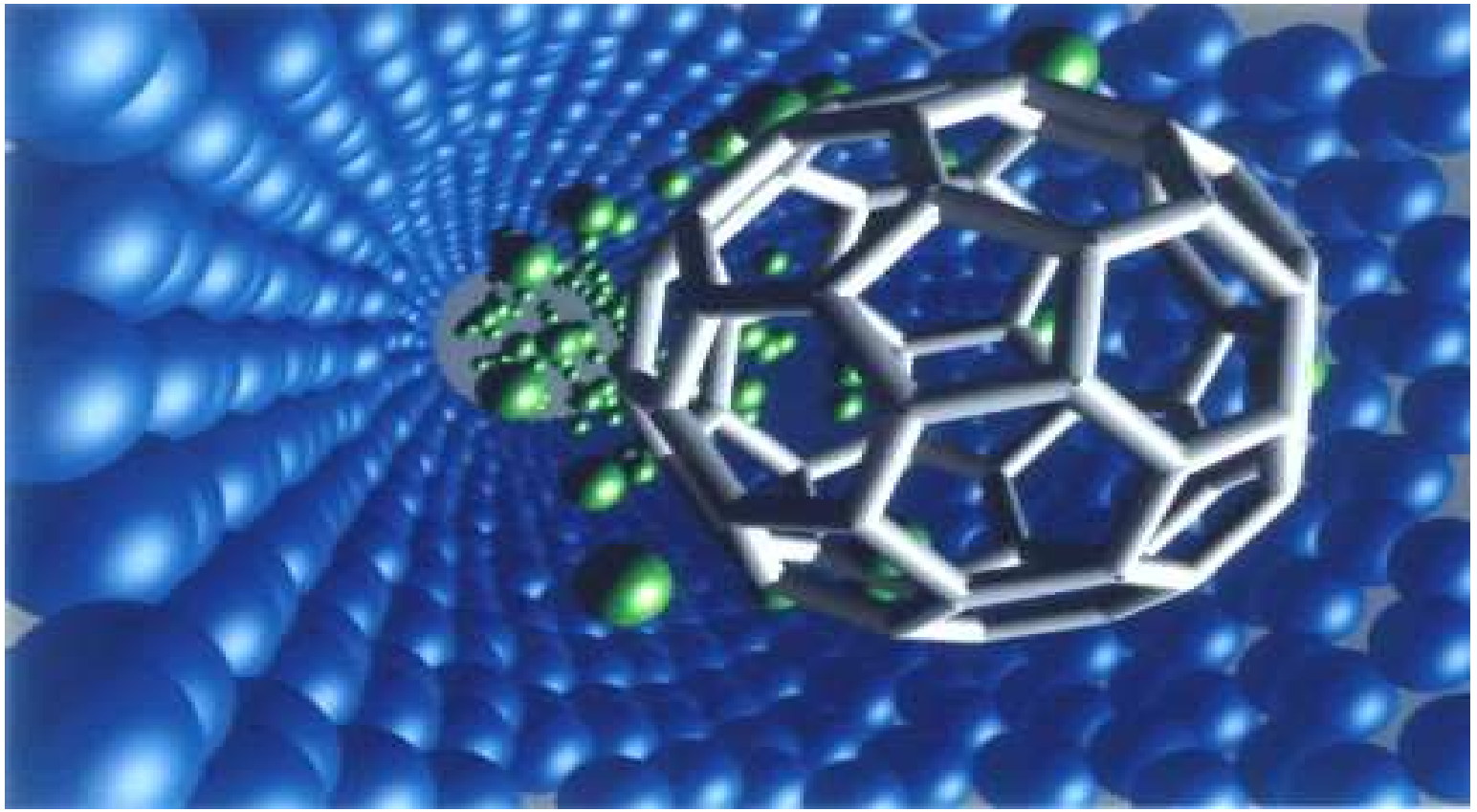
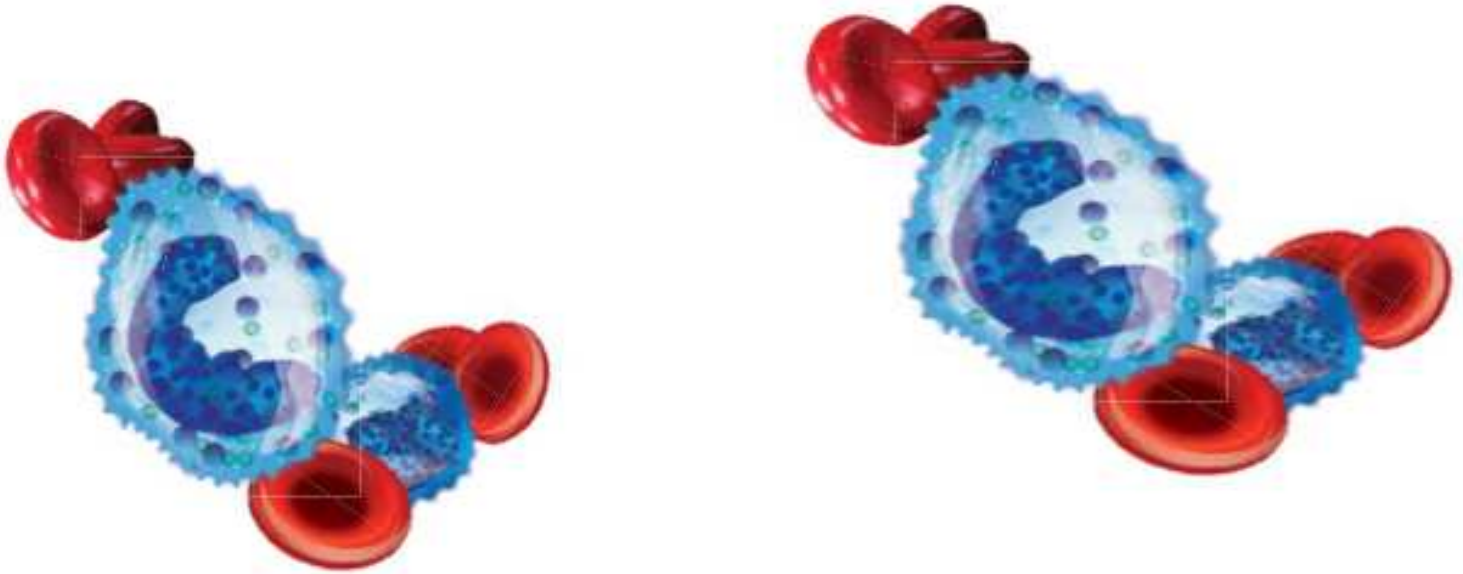
Diagnostica rapida delle infezioni del paziente critico

mediante sistemi innovativi in Multiplex PCR e Microarray



Patrizia Pecile
Laboratorio Microbiologia e Virologia
AOUCareggi, Firenze



“Poiché la letalità attribuibile alla sepsi è alta, la

pronta ed accurata

evidenza dei batteri e miceti nel sangue (o nella sede primaria dell’infezione) è importante per la cura del paziente”

CLSI (NCCLS) Gennaio 2007

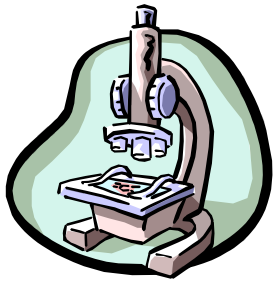
Diagnosi di sepsi

Gold standard → emocoltura → risultato ottimale

- **precedente terapia antimicrobica**
- **momento del prelievo**
- **intervallo e numero dei campioni**
- **tecniche sterili**
- **volume del prelievo**
- **rapporto inoculo/brodo**
- **caratteristiche del mezzo di coltura**
- **tempo ed atmosfera di incubazione**
- **agitazione**
- **evidenziazione dello sviluppo**
- **interpretazione del risultato**

30% falsi negativi

The workflow of blood cultures



Sampling

Pos. BC

Gram stain results

Isolated colonies

Preliminary AST results

Final results (ID & AST)

0 10 18 30 36 48 54 60

Time (hours)

- Tempo identificazione
- Tempo prove di sensibilità

- Terapia empirica
- Insorgenza resistenze
- > mortalità
- > costi

Physicians embraced an empirical approach to the management of many infectious diseases, favouring overuse of antibiotics.

