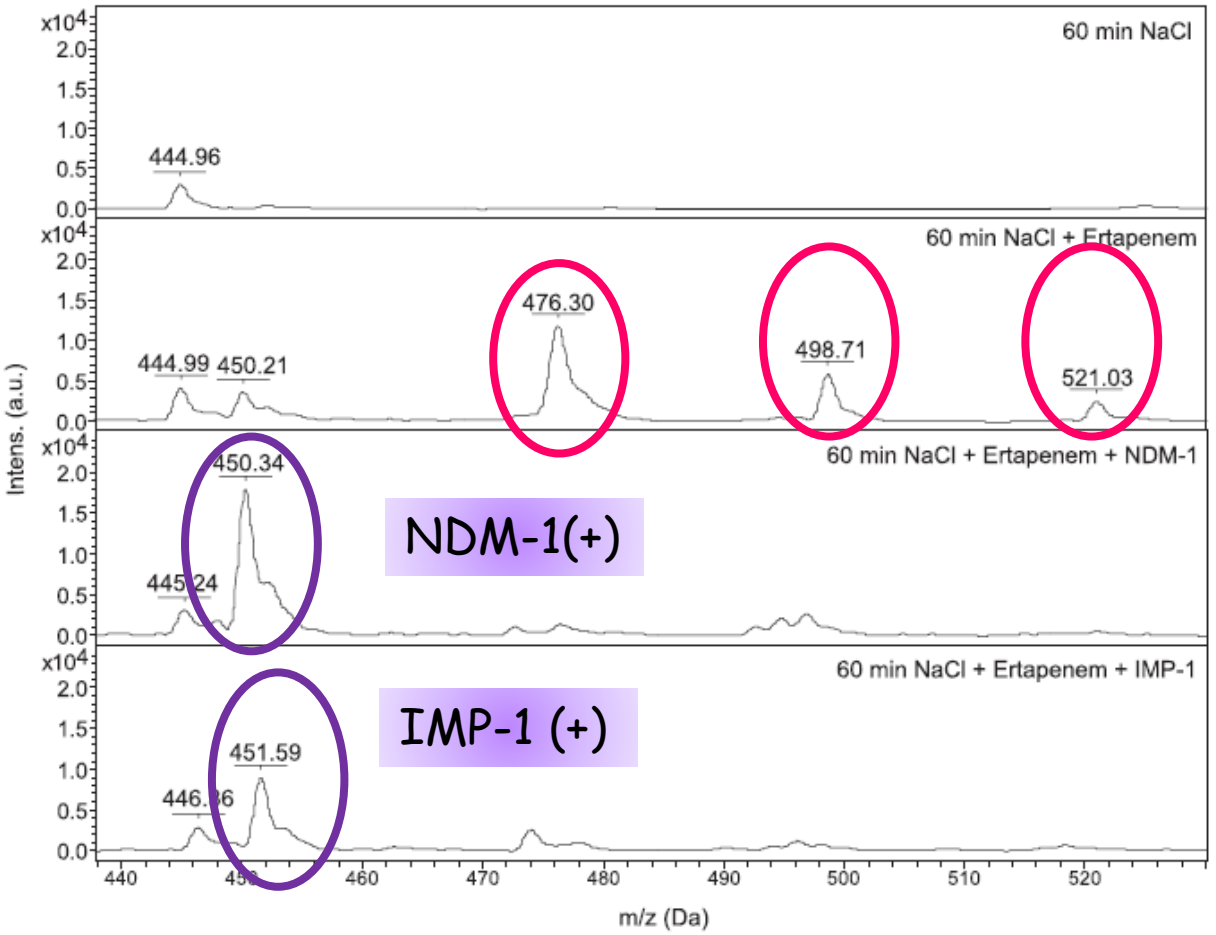


FIGURE 1 | Detection of β-lactamase producers by MALDI-TOF MS based on the hydrolysis of the target β-lactic, as visualized by peak disappearance.

AB'ye ait pikler azalır; hidroliz ürünlerine ait pikler oç

karbapenemaz

II. Antibiyotikteki değişikliklerin saptanması
Hidroliz, dekarboksilasyon, asetilasyon



47 izolat (KPC, NDM, IMP, VIM)
Ertapenem ile 1-2.5 saat inkübasyon
Karbapenemaz +/- ayrımı: %100

Ertapenem 'in oluşturduğu pikler:
476 m/z (ertapenem without sodium)
498 m/z (monosodium salt)
521 m/z (disodium salt)

Kaybolur

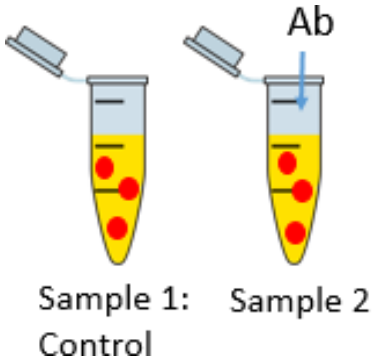
450 m/z (hydrolyzed and decarboxylated ertapenem)

Using Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry To Detect Carbapenem Resistance within 1 to 2.5 Hours[▽]

Irene Burckhardt* and Stefan Zimmermann

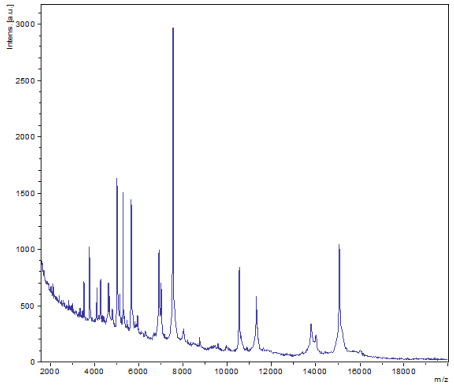
3. MBT-ASTRA:
MALDI Biotyper-Antibiotic susceptibility test rapid assay

Semikantitatif bir yöntem:
Saatler içinde duyarlı/dirençli sonucu



Incubation

Protein extraction +
Internal standard



Suceptible:
Proteins =/↘

Resistant:
Proteins ↗

Quantitative Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Rapid Resistance Detection

