Su iniziativa di

GIORNATA MONDIALE DELLA SEPSI

Diagnosi e gestione clinica della sepsi

Nuove metodologie diagnostiche rapide nelle sepsi batteriche

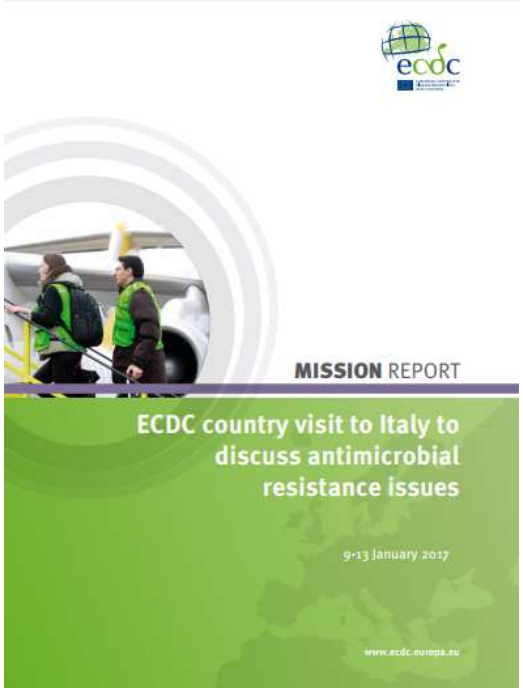
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ROMA, 13 SETTEMBRE 2018

Introduction

- Incidence and number of deaths related to BSI are increasing
- Lethality often due to inadequate antimicrobial therapy administered within the first 24 h
  - **Each hour of delay over 6 hours following septic shock in antibiotic administration decreases the survival rate of more than 7%**
- Empirical antimicrobial therapy may favour selection and spread of antimicrobial resistant pathogens



Dalla visita dell'ECDC

The AMR situation in Italian hospitals and regions poses a major public health threat to the country. The levels of carbapenem-resistant Enterobacteriaceae (CRE) and *Acinetobacter baumannii* have now reached hyper-endemic levels and, together with meticillin-resistant *Staphylococcus aureus* (MRSA), this situation causes Italy to be one of the Member States with the **highest level of resistance in Europe**.

Introduction – microbiological data

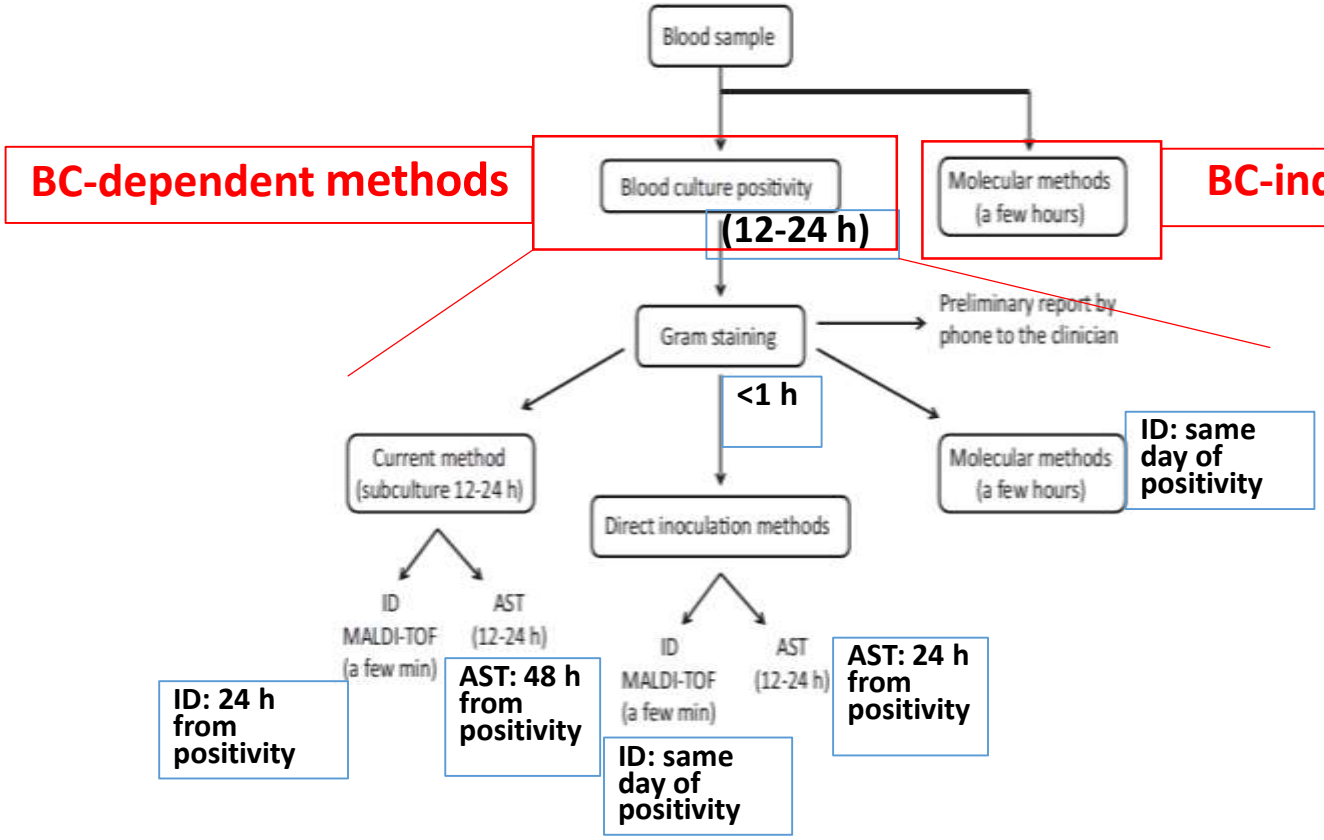


Table 4. Number of isolates and percentages of the ten most frequently isolated microorganisms in ICU-acquired bloodstream infection (BSI) episodes by country, EU/EEA, 2016

Microorganism	Belgium (n=66)	Czech Republic (n=102)	Estonia (n=78)	France (n=3 058)	Germany (n=2 626)	Hungary (n=59)	Italy (n=979)	Lithuania (n=89)	Luxembourg (n=31)	Malta (n=14)	Poland (n=48)	Portugal (n=308)	Slovakia (n=19)	Spain (n=1 079)	United Kingdom (n=103)	Total (n=8 659)
Coagulase-negative staphylococci	28.3	29.3	15.7	18.8	25.3	31.6	17.3	38.1	13.3	0.0	22.2	22.2	0.0	29.3	19.8	20.5
Klebsiella spp.	10	15.2	25.7	12.1	8.4	10.5	17.7	8.3	16.7	14.3	5.6	5.6	27.7	15.6	17.6	14.1
Enterococcus spp.	13.3	5.4	24.3	11.9	20.2	7.0	11.6	4.8	3.3	14.3	11.1	11.1	19.1	13.8	11.0	12.4
Pseudomonas aeruginosa	6.7	7.6	8.6	11.8	4.4	19.3	10.4	2.4	6.7	14.3	27.8	27.8	8.5	9.0	3.3	11.1
Escherichia coli	18.3	7.6	7.1	11.0	9.4	7.0	9.0	8.3	10.0	14.3	0.0	0.0	10.6	7.2	11.0	9.7
Staphylococcus aureus	8.3	12.0	1.4	10.2	14.9	3.5	9.0	7.1	16.7	7.1	5.6	5.6	4.3	5.7	9.9	8.7
Enterobacter spp.	5.0	3.3	7.1	11.2	5.3	5.3	6.6	6.0	20.0	35.7	5.6	5.6	0.0	4.0	5.5	8.5
Candida spp.	8.3	10.9	8.6	8.8	8.5	3.5	5.8	2.4	10.0	0.0	0.0	0.0	0.0	8.4	17.6	8.0
Acinetobacter spp.	0.0	4.3	1.4	1.4	0.8	12.3	9.2	19	3.3	0.0	22.2	22.2	23.4	2.4	1.1	3.6
Serratia spp.	1.7	4.3	0.0	2.8	2.8	0.0	3.4	3.6	0.0	0.0	0.0	0.0	6.4	4.6	3.3	3.3

Gram-negative are now more prevalent than Gram-positive, fungi keep to increase (with local differences due to patient population)

Standard methods and Rapid Diagnostic Tests in Sepsis



Empiric therapy

Oriented therapy
(Identification (ID)

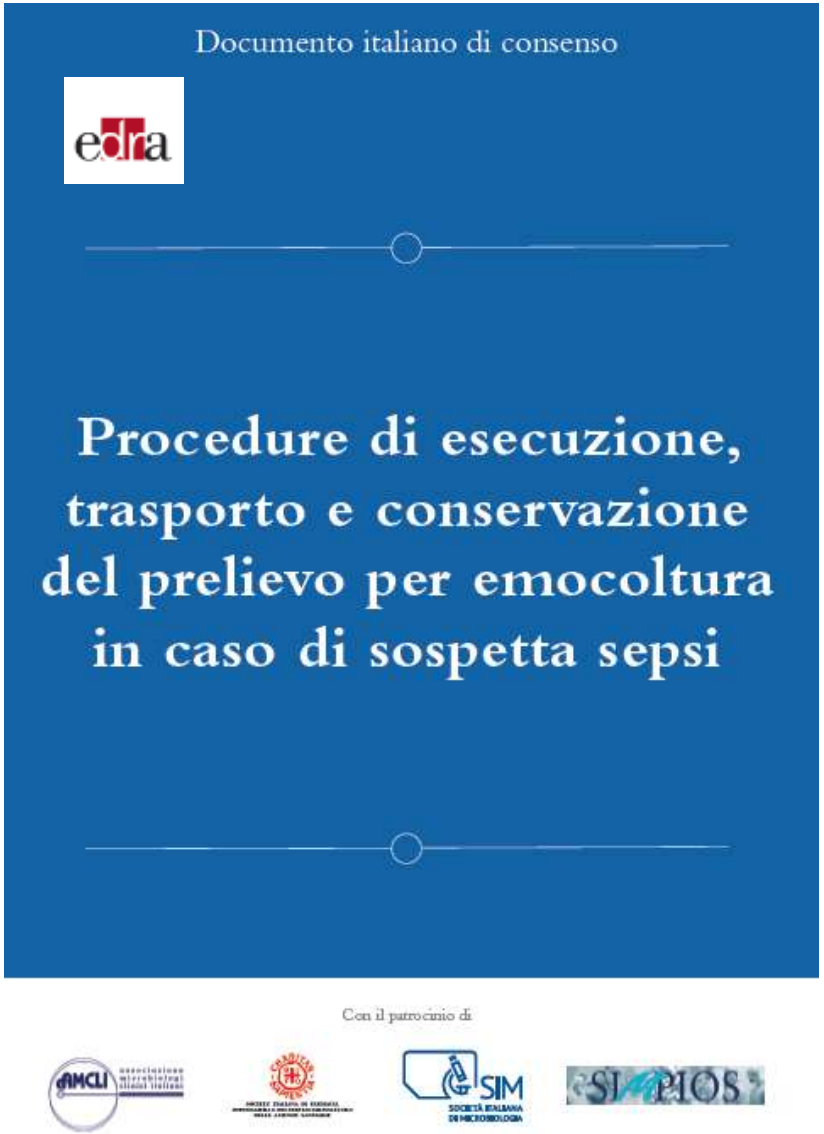
Targeted therapy
(Antimicrobial Susceptibility Test (AST)

