KAN DOLAŞIMI ENFEKSİYONLARININ TANISINDA SENDROMİK YAKLAŞIM

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

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]inberger,[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

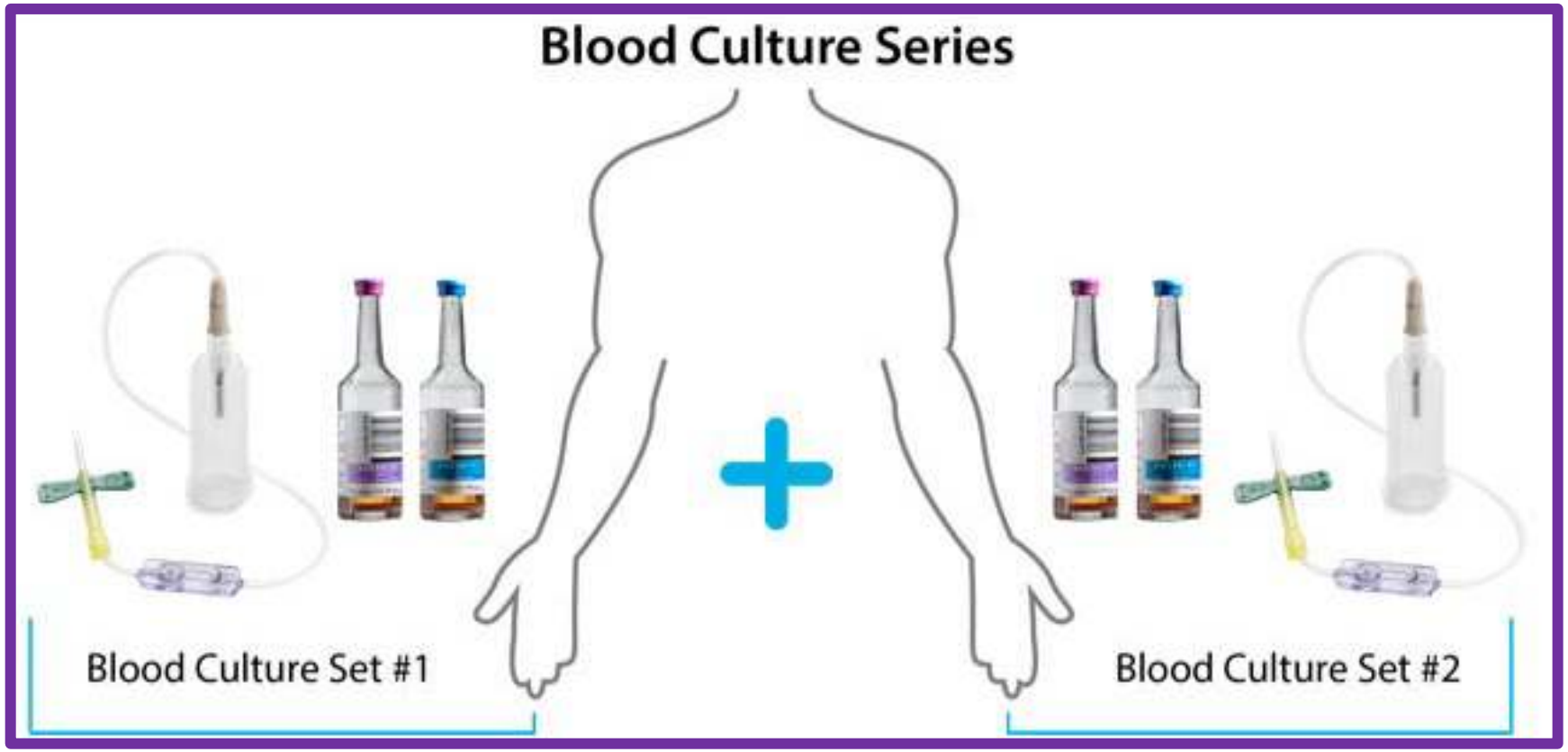
Karina Siewers BSc[1,2] | S M Osama Bin Abdullah MD[3] |

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:

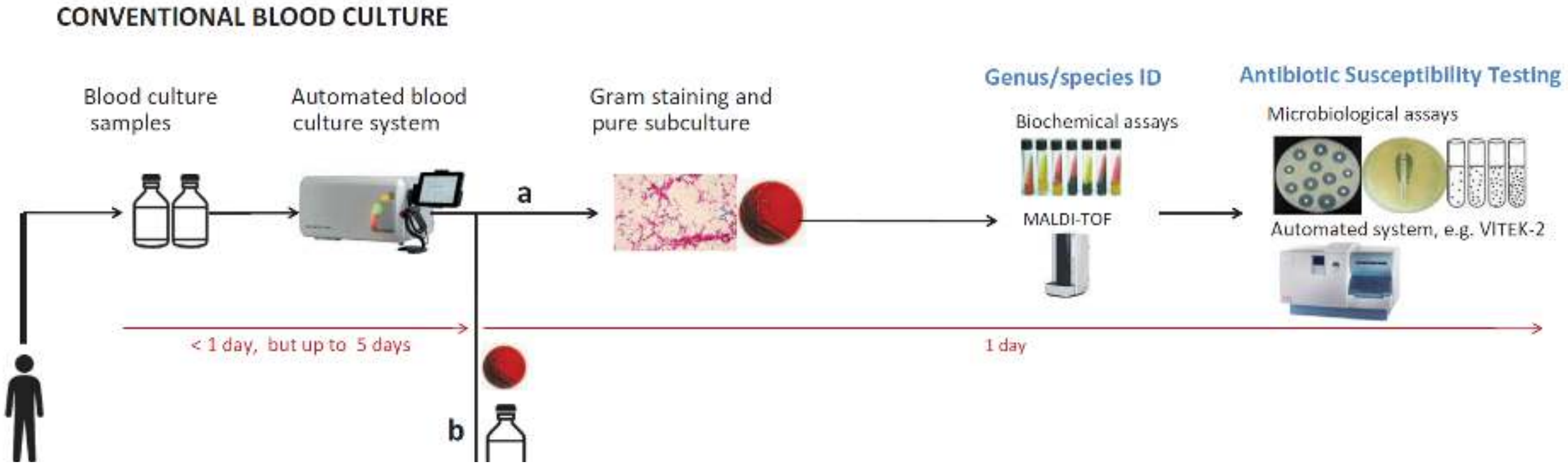
40-80 ml

2-4 set kan kültürü

GRAM BOYAMA

Kan kültürü SİNYAL verdikten sonra

When a blood culture is positive,
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 24-48 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

Üreme sinyali alınan
kan kültürü şişesinden

2

Direkt kandan

Pozitif sinyal veren Kan kültürü şişelerinden tanımlama

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

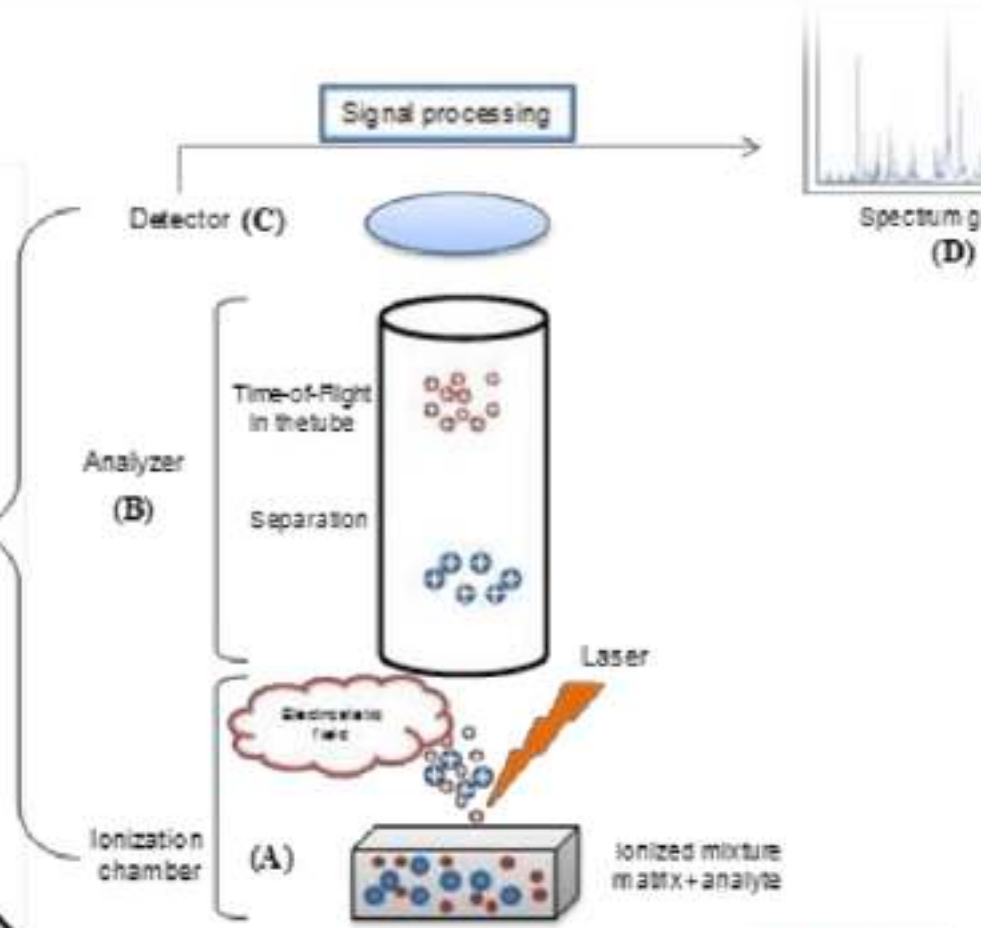
Tam kandan tanımlama

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

Pozitif kan kültüründen direkt tanımlama

Pozitif KK şişesinden tanımlama

1 MALDI-TOF MS



HIZLI

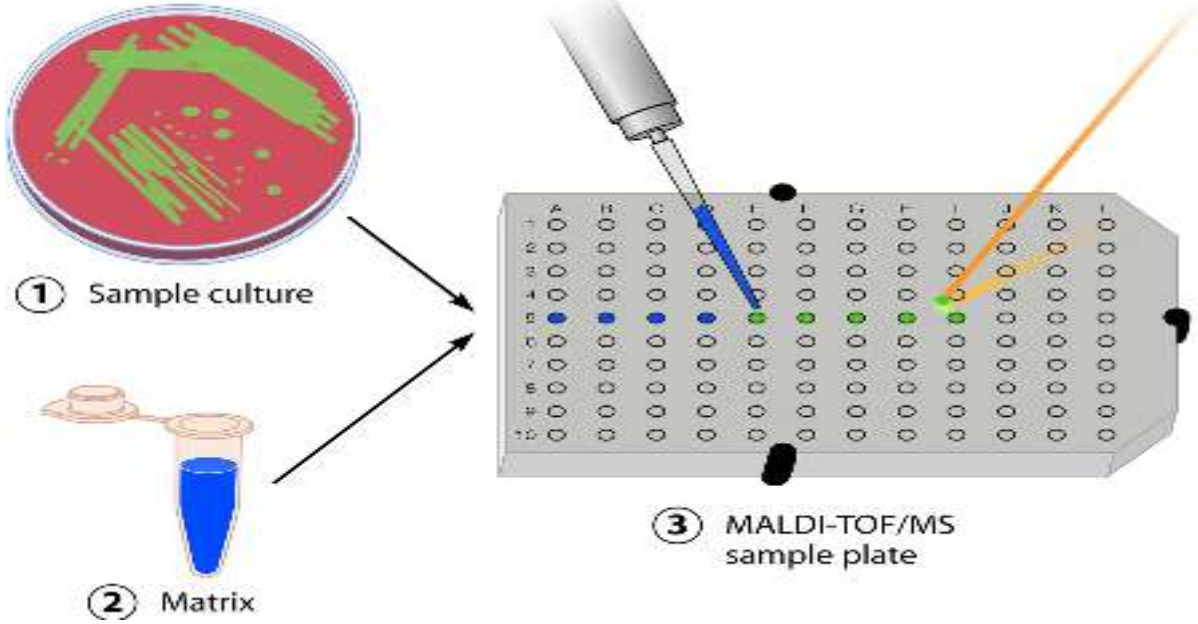
Workflow step	TIME (min)		
	5 izolat	24 izolat	96 izolat
Application on plate	1	5	16
Application matrix	1	3	10
Drying	2	2	7
Read in system	5	12	43
Time to result			
TOTAL	**9**	**22**	**76**

Dakikalar...

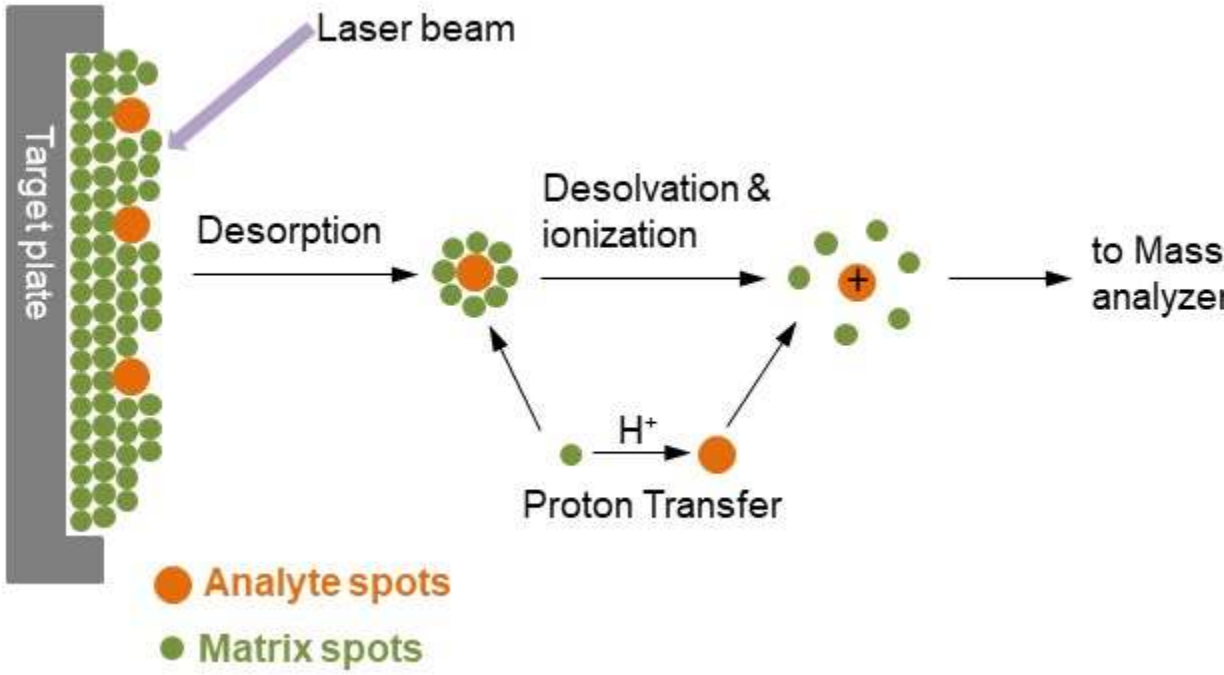
Çalışma Prensipi nedir?

