Bakteriyolojik Tanı Yöntemlerinde Gelişmeler

Dr Gökhan AYGÜN

CTF Tıbbi Mikrobiyoloji AD

İnfeksiyon hastalığında laboratuvar

- İnfeksiyon şüphesi olan hastada hiçbir laboratuvar incelemesi anamnez ve fizik muayenenin yerini alamaz !!!

İnfeksiyon Hastalıklarında laboratuvar

- Bir odak belirlenemeyen infeksiyon şüpheli hastalarda laboratuvar (İnfeksiyona yatkınlık? İnfeksiyonu öngörmek?)

- Etkeni saptamakta laboratuvar

Ciddi Bakteriyal İnfeksiyon?Sepsis ?

- Mikrobik yapılar:Endotoksin, teikoik asit,…

- İmmünolojik Tanı:

CRP, Prokalsitonin,IL-6,IL-8,….

Tanı ve prognoz yönünden yol gösterici!

Medicine®

OBSERVATIONAL STUDY

OPEN

Diagnostic Accuracy of Procalcitonin for Predicting Blood Culture Results in Patients With Suspected Bloodstream Infection

An Observational Study of 35,343 Consecutive Patients (A STROBE-Compliant Article)

Nejla ... *D,* ... *ia, MSc,*

2.2 ng/mL [IQR 0.6–12.2]), and the lowest procalcitonin concentration was observed in patients with negative blood cultures (median 0.3 ng/mL [IQR 0.1–1.1]). With optimal thresholds ranging from ≤0.4 to ≤0.75 ng/mL, procalcitonin had a high diagnostic accuracy for excluding all pathogen categories with the following negative predictive values: Gram-negative bacteria (98.9%) (including enterobacteria [99.2%], nonfermenting Gram-negative bacilli [99.7%], and anaerobic bacteria [99.9%]), Gram-positive bacteria (98.4%), and fungi (99.6%). A procalcitonin concentration ≥10 ng/mL was associated with a high risk of Gram-negative (odds ratio 5.98; 95% CI, 5.20–6.88) or Gram-positive (odds ratio 3.64; 95% CI, 3.11–4.26) bacteremia but dramatically reduced the risk of PCBCs or fungemia. In this large real-life setting experience with more than 35,000 patients, procalcitonin was highly effective at excluding bloodstream infections regardless of pathogen categories. The results from our study are limited by its cross-sectional design and deserve to be validated in prospective longitudinal studies.

(*Medicine* 94(44):e1774)

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OBSERVATIONAL STUDY

OPEN

Diagnostic Accuracy of Procalcitonin for Predicting Blood Culture Results in Patients With Suspected Bloodstream Infection

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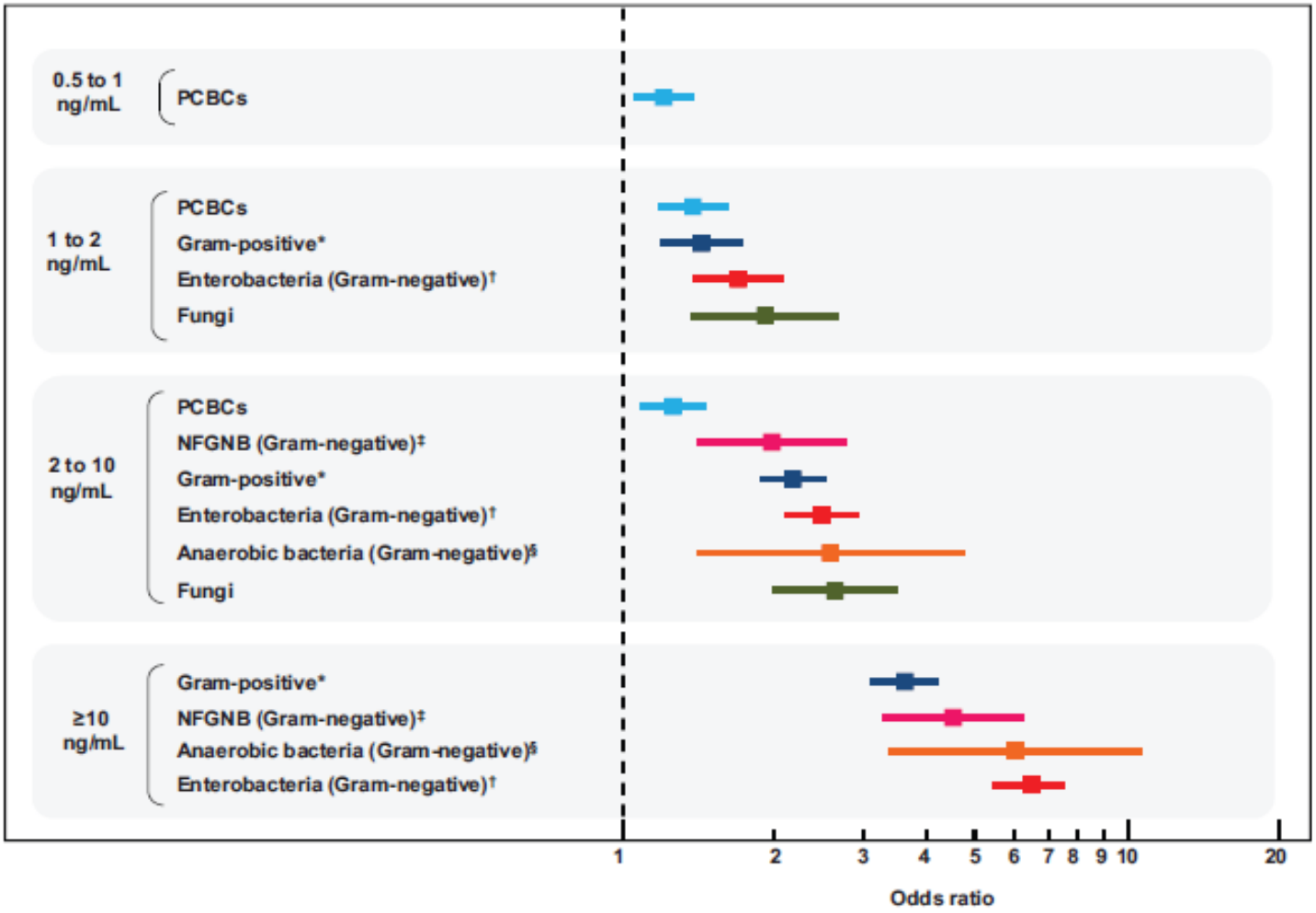

Sepsise yatkınlık tanımı

- PIRO
- Predisposition

- MBL gen polimorfizmi
- TLR gen polimorfizmi
- …….

Sepsise yatkınlık tanımı

Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious disease

Lancet Infect Dis 2005; 5: 156–64

Nicolas WJ Schröder and Ralf R Schumann

Reference	Association studied	SNP studied	Number of patients	Number of controls	Results: patients vs controls (%)	p value
Arbour et al[81]	Hyporesponsiveness to inhaled lipopolysaccharide	Asp299Gly Thr399Ile	31	57	22·6 vs 5·8 Cosegregating	0·029
Read et al[82]	Meningococcal disease	Asp299Gly	1047	879	5·9 vs 6·5 Not significant	Not significant
Allen et al[83]	Meningococcal disease	Asp299Gly Thr399Ile	252	251	20·2 vs 20·3 Not significant	0·9
Tal et al[84]	Respiratory syncytial virus infection	Asp299Gly Thr399Ile	99	82	20·2 vs 4·9	0·003
Feterowski et al[85]	Sepsis after surgery	Asp299Gly Thr399Ile	153	154	6·5 vs 12·3 Not significant	0·12
Lorenz et al[86]	Septic shock	Asp299Gly Thr399Ile	91	73	5·5 vs 0 Asp299Gly only	0·05
Child et al[87]	Severe inflammatory response syndrome	Asp299Gly Thr399Ile	94	–	Non-significant trend towards higher mortality	0·08
Agnese et al[88]	Bacterial infection (Gram-negative)	Asp299Gly Thr399Ile	77	39	18·0 vs 12·8 Significant when stratified for SNP or no SNP	0·004

Biomarkers: The Future

Steven P. LaRosa, MD[a,b,*], Steven M. Opal, MD[a,c]

Crit Care Clin 27 (2011) 407–419
doi:10.1016/j.ccc.2010.12.012

Table 1
Selected gene association studies with susceptibility to infection and sepsis

Gene Product	SNP/Amino Acid Changes	Clinical Findings
TLR4	Asp299Gly in peptide sequence	Possible increased susceptibility to gram-negative bacteria and aspergillosis, lower risk of legionellosis
TLR2	SNPs in TLR2 gene coding regions	Associated with increased risk of infection from gram-positive bacteria
CD14	C-159T promoterpolymorphism	TT homozygotes at position −159 reported to have increased levels of soluble CD14 and increased risk of septic mortality
MBL	SNPs in exon 1 of MBL gene	Associated with low MBL levels and increased risk of severe infection
IL-6	−174 G/Cpolymorphism	Conflicting reports that this promoter polymorphism alters IL-6 levels and increases incidence of sepsis
TNF-α	G-A polymorphism at position −308	Polymorphism in promoter region of TNF-α gene associated with increased risk of sepsis in some studies
Protein C	−1654C/T or −1641G/A	Polymorphisms in this noncoding region associated with increased risk of death from sepsis
PAI-1	SNP in promoter region	Increased production of PAI-1 leads to reduced fibrinolysis and poor outcome in meningococcal

İnfeksiyon Hastalıklarında laboratuvar

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- Etkeni saptamakta laboratuvar

Etkeni tanımlamada temel yenilikler

- Kültür yöntemlerinde gelişmeler
- İmmünolojik yaklaşım (sitokin temelli)
- Moleküler Biyoloji
- MALDI-TOF
- Otomatizasyon
- Diğer yenilikler?

