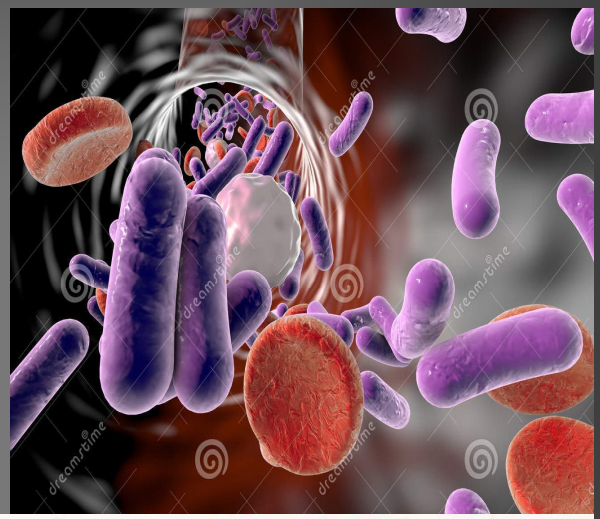
Técnicas de diagnóstico microbiológico rápido de la bacteriemia

Jordi Vila
Department of Clinical Microbiology
Hospital Clinic
Barcelona, Spain



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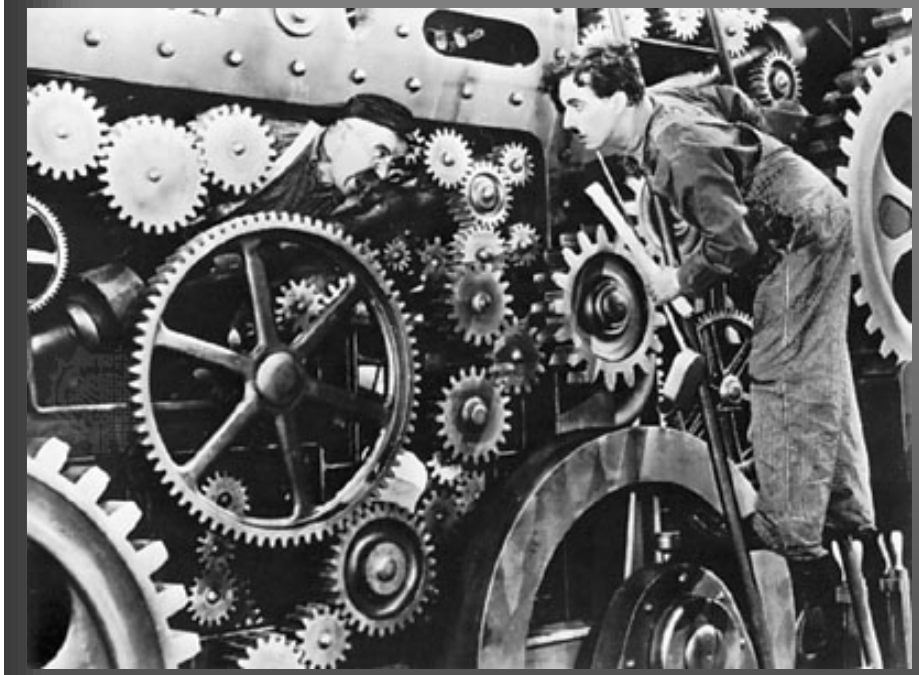
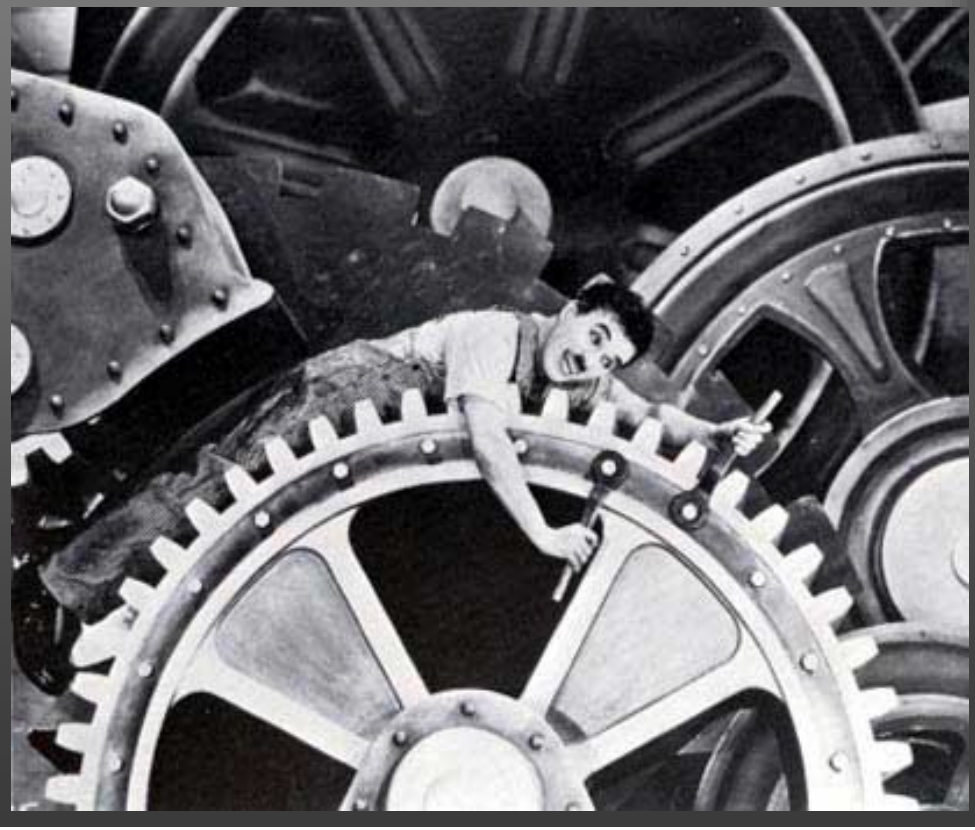
Conflict of interest declaration

