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MELTEM
YALINAY

3. Ulusal
Klinik Mikrobiyoloji
Kongresi-2015

18-22 Kasım 2015
Titanic Kongre Merkezi
Belek, Antalya

HASTANE ENFEKSİYONLARININ TANISINDA YÜKSEK TEKNOLOJİ: YENİ TANI ARAÇLARI VE YENİ KONSEPTLER

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Gazi Üniversitesi Tıp Fakültesi Tıbbi Mikrobiyoloji Anabilim Dalı

Kütle spektrometreleri

- **MALDI-TOF/MS** (matrix associated laser desorption and ionization-time of flight mass spektrometry) (Matriks ile desteklenmiş lazer desorpsiyon/iyonizasyon uçuş zamanı kütle spektrometresi)

- **PCR/ESI-MS** (PCR electrospray ionisation mass spectrometry) (PZR-elektrosprey iyonizasyon kütle spektrometresi)

MALDI-TOF/MS

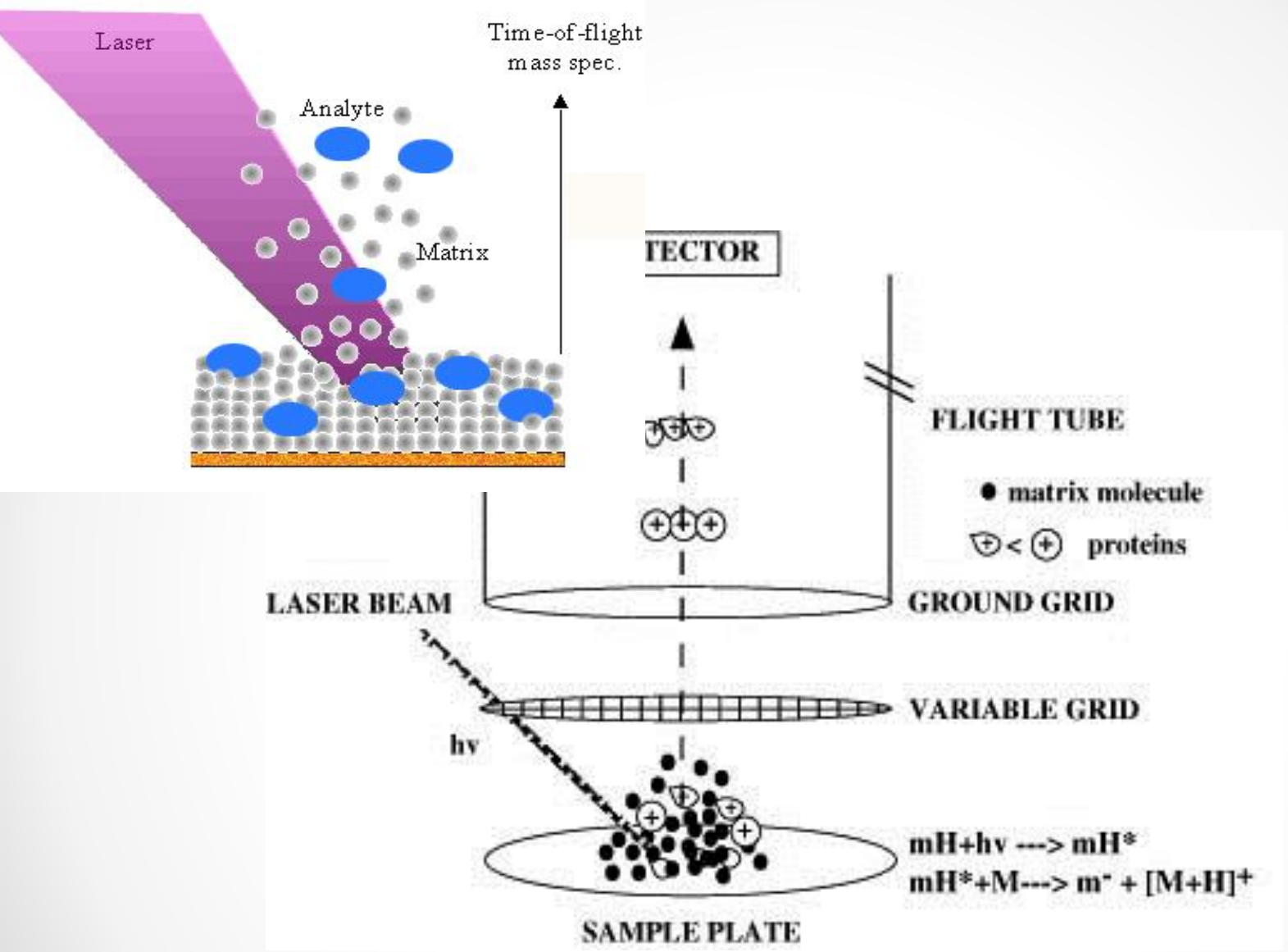
MALDI-TOF/MS, mikroorganizmanın tam bir kolonisi veya bakteriyel protein ekstraktının oldukça korunmuş proteinlerin proteomik profillenmesinden oluşan direkt iyonizasyonuna dayanmaktadır ve bu spektral imzayı referans suşlardan toplanan veri tabanı ile karşılaştırmakdır.

Prensip

- MALDI kütle spektrometrede kullanılan bir **iyonizasyon tekniği**
- Biyomoleküllerin (protein, peptid ve şeker gibi) büyük organik moleküllerin (polimer, dendrimer, makromolekül gibi) analizi
- İyonizasyon **lazer atışı**
- **Matriks** biyomolekülün amacı; direkt lazer atışının tahribinden korumak ve iyonizasyonu kolaylaştırmak

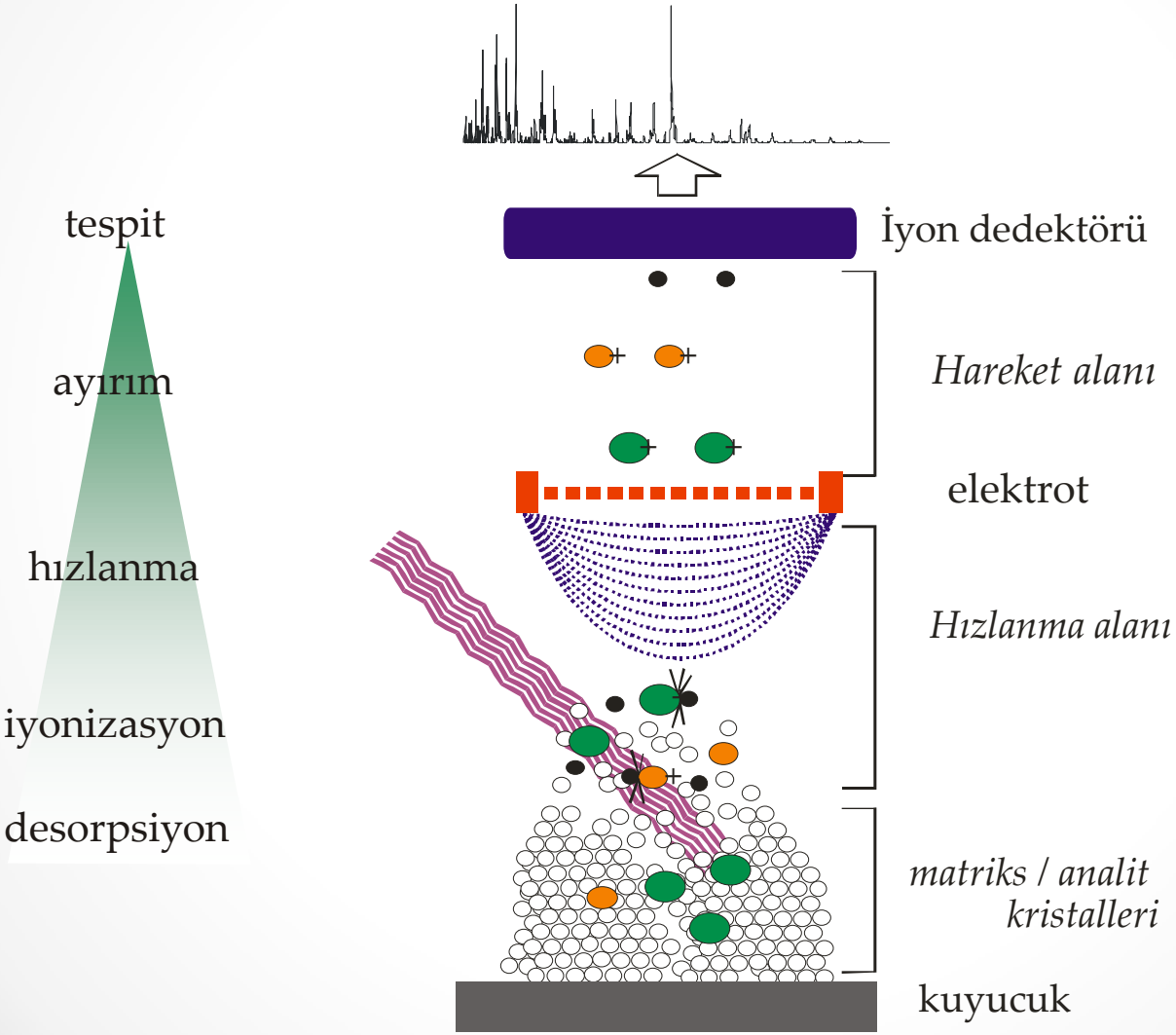
Matriks

- UV absorbe eden matriks (Hillenkamp ve Karas)
- Matriks ve polimer moleküler düzeyde uygun bir çözücü (formik asit, trifloroasetik asit, asetik asit v.b.) içinde karıştırılır.
- Çözücü polimerin agregasyonunu engeller.
- Örnek/matriks karışımı örnek prop ucuna yerleştirilir.
- Vakum koşullarıyla çözücü uzaklaştırılarak matriks molekülleri içinde homojen olarak yayılmış polimer moleküllerini bırakır.

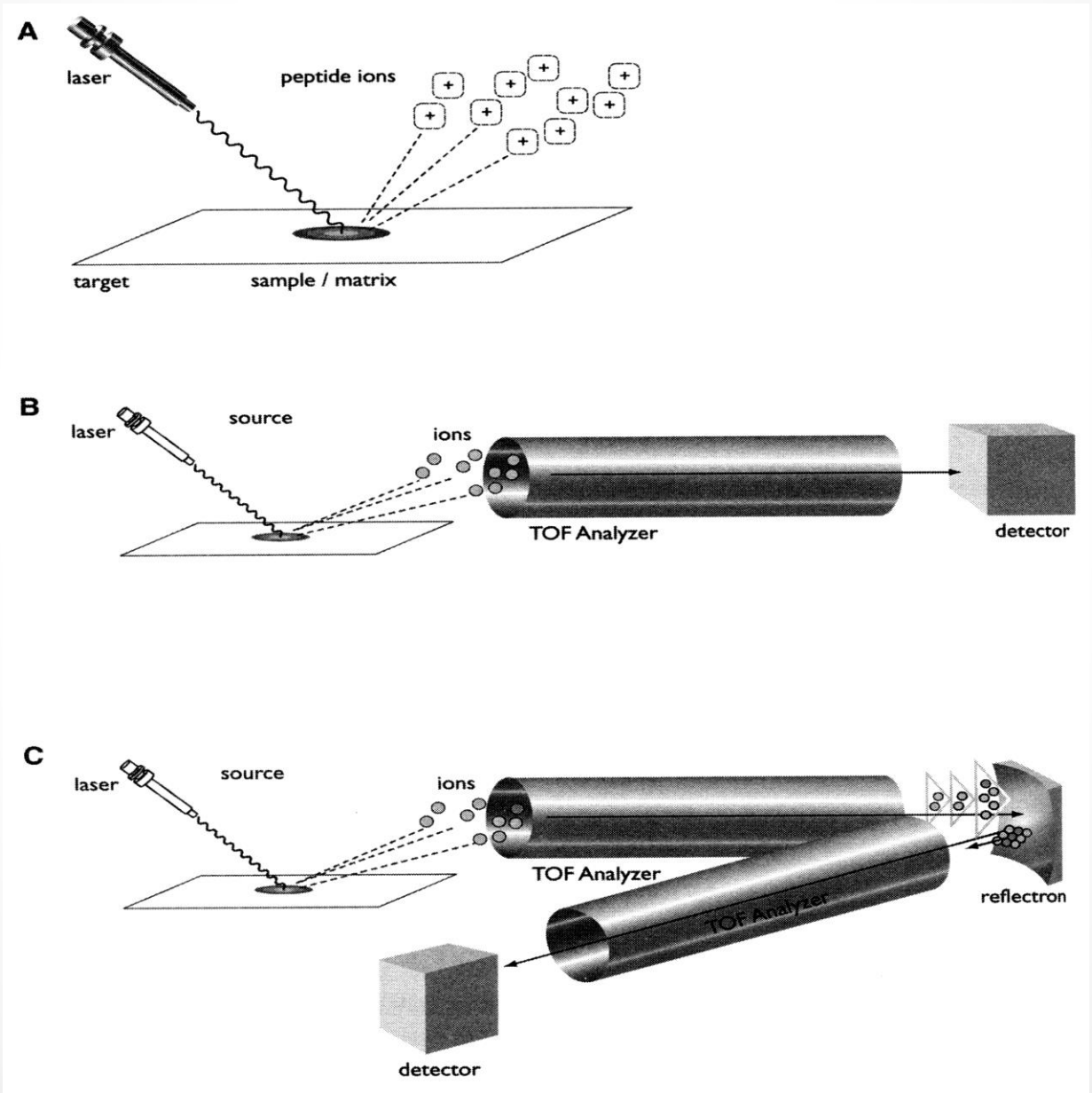

Laser
Time-of-flight
mass spec.
Analyte
Matrix
TECTOR
FLIGHT TUBE
matrix molecule
proteins
LASER BEAM
GROUND GRID
VARIABLE GRID
hv
mH+hv ---> mH*
mH*+M---> m⁻ + [M+H]⁺
SAMPLE PLATE

Lazer vuruş atımları uygun frekansa ayarlanınca, kısmen buharlaşmış ve buhar fazında intakt polimeri taşıyan ve polimer zincirlerinin elektrik yüklenmesine neden olan matrikse enerji aktarılır.