Clinical Laboratory Testing - Today

30-50 lab tests

RIAs for hormones

Intro of molecular testing

>5000 lab tests

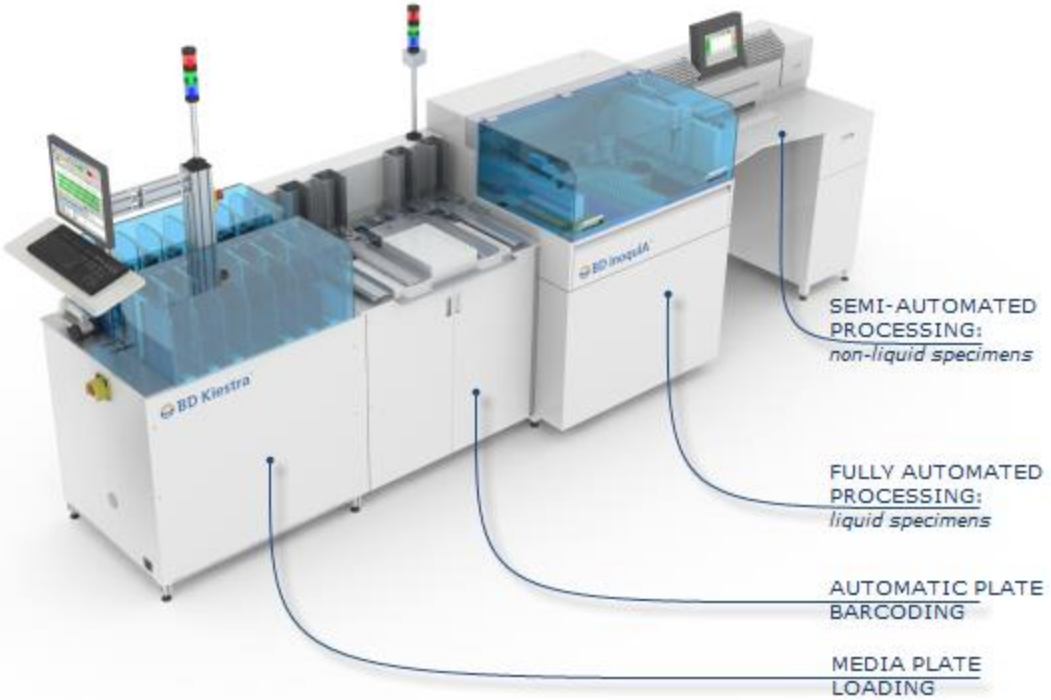
1970 1980 1990 2000 2010

Intro of automated instruments

Immunoassay automation

Major expansion of molecular testing

Michael Laposata, AACC 2010

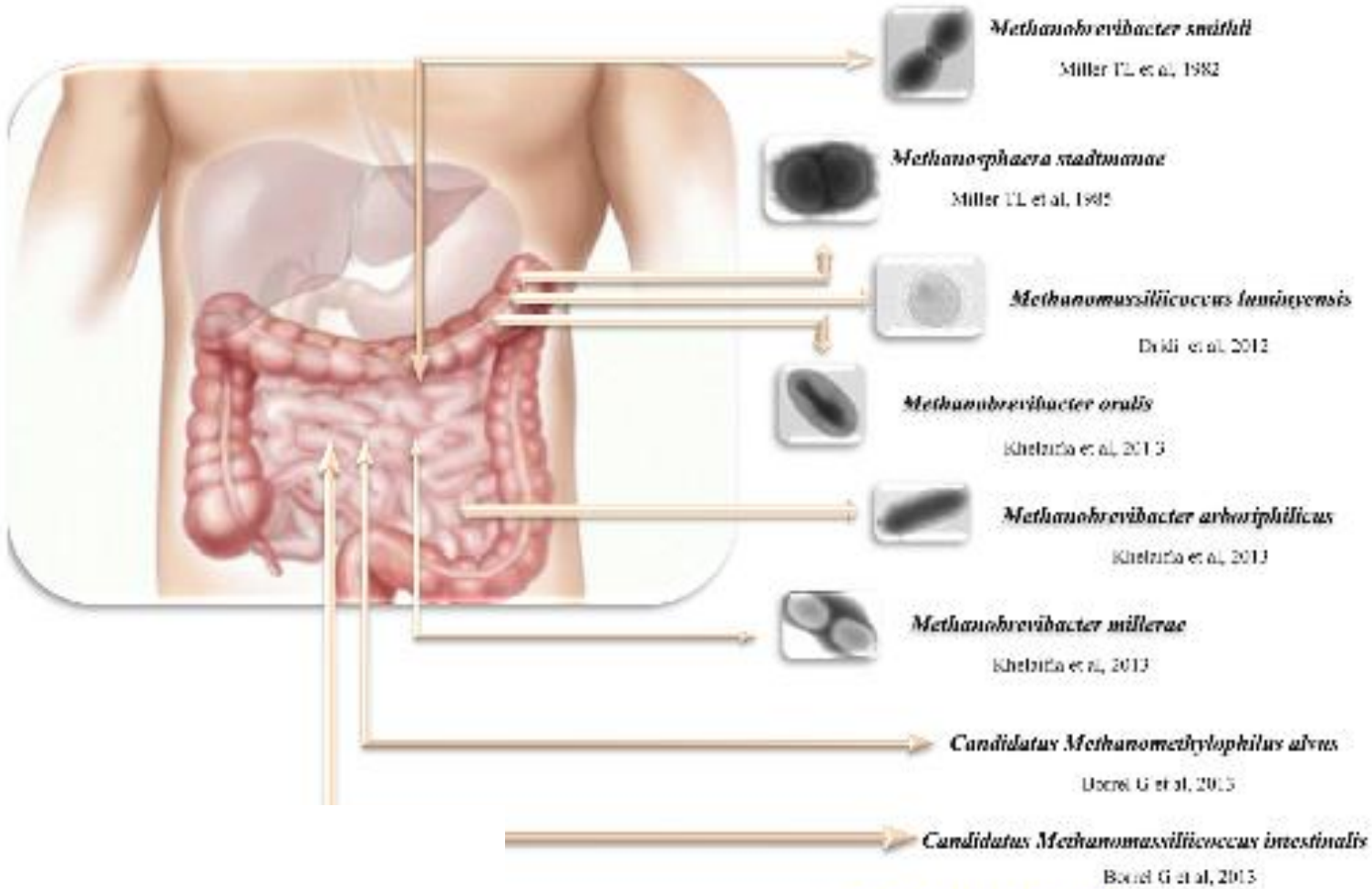

BD Kiestra
BD InoqulA
SEMI-AUTOMATED PROCESSING:
non-liquid specimens
FULLY AUTOMATED PROCESSING:
liquid specimens
AUTOMATIC PLATE BARCODING
MEDIA PLATE LOADING


BIOMERIEUX


BD
Phoenix 100

Kromojenik besiyerleri

Archea kültürleri



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Current and Past Strategies for Bacterial Culture in Clinical Microbiology

Jean-Christophe Lagier, Sophie Edouard, Isabelle Pagnier, Oleg Mediannikov, Michel Drancourt, Didier Raoult

Hücreiçi bakteri kültürleri-protozoolar

CMR
JournalsASM.org

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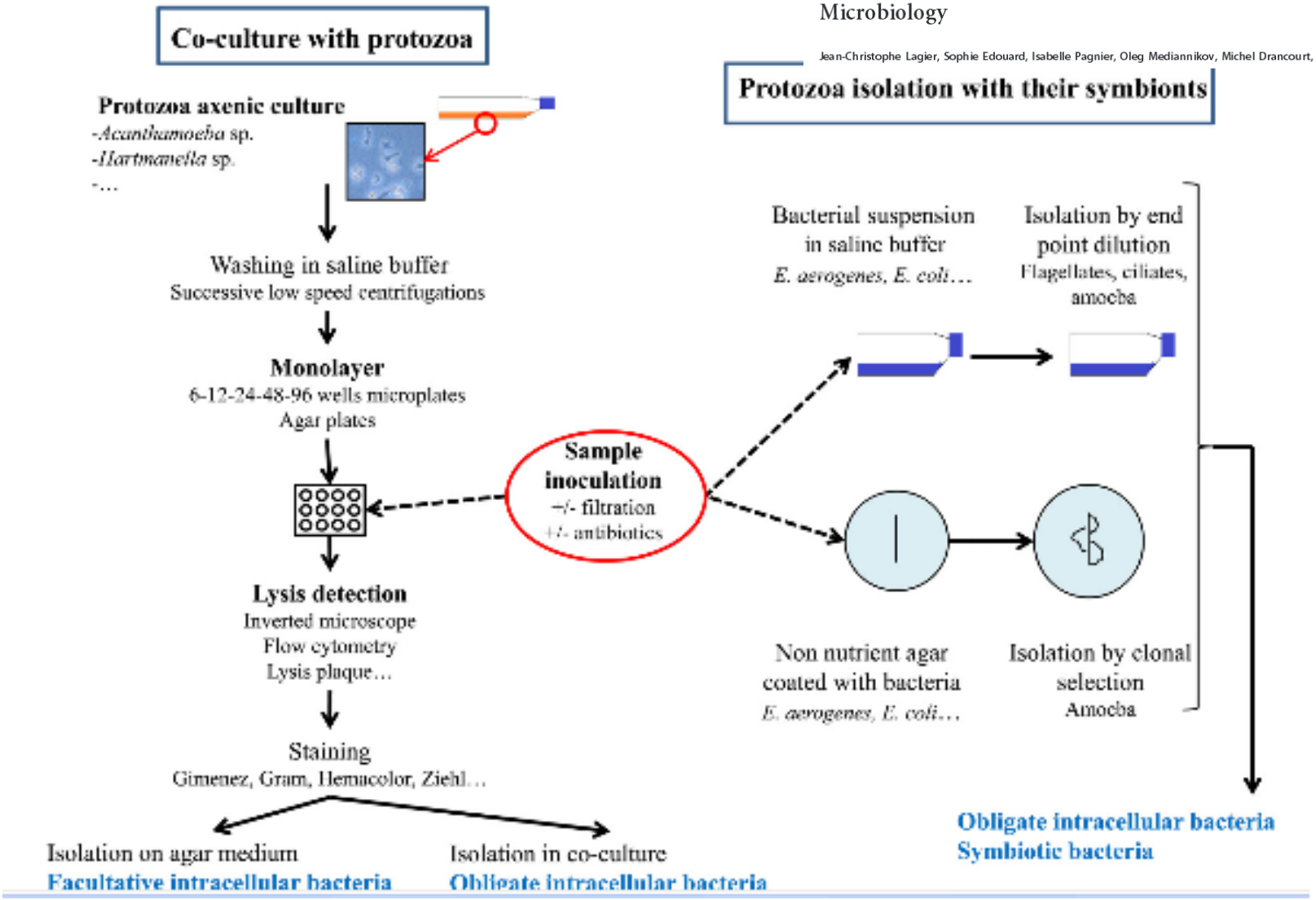