Modified from Florio w. *et al*. 2017 Critical reviews in Microbiology

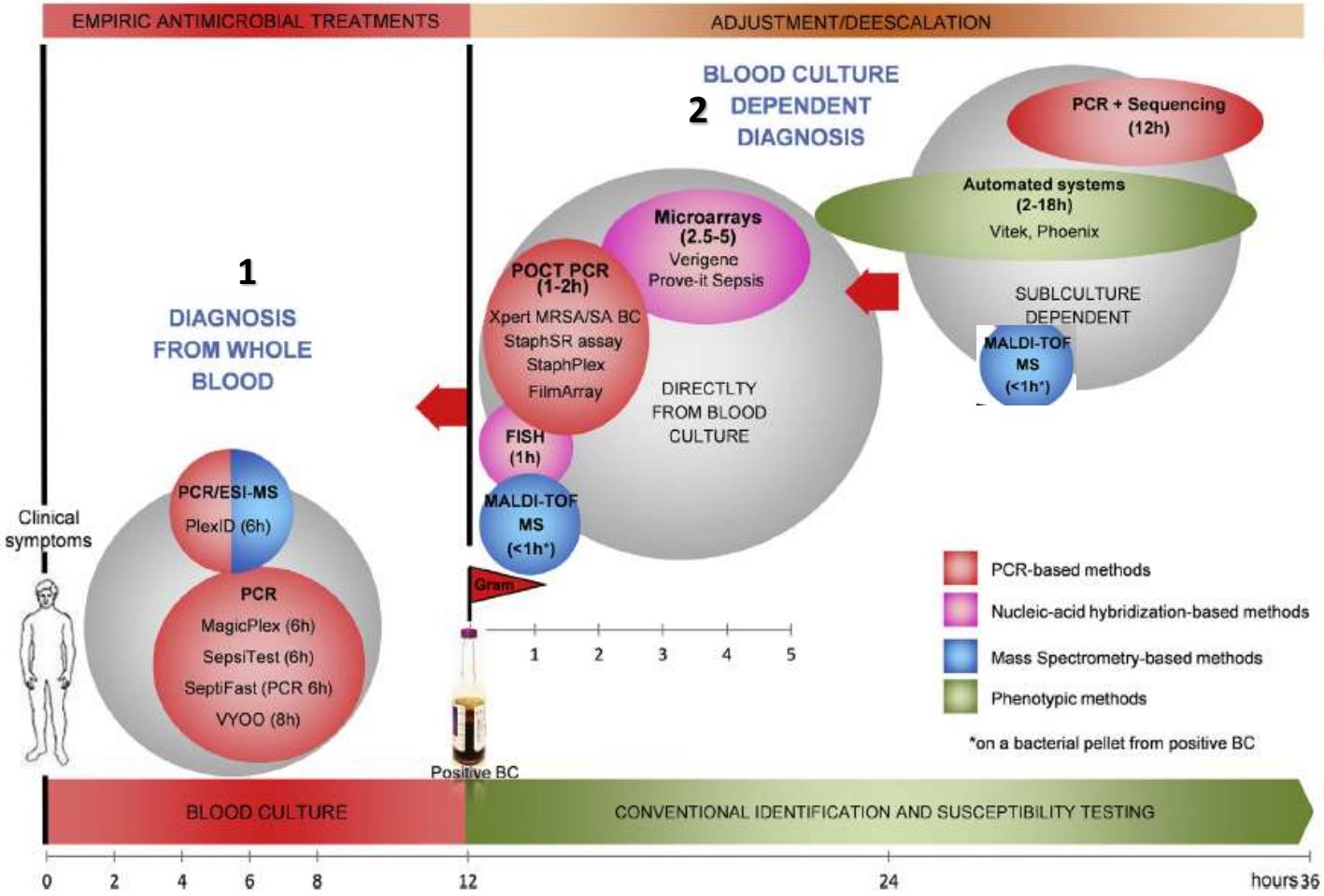
Blood culture: pre-analytical step

Particular care should be taken in:

- **TIMING OF BC SPECIMEN COLLECTION**
- **BLOOD CULTURE COLLECTION**
  - **Skin Preparation**
  - **Sampling Site**
  - **Sampling strategy**
  - **Impact of Volume Sampled on BCs Yield**
- **TIME TO TRANSPORTATION**



Rapid Diagnostic Test (RDT) for BSI



1. Directly from whole blood:

- Molecular methods

2.Blood culture positive-dependent methods:

- Directly from blood culture
- Subculture dependent

Opota et al, Clin Microbiol Infect 2015

Methods directly from whole blood (BC-independent)

PCR-based

3-8 h

R genes



Table 2
Commercially available tests for pathogen detection directly from blood [7,9,13–15,23–25]

System (Manufacturer)	Principle	TAT (h)	Blood (mL)	Panel	Resistance genes	LoD (CFU/mL)	Sensitivity (%)	Specificity (%)	Cost	
									Instrument	Test
SepsiTest (Molzym)	Broad-range PCR + sequencing	8–12	2 × 1	>345 bacteria and fungi	—	20–460	21–87	85–96	€€	€€€
SeptiFast (Roche Molecular Diagnostics)	Multiplex real-time PCR	4–6	1.5	9 GP, 10 GN, 6 fungi	*mecA*	3–30	25–86	74–97	€€	€€€
MagicPlex Seegene)	Multiplex real-time PCR	3–5	1	73 GP, 12 GN, 6 fungi	*mecA*, *vanA*, *vanB*	30	37–65	66–92	€€	€€€
VYOO (Analytic Jena)	Multiplex PCR + gel electrophoresis	7–8	5	14 GP, 18 GN, 7 fungi	*mecA*, *vanA*, *vanB*, bla_{SHV}	3–10	60	70–75	€€	€€€
Iridica (Abbott Molecular)	Multiplex PCR + ESI-MS	6–8	5	>750 bacteria and fungi	*mecA*, *vanA*, *vanB*, bla_{KPC}	4–16	50–91	79	€€€€	€€€
T2Candida panel (T2 Biosystems)	PCR + magnetic resonance technology	3–4	4	5 *Candida*	—	1	91–100	98	€€€	€€€

GN, Gram-negative bacteria; GP, Gram-positive bacteria; LoD, limit of detection (in CFU/mL); TAT, turnaround time (in hours).

Major disadvantages

- **High costs (mainly for test)**
- **ID achieved for pathogens covered by each panel (conventional tests are needed**
- **Low sensitivity and specificity**…except for T2 Bacteria panel

Rello et al, Clin Microbiol Infect 2018. in press.

J Antimicrob Chemother 2018; 73 Suppl 4: iv20–iv26
doi:10.1093/jac/dky049

Journal of Antimicrobial Chemotherapy

T2Bacteria magnetic resonance assay for the rapid detection of ESKAPEc pathogens directly in whole blood

Giulia De Angelis[1]†, Brunella Posteraro[2]†, Elena De Carolis[1], Giulia Menchinelli[1], Francesco Franceschi[3], Mario Tumbarello[4], Gennaro De Pascale[5], Teresa Spanu[1] and Maurizio Sanguinetti[1]*

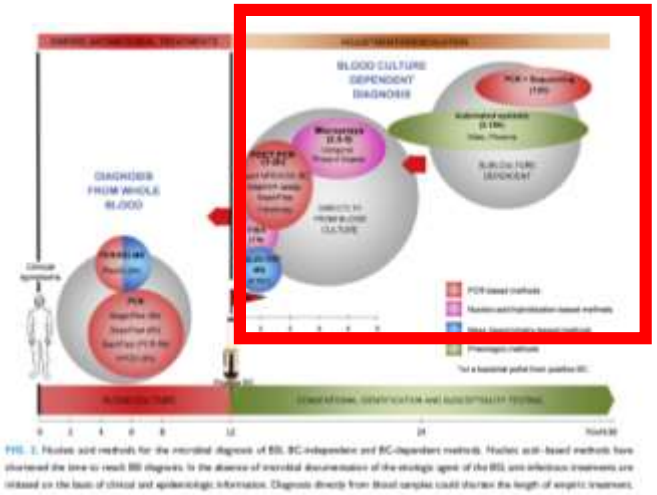


- **Positive results more than 4.5 times faster** (5.5 hours vs. 25 hours for BC).
- **Negative results 20 times faster** (6.1 hours vs. 120 hours for BC).
- 89.5% **sensitivity** and 98.4% **specificity** in comparison with clinical criterion of a true infection.

Table 4. Performance of the T2Bacteria Panel in clinical blood samples according to the true-infection criterion used as the gold standard assessed by detection channel

	T2Bacteria results by detection channel						
	Ab	Eci	Efm	Kp	Pa	Sa	Total
Matched positives, *n*	1	6	5	1	2	2	17
Matched negatives, n	135	128	134	138	135	138	808
T2Bacteria overdetections[a], n	4	5	1	0	3	0	13
T2Bacteria misses, *n*	0	1	0	1	0	0	2
Overall agreement, %	97.1	95.7	99.3	99.3	97.9	100.0	98.2
Sensitivity, %	100.0	85.7	100.0	50.0	100.0	100.0	89.5
Specificity, %	97.1	96.2	99.3	100.0	97.8	100.0	98.4
PPV, %	20.0	54.5	83.3	100.0	40.0	100.0	56.7
NPV, %	100.0	99.2	100.0	99.3	99.3	100.0	99.8