I have the following potential conflicts of interest to report

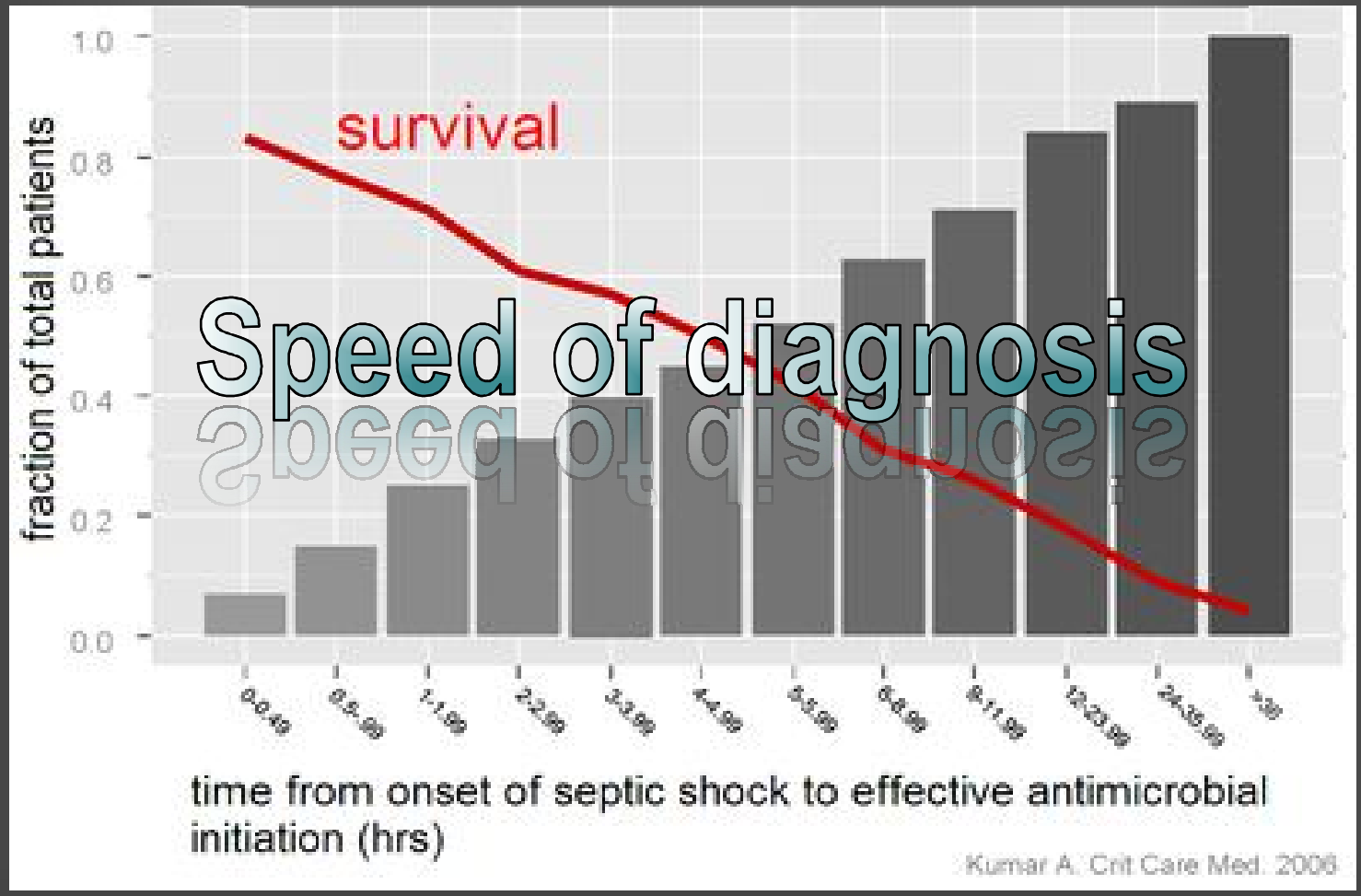
Type of Affiliation / Financial Interest	Name of Commercial Company
Receipt of grants / research supports:	Cepheid, STAT Dx, Astra-Zeneca, ABAC Therapeutics
Receipt of honoraria or consultation fees:	Phillips Diagnostics; Roche Diagnostics, Sideromics
Participation in a company sponsored speaker's bureau	MSD