Hücreiçi bakteri kültürleri-hücre kültürleri

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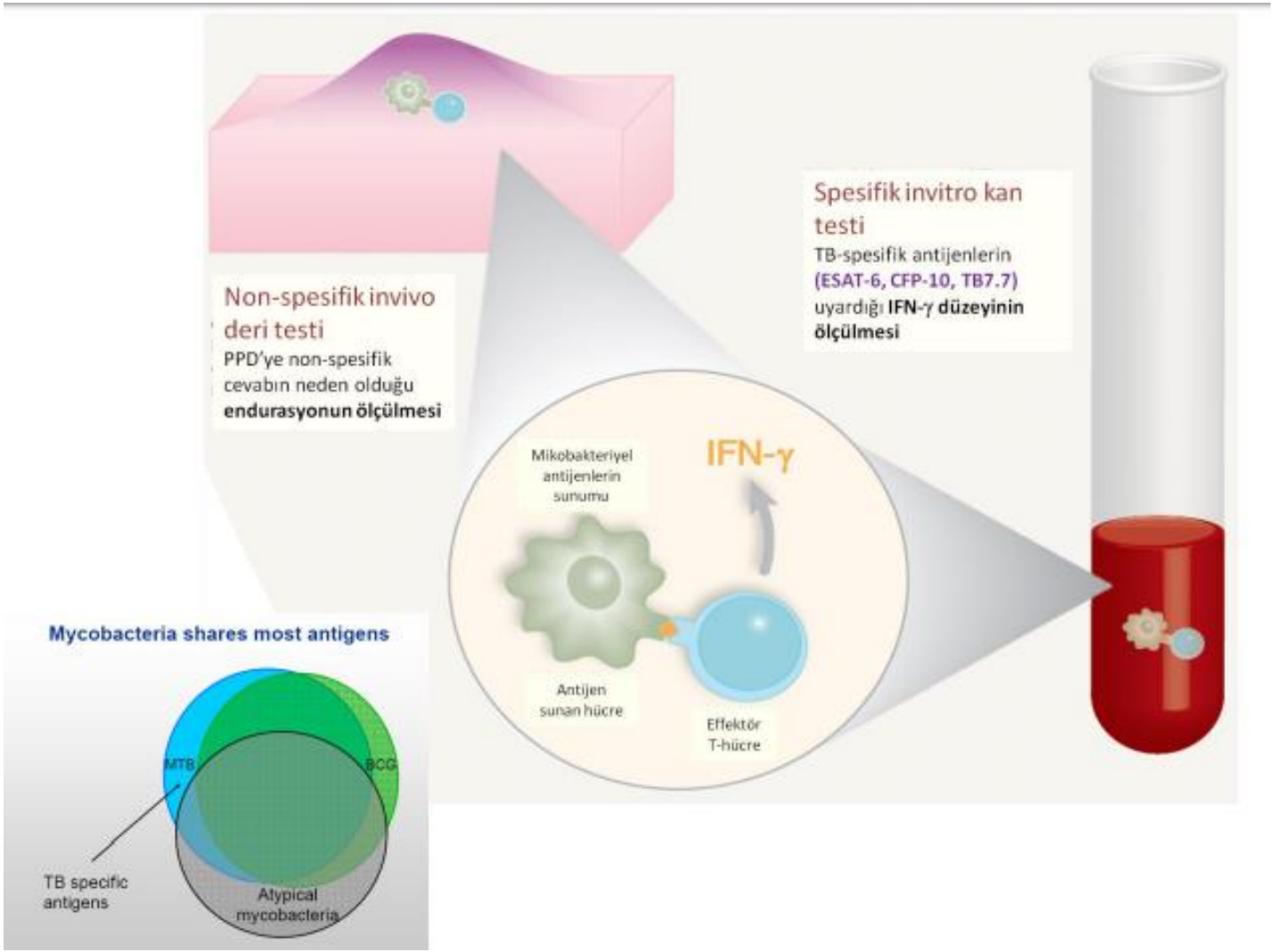
Current and Past Strategies for Bacterial Culture in Clinical Microbiology

Jean-Christophe Lagier, Sophie Edouard, Isabelle Pagnier, Oleg Mediannikov, Michel Drancourt, Didier Raoult

TABLE 4 The most susceptible cells line used for the culture of intracellular bacteria

Bacterium	Cell line(s) (culture temp [°C])		
	Mammalian	Arthropod	Fish and amphibian
C. burnetii	HEL, Vero (35)	*Ixodes scapularis* cell line-IDE8 (34)	
Spotted fever group *Rickettsia*	L929, Vero (32)	*Dermacentor* sp. cell lines DAE3 and DALBE3 (34); *Ixodes scapularis* cell line ISE6 (34); *Aedes albopictus* cell linesAa23, AeAl2, C7/10, and C6/36 (22–32); *Anopheles gambiae* cell line Sua5B (22–25)	
Typhus group *Rickettsia*	L929, Vero (35)	*Aedes albopictus* cell line AeAl2 (28	
Rickettsia felis	Vero (32)	*Ixodes scapularis* cell line ISE6 (32), *Aedes albopictus* cell lines Aa23 and C6/36 (22–25), *Anopheles gambiae* cell line Sua5B (22–25)	XTC (28)
Orientia tsutsugamushi	L929 (32)		
Anaplasma phagocytophilum	HL60 (37)	*Ixodes scapularis* cell lines ISE6 and IDE8 (34)	
Ehrlichia chaffeensis	DH82 (37)	*Ixodes scapularis* cell line ISE6 (34)	
Ehrlichia canis	DH82 (37)	*Ixodes scapularis* cell line ISE6/IDE8 (34) *Ixodes ricinus* cell line IRE/CTVM18 (34)	
Wolbachia pipientis	HEL (28 and 37)	*Aedes albopictus* cell lines Aa23 and C6/36 (26–28)	
Tropheryma whipplei	MRC5, HEL (35)		
Chlamydia trachomatis	McCoy, HeLa 229 (35)		
Chlamydia pneumoniae	HL, HEp-2 (35)		

IGRA testleri



MAJOR ARTICLE

Clinical Infectious Diseases 2013;56(12):1742–51

Specific Interferon γ Detection for the Diagnosis of Previous Q Fever

Teske Schoffelen,[1] Leo A. B. Joosten,[1] Tineke Herremans,[2] Anton F. J. de Haan,[3] Anne Ammerdorffer,[1] Hans C. Rümke,[4] Clementine J. Wijkmans,[5] Hendrik I. Jan Roest,[6] Mihai G. Netea,[1] Jos W. M. van der Meer,[1] Tom Sprong,[1,7,8,a] and Marcel van Deuren[1,a]

[1]Department of Medicine, Radboud University Nijmegen Medical Centre, and Nijmegen Institute for Infection, Inflammation and Immunity (N4i), Nijmegen; [2]National Institute for Public Health and the Environment, Centre for Infectious Disease Control, Bilthoven; [3]Department of Epidemiology, Biostatistics and HTA, Radboud University Nijmegen Medical Centre, Nijmegen; [4]Vaxinostics BV, Rotterdam; [5]Municipal Public Health Service Hart voor Brabant, 's-Hertogenbosch; [6]Department of Bacteriology and TSEs, Central Veterinary Institute, part of Wageningen UR, Lelystad; and Departments of [7]Internal Medicine and [8]Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands

(See the Editorial Commentary by Graves on pages 1752–3.)

Background. Current practice for diagnosis of Q fever, caused by the intracellular pathogen *Coxiella burnetii*, relies mainly on serology and, in prevaccination assessment, on skin tests (STs), which both have drawbacks. In this study, *C. burnetii*–specific interferon γ (IFN-γ) production was used as a new diagnostic tool for previous Q fever, circumventing most of these drawbacks. Our aim was to compare this test to serology and ST.

Methods. One thousand five hundred twenty-five individuals from an endemic area with a risk for chronic Q fever were enrolled. IFN-γ production was measured after in vitro stimulation of whole blood with *C. burnetii* antigens. Various formats using different *C. burnetii* antigens were tested. Serology and ST were performed in all individuals.

Results. In all assay formats, *C. burnetii*–specific IFN-γ production was higher ($P < .0001$) in seropositive or ST-positive subjects than in seronegative and ST-negative subjects. Whole blood incubated for 24 hours with *C. burnetii* Nine Mile showed optimal performance. After excluding subjects with equivocal serology and/or borderline ST results, IFN-γ production was 449 ± 82 pg/mL in the positive individuals (n = 219) but only 21 ± 3 pg/mL in negative subjects (n = 908). Using Bayesian analysis, sensitivity and specificity (87.0% and 90.2%, respectively) were similar to the combination of serology and ST (83.0% and 95.6%, respectively). Agreement with the combination of serology and ST was moderate (84% concordance; $\kappa = 0.542$).

Conclusions. Specific IFN-γ detection is a novel diagnostic assay for previous *C. burnetii* infection and shows similar performance and practical advantages over serology and ST. Future studies to investigate the clinical value in practice are warranted.

Moleküler Biyoloji

- PCR (isotermal-LAMP,helikaz- HDA)

  TMA

  “Multiplex” PCR

  Otomatize sistemler (sample to result)

  “Microarray” teknolojileri

  Sekans metodları *

PCR

- Bakteriyemi hızlı tanısı
- Pnömoni tanısı
- Genital sistem infeksiyonları tanısı
  (gonokok, C.trachomatis)
- Enterik patojenler
  (Salmonella, Shigella, Campylobacter, stx1/stx2)
- Clostridium difficile tanısı
- Spesifik patojen tanıları
  (S.aureus, S.pyogenes, Grup-B streptokok, M.tuberculosis)
- Direnç
  VRE, MRSA, Rif R M.tuberculosis

Rapid Diagnosis of Infection in the Critically Ill, a Multicenter Study of Molecular Detection in Bloodstream Infections, Pneumonia, and Sterile Site Infections*

Jean-Louis Vincent, MD, PhD, FCCM[1]; David Brealey, MD[2]; Nicolas Libert, MD[3]; Nour Elhouda Abidi, MD[4]; Michael O'Dwyer, MD[5]; Kai Zacharowski, MD[6]; Malgorzata Mikaszewska-Sokolewicz, MD[7]; Jacques Schrenzel, MD[8]; François Simon, MD[9]; Mark Wilks, PhD[5]; Marcus Picard-Maureau, PhD[10]; Donald B. Chalfin, MD, MPH[11]; David J. Ecker, PhD[11]; Rangarajan Sampath, PhD[11]; Mervyn Singer, MD[2]; the Rapid Diagnosis of Infections in the Critically Ill Team

Conclusions: Polymerase chain reaction/electrospray ionization-mass spectrometry provides rapid pathogen identification in critically ill patients. The ability to rule out infection within 6 hours has potential clinical and economic benefits. (*Crit Care Med* 2015; 43:2283–2291)