Bissonnette L and Bergeron MG. CMI 2010.

Far prima.....

Raggiungere l'obbiettivo è possibile, mediante nuove tecnologie, automazione e test rapidi in grado di migliorare il flusso di lavoro provvedendo nello stesso tempo a mantenere l'alta qualità dei risultati.

Camporese A. Inf Med 2004;12:118-125

TECNICHE MOLECOLARI

da materiale

Test molecolari diretti

1

SeptiFast Test®
Roche

2

Magicplex Sepsis Real-time Test Seegene

5 – 6 ore

Diagnosi molecolare di sepsi con SeptiFast ® Roche Diagnostics

permette la diagnosi eziologica di sepsi in circa 5 ore

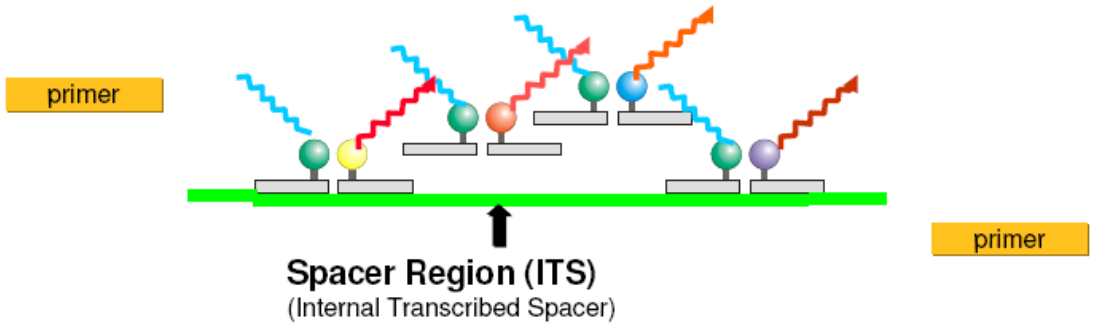
- **Il sistema è una PCR *real-time* di tipo multiplex in grado di rilevare ed identificare a livello di specie un pannello di 25 patogeni batterici e fungini, complessivamente responsabili di più del 90% dei casi di sepsi microbiologicamente confermati**

utilizza primer specifici per la regione Internal Transcribed Spacer ITS e *coppie di sonde FRET* marcate con 4 fluorofori differenti per la rilevazione contemporanea di più sequenze.
Attraverso l'analisi di melting è possibile caratterizzare il prodotto di amplificazione per l'identificazione di specie, sfruttando la specificità delle temperature di melting per sequenze diverse

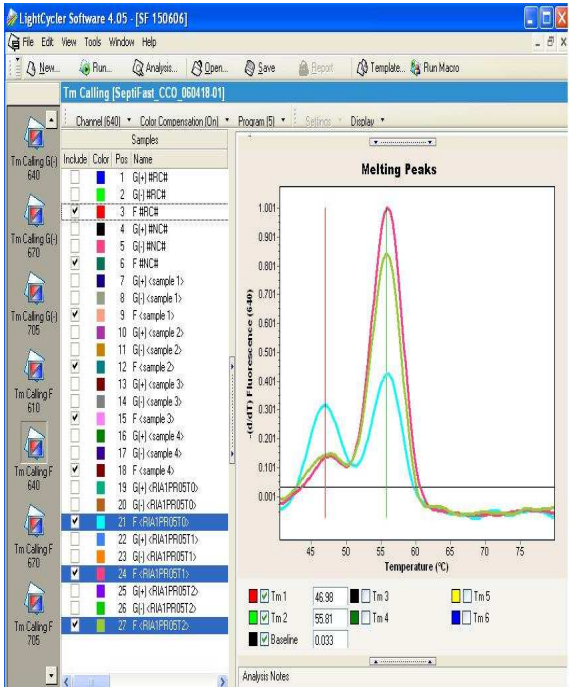
LightCycler® Septi*Fast* Test

Regione target – Internal Transcribed Spacer

si trova tra i geni per rRNA 16S e 23S dei batteri o 18S e 28S dei funghi.



- **Presente in copie multiple > sensibilità analitica**
- **Ben conosciuta**
- **Adatta per identificazione di specie**



Microrganismi evidenziabili

Gram (-)	Gram (+)	Fungi
Escherichia coli	Staphylococcus aureus	Candida albicans (tropicalis & parapsilosis)
Klebsiella pneumoniae/ oxytoca	CoNS (S. epidermidis, haemolyt.)	Candida krusei & glabrata)
Serratia marcescens	Strep. pneumoniae	Aspergillus fumigatus
Enterobacter cloacae/aerogenes	Strep. viridans (“group”)	
Proteus mirabilis	Streptococcus spp.	
Pseudomonas aeruginosa	Strept. pyogenes	
Acinetobacter baumannii	Strept. agalactiae	
Stenotrophomonas maltophilia	Strept. sanguinis	
	Enterococcus faecium	
	Enterococcus faecalis	

MRSA (mecA)

25 microrganismi rilevati $\Rightarrow$ ~ 85-90% dei microrganismi isolati

risultati

SeptiFast Identification Software 1.0.5.34 14/09/2006 11:56:01 page 1

Imported LC-File:
SF 150606

Last modified date: 14/09/2006 11:45:06
Operator: lab
LC Instrument-ID: LC_15366
LCS Version: LCS4 4.0.5.415
Macro: SeptiFast_1.0_04469046001
CCC File Name: SeptiFast_CCO_060418-01