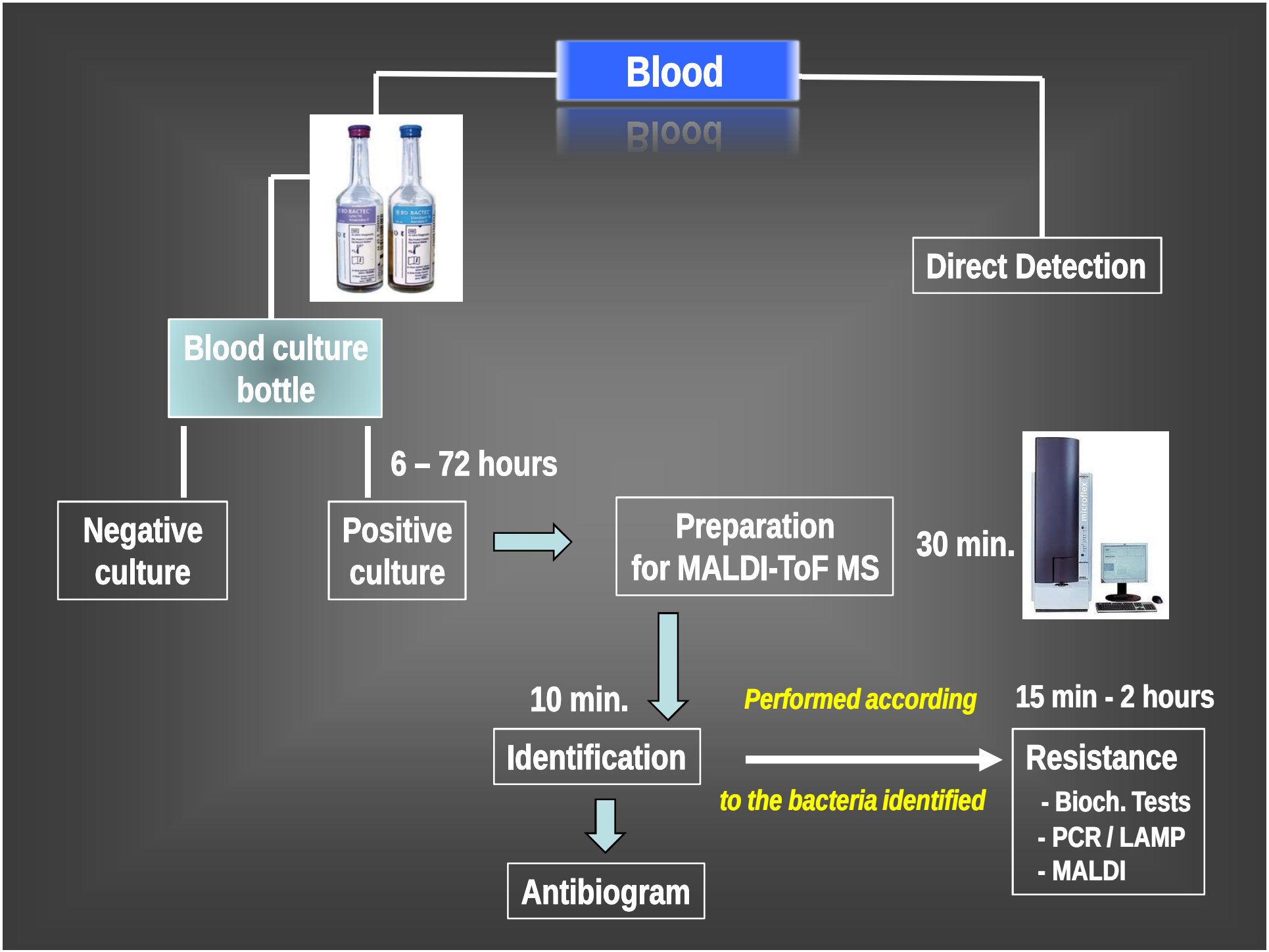
Changing clinical microbiology



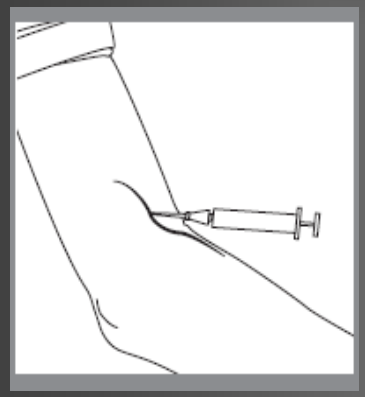


Delay in the administration of adequate empiric antibiotic treatment (Sepsis)