MALDI-TOF MS: Temel Prensipler

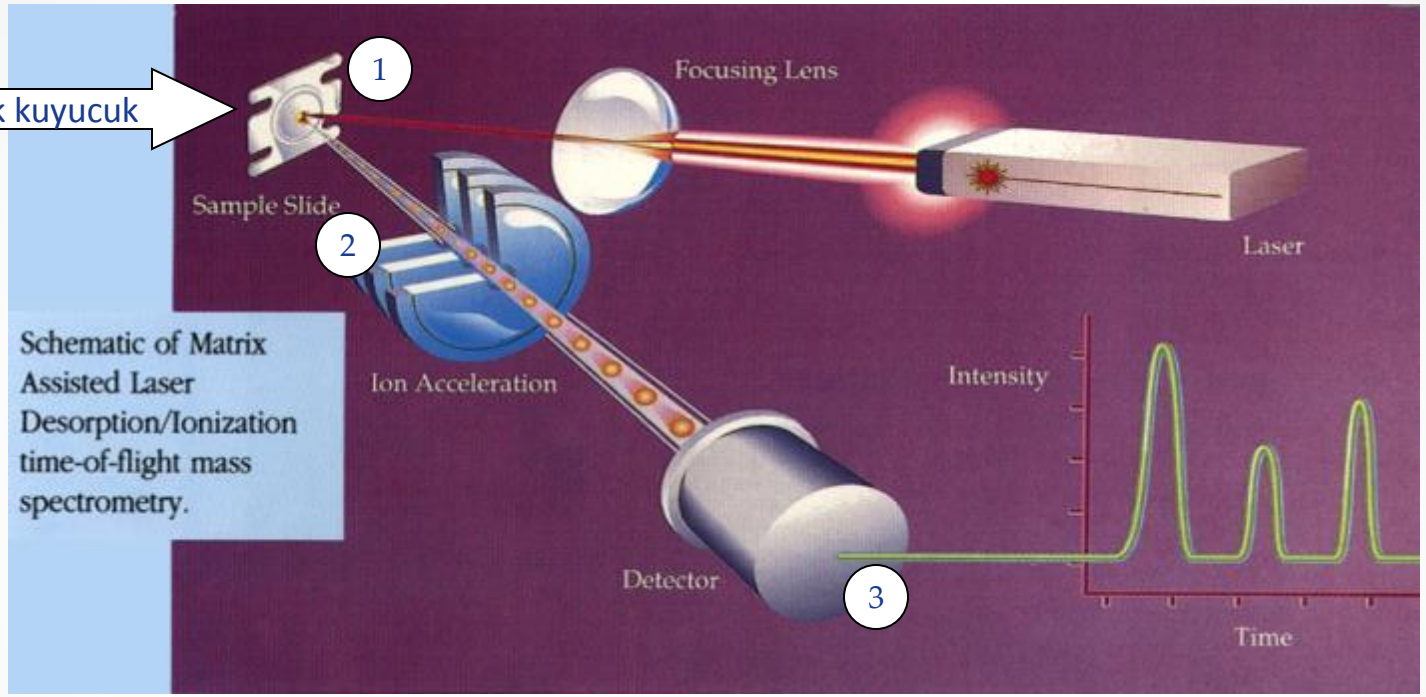


MALDI-TOF Kütle spektrometre



MALDI-TOF MS: Teknoloji

Tek kuyucuk



1. Lazer ışını numune ve matriksin buharlaşmasına neden olur
2. Oluşan iyonlar yüksek voltaj kaynağı tarafından hızlandırılır ve yüklerine göre ayrılacakları tüp içerisinde hareket ederler
3. Tüp sonunda tespit yapılır

1- Örnek Hazırlığı

1µL-Öze ile
Tek koloni alınır

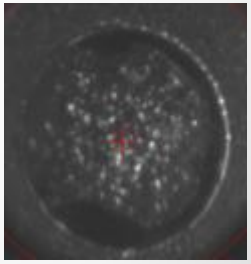
Tek kuyucuk

Mikobakteriler için
50 µl
TrifloroAsetikAsit ile
inaktivasyon

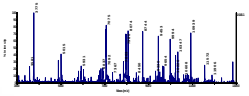
Bakteri

1µL HCCA matrix

Kurumaya bırakılır

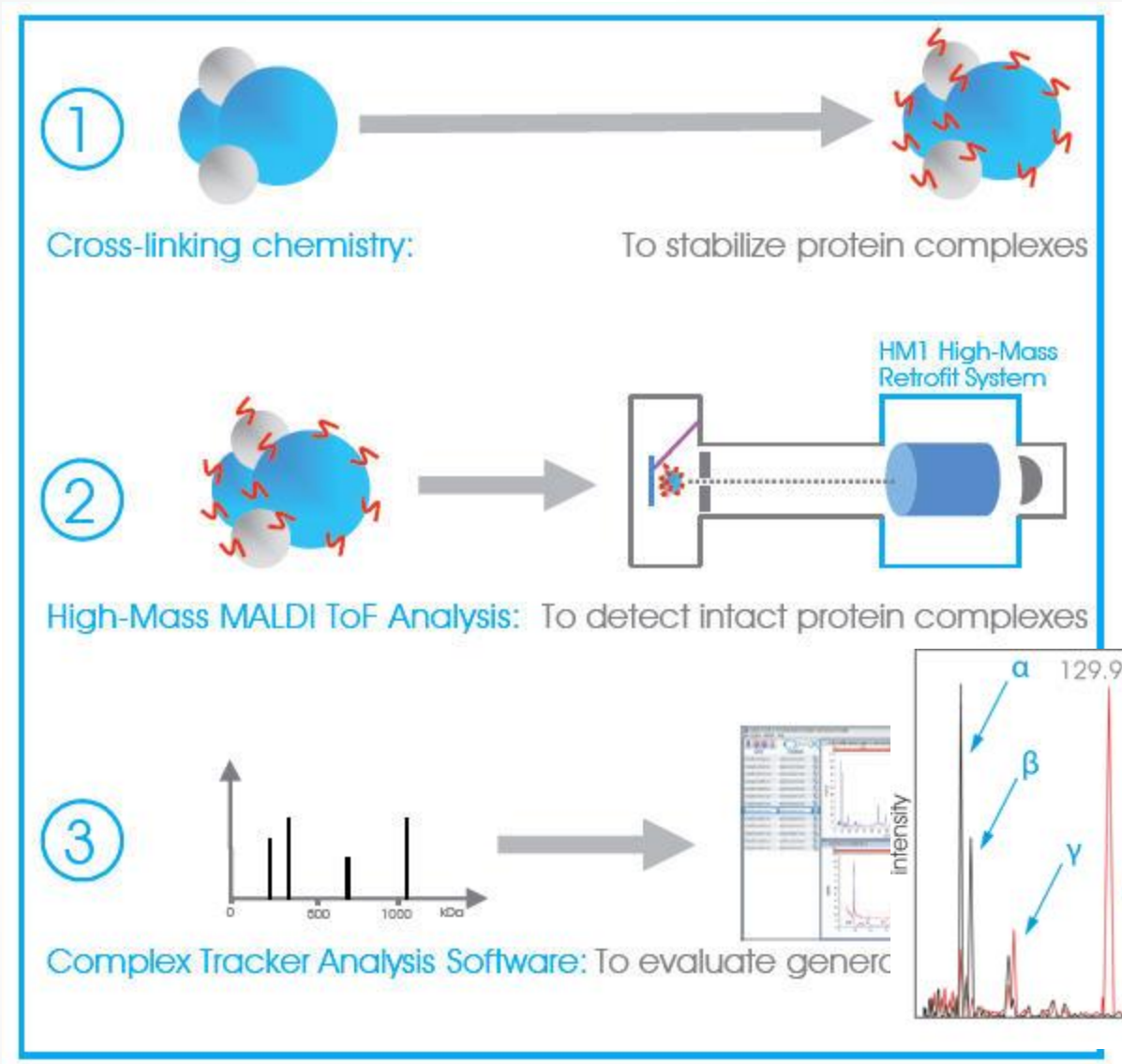


2- Ölçüm



Veri
Analizi

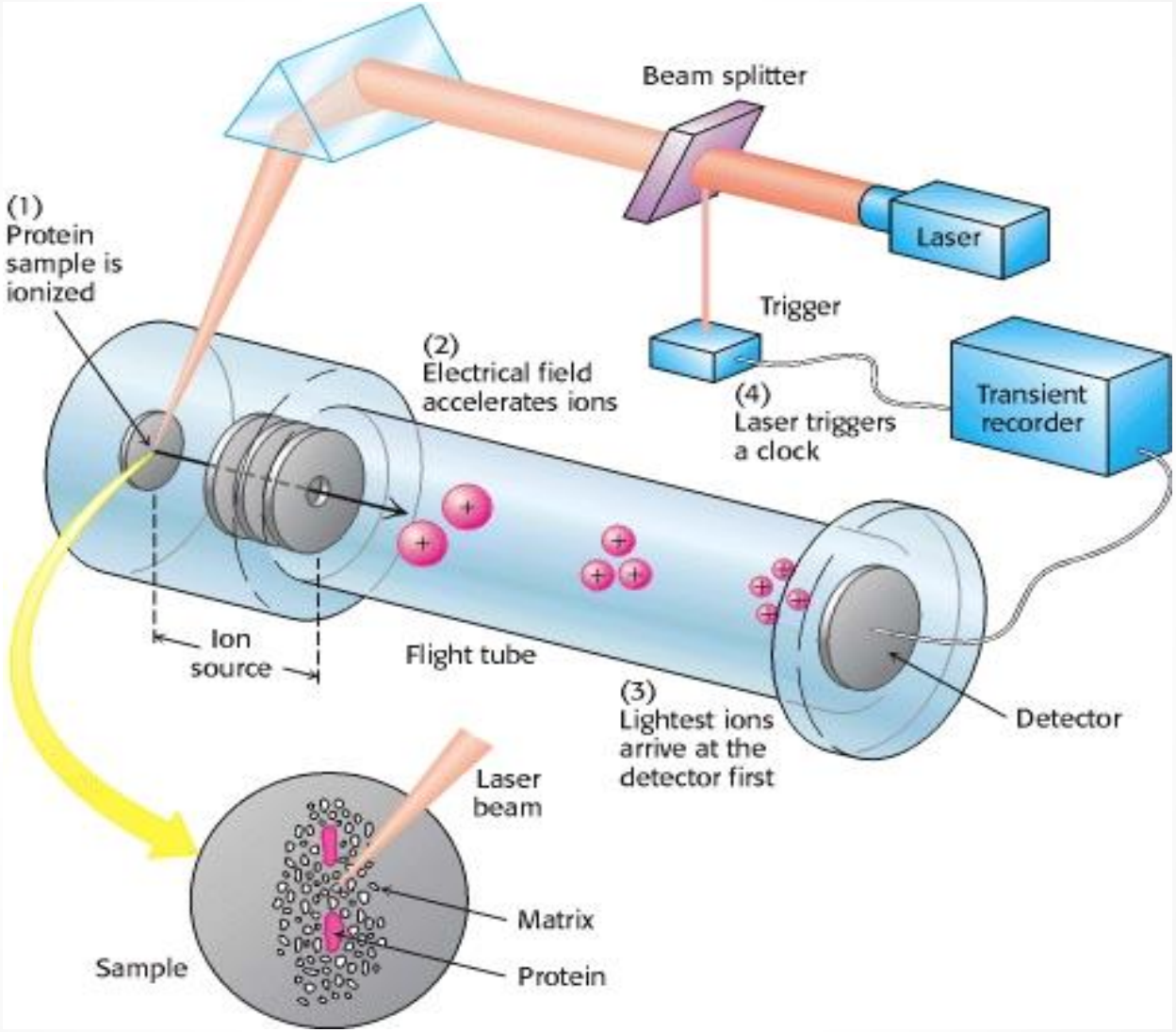
3- Analiz - Tanımlama



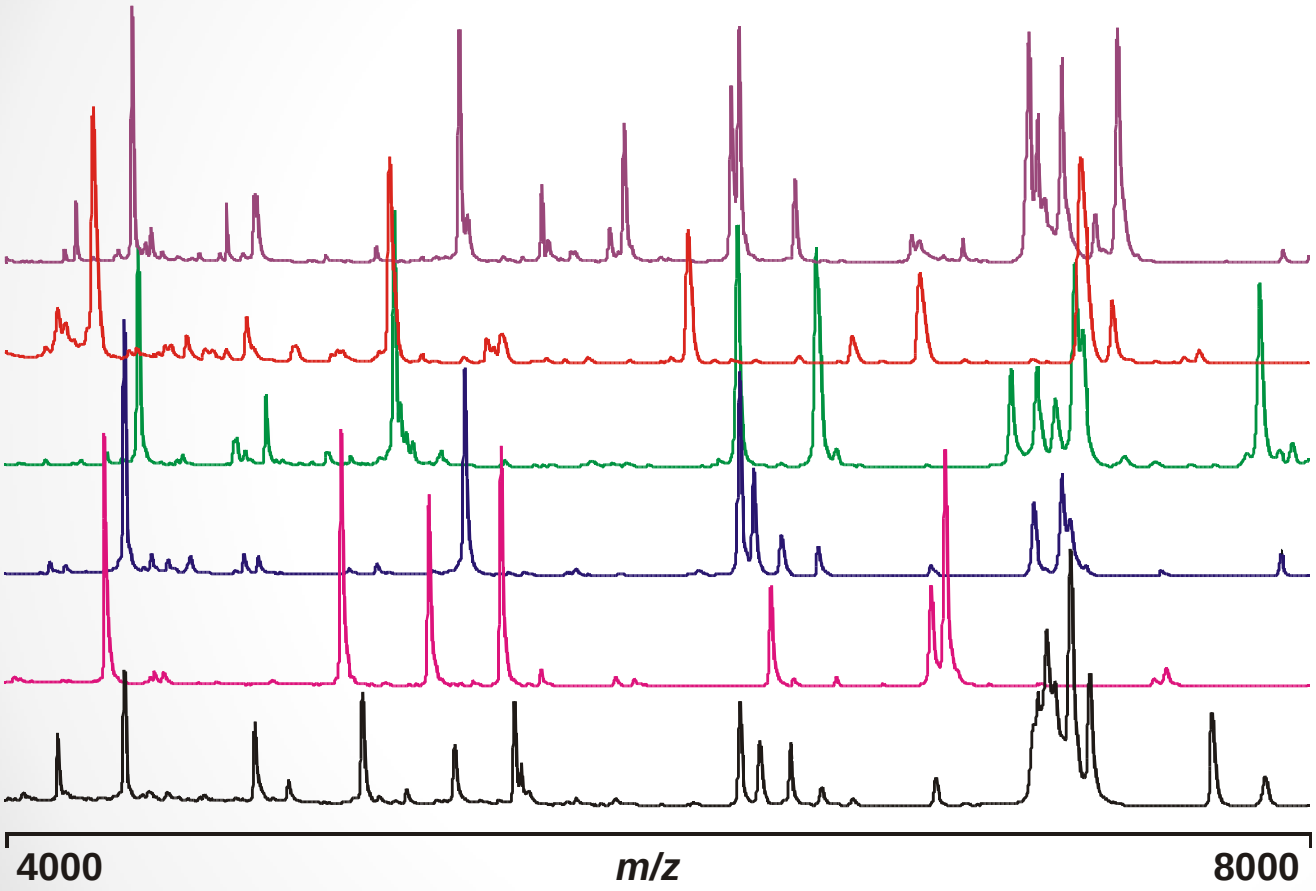
Protein Complex Analysis by High-Mass MALDI ToF MS

Biraz fizik…

- Lazer vuruşları ile oluşan dijitalize veriler **TOF kütle spektrumu** oluşturacak şekilde toplanır.
- TOF kütle spektrumu zamanın bir fonksiyonu olarak saptayıcı sinyalin bir kaydıdır.
- Kütlenin (m) bir molekülünün uçuş zamanı ve bu mesafeyi geçerken yüklendiği akım (z) $(m/z)^{1/2}$ye orantılıdır.
- Zaman ve $(m/z)^{1/2}$ arasındaki ilişki **iyonların kütlelerini** hesaplamada kullanılabilir.