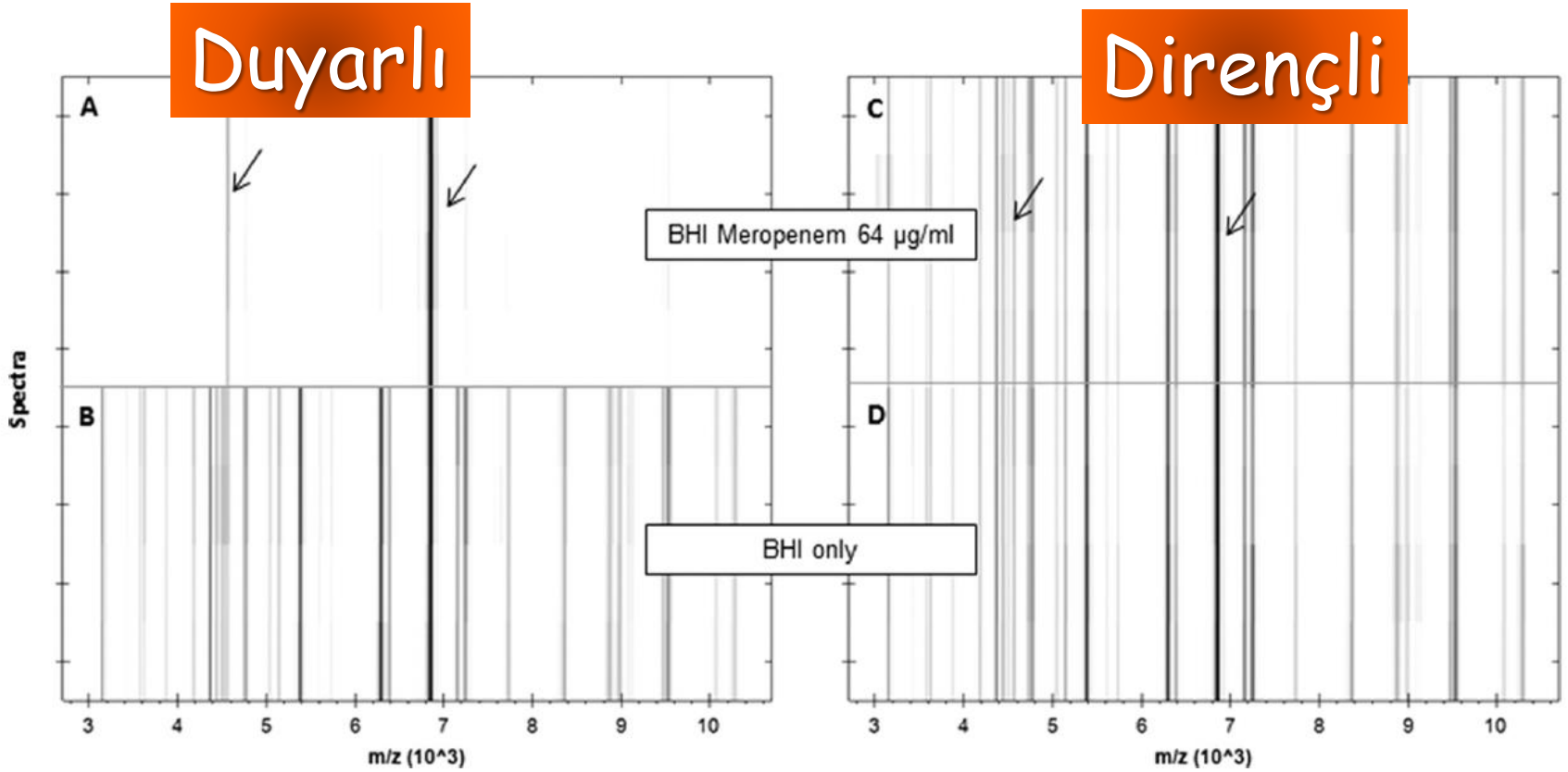
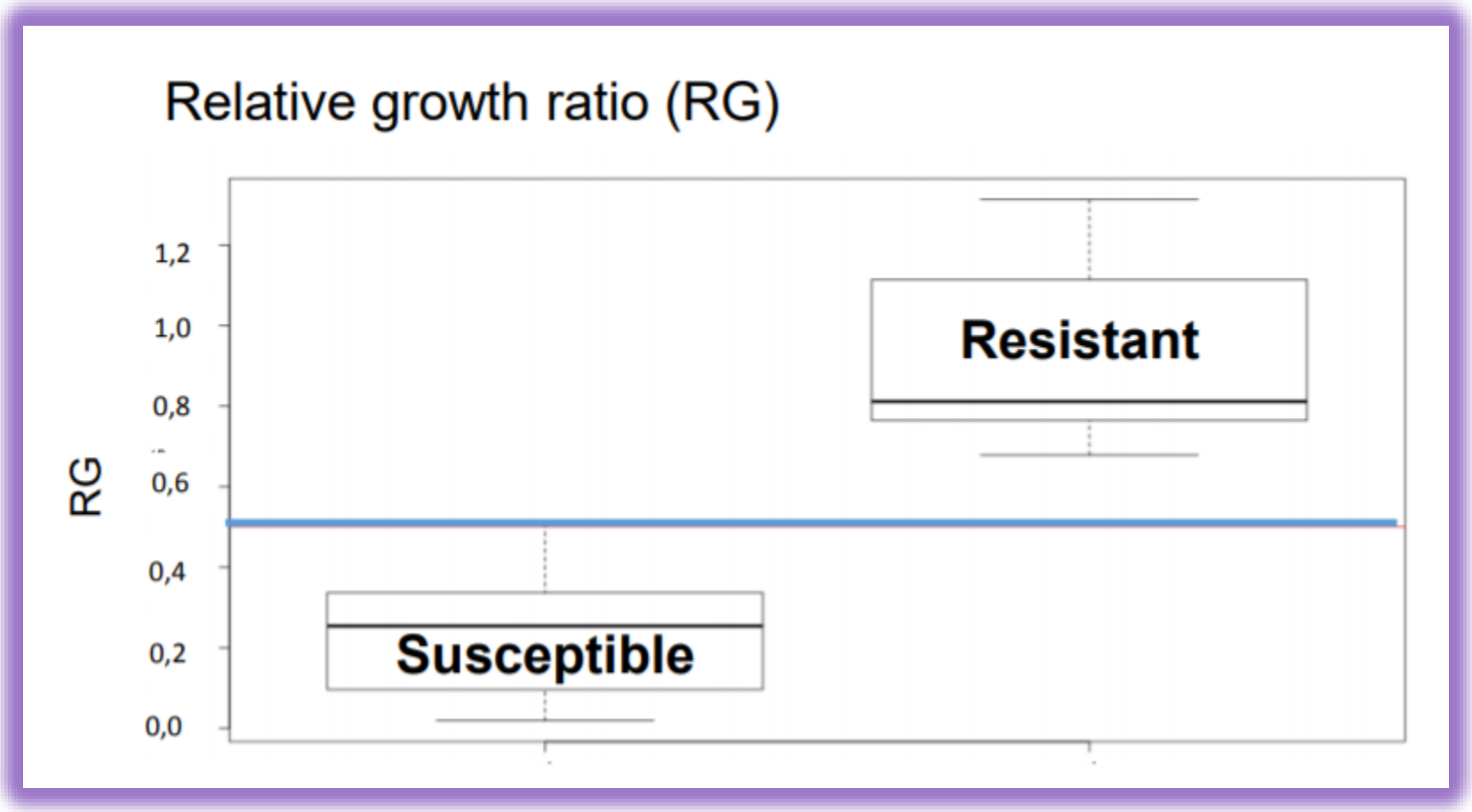


FIG 1 Pseudogel views of the mass range between 3 and 10 kDa of a susceptible (A, B) and a resistant (C, D) *K. pneumoniae* strain after incubation in the absence (lower panels) or presence (upper panels) of meropenem (64 µg/ml) for 1 h. For each incubation, four spectra acquired from two different spots are shown. Internal standard peaks are marked by arrows.



$$RG: \frac{AUC + Ab}{AUC - Ab}$$

- AB varlığında üreme ile Absiz ortamda üreme kıyaslama
- AUC hesaplanır
- Yüksek AUC: üremeyi gösterir yani direnci gösterir

4) DOT-MGA: Direct on target microdroplet Growth Assay

- Eşik değer konsantrasyonunda AB direkt target üzerine uygulanır
- 6 µl (3 µl AB + 3 µl bakt süsp (Son inokulum ~ $5x10^5$ CFU/ml)
- Optimum: 4-5 saat