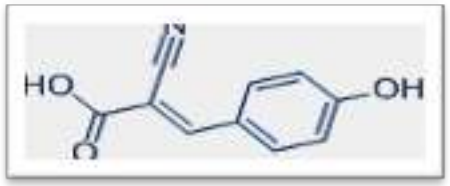
Matrix Assisted Laser Desorption

Ionization-Time of Flight/Mass Spectrometry



PEPTİT PARMAK İZİ

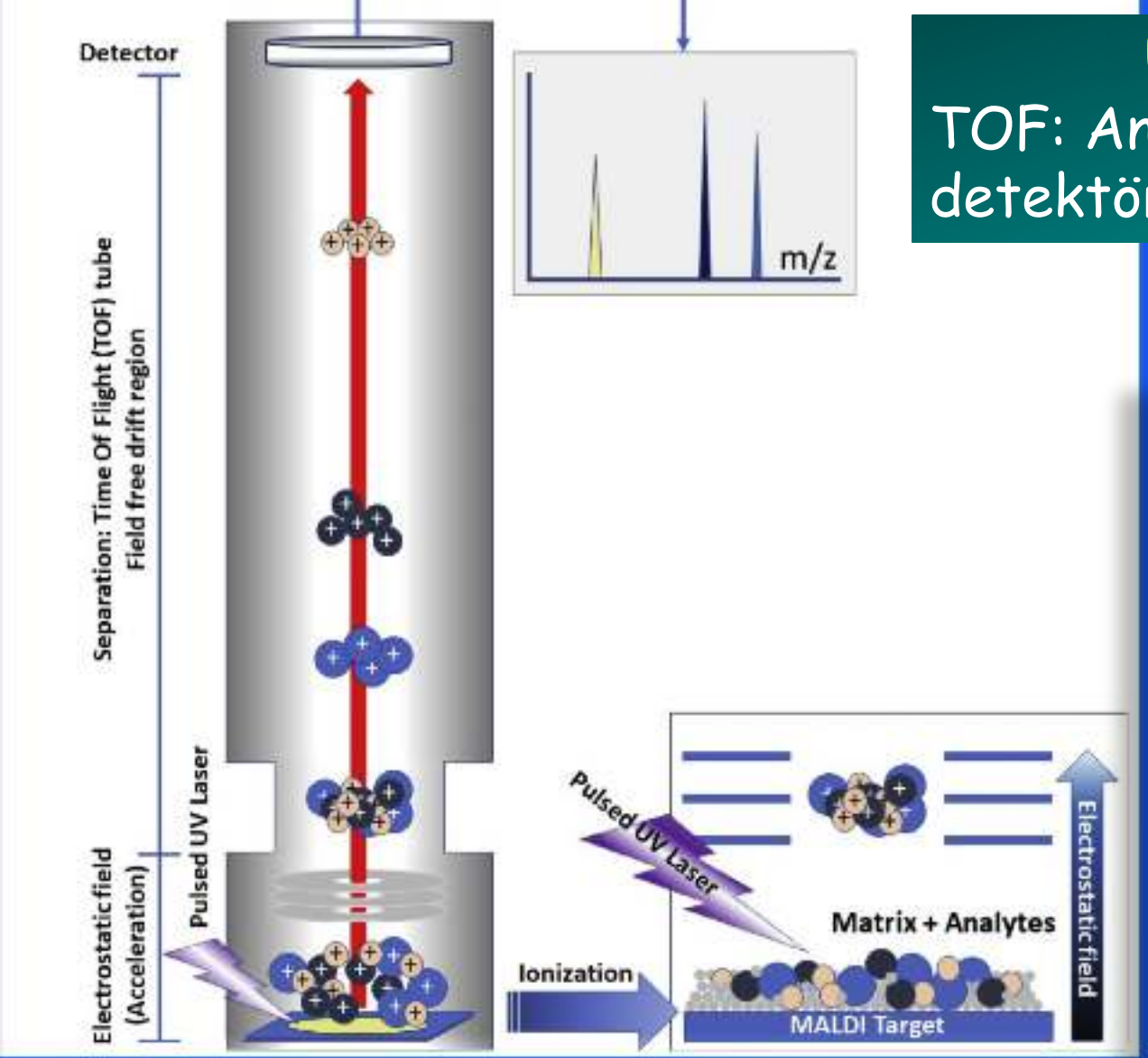


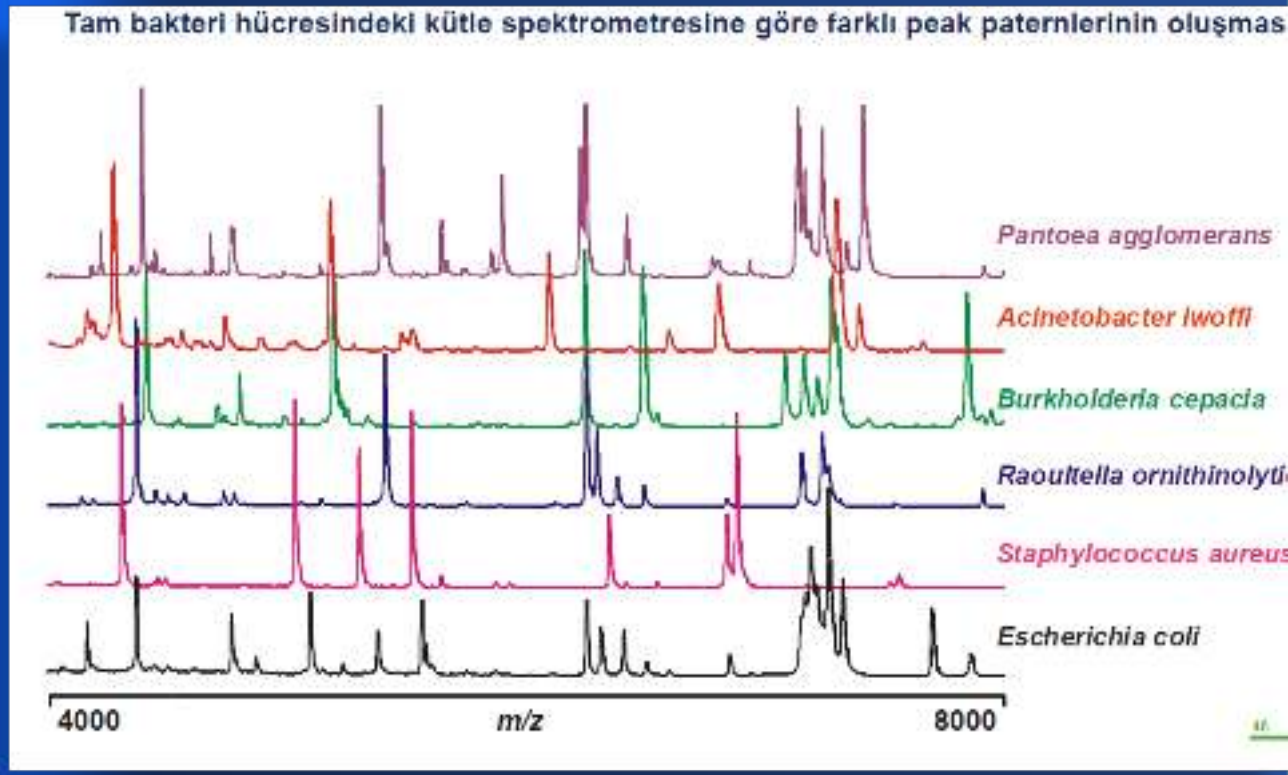


α-cyano-4-hydroxycinnamic acid

Uçuş tüpü

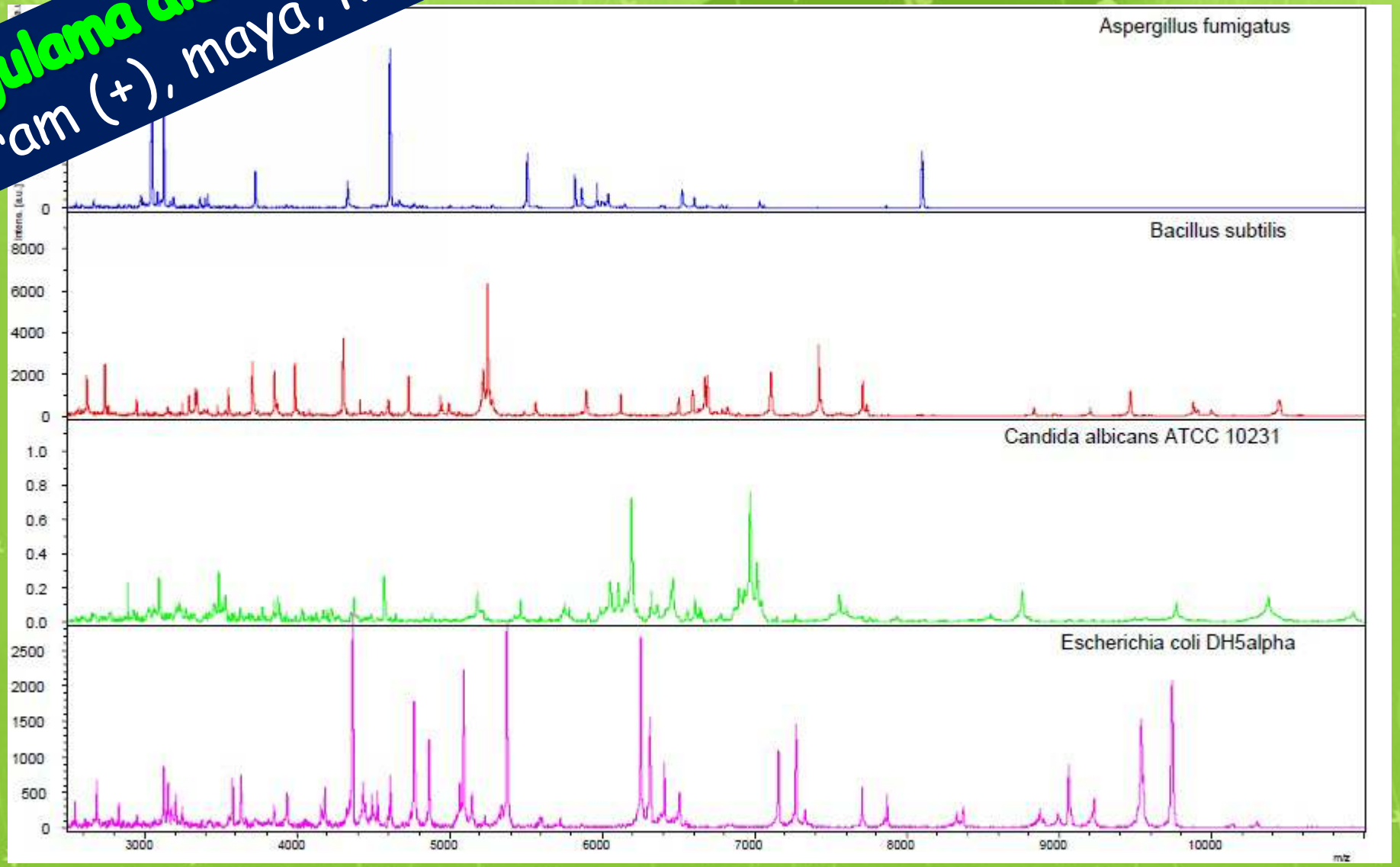
TOF: Analitlerin detektöre ulaşma zamanı





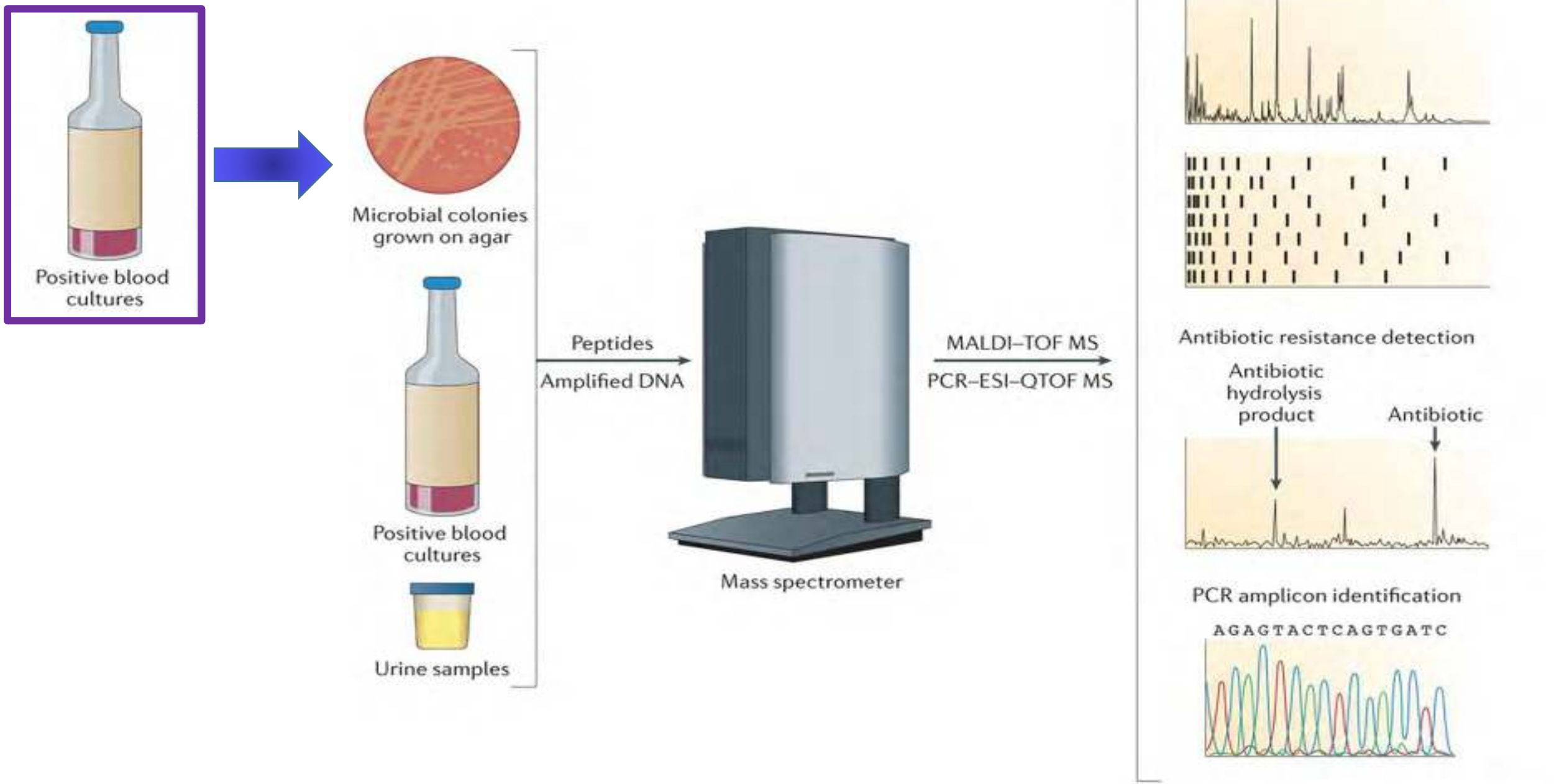
Yaygın uygulama alanı var:

Gram (-), Gram (+), 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 besiyerinde kısa inkübasyon* m.g. üreme

   Ucuz ve basit

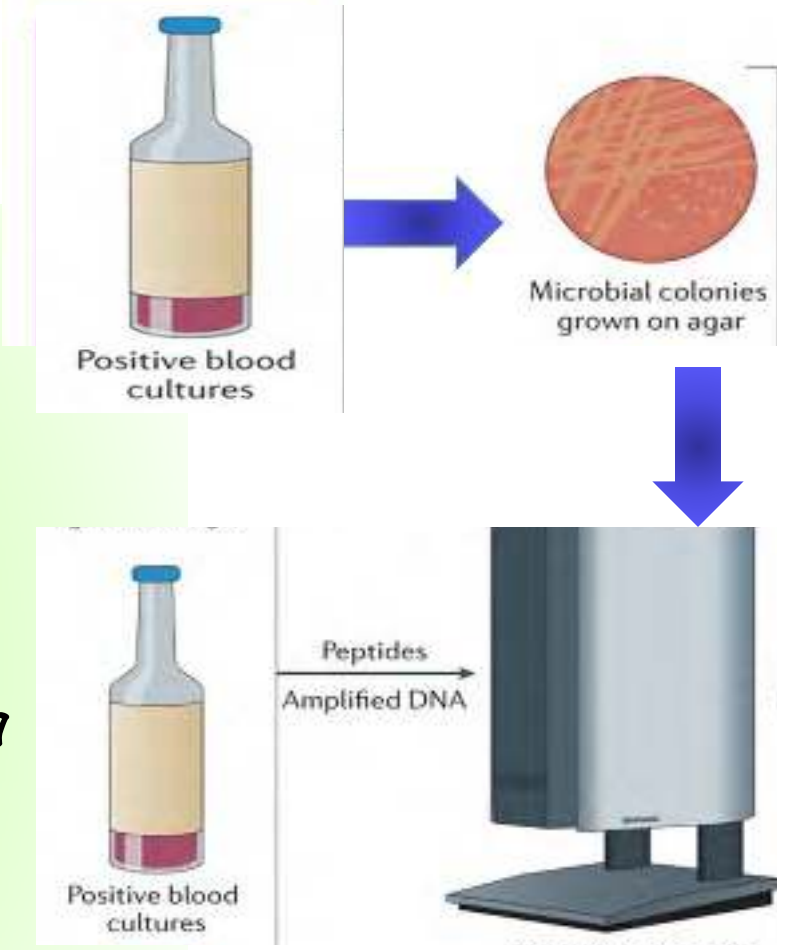
   Gr (–) için: 2 saat

   Gr (+) için: 6 saat

   Anaerop başarısız: 4-6 sa %50 tanımlama

2) *Direkt kan kültürü sıvı besiyerinden tanımlama*

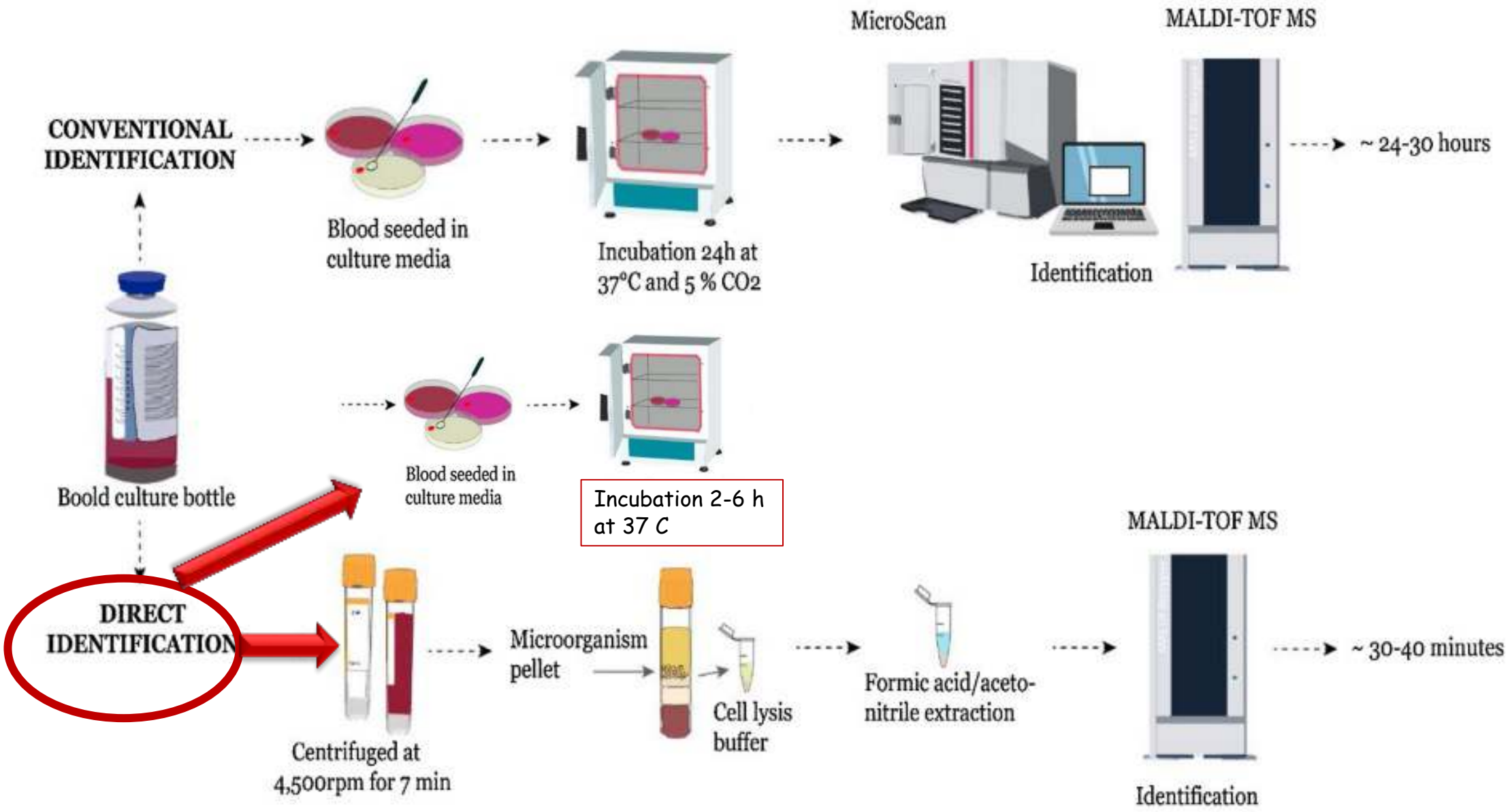
   Lizis, santrifügasyon, fitrasyon, protein ekstraksiyonu



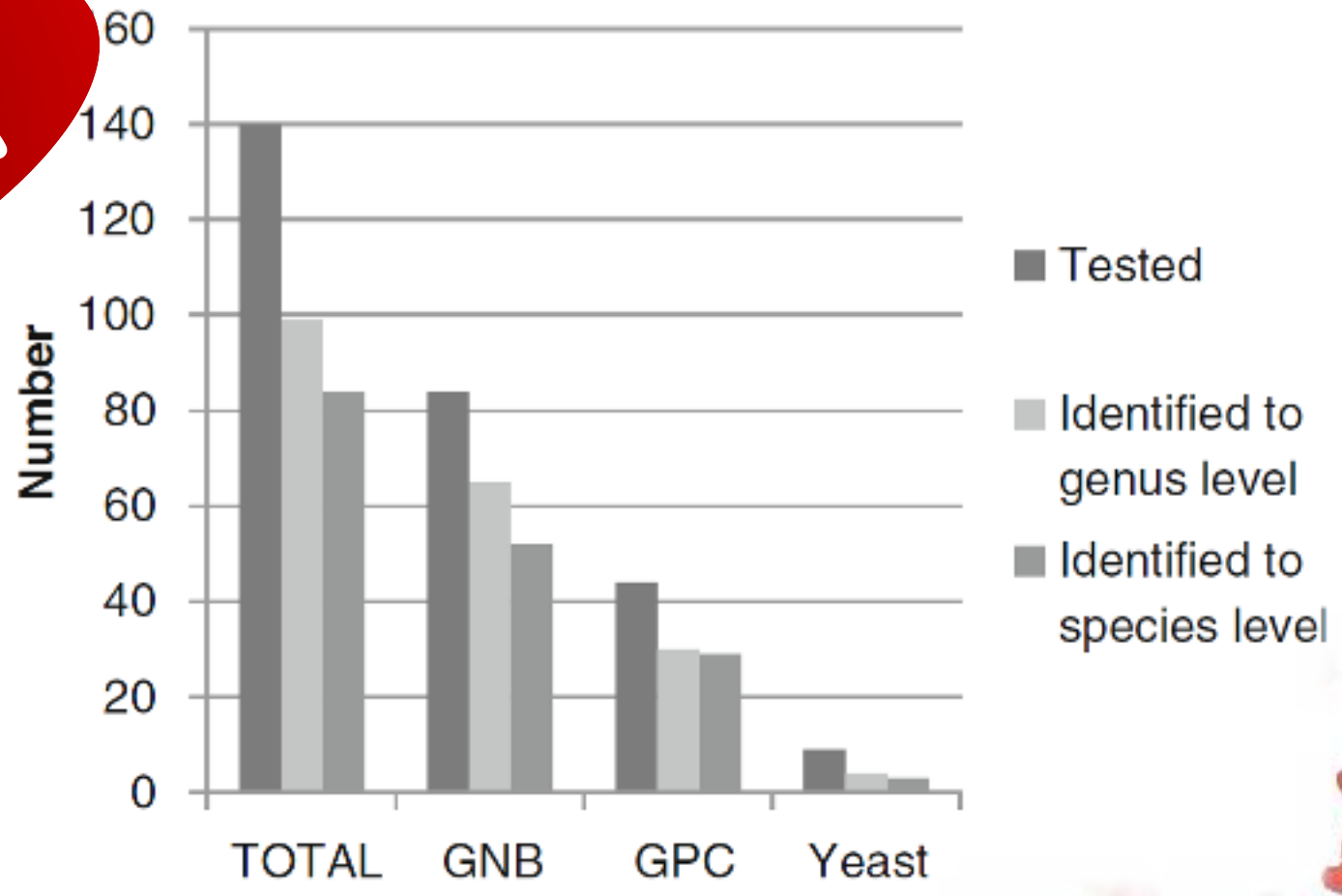
Bruker SepsiTyper®
bioMerieux Vitek®MS Blood Culture kits