Rapid Diagnosis of Infection in the Critically Ill, a Multicenter Study of Molecular Detection in Bloodstream Infections, Pneumonia, and Sterile Site Infections*

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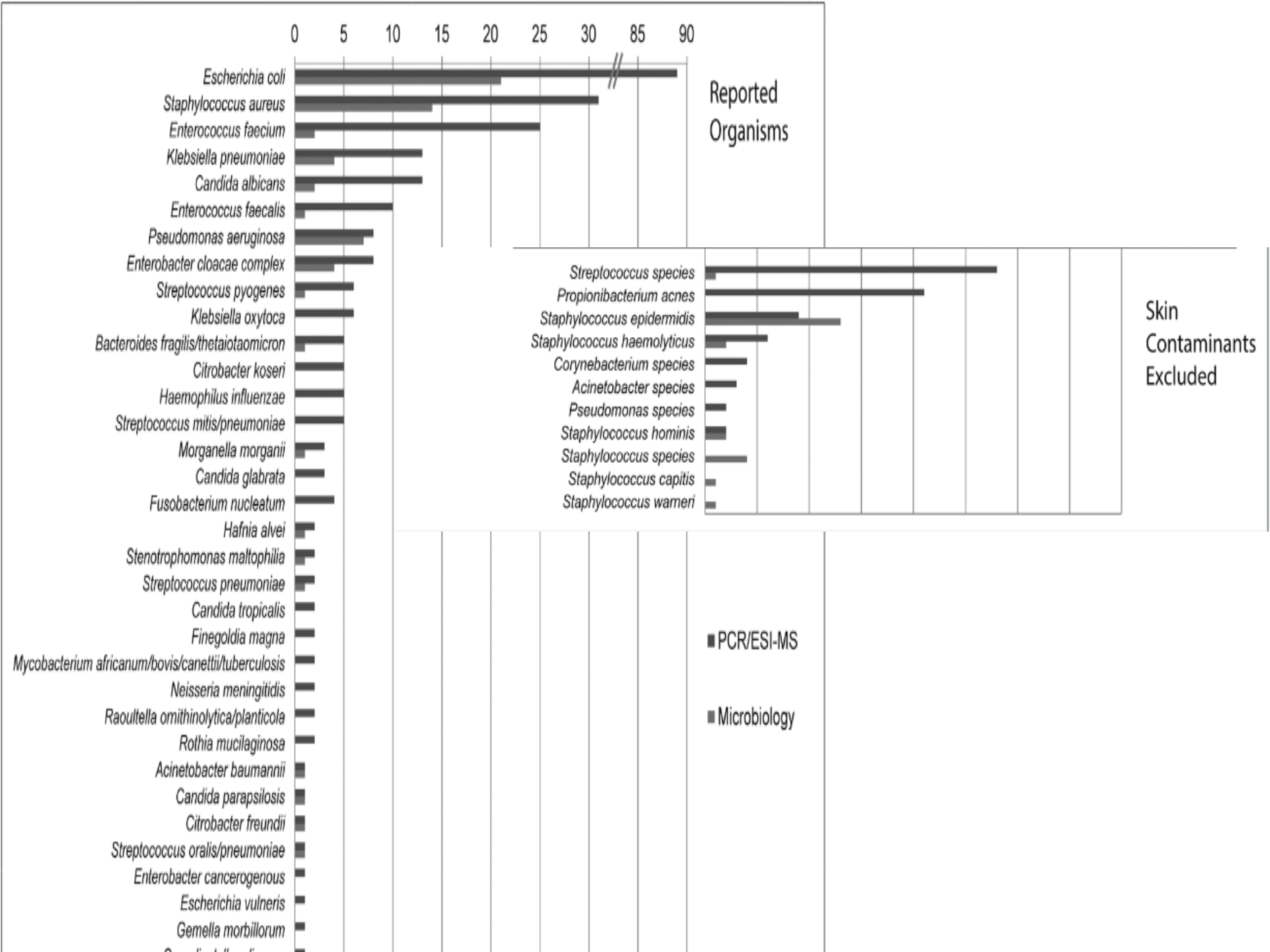
TABLE 2. Bloodstream Infection Assay Performance

		Culture				
		+	–	Total	Sensitivity	81% (95% CI, 70–89%)
Polymerase chain reaction/ electrospray ionization-mass spectrometry	+	55	173	228	Specificity	69% (95% CI, 65–73%)
	–	13	384	397	Positive predictive value	24% (95% CI, 19–30%)
	Total	68	557	625	Negative predictive value	97% (95% CI, 94–98%)


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 omithinolytica/planticola
Rothia mucilaginosa
Acinetobacter baumannii
Candida parapsilosis
Citrobacter freundii
Streptococcus oralis/pneumoniae
Enterobacter cancerogenous
Escherichia vulneris
Gemella morbillorum
Reported Organisms
Streptococcus species
Propionibacterium acnes
Staphylococcus epidermidis
Staphylococcus haemolyticus
Corynebacterium species
Acinetobacter species
Pseudomonas species
Staphylococcus hominis
Staphylococcus species
Staphylococcus capitis
Staphylococcus warneri
Skin Contaminants Excluded
PCR/ESI-MS
Microbiology

PCR ile tanımlama

Minerva Anestesiol. 2015 May 29. [Epub ahead of print]

Comparison of blood culture and multiplex real-time pcr for the diagnosis of nosocomial sepsis.

Dinç F[1], Akalın H, Özakın C, Sınırtaş M, Kebabçı N, İşçimen R, Kelebek Girgin N, Kahveci F.

Author information

Abstract

BACKGROUND: Causative microorganisms cannot be isolated in many cases of suspected sepsis. Multiplex real-time PCR generates results more rapidly than conventional blood culture systems.

METHODS: In this study, we evaluated the diagnostic performance of multiplex real-time PCR (LightCycler SeptiFast), and compared with blood cultures and cultures from focus of infection in nosocomial sepsis.

RESULTS: Seventy-eight nosocomial sepsis episodes in 67 adult patients were included in this study. The rates of microorganism detection by blood culture and PCR were 34.2% and 47.9%, respectively. Sixty-five microorganisms were detected by both methods from 78 sepsis episodes. Nineteen of these microorganisms were detected by both blood culture and PCR analysis from the same sepsis episode. There was statistically moderate concordance between the two methods (kappa=0.445, p<0.001). There was no significant agreement between the blood culture and PCR analysis in terms of microorganism detected (kappa=0.160, p=0.07). Comparison of the results of PCR and cultures from focus of infection revealed no significant agreement(kappa=0.110, p=0.176). However, comparison of the results of PCR and blood cultures plus cultures from focus of infection(positive blood culture and/or positive culture from focus of infection) showed poor agreement(kappa=0.17, p=0.026). When the blood culture was used as the gold standart, the sensitivity, specificity, positive and negative predictive value of PCR in patients with bacteremia was 80%, 69%, 57% and 87%, respectively.

CONCLUSIONS: The SeptiFast may be useful when added to blood culture in the diagnosis and management of sepsis.

TABLE 2

Comparison of FDA-cleared molecular methods for detection of microorganisms in positive blood culture broths

Test	Targets	Sensitivity (%)	Specificity (%)	Time to result (h)	Format and setup
Verigene BC-GP	12 Gram-positive genus or species targets and 3 resistance markers (*mecA*, *vanA*, *vanB*)	92-100	98-100	2.5	On-demand, microarray, automated sample processor, manual transfer of array to analyzer
Verigene BC-GN	8 Gram-negative genus or species targets and 6 resistance markers (KPC, NDM, CTX-M, VIM, IMP, OXA)	81-100	98-100	2	On-demand, microarray, automated sample processor, manual transfer of array to analyzer
FilmArray BCID	8 Gram-positive, 11 Gram-negative, and 5 yeast genus or species targets, 4 resistance markers (*mecA*, *vanA/B*, KPC, NDM)	88-100	94-100	1	On-demand, parallel miniaturized singleplex RT-PCR, full sample-to-result capability
GeneOHM StaphSR	*S. aureus*, MRSA	50-100	98-99	2	Batch, RT-PCR, offline manual sample lysis, extraction, and RT-PCR setup
Xpert MRSA/SA Blood Culture	*S. aureus*, MRSA	69-100	98-100	1	On-demand, RT-PCR, full sample-to-result capability
Septifast[a]	6 Gram-positive, 8 Gram-negative, and 5 yeast targets and *A. fumigatus*	42-79	67-97	6	Batch, 1.5-10 ml whole blood; offline extraction and setup of 3 parallel RT-PCRs

[a] Not cleared by FDA for clinical use. Data are from direct analysis of whole blood.

Beacon-based (bbFISH®) technology for rapid pathogens identification in blood cultures

Christina Sakarikou[2], Martina Parisato[3], Giuliana Lo Cascio[3†] and Carla Fontana[1,2*†]

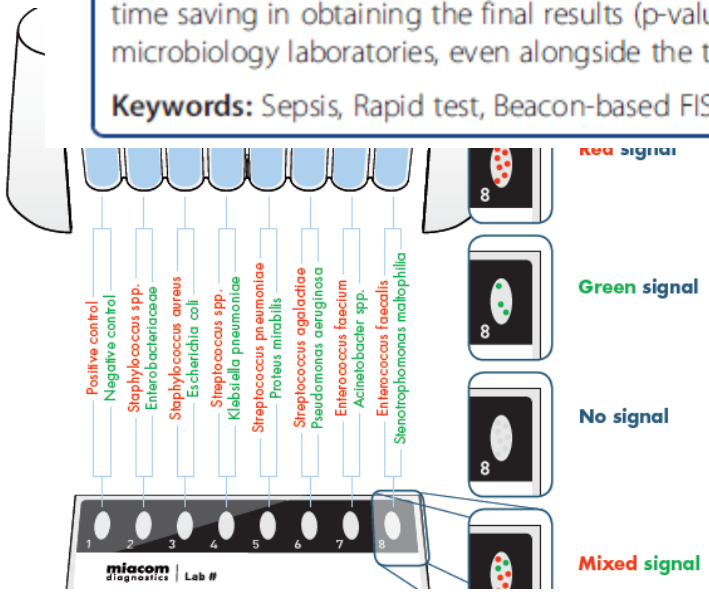
Abstract

Background: Diagnosis and treatment of bloodstream infections (BSI) are often hampered by the delay in obtaining the final results of blood cultures. Rapid identification of pathogens involved in BSI is of great importance in order to improve survival of septic patients. Beacon-based fluorescent in situ hybridization (hemoFISH® Gram positive and hemoFISH® Gram negative test kits, miacom diagnostics GmbH Düsseldorf, Germany) accelerates the identification of most frequent bacterial pathogens of sepsis.