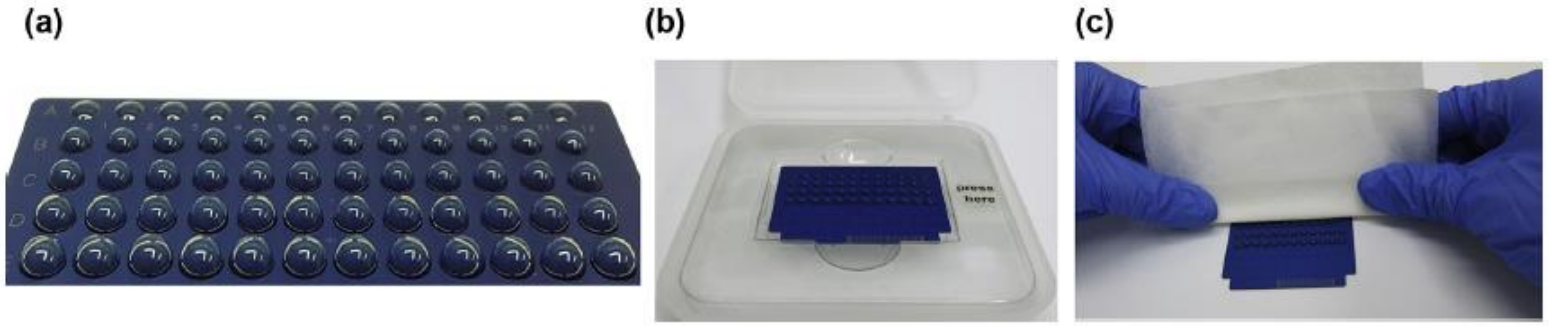


Fig. 1. The experimental setup. (a) Detail of a MALDI-TOF MS target with microdroplets before incubation. Rows A, B, C, D and E contain microdroplets with total volumes of 2, 4, 6, 8 and 10 µL, respectively. Columns 1 to 3, susceptible *Klebsiella pneumoniae* isolate with meropenem; columns 4 to 6, growth control of susceptible *K. pneumoniae* isolate without antibiotic; columns 7 to 9, resistant *K pneumoniae* isolate with meropenem; columns 10 to 12, growth control of resistant *K. pneumoniae* isolate without antibiotic. (b) MALDI-TOF MS target with microdroplets in a 'humidity chamber'. (c) Separation of nutrient broth from microbial cells by capillary effects using a tissue wipe as an absorptive material.

- Kan kültüründen direkt uygulama da mümkün
- Direnç mekanizmasından bağımsız sonuç verir

Use of target transport box as „humidity chamber“

MSP 96 target box

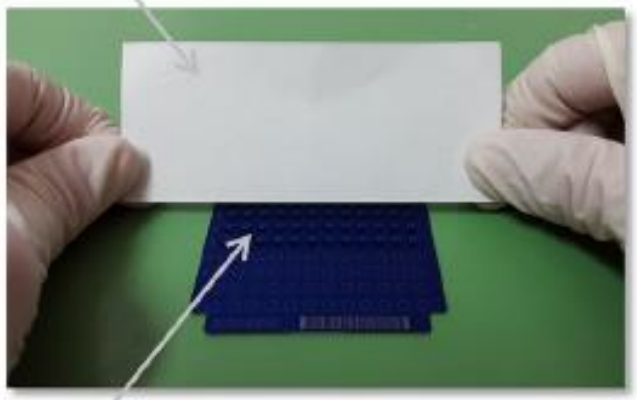
Inoculated MBT Biotarget 96

Water

Incubation at 35±1°C

Separation of broth from microbial cells by “touching” of microdroplets with an absorptive strip

Filter paper

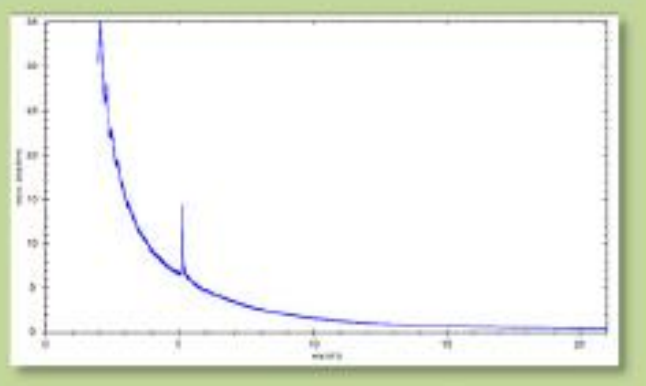


Microdroplets

Adding 1 µl matrix, followed by

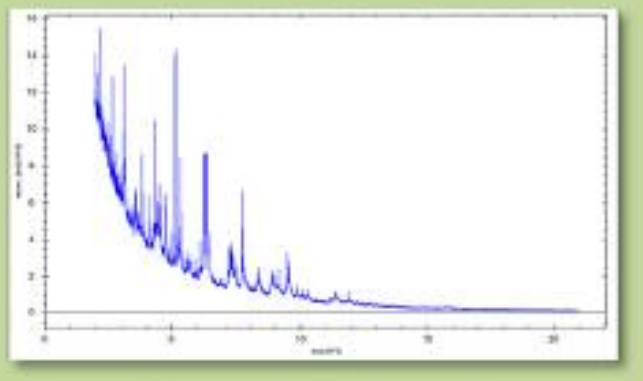
MALDI-TOF MS measure-ment

Evaluation of acquired spectra



Sample with antibiotic

Duyarlı: No ID (skor< 1.7)



Sample with antibiotic

Dirençli: ID (skor ≥ 1.7)

2019

Rapid Detection of Extended-Spectrum β-Lactamases (ESBL) and AmpC β-Lactamases in *Enterobacterales*: Development of a Screening Panel Using the MALDI-TOF MS-Based Direct-on-Target Microdroplet Growth Assay

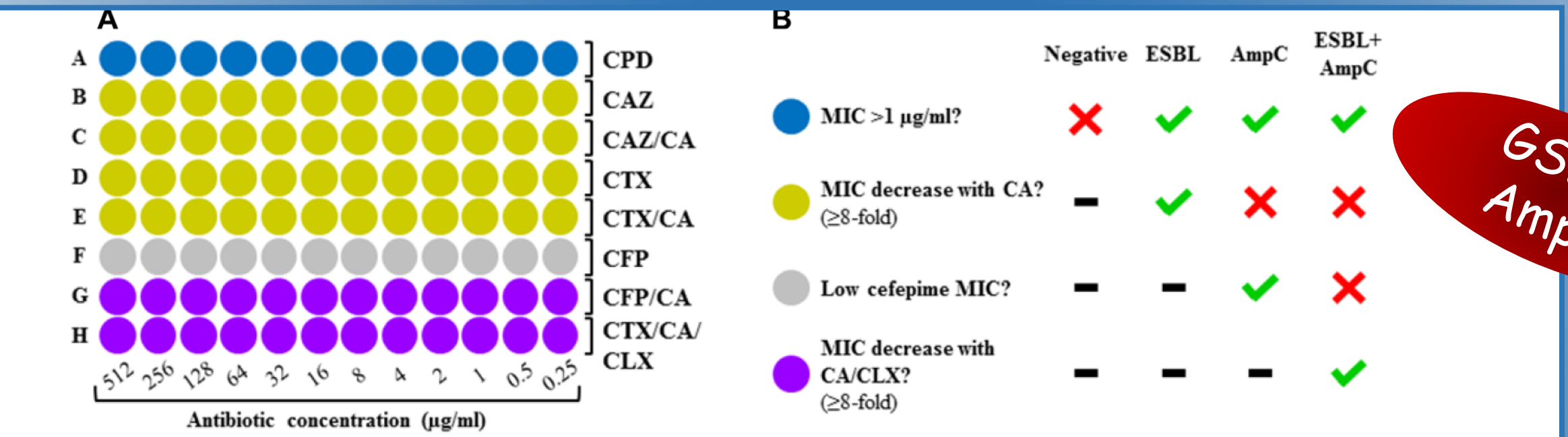
Carlos L. Correa-Martínez[1†], Evgeny A. Idelevich[1†], Katrin Sparbier[2], Markus Kostrzewa[2]

GSBL, AmpC ve karbapenemaz aktivitesinin saptanması için de modifiye edildi

2020

Development of a MALDI-TOF MS-based screening panel for accelerated differential detection of carbapenemases in *Enterobacterales* using the direct-on-target microdroplet growth assay

Carlos L. Correa-Martínez[1,2,5], Evgeny A. Idelevich[1,5], Katrin Sparbier[3], Thorsten Kuczius[2], Markus Kostrzewa[3] & Karsten Becker[1,4*]