Pozitif KK şişesinden tanımlama

1 MALDI-TOF MS

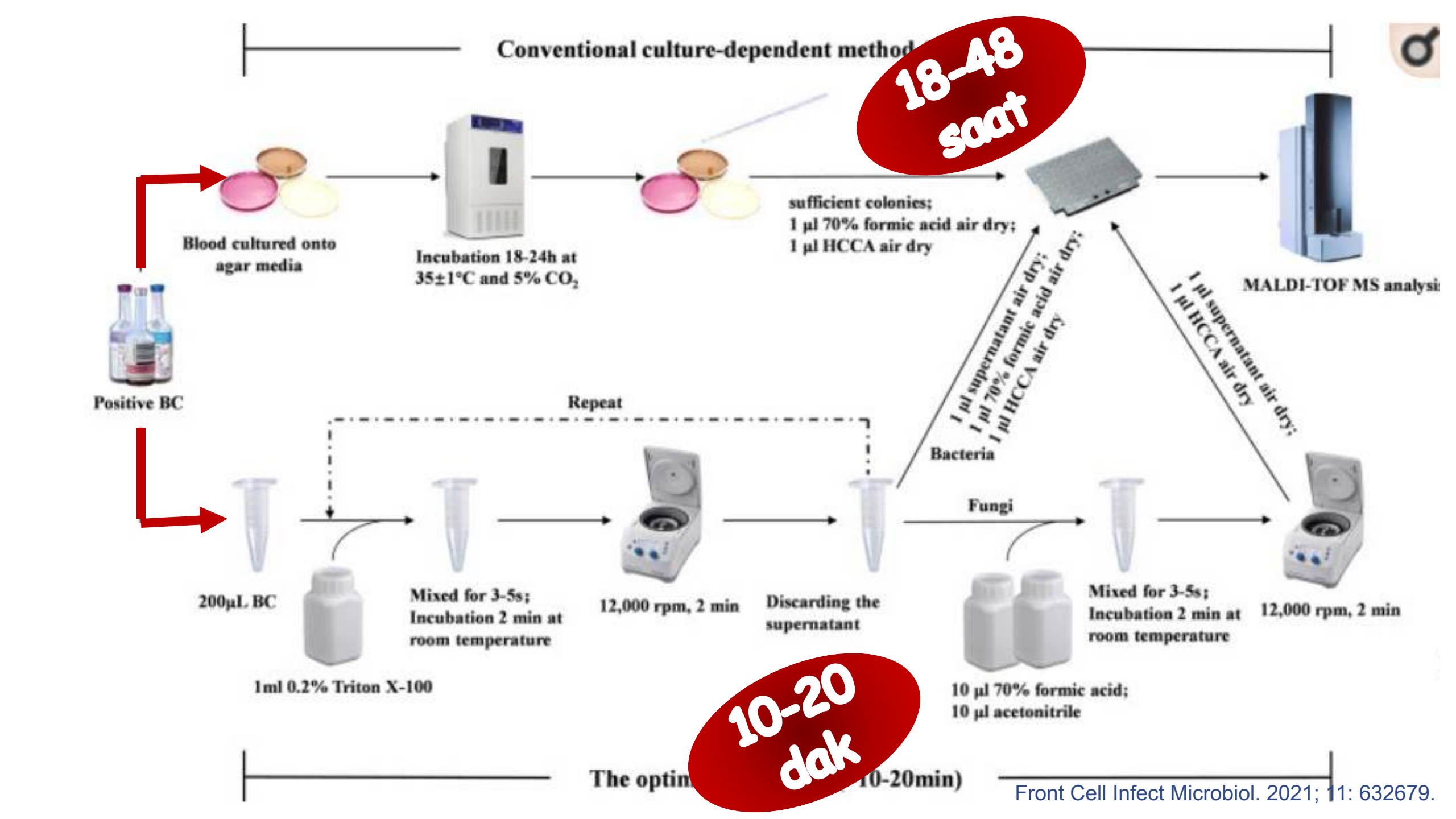


Direkt kan kültüründen



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

Direkt kan kültürü şişesinden
MALDI-TOF ile tanımlama yapmak
ne kadar süre kazandırıyor?



Front Cell Infect Microbiol. 2021; 11: 632679.

Blood culture
bacterial pellet

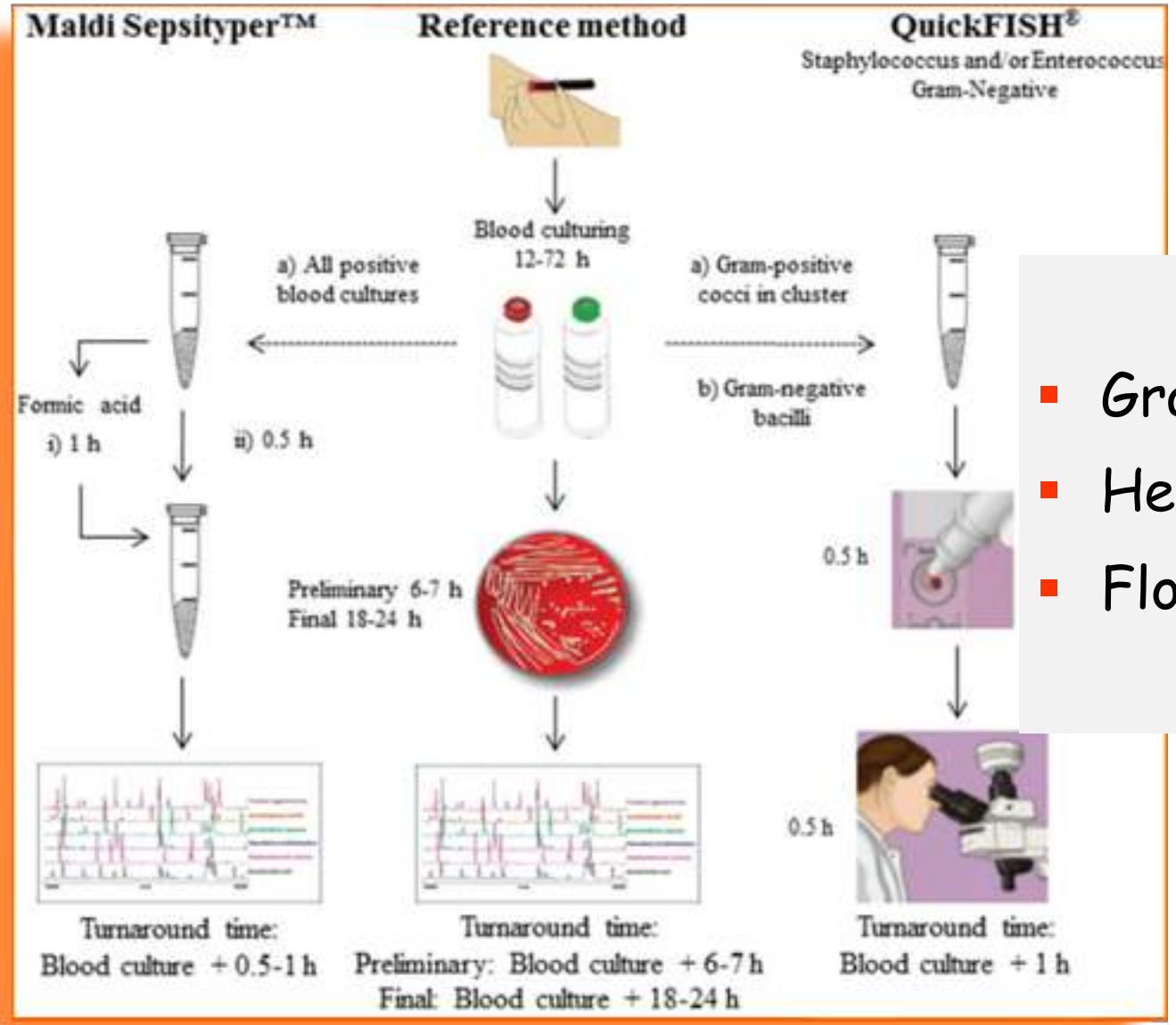
Gram staining ➡ **≤1h**
> 100% accurate

MALDI-TOF MS ➡ **≤1h**
> 99% accurate

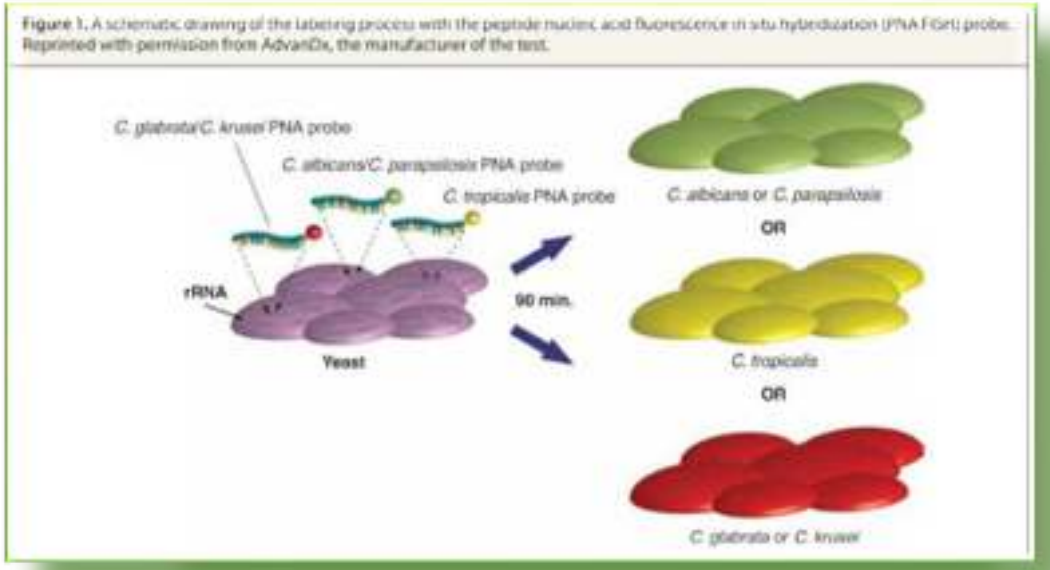
Pozitif KK şişesinden tanımlama

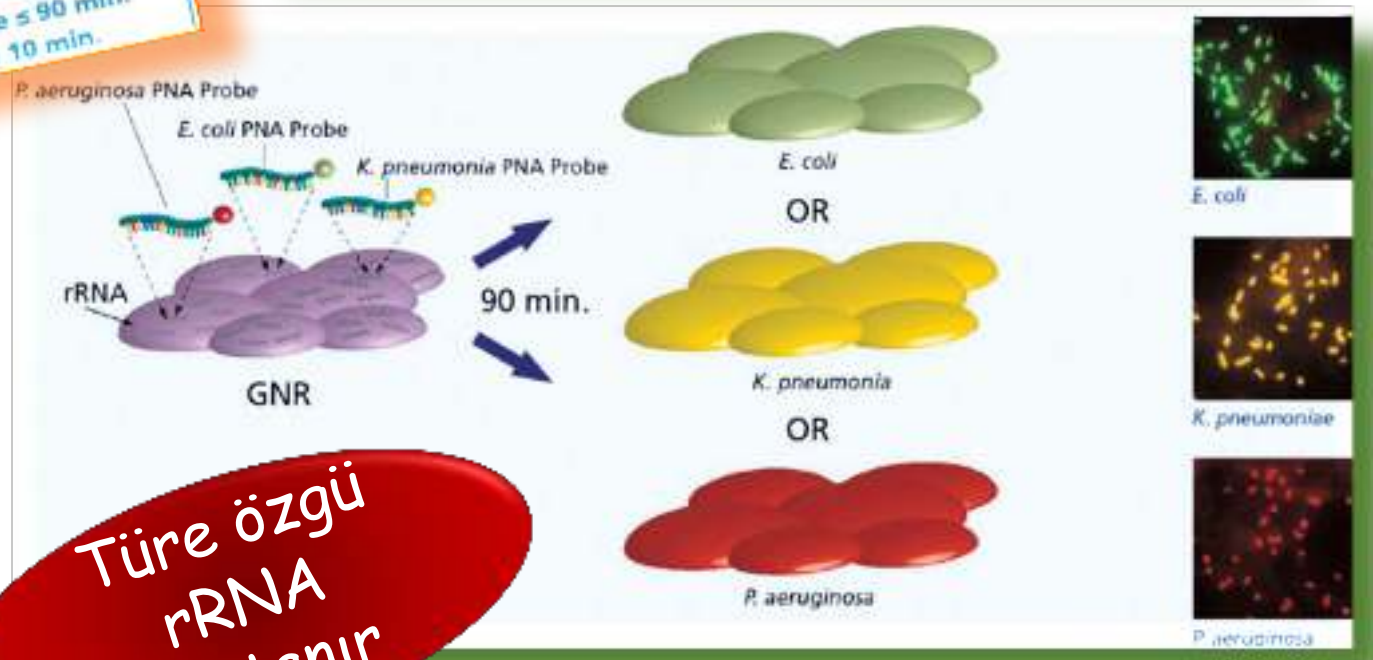
2

Floresan insitu hibridizasyon



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





Once a blood culture turns positive

⬇

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 ate MSSA from sidentified as n occur with mixed th prolonged times nen 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		(*C. tropicalis*); Sp: 100%	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*

QuickFISH

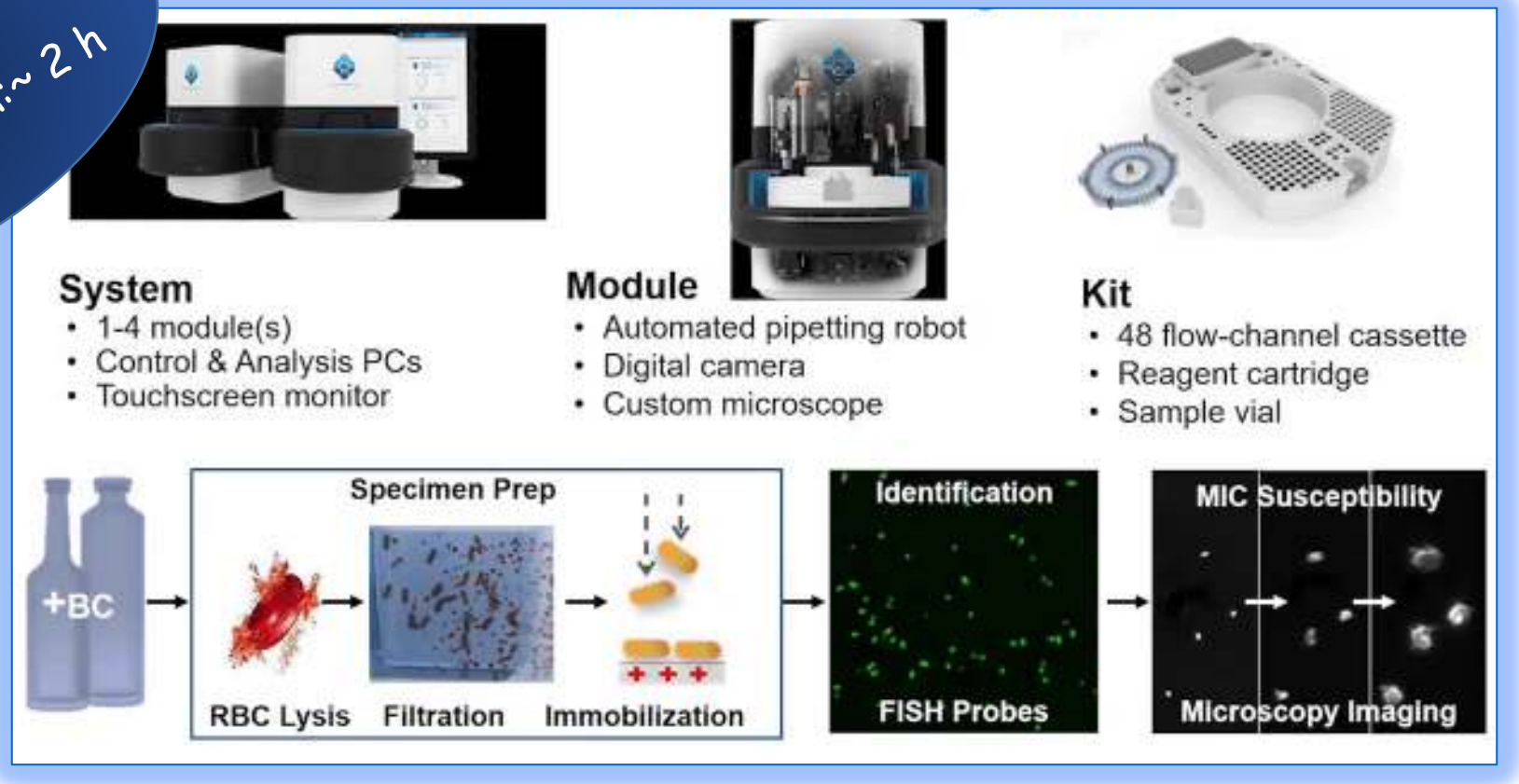
- 20-30 dak
- Panel: sınırlı mo
- Direnç saptamaz
- MRSA/MSSA ayrımı yok
- C.glabrata/krusei ayrımı yok

PNA FISH

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

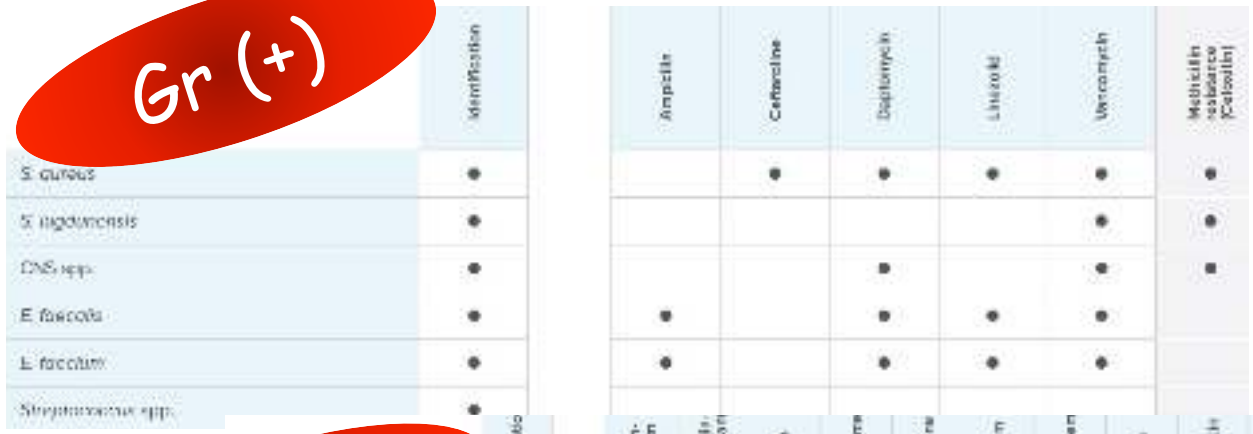
Accelerate Pheno™ System

AST : ~ 7 h
Identification:~ 2 h



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

Gr (+)



Gr (-)

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. sefalo
- P.aeruginosa: sonuçları iyi değil

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 susceptibility testing by time lapse record o bacterial grow	*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 .): *ginosa*): *mannii*): *ella* spp.): : 97.3%; op.): *s* spp.): *acter* 00% %; Se %; Sp:	Only platform to provide MIC-based resistance information (rather than resistance genes information that may not correlate with phenotypic resistance)	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, Streptokok spp: AST yok
- Tanımlama: 90 dak
- AST: 6.5 saat
- Panel kısıtlı
- Pnömokok-diğer Strep ???