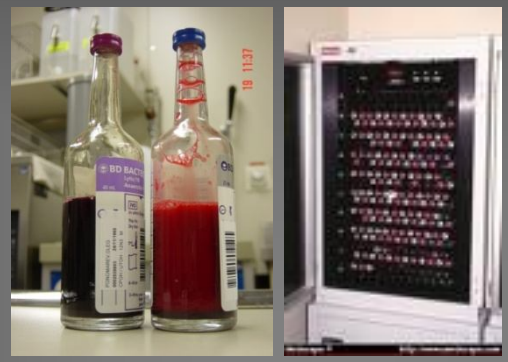



Blood
Direct Detection
Blood culture bottle
Negative culture
Positive culture
6 – 72 hours
Preparation for MALDI-ToF MS
30 min.
10 min.
Identification
Antibiogram
Performed according
to the bacteria identified
15 min - 2 hours
Resistance
- Bioch. Tests
- PCR / LAMP
- MALDI

Direct testing of positive blood cultures by MALDI-TOF



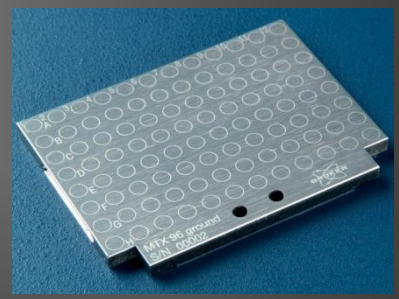
Sampling



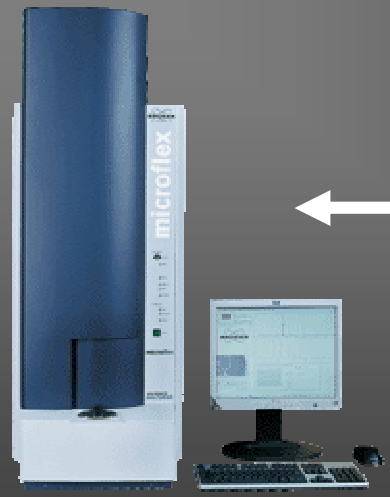
Incubation of blood culture bottles

WHEN POSITIVE

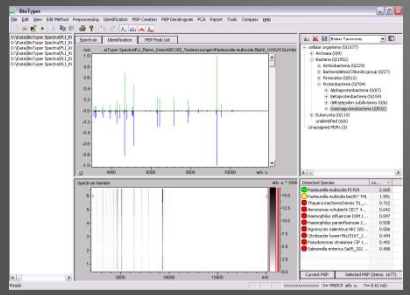
Preparation of a bacterial pellet



Deposition of bacterial pellet on MALDI microplate



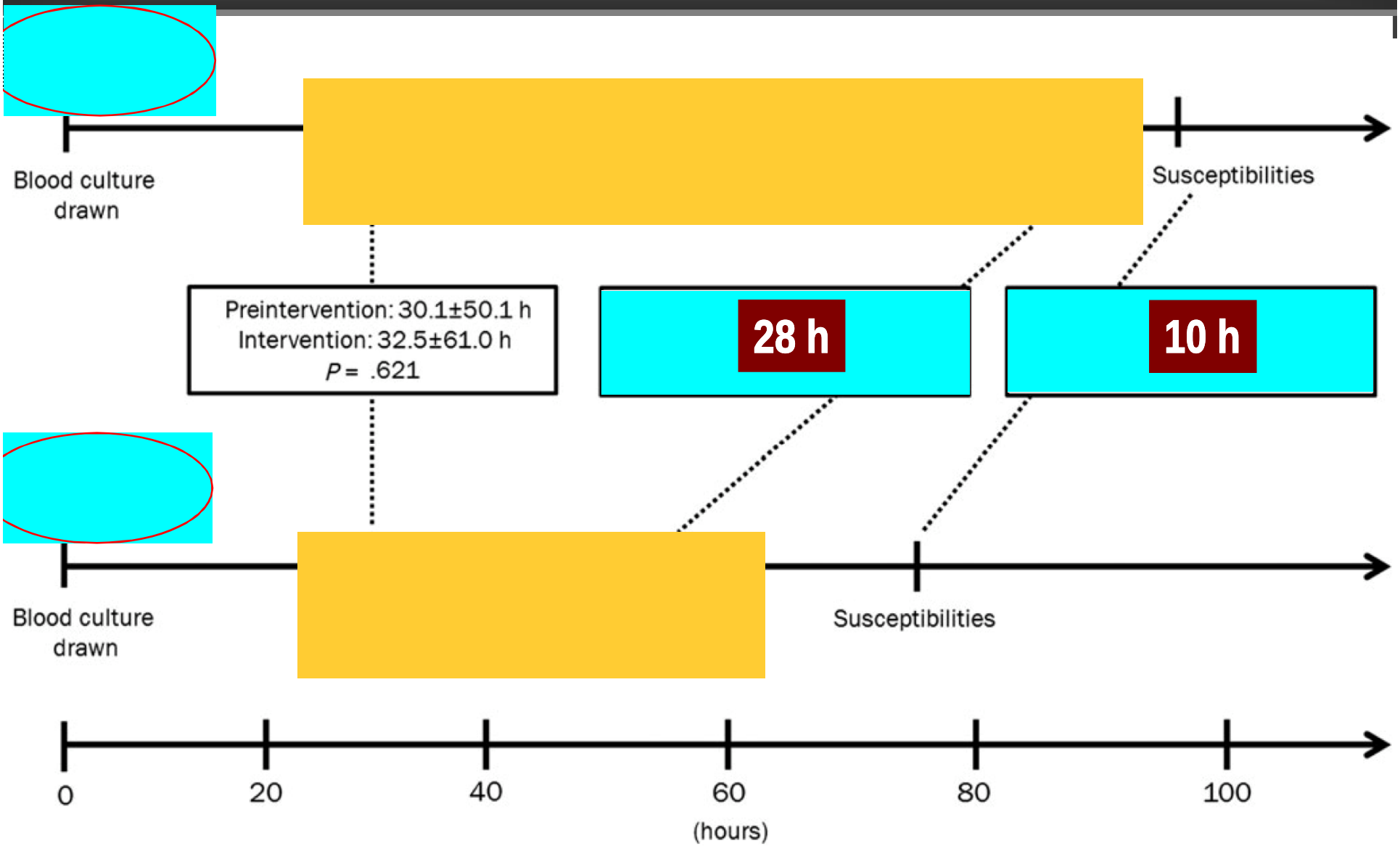
Acquisition of the proteic profile



Comparison with a database

TAT
Circa 30 min.

Huang A, et al. **Impact of Rapid Organism Identification via Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Combined With Antimicrobial Stewardship Team Intervention in Adult Patients With Bacteremia and Candidemia.** *Clin Infect Dis 2013; 57: 1237–45*



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Clinical outcomes			
Time to microbiological clearance, d	3.3 ± 4.8	3.3 ± 5.7	.928