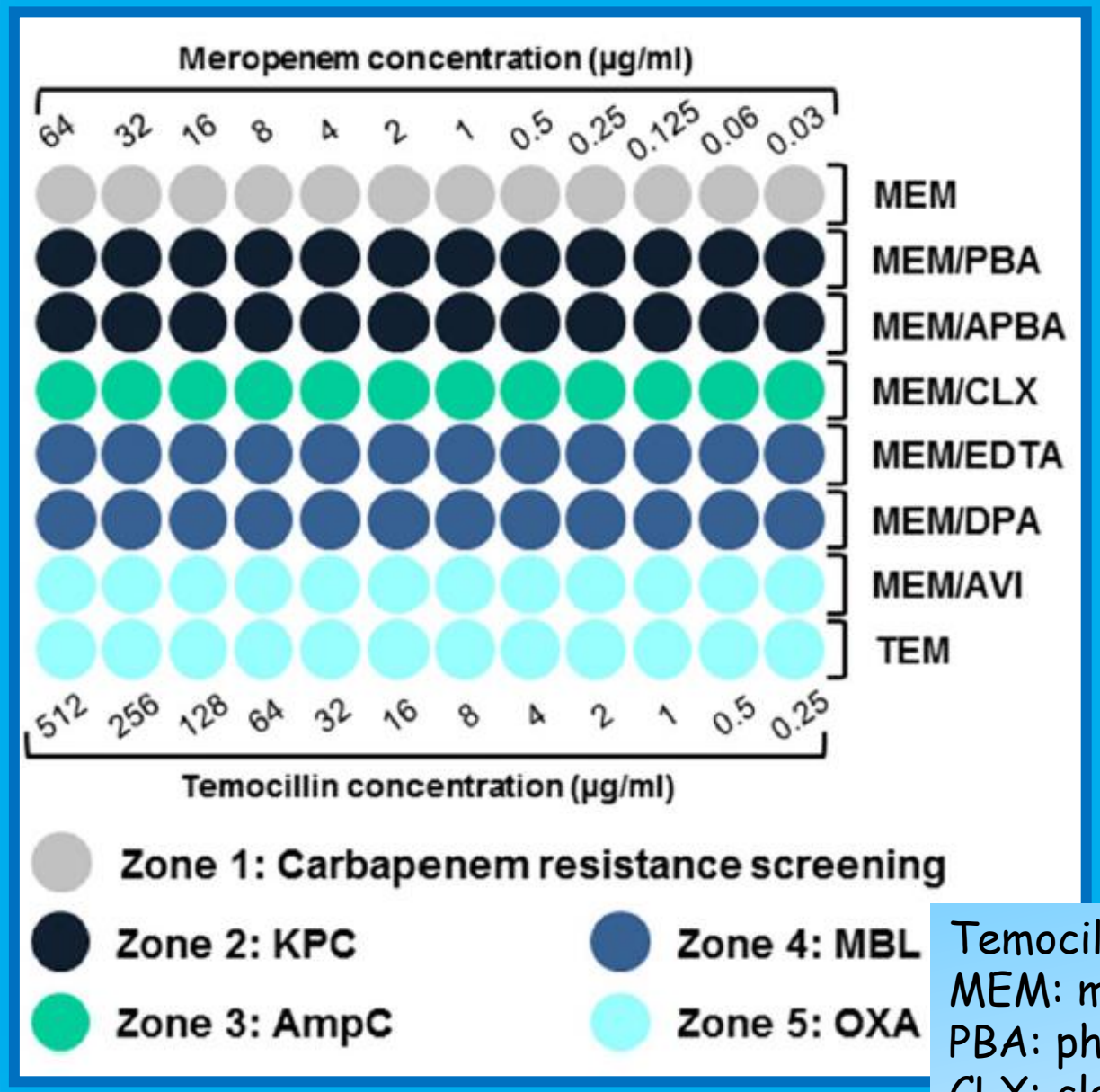


(A) Layout of the DOT-MGA screening panel. Blue zone, resistance screening; yellow zone, detection of ESBL; gray zone, detection of AmpC; purple
tion of ESBL + AmpC. CPD, cefpodoxime; CAZ, ceftazidime; CTX, cefotaxime; CFP, cefepime; CA, clavulanic acid; CLX, cloxacillin. **(B)** Interpretation

GSBL, AmpC

4 zon içerir:
Mavi: Sefpodoksim: 3rd jen sefalo direnci
Sarı: Sefotaksim, seftazidim ± GSBL inh: GSBL saptanması
Gri: Sefepim: AmpC saptanması
Mor: sefepim+GSBL inh
sefotaksim+GSBL inh + AmpC inh: AmpC+ maskelenmiş GSBL

PZR ile (+) % uyumu:
GSBL: 94.4 %
AmpC: 94.4 %
ESBL+AmpC : 100%



Hangi Karbapenemaz?

Targets were incubated for 3 or 4 hours at 35 ± 1 °C.

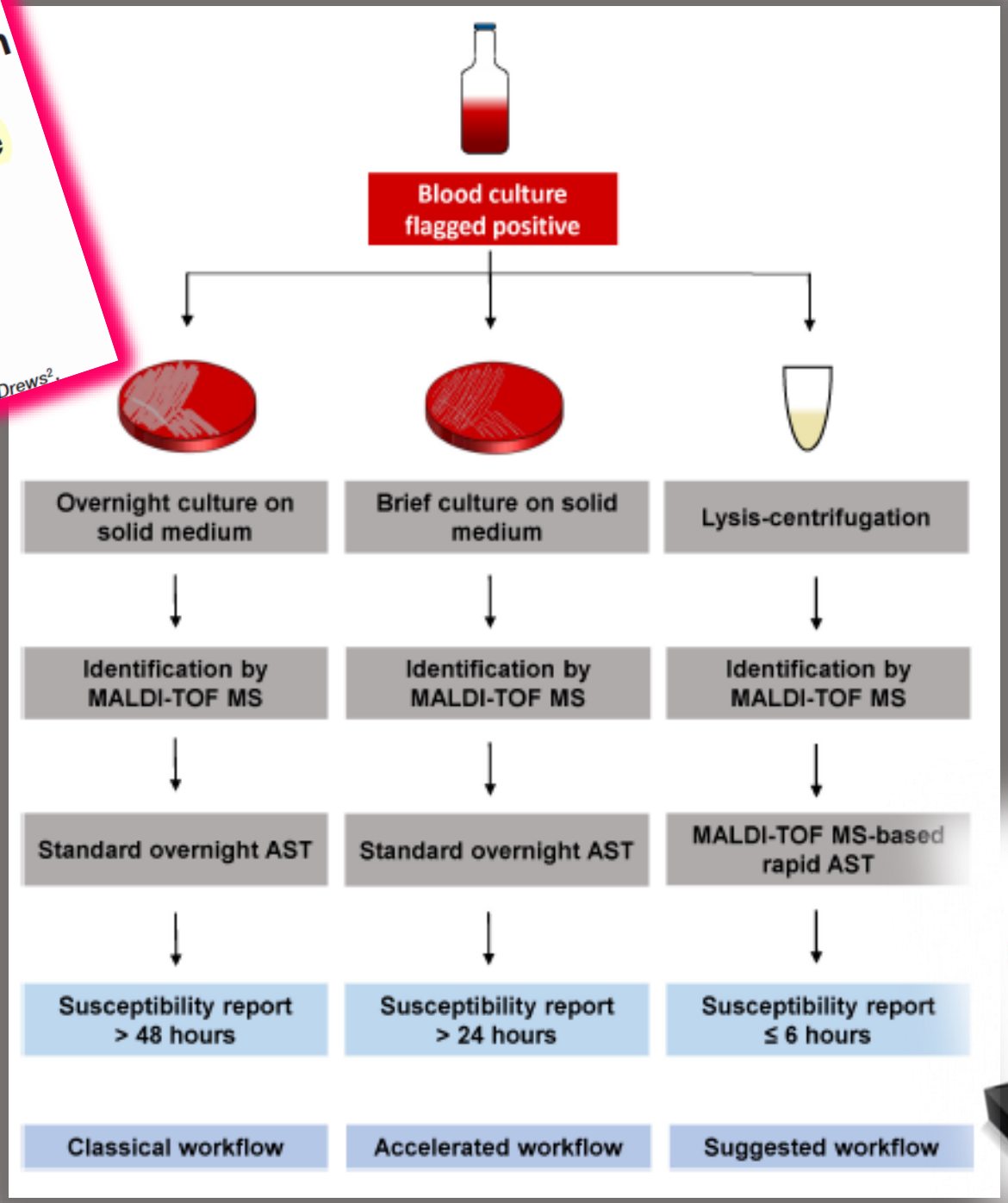
Temocillin MIC > 128 µg/ml is compatible with OXA production
MEM: meropenem
PBA: phenylboronic acid; APBA: aminophenylboronic acid
CLX: cloxacillin
AVI: avibactam