Pozitif KK şişesinden tanımlama

3

Mikroarray

- **Verigene™ system (Luminex, USA)**

13 Gr(+)
9 Gr(-)
9 Direnç geni

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

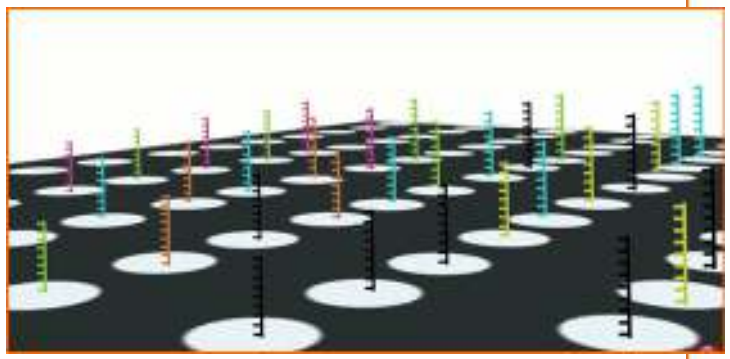
*Escherichia coli**
Klebsiella pneumoniae
Klebsiella oxytoca
Pseudomonas aeruginosa
*Serratia marcescens***

Genus

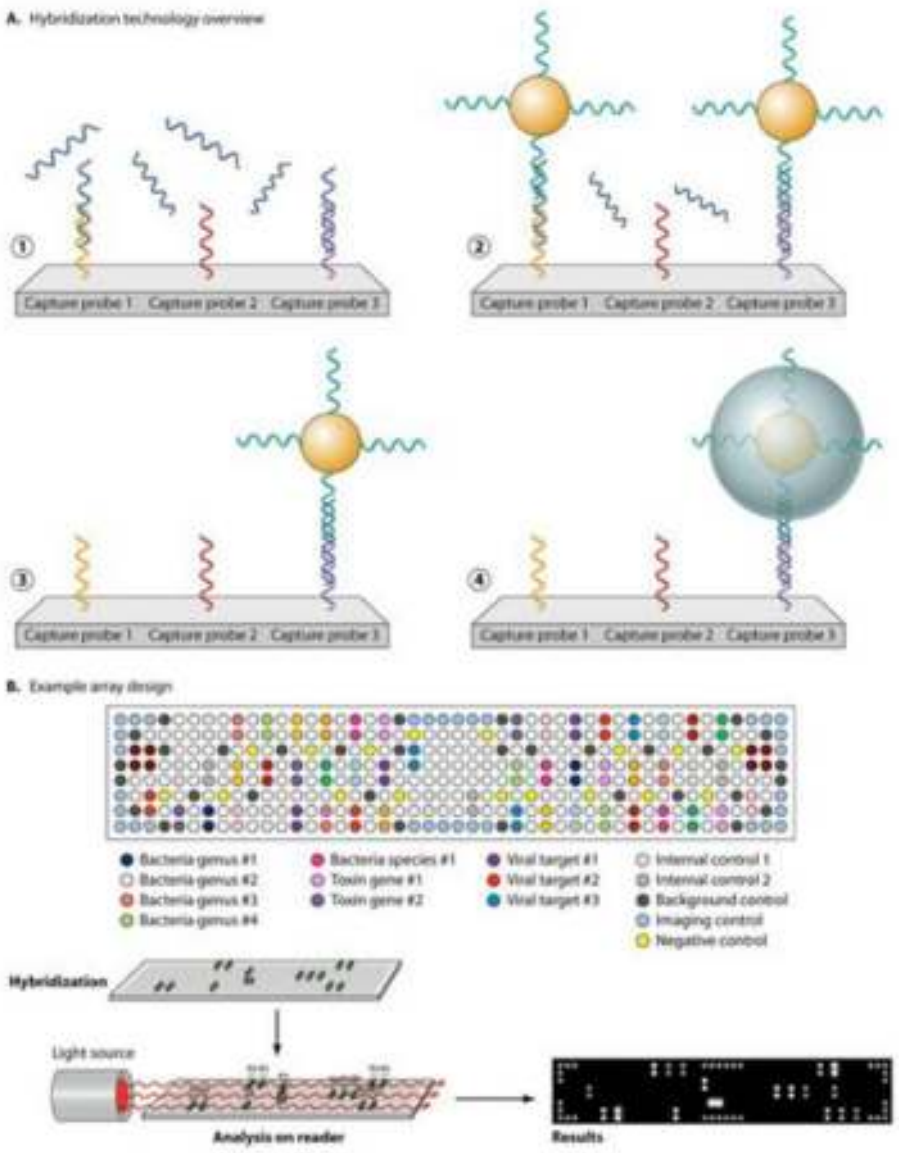
Acinetobacter spp.
Citrobacter spp.
Enterobacter spp.
Proteus spp.

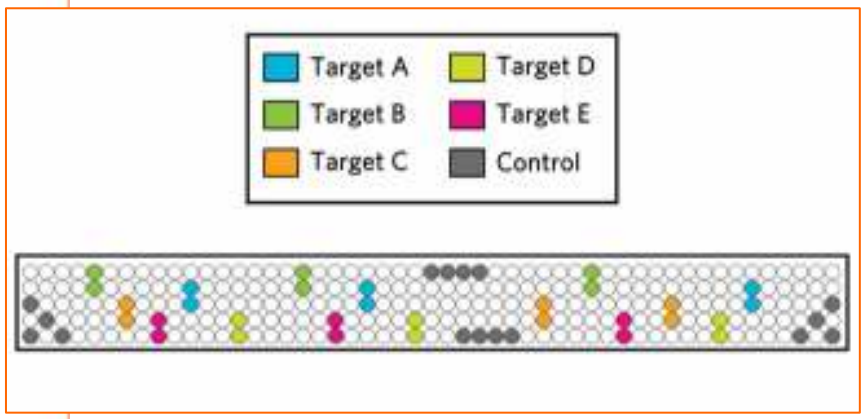
Resistance

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



Verigene solid-phase microarray.





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:* E (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

4

NA amplifikasyon

FilmArray®

(BioFire Diagnostics, Salt Lake City, USA)

- Nested multiplex PCR
- DNA is analyzed through melting curves

1 saat

TEK PANEL

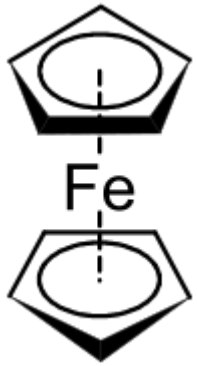
Manufacturer, assay	Methodology	Organisms panel	Direct genotypic or phenotypic resistance information	Turn around time hands-on time throughput limit of detection (LOD)[a]	FDA sensitivity and specificity information (in comparison with culture)[a]	Advantages	Drawbacks
BioFire, FilmArray Blood Culture 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

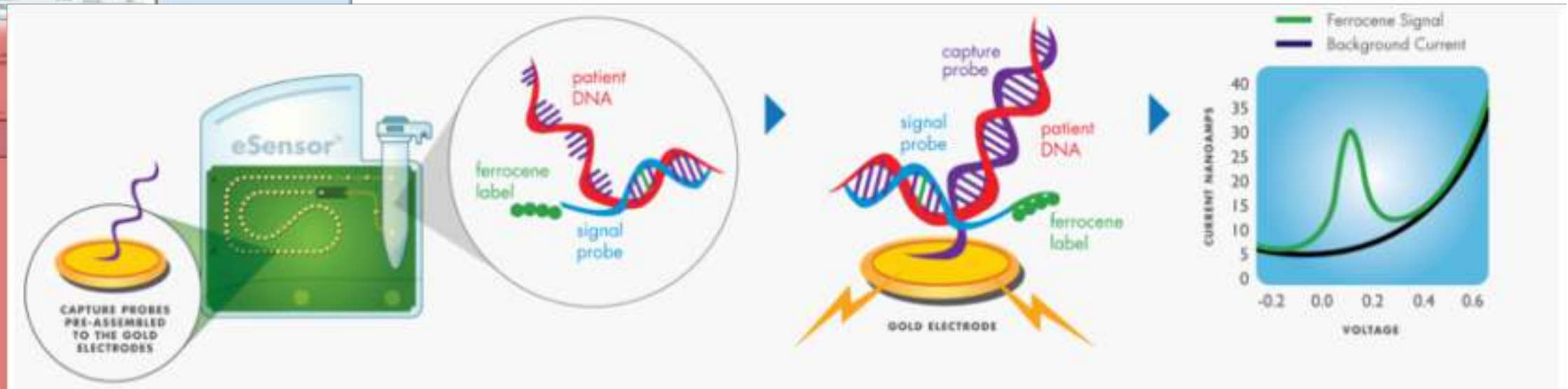
FilmArray

- TAT: 1 saat
- Geniş Panel
  - 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*

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*, *mecC*), Vancomycin (*vanA*, *vanB*	90 min 2 min hands-on time May process 3, 6, 12, 18,	*Bacteria* Se (*Bacillus cereus* group): 98.3%; Se (*B. subtilis*	Very expanded organism panel Provides expanded	Relatively low sensitivity for *Corynebacterium* spp. Lower sensitivity for *vanA* detection in *feacalis* (88%) negatives can occur with mixed tures

Manufacturer, assay	Methodology	Organisms panel	Direct genotypic or phenotypic resistance information
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), Carbapenem (KPC, IMP, NDM, OXA, VIM)

cks
ccur with mixed
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

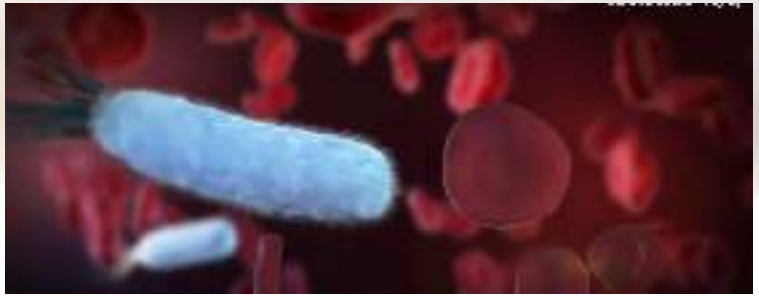
FDA-cleared multiplex blood culture panels include

- 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

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

1

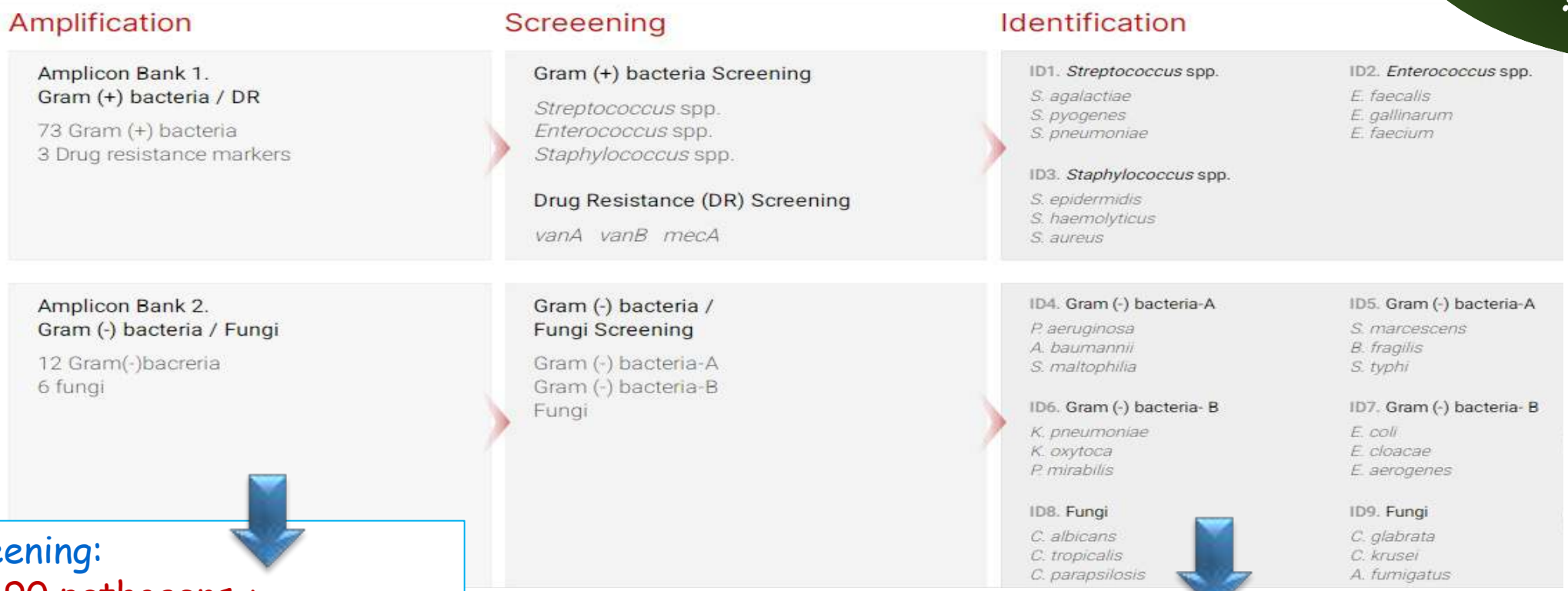
NA amplifikasyon

Magicplex™ system (Seegene, G.Kore)

- Multipleks Realtime-PCR
- 1 ml kan
- TAT: 3-6 h
- Duy⬇ (%29-47); Özg:⬆ (%95)

Magicplex™ system

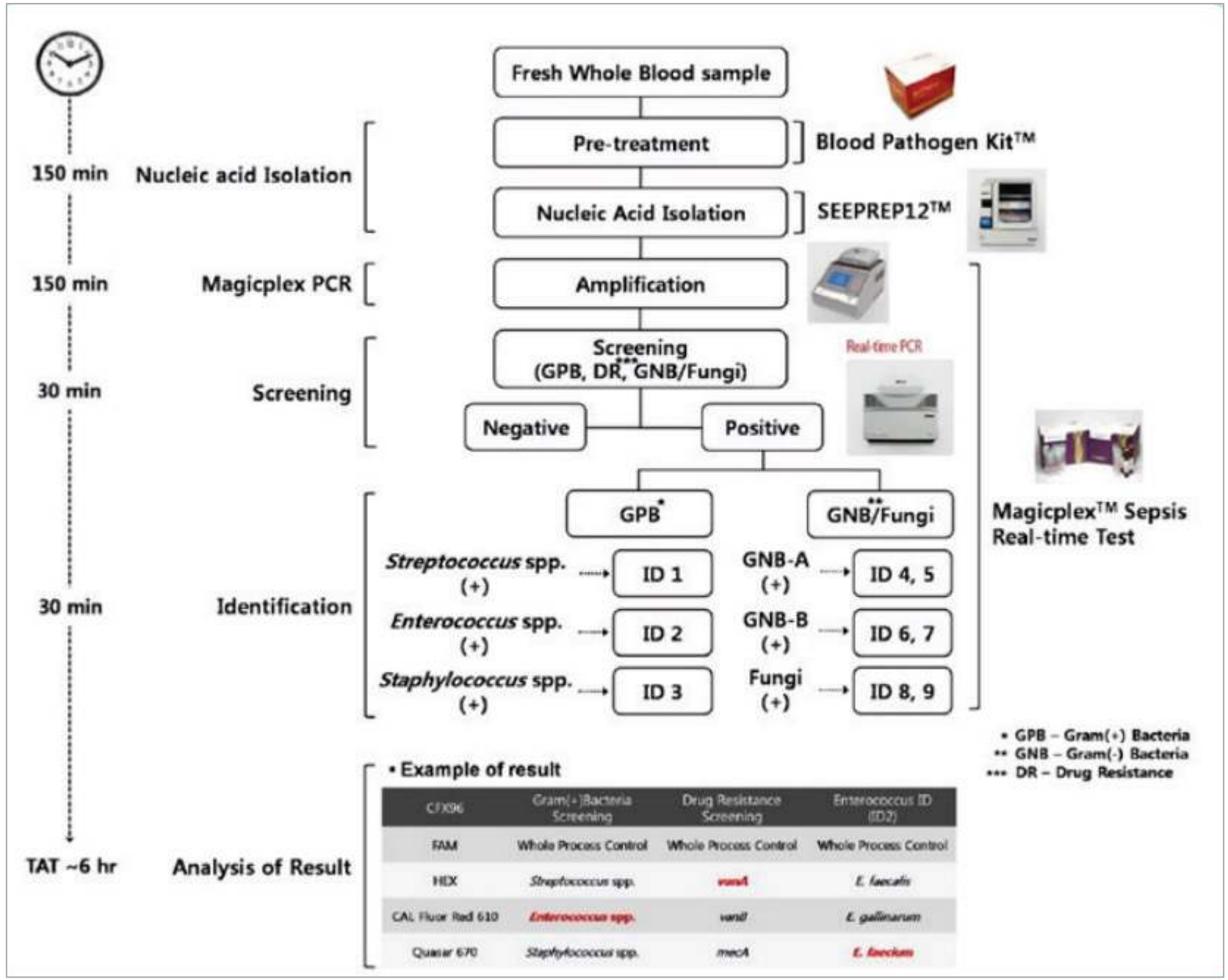
Gr(-) direnç ??



Screening:

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

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

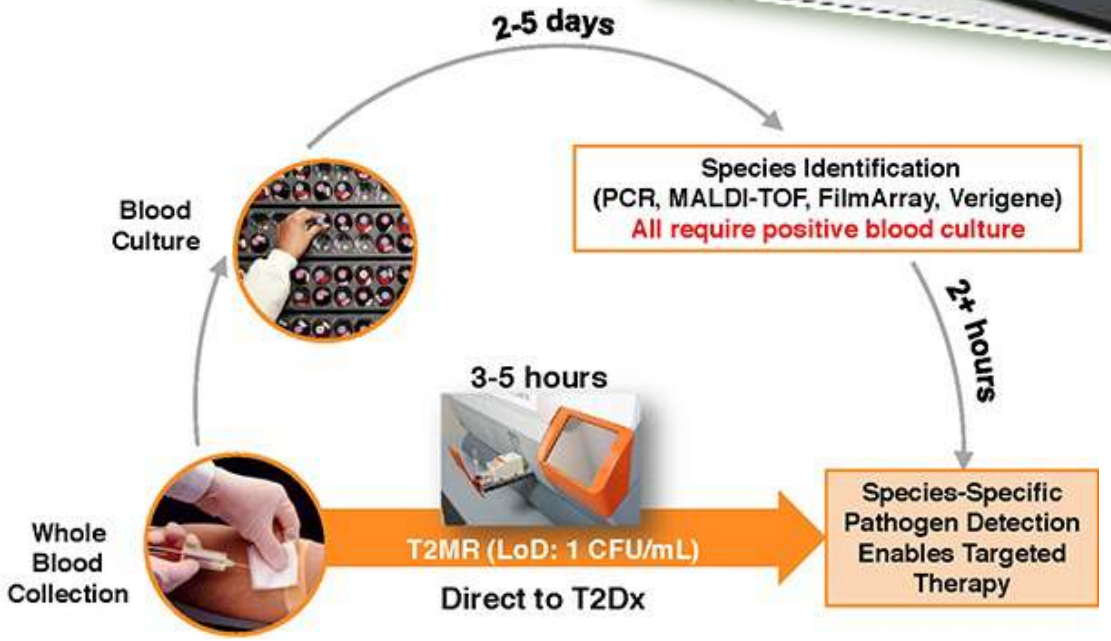

Fresh Whole Blood sample
Pre-treatment
Blood Pathogen Kit™
Nucleic Acid Isolation
SEEPREP12™
150 min
Nucleic acid Isolation
150 min
Magicplex PCR
Amplification
Real-time PCR
30 min
Screening
Screening (GPB, DR***, GNB/Fungi)
Negative
Positive
GPB*
GNB/Fungi**
Magicplex™ Sepsis Real-time Test
30 min
Identification
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
* GPB – Gram(+) Bacteria
** GNB – Gram(-) Bacteria
*** DR – Drug Resistance
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