Beam splitter
Laser
(1)
Protein
sample is
ionized
Trigger
(2)
Electrical field
accelerates ions
(4)
Laser triggers
a clock
Transient
recorder
Ion
source
Flight tube
(3)
Lightest ions
arrive at the
detector first
Detector
Laser
beam
Matrix
Sample
Protein

Tam bakteri hücresindeki kütle spektrometresine göre farklı kromatogramların oluşması



Pantoea agglomerans

Acinetobacter lwofii

Burkholderia cepacia

Raoultella ornithinolytica

Staph.aureus

E.coli

Analiz - Tanımlama

Mikroorganizma tanımlaması

Algoritma hesaplaması

Veritabanı ile karşılaştırma

Biyoinformatik Analiz

- BioTyper (Bruker Daltonics Inc., Bremen, Almanya)
- Saramis (AnagnosTec GmbH, Potsdam-Golm, Almanya)

Abiotrophia	Bordetella	Corynebacterium	Fusarium	Lactobacillus	Nocardia	Propionibacterium	Staphylococcus
Achromobacter	Brettanomyces	Cronobacter	Fusobacterium	Lactococcus	Obesumbacterium	Proteus	Stephanoascus
Acinetobacter	Brevibacillus	Cryptococcus	Gardnerella	Leclercia	Ochrobactrum	Prototheca	Streptococcus
Actinobacillus	Brevibacterium	Cupriavidus	Gemella	Lecythophora	Oerskovia	Providencia	Suttonella
Actinomyces	Brevundimonas	Debaryomyces	Geobacillus	Legionella	Oligella	Pseudoflavonifractor	Tatumella
Aerococcus	Brochothrix	Delftia	Geotrichum	Leifsonia	Paecilomyces	Pseudomonas	Tetragenococcus
Aeromonas	Budvicia	Dermabacter	Globicatella	Leptotrichia	Paenibacillus	Psychrobacter	Thermoanaerobacterium
Aggregatibacter	Burkholderia	Dermacoccus	Gluconobacter	Leuconostoc	Pantoea	Rahnella	Trichosporon
Alcaligenes	Buttiauxella	Dietzia	Gordonia	Listeria	Parabacteroides	Ralstonia	Trueperella
Alicyclobacillus	Campylobacter	Edwardsiella	Granulicatella	Lysinibacillus	Paracoccus	Raoultella	Vagococcus
Alloiococcus	Candida	Eggerthella	Gr[illegible]ntia	Macrococcus	Parvimonas	Rhizobium	Veillonella
Alternaria	Capnocytophaga	Eikenella	Haemophilus	Malassezia	Pasteurella	Rhodococcus	Vibrio
Anaerobiospirillum	Cardiobacterium	Elizabethkingia	Hafnia	Mannheimia	Pectinatus	Rhodotorula	Virgibacillus
Aneurinibacillus	Carnobacterium	Empedobacter	Helcococcus	Methylobacterium	Pectobacterium	Riemerella	Weeksella
Arcanobacterium	Cedecea	Enterobacter	Helicobacter	Microbacterium	Pediococcus	Rothia	Weissella
Arcobacter	Cellulosimicrobium	Enterococcus	Histophilus	Micrococcus	Penicillium	Saccharomyces	Williopsis
Arthrobacter	Chryseobacterium	Erwinia	Kingella	Mobiluncus	Peptoniphilus	Salmonella	Xanthomonas
Asaia	Citrobacter	Erysipelothrix	Klebsiella	Moellerella	Peptostreptococcus	Serratia	Xenorhabdus
Aspergillus	Cladosporium	Escherichia	Kloeckera	Moraxella	Photobacterium	Shewanella	Yersinia
Bacillus	Clavibacter	Eubacterium	Kluyvera	Morganella	Pichia	Sphingobacterium	Yokenella
Bacteroides	Clostridium	Ewingella	Kocuria	Mycobacterium	Plesiomonas	Sphingobium	Zygosaccharomyces
Bergeyella	Collinsella	Facklamia	Kodamaea	Myroides	Porphyromonas	Sphingomonas	
Bifidobacterium	Comamonas	Finegoldia	Kytococcus	Neisseria	Prevotella	Sporobolomyces	

753 Tür

"MALDI-TOF"	8396	15062
"MALDI-TOF" "bacteria"	1393	3410
"MALDI-TOF" "fungi"	847	2432
"MALDI-TOF" "virus"	249	444

http://www.ncbi.nlm.nih.gov/sites/entrez

Kasım 2010

Kasım 2015

- MALDI-MS mikroorganizma analizi için önemli bir tanı aracı
- MALDI-MS
  - hastalık monitorizasyonu
  - tanısı
  - kanın taranması
- Bakteri, maya ve filamentöz mantarların, virus, viral vektörler ve doğru ve hızlı tanımlanması
- Taksonomi ve epidemiyoloji

Kan Kültürden Direkt İdentifikasyon

asm2012

American Society for Microbiology
112th General Meeting
San Francisco, CA
June 16-19, 2012
4424

Rapid Identification of Bacteria and Yeasts from Positive Blood Culture Bottles by Using a Lysis-Filtration Method and MALDI-TOF Mass Spectrum Analysis with SARAMIS Database

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[1]Emory University School of Medicine, Atlanta, GA, [2]bioMérieux, Inc., Durham, NC, [3]Grady Memorial Hospital, Atlanta, GA.

EMORY UNIVERSITY

Grady Health System

INTRODUCTION

Rapid identification of bloodstream infections after a positive blood culture result would greatly improve patient care. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) can be used to identify (ID) microorganisms. The VITEK® MS RUO System with SARAMIS™ database by bioMérieux is a research use only MALDI-TOF MS system for rapid detection of bacterial and yeast isolates. MALDI-TOF mass spectrometry (MS) has the potential to serve as a fast and reliable method for identifying microorganisms. This study aims to evaluate the performance of a novel filtration-based method[4] for processing positive BacT/ALERT® blood culture broth for immediate identification by MALDI-TOF MS.

MATERIALS AND METHODS

BacT/ALERT® anaerobic (SN) and standard aerobic (SA) non-charcoal based blood culture bottles that were flagged as positive by bioMérieux's BacT/ALERT 3D system were included in the study. If possible, the bottles were processed the same day they flagged as positive, but older bottles were included as well. A bottle was considered to have a valid MALDI result if at least one spot gave a SARAMIS rating of 75% or more and other spots were not contradictory. Bottles that did not generate a MALDI ID on the first attempt were automatically repeated; either bottles, later found to have inconsistent results compared to VITEK 2 results were repeated as well. If a bottle had inconsistent results but could not be repeated it was eliminated from consideration. If no MALDI ID was generated on initial testing, or if there was a discrepancy compared to the reference ID, a bottle was only processed two times. Both bottle types were processed identically. Samples and reagents were brought to room temperature before use.

SAMPLE PREPARATION

Figure 1: Sample Preparation

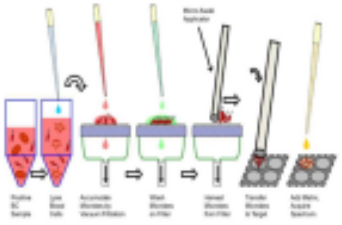

Figure 2: Lysate Application, Completed Spot for Analysis, and manifold and reservoir

SAMPLE PREPARATION

- A 2.0 ml sample of positive blood culture broth was added to 1.0 ml of lysis buffer (0.6% Brij-97 in 0.4M CAPS, 0.2µ filtered, pH 11.7), vortexed for 5 seconds, and allowed to incubate for 2 minutes at room temperature. The resulting lysate was passed in a constant stream through a 25mm 8.45µm filter (Millipore Ekspress PLUS®, shiny side down) for 40 seconds. If the liquid backed up, the sample addition was slowed in order to keep the sample application area to roughly 1 cm².
- The microbial cells remaining on the filter membrane after 40 seconds were washed 3 times with wash buffer (20 mM No phosphate, 8.95% Brij-97, 0.45% NaCl, 0.2µ filtered, pH 7.2) and then three times with deionized water. For each wash, enough buffer/water was added to the membrane to completely cover the membrane without flowing over. All liquid was allowed to pass through the membrane before subsequent washes.
- Once the microorganisms had been washed, they were removed by firmly scraping the membrane with a polyester fabric-tipped micro-swab (Texwipe CleanTips® Swabs, cat. No. TXTS48). Organisms were then directly applied to disposable MALDI target plates (Shimadzu Biotech, cat. No. 220-000889-FIM1) and immediately covered with 1 µL of CHCA matrix. When a blood culture bottle was repeated, the volume of blood culture broth and corresponding buffers was doubled. All other procedures remained the same.

RESULTS

- A total of 250 bottles were included in the study, comprising 225 monomicrobic, 20 polymicrobic, and 5 that were negative on subculture. With all bottles included, the MALDI-TOF MS was able to identify 73.6% of positive-flagged cultures to the species level, 19.1% gave no ID, while 3.1% were incorrect (Table 2).
- There were 225 confirmed-positive bottles containing a single organism, and MALDI was able to identify 77.8% to the species level and 3 organisms (1.3%) were only identified to the genus level. 17.8% of bottles processed with a single organism present did not generate a MALDI ID, and 3.1% were incorrect (Table 1). Thus, for monomicrobic cultures, if MALDI was able to generate an ID (185) it was correct to the species level 94.6% of the time.
- Among monomicrobic cultures, 86.4% of gram negative bacteria, 76.8% of gram positive bacteria, and 88.2% of yeasts were correctly identified by MALDI to at least the family level (Table 3).
- Twenty-eight bottles had multiple organisms; one organism of the mixture in each of 13 bottles was identified to the species level (46.4%), and 3 were identified only to genus level (10.7%). Eleven bottles (39.3%) gave no MALDI ID, and 1 bottle had an incorrect result (3.6%). No more than one organism was identified from each bottle.
- Including both monomicrobic and polymicrobic cultures, when MALDI was able to generate an ID (282) it was correct to the species level 93.1% of the time, and 4.6% of IDs were incorrect.

RESULTS

Table 1: Results of All Single-Organism Identifications

Organism	# Analyzed	# Species ID consistent with reference	# Genus ID consistent with reference	# No MALDI ID	# Incorrect
Staphylococcus (CNS)[2]	64	49[2]	0	14	1
Staphylococcus aureus (MSSA)	23	22	0	0	1
Staphylococcus aureus (MRSA)	22	21	0	1	0
Escherichia coli	16	14[2]	1	1	0
Klebsiella pneumoniae	16	16	0	0	0
Streptococcus pneumoniae	11	2	0	9	0
Acinetobacter baumannii	7	3	0	3	1
Candida parapsilosis	7	7	0	0	0
Candida albicans	6	5[2]	0	0	1
Streptococcus agalactiae	6	5	0	1	0
Corynebacterium	5	0	0	4	1
Enterococcus faecium	5	4[2]	1	0	0
Candida glabrata	3	2	0	1	0
Enterobacter aerogenes	3	3	0	0	0
Enterobacter cloacae	3	2[2]	1	0	0
Enterococcus faecalis	3	3	0	0	0
Streptococcus, Viridans Group	3	2[2]	0	1	0
Haemophilus influenzae	2	1	0	1	0
Proteus mirabilis	2	2	0	0	0
Propionibacterium acnes	2	2[2]	0	0	0
Salmonella sp	2	2[2]	0	0	0
Actinomyces meyer	1	0	0	1	0
Bacillus sp	1	0	0	1	0
Bacteroides fragilis	1	1	0	0	0
Candida sp	1	0	0	0	1
Candida tropicalis	1	1	0	0	0
Pseudomonas sp	1	1[2]	0	0	0
Lactobacillus sp	1	0	0	1	0
Morganella morganii	1	0	0	0	1
Micrococcus sp	1	1[2]	0	0	0
Pseudomonas sp	1	0	0	1	0
Pseudomonas aeruginosa	1	1	0	0	0
Serratia marcescens	1	1	0	0	0
Streptococcus, Group B	1	1[2]	0	0	0
Streptococcus, Group G	1	1[2]	0	0	0
Total (%)	225	175 (77.8)	3 (1.3)	48 (17.8)	7 (3.1)

[2]Reference ID consisted of routine laboratory workup, which was not to species level where indicated (see footnote 1)

[4] "PROCEDURE FOR PREPARING BLOOD CULTURES FOR DIRECT IDENTIFICATION OF MALDI-TOF MS", bioMérieux research use only (RUO) protocol, February 13, 2012

RESULTS

Table 2: Results from all Positive Blood Culture Bottles Processed

	All Blood Culture Bottles
# Correct Species (%)	185[1] (73.6)
# Correct only to Genus/Family (%)	6 (2.3)
# No MALDI ID, subculture positive (%)	51 (19.7)
# No Growth and No MALDI ID (%)	6 (2.3)
# Incorrect ID (%)	8 (3.1)
Total # Samples	259

[1]Where all three organisms were identified from bottles with multiple organisms to the species level. Two organisms were identified to the species level with "low discrimination". One MALDI gave multiple species results, one of which was correct. Fifty-three of those organisms were determined to have "essential agreement" to the species level. Organisms were considered to have "essential agreement" if the MALDI ID and conventional method were in agreement at the family and genus levels, but only the MALDI provided a species level identification.

Table 3: Characteristics of Monomicrobic Positive Blood Culture Bottles Processed

	Correct/Total (%)
# Gram Negative bacteria	51/59 (86.4%)
# Gram Positive bacteria	113/149 (75.8%)
# Yeast	15/17 (88.2%)
Total # Samples	225

[1]organisms were counted as correct if they matched at family and genus levels. Organisms that had low discrimination or essential agreement were also included in the total correct.