Length of hospitalization, d[a]	14.2 ± 20.6	11.4 ± 12.9	.066
Recurrence of same BSI	15 (5.9)	5 (2.0)	.038
30-day readmission with same BSI	9 (3.5)	4 (1.6)	.262
Treatment-related outcomes			

Blood

Classical

Blood culture

Gram stain

Culture

ID/Antibiogram

Semi-molecular

Blood culture

Gram stain

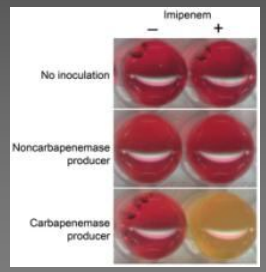
MALDI-TOF → Detection of MRSA

Detection of β-lactamases

Detection of resistance from positive BC

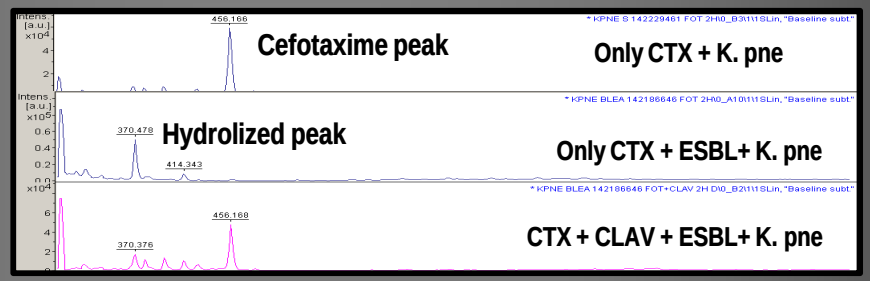
ESBL and carbapenemases

- **Phenotypic tests:**
  - ESBL NDP
  - CARBA NP
  - Blue CARBA TEST



- TAT < 2h
- Lower sensit. for OXA-48

- **Detection of enzymatic activity by MALDI-TOF**
  - Carbapenemases
  - ESBLs



- TAT < 2-4h
- Lower sensit. for OXA-48

- **Detection by NAAT**
  - Xpert-CARBA-R - RT-PCR
    - KPC, NDM, VIM, OXA-48, IMP
  - Check-point - Microarrays
    - KPC, VIM, IMP, NDM, OXA-48 and ESBL (TEM, CTXM and SHV)
  - Eazyplex - LAMP
    - NDM, VIM, KPC, OXA-48 and ESBLs families CTX-M-1, CTX-M-9

Zboromyrska Y: Rapid detection of b-lactamases directly from positive blood cultures using a loop-mediated isothermal amplification (LAMP)-based assay