Resistance mechanism	Detection method (incubation time)							
	DOT-MGA (4 h)		DOT-MGA (3 h)		BMD (18 h)		CDT (18 h)	
	PPA	NPA	PPA	NPA	PPA	NPA	PPA	NPA
Carbapenem resistance	100%	100%	70%	100%	80%	80%	90%	10%
KPC	— *	100%	— *	95%	— *	90%	— *	65%
AmpC	33.3%	88.2%	33.3%	94.1%	33.3%	82.4%	33.3%	82.4%
MBL	100%	100%	50%	100%	75%	100%	75%	100%
OXA	100%	100%	71.4%	100%	85.7%	84.6%	100%	53.9%

Table 2. Detection performance of DOT-MGA, BMD and CDT on clinical isolates compared to PCR. *No PPA available as KPC was not detected in any of the tested clinical isolates.

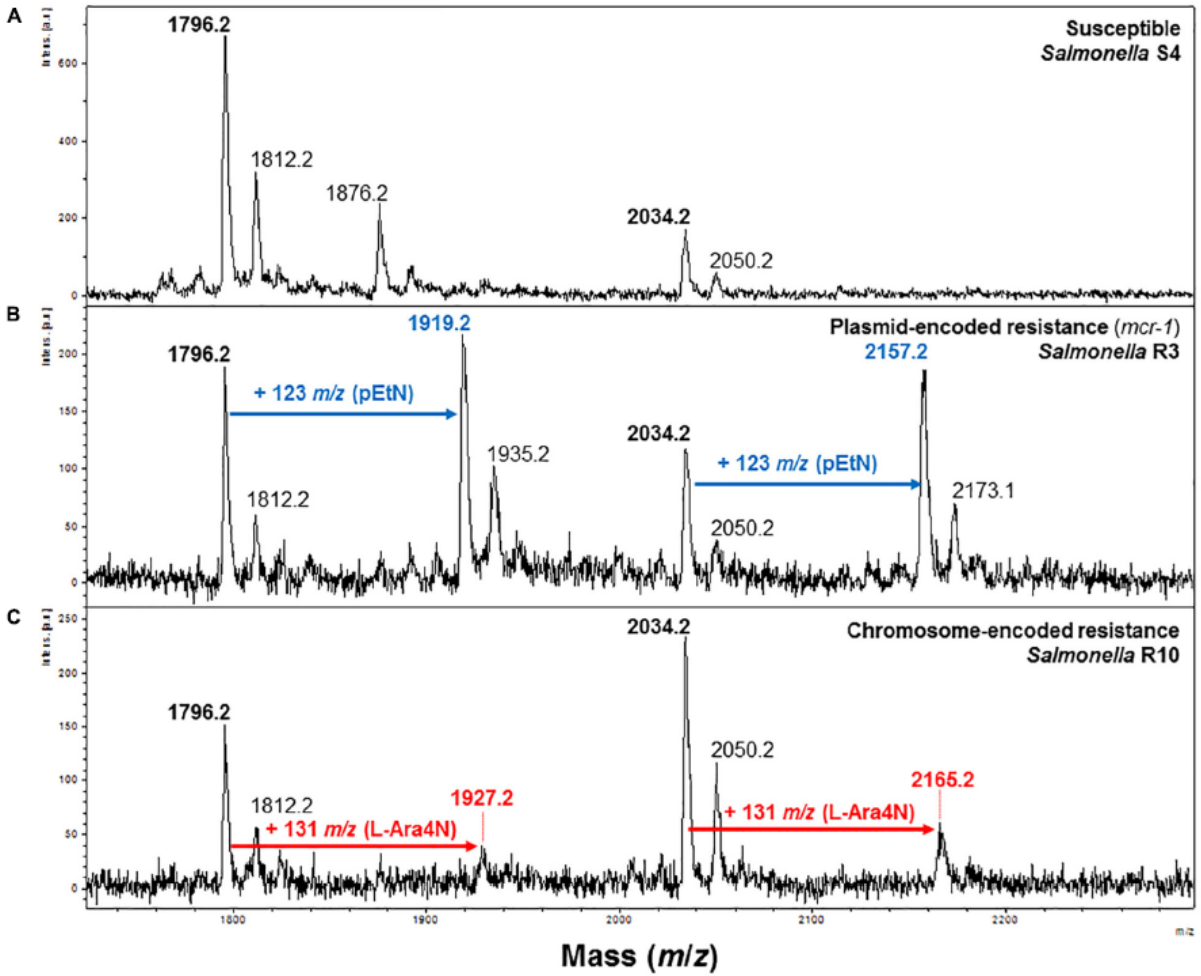
Detection of Methicillin Resistance in *Staphylococcus aureus* From Agar Cultures and Directly From Positive Blood Cultures Using MALDI-TOF Mass Spectrometry-Based Direct-on-Target Microdroplet Growth Assay

Ilka D. Nix[1], Evgeny A. Idelevich[1], Luise M. Storck[1], Katrin Sparbier[2], Oliver Drews[2],



Detection of Colistin Resistance in *Salmonella enterica* Using MALDIxin Test on the Routine MALDI Biotyper Sirius Mass Spectrometer

*Laurent Dortet[1,2,3,4], Rémy A. Bonnin[2,3,4], Simon Le Hello[5], Laetitia Fabre[5], Richard Bonnet[4,6], Markus Kostrzewa[7], Alain Filloux[1] and Gerald Larrouy-Maumus[1]**