Run Flags

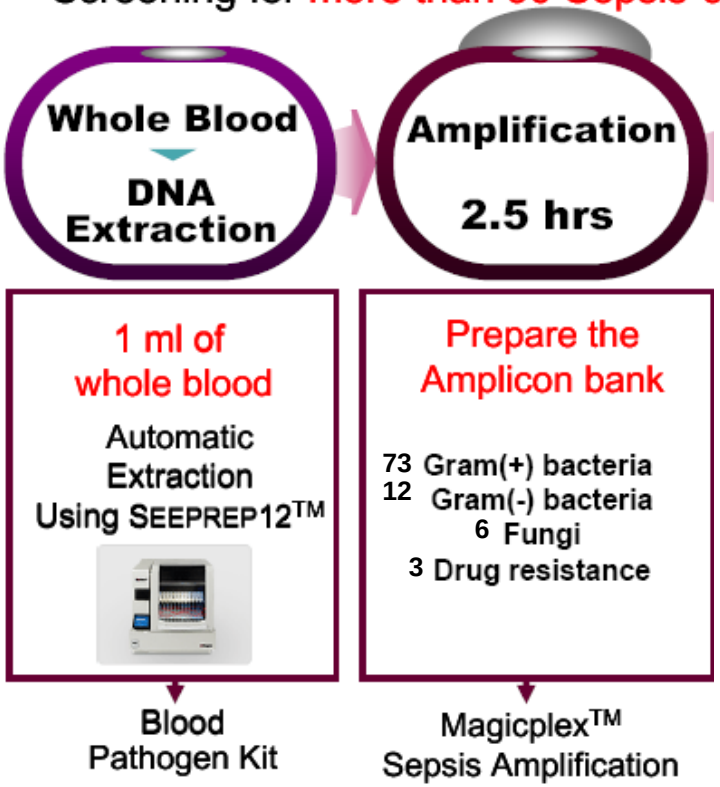
Assay Flags

Assay		Flags
G(+)		
G(-)		
F		

Specimen	Assay	Data	Results
SeptiFast RIA1SM04T0	G(+)		⊖
	G(-)		⊖
	F		⊖
SeptiFast RIA1SM04T1	G(+)		⊖
	G(-)		
	F		⊖
SeptiFast RIA1SM04T2	G(+)		⊖
	G(-)	ch640 t66.69 h0.05	E. cloacae/aerogenes
	F		⊖
SeptiFast RIA1SM04T3	G(+)		⊖
	G(-)		⊖
	F		⊖
SeptiFast RIA1PR05T0	G(+)	ch640 t61.00 h0.10 cp21.92	S. aureus
	G(-)	ch640 t64.72 h0.03	E. cloacae/aerogenes
	F	ch640 t55.81 h0.39	C. albicans
SeptiFast RIA1PR05T1	G(+)	ch640 t61.44 h0.44 cp21.88	S. aureus
	G(-)		⊖
	F	ch640 t55.98 h0.97	C. albicans
SeptiFast RIA1PR05T2	G(+)		⊖
	G(-)		⊖
	F	ch640 t55.87 h0.81	C. albicans