Tam kandan tanımlama

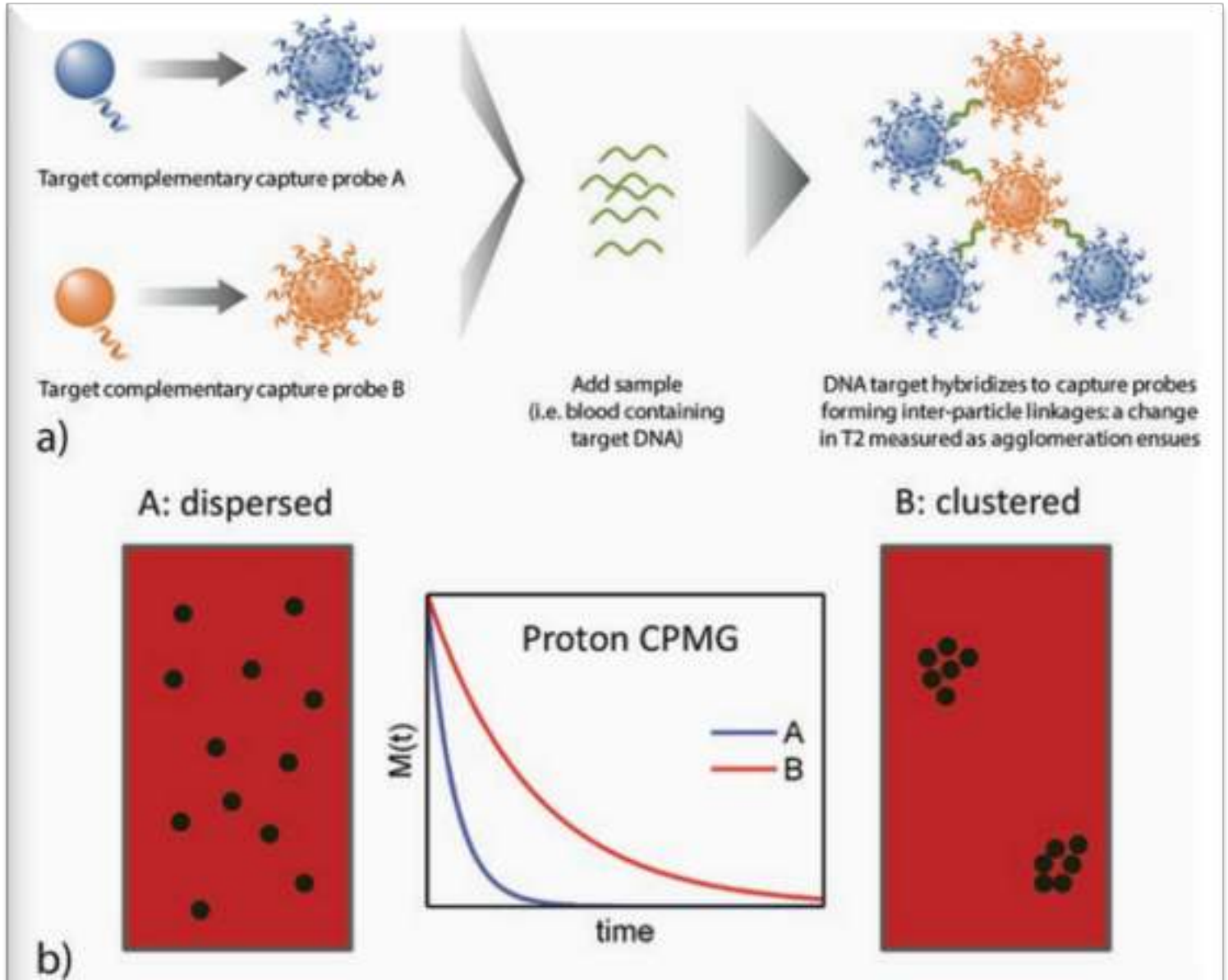
2 T2 magnetik rezonans

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

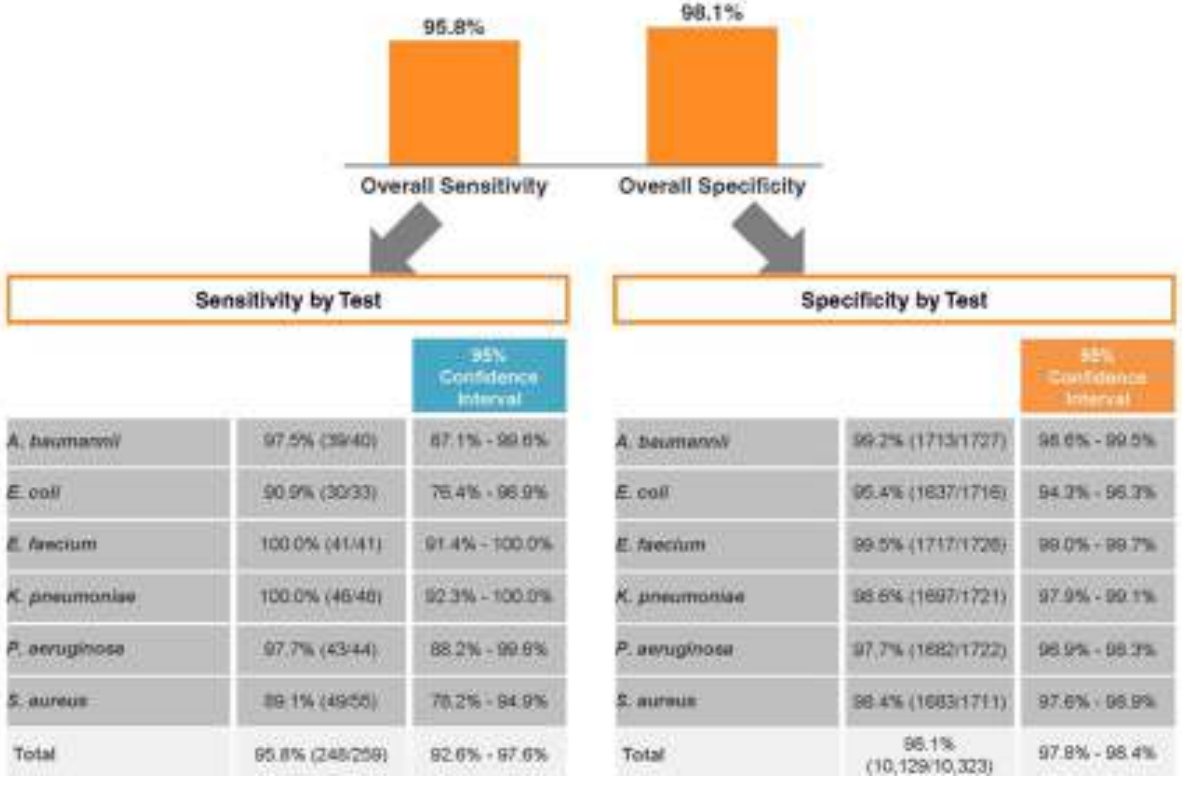
Sensitivity: 90%[1,*]
Specificity: 98%[1,*]

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

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*.



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

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 'Breakthrough Device' designation
	T2Cauris™ (T2Biosystems)	5	*Candida auris*, *Candida duobushaemulonii*, *Candida haemulonii*	—	Fully automated Trained personnel	89%/98%	None yet

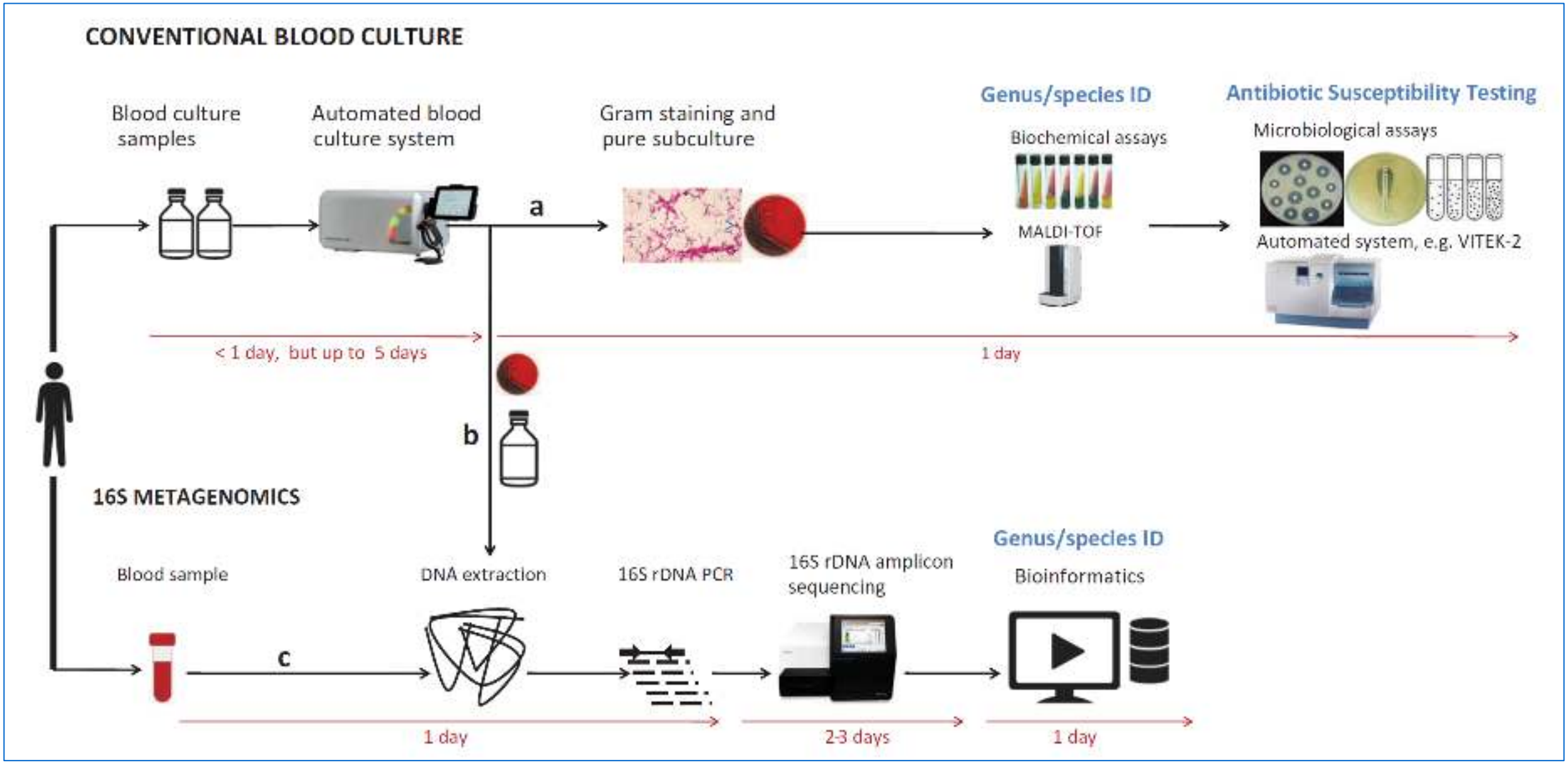
Abbreviations: CE, European Conformity; FDA, US Food and Drug Administration; TAT, turnaround time; NA, Not available.
[a] *Acinetobacter baumanni* is CE marked only and not FDA approved, so is not in the panel in the USA.

3

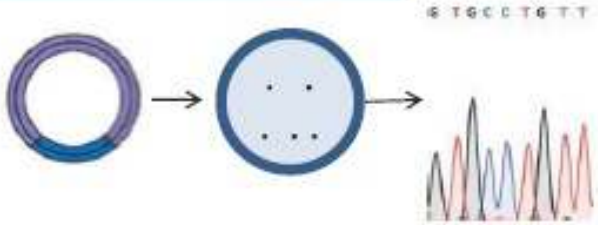
Metagenomik

- 16 s metagenomik
- Shotgun metagenomik

▪ 16 s metagenomik



First generation sequencing
Sanger sequencing



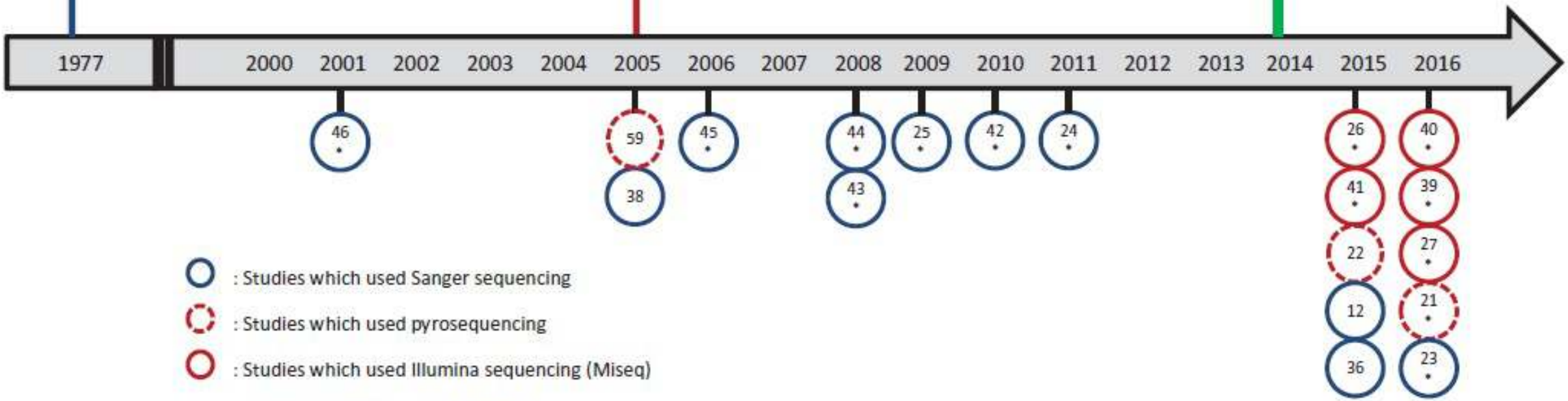
Population coverage: low
False positivity rate: low

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



○ : Studies which used Sanger sequencing

◌ : Studies which used pyrosequencing

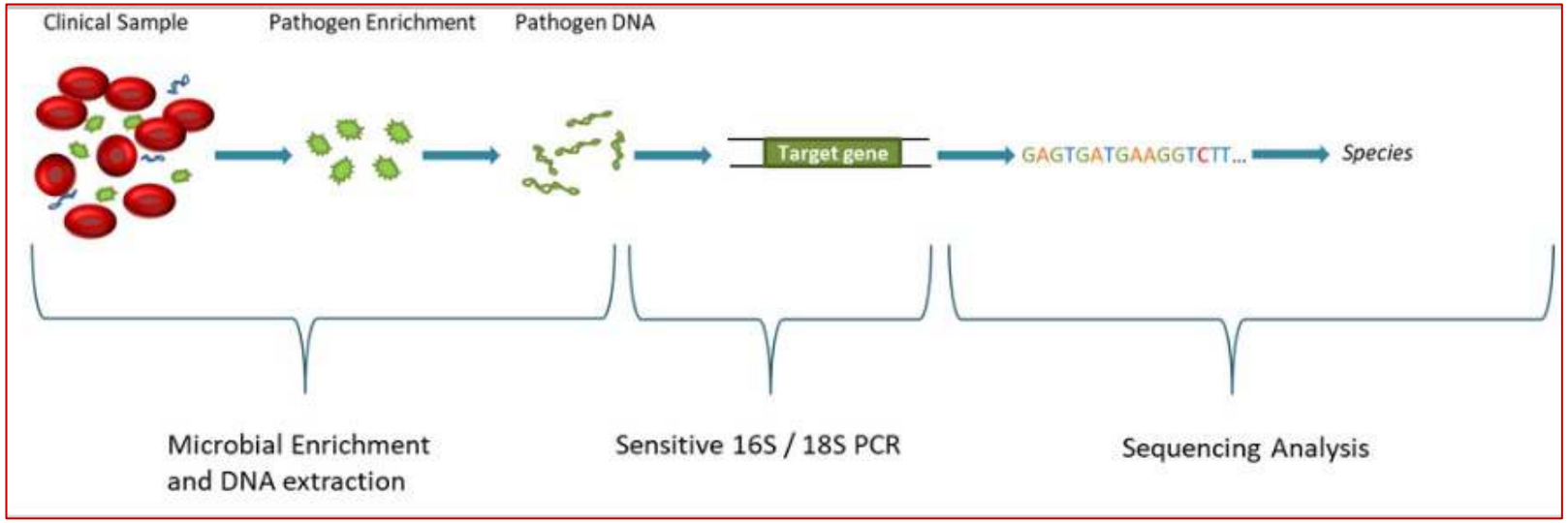
○ : Studies which used Illumina sequencing (Miseq)

* : Studies conducted on whole blood

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

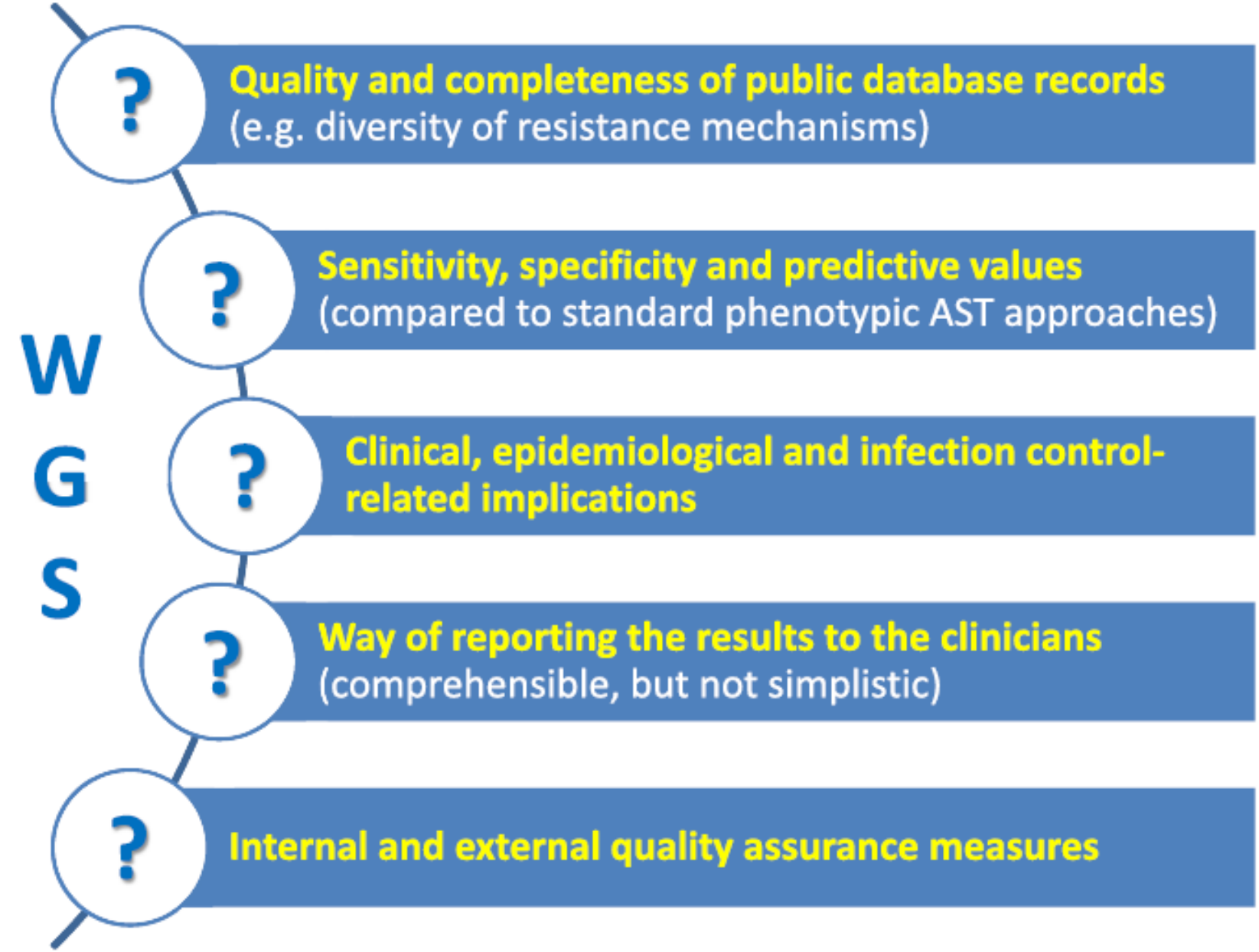


Fig. 2. Unsolved questions standing in the way of establishing whole genome sequencing (WGS) as approach for routine antimicrobial susceptibility testing (AST).