Direct BC positive-dependent methods



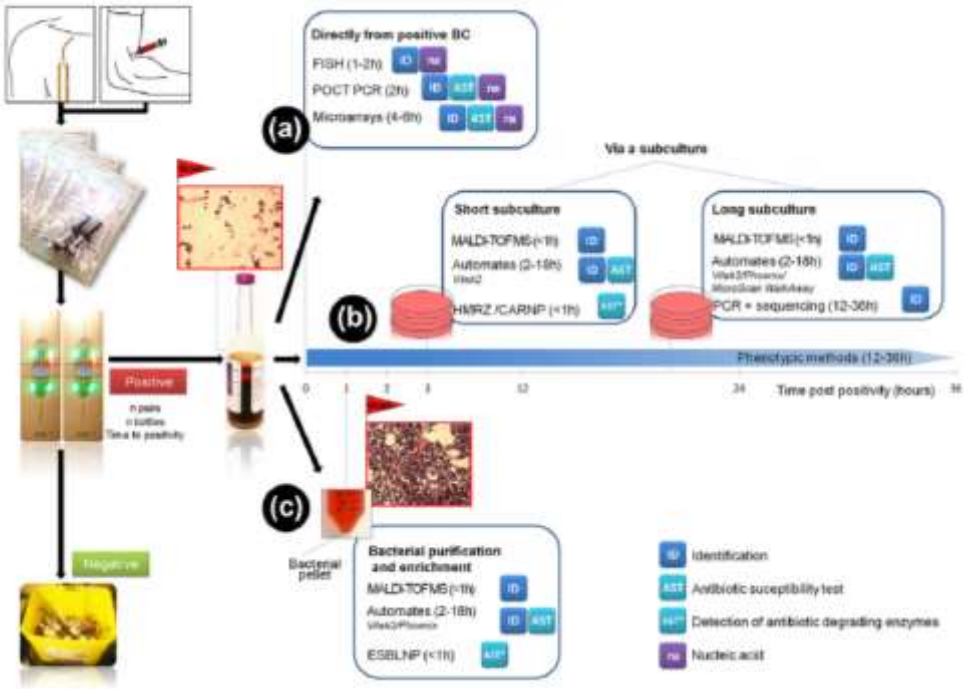
- Directly from blood culture

  (a) Directly from positive BC (t 0’); **ID by molecular methods (Microarray-based; PCR-based; FISH)**

  (c) Directly from positive BC from bacterial pellet (t 1 h); **ID by MALDI TOF**

- Subculture-dependent (solid media):

  (b) short (t 3 h); **ID by MALDI TOF**



Opota et al, Clin Microbiol Infect 2015, 21:313-22

Direct methods from positive BCs



from bacterial pellet

Table 1
Methods applied on positive BCs [8,13–20,28–30]

Kit/system (manufacturer)	Principle	TAT (h)	Panel	Resistance genes or antibiotics	Sensitivity (%)	Specificity (%)	Cost	
							Instrument	Test
Microflex LT (Bruker Daltonics) Vitek MS (bioMérieux)	MALDI-TOF	0.2–0.5	Not limited	—	75[a] 92[b]	88[a] 81[b]	€€€	€
PNA-FISH/*Quick*FISH (AdvanDx)	Fluorescent *in situ* hybridization with PNA probes	1.5–3/0.5	4 different panels (*S. aureus*/CoNS; *E. faecalis*/*Enterococcus* spp.; *E. coli*/*K. pneumoniae*/*P. aeruginosa*; *Candida* spp.)	—	88–100	97–100	€	€€
Xpert MRSA/SA Blood Culture (Cepheid)	Multiplex real-time PCR	1	*S. aureus*/CoNS	*mecA*	98–99	99–100	€€	€€
GeneOhm StaphSR (BD)	Multiplex real-time PCR	1–1.5	*S. aureus*/CoNS	*mecA*	94–99	96–98	€€	€€
Prove-it Sepsis (Mobidiag)	Broad-range PCR + microarray-based detection	3	60 bacteria, 13 fungi	*mecA*, *vanA*, *vanB*	96–99	98–100	€	€€
FilmArray Blood Culture Identification [BCID] (bioMérieux)	Multiplex real-time PCR	1	8 GP, 11 GN, 5 *Candida*	*mecA*, *vanA/B*, bla_{KPC}	80–97	91–100	€€	€€€
Verigene Gram-Positive Blood Culture [BC-GP] (Luminex)	Multiplex PCR + solid-microarray detection	2.5	11 GP	*mecA*, *vanA*, *vanB*	93–100	94–100	€€	€€€
Verigene Gram-Negative Blood Culture [BC-GN] (Luminex)	Multiplex PCR + solid-microarray detection	2	9 GN	bla_{CTX-M}, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA-48}, bla_{OXA-23}, $bla_{OXA-24/40}$, bla_{OXA-58}	89–100	93–100	€€	€€€
Sepsis Flow Chip (Master Diagnostica)	Microarray-based assay	3	7 GP, 10 GN, 1 *Candida*	*mecA*, *vanA/B*, bla_{CTX-M}, bla_{SHV}, bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{OXA-48}, bla_{OXA-23}, $bla_{OXA-24/40}$, bla_{OXA-51}, bla_{OXA-58}	93	100	€	€€€
Accelerate Pheno System (Accelerate Diagnostics)	FISH (ID) Morphokinetic cellular analysis (AST)	1.5 (ID) 7 (AST)	6 GP, 8 GN, 2 *Candida*	CFT, DAP, ERY, LZD, VAN (*S. aureus*); DAP, VAN (CoNS); AMP, DAP, LZD (*Enterococcus* spp.); AMK, ATM, CAZ, CIP, CRO, ERT, FEP, GE, MEM, SAM, TOB, TZP (*E. coli*; *Klebsiella* spp.; *Proteus* spp.), AMK, ATM, CAZ, CIP, CRO, ERT, FEP, GE, MEM, SAM, TOB, TZP (*Enterobacter* spp., *Citrobacter* spp., *Serratia* spp.), AMK, CAZ, CIP, FEP, GE, MEM, TOB, TZP (*P. aeruginosa*); AMK, TZP (A. baumannii)	89–96 (ID) 91–95 (AST)[c]	99–100 (ID)	€€	€€€

Rello et al, Clin Microbiol Infect 2018. in press.

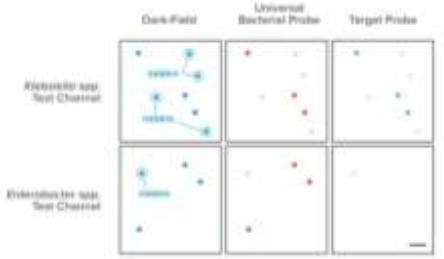
Main advantages

- rapid TAT for ID (1 to 3 h)
- High sensitivity and specificity
- **High performance in polymicrobial BSI (no MALDI TOF)**
- Information on specific resistance genes

Main disadvantages

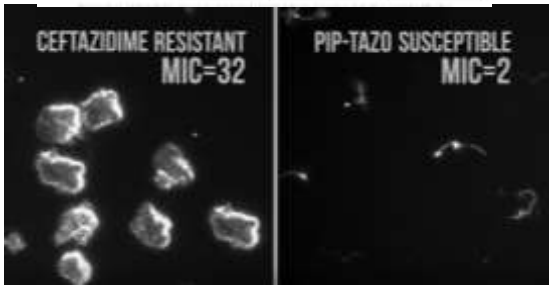
- Need to wait for BC positivity (time of BC incubation)
- relatively high cost
- Are “add-on” tests, conventional testing are required

Rapid ID and AST from positive blood cultures – Accelerate Pheno System



PRINCIPLE:

- **ID**: **Fluorescence *in situ* hybridization (FISH)** technology.
  - **1.5 h ID**.



- **AST**: **Morphokinetic cellular analysis**. Report MIC results
  - **7 h AST**

8

12

6

8

2

Organism[a]	AMP	CFT	DAP	ERY	LZD	VAN	MRS	MLSb	AMK	SAM	ATM	CAZ	FEP	CRO	CIP	ETP	GEN	MEM	TZP	TOB
AST for Gram-positive bacteria																				
CNS			X			X	X	X												
EFS	X		X		X	X														
EFM	X		X		X	X														
SAU		X	X	X	X	X	X													
SLU						X	X	X												
STR																				
AST for Gram-negative bacteria																				
ABA									X										X	
CIT									X		X	X	X	X	X	X	X	X	X	X
ENT									X		X	X	X	X	X	X	X	X	X	X
ECO									X	X	X	X	X	X	X	X	X	X	X	X
KLE									X	X	X	X	X	X	X	X	X	X	X	X
PRO									X	X	X	X	X	X	X	X	X	X	X	X
PAE									X			X	X		X		X	X	X	X
SMA									X		X	X	X	X	X	X	X	X	X	X
No antifungal susceptibility testing																				
CAL																				
CGL																				

[a]CNS, coagulase-negative Staphylococcus spp.; EFS, Enterococcus faecalis; EFM, Enterococcus faecium; SAU, Staphylococcus aureus; SLU, Staphylococcus lugdunensis; STR, Streptococcus spp.; ABA, Acinetobacter baumannii; CIT, Citrobacter spp.; ENT, Enterobacter spp.; ECO, Escherichia coli; KLE, Klebsiella spp.; PRO, Proteus spp.; PAE, Pseudomonas aeruginosa; SMA, Serratia marcescens; CAL, Candida albicans; CGL, Candida glabrata.

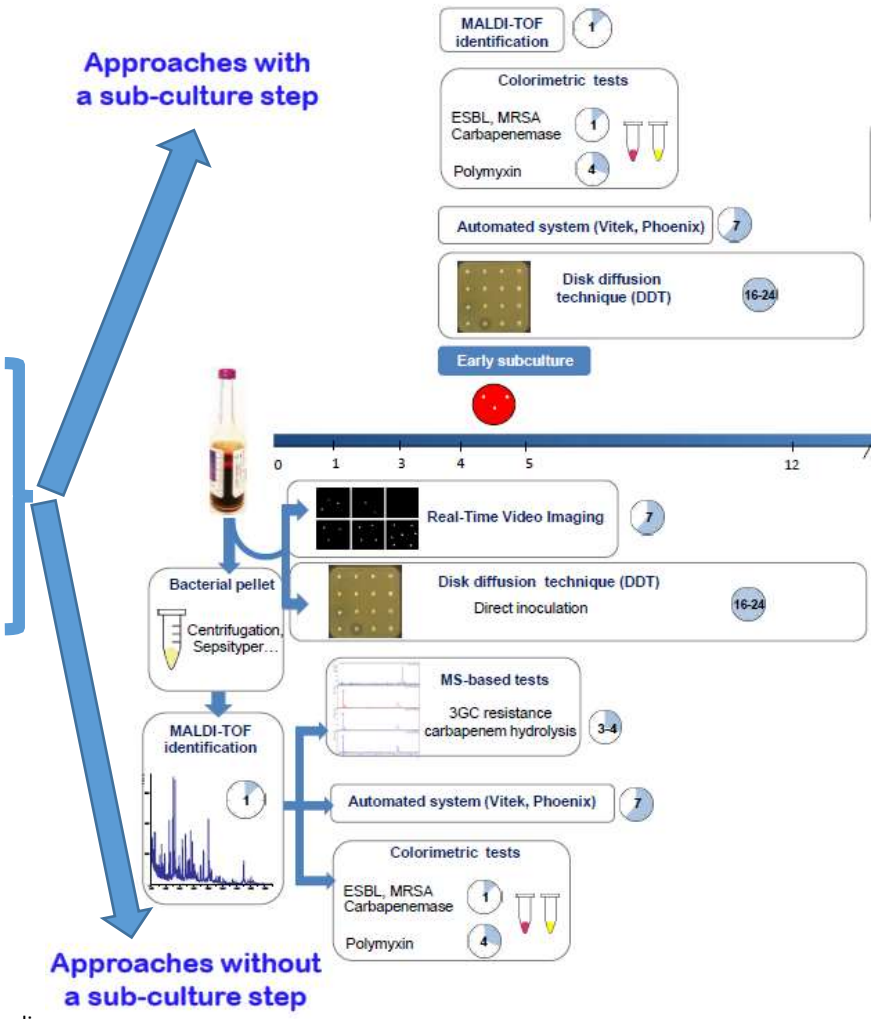
[b]AMP, ampicillin; CFT, ceftaroline; DAP, daptomycin; ERY, erythromycin; LZD, linezolid; VAN, vancomycin; MRS, methicillin-resistant Staphylococcus; MLSb, macrolide-lincosamide-streptogramin B resistance; AMK, amikacin; SAM, ampicillin-sulbactam; ATM, aztreonam; CAZ, ceftazidime; FEP, cefepime; CRO, ceftriaxone; CIP, ciprofloxacin; ETP, ertapenem; GEN, gentamicin; MEM, meropenem; TZP, piperacillin-tazobactam; TOB, tobramycin. "X" represents that AST is performed for the given antimicrobial agent when the given organism is identified.