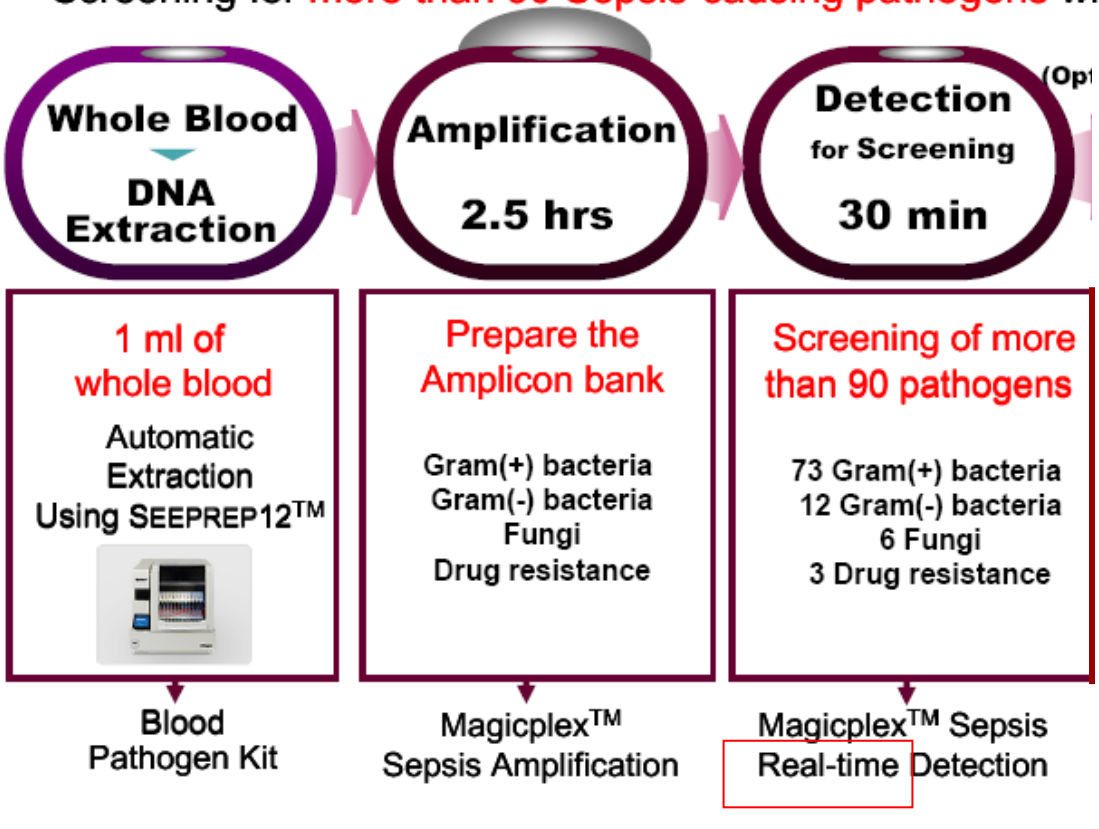
Process of Magicplex™ Sepsis Test

Screening for more than 90 Sepsis-causing pathogens within 6 hrs



Process of Magicplex™ Sepsis Test

Screening for more than 90 Sepsis-causing pathogens within 6 hrs



Gram +

Gram- A

Gram- B

funghi

R

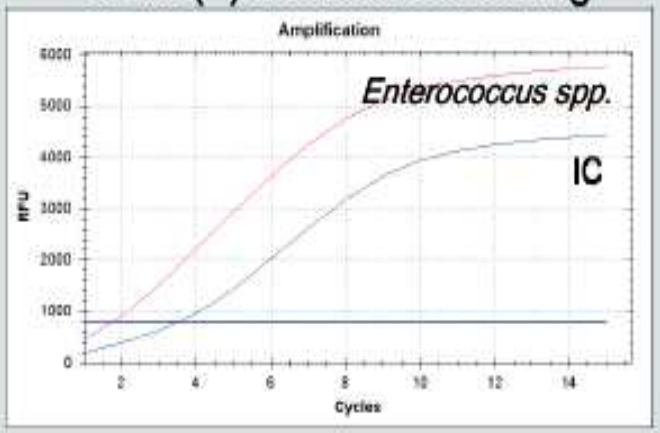
Process of Magicplex™ Sepsis Test

Screening for more than 90 Sepsis-causing pathogens within 6 hrs

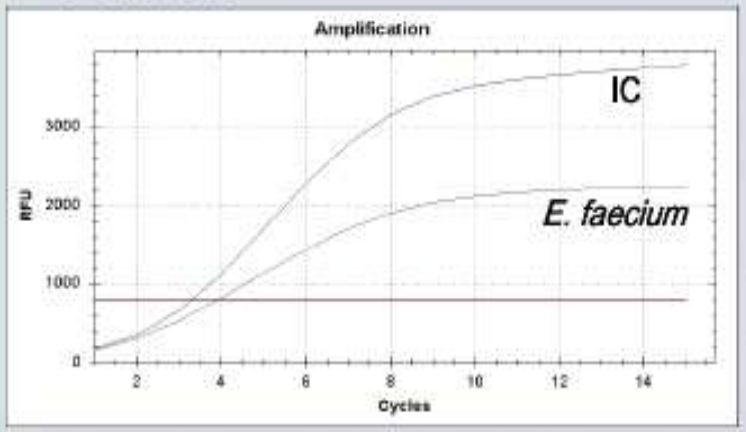
Whole Blood → DNA Extraction	Amplification 2.5 hrs	Detection for Screening 30 min (Option)	Detection for Identification 30 min
1 ml of whole blood Automatic Extraction Using SEEPREP12™	Prepare the Amplicon bank Gram(+) bacteria Gram(-) bacteria Fungi Drug resistance	Screening of more than 90 pathogens 73 Gram(+) bacteria 12 Gram(-) bacteria 6 Fungi 3 Drug resistance	ID of 27 Pathogens 9 Gram (+) bacteria - 3 Staphylococci - 3 Streptococci - 3 Enterococci 12 Gram (-) bacteria 6 Fungi
Blood Pathogen Kit	Magicplex™ Sepsis Amplification	Magicplex™ Sepsis Real-time Detection	Magicplex™ Sepsis-ID Real-time Detection
	PCR	PCR RT	PCR RT

In case of Vancomycin-resistant *Enterococcus faecium* infection

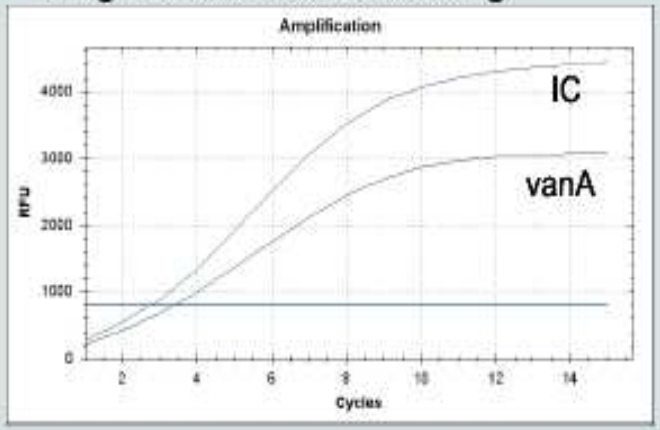
Gram (+) bacteria Screening



Enterococcus ID



Drug resistance Screening



	Gram (+) bacteria Screening	Drug resistance	ID 2 (*Enterococcus* ID)
FAM	Internal control	Internal control	Internal control
HEX	*Streptococcus spp.*	vanA	*E. faecalis*
Cal Red 610	***Enterococcus spp.***	vanB	*E. gallinarum*
Quasar 670	*Staphylococcu spp.*	mecA	***E. faecium***

- **Clinical evaluation of the Magicplex Sepsis Real-time Test (Seegene) to detect Candida DNA in pediatric patients**
- **J Serra***, **E Rosello**, **C Figueras**, **M Pujol**, **Y Peña**, **P Céspedes**, **JL Dapena**, **C Díaz-Heredia**, **MG Codina** and **A Andreu**

In this study the evaluated PCR system provides a rapid technique for Candida detection.
It can be considered an effective diagnostic tool for diagnosing invasive candidiasis along with conventional cultures.

Critical Care 2012, **16**(Suppl 3):P42

22nd European Congress of Clinical Microbiology and Infectious Diseases
(ECCMID)
31.03.2012 - 03.04.2012

- **Molecular diagnosis of sepsis and joint infections**

Monday, April 02, 2012, 12:30 - 13:30

Evaluation of new real-time PCR test for the detection of bacterial and fungal pathogens in patient with suspected bacteraemia

E. Raukas*, A. Leit-Teetlaus, Ü Laaring, R. Pulk (Pärnu, EE)

A new commercial multiplex RT PCR based assay (Magicplex Sepsis Real-time Test, Seegene) for screening more than 90 and direct detection of 27 different bacterial and fungal pathogens in a whole blood was compared to standard blood culture (BC)

……

The PCR method and BC suplement each other.
The positivity rate of PCR was higher than BC's , but it's detection menu is more limited.
Only PCR was able to detect fungi. **A single PCR test does not seem to give sufficient results in all cases.**

JCM
Journals.ASM.org

Diagnostic Performance of a Multiple Real-Time PCR Assay in Patients with Suspected Sepsis Hospitalized in an Internal Medicine Ward

Leonella Pasqualini,[a] Antonella Mencacci,[b] Christian Leli,[b] Paolo Montagna,[b] Angela Cardaccia,[b] Elio Cenci,[b] Ines Montecarlo,[a] Matteo Pirro,[a] Francesco di Filippo,[a] Emma Cistaro,[a] Giuseppe Schillaci,[a] Francesco Bistoni,[b] and Elmo Mannarino[a]

Internal Medicine, Angiology and Arteriosclerosis Diseases Section, Department of Clinical and Experimental Medicine, University of Perugia, Perugia, Italy,[a]* and Microbiology Section, Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Perugia, Italy[b]

. In the subgroup of patients ($n = 191$) who had been receiving antibiotic treatment for ≥24 h, SF identified more pathogens (16 versus 6; $P = 0.049$) compared to BC. These results suggest that, in patients with suspected sepsis, hospitalized in an internal medicine ward, SF could be a highly valuable adjunct to conventional BC, particularly in patients under antibiotic treatment.

Cost analysis of real-time polymerase chain reaction microbiological diagnosis in patients with septic shock.

Alvarez J, Mar J, Varela-Ledo E, Garea M, Matinez-Lamas L, Rodriguez J, Regueiro B.
Department of Anesthesia and Microbiology, University Hospital, Spain.

- ……..statistically significant differences (P <0.05), giving rise to an average net saving of €9970 per patient. The mortality rate was similar in both groups. The main finding of this study was the significant economic saving afforded by the use of the LCS technique, due to the shortening of intensive care unit stay

  and the use of fewer antibiotics.

  LightCycler SeptiFast

Anaesth Intensive Care. 2012 Nov;40(6):958-63

The value of adding Septifast**(R) to a score for predicting complicated bloodstream infections caused by Gram-positive bacteria or Candida species.**

Fernández-Cruz A, Marín M, Kestler M, Alcalá L, Rodriguez-Créixems M, Bouza E.
Department of Clinical Microbiology and Infectious Diseases; Hospital General Universitario Gregorio Marañón, Instituto de Investigación Sanitaria del Hospital Gregorio Marañón, Universidad Complutense, Madrid, Spain.

- Patients with a positive Septifast® result between days 3 and 7 after a positive bloodculture have an almost 8-fold higher risk of developing complicated bloodstream infections . A score combining clinical data with

  Septifast® may improve exclusion of complicated bloodstream infections.

J Clin Microbiol. 2013 Jan 30

Routine use of a real-time polymerase chain reaction method for detection of bloodstream infections in neutropaenic patients.

Paolucci M, Stanzani M, Melchionda F, Tolomelli G, Castellani G, Landini MP, Varani S, Lewis RE, Sambri V.
Unit of Microbiology, Department of Specialistic, Diagnostic and Experimental Medicine, University of Bologna, Bologna, Italy.

PCR results were available earlier (median 3 days for bacteria, 5 days fungal pathogens; P ≤ 0.01).