Results: In this study a total of 558 blood culture (377 blood culture positive and 181 negative) were tested with the hemoFISH® method and the results were evaluated in comparison with the traditional culture based methods. The overall sensitivity and specificity of the hemoFISH® tests were 94.16% and 100%, while, the PPV and NPV were 100 and 89.16%, respectively. As the hemoFISH® results were obtained within 45 mins, the time difference between the final results of the traditional culture method and the hemoFISH® assay was about two days.

Conclusions: Considering the good sensitivity and specificity of the hemoFISH® assays as well as the significant time saving in obtaining the final results (p-value 0.0001), the introduction of the system could be rialable in the microbiology laboratories, even alongside the traditional systems.

Keywords: Sepsis, Rapid test, Beacon-based FISH, hemoFISH



Sakarikou *et al. BMC Microbiology* 2014, **14**:99
http://www.biomedcentral.com/1471-2180/14/99

Targeting antimicrobial-resistant bacterial respiratory tract pathogens: it is time to 'get smart'

Boeun Lee and Helen W. Boucher

KEY POINTS

- Guidelines recommend increased efforts to encourage pathogen-directed therapy to control pneumonia caused by antibiotic-resistant bacteria.
- Rapid molecular assays have shown promise to expedite pathogen-directed therapy; however, the clinical impact and cost effectiveness of these 'point-of-care' molecular assays require further study.
- Increased efforts to improve antibiotic usage with the optimal drug, at the right time, with the best dose for the most effective duration, are needed to tackle antimicrobial resistance.
- New antibiotics to treat carbapenem-resistant GNB are desperately needed, and no convincing evidence exists to support combination therapy.
- Aerosolized therapy may provide additive benefits to parenteral therapy by delivering high local concentrations with low systemic absorption and less toxicity.

Targeting antimicrobial-resistant bacterial respiratory tract pathogens: it is time to 'get smart'

Boeun Lee and Helen W. Boucher

Table 1. Rap

Technology	Details/microbiology	Turnaround time	Sensitivity/specificity
Biofire Filmarray assays [48]	Nested RT-PCR followed by multiplex PCR 20 respiratory pathogens including 17 viruses, *B. pertussis*, *M. pneumonia* and *C. pneumoniae*	1 h	84–100%/89–100%
Curetis Unyvero P50 Pneumonia Application [49,50]	Multiplex PCR Can directly use all native samples irrespective of collection methods Detects 17 bacterial and fungal (PCP) pathogens as well as 22 antibiotic resistance markers	5.2 h	80.6%/96%
Respifinder SMART 22 v2.0 [51]	Multiplex PCR Allows analysis of up to 25 targets in a single reaction 18 viral + 4 bacterial RTI	4 h	86% (20–100%)/98.8% (97–100%)
The respiratory MWS r-gene [52]	Real time PCR Detect 40 pathogens including 8 respiratory viruses, *C. pneumoniae*, *L. pneumophilia*, and *M. pneumoniae*	1.5–2.5 h	No data
Randox respiratory pathogen biochip array [53]	Multiplex PCR Detects 15 viral pathogens and 7 bacterial pathogens	Within 3–6 h	No data
Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF)	Mass spectrometry	1–1.5 days faster than routine cultures	Under investigation; sensitivity >95% (lower in respiratory specimens?)

Comparison of next-generation sequencing methods[59][60]

Method	Read length	Accuracy (single read not consensus)	Reads per run	Time per run	Cost per 1 million bases (in US$)	Advantages	Disadvantages
Single-molecule real-time sequencing (Pacific Biosciences)	10,000 bp to 15,000 bp avg (14,000 bp N50); maximum read length >40,000 bases[61][62][63]	87% single-read accuracy[64]	50,000 per SMRT cell, or 500–1000 megabases[65][66]	30 minutes to 4 hours[67]	$0.13–$0.60	Longest read length. Fast. Detects 4mC, 5mC, 6mA.[68]	Moderate throughput. Equipment can be very expensive.
Ion semiconductor (Ion Torrent sequencing)	up to 400 bp	98%	up to 80 million	2 hours	$1	Less expensive equipment. Fast.	Homopolymer errors.
Pyrosequencing (454)	700 bp	99.9%	1 million	24 hours	$10	Long read size. Fast.	Runs are expensive. Homopolymer errors.
Sequencing by synthesis (Illumina)	50 to 300 bp	99.9% (Phred30)	up to 6 billion (TruSeq paired-end)	1 to 11 days, depending upon sequencer and specified read length[69]	$0.05 to $0.15	Potential for high sequence yield, depending upon sequencer model and desired application.	Equipment can be very expensive. Requires high concentrations of DNA.
Sequencing by ligation (SOLiD sequencing)	50+35 or 50+50 bp	99.9%	1.2 to 1.4 billion	1 to 2 weeks	$0.13	Low cost per base.	Slower than other methods. Has issues sequencing palindromic sequences.[70]
Chain termination (Sanger sequencing)	400 to 900 bp	99.9%	N/A	20 minutes to 3 hours	$2400	Long individual reads. Useful for many applications.	More expensive and impractical for larger sequencing projects. This method also requires the time consuming step of plasmid cloning or PCR.

OPEN ACCESS Freely available online

PLOS ONE

Genome-Wide Sequencing of Cellular microRNAs Identifies a Combinatorial Expression Signature Diagnostic of Sepsis

Yuqian Ma[1,3¤], David Vilanova[4], Kerem Atalar[1,3], Olivier Delfour[4], Jonathan Edgeworth[3,5], Marlies Ostermann[3,5], Maria Hernandez-Fuentes[1,3], Sandrine Razafimahatratra[4], Bernard Michot[4], David H. Persing[6], Ingrid Ziegler[7], Bianca Törös[8], Paula Mölling[8], Per Olcén[8], Richard Beale[2,3,5], Graham M. Lord[1,3,5]*

Abstract

Rationale: Sepsis is a common cause of death in the intensive care unit with mortality up to 70% when accompanied by multiple organ dysfunction. Rapid diagnosis and the institution of appropriate antibiotic therapy and pressor support are therefore critical for survival. MicroRNAs are small non-coding RNAs that play an important role in the regulation of numerous cellular processes, including inflammation and immunity.

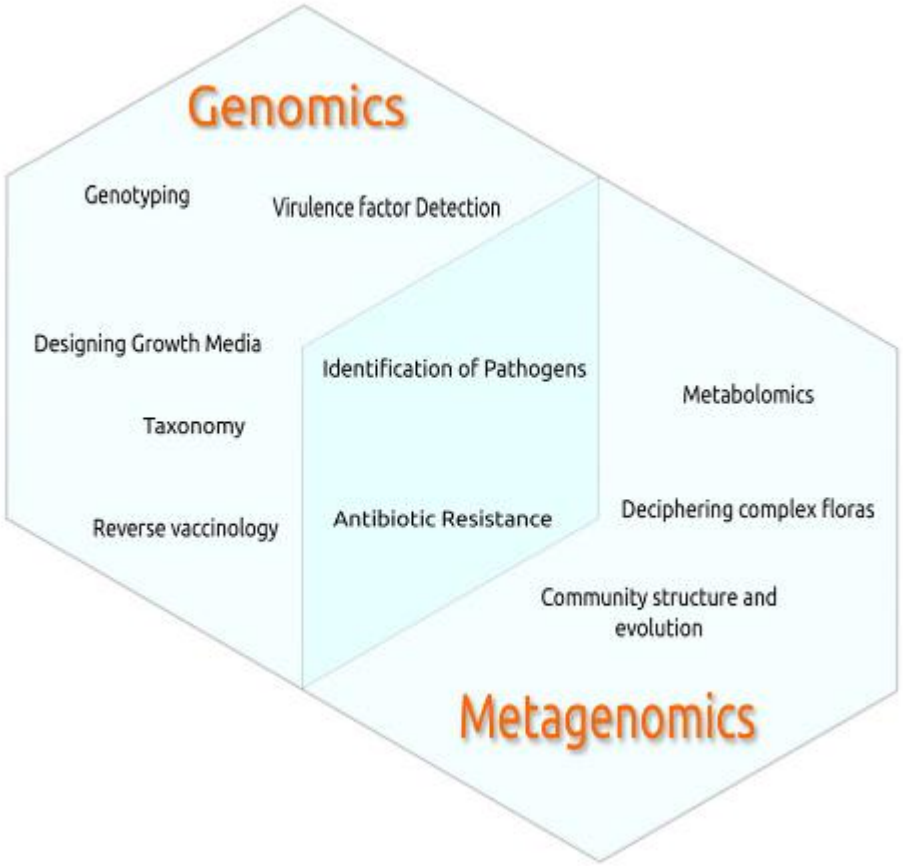
Objectives: We hypothesized changes in expression of microRNAs during sepsis may be of diagnostic value in the intensive care unit (ICU).

Methods: Massively parallel sequencing of microRNAs was utilised for screening microRNA candidates. Putative microRNAs were validated using quantitative real-time PCR (qRT-PCR). This study includes data from both a training cohort (UK) and an independent validation cohort (Sweden). A linear discriminant statistical model was employed to construct a diagnostic microRNA signature.

Results: A panel of known and novel microRNAs were detectable in the blood of patients with sepsis. After qRT-PCR validation, microRNA miR-150 and miR-4772-5p-iso were able to discriminate between patients who have systemic inflammatory response syndrome and patients with sepsis. This finding was also validated in independent cohort with an average diagnostic accuracy of 86%. Fractionating the cellular components of blood reveals miR-4772-5p-iso is expressed differentially in monocytes. Functional experiments using primary human monocytes demonstrate that it expressed in response to TLR ligation.

Conclusions: Taken together, these data provide a novel microRNA signature of sepsis that should allow rapid point-of-care diagnostic assessment of patients on ICU and also provide greater insight into the pathobiology of this severe disease.