International Journal of Antimicrobial Agents 2015; 45: 355

Protocol

25 µl of + BC

↓

+ 500 µl of resuspension and lysis buffer

↓

99ºC for 2 min

↓

Centrifuged

↓

1,400 x g for 30 sec

↓

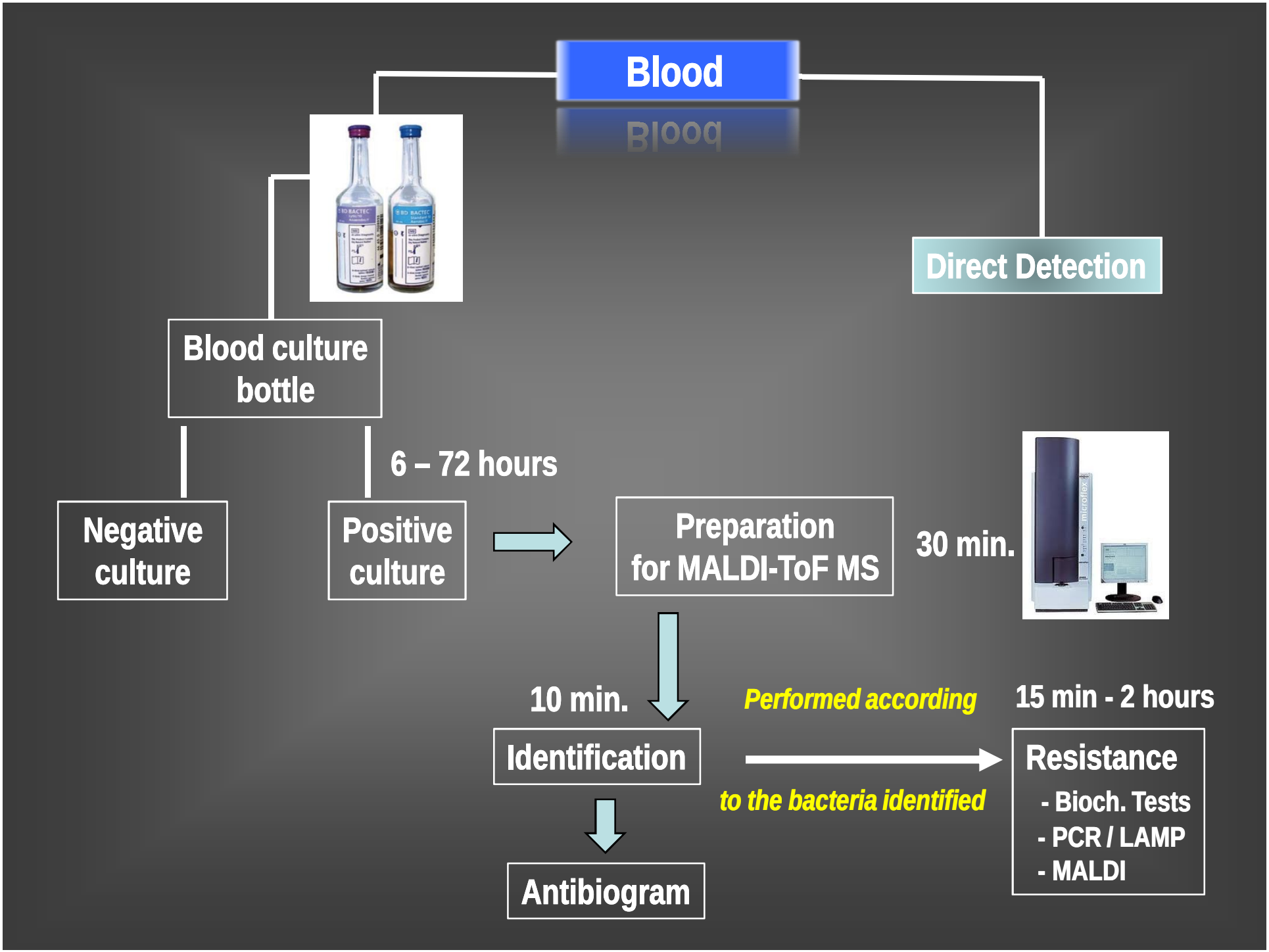
25 µl to perform the Eazyplex assay

Table 1
Bacterial strains harbouring different resistance markers used in the study.

Bacterial species	No. of isolates	Resistance mechanism
Escherichia coli	3	CTX-M-1
Klebsiella pneumoniae	1	CTX-M-1
K. pneumoniae	1	CTX-M-32
E. coli	2	CTX-M-14
E. coli	1	CTX-M-27
E. coli	2	CTX-M-9
Enterobacter asburiae	1	KPC and CTX-M-15
K. pneumoniae	1	KPC-9
K. pneumoniae	2	KPC-2
K. pneumoniae	1	KPC-3
Acinetobacter baumannii	2	NDM-1
E. coli	1	NDM-1 and CTX-M-15
A. baumannii	1	NDM-2
E. coli	1	NDM-5
K. pneumoniae	2	OXA-48
K. pneumoniae	3	OXA-48 and CTX-M-15
Enterobacter cloacae	1	VIM-1
Klebsiella oxytoca	2	VIM-1
E. coli	1	VIM-1
Pseudomonas aeruginosa	1	VIM-2