CONCLUSION

This study demonstrates the effectiveness of this new Lysis-Filtration method for isolating and identifying microorganisms from positive BacT/ALERT® blood culture bottles in a clinical setting. Approximately 80% of monomicrobic cultures were correctly identified. The differing levels of biomass within some positive blood cultures, as well as autolysis of some species, may contribute to the number of bottles that were unable to generate a MALDI result. The lysis buffer used eliminates blood cells while leaving microorganisms intact to undergo rapid analysis by MALDI-TOF MS. This method is advantageous as it does not require centrifugation and produces a clean, concentrated sample of microorganisms in less than 10 minutes.

For more information and/or question please contact:
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yfwang@emory.edu

Table 2: Results from all Positive Blood Culture Bottles Processed

	All Blood Culture Bottles
# Correct Species (%)	188^ (72.6)
# Correct only to Genus/Family (%)	6 (2.3)
# No MALDI ID, subculture positive (%)	51 (19.7)
# No Growth and No MALDI ID (%)	6 (2.3)
# Incorrect ID (%)	8 (3.1)
Total # Samples	259

Table 3: Characteristics of Monomicrobic Positive Blood Culture Bottles Processed

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# Gram Negative bacteria	51/59 (86.4%)
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ANNALS OF LABORATORY MEDICINE

Evaluation of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry-Based VITEK MS System for the Identification of *Acinetobacter* Species from Blood Cultures: Comparison with VITEK 2 and MicroScan Systems

Seung Yeob Lee, M.D., Jong Hee Shin, M.D., Soo Hyun Kim, M.D., Myung Geun Shin, M.D., Soon Pal Suh, M.D., and Dong Wook Ryang, M.D.

Department of Laboratory Medicine, Chonnam National University Medical School, Gwangju, Korea

Background: *Acinetobacter* species are the leading cause of bloodstream infection (BSI), but their correct identification is challenging. We evaluated the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS)-based VITEK MS (bioMérieux, France), and two automated systems, VITEK 2 (bioMérieux) and MicroScan (Siemens, USA) for identification of *Acinetobacter* BSI isolates.

Methods: A total of 187 BSI isolates recovered at a university hospital in Korea between 2010 and 2012 were analyzed. The identification results obtained using VITEK MS and two automated systems were compared with those of *rpoB* sequencing.

Results: Of 187 isolates analyzed, 176 were identified to the species level by *rpoB* sequencing: the *Acinetobacter baumannii* group (ABG; 101 *A. baumannii*, 43 *A. nosocomialis*, 10 *A. pittii* isolates) was most commonly identified (82.4%), followed by *Acinetobacter* genomic species 13BJ/14TU (5.3%), *A. ursingii* (2.1%), *A. soli* (2.1%), *A. bereziniae* (1.1%), and *A. junii* (1.1%). Correct identification rates to the species group (ABG) level or the species level was comparable among the three systems (VITEK MS, 90.3%; VITEK 2, 89.2%; MicroScan, 86.9%). However, VITEK MS generated fewer misidentifications (0.6%) than VITEK 2 (10.8%) and MicroScan (13.1%) ($P<0.001$). In addition, VITEK MS demonstrated higher specificity (100%) for discrimination between ABG and non-ABG isolates than the other systems (both, 31.8%) ($P<0.001$).

Conclusions: The VITEK MS system is superior to the VITEK 2 and MicroScan systems for identification of *Acinetobacter* BSI isolates, with fewer misidentifications and better discrimination between the ABG and non-ABG isolates.

Key Words: VITEK MS, VITEK 2, MicroScan, *Acinetobacter*, Identification

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LETTER TO THE EDITOR

Differentiation of *Acinetobacter* Genomic Species 13BJ/14TU from *Acinetobacter haemolyticus* by Use of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS)

Benjamin E. W. Toh,[a] Hosam M. Zowawi,[a,b,c] Lenka Krizova,[e] David L. Paterson,[a] Witchuda Kamolvit,[a,d] Anton Y. Peleg,[f,g] Hanna Sidjabat,[a] Alexandr Nemec,[e] Valentin Pflüger,[h] Charlotte A. Huber[a]

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BioMed Research International
Volume 2015, Article ID 535027, 8 pages
http://dx.doi.org/10.1155/2015/535027

Research Article

Insufficient Discriminatory Power of Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry Dendrograms to Determine the Clonality of Multi-Drug-Resistant *Acinetobacter baumannii* Isolates from an Intensive Care Unit

John Hoon Rim,[1] Yangsoon Lee,[2] Sung Kuk Hong,[1] Yongjung Park,[1] MyungSook Kim,[1] Roshan D'Souza,[1] Eun Suk Park,[3] Dongeun Yong,[1] and Kyungwon Lee[1]

While pulsed-field gel electrophoresis (PFGE) is recognized as the gold standard method for clonality analysis, MALDI-TOF MS has recently been spotlighted as an alternative tool for species identification. Herein, we compared the dendrograms of multi-drug-resistant (MDR) *Acinetobacter baumannii* isolates by using MALDI-TOF MS with those by using PFGE. We used direct colony and protein extraction methods for MALDI-TOF MS dendrograms. The isolates with identical PFGE patterns were grouped into different branches in MALDI-TOF MS dendrograms. Among the isolates that were classified as very close isolates in MALDI-TOF MS dendrogram, PFGE band patterns visually showed complete differences. We numeralized similarity among isolates by measuring distance levels. The Spearman rank correlation coefficient values were 0.449 and 0.297 between MALDI-TOF MS dendrogram using direct colony and protein extraction method versus PFGE, respectively. This study is the first paper focusing solely on the dendrogram function of MALDI-TOF MS compared with PFGE. Although MALDI-TOF MS is a promising tool to identify species in a rapid manner, our results showed that MALDI-TOF MS dendrograms could not substitute PFGE for MDR *Acinetobacter baumannii* clonality analysis.

RESEARCH ARTICLE

Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass-Spectrometry (MALDI-TOF MS) Based Typing of Extended-Spectrum β-Lactamase Producing *E. coli* – A Novel Tool for Real-Time Outbreak Investigation

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Abstract

Epidemiologically linked clusters are confirmed by typing strains with molecular typing such as pulsed-field gel electrophoresis (PFGE). We compared six extended-spectrum β-lactamase producing *E. coli* of a PFGE-related cluster with Matrix-assisted laser desorption/ionization-time of flight mass-spectrometry based typing that confirmed relatedness faster and more cost-effective, but as reliable as PFGE.

Detection of carbapenemase activity directly from blood culture vials using MALDI-TOF MS: a quick answer for the right decision

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Objectives: Recently, matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) was successfully applied for the detection of carbapenemase activity directly from Gram-negative colonies. Based on this principle, we evaluated the performance of MALDI-TOF MS for rapid detection of carbapenemase activity directly from positive blood culture vials.
Methods: A total of 100 blood culture vials were randomly selected. MALDI-TOF MS carbapenemase assay results were confirmed by the detection of carbapenemase-encoding genes.
Results: A total of 110 bacterial isolates were recovered. The MALDI-TOF MS carbapenemase assay identified 21 of 29 (72.4%) of the carbapenemase-producing isolates directly from the blood culture vials, especially those encoding KPC-2 (100%) and SPM-1 (100%), after a 4 h incubation period. Although the majority of OXA- 23-producing Acinetobacter baumannii isolates were not identified on day 1, all isolates were identified as carbapenemase producers directly from the colony on the next day.
Conclusions: The MALDI-TOF MS carbapenemase assay is a feasible and rapid test to identify carbapenemase activity directly from blood culture vials. It may contribute to faster readjustment of empirical antimicrobial therapy and implementation of infection control measures.

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Case study

European Journal of Microbiology and Immunology 5 (2015) 2, pp. 177–179
DOI: 10.1556/1886.2015.00008

NOSOCOMIAL INFECTION DUE TO *ENTEROCOCCUS CECORUM* IDENTIFIED BY MALDI-TOF MS AND VITEK 2 FROM A BLOOD CULTURE OF A SEPTIC PATIENT

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We report the case of a nosocomial infection due to *Enterococcus cecorum* isolated from a blood culture of a 75-year-old septic male patient. Matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF MS) and Vitek 2 succeeded in identification of the isolate.

Keywords: *Enterococcus cecorum,* nosocomial infection, MALDI-TOF, mass spectrometry, Vitek 2

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Impact of Antimicrobial Stewardship Intervention on Coagulase-Negative *Staphylococcus* Blood Cultures in Conjunction with Rapid Diagnostic Testing

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Rapid diagnostic testing with matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) decreases the time to organism identification by 24 to 36 h compared to the amount of time required by conventional methods. However, there are limited data evaluating the impact of MALDI-TOF with real-time antimicrobial stewardship team (AST) review and intervention on antimicrobial prescribing and outcomes for patients with bacteremia and blood cultures contaminated with coagulase-negative *Staphylococcus* (CoNS). A quasiexperimental study was conducted to analyze the impact of rapid diagnostic testing with MALDI-TOF plus AST review and intervention for adult hospitalized patients with blood cultures positive for CoNS. Antibiotic prescribing patterns and clinical outcomes were compared before and after implementation of MALDI-TOF with AST intervention for patients with CoNS bacteremia and CoNS contamination. A total of 324 patients with a positive CoNS blood culture were included; 246 were deemed to have contaminated cultures (117 in the preintervention group and 129 in AST the intervention group), and 78 patients had bacteremia (46 in the preintervention group and 32 in the AST intervention group). No differences in demographics were seen between the groups, and similar rates of contamination occurred between the preintervention and AST intervention groups (64.3% versus 72.6%, $P = 0.173$). Patients with bacteremia were initiated on optimal therapy sooner in the AST intervention group (58.7 versus 34.4 h, $P = 0.030$), which was associated with a similarly decreased mortality (21.7% versus 3.1%, $P = 0.023$). Patients with CoNS-contaminated cultures had similar rates of mortality, lengths of hospitalization, recurrent bloodstream infections, and 30-day hospital readmissions, but the AST intervention group had a decreased duration of unnecessary antibiotic therapy (1.31 versus 3.89 days, $P = 0.032$) and a decreased number of vancomycin trough assays performed (0.88 versus 1.95, $P < 0.001$). In patients with CoNS bacteremia, rapid pathogen identification integrated with real-time stewardship interventions improved timely organism identification and initiation of antibiotic therapy. Patients in the AST group with blood cultures contaminated with CoNS had decreased inappropriate antimicrobial prescribing and decreased unnecessary serum vancomycin trough assays.

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Yeast Identification Algorithm Based on Use of the Vitek MS System Selectively Supplemented with Ribosomal DNA Sequencing: Proposal of a Reference Assay for Invasive Fungal Surveillance Programs in China

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Sequence analysis of the internal transcribed spacer (ITS) region was employed as the gold standard method for yeast identification in the China Hospital Invasive Fungal Surveillance Net (CHIF-NET). It has subsequently been found that matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is potentially a more practical approach for this purpose. In the present study, the performance of the Vitek MS v2.0 system for the identification of yeast isolates collected from patients with invasive fungal infections in the 2011 CHIF-NET was evaluated. A total of 1,243 isolates representing 31 yeast species were analyzed, and the identification results by the Vitek MS v2.0 system were compared to those obtained by ITS sequence analysis. By the Vitek MS v2.0 system, 96.7% ($n = 1,202$) of the isolates were correctly assigned to the species level and 0.2% ($n = 2$) of the isolates were identified to the genus level, while 2.4% ($n = 30$) and 0.7% ($n = 9$) of the isolates were unidentified and misidentified, respectively. After retesting of the unidentified and misidentified strains, 97.3% ($n = 1,209$) of the isolates were correctly identified to the species level. Based on these results, a testing algorithm that combines the use of the Vitek MS system with selected supplementary ribosomal DNA (rDNA) sequencing was developed and validated for yeast identification purposes. By employing this algorithm, 99.7% (1,240/1,243) of the study isolates were accurately identified with the exception of two isolates of *Candida fermentati* and one isolate of *Cryptococcus gattii*. In conclusion, the proposed identification algorithm could be practically implemented in strategic programs of fungal infection surveillance.

The Brazilian Journal of
INFECTIOUS DISEASES

www.elsevier.com/locate/bjid

Original Article

Influence of microbiome species in hard-to-heal wounds on disease severity and treatment duration

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Bacterial strains
MALDI-TOF
Sequencing
Superficial wounds

ABSTRACT

Background: Infections, mostly those associated with colonization of wound by different pathogenic microorganisms, are one of the most serious health complications during a medical treatment. Therefore, this study is focused on the isolation, characterization, and identification of microorganisms prevalent in superficial wounds of patients ($n = 50$) presenting with bacterial infection.

Methods: After successful cultivation, bacteria were processed and analyzed. Initially the identification of the strains was performed through matrix-assisted laser desorption/ionization time-of-flight mass spectrometry based on comparison of protein profiles (2–30 kDa) with database. Subsequently, bacterial strains from infected wounds were identified by both matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and sequencing of 16S rRNA gene 108.

Results: The most prevalent species was *Staphylococcus aureus* (70%), and out of those 11% turned out to be methicillin-resistant (*mecA* positive). Identified strains were compared with patients' diagnoses using the method of artificial neuronal network to assess the association between severity of infection and wound microbiome species composition. Artificial neuronal network was subsequently used to predict patients' prognosis ($n = 9$) with 85% success.