Sekans teknolojisindeki gelişmelerin yansımaları



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Review

Genomics and metagenomics in medical microbiology

Roshan Padmanabhan, Ajay Kumar Mishra, Didier Raoult, Pierre-Edouard Fournier *

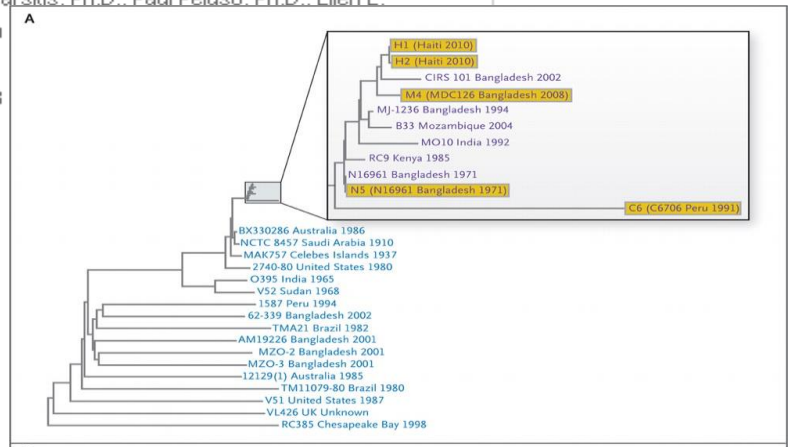
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

The Origin of the Haitian Cholera Outbreak Strain

Chen-Shan Chin, Ph.D., Jon Sorenson, Ph.D., Jason B. Harris, M.D., William P. Robins, Ph.D., Richelle C. Charles, M.D., Roger R. Jean-Charles, M.D., James Bullard, Ph.D., Dale R. Webster, Ph.D., Andrew Kasarskis, Ph.D., Paul Peluso, Ph.D., Ellen E. Paxinos, Ph.D., Yoshiharu Yamaichi, Ph.D., Stephen B. Calderwood, M.D., John Matthew K. Waldor, M.D., Ph.D.

N Engl J Med 2011; 364:33-42 | January 6, 2011 | DOI: 10.1056/NEJMoa1012928



METHODS

We used third-generation single-molecule real-time DNA sequencing to determine the genome sequences of 2 clinical *Vibrio cholerae* isolates from the current outbreak in Haiti, 1 strain that caused cholera in Latin America in 1991, and 2 strains isolated in South Asia in 2002 and 2008. Using primary sequence data, we compared the genomes of these 5 strains and a set of previously obtained partial genomic sequences of 23 diverse strains of *V. cholerae* to assess the likely origin of the cholera outbreak in Haiti.

Full Text of Methods...

RESULTS

Both single-nucleotide variations and the presence and structure of hypervariable chromosomal elements indicate that there is a close relationship between the Haitian isolates and variant *V. cholerae* El Tor O1 strains isolated in Bangladesh in 2002 and 2008. In contrast, analysis of genomic variation of the Haitian isolates reveals a more distant relationship with circulating South American isolates.

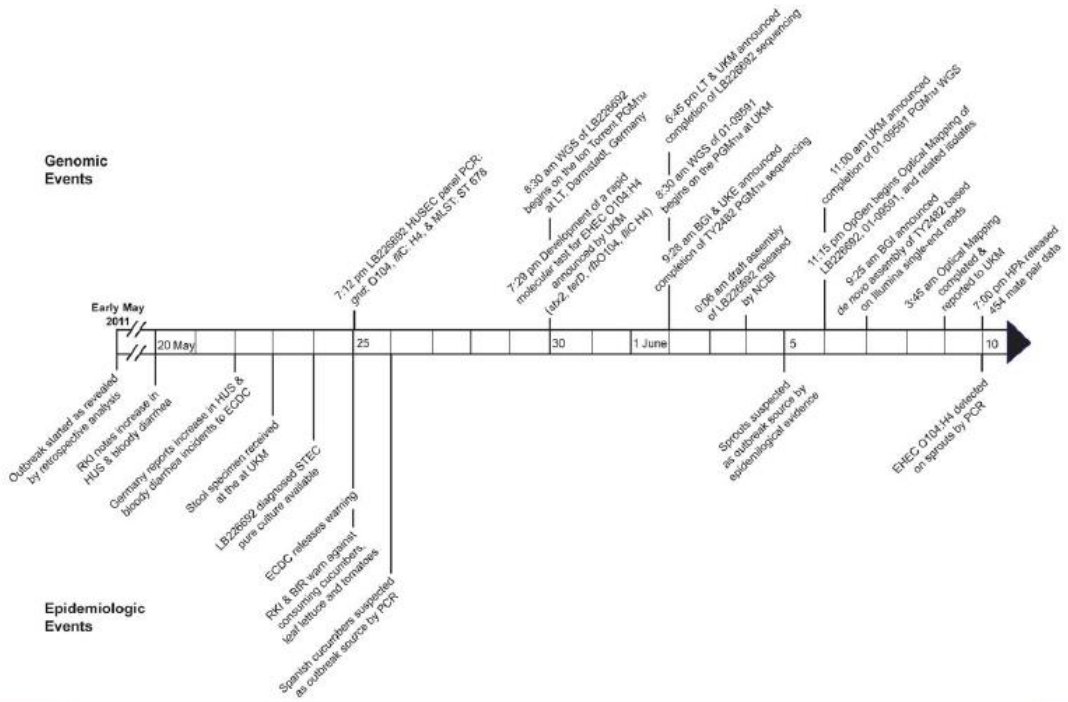
OPEN ACCESS Freely available online

PLoS one

Prospective Genomic Characterization of the German Enterohemorrhagic *Escherichia coli* O104:H4 Outbreak by Rapid Next Generation Sequencing Technology

Alexander Mellmann[1☯], Dag Harmsen[2☯], Craig A. Cummings[3☯], Emily B. Zentz[4], Shana R. Leopold[1], Alain Rico[5], Karola Prior[2], Rafael Szczepanowski[2], Yanmei Li[3], Wenlan Zhang[1], Stephan E. Mellmann[3], John K. Henkhaus[4], Benjamin Leopold[4], Moore[4], Simone Guenther[5], Jonathan

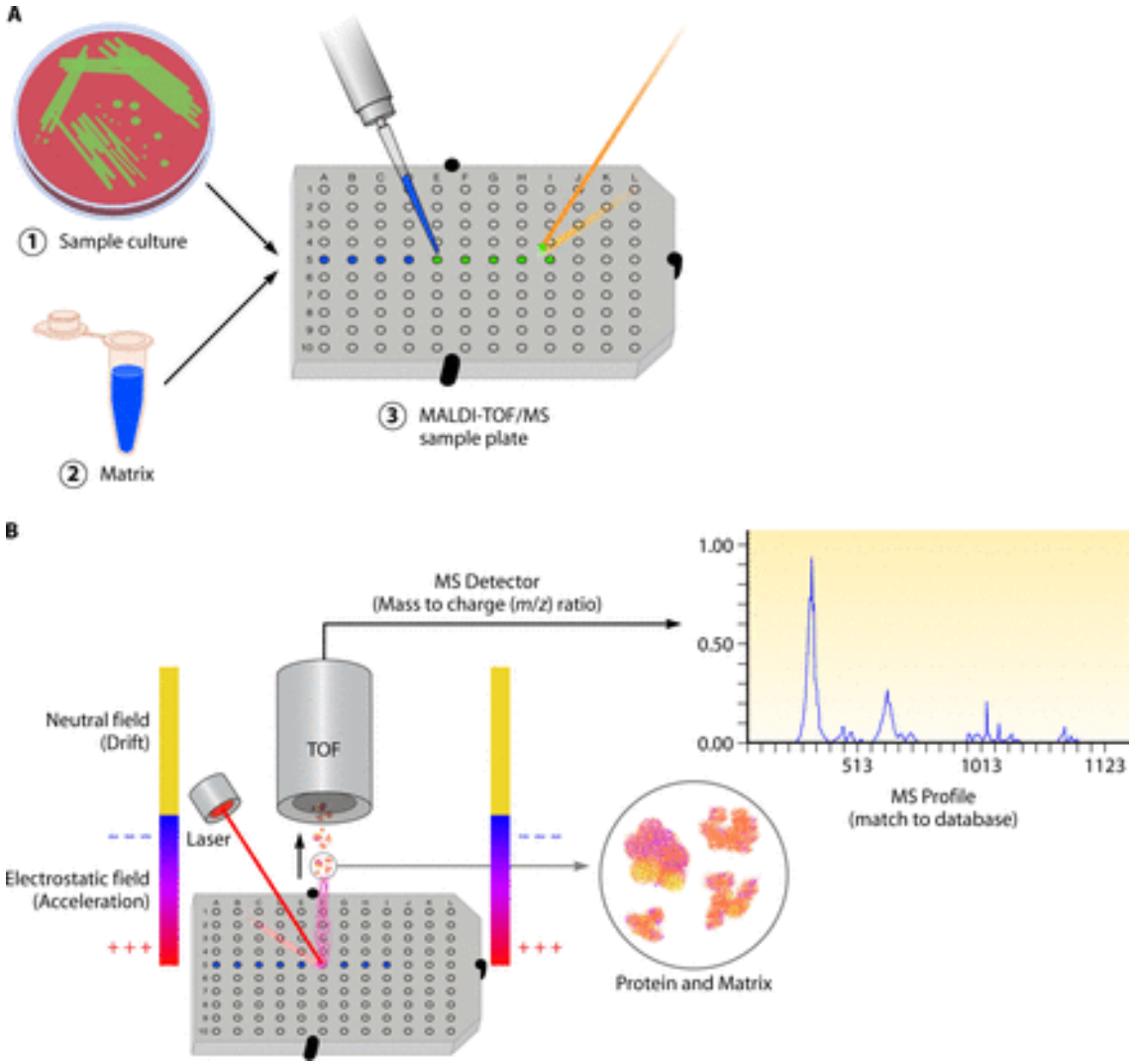
1 Institute of Hygiene, University Münster, Münster, Germany, California, United States of America, 4 OpGen, Gaithersburg, Wernigerode Branch, Wernigerode, Germany, 7 Ion Torrent



Abstract

An ongoing outbreak of exceptionally viru has caused over 830 cases of hemolytic ur has not been detected in animals, has rare characteristics of this strain in the early Technologies Ion Torrent PGM™ sequence historic O104:H4 HUS isolate from 2001 (01- the newly introduced PGM™ within 62 hou pathogenic *E. coli*, enteroaggregative *E. co* core *E. coli* genes indicate that the HUS-cau 55989 show a close relationship but are c outbreak strain differs from the 2001 strai 55989 and EHEC O104:H4 strains evolved fr loss of chromosomal and plasmid-encoded virulence factors, a highly pathogenic hybrid of EAEC and EHEC emerged as the current outbreak clone. In conclusion, rapid next-generation technologies facilitated prospective whole genome characterization in the early stages of an outbreak.

MALDI-TOF


A
1 Sample culture
2 Matrix
3 MALDI-TOF/MS sample plate
B
MS Detector (Mass to charge (m/z) ratio)
1.00
0.50
0.00
513
1013
1123
MS Profile (match to database)
Neutral field (Drift)
TOF
Laser
Electrostatic field (Acceleration)
+++
+++
Protein and Matrix

Sınırlılıkları

- Çok sayıda bakteri gerekli
- Karışık koloni varlığında sonuç veremiyor,
- Shigella – E.coli ayırt edemiyor,
- Salmonella türlerini tanımlayamıyor, (Salmonella spp.)
- S.pneumoniae ile S. mitis/oralis gibi yakın türleri ayırt etmekte güçlük çekiyor.