Global concordance: 100% using the Protocol described

TAT approx. 20 min.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Resistance
- Bioch. Tests
- PCR / LAMP
- MALDI

Technologies for the diagnosis of sepsis

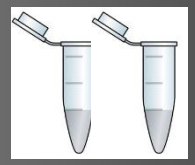
	SepsiTest*	Prove-it Sepsis*	Septifast*	Magicplex*	Vyoo*	Filmarray	Verigene	Plex-ID*
Methods	Broad rt-PCR + sequencing	mPCR + microarray	rt-PCR	rt-PCR	mPCR + electrophor.	RT-PCR	Microarray + signal amplif.	Broad PCR ESI-TOF MS
Nº microorg.	>300	83	25	>90	39	24	3 Panels (30)	Up to 800
Resistant markers	--	*vanA/B, mecA*	---	*vanA, vanB, mecA*	*mecA, van A/B, SHV, CTX-M*	*vanA, vanB, mecA, KPC*	*vanA, vanB, mec* + 6 Carbap.	*vanA, vanB, mecA, KPC*
Sensitivity	21-85	94	43-91	37-65	60	88-100	50-100	47-91
Specificity	58-95	98	88-96	77-92	75	>98	98-100	98-99
Time to results	8-12	3	6	6	8	1	2.5	6

*** They have been applied directly from blood samples**

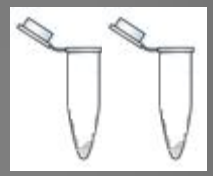
Diagnosis of catheter-associated bacteremia

1. Centrifuge 4 mL of blood collected in EDTA-tube at 1,500 rpm for 5 min.

2. Transfer supernatant to 2 eppendorf tubes (1.5 mL)

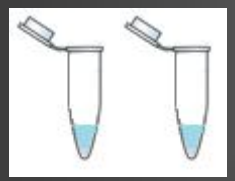


3. Centrifuge at 13,000 rpm for 2 min.



4. Discard the supernatant

5. Add 100 µl of saline solution and resuspend the pellet and carry out the detection following the instructions



200 µl

www.cepheid.com

- Identification of *S. aureus*
- Identification of CNS
- Resistance to methicillin

BC / GeneXpert	Positive	Negative	Total
Positive	14	6	20
Negative	2	70*	72
Total	16	76	92

Concordance between BC and GeneXpert:

- 4 *S. aureus*
- 10 *CNS*

* Including 16 blood cultures contaminated by CoNS

Sensitivity of GeneXpert 87.5%
Specificity of GeneXpert 92.1%

PPV of GeneXpert 70.6%
NPV of GeneXpert 98.6%

Zboromyrska Y. et al. PLoS one (2016) 11:e0161684

ESI Mass Spectrometry

PCR and Mass Spectrometry (Detection of nucleic acids)

6-8 h turnaround time
30-40 mins hands-on-time

O'Dwyer MJ: Detection of microbial DNA but not cultures bacteria is associated with increased mortality in patientes with suspected sepsis – a prospective multi-centre European observational study

Clinical Microbiology and Infection 2017; 208-e1

Predictor	Univariable		Multivariable	
	p	OR (95% CI)	p	OR (95% CI)
Age (per year)	<0.0001	1.05 (1.03–1.07)	<0.0001	1.05 (1.03–1.07)
SOFA score (per unit)	<0.0001	1.15 (1.09–1.22)	<0.0001	1.15 (1.08–1.23)
History of cancer	0.02	1.8 (1.1–2.8)	0.02	1.8 (1.08–3.15)
Immune suppression	0.04	1.9 (1.1–3.6)	0.14	1.8 (0.8–3.7)
Positive blood culture	0.74	1.1 (0.6–2.1)		
Cardiovascular disease	0.5	1.3 (0.7–2.3)		
Respiratory disease	0.7	1.3 (0.67–2.0)		
Diabetes mellitus	0.5	1.2 (0.72–2.0)		
Chronic kidney disease	0.7	1.1 (0.6–2.0)		
Cirrhosis	0.6	1.4 (0.6–2.7)		
History of smoking	0.5	1.3 (0.7–2.7)		

Presence of microbial DNA in patients with suspected sepsis might define a patient group at higher risk of death.

Limitations:

- No influence in treatment
- No standardization among centres

Is this DNA indicative of a pathogenic finding?