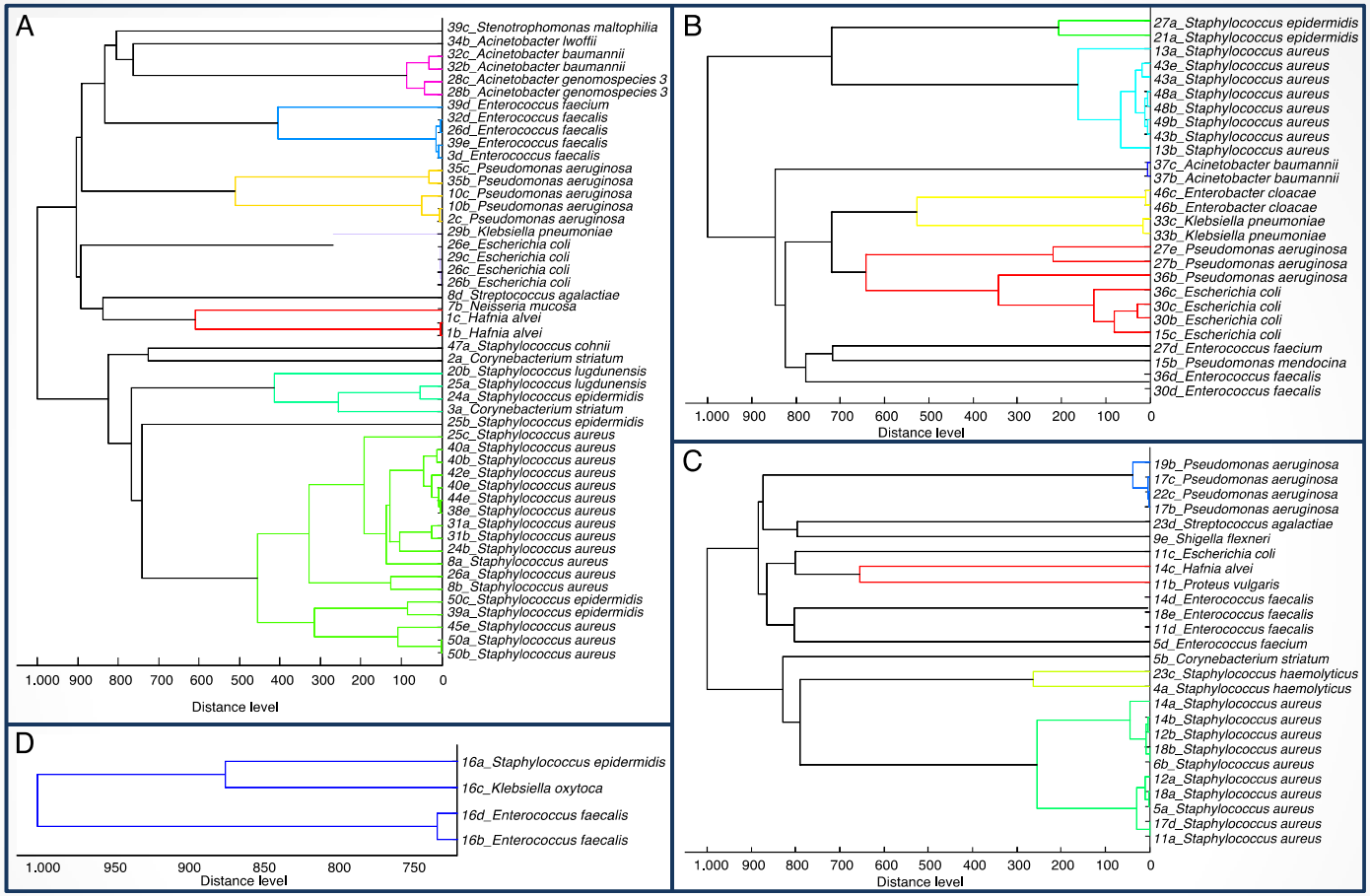


Fig. 2 – Dendrograms from protein mass profiles of microorganisms in different groups based on treatment duration. Created in MALDI Biotyper™. (A) Treatment duration less than four weeks. (B) Treatment duration 4–7 weeks. (C) Treatmen duration eight and more weeks. (D) Exitus.

Avantaj

- Fenotipe yansıyan özellikler
- Hızlı
- Fazla sayıda örnek
- Maliyet etkin
- Sarf malzemeleri ucuz
- Duyarlılık, özgüllük ve pozitif negatif prediktif değer ↑
- Güvenilir

Dezavantaj

- Kurulum pahallı
- Koloni olmalı
- Teknik donanım
- Veriler biyoinformatik programla kısıtlı

PCR-ESI/MS

- PCR-ESI/MS ve MALDI-TOF/MS türe spesifik spektra ile mikroorganizmaları tanımlamaktadır.

- PCR-ESI/MS, bakteriyel, fungal ve viral genomlarda korunmuş ve türe spesifik bölgelere odaklanan birçok lokustan oluşan PCR **amplikonlarının kütle/akım oranını (m/z)** ölçerek mikroorganizmaların veri tabanındaki karşılaştırmalı analizlerle baz kompozisyonlarını tanımlar.


iridica.

- Nükleik asit ekstraksiyonu (direkt klinik örnek)
- Amplifikasyon broad range PCR
- Elektrosprey iyonizasyon kütle spektrometresi
- Patojen veri tabanı analizi

PCR/ESI-MS: Polymerase chain reaction/ionization-mass spectrometry

NUCLEIC ACID EXTRACTION

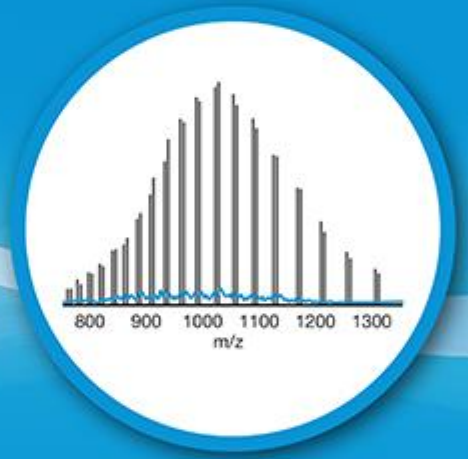
Pathogen nucleic acids extracted from clinical samples

AMPLIFICATION

Broad-range primers bind to conserved regions in bacteria, viruses or fungi

Variable pathogen-specific regions flanked by the conserved regions are amplified

MASS SPECTROMETRY



ESI-MS analysis provides information on the base composition of the amplicon

SAMPLE

'Same-shift' pathogen identification is an achievable goal

Ecker et al. Expert Rev Mol Diagn 2010;10:399–415

THE IRIDICA PLATFORM: HOW IT WORKS

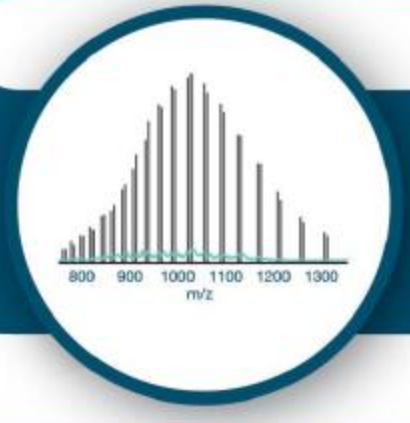
AN URGENT NEED FOR IDENTIFYING INFECTIONS EARLY

Abbott's IRIDICA platform, now available in Europe and other CE-Mark re has the potential to change the way serious infections are diagnosed. The ne identify more than 1,000 infection-causing pathogens in less than six hours source of infections sooner, doctors can improve care for the critically ill.

SAMPLE

A lab technician collects a patient specimen. Genetic material from this specimen is extracted and used for further testing. While most of the genetic material in the specimen is of human origin, some of it belongs to the pathogen that is making the person sick.

Multiple copies of the pathogen's genetic material are generated using a process called polymerase chain reaction (PCR).



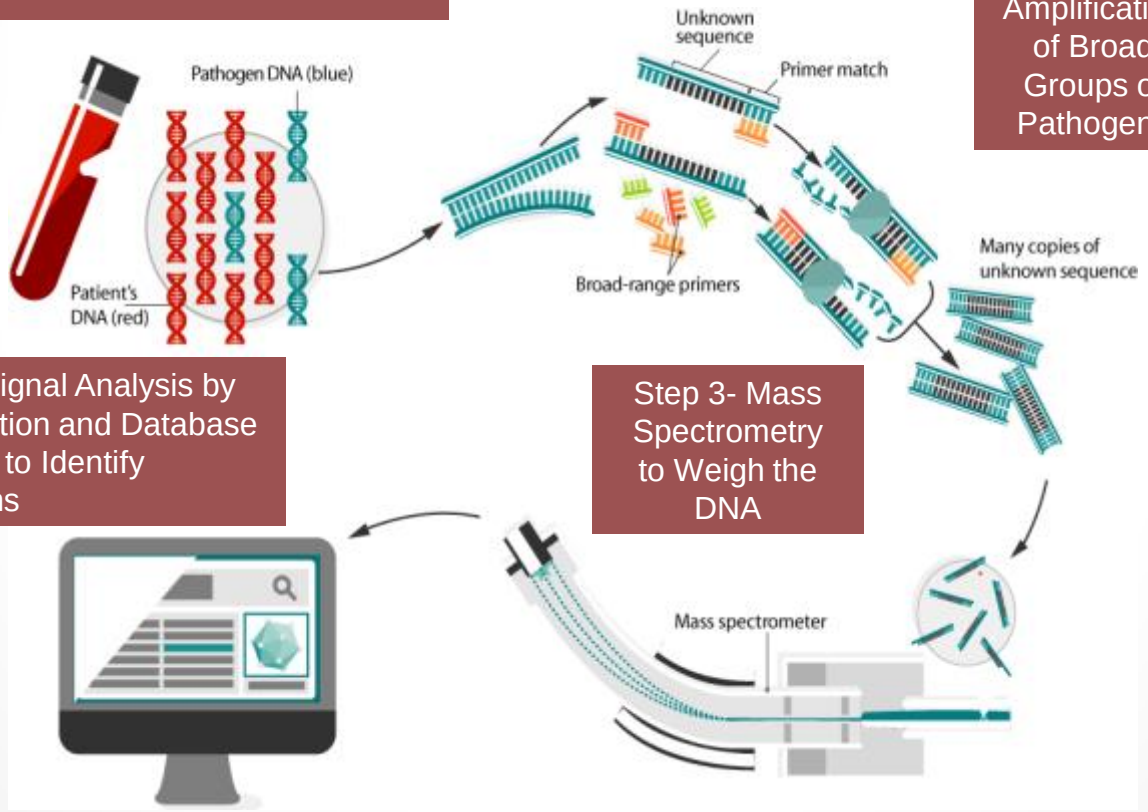
A device called a mass spectrometer is used to determine the molecular weight of the amplified genetic material.

Sophisticated mathematical algorithms

PCR/ESI-MS

Step 1- Nucleic Acid Extraction from Direct Specimens: Blood, BAL, ETA, Tissues

Step 2- Unique Primer Design for Amplification of Broad Groups of Pathogens



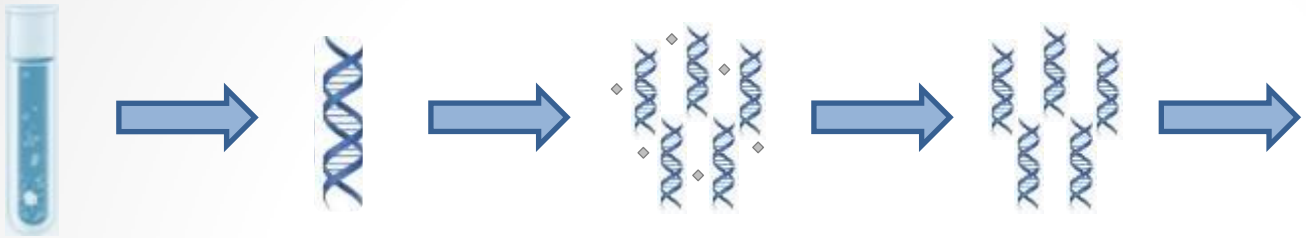
Step 3- Mass Spectrometry to Weigh the DNA

Step 4- Signal Analysis by Triangulation and Database Matching to Identify Pathogens

The IRIDICA technology combines two Nobel-prize winning technologies: PCR and ESI/MS

IRIDICA iş akışı

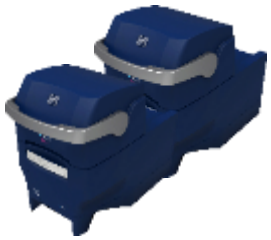
Sample preparation (BB + SP) | **DNA amplification (TC)** | **Desalting (DS)** | **Determining DNA masses (MS)**



sample | isolated DNA | amplified DNA | desalted DNA

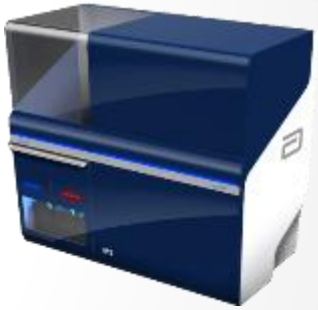
Bead Beater (BB)

Sample Prep (SP)



Thermal Cycler (TC)

Desalter (DS)



Mass Spectrometer (MS)

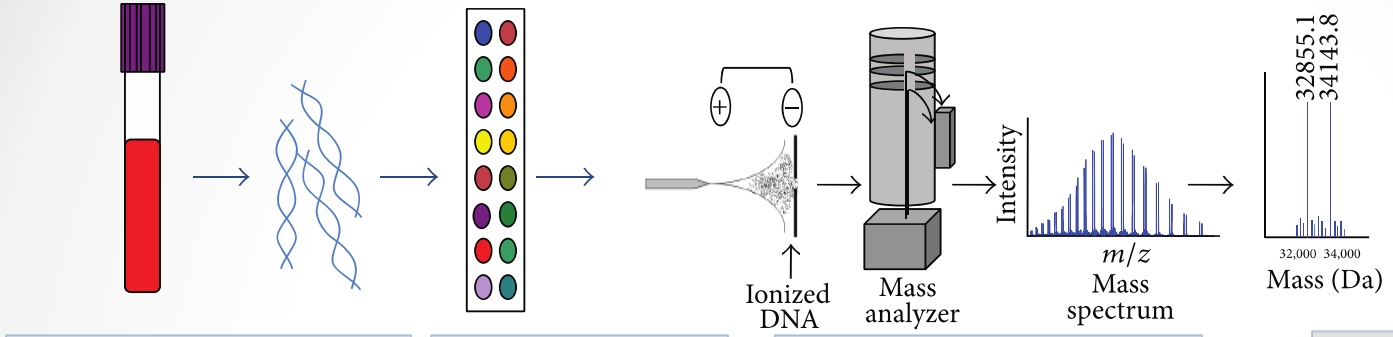
Hands on time:

~ 15 min	~ 10 min	~ 1 min	~ 5 min	~ 1 min

Walk away time:

~ 5 min	~ 110 min	~ 140 – 200 min	~ 35 min	~ 30 min*

*Hands on time and walk away time based on in-house data // * To first sample result*



DNA extraction from whole blood (or other direct specimens)

Amplification using several conserved primers

Desalting and analysis with ESI-MS

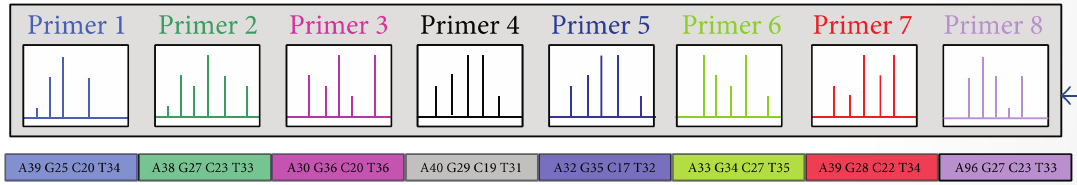
Deduction of base composition based on the molecular mass

Both strands of DNA are analyzed. As they are complementary, only one base composition is possible

[A33 G29 C40 T7]

→[A19 G40 C9 T40]

[A22 G11 C45 T30]