BC-dependent Rapid Susceptibility Testing

Molecular and phenotypic methods implemented in microbiology laboratories to reduce time to result in reporting AST from blood cultures (BC)

- Molecular rapid susceptibility tests, limited to the detection of a few specific resistance genes (e.g., *mecA* in *Staphylococcus aureus Van A/B* per *Enterococcus,* or *bla*KPC in *Enterobacteriaceae*).
- Phenotypic rapid susceptibility tests (such as disk diffusion and microdilution, or colorimetric tests), directly from positive BC or from early subcultures

- Time to results: 24 h earlier then conventional AST!



Barnini et al. 2016; Bruins et al. 2004; de Cueto et al. 2004; Funke and Funke-Kissling 2004; Gherardi et al. 2012; Idelevich et al, 2016; Lupetti et al. 2010a, 2013; Machen et al. 2014; Mitchell and Alby 2017

Dubourg et al, Clin Microbiol Infect 2018, doi: 10.1016/j.cmi.2018.03.031

Positive BC-dependent methods and clinical impact

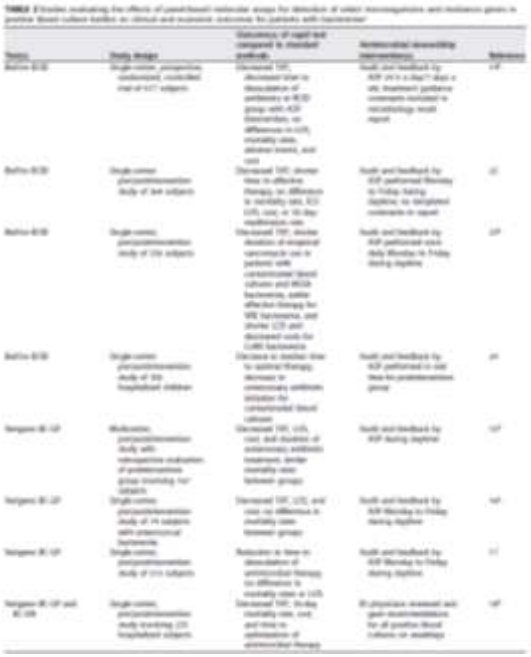
3 FDA-approved molecular methods

- FilmArray BCID
- Verigene Gram-positive blood culture
- Verigene Gram negative blood culture

Ramanan P et al, Clin Microbiol Rev 2018 31:e00024-17.

Test(s)	Study design	Outcome(s) of rapid test compared to standard methods	Antimicrobial stewardship intervention(s)	Reference
Verigene BC-GP	Single-center, retrospective, pre/postintervention study involving 147 subjects with MSSA or VRE bacteremia	Decreased TAT and time to optimal antibiotics for MSSA and VRE bacteremia, no differences in LOS or mortality rates	Microbiology report included treatment guidance comments	19
Verigene BC-GN	Single-center, retrospective, pre/postintervention study involving 195 subjects	Decreased TAT, ICU LOS, 30-day mortality rate, mortality rate associated with MDR pathogens, and cost	Audit and feedback by ASP daily	20[a]
Verigene BC-GN	Single-center, retrospective study with theoretical evaluation of antimicrobial therapy-related outcomes involving 132 subjects	Shorter times to effective and optimal antibiotic therapy	Theoretical ASP intervention by ID physician and pharmacist	21
Rapid blood culture diagnostics such as MALDI-TOF MS, PNA-FISH, PCR, and microarray technologies	Meta-analysis of 31 studies involving 5,920 subjects with bacteremia	Decreased mortality risk in the presence but not in the absence of ASP; lower mortality rates for infections by Gram-positive and Gram-negative organisms but not yeast; decreased time to effective therapy and LOS	Various	25

[a]Adapted from reference 8. TAT, turnaround time; ICU, intensive care unit; LOS, length of stay; ASP, antimicrobial stewardship program; MSSA, methicillin-susceptible Staphylococcus aureus; VRE, vancomycin-resistant Enterococcus species; MDR, multidrug resistant; ID, infectious disease.
[b]This study was included in the meta-analysis (25).



POSITIVE clinical and economical impact → **Decrease of:**

- **Turn around time**
- **hospital length of stay**
- **time to de-escalation and optimal therapy**
- **mortality**
- **costs**

How to use rapid tests for BSI

- To realize maximal benefits (better outcomes), RDTs should be performed in specialized clinical microbiology lab opened 24 h/7 days, coupled with diagnostic and antimicrobial stewardship programs (= real time intervention)

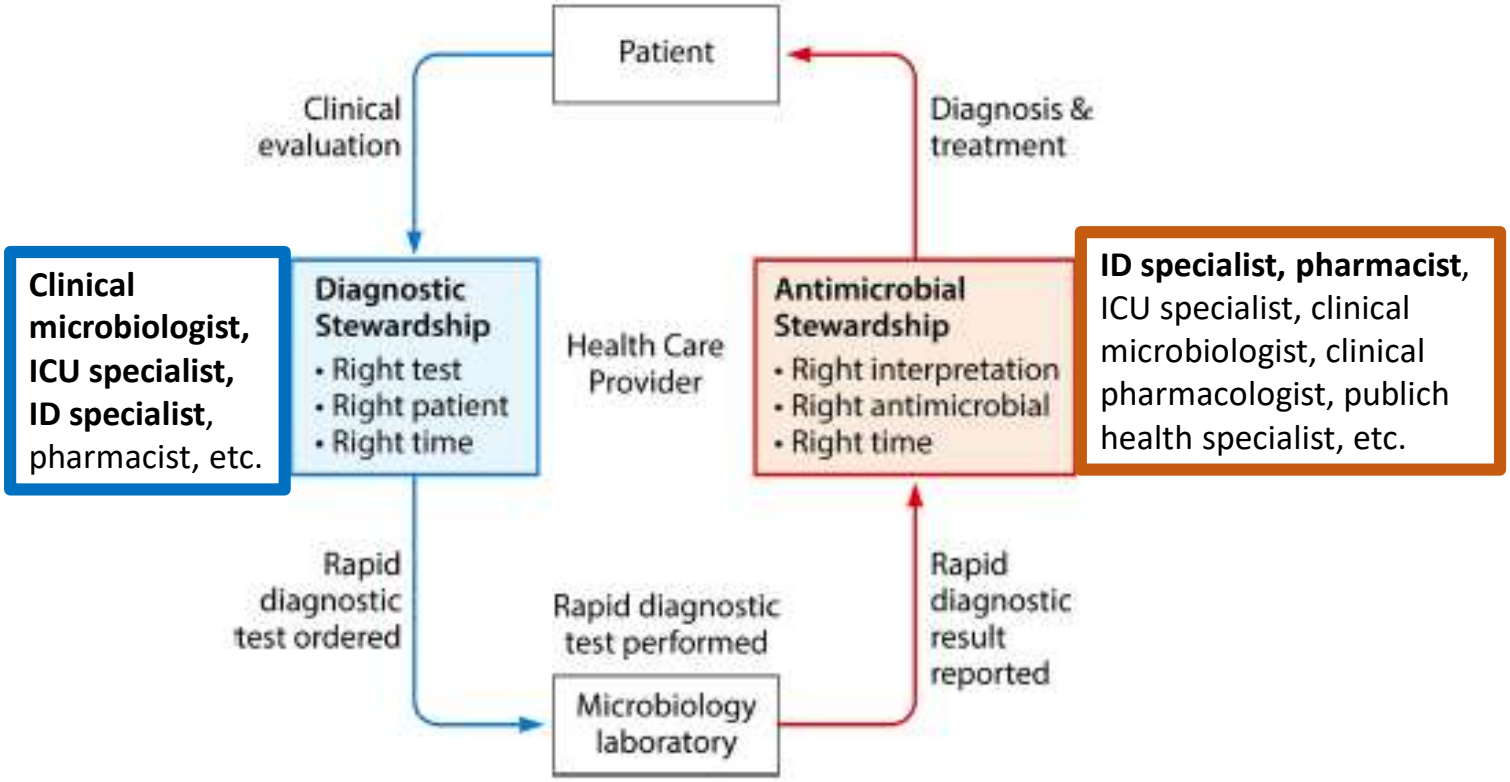
→ **RESOURCE!**

- The choice of the rapid method mainly relies on the micro lab organization, staff availability, cost-effectiveness, clinical needs.

MULTIDISCIPLINARITY

Important role of clinical microbiology laboratory:

- helping clinicians in the diagnosis and treatment of infectious diseases.
- supporting infection control specialists.



Messacar K et al. 2017. J Clin Microbiol 55:715–723

THANKS!