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

Metagenomics based assays for the diagnosis of bloodstream infections from whole blood

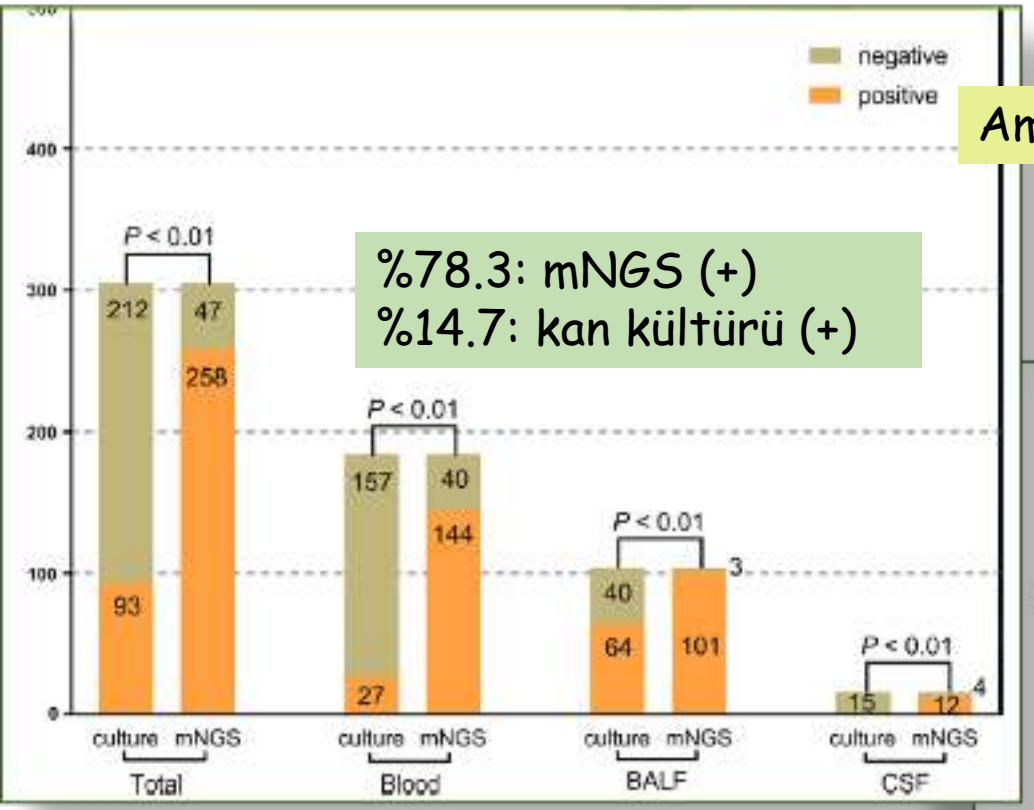
Technology	Assay (manufacturer)	TAT (h)	Organisms detected	Resistance genes detected	Complexity—Personnel experience level	Sensitivity/specificity	FDA clearance/ CE marked
PCR of 16S/18S regions, followed by sequencing	SepsiTest™ (Molzym)	8–12	Over 345 Bacteria and 8 fungi	—	Partially automated Specially trained personnel	48%/86%	CE marked
PCR of 16S/18S regions, followed by sequencing	Micro-Dx™ (Molzym)	7	Over 200 bacterial and 65 fungal genera	—	Partially automated Specially trained personnel	Performance on whole blood not provided	CE marked
PCR of 16S/28S regions, followed by sequencing	Hybcell Pathogens DNA (CubeDx)[a]	3	Bacteria: 56 species and 11 genera Fungi: 19 species + 5 genera	*vanA|B*, *mecA|C*, *CTX-M*, *KPC*, *OXA-48*, *NDM*, *IMP*	Partially automated Specially trained personnel	63%/83%	CE marked
Untargeted NGS	iDTECT® Dx Blood (PathoQuest) [NGS platform: MiSeq (Illumina)]	NA	Over 1200 pathogens (bacteria and viruses)	—	Not provided Specially trained personnel	(Negative predictive value: 98.4%)	CE marked
Untargeted NGS	Karius NGS plasma Test™ (Karius) [NGS platform: NextSeq (Illumina)]	53[b,c]	Over 1200 pathogens (bacteria, fungi, DNA viruses[b] and parasites)	—	High complexity test Specially trained personnel	93%/63%	FDA approval not required

Abbreviations: CE, European Conformity; FDA, US Food and Drug Administration; ID, identification; NA, not available; TAT, turnaround time.

[a] Specific cartridges for bacteria and fungi are also available (Hybcell Bacteria, Hybcell Fungi).

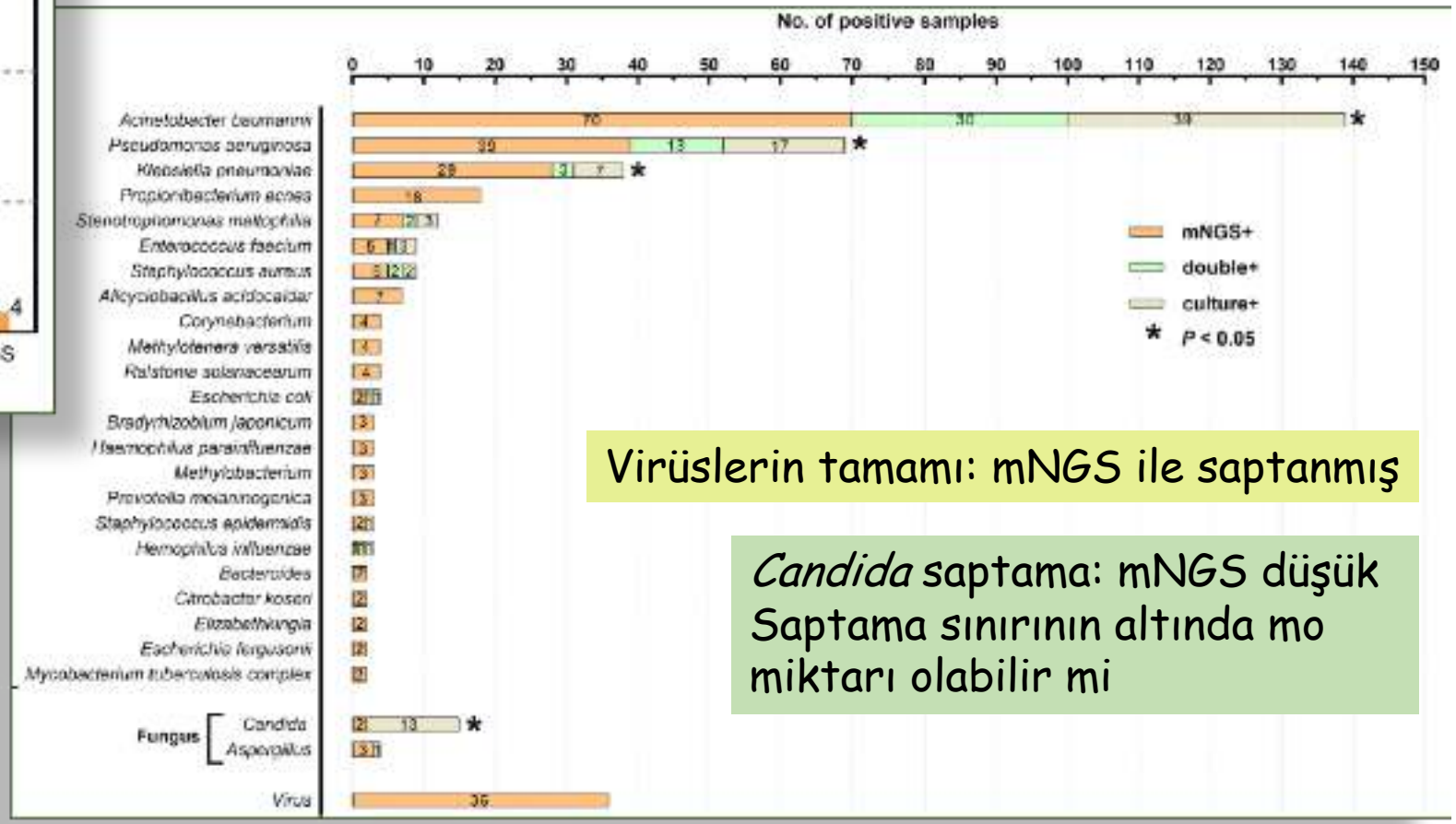
[b] Including sample shipment.

[c] The test does not detect RNA viruses.



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

Ampirik AB tedavisi .....



Virüslerin tamamı: mNGS ile saptanmış

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

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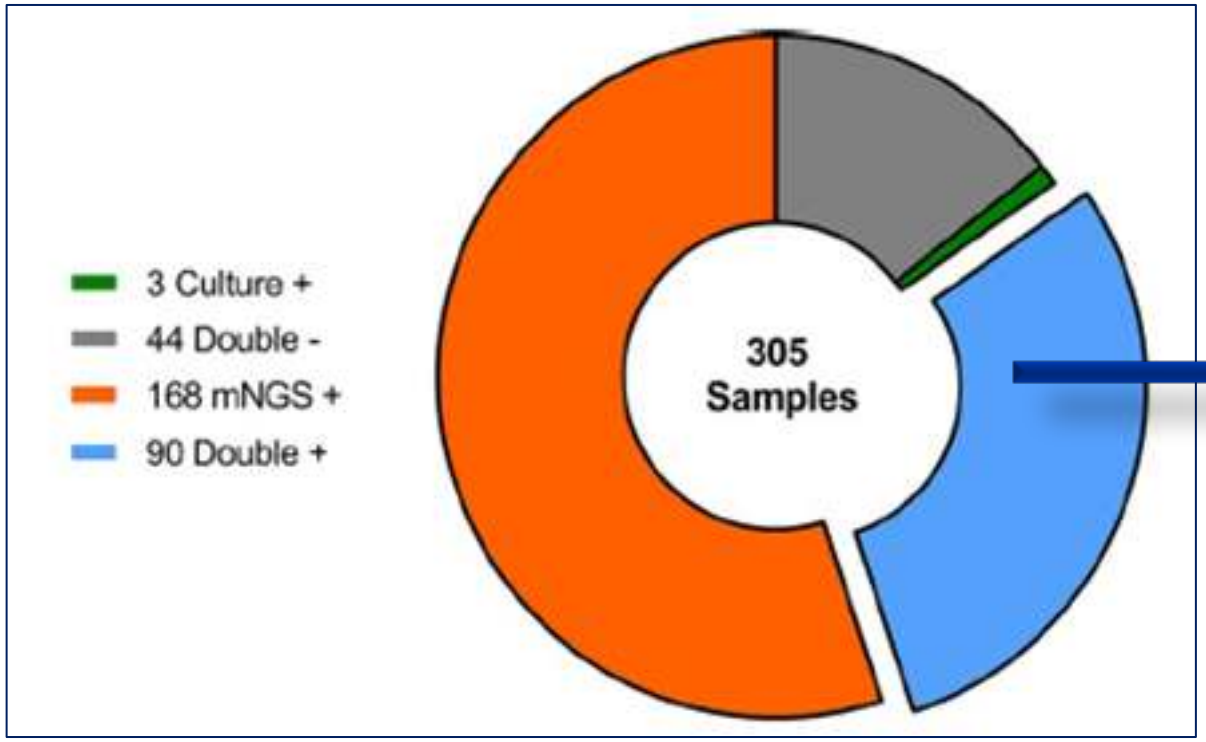
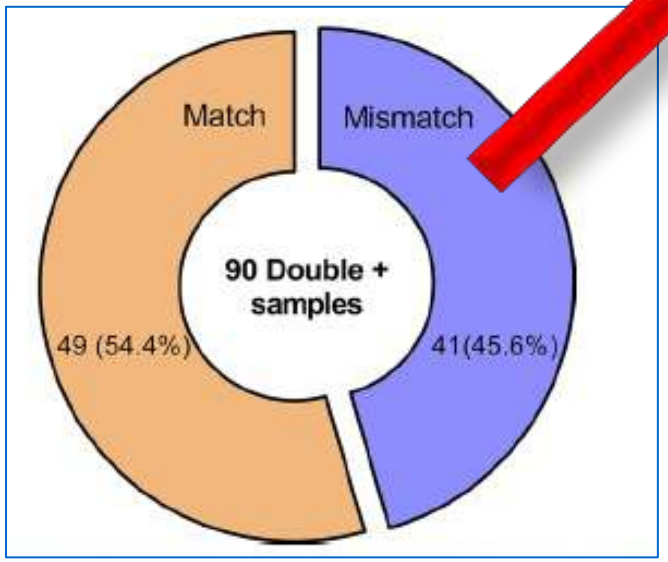
Uyumsuz sonuçlar için
3. yönteme ihtiyaç var





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

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

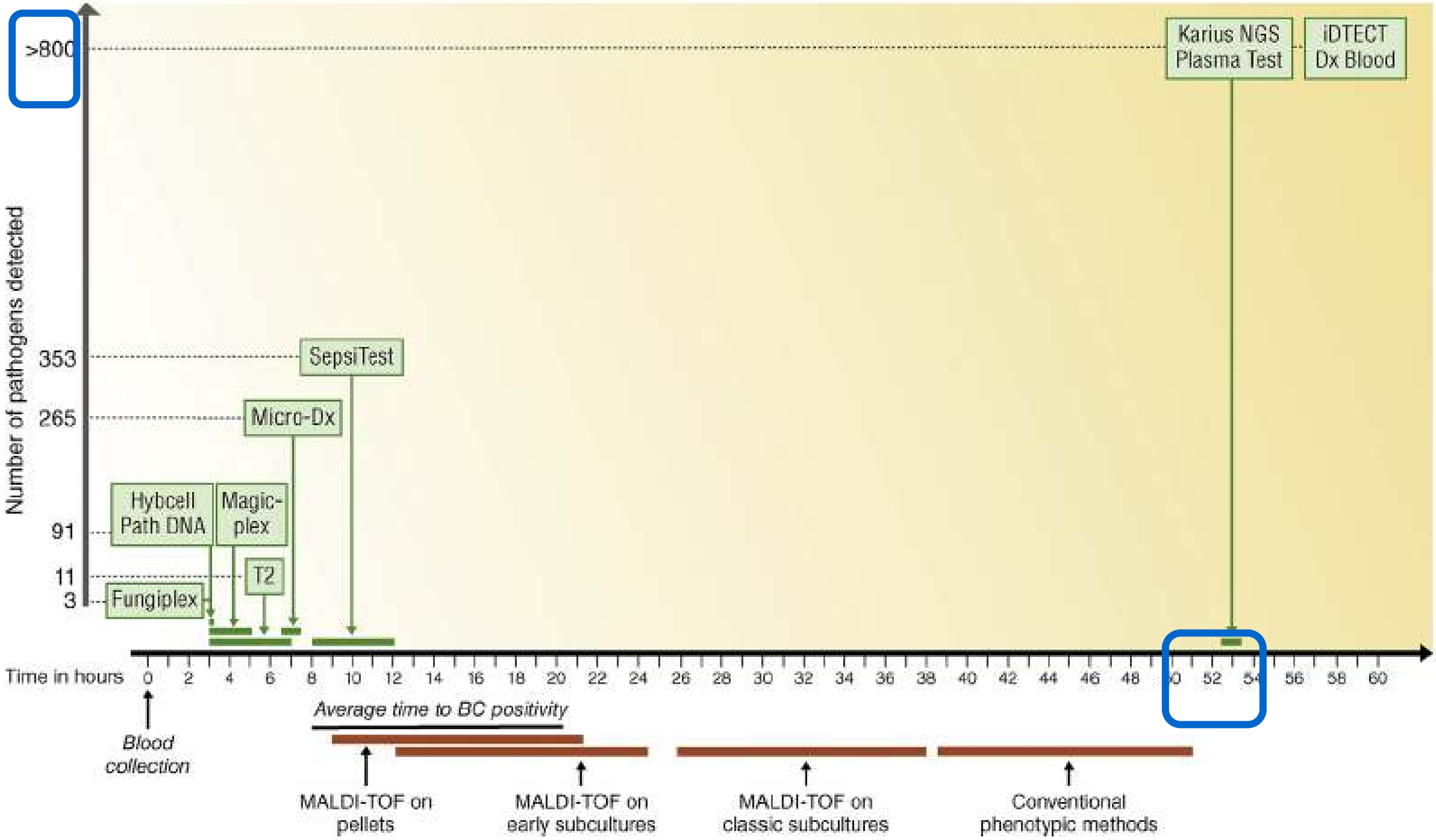
Rong Zhang[1], Yan Zhuang[1], Zheng-hui Xiao[2], Cai-yun Li[3], Fan Zhang[1], Wei-qing Huang[1], Min Zhang[1], Xiao-ming Peng[1] and Chao Liu[3]**

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
91
Hybcell Path DNA
Magic-plex
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

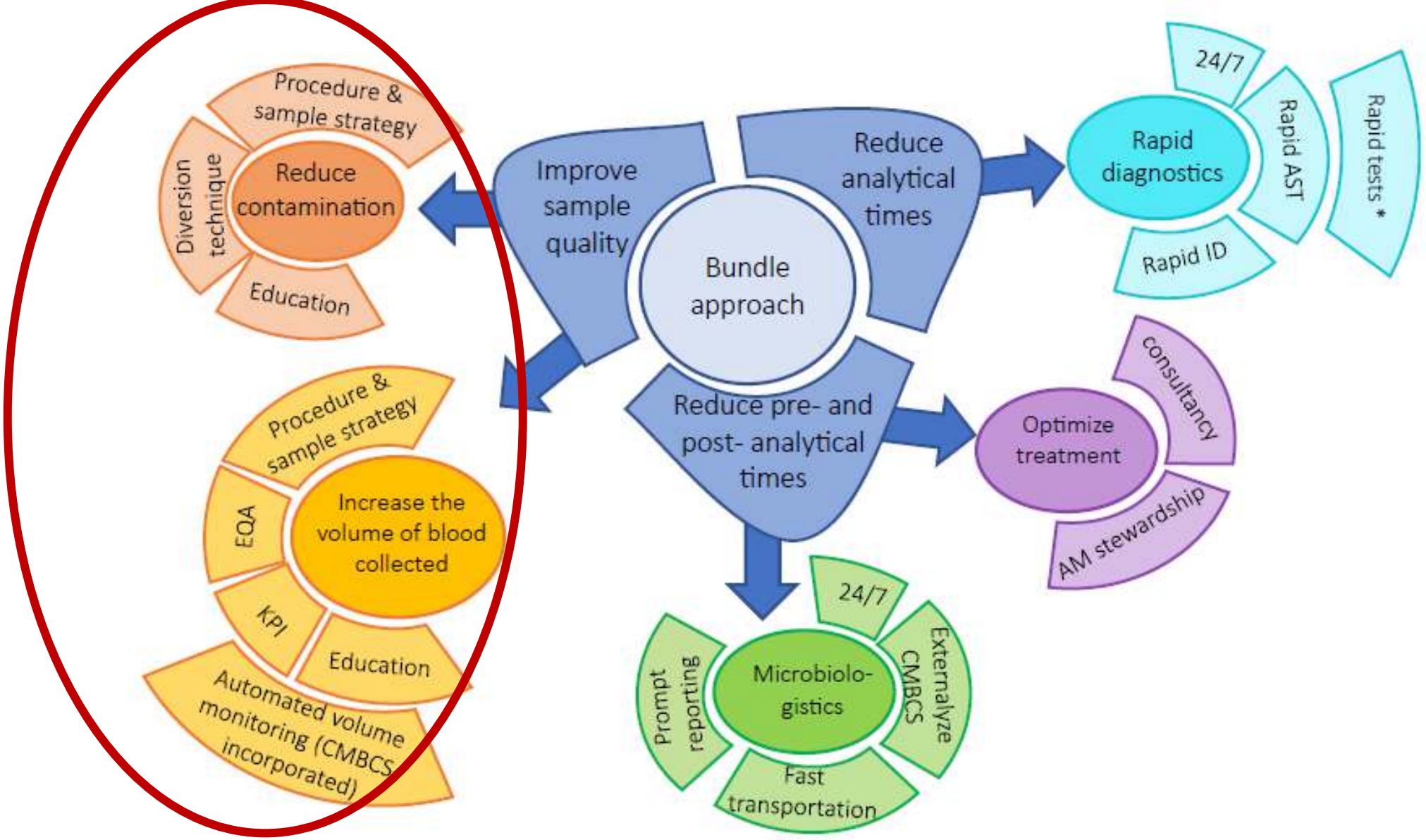


Fig. 1. Summary of all the actions to improve the bloodstream infection pathogen diagnostics. Types of actions belong to three complementary axes and actions aim to manage sample quality, times before and after analysis and analytical times. Each action per se is associated with a limited improvement 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.