The system gathers all the information obtained from each primer pair

Identified organisms:
(1) *Escherichia coli*
(2) *Staphylococcus aureus*

An unequivocal identification is achieved

IRIDICA Bakteri

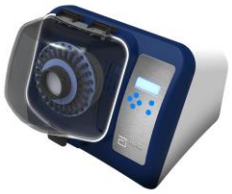
Whole Blood: 5 ml
No diluent

Sterile Fluids: 0.5 ml
Diluent, SF: 4.5 ml

Tissues: 35 mg
Sample Diluent : 4.975 ml

BAL/ETA: 0.1 ml
Sample Diluent: 4.9 ml

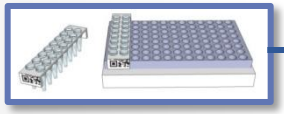
Lysis Buffer + Extraction Control + Beads



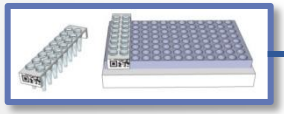
Bead Beater

Automated DNA Extraction and PCR Setup Platform

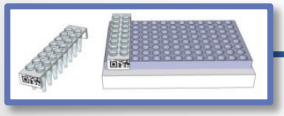
BAC BSI



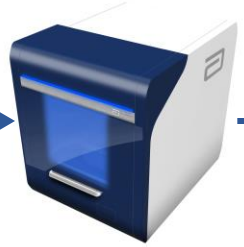
BAC SF&T



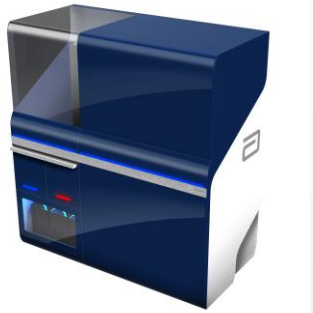
BAC LRT



Thermocycler



Desalter

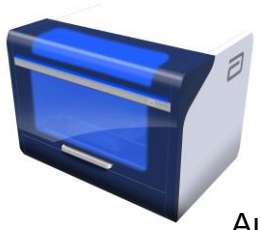


Mass Spectrometer, Database and Analysis Computer

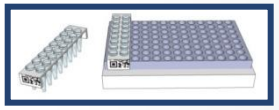
IRIDICA Virus

500 µL plasma + Lysis/Extraction Control Mix

QIAamp/QIAvac System*



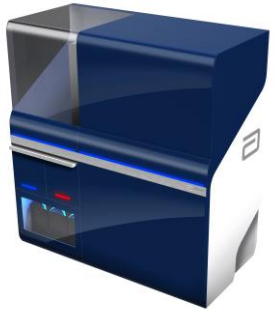
Automated DNA Extraction and PCR Setup Platform



Broad-spectrum PCR primers (8wells/sample)

Thermocycler

Desalter



Mass spectrometer, Database and Analysis Computer

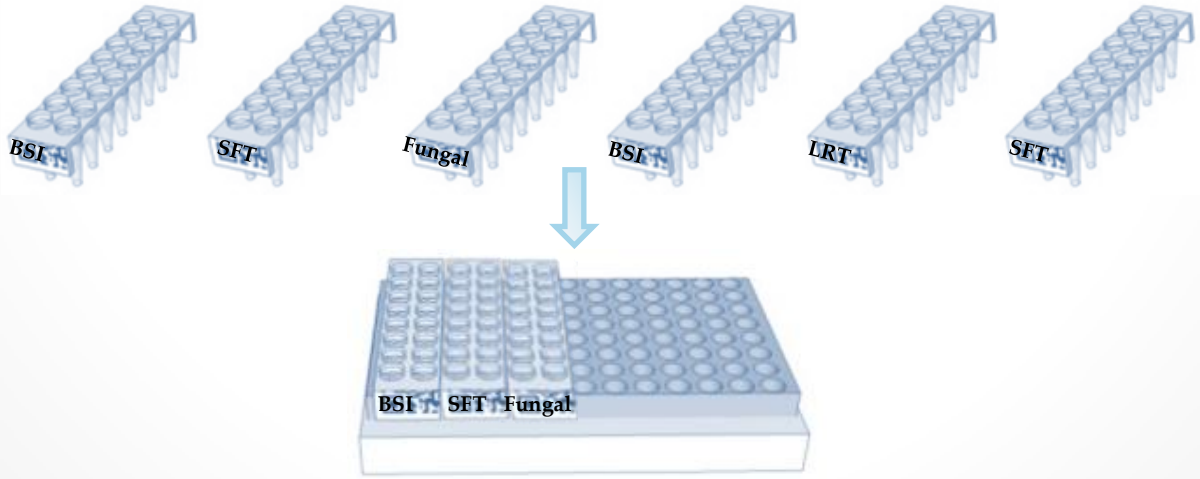
* Not part of the IRIDICA system, not provided by Abbott Diagnostics

Örnekte paralel çalışma

EDTA whole blood
Sterile fluid
Sterile tissues
BAL
ETA

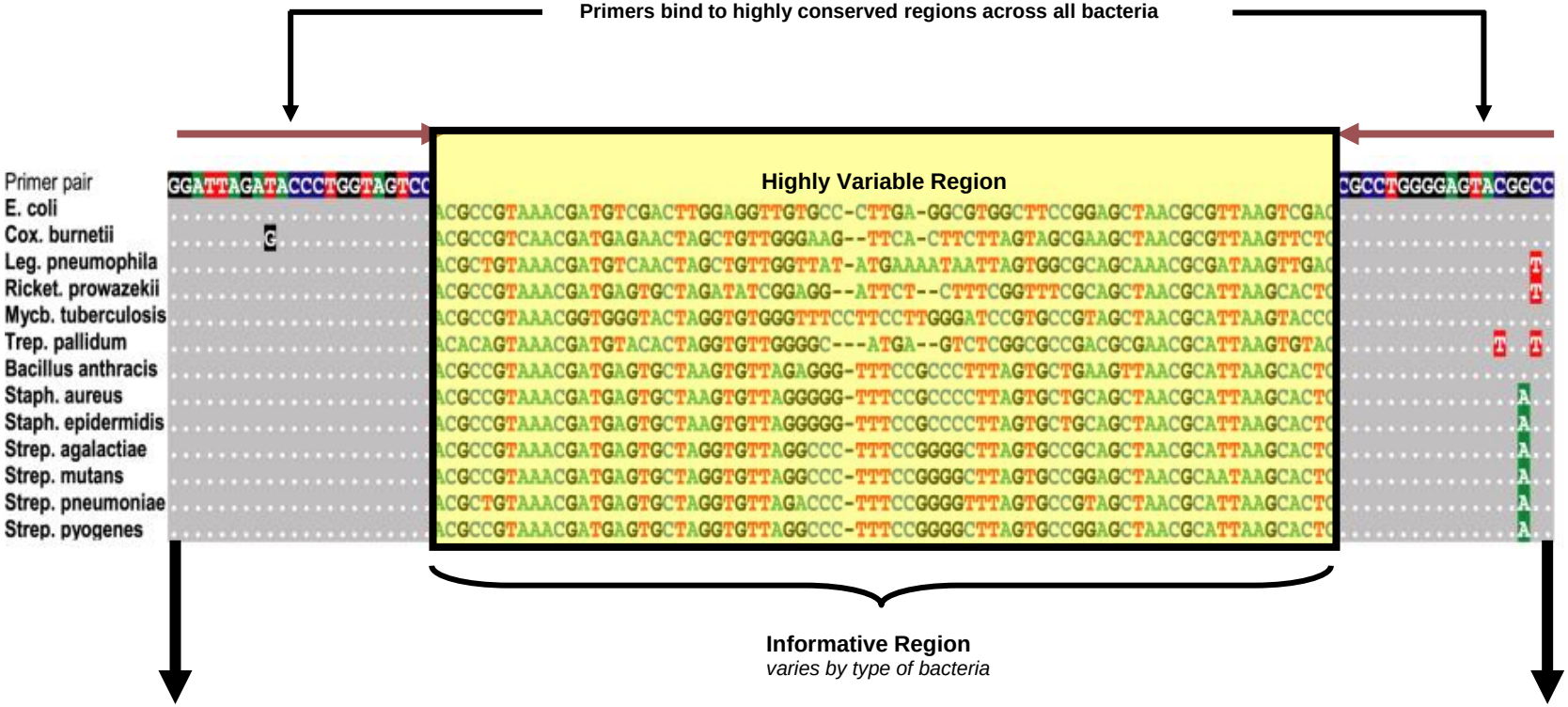
Bacteria
Fungi
Antibiotic Resistance

Identical DNA Extraction Kit & Procedure

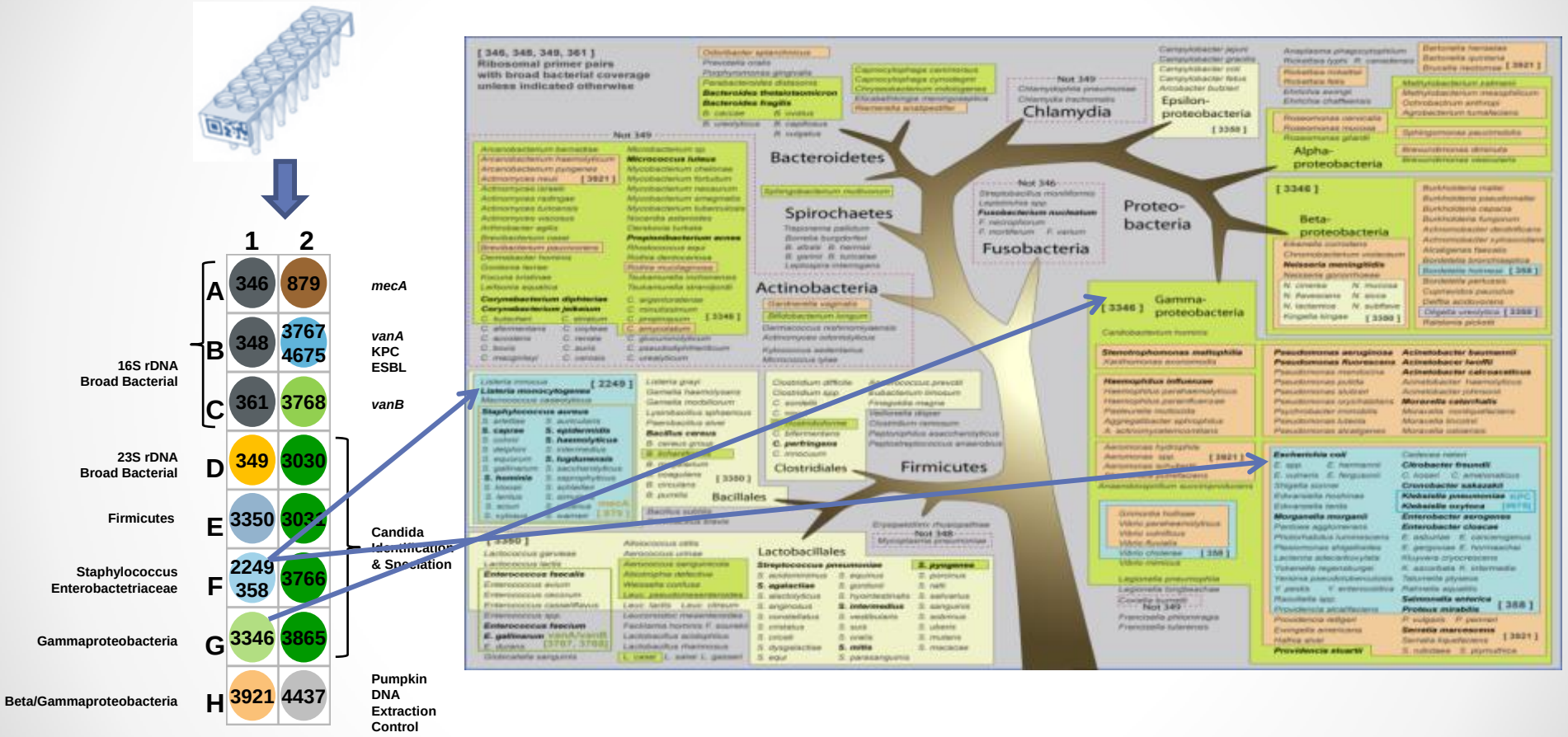


- Patojenin hızlı tanımlanması (6 saat)
- Direkt örneğin test edilmesi (klinik örnek-tam kan, steril sıvılar, steril dokular, plasma, BAL, ETA)
- Bakteri, mantar, virus birçok patojen saptanması
- Direnç tipleme
- Polimikrobiyal enfeksiyonlarda bir patojenden fazla tanımlama

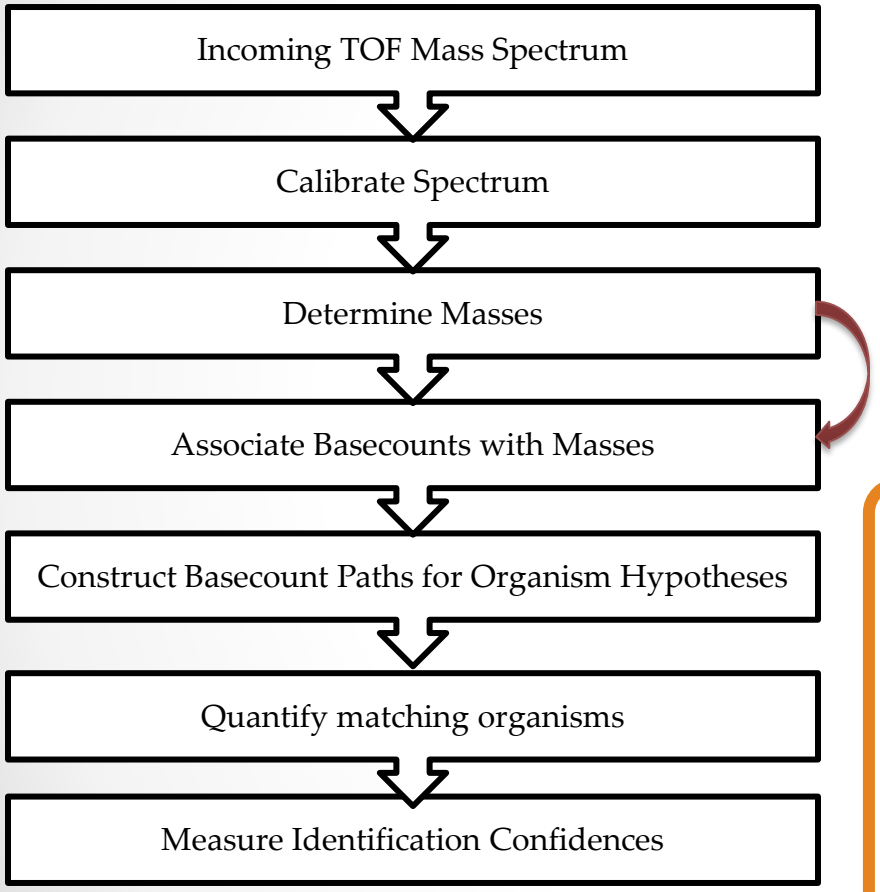
Geniş amplifikasyon(1)