Introduction – microbiological data



Studio di prevalenza su infezioni correlate all'assistenza e uso di antibiotici negli ospedali per acuti

Rapporto regionale 2016

Gram-positive = 47.9%

Gram-negative = 44.7%

Yeast = 7.5%

Tabella A.14. Microrganismi associati a batteriemia

Microrganismi	n	%
Totale	*94*	
Staphylococcus aureus	11	11,7
Staphylococcus epidermidis	11	11,7
Klebsiella pneumoniae	10	10,6
Escherichia coli	9	9,6
Enterococcus faecalis	8	8,5
Pseudomonas aeruginosa	5	5,3
altri Stafilococchi coagulasi-negativi (CNS)	5	5,3
Proteus mirabilis	4	4,3
Acinetobacter baumannii	3	3,2
altri streptococchi spp.	3	3,2
Candida albicans	3	3,2
Candida parapsilosis	3	3,2
Haemophilus influenzae	2	2,1
Stenotrophomonas maltophilia	2	2,1
Enterococcus faecium	2	2,1
Staphylococcus haemolyticus	2	2,1
Enterobacter cloacae	2	2,1
Klebsiella oxytoca	2	2,1
altri gram-negativi, non enterobacteriaceae	1	1,1
Stafilococchi coagulasi-negativi, non specificati	1	1,1
Staphylococcus spp. non specificato	1	1,1
Streptococcus spp. non specificato	1	1,1
Citrobacter freundii	1	1,1
Serratia marcescens	1	1,1
Candida glabrata	1	1,1

Directly from whole blood Molecular PCR-based methods



TABLE 1. Main commercially available systems for identification of microbes directly from blood samples

System	Method	Time to result (hours)	Blood volume (mL)	Microorganism coverage	Resistance and virulence markers	Sensitivity, specificity, and correlation with conventional methods (%)	Comments	Ref.
SepsiTest Molzym, Bremen, Germany	Broad-range PCR + sequencing	6	1–10[a]	>345 bacteria (Gram positive and Gram negative) and fungi	0	21–87, 85–96, NR	Pros: can be used in other sterile samples; Cons: variable sensitivity and specificity	[43,56,58–61]
SeptiFast Roche Molecular System, Basel, Switzerland	Multiple broad-range real-time PCR	3.5–5	1.5	6 Gram positive, 8 Gram negative, 5 fungi	*mecA*[b]	43–95, 60–100, 43–83	Pros: time to result; Cons: variable sensitivity and specificity, no quantification	[35–53,74]
MagicPlex Seegene, Seoul, Korea	Multiple PCR + multiplex real-time PCR	3–5	1	21 bacteria (Gram positive and Gram negative) at species level (90 at genus level), 6 fungi	*mecA*, *vanA/B*	37–65, 77–92, 73	Pros: fast; Cons: limited number of studies, succession of reaction and device, no quantification	[55,56]
VYOO SIRS-Lab, Jena, Germany	Multiplex PCR + electrophoresis	8	5	14 Gram positive, 18 Gram negative, 7 fungi	0	NR, NR, 70	Pros: highly sensitive; Cons: limited number of studies, several manual steps	[59,63,75]
PLEX-ID, Abbott Molecular, Carlsbad, CA, USA	Multiplex broad-range PCR/ESI-MS	6	1.25–5[c]	Up to 800 (Gram positive, Gram negative, fungi)	*mecA*, bla_{KPC}, *vanA/B*	50–91[d], 98–99, 79–97	Pros: universal, detection of mixed bacterial populations, semiquantitative; Cons: no interventional studies	[11,76,77]

ESI-MS, electrospray ionization mass spectrometry.
[a]With an additional kit.
[b]With an additional test for patient with positive detection of *Staphylococcus aureus*.
[c]For the latest version.
[d]Depending on the sample volume (1.25 versus 5).

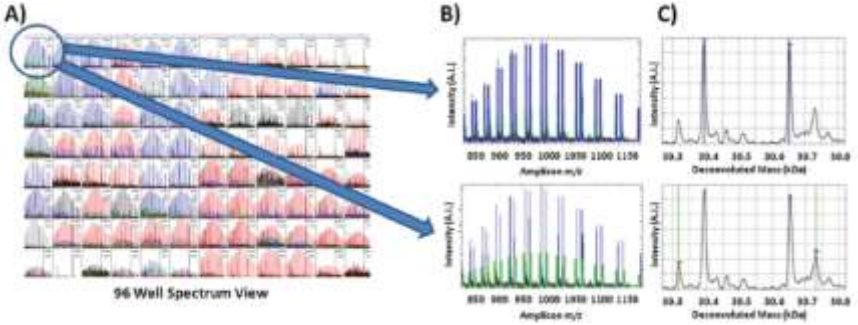
Blood Culture Assay	Pathogens Detected	Resistance Markers	Turnaround Time (After Blood Cultures Turn Positive)
Light Cycler® SeptiFast	*S. aureus*, CoNS, *Streptococcus* spp., *S. pneumoniae*, *E. faecalis*, *E. faecium*, *E. coli*, *K. pneumoniae*, *K. oxytoca*, *P. aeruginosa*, *Enterobacter cloacae/aerogenes*, *S. marcescens*, *A. baumanii*, *Proteus mirabilis*, *Stenotrophomonas maltophilia*, *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *Aspergillus fumigatus*	No	6 h

Opota et al, Clin Microbiol Infect 2015

Molecular methods directly from whole blood

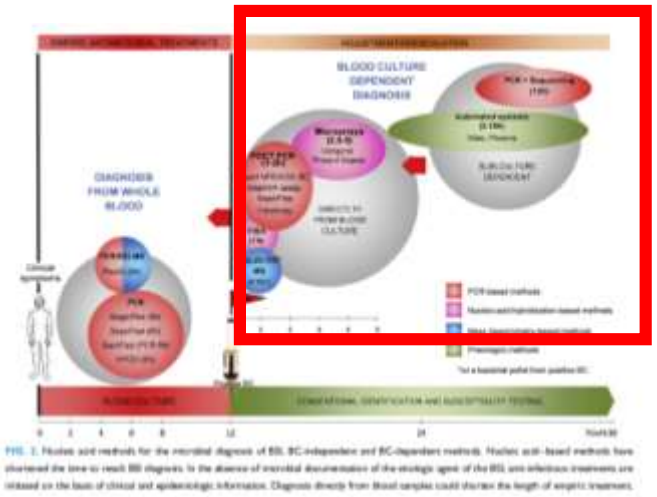
PCR combined with Electrospray ionization (ESI)-MS

- **broad-spectrum PCR combined with ESI-MS** to potentially identify any microorganism present in a clinical specimen.
- Using MS, the **mass of each PCR amplicon is determined based on nucleotide base composition** and **compared to a database**, achieving the identification of the pathogen.
- **identify more than 800 species of bacteria** with a **TAT of 6–8 h**
- Recently, **a platform based on the PCR/ESI-MS** technology, called **IRIDICA** (Ibis Biosciences, Carlsbad, CA), that includes **automated DNA extraction, PCR amplification, amplicon purification, and ESI-MS analysis**, has been developed (The IRIDICA system can also detect antibiotic resistance markers: *mecA*, *vanA*, *vanB*, and bla_{KPC})

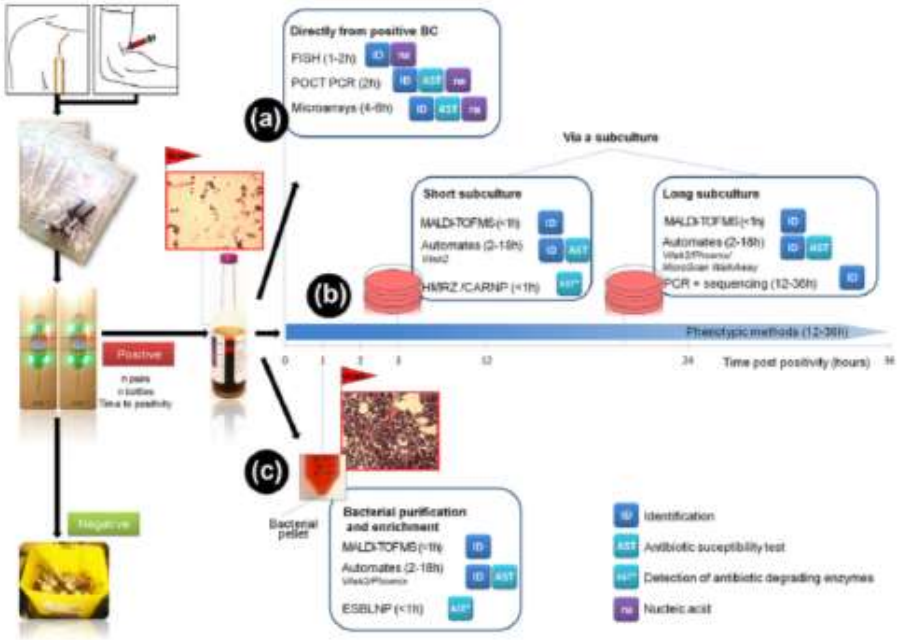


Kaleta et al. JCM 2011; Jordana-Lluch et al. PLoS One 2013, 2015; Laffler et al. JCM 2013; Bacconi et al. JCM 2014; Metzgar et al. PLoS One 2016

Culture-dependent microbiological diagnosis



- Directly from blood culture
  - **(a)** Directly from positive BC (t 0')
  - **(c)** Directly from positive BC from bacterial pellet (t 1 h)
- Subculture-dependent (solid media):
  - **(b)** short (t 3 h)
  - long subculture (t 18-24 h) (<u>conventional</u>)



Opota et al, Clin Microbiol Infect 2015, 21:313-22

Blood culture positive-dependent methods
Directly from positive BC - Molecular methods

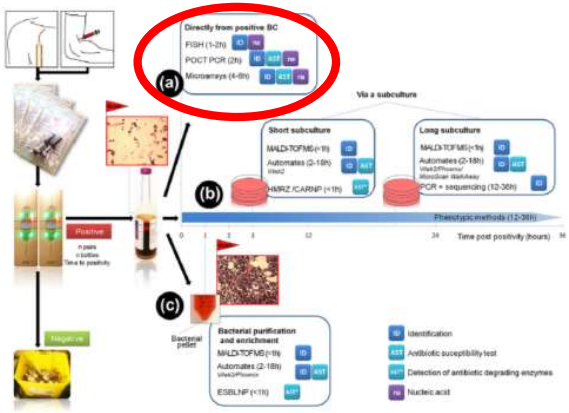
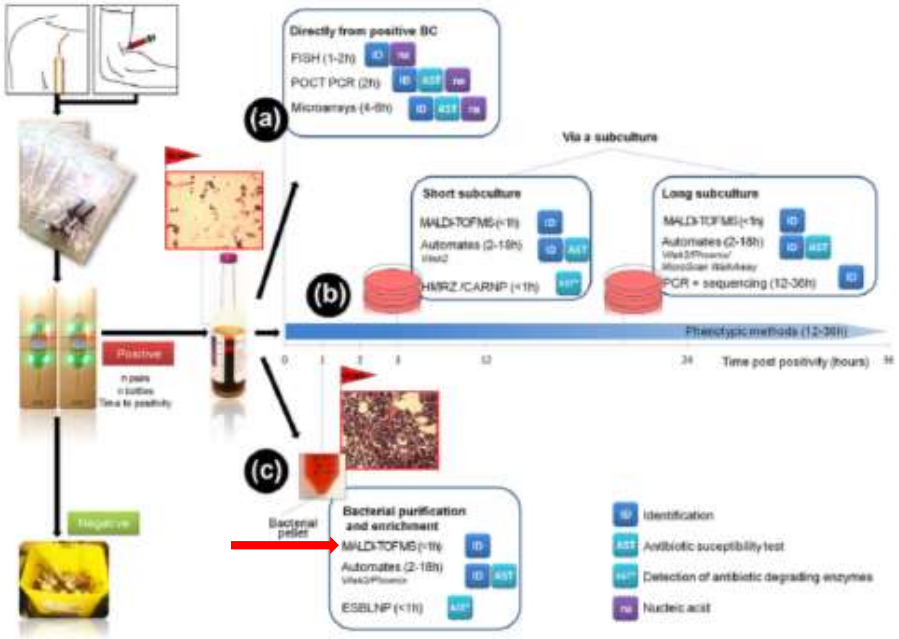