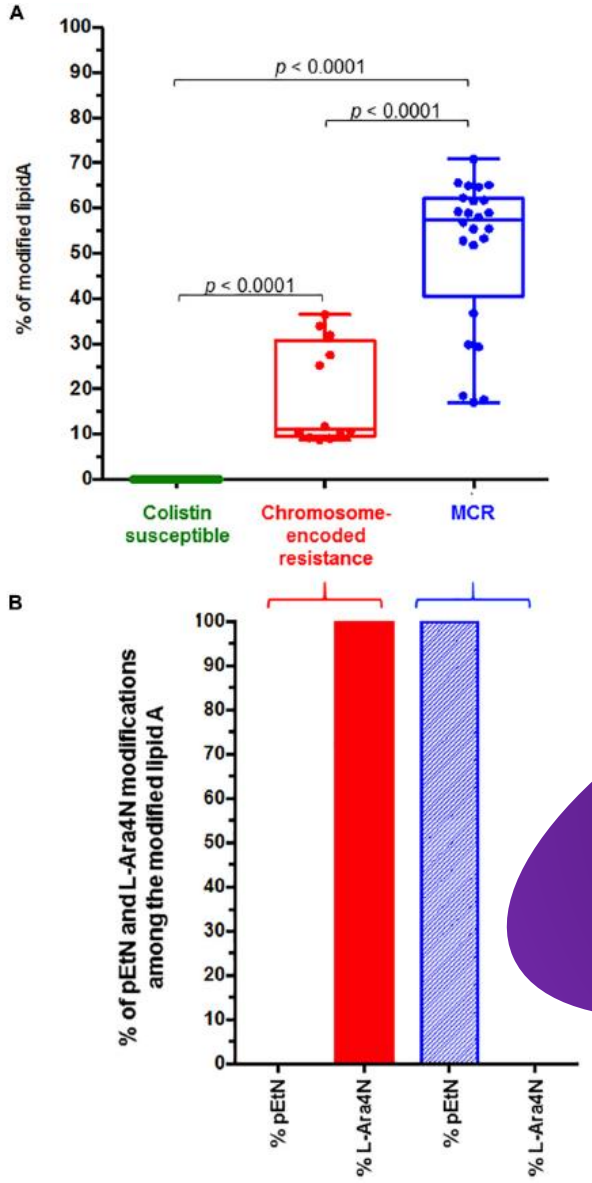
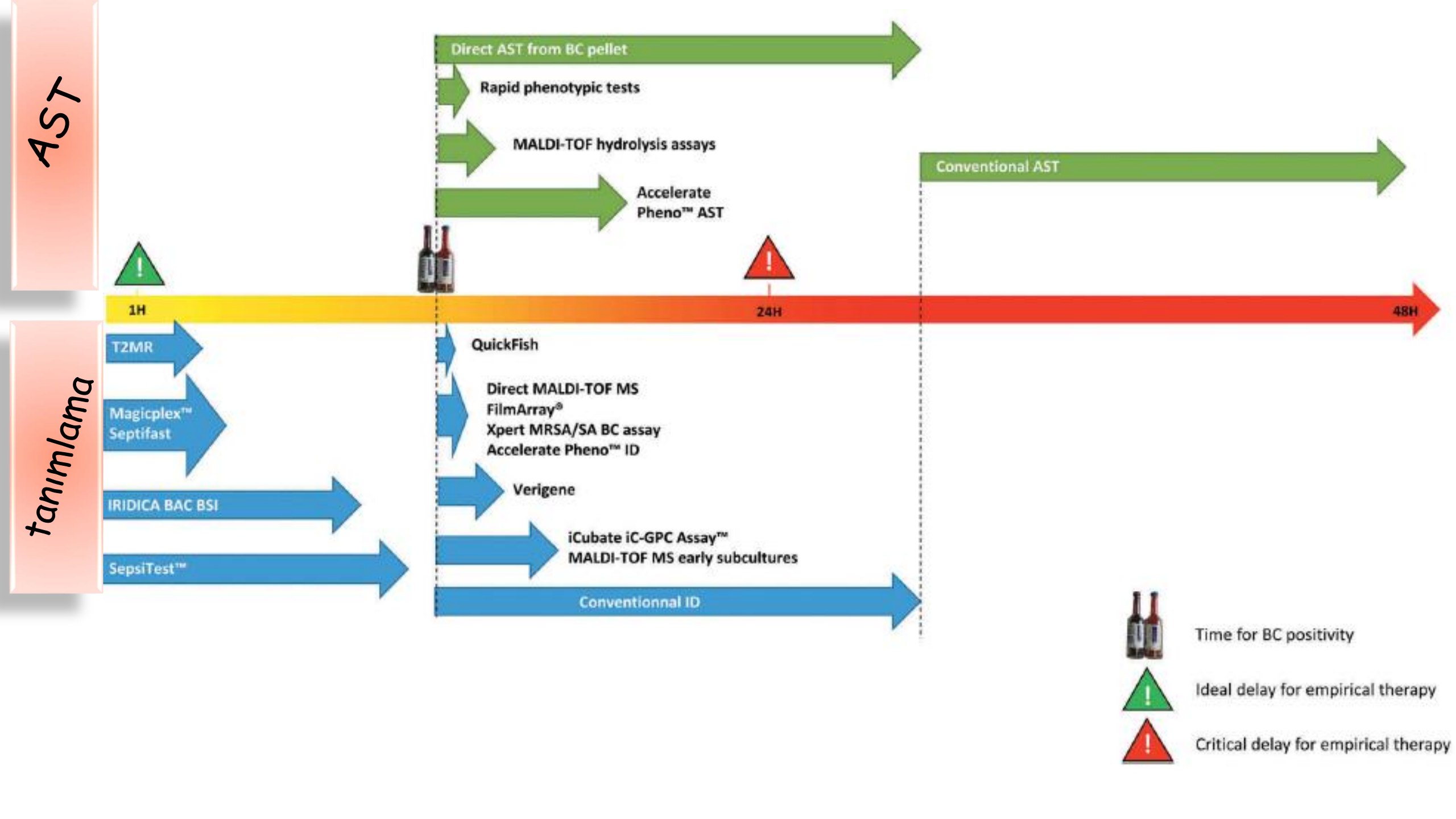


FIGURE 2 | (A) Representation of the percentage of the modified lipid A for colistin susceptible and colistin resistant *S. enterica* isolates. The global percentage of modified lipid A (L-Ara4N + pETN modified lipid A / native lipid A) is represented for colistin susceptible strains (*n*=11), colistin chromosome-encoded resistant *S. enterica* isolates (*n*=4) and MCR-producing *S. enterica* isolates (*n*=8). All experiments were performed in triplicate. **(B)** Representation of the percentage of L-Ara4N and pETN modified lipid A among the global modified lipid A for colistin resistant

15 dakika

Front. Microbiol. 11:1141.
doi: 10.3389/fmicb.2020.01141


AST
tanımlama
Direct AST from BC pellet
Rapid phenotypic tests
MALDI-TOF hydrolysis assays
Accelerate Pheno™ AST
Conventional AST
1H
24H
48H
T2MR
Magicplex™ Septifast
IRIDICA BAC BSI
SepsiTest™
QuickFish
Direct MALDI-TOF MS
FilmArray®
Xpert MRSA/SA BC assay
Accelerate Pheno™ ID
Verigene
iCubate iC-GPC Assay™
MALDI-TOF MS early subcultures
Conventionnal ID
Time for BC positivity
Ideal delay for empirical therapy
Critical delay for empirical therapy

SONUÇ

Moleküler yöntemlerin kısıtlılıkları neler?

- Pahalı
- Panele dahil olan patojen sayısı sınırlı

  Az görülen patojenleri saptamıyor
- Her örneğe çalışılmıyor, hasta alt grupları
- Seçili direnç genlerine bakılması:
  - mecA iyi
  - Diğerleri: tek direnç mekanizması yok.
  - Sonucun negatif çıkması direnci ekarte ettirmez

Moleküler yöntemler kültür ve fenotipik AST'nin yerini alamaz

ID ve AST yine de yapılmalı

Tanımlama:

- Panelde olmayan etkenler var
  Hedefte olmayan mo.ları saptayamaz
- **«non-viable DNA» saptanabilir**
- Bazı etkenlerin birbirinden ayrılmasında sıkıntılar
  - *S.pneumoniae-S.mitis/oralis*