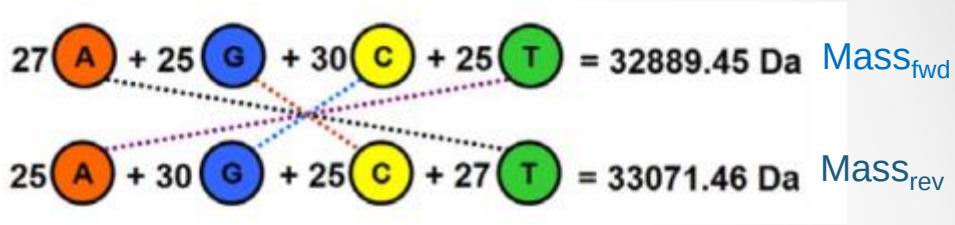


Geniş amplifikasyon(2)

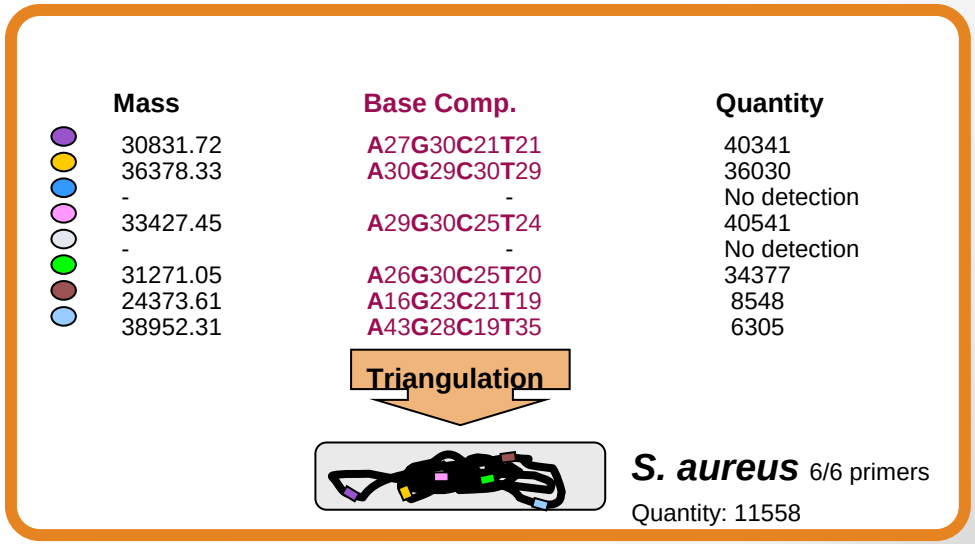


PCR amplikonlarının kütle spektrometresi, sinyal işlenmesi ve organizma tanımlanması

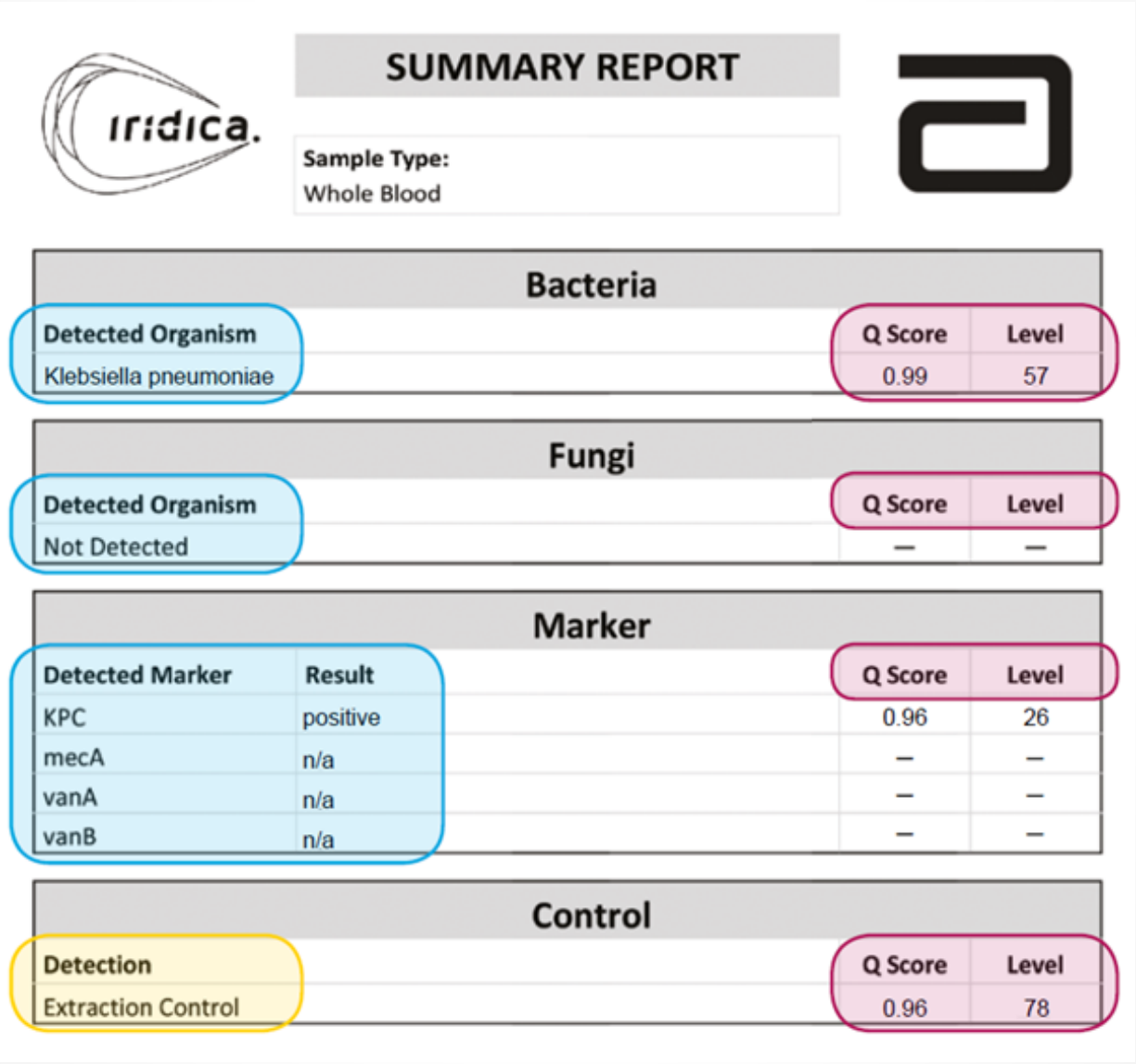




Triangulation Across Multiple Primers



IRIDICA BAC Assay Örnek Raporu



iridica.

SUMMARY REPORT

Sample Type:
Whole Blood

Bacteria

Detected Organism	Q Score	Level
Klebsiella pneumoniae	0.99	57

Fungi

Detected Organism	Q Score	Level
Not Detected	–	–

Marker

Detected Marker	Result	Q Score	Level
KPC	positive	0.96	26
mecA	n/a	–	–
vanA	n/a	–	–
vanB	n/a	–	–

Control

Detection	Q Score	Level
Extraction Control	0.96	78

- >780 bakteri
- >200 mantar
- >130 virus türleri
- Antibiyotik direnci
  - mecA
  - vanA
  - van B
  - kpC

IRIDICA Test Menü

IRIDICA ASSAY		Assay Type	Coverage	Selected Diseases/Patient groups
BAC BSI	1	*Blood stream infection*	780 Bacteria and *Candida*, 4 Antibiotic Resistance Markers: *mecA*, *vanA*, *vanB* and *kpc*	Sepsis
BAC SFT	2	*Sterile fluids and tissues*		Joint prosthetics infection
BAC LRT	3	*Lower respiratory tract*	Identical coverage with semi-quantitative threshold	Pneumonia, IC patients
Fungal	4	*Fungal*	> 200 fungi and yeast	Pneumonia, IC Patients
Viral IC	5	*Viral-Immunocompromised*	13 viral reporting groups covering > 130 viral species	IC Patients

The RADICAL Study

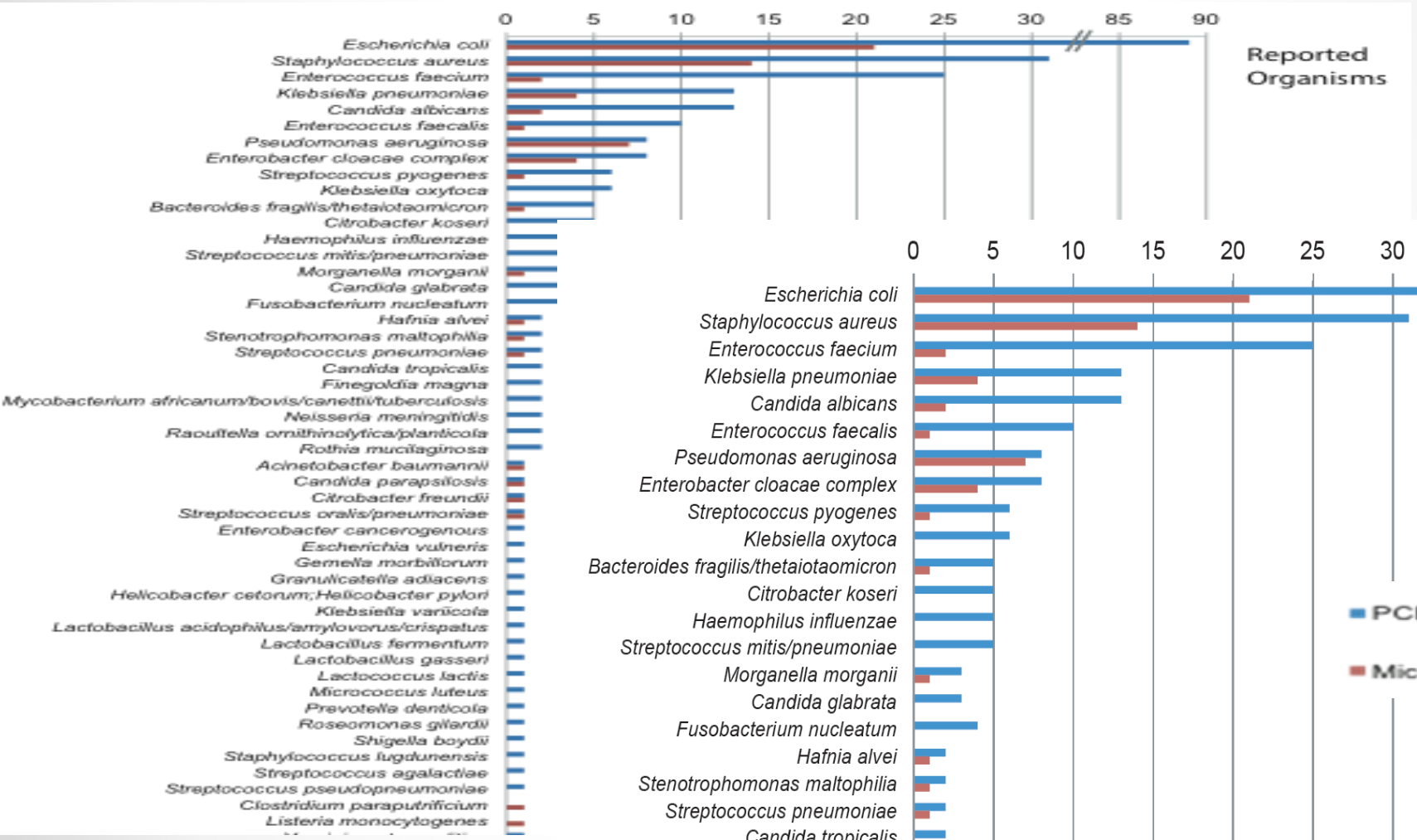
- **RApid Diagnosis of Infections in the CriticAlly ILL (RADICAL)**
- Multi-center observational study **including** 540 patients
  1. Demonstrate analytical performance
  2. Imputed clinical and economic value
- Evaluation of PCR/ESI-MS vs. Culture

Study Center
University College London Hospitals, UK
Service de Santé des Armées, France
Hôpitaux Universitaires de Genève, Switzerland
Barts Health, UK
Hôpital Erasme, Brussels, Belgium
Universitätsklinikum Frankfurt, Germany
Szpitala Dzieciątka Jezus, Warsaw, Poland

Performance against culture in RADICAL:

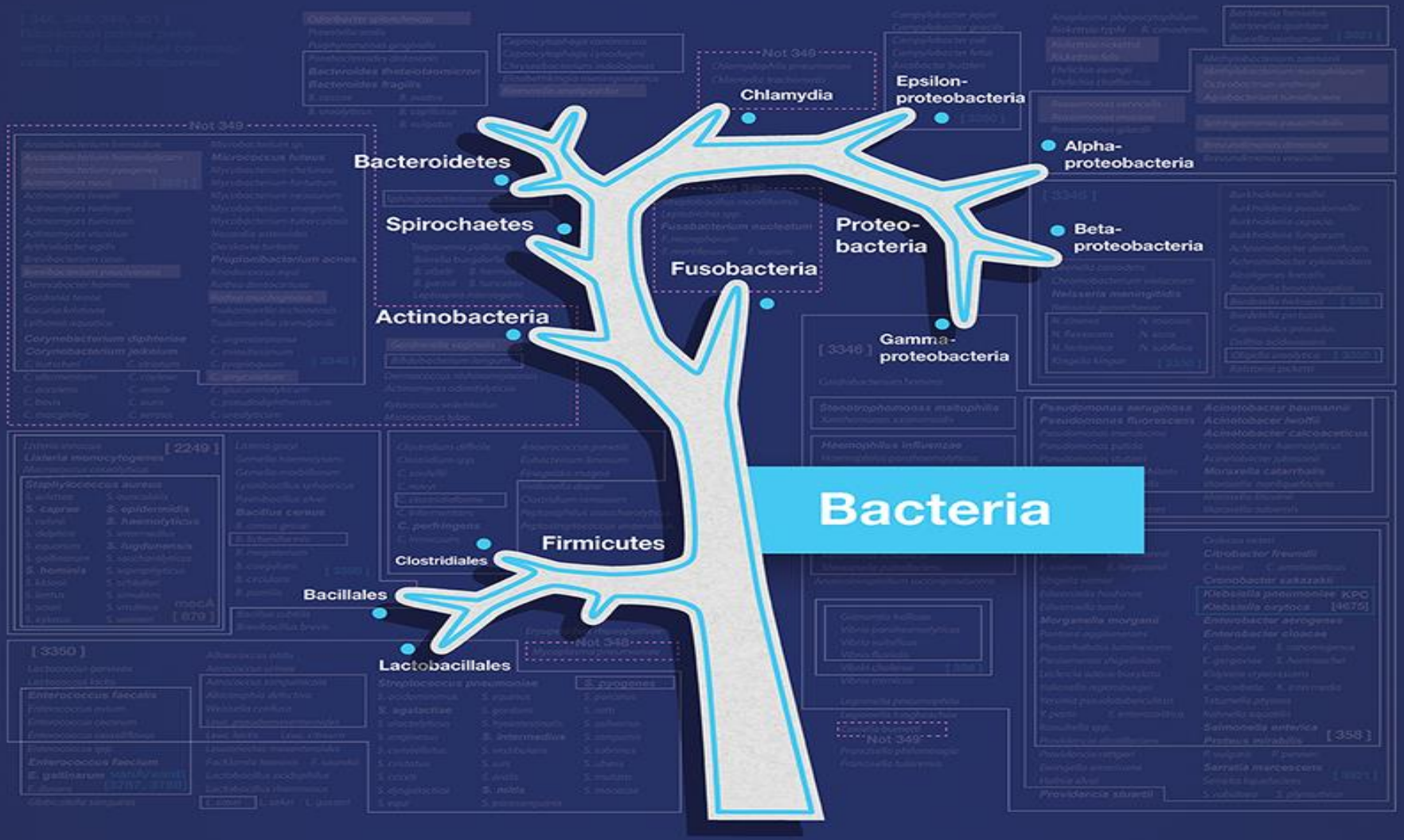
- **Sensitivity: >81%**
- **NPV: 97% for blood stream infections**