- ……the frequent failure of instrumental control procedures, the relatively poor sensitivity of the test, and the lack of phenotypic data on antimicrobial susceptibility associated with its high costs suggest that this assay cannot replace the blood cultures.

Diagn Microbiol Infect Dis. 2013 Feb;75(2):130-4.

SEPTIFAST

Diagnostic Microbiology and Infectious Disease 73 (2012) 308–311

Contents lists available at SciVerse ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Diagnostic utility of LightCycler SeptiFast and procalcitonin assays in the diagnosis of bloodstream infection in immunocompromised patients

M.V. Mauro[a,*], P. Cavalcanti[a], D. Perugini[a], A. Noto[b], D. Sperfi[c], C. Giraldi[a]

JCM 2012

Journals.ASM.org

9, No. 6

Multiplex PCR for Rapid and Improved Diagnosis of Bloodstream Infections in Liver Transplant Recipients

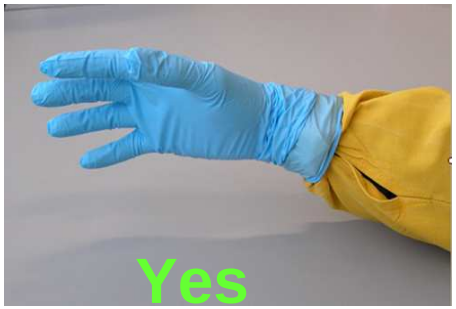
Peter-Michael Rath,[a] Fuat Saner,[b] Andreas Paul,[b] Nils Lehmann,[c] Eike Steinmann,[d] Jan Buer,[a] and Joerg Steinmann[a]

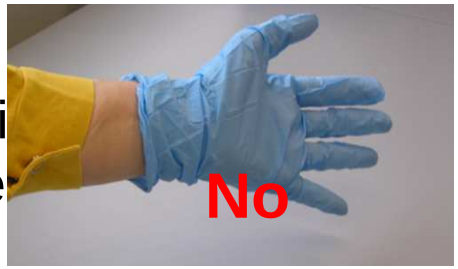
- **TTR ≤6h (theoretical)**
- **Good concordance with BC (67%)**
- **Increased sensitivity (antibiotic exposure)**

- **False negatives (limited pathogen coverage)**
- **Add-on value, not an alternative to culture**
- **High cost**

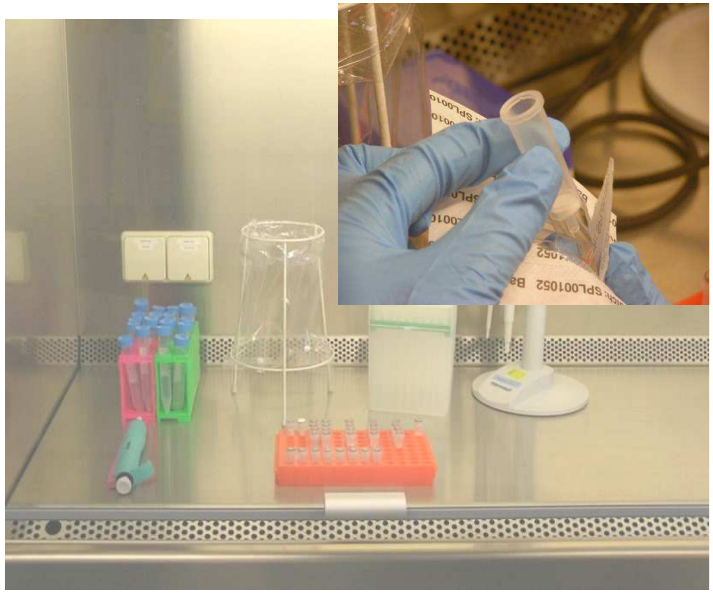
Attrezzatura di laboratorio

- Indossare un **camice monouso**
- Indossare guanti antistatici durante l'intero flusso di lavoro.
- Evitare di toccare il palmo e le dita dei guanti indossandoli.
- Evitare l'esposizione della pelle. Indossare i guanti sopra le maniche del camice da laboratorio - in alternativa indossare dei **manicotti coprimaniche**
- In caso di contaminazione, sostituire immediatamente i guanti o trattarli con un reagente di decontaminazione del DNA





Spazi di lavoro



- Le superfici all'interno della cappa a flusso laminare / cabina PCR devono essere pulite con reagente di decontaminazione DNA
- Decontaminare con lampada UV light

- Tenere sotto cappa solo materiale indispensabile e in modo ordinato
- Aprire il materiale monouso esclusivamente sotto cappa a flusso laminare o in cabina PCR
- Non utilizzare materiale comune ad altri esperimenti di laboratorio o usato fuori cappa/cabina PCR
- Fare attenzione a non toccare le superfici del materiale monouso che entrano in contatto col campione (bordo o la filettatura delle vials)
- Durante le procedure, disporre tutti gli elementi sotto il flusso laminare in un ordine logico (evitare il pipettamento incrociato).
- Se non è possibile lasciarle i materiali in cappa/cabina PCR conservarli in un luogo dedicato diverso da quello dove si tiene il materiale per altre metodiche

Risultati ottimali

- **Personale dedicato**
- **Strutture adeguate (lab. microbiologia di riferimento)**
- **Servizio su 24 h (12h)**
- **Conoscenza epidemiologia locale delle “R”**

- SMN
- NSG
- SMA

Laboratorio Microbiologia e Virologia
AOUC

AOUC

3000 posti letto
11 rianimazioni
6 TI

BSL

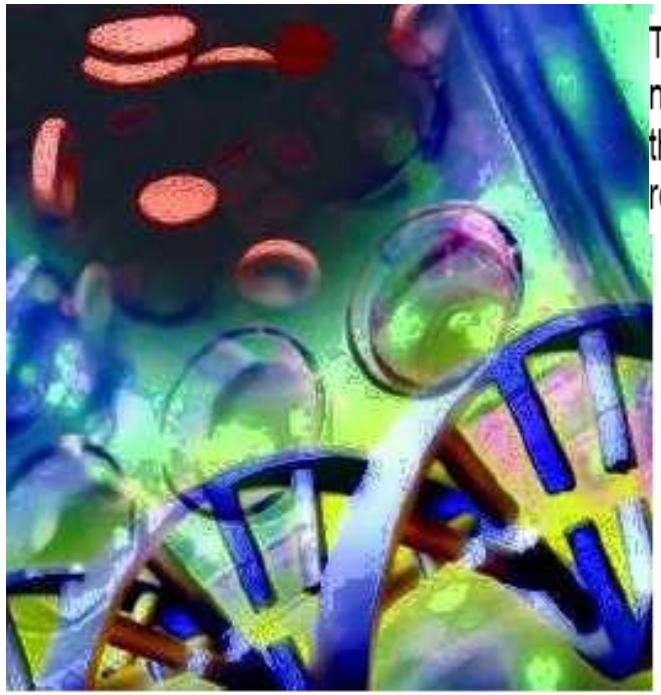
FVdA

MEYER

TECNICHE MOLECOLARI

da emocoltura positiva

hyplex® BloodScreen



The two hyplex BloodScreen PCR modules **BSP** (gram-positive) and **BSN** (gram-negative pathogens) each contain a mixture of oligonucleotide primers, which makes the simultaneous amplification of various specific DNA ranges in a single PCR reaction possible.