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

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

Duyarlılık:

- 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

Her hastane etken dağılımını ve direnç profilini saptamalı

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
doi:10.1093/jac/dkz548 Advance Access publication 4 February 2020

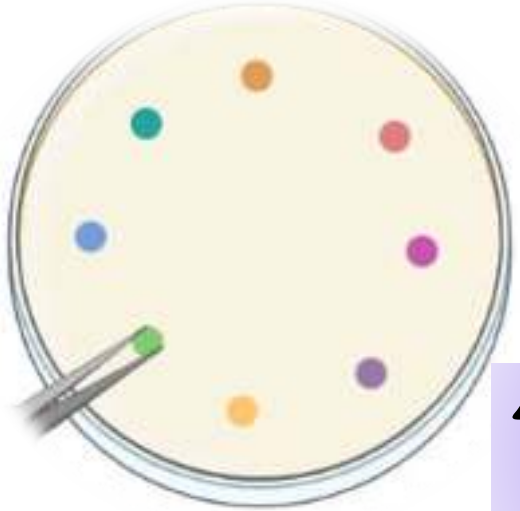
Journal of Antimicrobial Chemotherapy

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 antimicrobial susceptibility testing (RAST) directly from positive 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
SOP 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	18	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	16	15-17	16	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 antimicrobial susceptibility testing (RAST) directly from positive 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

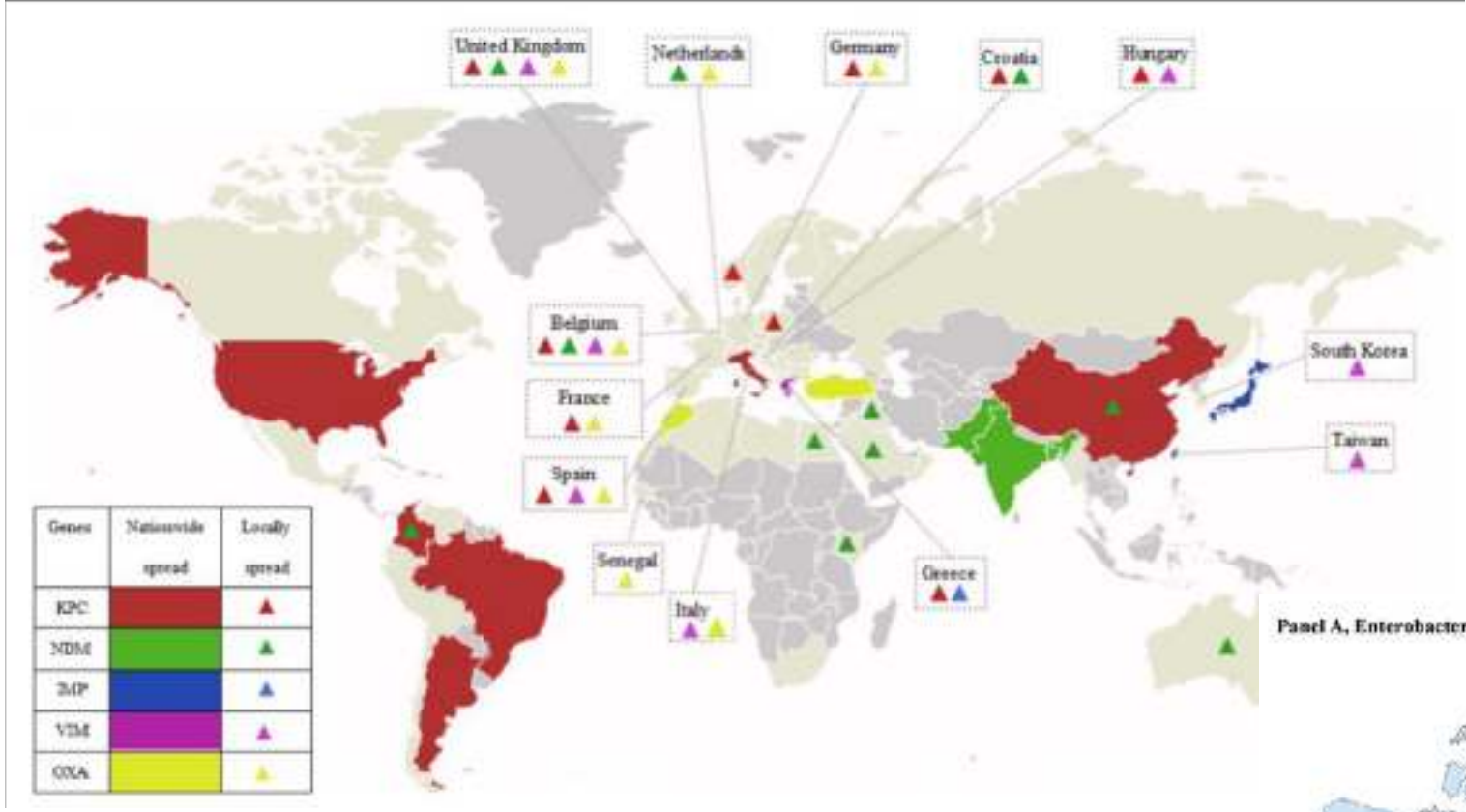
Annual report 2020

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*	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*	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?

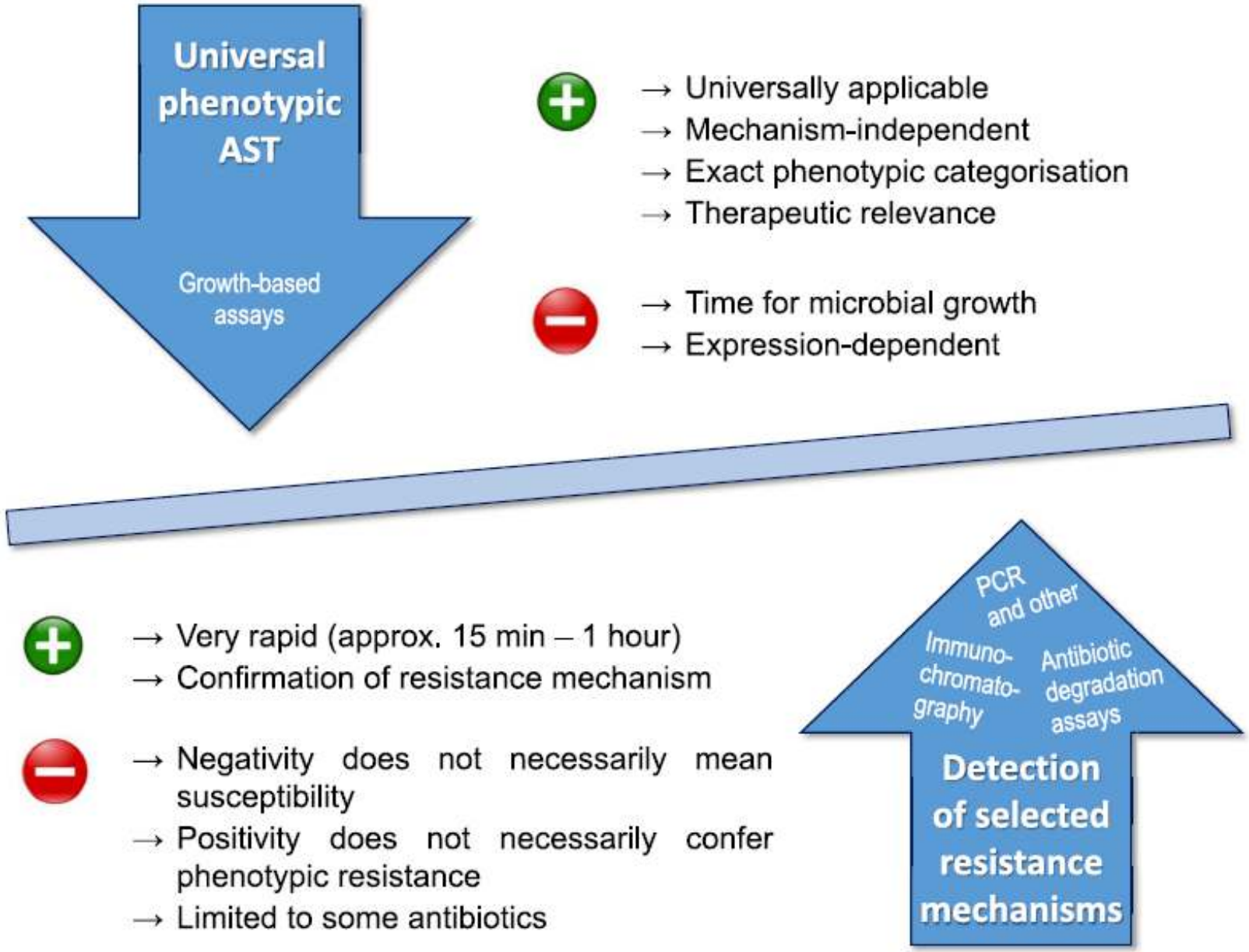


Fig. 1. Detection of particular resistance mechanisms vs. universal phenotypic susceptibility testing. AST, antimicrobial susceptibility testing.

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. maltophilia*, *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*, *blaCTX-M*, *blaKPC*, *blaOXA-48*, *blaIMP*, *blaNDM*, *blaVIM*, *blaSME*	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	95–97.5/93–99.5 (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

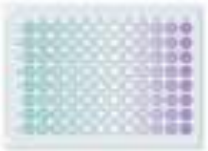
MIK

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

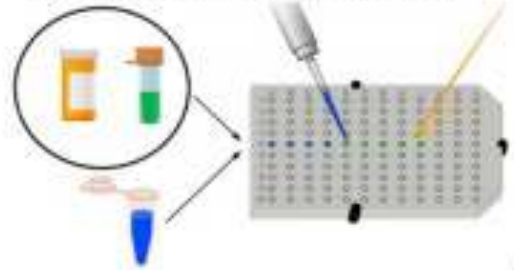
several days

** 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)*

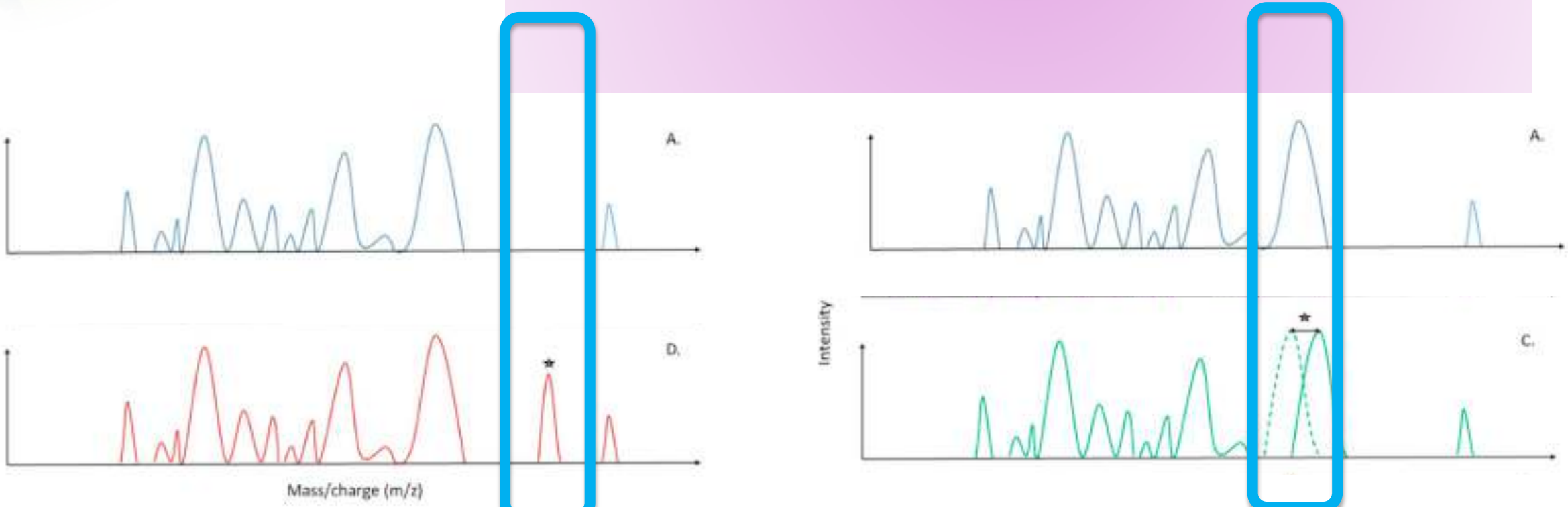
MALDI-TOF MS
AST

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



II. ANTİBİYOTİKteki değişikliklerin saptanması

Hidroliz, dekarboksilasyon, asetilasyon

B-laktamaz

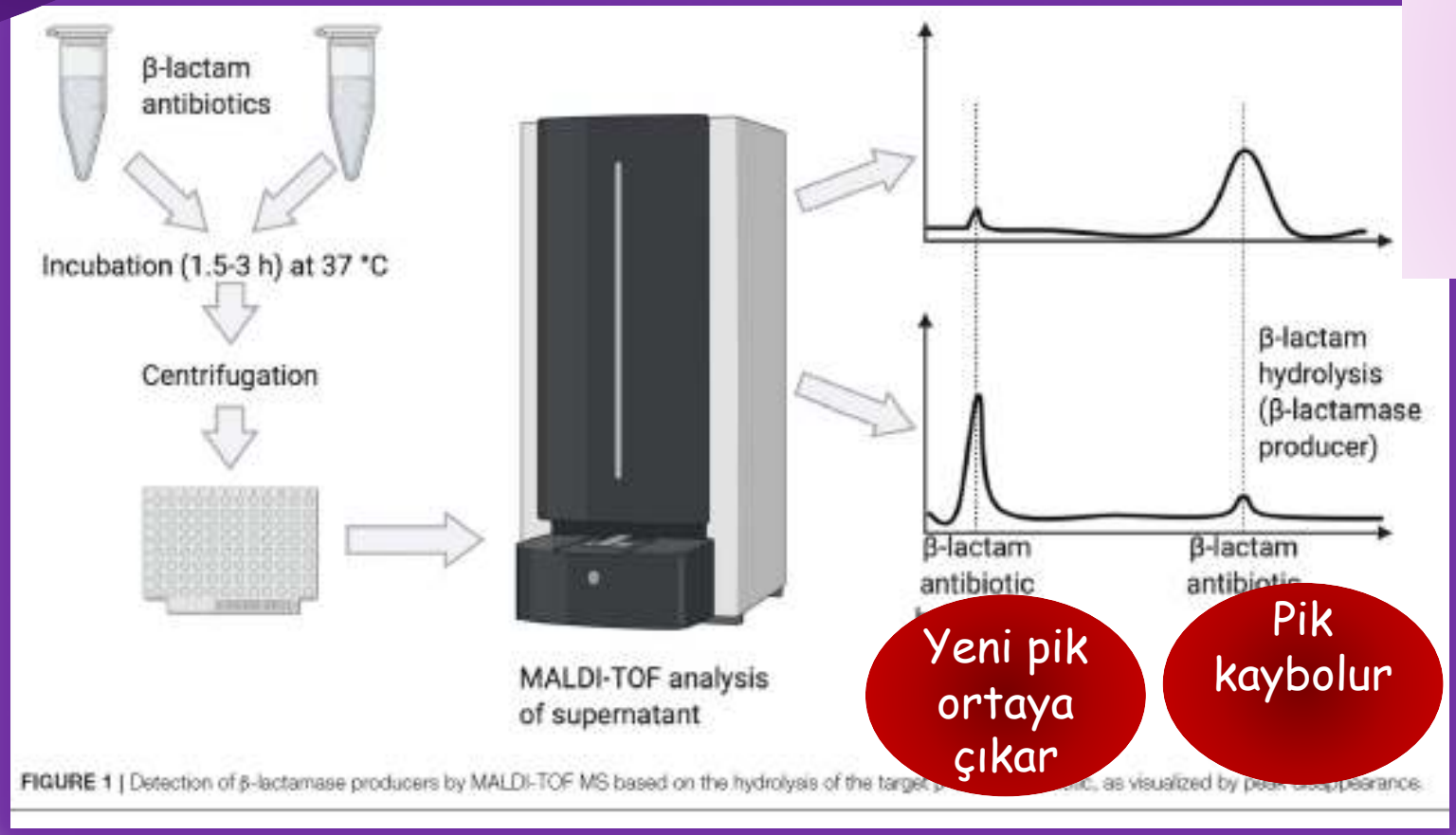
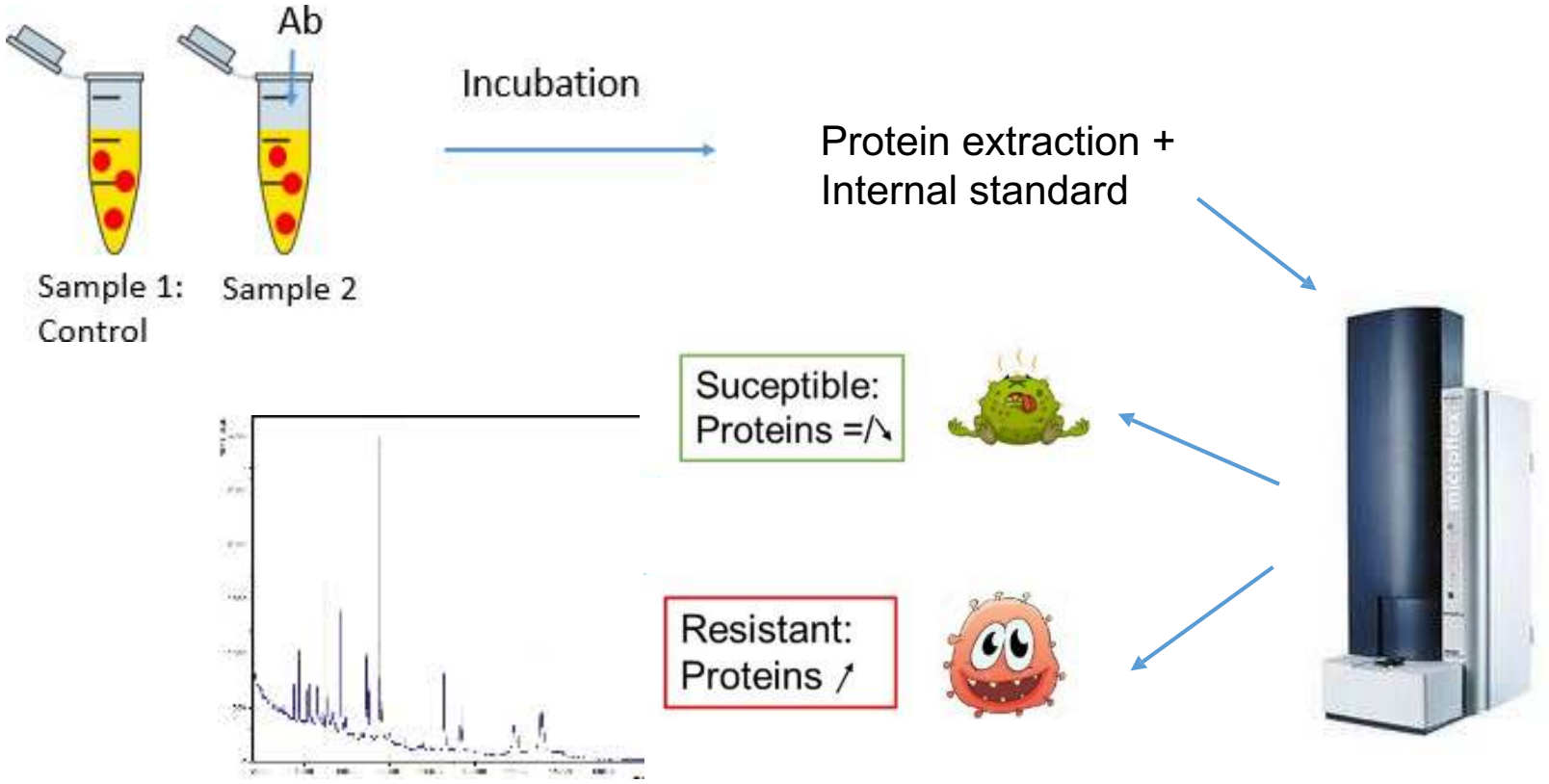