Brealey et al., Critical Care 2014, Volume 18 Suppl 2


0
5
10
15
20
25
30
85
90
Escherichia coli
Staphylococcus aureus
Enterococcus faecium
Klebsiella pneumoniae
Candida albicans
Enterococcus faecalis
Pseudomonas aeruginosa
Enterobacter cloacae complex
Streptococcus pyogenes
Klebsiella oxytoca
Bacteroides fragilis/thetaiotaomicron
Citrobacter koseri
Haemophilus influenzae
Streptococcus mitis/pneumoniae
Morganella morganii
Candida glabrata
Fusobacterium nucleatum
Hafnia alvei
Stenotrophomonas maltophilia
Streptococcus pneumoniae
Candida tropicalis
Finegoldia magna
Mycobacterium africanum/bovis/canettii/tuberculosis
Neisseria meningitidis
Raoultella ornithinolytica/planticola
Rothia mucilaginosa
Acinetobacter baumannii
Candida parapsilosis
Citrobacter freundii
Streptococcus oralis/pneumoniae
Enterobacter cancerogenous
Escherichia vulneris
Gemella morbillorum
Granulicatella adiacens
Helicobacter cetorum;Helicobacter pylori
Klebsiella varicola
Lactobacillus acidophilus/amylovorus/crispatus
Lactobacillus fermentum
Lactobacillus gasseri
Lactococcus lactis
Micrococcus luteus
Prevotella denticola
Roseomonas gilardii
Shigella boydii
Staphylococcus lugdunensis
Streptococcus agalactiae
Streptococcus pseudopneumoniae
Clostridium paraputrificum
Listeria monocytogenes
Reported Organisms
PCR/ESI-MS
Microbiology

Bacterial Tree of Life

IRIDICA primers are designed to cover the phylogenetic breadth of bacterial species



This illustration depicts the theoretical detection but not the intended actual reporting of specific organisms by IRIDICA BAC Assays.

PLOS | ONE

RESEARCH ARTICLE

Evaluation of the Broad-Range PCR/ESI-MS Technology in Blood Specimens for the Molecular Diagnosis of Bloodstream Infections

Elena Jordana-Lluch[1,2,5], Montserrat Giménez[1,2], Mª Dolores Quesada[1], Belén Rivaya[1], Clara Marcó[1], Mª Jesús Domínguez[3], Fernando Arméstar[4], Elisa Martró[1,5,6]*, Vicente Ausina[1,2,5]

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The overall positive and negative agreement of IRIDICA with blood culture in the analysis by specimen was 74.8% and 78.6%, respectively, rising to 76.9% and 87.2% respectively, when compared with the clinical infection criterion. Interestingly, IRIDICA detected 41 clinically significant microorganisms missed by culture, most of them from patients under antimicrobial treatment. Of special interest were the detections of one *Mycoplasma hominis* and two *Mycobacterium simiae* in immunocompromised patients. When ICU patients were analyzed separately, sensitivity, specificity, positive and negative predictive values compared with blood culture were 83.3%, 78.6%, 33.9% and 97.3% respectively, and 90.5%, 87.2%, 64.4% and 97.3% respectively, in comparison with the clinical infection criterion.

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BioMed Research International
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Review Article

Improving the Diagnosis of Bloodstream Infections: PCR Coupled with Mass Spectrometry

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The reference method for the diagnosis of bloodstream infections is blood culture followed by biochemical identification and antibiotic susceptibility testing of the isolated pathogen. This process requires 48 to 72 hours. The rapid administration of the most appropriate antimicrobial treatment is crucial for the survival of septic patients; therefore, a rapid method that enables diagnosis directly from analysis of a blood sample without culture is needed. A recently developed platform that couples broad-range PCR amplification of pathogen DNA with electrospray ionization mass spectrometry (PCR/ESI-MS) has the ability to identify virtually any microorganism from direct clinical specimens. To date, two clinical evaluations of the PCR/ESI-MS technology for the diagnosis of bloodstream infections from whole blood have been published. Here we discuss them and describe recent improvements that result in an enhanced sensitivity. Other commercially available assays for the molecular diagnosis of bloodstream infections from whole blood are also reviewed. The use of highly sensitive molecular diagnostic methods in combination with conventional procedures could substantially improve the management of septic patients.

JCM

PCR-Electrospray Ionization Mass Spectrometry for Direct Detection of Pathogens and Antimicrobial Resistance from Heart Valves in Patients with Infective Endocarditis

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Microbiological diagnosis is pivotal to the appropriate management and treatment of infective endocarditis. We evaluated PCR-electrospray ionization mass spectrometry (PCR/ESI-MS) for bacterial and candidal detection using 83 formalin-fixed paraffin-embedded heart valves from subjects with endocarditis who had positive valve and/or blood cultures, 63 of whom had positive valvular Gram stains. PCR/ESI-MS yielded 55% positivity with concordant microbiology at the genus/species or organism group level (e.g., viridans group streptococci), 11% positivity with discordant microbiology, and 34% with no detection. PCR/ESI-MS detected all antimicrobial resistance encoded by *mecA* or *vanA/B* and identified a case of *Tropheryma whipplei* endocarditis not previously recognized.

JCM
Journals.ASM.org

Rapid Identification of Bacteria and *Candida* Pathogens in Peritoneal Dialysis Effluent from Patients with Peritoneal Dialysis-Related Peritonitis by Use of Multilocus PCR Coupled with Electrospray Ionization Mass Spectrometry

Yu-Tzu Chang,[a] Hsuan-Chen Wang,[b] Ming-Cheng Wang,[a] An-Bang Wu,[a] Junne-Ming Sung,[a] H. Sunny Sun,[c] Ih-Jen Su,[b] Wei-Chih Kan,[d] Chih-Chiang Chien,[d] Jyh-Chang Hwang,[d] Hsien-Yi Wang,[d] Chin-Chung Tseng,[a] Chi-Jung Wu[a,b]

Department of Internal Medicine, National Cheng Kung University Hospital and College of Medicine, Tainan, Taiwan[a]; National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Tainan, Taiwan[b]; Institute of Molecular Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan[c]; Department of Nephrology, Chi-Mei Medical Center, Tainan, Taiwan[d]

PCR coupled with electrospray ionization mass spectrometry (PCR/ESI-MS) was compared with culture for pathogen detection in peritoneal dialysis (PD)-related peritonitis. Of 21 samples of PD effluent, PCR/ESI-MS identified microorganisms in 18 (86%) samples, including *Mycobacterium tuberculosis* in 1 culture-negative sample. Of 15 double-positive samples, PCR/ESI-MS and culture reached levels of agreement of 100% (15/15) and 87.5% (7/8) at the genus and species levels, respectively. PCR/ESI-MS can be used for rapid pathogen detection in PD-related peritonitis.

JCM
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Improved Sensitivity for Molecular Detection of Bacterial and *Candida* Infections in Blood

Andrea Bacconi,[a] Gregory S. Richmond,[a] Michelle A. Baroldi,[a] Thomas G. Laffler,[a] Lawrence B. Blyn,[a] Heather E. Carolan,[a] Mark R. Frinder,[a] Donna M. Toleno,[a] David Metzgar,[a] Jose R. Gutierrez,[a] Christian Massire,[a] Megan Rounds,[a] Natalie J. Kennel,[a] Richard E. Rothman,[b] Stephen Peterson,[b] Karen C. Carroll,[c] Teresa Wakefield,[c] David J. Ecker,[a] Rangarajan Sampath[a]

Ibis Biosciences, Inc., Carlsbad, California, USA[a]; Department of Emergency Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA[b]; The Johns Hopkins Hospital Clinical Microbiology Laboratory, Baltimore, Maryland, USA[c]

The rapid identification of bacteria and fungi directly from the blood of patients with suspected bloodstream infections aids in diagnosis and guides treatment decisions. The development of an automated, rapid, and sensitive molecular technology capable of detecting the diverse agents of such infections at low titers has been challenging, due in part to the high background of genomic DNA in blood. PCR followed by electrospray ionization mass spectrometry (PCR/ESI-MS) allows for the rapid and accurate identification of microorganisms but with a sensitivity of about 50% compared to that of culture when using 1-ml whole-blood specimens. Here, we describe a new integrated specimen preparation technology that substantially improves the sensitivity of PCR/ESI-MS analysis. An efficient lysis method and automated DNA purification system were designed for processing 5 ml of whole blood. In addition, PCR amplification formulations were optimized to tolerate high levels of human DNA. An analysis of 331 specimens collected from patients with suspected bloodstream infections resulted in 35 PCR/ESI-MS-positive specimens (10.6%) compared to 18 positive by culture (5.4%). PCR/ESI-MS was 83% sensitive and 94% specific compared to culture. Replicate PCR/ESI-MS testing from a second aliquot of the PCR/ESI-MS-positive/culture-negative specimens corroborated the initial findings in most cases, resulting in increased sensitivity (91%) and specificity (99%) when confirmed detections were considered true positives. The integrated solution described here has the potential to provide rapid detection and identification of organisms responsible for bloodstream infections.

JCM
Journals.ASM.org

CASE REPORT

Identification of Occult *Fusobacterium nucleatum* Central Nervous System Infection by Use of PCR-Electrospray Ionization Mass Spectrometry

Sudha Nagalingam,[a] Michelle Lisgaris,[a] Benigno Rodriguez,[a] Michael R. Jacobs,[b] Michael Lederman,[a] Robert A Salata,[a] Andrea M. Hujer,[a,f] Atis Muehlenbachs,[c] Marlene DeLeon-Carnes,[c] John J. Farrell,[d] Rangarajan Sampath,[e] Robert A. Bonomo[f,g]

Department of Medicine, Division of Infectious Disease and HIV Medicine, University Hospitals Case Medical Center, Case Western Reserve University, Cleveland, Ohio, USA[a]; Department of Pathology, University Hospitals Case Medical Center, Case Western Reserve University, Cleveland, Ohio, USA[b]; Infectious Diseases Pathology Branch, Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, Georgia, USA[c]; Department of Medicine, University of Illinois School of Medicine, Peoria, Illinois, USA[d]; Ibis Biosciences, an Abbott Company, Carlsbad, California, USA[e]; Research Service, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, Ohio, USA[f]; Departments of Pharmacology, Microbiology and Molecular Biology, Case Western Reserve University, Cleveland, Ohio, USA[g]

Anaerobic bacteria are often difficult to detect, especially after the initiation of antibiotics. We describe the application of PCR-electrospray ionization mass spectrometry (PCR/ESI-MS) using a sample of cerebrospinal fluid to identify an anaerobic Gram-negative bacillus, *Fusobacterium nucleatum*, in a patient with "culture-negative" meningitis and cerebral abscesses.

Published in final edited form as:

J Virol Methods. 2015 March ; 214: 43–45. doi:10.1016/j.jviromet.2015.01.002.

Prospective comparison of RT-PCR/ESI-MS to Prodesse ProFlu Plus and Cepheid GenXpert for the detection of Influenza A and B viruses

Justin Hardick[a,*], **Andrea Dugas**[b], **Joshua Goheen**[a], **Richard Rothman**[b], and **Charlotte Gaydos**[a]

[a] Johns Hopkins University School of Medicine, Division of Infectious Diseases, United States

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Abstract

RT-PCR/ESI-MS has previously demonstrated the capability to detect and identify respiratory viral pathogens in nasopharyngeal swabs. This study expands on previous research by performing a prospective evaluation of RT-PCR/ESI-MS to detect and identify Influenza A and B viruses compared to Prodesse ProFlu Plus and combined ProFlu Plus and Cepheid Xpert Flu. ProFlu Plus was also used as a gold standard for comparison for respiratory syncytial virus detection. Using ProFlu Plus as a gold standard, RT-PCR/ESI-MS had sensitivity and specificity of 82.1% (23/28) and 100% (258/258), respectively, for Influenza A, 100% (16/16) and 99.6% (269/270), respectively for Influenza B, and 88.6% (39/44) and 99.6% (241/242) for any Influenza virus. Using matching results from ProFlu Plus and Xpert Flu as a gold standard, RT-PCR/ESI-MS had 85.2% (23/27) and 100% (259/259) sensitivity and specificity respectively for Influenza A, 100% (14/14) and 99.6% (270/272), respectively for Influenza B virus. Overall, RT-PCR/ESI-MS was not as sensitive as the combined gold standard of ProFlu Plus and Xpert Flu, although it has the capability of detecting other respiratory viruses.

Avantaj

- Direkt hasta örneğinden çalışılması
- Fenotipe yansıyan özellikler
- Hızlı
- Bakteri, mantar, antimikrobiyal direnç
- Virus
- Duyarlılık, özgüllük ve pozitif negatif prediktif değer
- Güvenilir

Dezavantaj

- Kurulum pahallı
- Teknik donanım
- Veriler biyoinformatik programla kısıtlı

SONUÇ

- Bruker MALDI Biotyper MALDI-TOF/MS yazılımı ise patern karşılaştırmalı algoritmalarla bakteri ve mantarların tanımlanmasında elverişlidir.

- PCR- ESI/MS yazılım programı (Abbott PLEX-ID) ile mantarlar, virusler, bakteriler ve antibiyotik dirençli mikroorganizmaların ve direkt kan kültür şişelerinden patojenlerin tanımlanmasında kullanışlıdır.