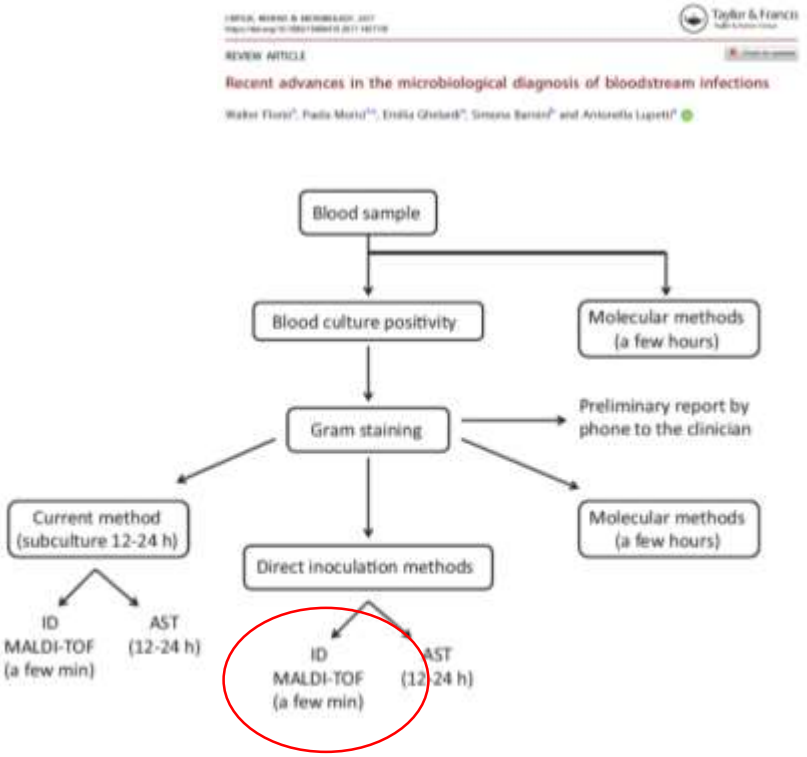
TABLE 2. Characteristics of the commercially available systems for the identification of microbes from positive blood culture

System (Manufacturer)	Methods	Time to result	Microorganism coverage	Resistance and virulence markers	Sensitivity Specificity Correlation with conventional methods (%)	Comments	References
PNA FISH and QuickFISH (AdvanDx, Woburn, MA, USA)	FISH	<1–3 hours	4 Gram positive 4 Gram negative 5 Fungi	0	97–100 90–100 96–99	+: rapid, sensitive and specific –: dependent on the choice of the probes to be tested, no resistance marker	[38,41,42,75–80]
AccuProbe (Gen-Probe, San Diego, CA, USA)	FISH	<1 hour	*Staphylococcus aureus* *Enterococcus* spp. *Streptococcus pneumoniae* *Streptococcus* group A *Streptococcus* group B	0	80.8–100 98.7–100 nr	+: high specificity –: variable sensitivity, no resistance marker, limited number of publications	[44,45]
Verigene (Nanosphere, Northbrook, IL, USA)	Microarray	2.5 hours	12 Gram positive 9 Gram negative	*mecA*, *vanA/B*, KPC, NDM, CTX-M,VIM, IMP,OXA12	81–100 98–100 nr	+: detection of resistance markers, good specificity –: variable sensitivity, narrow range of pathogens detected	[47,81–89]
Prove-it Sepsis (Mobidiag, Esbo, Finland)	Microarray	3.5 hours	60 bacteria 13 fungi	*mecA*	95% 99% nr	+: sensitive and specific –: limited number of publications	[49]
FilmArray (Idaho Technology, Salt Lake City, UT, USA)	Multiplex PCR	1 hour	8 Gram positive 11 Gram negative 5 Fungi	*mecA*, *vanA/B*, KPC	97–95 91–98 nr	+: rapid, sensitive and specific –: narrow range of pathogens detected	[56,90–92]
Xpert MRSA/SA BC (Cepheid, Sunnyvale, CA, USA)	Real-time PCR	1 hour	*S. aureus*	*mecA*	100 99–100 nr	+: rapid, sensitive and specific –: expensive	[52,53,93–95]
StaphSR assay (BD GeneOhm, San Diego, CA, USA)	Multiplex PCR	1–2 hours	*S. aureus*	*mecA*	96–100 95–98 nr	+: rapid, sensitive and specific –: expensive	[96,97]
StaphPlex (Genaco Biomedical Products, Huntsville, AL, USA)	Multiplex PCR + Microarray	5 hours	*S. aureus*	*mecA* (+ PVL)	100 95–100 92	–: limited number of publications	[98]

Opota et al, Clin Microbiol Infect 2015

Directly from positive BC from bacterial pellet ID by MALDI TOF





Different sample preparation protocols

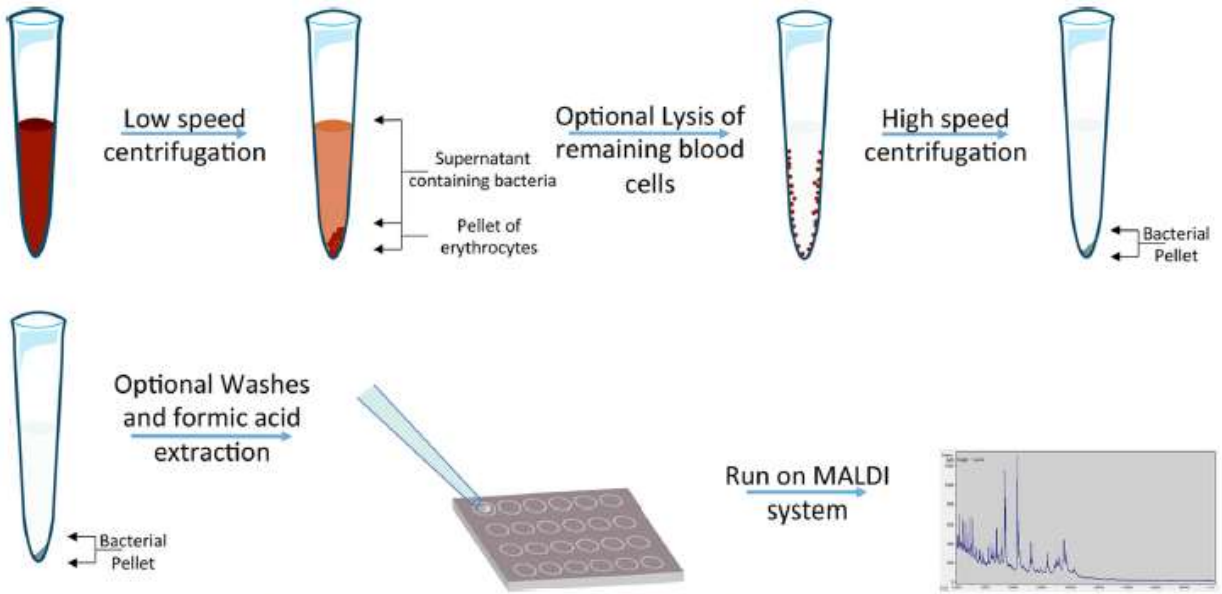


FIG 1 Procedures between studies to perform identification directly from positive blood cultures using MALDI-TOF MS can differ greatly but usually follow a similar workflow. A sample of blood culture is drawn from the bottle and is centrifuged to pellet the erythrocytes. In some studies, after the supernatant is collected, an optional lysis of remaining blood culture cells is performed to further clean the sample to produce less background. High-speed centrifugation is performed to pellet the remaining bacteria. This pellet can be washed and centrifuged a few times to improve the purification of bacterial pellets. Once purified, either an extraction can be performed or the bacteria can be added to a target plate and tested on a MALDI system for identification.

to separate bacterial and host proteins through cell lysis and/or differential centrifugation steps (Sepsityper lysis, lysis filtration, centrifugation at low and then at high speed, etc)

25-30 min of hands-on time

Faron ML, JCM 2017, 55:3328 –3338

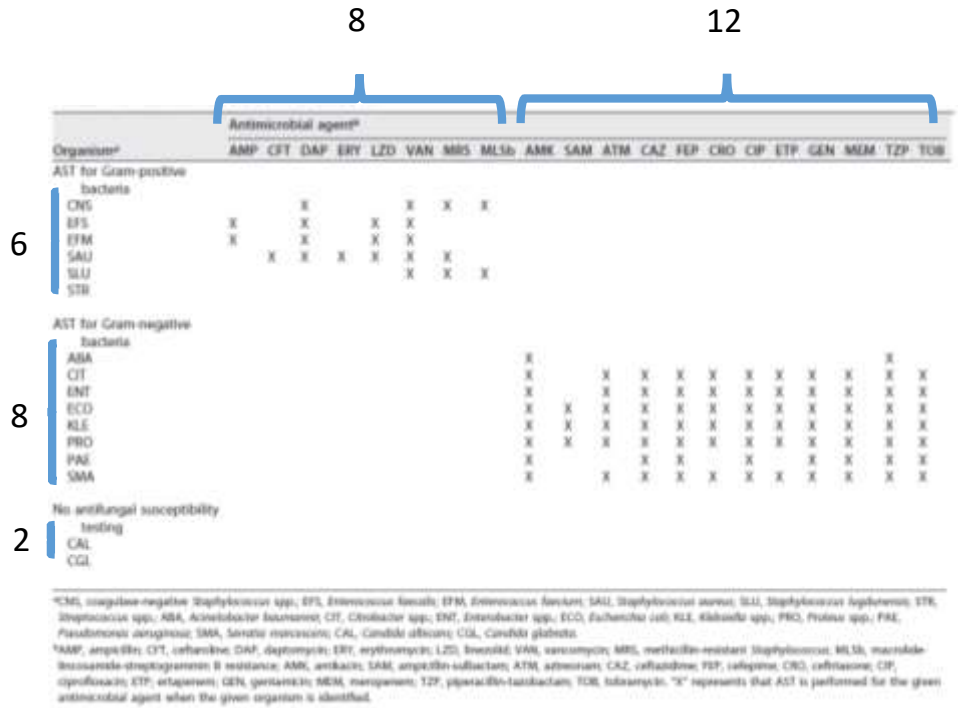
Directly from positive BC from bacterial pellet - ID by MALDI TOF