Desmet S: Broad-range PCR coupled with electrospray ionization time of flight mass spectrometry for detection of bacteremia and fungamia in patients with neutropenic faver

Journal of Clinical Microbiology 2016; 54: 2513

TABLE 2 Microorganisms detected by BC only, PCR/ESI-MS only, and detected by both methods for definite and probable BSI

Organism reported	No. of microorganisms detected			
	BC only	PCR/ESI-MS only	BC and PCR/ESI-MS	Total
Definite BSI				
Gram negatives	6	1	1	8
Escherichia coli	4	0	1	5
Fusobacterium nucleatum	0	1	0	1
Klebsiella oxytoca	1	0	0	1
Klebsiella pneumoniae	1	0	0	1
Gram positives	13	1	12	26
Clostridium ramosum	1	0	0	1
Enterococcus faecium	1	0	2	3
Enterococcus faecalis	1	0	1	2
Gemella spp.	2	0	0	2
Rothia mucilaginosa	1	0	2	3
Staphylococcus epidermidis[a]	4	0	5	9
Staphylococcus haemolyticus[a]	1	0	1	2
Staphylococcus hominis[a]	2	0	0	2
Streptococcus mitis	0	0	1[b]	1
Streptococcus pneumoniae	0	1	0	1
Fungi	0	0	2	2
Candida kefyr	0	0	1[c]	1
Saccharomyces cerevisiae	0	0	1[c]	1
Total	19	2	15	36
Probable BSI				
Brevibacterium paucivorans/Corynebacterium auris[a]	0	1	0	1
Corynebacterium tuberculostearicum[a]	0	1	0	1
Gordonia polyisoprenivorans[a]	0	2	0	2
Micrococcus luteus	1	0	0	1
Propionibacterium acnes[a]	0	2	0	2
S. epidermidis[a]	3	0	3	6
S. haemolyticus[a]	0	1	0	1
S. hominis[a]	4	0	0	4
Total	8	7	3	18

[a] Reported as potential contaminant by PCR/ESI-MS BAC BSI assay.
[b] PCR/ESI-MS reported *Streptococcus pneumoniae*.
[c] PCR/ESI-MS reported "fungus detected, no ID provided."

PCR/ESI MS

Sens. 47%

Spec. 93%

- **Differences:**
  - Febrile neutropenic patients lack of neutrophils containing mDNA might results in a lower [mDNA]
  - Eight bottles collected for BC vs 5 ml for PCR/ESI MS
  - Blood for PCR/ESI MS was collected through one lumen of the catheter whereas for BC it was from all lumens and peripheric blood vein

Rapid tests based on molecular methods to diagnose sepsis

Rapid tests compared to BC:

- Advantages
  - Patients receiving antibiotic
  - Detection of fungemia caused mainly by *Aspergillus*
  - More rapid identification than the BC.
- Disadvantages
  - Bacteraemia may be missed due to lower sample volume or pathogen not included into the test panel
  - No possibility to determine antimicrobial susceptibility only some resistant determinants
  - High cost

Rapid tests based on molecular methods to diagnose sepsis

These tests should be improved by:

- Include identification plus susceptibility testing
- Increase the volume of blood analyzed
- Adapt to other clinical sample
- Provide results in less than 1 hour in an automatic system
- To perform a quantitation of the DNAmeia?

Balker B: Detection cost-effectiveness analysis of multiplex PCR with magnetic resonance detection versus empiric or blood culture-directed therapy for management of suspected candidemia

Journal of Clinical Microbiology 2016; 54: 728

Table 2. Overall Sensitivity and Specificity of the T2 Magnetic Resonance Method

Sensitivity	No.	%	95% CI
[illegible]			
Overall per assay[a]	234/257	91.1	86.9–94.2
Per *Candida* species[a]			
C. albicans/tropicalis	96/104	92.3	85.4–96.6
C. parapsilosis	49/52	94.2	84.1–98.8
C. krusei/glabrata	89/101	88.1	80.2–93.7
Specificity			
[illegible]			
Per species[a]			
C. albicans/tropicalis	[illegible]	[illegible]	[illegible]
C. parapsilosis	[illegible]	[illegible]	[illegible]
C. krusei/glabrata	[illegible]	[illegible]	[illegible]

se of BSI in the ICUs

1% dependent of patient population

robiology

andidadiasis are often receiving

dida [CID (2015) 60: 892]

36.7% of the resolved upon initial

2DT when the prevalence was varied over a range from 1% to 30%, but T2DT was less costly than EET when the prevalence remained at 7.2% and more costly than BCDT when the prevalence was 2.4%. This suggests that the optimal use of T2DT may be in a moderate-risk setting where the IC prevalence is around 5% and empirical or prophylactic antifungal therapy is prescribed routinely.

CONCLUSIONES

- Existen evidencias clínicas que demuestran que una rápida identificación y determinación de la sensibilidad antimicrobiana de bacterias causantes de sepsis ayuda a la implementación de una terapia antimicrobiana adecuada y por ello a reducir la mortalidad.
- La espectrometría de masas MALDI-ToF para identificación de bacterias/ hongos de HC positivos tiene un impacto clínico evidente.
- La combinación de métodos fenotípicos y genotípicos cuando sea posible es deseable para detectar determinantes de resistencia a partir de hemocultivos positivos.
- Ninguno de los métodos moleculares puede a día de hoy re-emplazar al hemocultivo.
- Los resultados generados a través de las pruebas de diagnóstico rápido deben ser comunicados al equipo PROA lo más rápidamente posible para que tengan un impacto clínico elevado.