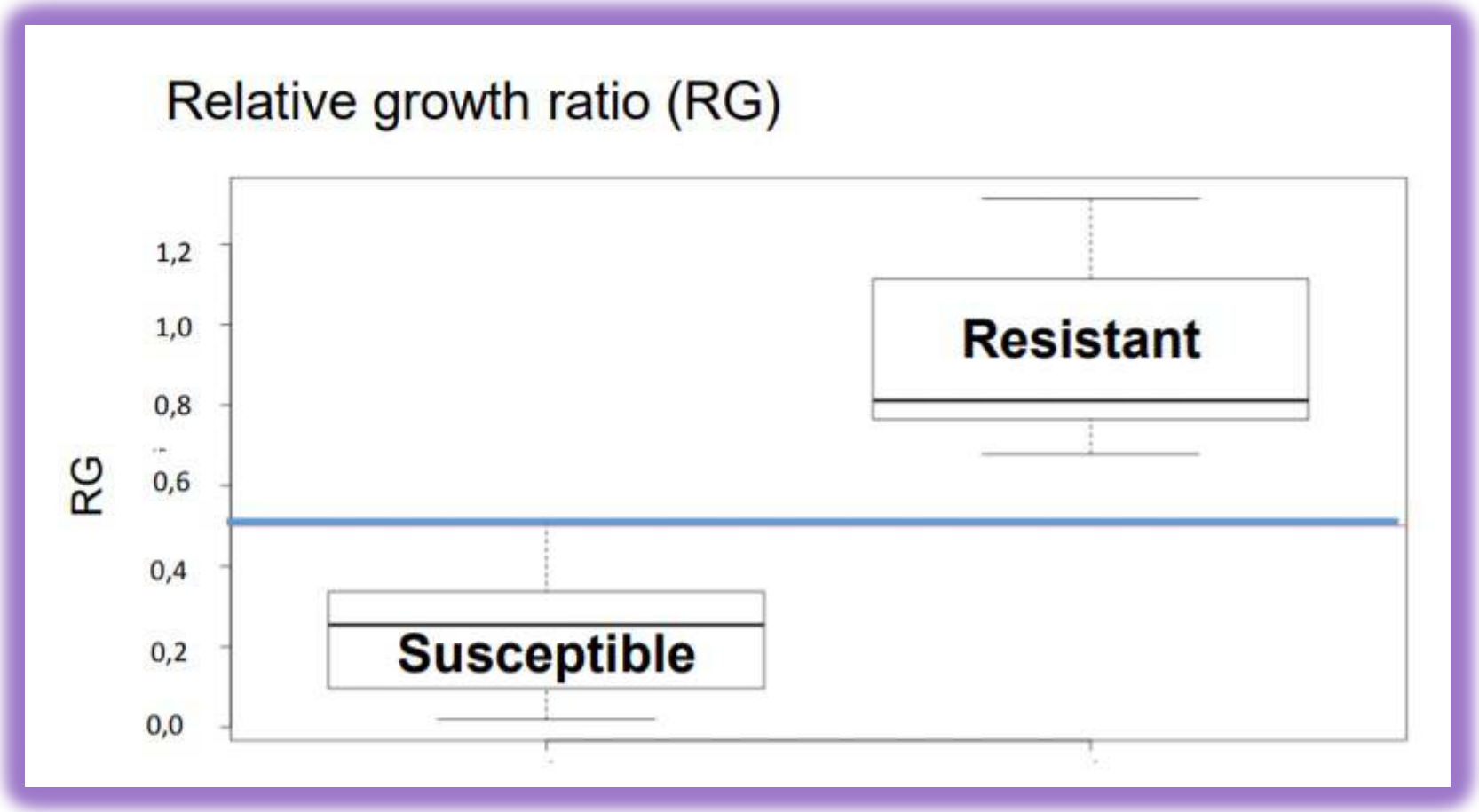
FIGURE 1 | Detection of β-lactamase producers by MALDI-TOF MS based on the hydrolysis of the target β-lactam antibiotic, as visualized by peak disappearance.

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

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

Semikantitatif bir yöntem:
Saatler içinde duyarlı/dirençli sonucu





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

- 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

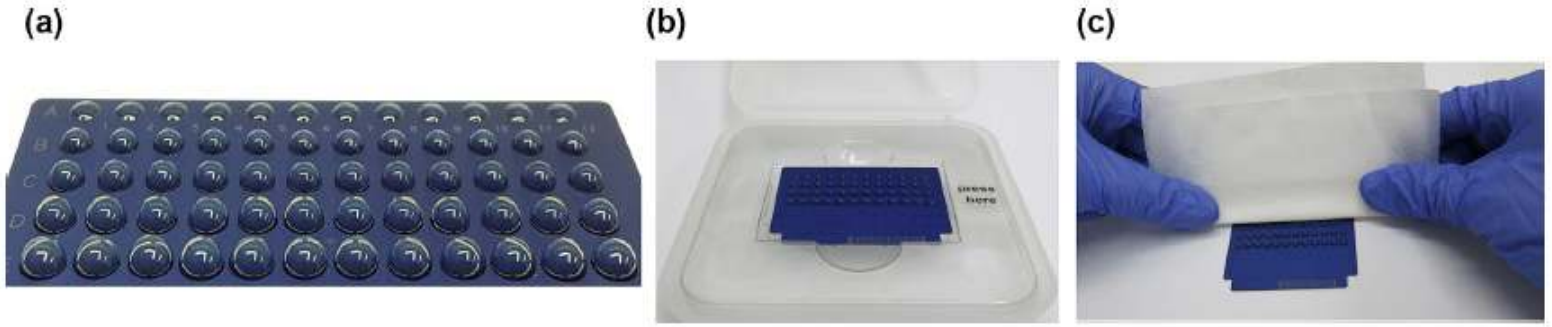


Fig. 1. The experimental setup. (a) Detail of a MALDI-TOF MS target with microdroplets before incubation. Rows A, B, C, D and E contain microdroplets with total volumes of 2, 4, 6, 8 and 10 µL, respectively. Columns 1 to 3, susceptible *Klebsiella pneumoniae* isolate with meropenem; columns 4 to 6, growth 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

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-Martinez[1†], Evgeny A. Idelevich[1†], Katrin Sparbier[2], Markus Kostrzewa[3]

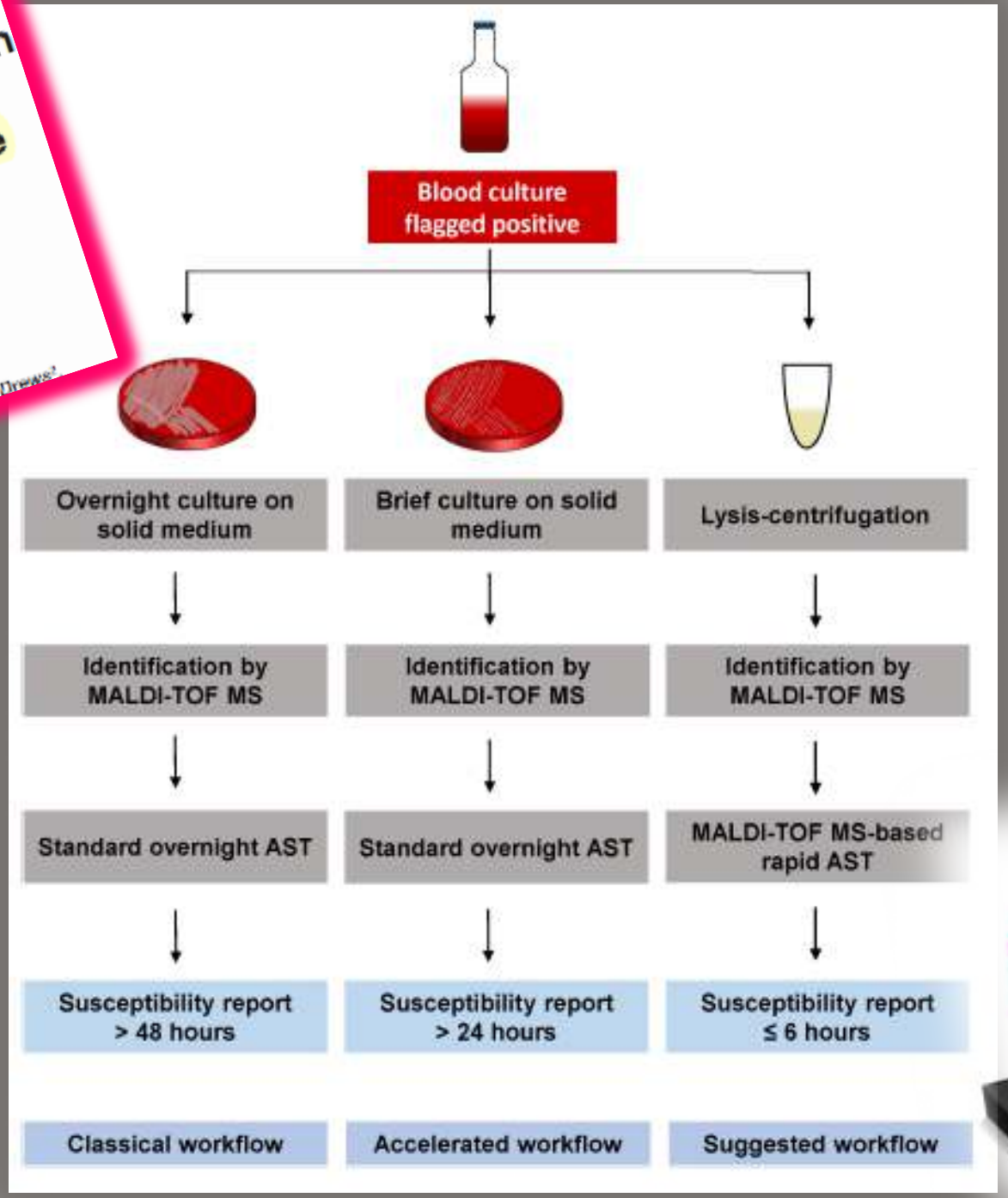
GSBL, AmpC ve karbapenemaz aktivitesinin saptanması için de modifiye edildi

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

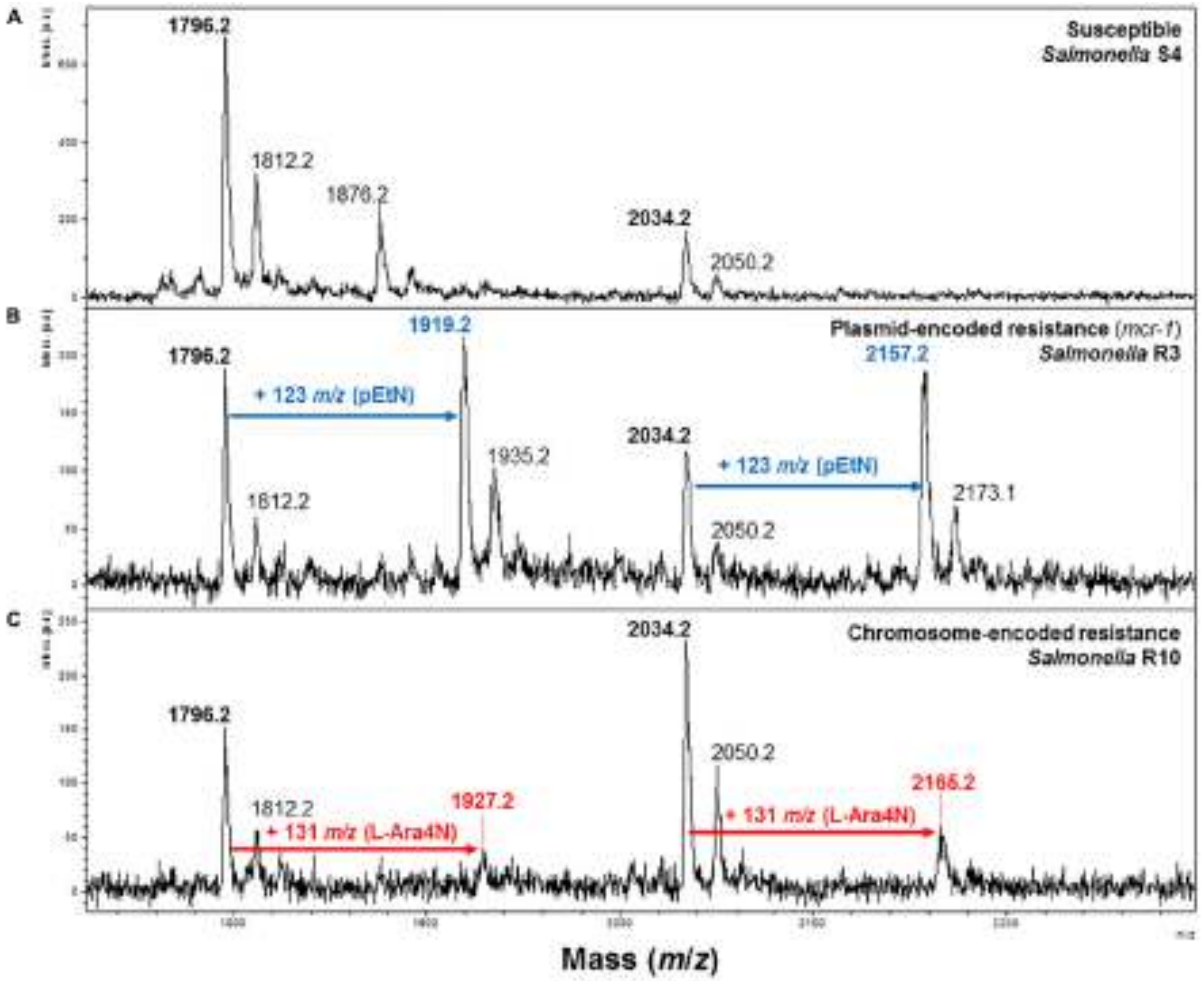
Carlos L. Correa-Martínez[1,2,5], Evgeny A. Idelevich[1,5], Katrin Sparbier[3], Thorsten Kuczius[2], Markus Kostrzewa[3] & Karsten Becker[1,4*]

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



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

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



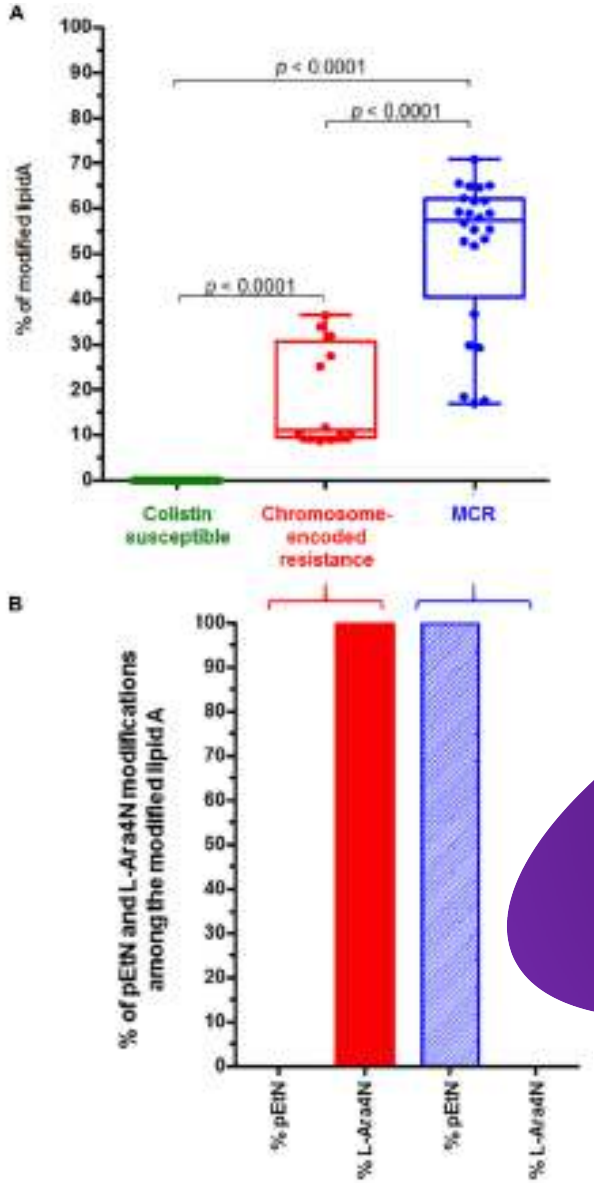


FIGURE 2 | (A) Representation of the percentage of the modified lipid A for colistin susceptible and colistin resistant *S. enterica* isolates. The global percentage of modified lipid A (L-Ara4N + pETN modified lipid A / native lipid A) is represented for colistin susceptible strains (n=11), colistin 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

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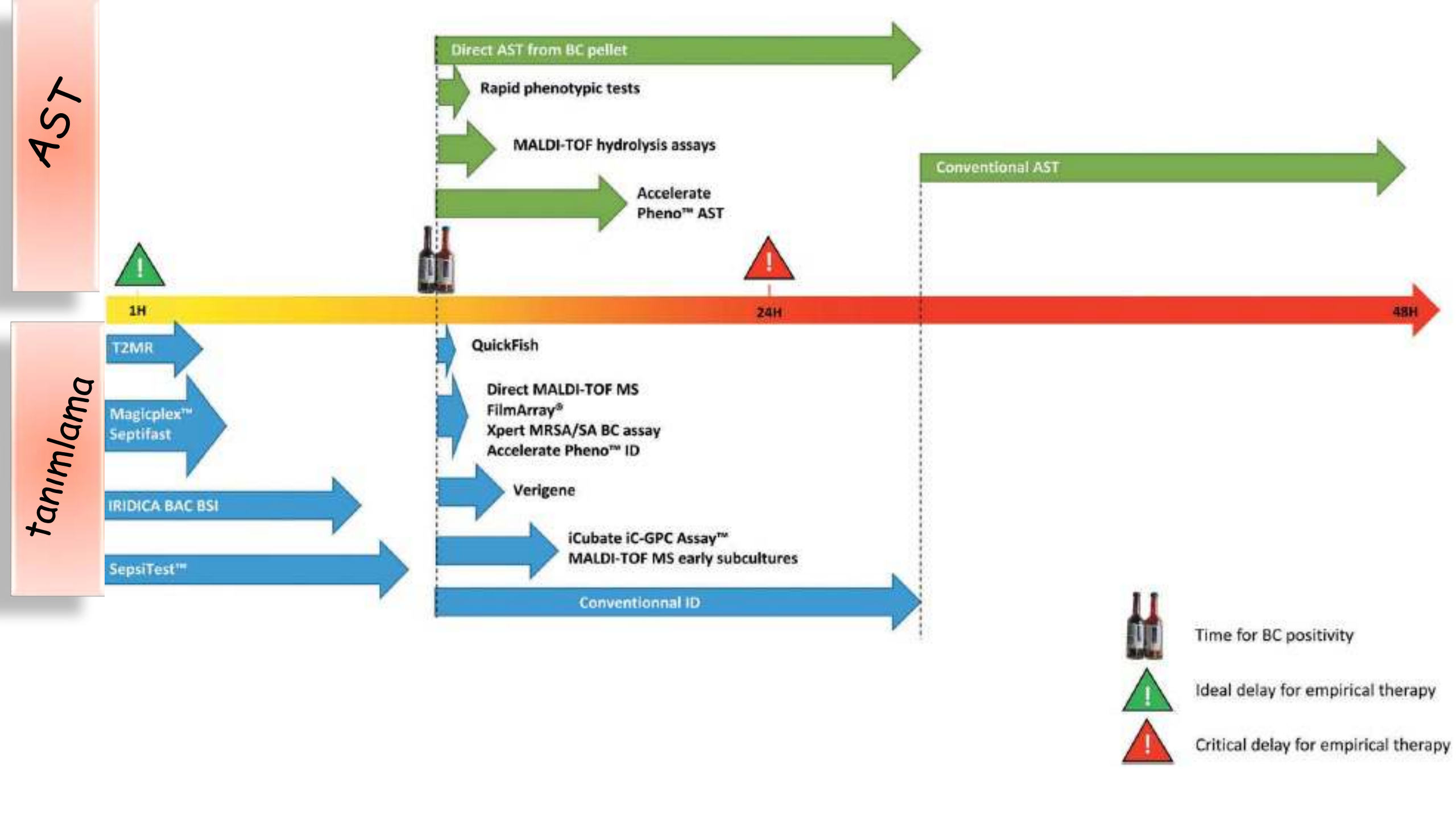
AST
tanımlama
Direct AST from BC pellet
Rapid phenotypic tests
MALDI-TOF hydrolysis assays
Accelerate Pheno™ AST
Conventional AST
1H
24H
48H
T2MR
Magicplex™ Septifast
IRIDICA BAC BSI
SepsiTest™
QuickFish
Direct MALDI-TOF MS
FilmArray®
Xpert MRSA/SA BC assay
Accelerate Pheno™ ID
Verigene
iCubate iC-GPC 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