Disadvantages:

- Performance better for gram negative than gram positive (58%-86% for Gram-positive, 84%-98% for Gram-negative rods)
- Polymicrobial infections (generally only one species is identified)
- For organizational reasons due to additional processing time

French K et al, PLoS One 2016

Rapid ID and AST from positive blood cultures by Accelerate Pheno System



*CNS, coagulase-negative Staphylococcus spp.; EFS, Enterococcus faecalis; EFM, Enterococcus faecium; SAU, Staphylococcus aureus; SLU, Staphylococcus lugdunensis; STR, Streptococcus spp.; ABA, Acinetobacter baumannii; CIT, Citrobacter spp.; ENT, Enterobacter spp.; ECO, Escherichia coli; KLE, Klebsiella spp.; PRO, Proteus spp.; PAE, Pseudomonas aeruginosa; SMA, Serratia marcescens; CAL, Candida albicans; CGL, Candida glabrata.
†AMP, ampicillin; CFT, ceftaroline; DAP, daptomycin; ERY, erythromycin; LZD, linezolid; VAN, vancomycin; MRS, methicillin-resistant Staphylococcus; MLSb, macrolide-lincosamide-streptogramin B resistance; AMK, amikacin; SAM, ampicillin-sulbactam; ATM, aztreonam; CAZ, ceftazidime; FEP, cefepime; CRO, ceftriaxone; CIP, ciprofloxacin; ETP, ertapenem; GEN, gentamicin; MEM, meropenem; TZP, piperacillin-tazobactam; TOB, tobramycin. "X" represents that AST is performed for the given antimicrobial agent when the given organism is identified.

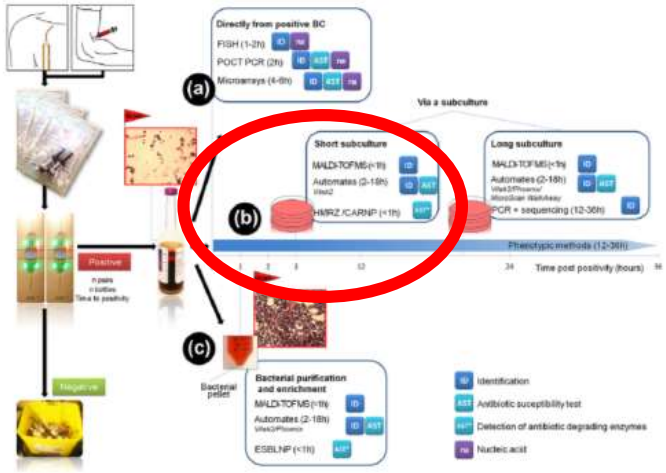
Charnot-Katsikas et al, 2018, J Clin Microbiol 56:e01166-17; Lutgring et al, 2018, J Clin Microbiol 56:e01672-17; Marschal et al, 2017, J Clin Microbiol 55:2116 –2126; Panchioli et al, 2018, Clin Microbiol 56:e01329-17

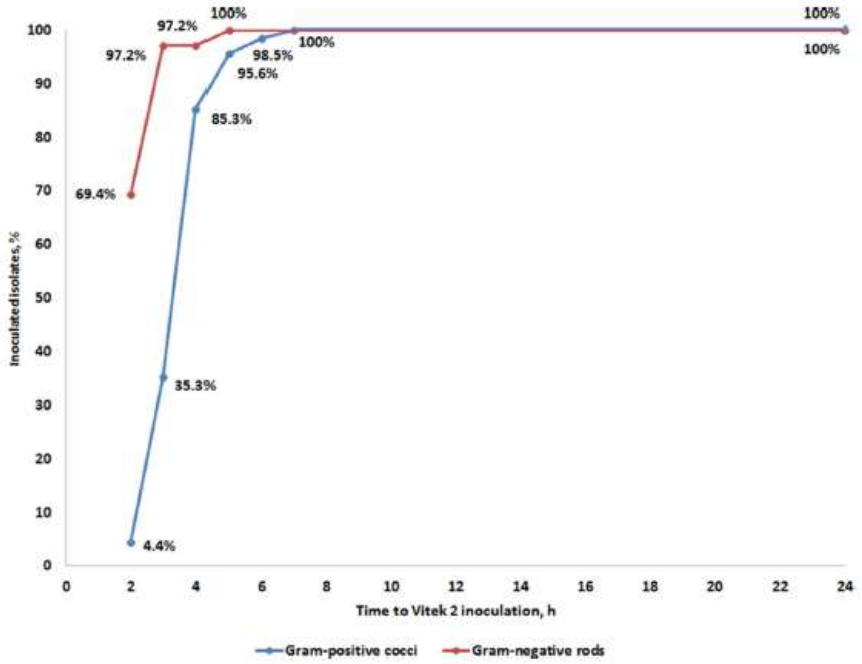
Some disadvantages

- High costs
- Identification achieved to genus-level for some organisms (such as *Streptococcus* and *Staphylococcus* spp), or no identification due to uncovered pathogens or invalid runs
- certain organism-antimicrobial combinations did not perform well (such as ceftazidime for *P. aeruginosa*, or TZP for *Klebsiella*)
- the system can process only one sample at a time per module, although it can currently accommodate up to 4 modules
- The system currently does not include ID and AST for Gram-positive rods, anaerobes, or AST for yeast

Subculture dependent method
MALDI TOF after short-term incubation (about 3 hrs)

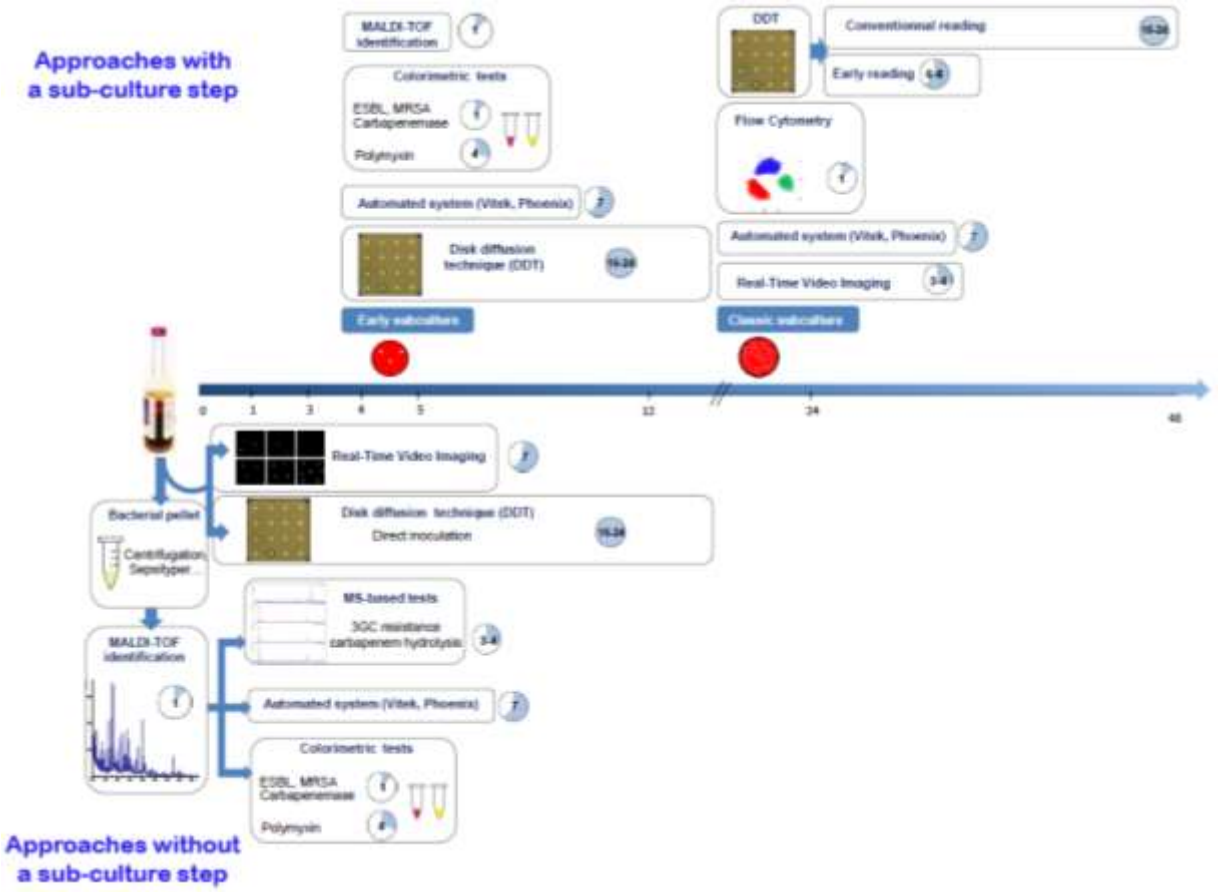
- Low cost, simple and rapid (2/4 h for Gram-neg, 4/5 h for Gram-pos) method
- Used for direct ID by MALDI-TOF with high performance (≥ 80% for Gram positive and ≥ 95% for Gram negative)
- Easy implementation and integration into diagnostic routine
- Limitations:
  - Greater benefits for lab opened 24h/day
  - Polimicrobial cultures
  - Specific species or group of organisms (i.e., *Streptococci*, yeast, anaerobes)





Delport et al, *PLoS ONE* 2016; Dubourg et al, *Clin Microbiol Infect* 2018; Ha et al, *Ann Lab Med* 2018; Idelevich et al, *Clin Microbiol Infect* 2014; Idelevich et al, *J Cl Microbiol* 2014; Mauri et al, *BMC Infectious Diseases* 2017; Mitchell et al, *Eur J C Microbiol Infect Dis* 2017

- Phenotypic methods implemented in microbiology laboratories to reduce time to result in reporting AST from blood cultures (BC)



Dubourg et al, Clin Microbiol Infect 2018, doi: 10.1016/j.cmi.2018.03.031

….and molecular rapid AST methods (< 1 h) from pellets and/or short-time subculture

few examples:



Real-time PCR (Xpert Carba-R)

Smith et al, JMM 2016

For Gram-negative



Real-time PCR (Xpert MRSA BC)

Clerc et al, CMI 2014

For Gram-positive (MRSA)

New rapid ID and AST methods **under development – from whool blood**


frontiers in GENETICS 2015

The evolution of nanopore sequencing

*Yue Wang, Qiuping Yang and Zhimin Wang**

Nanopore NGS


SCIENTIFIC REPORTS

2017

OPEN Massively parallel digital high resolution melt for rapid and absolutely quantitative sequence profiling

Digital PCR and high resolution melt analysis


PLOS | MEDICINE

2015

RESEARCH ARTICLE

A Molecular Host Response Assay to Discriminate Between Sepsis and Infection-Negative Systemic Inflammation in Critically Ill Patients: Discovery and Validation in Independent Cohorts

Host response-targeted RT-qPCR Assay

Quantifies relative expression levels of 4 RNA biomarkers (CEACAM4, LAMP1, PLA2G7, PLAC8)


J Antimicrob Chemother 2018; 73 Suppl 4: ii20–ii26

doi:10.1093/jac/dkx049

Antimicrobial Chemotherapy

T2Bacteria magnetic resonance assay for the rapid detection of ESKAPEc pathogens directly in whole blood

Giulia De Angelis[1†], Brunella Posteraro[1†], Elena De Carolis[1], Giulia Menchinelli[1], Francesco Franceschi[2], Maria Tumbarello[3], Gennaro De Pascale[3], Teresa Spanu[1] and Maurizio Sanguinetti[1*]

T2 Magnetic Resonance Assay


Published in final edited form as:

Lab Chip. 2015 May 21; 15(10): 2297–2307. doi:10.1039/c5lc00211c.