Beklentiler

- Direkt örnekten tanımlama
- Antibiyotik direncini saptama
- Mikobakteri tiplendirme
- Klonalite çalışmaları
- MS ile protein dışı yapıların saptanması…

Eur J Clin Microbiol Infect Dis (2015) 34:1787–1795
DOI 10.1007/s10096-015-2413-y

ARTICLE

Rapid urine preparation prior to identification of uropathogens by MALDI-TOF MS

L. Veron[1] · S. Mailler[2] · V. Girard[2] · B. H. Muller[1] · G. L'Hostis[1] · C. Ducruix[1] · A. Lesenne[3] · A. Richez[4] · H. Rostaing[1] · V. Lanet[1] · S. Ghirardi[2] · A. van Belkum[2] · F. Mallard[1]

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Abstract Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-ToF MS) has been introduced in clinical routine microbiology laboratories. For the rapid diagnosis of urinary tract infections, culture-independent methods prior MALDI-mediated identification have been described. Here, we describe a comparison of three of these methods based on their performance of bacterial identification and their potential as a routine tool for microbiology labs : (i) differential centrifugation, (ii) urine filtration and (iii) a 5-h bacterial cultivation on solid culture media. For 19 urine

Conclusions

The results shown in the present study demonstrate that short culture is a very simple and efficient sample preparation method allowing the fast and reliable identification of uropathogens by MALDI. This approach appears to be most convenient since it is compatible with high throughput, it provides "same day" identification and it would result in earlier and better antibiotic selection. If faster results are required, we have shown that dual-filtration is a more user-friendly surrogate than the differential centrifugation protocol. The additional cost of this solution would be justified by the higher medical value of fast results.

most prevalent causative species. In the setting of a possible

Ghebremedhin et al.
Ann Clin Microbiol Antimicrob (2016) 15:5
DOI 10.1186/s12941-016-0120-x

Annals of Clinical Microbiology and Antimicrobials

RESEARCH Open Access

MALDI-TOF MS based carbapenemase detection from culture isolates and from positive blood culture vials

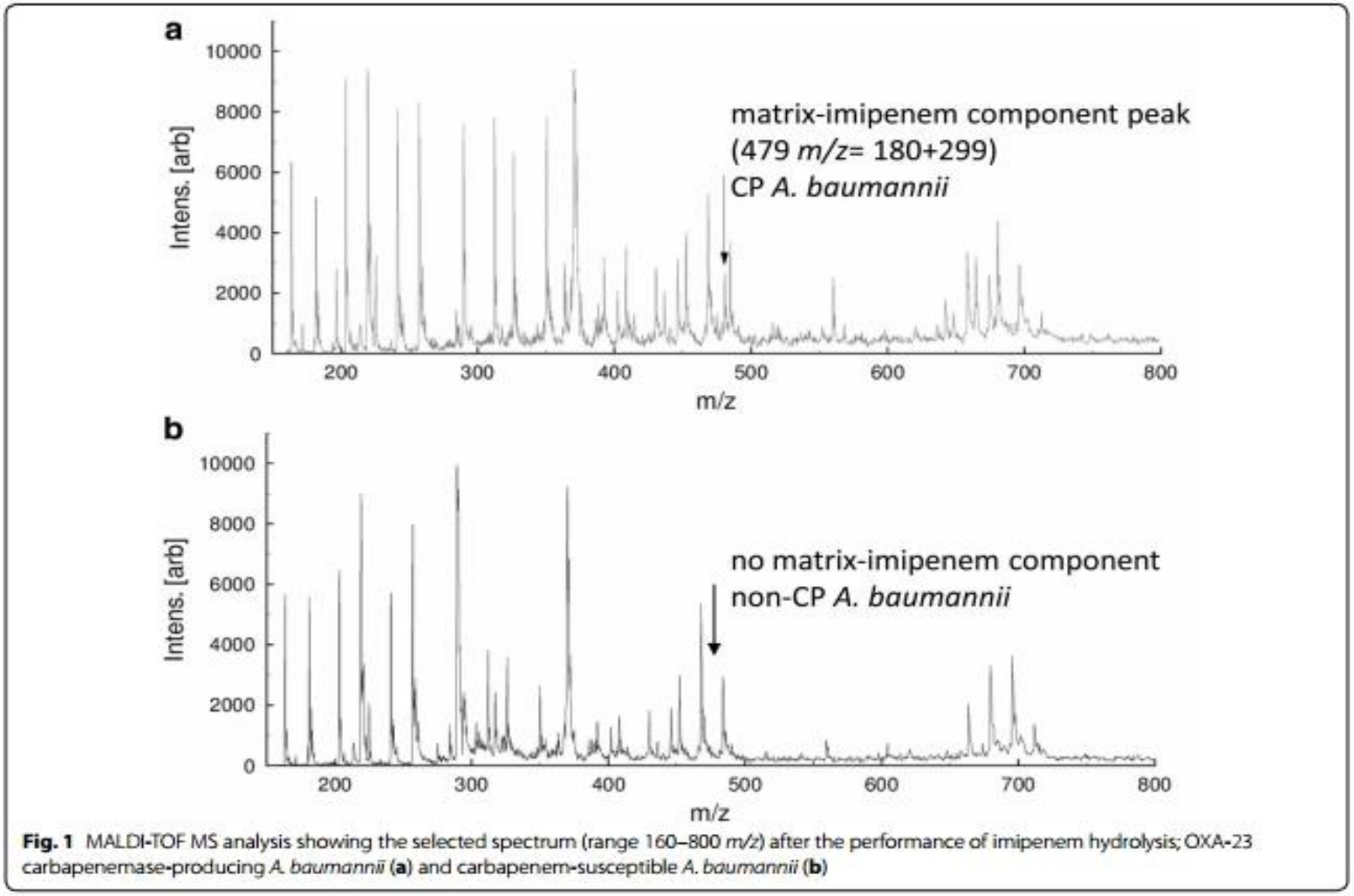


Fig. 1 MALDI-TOF MS analysis showing the selected spectrum (range 160–800 *m/z*) after the performance of imipenem hydrolysis; OXA-23 carbapenemase-producing *A. baumannii* (**a**) and carbapenem-susceptible *A. baumannii* (**b**)

PLOS | ONE

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

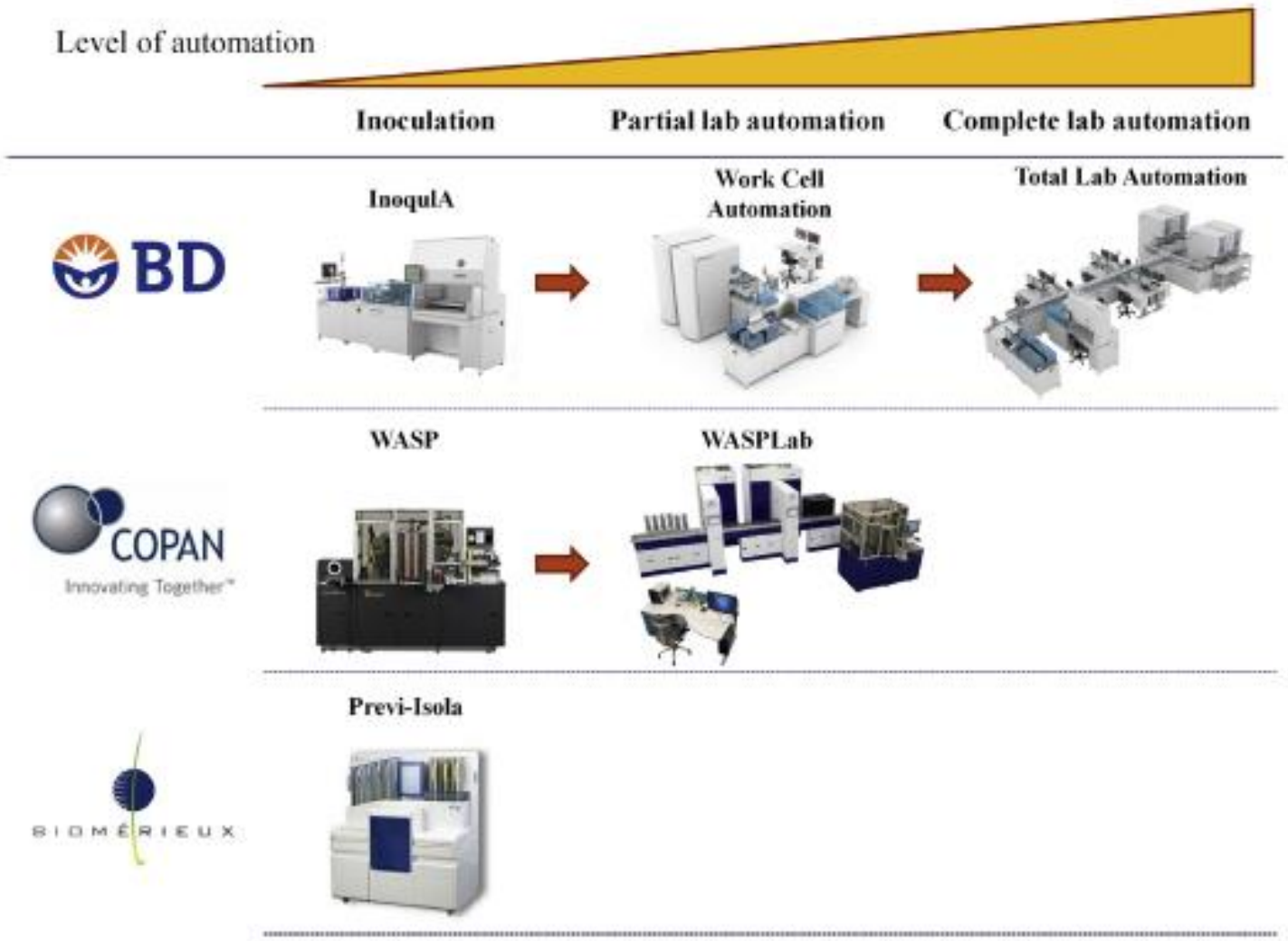
Adrian Egli[1,2]*, Sarah Tschudin-Sutter[3], Michael Oberle[4], Daniel Goldenberger[1], Reno Frei[1], Andreas F. Widmer[3]