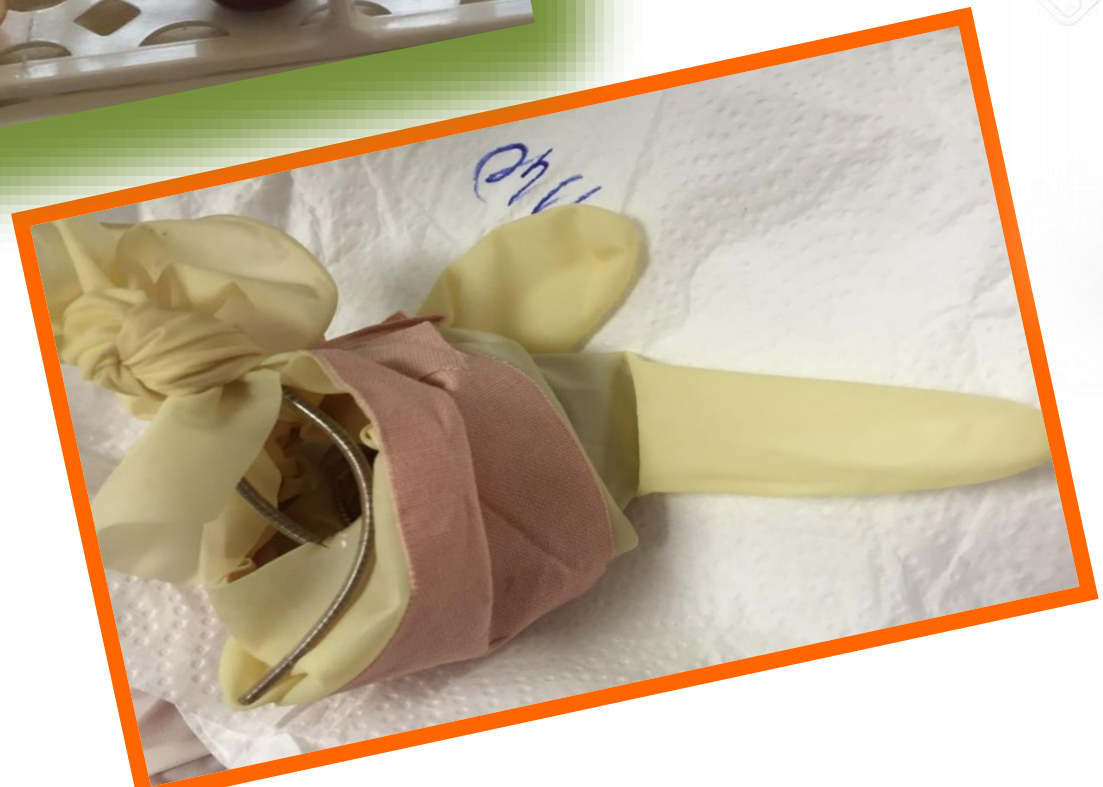
AST:

- Sadece panelde bulunan direnç genlerine bakar
  - Gram(+) ler için yeterli ama Gram (-): yetersiz
- Saptanan gen eksprese edilmiyor olabilir
- Direnç mekanizmaları arasında etkileşim olabilir
- Yeni direnç mekanizmaları gelişebilir
- Mutasyon meydana gelebilir

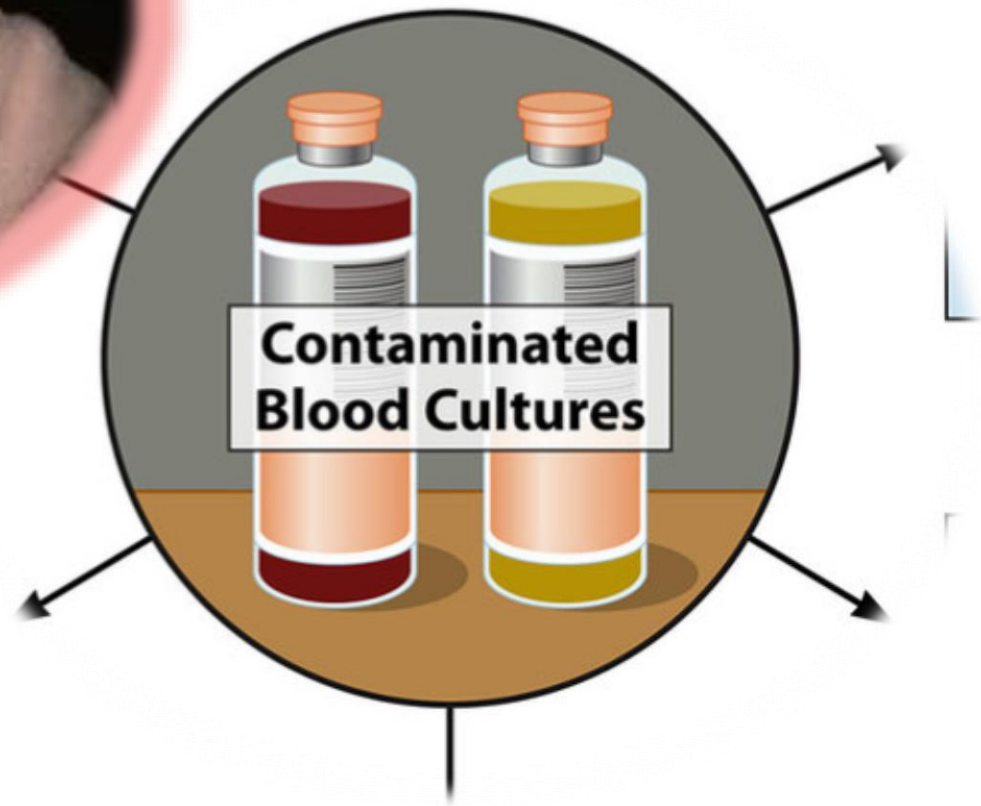

Öncelikle!!!

Her hastane

etken dağılımını ve direnç profilini saptamalı

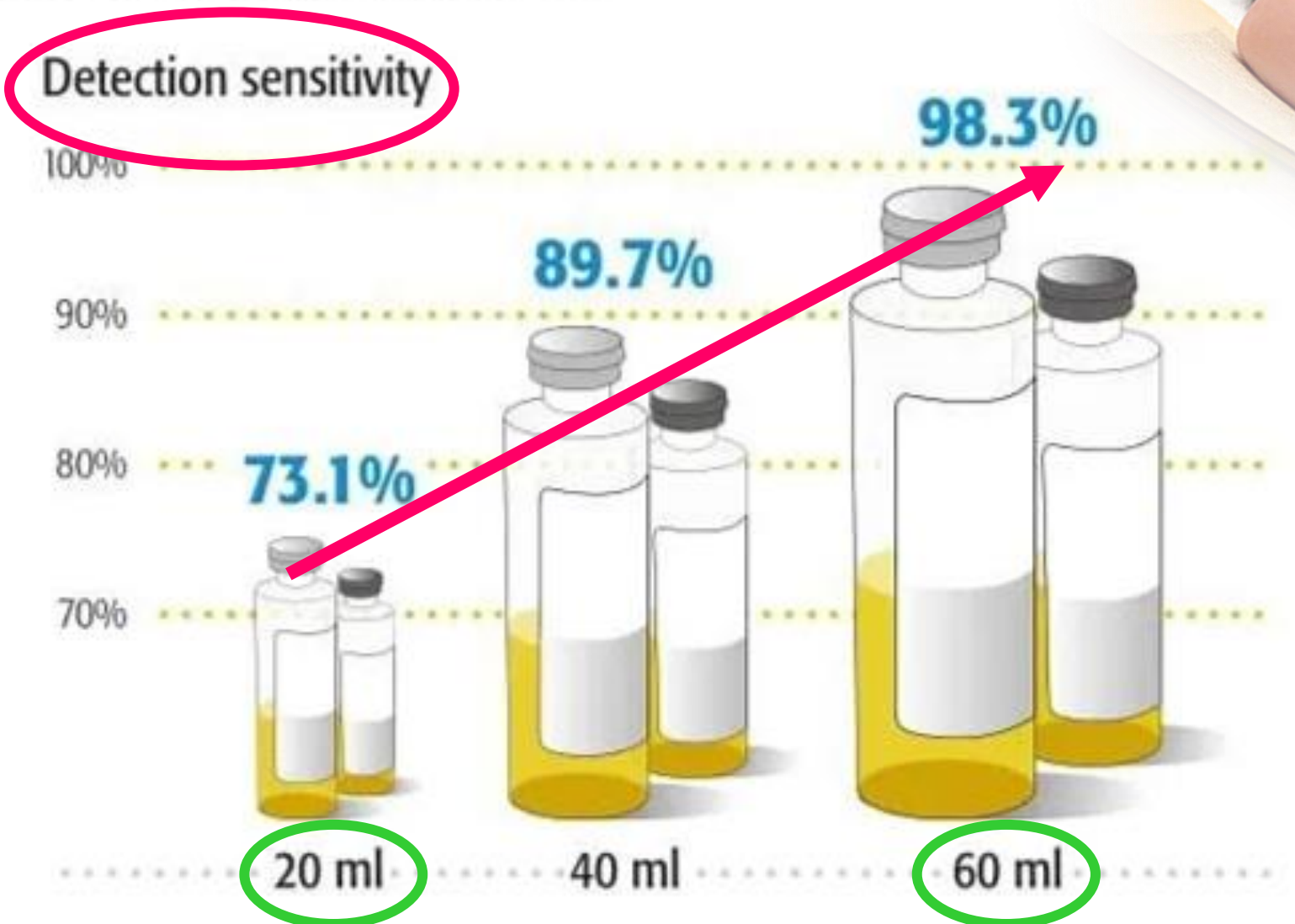



Kaliteli
örnek !