DNA extraction

hyplex® BloodScreen PCR module **BSN**	the species *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* as well as the genus *Klebsiella*
hyplex® BloodScreen hybridisation module	
hyplex® BloodScreen PCR module **BSP**	staphylococcal gene **mecA**, the species *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* as well as *Enterococcus faecalis* and *Enterococcus faecium*.
hyplex® BloodScreen hybridisation module	

5/6 h

JOURNAL OF CLINICAL MICROBIOLOGY, July 2004, p. 3147–3152 Vol. 42, No. 7
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.7.3147–3152.2004

Evaluation of the Hyplex BloodScreen Multiplex PCR–Enzyme-Linked Immunosorbent Assay System for Direct Identification of Gram-Positive Cocci and Gram-Negative Bacilli from Positive Blood Cultures

Nele Wellinghausen,[1]* Beate Wirths,[1] Andreas Essig,[1] and Lars Wassill[2]

Department of Medical Microbiology and Hygiene, University of Ulm, Ulm,[1] and Amplex Diagnostics GmbH, Munich,[2] Germany

***moniae* and the staphylococcal *mecA* gene. The Hyplex BloodScreen test showed an overall sensitivity of 10 for the identification of gram-negative bacilli and 96.6 to 100% for the identification of gram-positive cocci**

. . . . . **The result of the *mecA* gene detection module correlated with the result of the phenotypic oxacillin resistance testing in all 38 isolates of *Staphylococcus aureus* investigated. In conclusion, the Hyplex BloodScreen PCR-ELISA system is well suited for the direct and specific identification of the most common pathogenic bacteria and the direct detection of the *mecA* gene of *Staphylococcus aureus* in positive blood cultures.**

da BC pos estrazione

5/6 h

2004

- Prove-it™ Sepsis Prove-it™ Sepsis StripArray
- **Accurate DNA-based identification of 80 bacterial and fungal pathogens, and antibiotic resistance markers**

Prove-it™ Sepsis assay identifies sepsis-causing bacteria and fungi from positive blood culture in only 3,5 hours

Method: 1° step extraction
2° step broad-range PCR
3° step identification on a microarray.

Coverage: over 60 microrganisms and the *mecA* methicillin resistance marker in one assay.

Assay Workflow

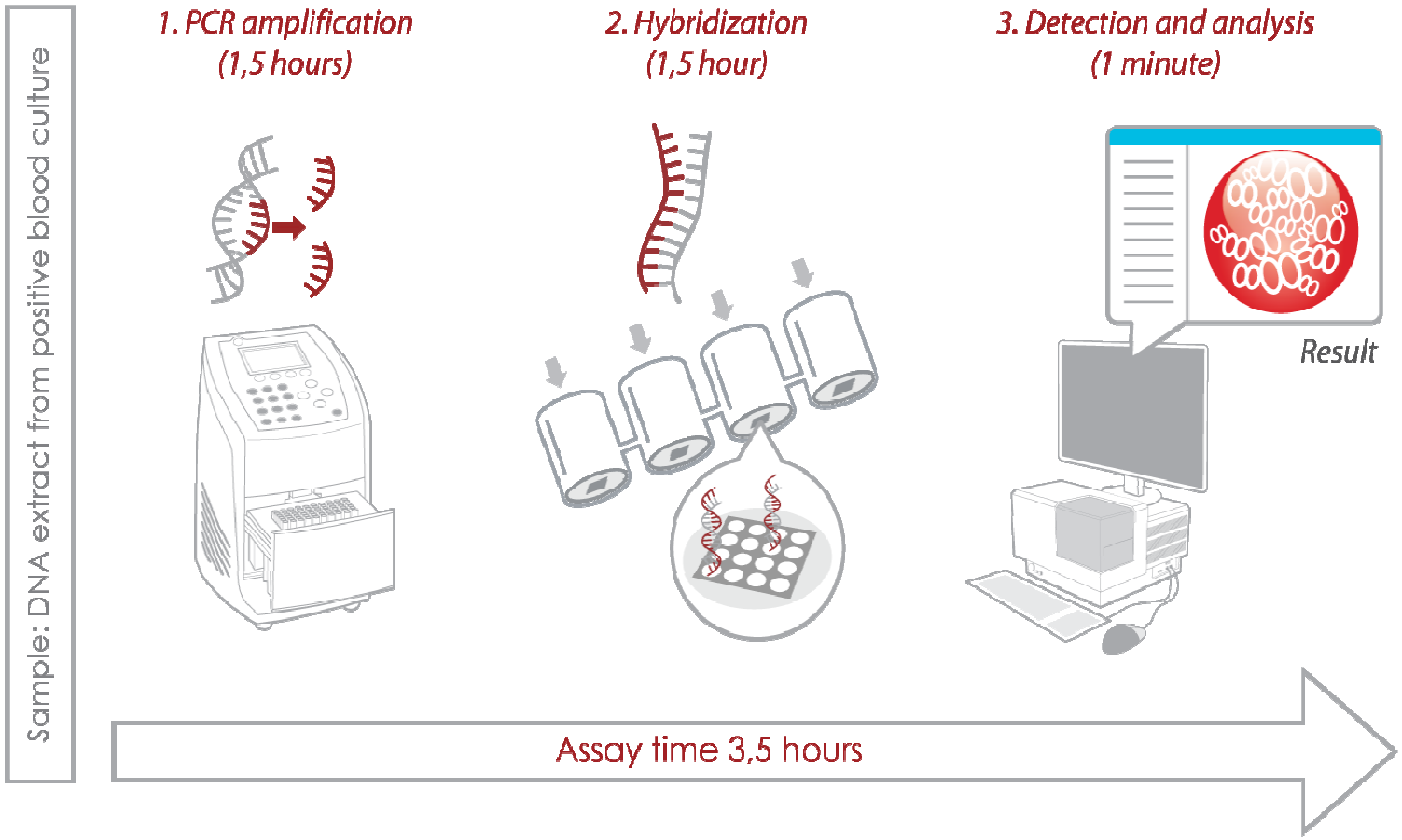


Table 1 (abstract P3)

Bacteria and an antibacterial resistance marker identified by the Prove-it™ Sepsis assay

Gram-negative	Gram-positive	Antibacterial resistance
Neisseria meningitidis	*Staphylococcus aureus*	Methicillin resistance marker mecA
Enterobacter aerogenes	*Staphylococcus epidermidis*	
Enterobacter cloacae	Coagulase-negative *Staphylococcus*[d]	
Escherichia coli	*Streptococcus pyogenes*	
Klebsiella oxytoca	*Streptococcus agalactiae*	
Klebsiella pneumoniae	*Streptococcus dysgalactiae* subsp. *equisimilis*	
Proteus mirabilis	*Streptococcus pneumoniae*	
Proteus vulgaris	*Enterococcus faecalis*	
Salmonella enterica subsp. *enterica*[a]	*Enterococcus faecium*	
Serratia marcescens	*Listeria monocytogenes*	
Enterobacteriaceae family[b]	*Clostridium perfringens*	
Acinetobacter baumannii		
Pseudomonas aeruginosa		
Stenotrophomonas maltophilia		
Haemophilus influenzae		
Campylobacter jejuni/coli		
Bacteroides fragilis group[c]		

Critical Care Volume 13 Suppl 4, 2009
Sepsis 2009
Amsterdam, the Netherlands, 11–14 November 2009

Performance evaluation and further development of the PCR and microarray-based Prove-it™ Sepsis assay

P Tissari[1], E Tarkka[1], S Mero[1], L Savolainen[1], M Vaara[1], A Zumla[2], J Huggett[2], C Carder[2], V Gant[2], A Aittakorpi[3], S Laakso[3], M Lindfors[3], P Piiparinen[3], N Kumlin[3], H Piiparinen[3], M Mäki[3]

[1]Division of Clinical Microbiology, Helsinki University Hospital Laboratory HUSLAB, Helsinki, Finland; [2]Department of Clinical Microbiology, University College London Hospitals NHS Foundation Trust, and University College London Medical School, Centre for Infectious Diseases and International Health, London, UK; [3]Mobidiag Ltd, Helsinki, Finland

3,318 blood cultures,

Sensitivity and specificity for Prove-it™ Sepsis were 94.7% and 98.7%, respectively. Of particular significance was the assay's faultless ability to differentiate MRSA from MSSA and from CNS.