1 Division of Clinical Microbiology, University Hospital Basel, Basel, Switzerland, 2 Infection Biology Lab, Department Biomedicine, University of Basel, Basel, Switzerland, 3 Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland, 4 Clinical Microbiology, Cantonal Hospital Aarau, Aarau, Switzerland

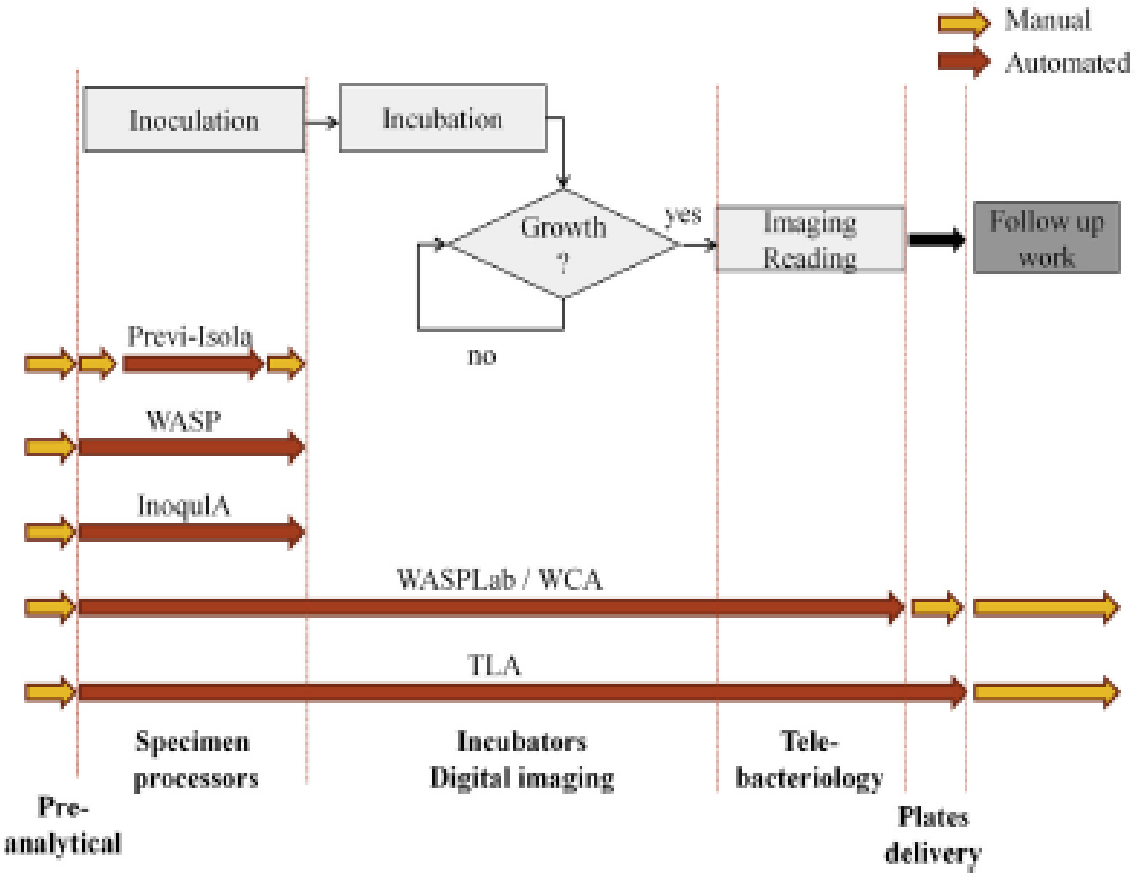
* a.egli@usb.ch

2014

Otomatize Laboratuvarlar



Otomatize Laboratuvarlar

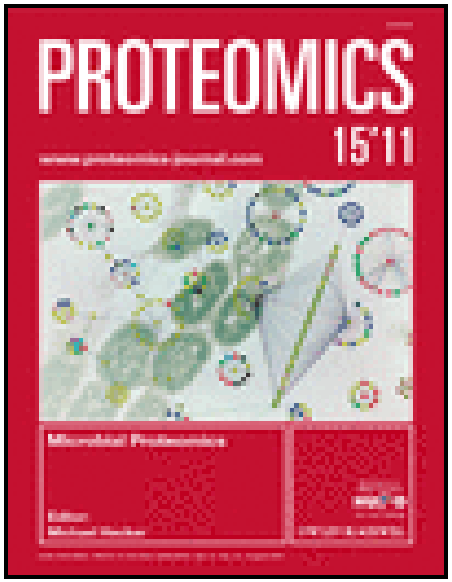


Omikler

- Genomik
- Proteomik (MS)
- Metabolomik

  lipidomik

  .....



ORIGINAL ARTICLE

Surveillance for lower airway pathogens in mechanically ventilated patients by metabolomic analysis of exhaled breath: a case-control study

Stephen J Fowler,[1,2] Maria Basanta-Sanchez,[1] Yun Xu,[3] Royston Goodacre[3]

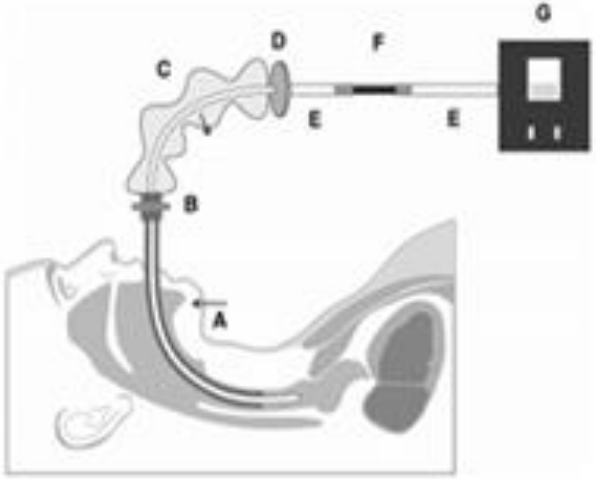


Figure 1 Schematic: breath sampling equipment for ventilated systems. Intratracheal air is sampled at 1 l/min (regulated by an air-sampling pump (G)) onto an adsorbent trap (F) via oxygen tubing (E), a bacterial/hydrophobic filter (D), and a suction catheter (C) placed in the endotracheal tube (A) via the standard access port (B).

Bakteriyolojide Yeni Yönelimler

Trends in Endocrinology & Metabolism

Special Issue: Systems Approach to Metabolic Disease

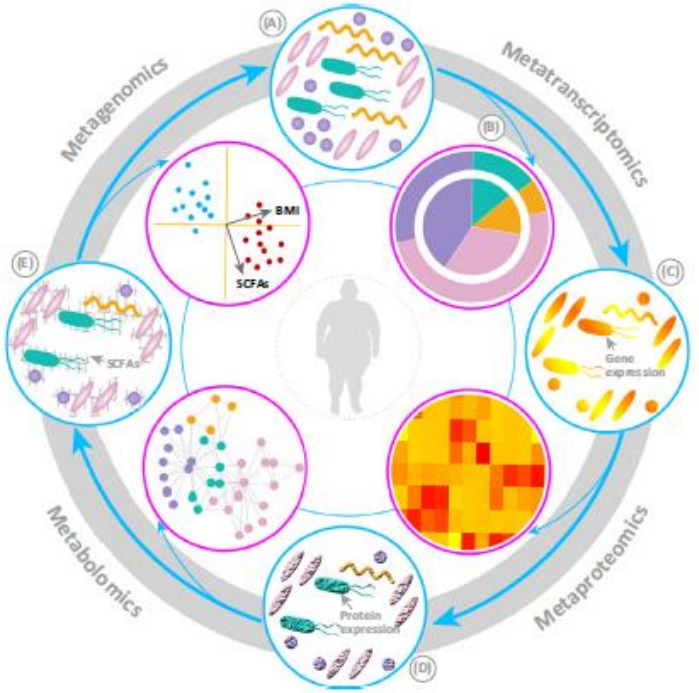
Review

Linking Microbiota to Human Diseases: A Systems Biology Perspective

Hao Wu,[1,‡] Valentina Tremaroli,[1,‡] and Fredrik Bäckhed[1,2,*]

CellPress

Integration of 'Omics' Techniques



Bakteriyolojide Yeni Fikirler

- Günümüzde İnfeksiyon Hastalıkları uzmanları mikropları öldüren değil onları anlayan, yöneten uzmanlar olmalıdır:
- “Mikropların Efendisi”

- Herşeyin nedeni mikroplar!!!

(ileriki yıllarda tüm hastalar İnfeksiyonculara gelecek!)

Yakın dönem sorunları

- Maliyet?
- Kafa karışıklığı !

HK ….üç bakteri PCR (+)

Solunum örneği….Multipleks PCR… üç etken (+)

- Tüm infeksiyon kültürümüz “kültür” temelli peki şimdi ne olacak!

Klinik Mikrobiyolojinin sonu

JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2002, p. 3889–3893 Vol. 40, No. 11
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.11.3889–3893.2002

GUEST COMMENTARY

Clinical Microbiology in the Year 2025

W. Michael Dunne, Jr.,[1]* J. Keith Pinckard,[1] and Lora V. Hooper[2]

Departments of Pathology and Immunology[1] and Molecular Biology and Pharmacology,[2] Washington University School of Medicine, St. Louis, Missouri 63110

EPILOGUE

Our take on the future provides a happy ending for the profession of clinical microbiology. Perhaps these predictions will come true such that the advanced diagnostic technology will continually rely on the basic principles and practice of culture and identification, as we know it today. Perhaps this is too shortsighted and technological development will advance at a far greater pace than predicted. Ours is not the only profession that is wrestling with the prospect of extinction. Surgical pathologists will insist that a skilled individual and a microscope can never be replaced by a microarray for the diagnosis of all disease states. Certainly, the diagnosis of a large number of infectious diseases (and diseases in general) will be made using molecular technology in the year 2025, but we contend that the proportion of microorganisms amenable

Klinik Mikrobiyoloji Laboratuvarı

Uygun Örnek ne olabilir, hangi testleri istemelisiniz. Laboratuvarla iletişim…

- Laboratuvar çalışmaları

Üreme etken/kolonizasyon…YORUM

Boyalı preparatlarla ufuk açıcı yaklaşım…

Sonucun kullanımının takibi

Kontrol önlemleri

Sonuç

- Teknolojik gelişmeler yakın gelecekte tanı ve tedavi gelişmeleri dışında ciddi algı değişimleri yaratacaktır.
- İHKMU bu değişime hazırlıklı olmalıdır.
- Hazırmıyız?

Teşekkür ederim …