Contaminated
Blood Cultures

Cumulative sensitivity of blood culture sets

Adapted from Lee *et al*. Detection of Bloodstream Infections in Adults: How Many Blood Cultures Needed? J Clin Microbiol. 2007; 45:3546-3548



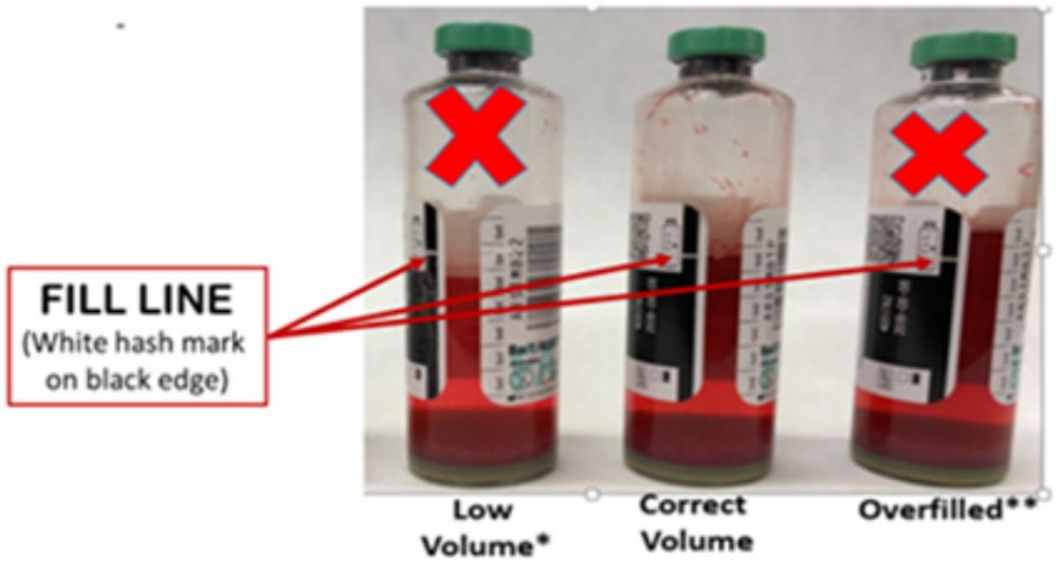
Controlled Evaluation of 5 versus 10 Milliliters of Blood Cultured in Aerobic BacT/Alert Blood Culture Bottles

MELVIN P. WEINSTEIN,[1,2]* STANLEY MIRRETT,[3] MICHAEL L. WILSON,[3,4]†

nonfermentative gram-negative rods, or yeasts. When both bottles were positive, the bottles inoculated with 10 ml of blood showed growth sooner ($P < 0.001$). Earlier detection with 10-ml inocula was especially notable for

Optimal Adult Fill Volume for Bottles = 10 ml/bottle

For children collect no more than 1% of total blood volume



- **Under**-filling can cause possible **false negative** results.
- **Over**-filling could also cause **inaccurate results**.
- **Hold bottle upright** and **watch volume closely** during draw!
- Pre-set vacuum pressure in the bottles will draw >10ml.

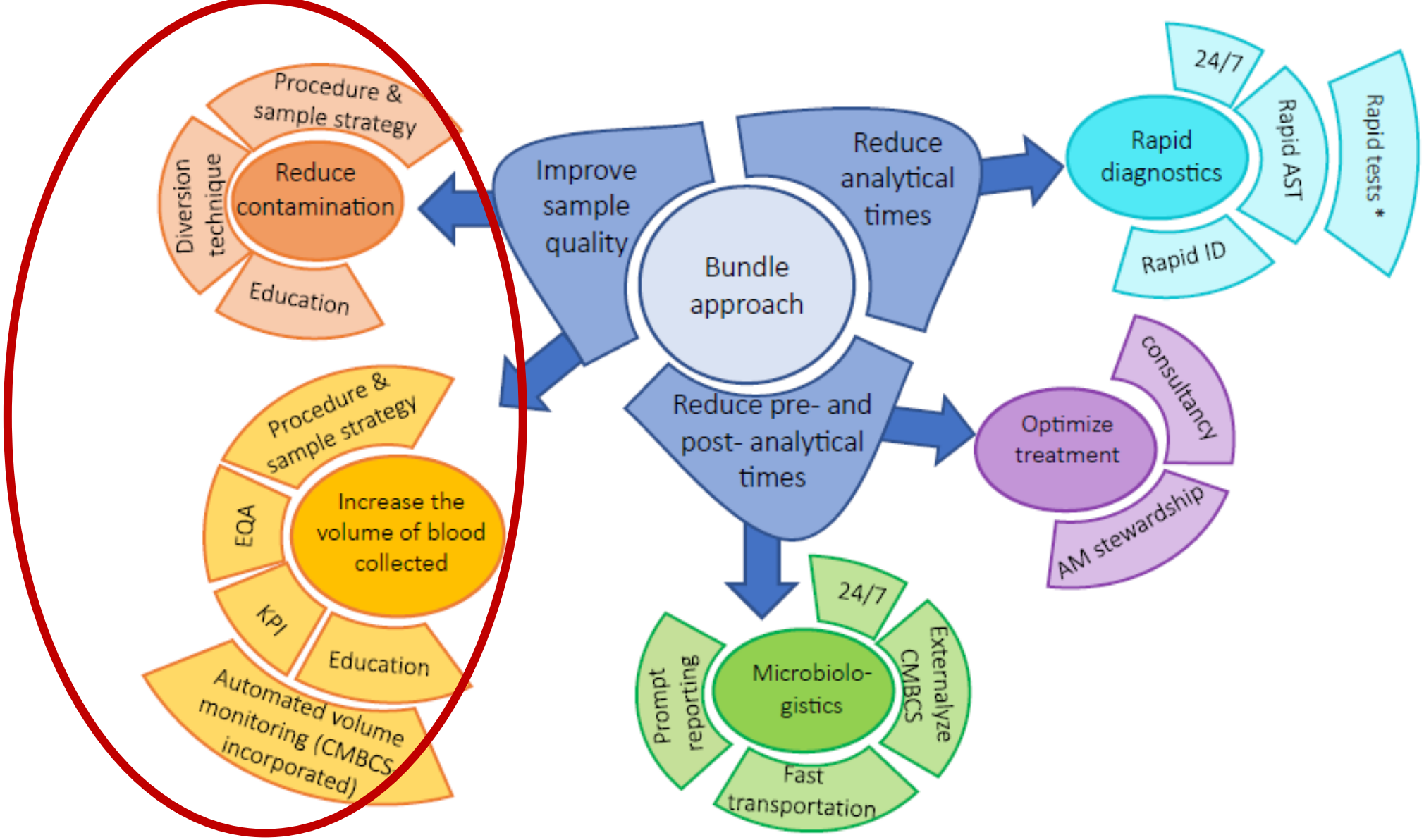


Fig. 1. Summary of all the actions to improve the bloodstream infection pathogen diagnostics. Types of actions belong to three complementary axes and actions aim to manage sample quality, times before and after analysis and analytical times. Each action per se is associated with a limited improvement but combination of several actions significantly improves diagnosis. Improvement is maximum when programme include actions on sampling quality, rapid diagnostics and logistics. KPI, key performance indicator; EQA, external quality assessment; CMBCS, continuous-monitoring blood culture system; AM stewardship, antimicrobial stewardship. *Rapid tests (e.g. *mecA* detection) may be needed in area of high level of resistance.