Prove-it sepsis assay

The Lancet, Volume 375, Issue 9728, Page 1780, 22 May 2010

Future diagnosis of sepsis

Chen a, Youxin Wang b , Wei Wang

…….DNA-based microarray platform (the **Prove-it sepsis assay**).
This assay yielded a clinical sensitivity of 94·7% (95% CI 93·6—95·7) and specificity of 98·8% (

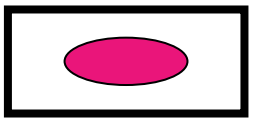
With its flexible design and persistent modification for difficulties related to PCR multiplexing capacity in polymicrobial bacteraemia, the Prove-it sepsis assay represents an advance in clinical diagnosis. However, further study with strict statistical analysis is needed before its widespread application.

1,6% falsi + 3% falsi -

da BC pos estrazione

4 h

SISTEMA VERIGENE NANOSPHERE



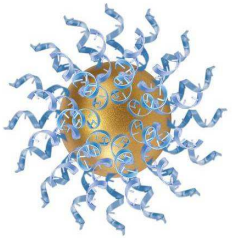
Carico campione e consumabili (<2 min)

Preparazione automatica del campione ed avanzamento del test (≤2.30h)

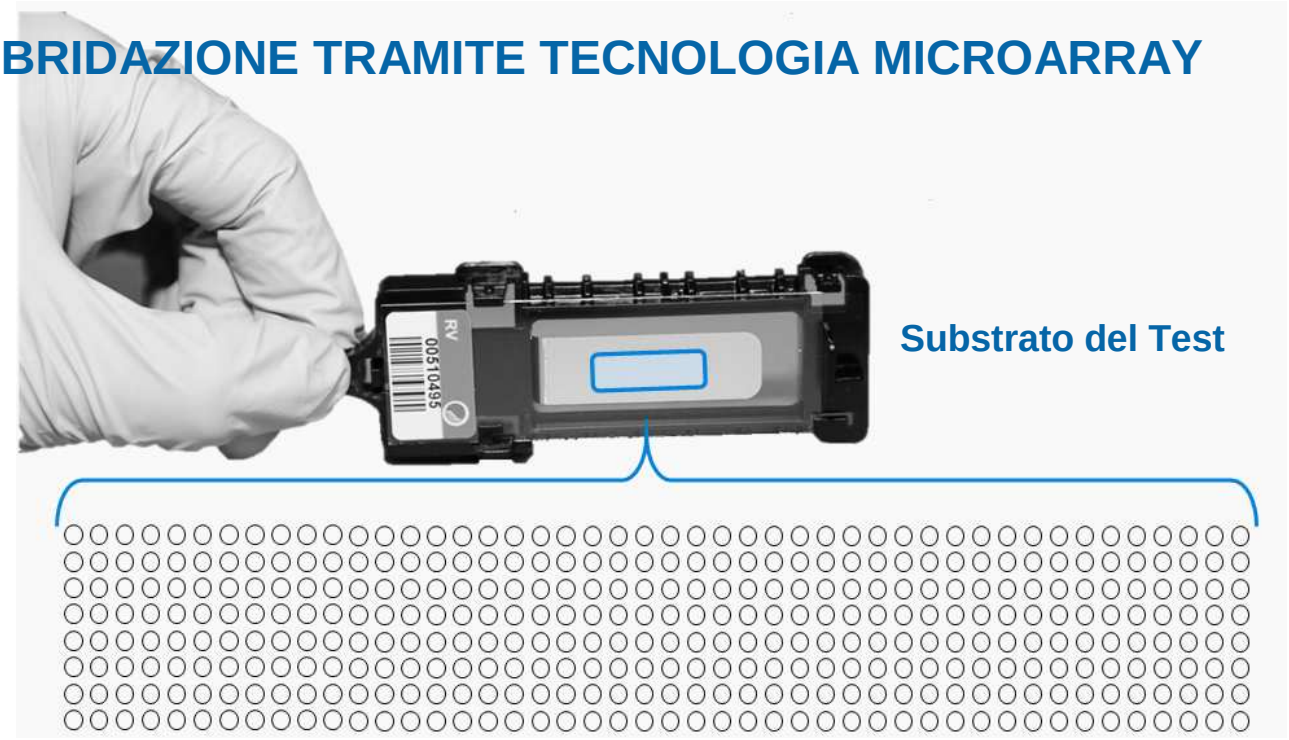
Analisi (<2min)

TECNOLOGIA

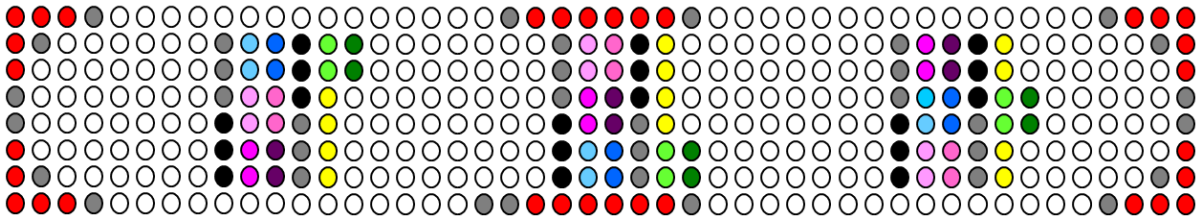
NANOPARTICELLE D‘ORO PER L‘AMPLIFICAZIONE DEL SEGNALE RILEVAMENTO MEDIANTE IBRIDAZIONE



IBRIDAZIONE TRAMITE TECNOLOGIA MICROARRAY



Substrato del Test



Empio di lettura del Microarray

Il test viene ripetuto diverse volte sul Microarray, se tutti i risultati sono concordi viene prodotto il referto

	Verigene Test	Targets	Sample Type	Status
sepsi	BC-GP	The most common gram-positive bloodstream infection-causing bacteria	Positive Blood Culture	CE-IVD.
	BC-GN	The most common gram-negative bloodstream infection-causing bacteria	Positive Blood Culture	Pending CE certification.
	BC-F	The most common bloodstream infection-causing fungi	Positive Blood Culture	In Development.