Direct detection and drug-resistance profiling of bacteremias using inertial microfluidics

Han Wei Hou[a,1,2], Roby P. Bhattacharyya[b,c,1], Deborah T. Hung[b,d,e,*], and Jongyoon Han[a,f,*]

[a]Department of Electrical Engineering & Computer Science, Massachusetts Institute of

Microfluidic chips


nature COMMUNICATIONS

ARTICLE

Received 23 Jan 2014 | Accepted 30 Sep 2014 | Published 13 Nov 2014 OPEN

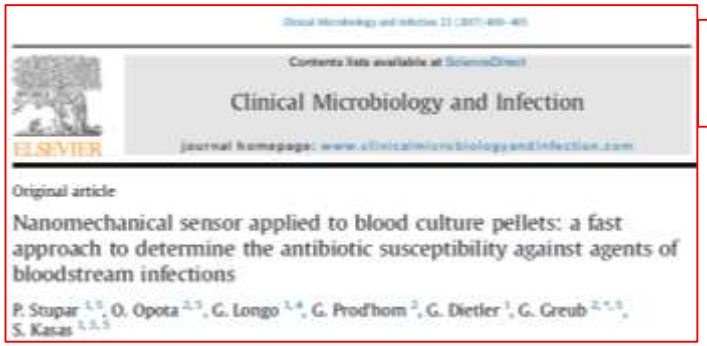
Rapid detection of single bacteria in unprocessed blood using Integrated Comprehensive Droplet Digital Detection

Dong-Ku Kang[1,2,3,4,5,*], M. Monsur Ali[1,2,3,4,5,*], Kaixiang Zhang[1,2,3,4,5,6], Susan S. Huang[7], Ellena Peterson[8], Michelle A. Digman[5,9,10], Enrico Gratton[5,9] & Weian Zhao[1,2,3,4,5]

Amplification-free technology

Droplet Digital Detection (IC 3D)

New rapid ID and AST methods **under development** – from positive blood cultures

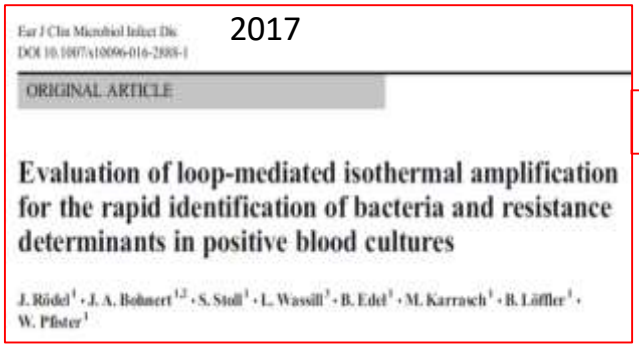

Clinical Microbiology and Infection

Original article

Nanomechanical sensor applied to blood culture pellets: a fast approach to determine the antibiotic susceptibility against agents of bloodstream infections

P. Stupar, O. Opota, G. Longo, G. Prod'hom, G. Dietler, G. Greub, S. Kasas

Nanomechanical sensors

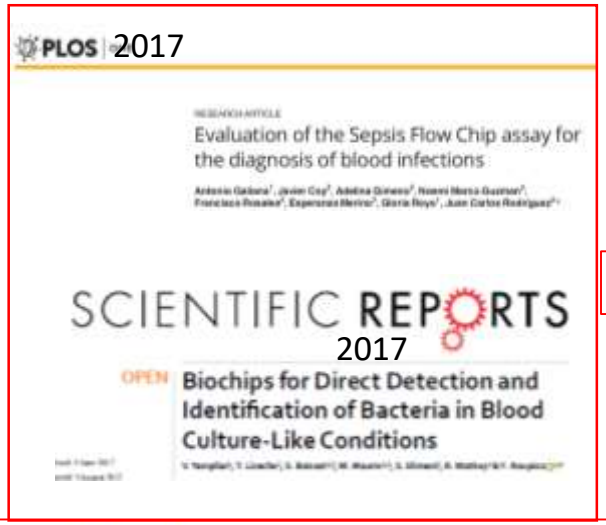

2017

ORIGINAL ARTICLE

Evaluation of loop-mediated isothermal amplification for the rapid identification of bacteria and resistance determinants in positive blood cultures

J. Rödel · J. A. Bohnert · S. Stoll · L. Wassill · B. Edel · M. Karrasch · B. Löffler · W. Pfister

LAMP


PLOS 2017

Evaluation of the Sepsis Flow Chip assay for the diagnosis of blood infections

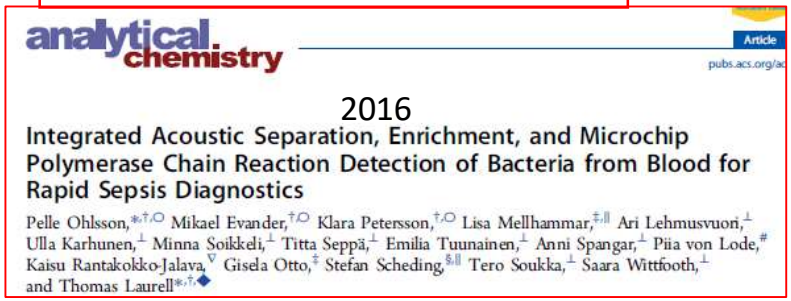
SCIENTIFIC REPORTS

2017

OPEN

Biochips for Direct Detection and Identification of Bacteria in Blood Culture-Like Conditions

Biochips


analytical chemistry

Article

pubs.acs.org/ac

2016

Integrated Acoustic Separation, Enrichment, and Microchip Polymerase Chain Reaction Detection of Bacteria from Blood for Rapid Sepsis Diagnostics

Pelle Ohlsson, Mikael Evander, Klara Petersson, Lisa Mellhammar, Ari Lehmusvuori, Ulla Karhunen, Minna Soikkeli, Titta Seppä, Emilia Tuunainen, Anni Spangar, Piia von Lode, Kaisu Rantakokko-Jalava, Gisela Otto, Stefan Scheding, Tero Soukka, Saara Wittfooth, and Thomas Laurell

CONCLUSIONS (1)

- MALDI TOF and mRDT can allow a rapid and accurate ID identification of microorganism (within 2-5 hrs).
- AST can be also reached in few hrs (anyway 24 h earlier then conventional tests).
- Always coupled with the conventional standard BC procedures for ID and AST.
- Interpretation of individual results should always be done in the light of a clinical evaluation.
- RDTs should be performed from high-specialized and trained staff, 24 h a day/7 days a week, coupled with stewardship programs (precise information flow system)

CONCLUSIONS (2)

MULTIDISCIPLINARITY

Important role of a clinical microbiology laboratory in:

- assisting clinicians in the diagnosis and treatment of infectious diseases.
- supporting infection control specialists.

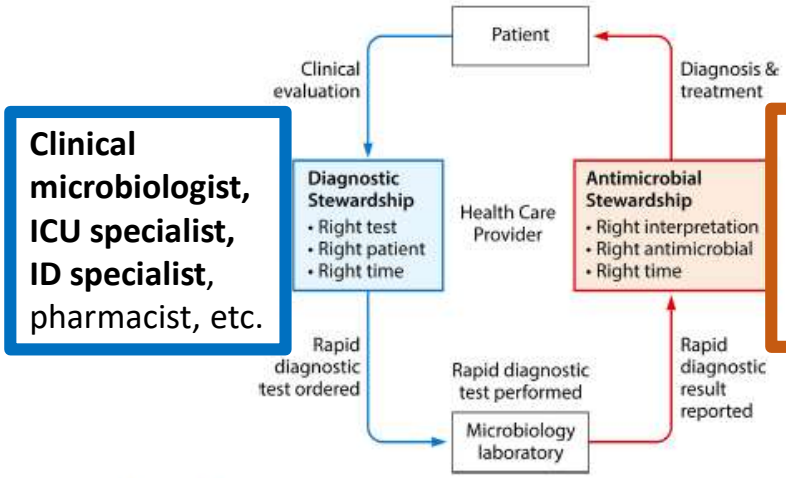
TABLE 1 Key diagnostic stewardship considerations for implementation of rapid infectious disease diagnostics

Goal	Key question	Key considerations and potential strategies
Right test	Is the test appropriate for the clinical setting?	Sensitivity and specificity Predictive values Testing volumes Diagnostic yield Laboratory feasibility Cost Clinical impact
Right patient	Will the clinical care of the patient be affected by the test result?	Laboratory test utilization committee Automatic laboratory reflex CPOE decision support Appropriate use criteria Indication selection Prior authorization Benchmarking Specimen rejection
Right time	Will the result be available in time to optimally affect care?	Time to specimen receipt Centralized vs point-of-care testing On-demand vs batched testing Specimen preparation time Run time Result reporting time

TABLE 2 Key antimicrobial stewardship considerations for implementation of rapid infectious disease diagnostics

Goal	Key question	Key considerations and potential strategies*
Right interpretation	Will the clinician understand the test result?	Result report language Selective reporting of relevant results AS prospective audit and feedback AS real-time decision support
Right antimicrobial	Will the clinician appropriately modify antimicrobials based on the test result?	Clinical practice guidelines EMR-based decision support with result reporting AS prospective audit and feedback AS real-time decision support
Right time	Will the clinician act upon the test result promptly?	EMR reporting Results called with readback reporting AS prospective audit and feedback AS real-time decision support

*AS, antimicrobial stewardship.



Clinical microbiologist, ICU specialist, ID specialist, pharmacist, etc.

ID specialist, pharmacist, ICU specialist, clinical microbiologist, clinical pharmacologist, publich health specialist, etc.

FIG 1 Roles of diagnostic and antimicrobial stewardship in the implementation of rapid molecular infectious disease diagnostics in the clinical setting.

Messacar K et al. 2017. J Clin Microbiol 55:715–723