GRAM-POSITIVE COCCI IN CLUSTERS	
Organism/Target	Total Agreement*
Staphylococcus spp.	99.7%
Staphylococcus aureus	100%
Staphylococcus epidermidis	98.8%
***mecA* (n= 223)**	**98.7%**
Staphylococcus lugdunensis	100%
Micrococcus spp.	99.1%

GRAM-POSITIVE RODS	
Organism	Total Agreement*
Listeria spp.	100%

GRAM-POSITIVE COCCI IN CHAINS/PAIRS	
Organism/Target	Total Agreement*
Enterococcus faecalis	99.3%
Enterococcus faecium	99.6%
***vanA* (n= 141)**	**97.9%**
***vanB* (n= 141)**	**100%**
Streptococcus spp.	99.6%
Streptococcus agalactiae	100%
Streptococcus pyogenes	100%
Streptococcus anginosus Group	100%
Streptococcus pneumoniae	100%

VERIGENE® BC-GN TEST

INDAGINE MULTIPLA DEI PIU' COMUNI BATTERI GRAM-NEGATIVI E DELLE RESISTENZE ASSOCIATE

Pannello proposto (in sviluppo)	Organismo/Gene	
Bacterial Targets	*Acinetobacter* spp. *Citrobacter* spp. *Enterobacter* spp. *Proteus spp.* *E. coli* *Klebsiella pneumoniae* *Klebsiella oxytoca* *Pseudomonas aerogenes* *Serratia marcescens*	
Resistance Marker	CTX-M	VIM
	KPC	IMP
	NDM	OXA (48/23/40/58)

AUO Careggi

N. di serie: 10009011
ID sessione: microbiologia

Vedere pacchetto applicativo inserito per dettagli sui risultati.

Campione	gran meno		
Test	BC-GN	**Elaborazione completata**	14·02·13, 12:07
Cartuccia	02107800	**Analisi completata**	14·02·13, 12:34

Elenco

✻	**K. pneumoniae**	Detected	✻	**KPC**	Detected

Dettagliato

	Acinetobacter	Not Detected		**Citrobacter**	Not Detected
	Enterobacter	Not Detected		**Proteus**	Not Detected
	E. coli	Not Detected		**P. aeruginosa**	Not Detected
	K. oxytoca	Not Detected	✻	**K. pneumoniae**	Detected
	S. marcescens	Not Detected		**OXA**	Not Detected
	CTX-M	Not Detected	✻	**KPC**	Detected
	NDM	Not Detected		**IMP**	Not Detected
	VIM	Not Detected			

AUO Careggi

N. di serie: 10009011
ID sessione: microbiologia

Vedere pacchetto applicativo inserito per dettagli sui risultati.

Campione	gram neg		
Test	BC-GN	**Elaborazione completata**	13-02-13, 16:14
Cartuccia	02107799	**Analisi completata**	13-02-13, 16:17

Elenco

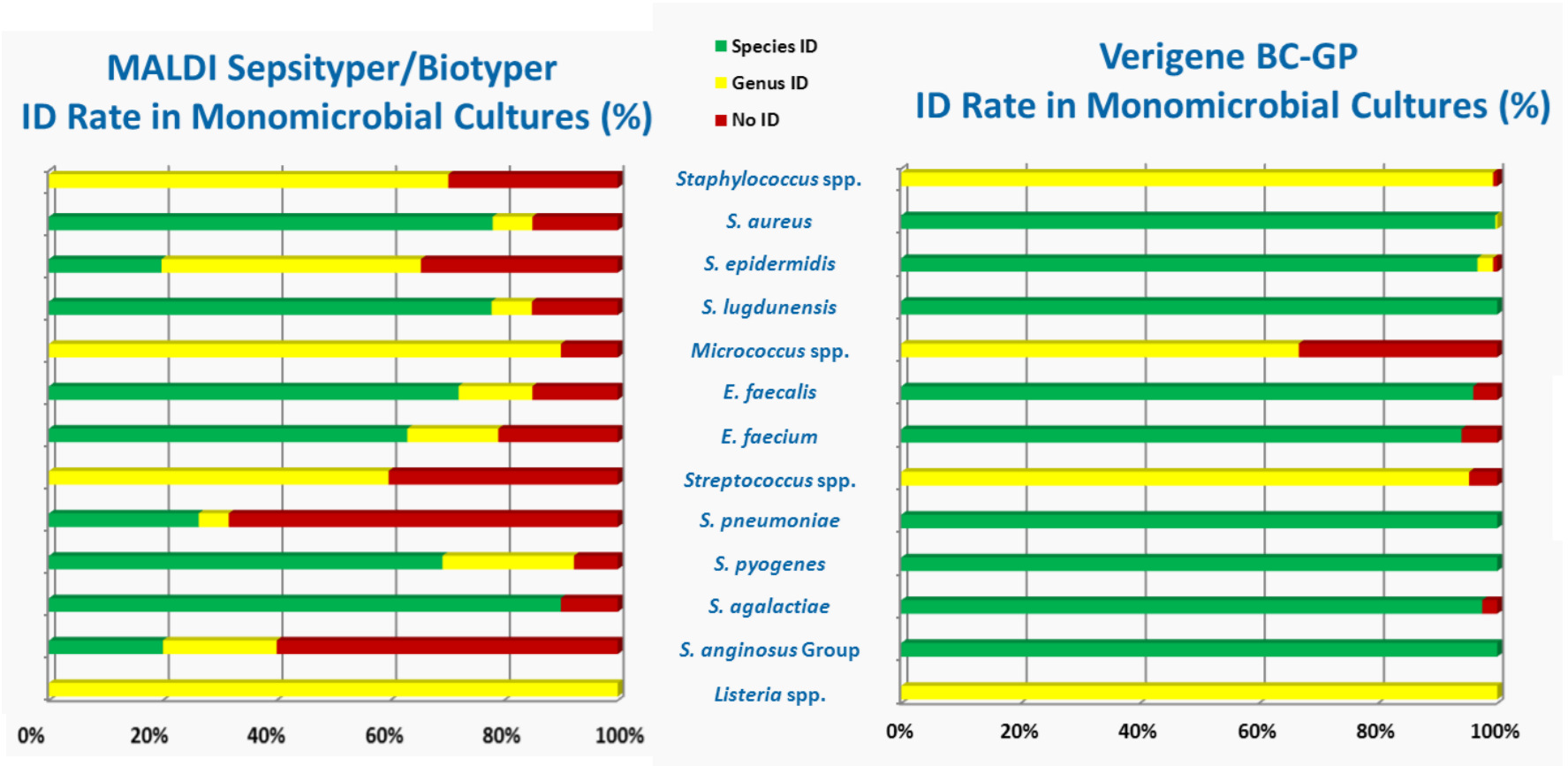
*	**Proteus**	Detected	*	**E. coli**	Detected
*	**CTX-M**	Detected	*	**KPC**	Detected

Dettagliato

	Acinetobacter	Not Detected		**Citrobacter**	Not Detected
	Enterobacter	Not Detected	*	**Proteus**	Detected
*	**E. coli**	Detected		**P. aeruginosa**	Not Detected
	K. oxytoca	Not Detected		**K. pneumoniae**	Not Detected
	S. marcescens	Not Detected		**OXA**	Not Detected
*	**CTX-M**	Detected	*	**KPC**	Detected
	NDM	Not Detected		**IMP**	Not Detected
	VIM	Not Detected			

DIAGNOSTICA SEPSI

ANALISI DELLA PERFORMANCE: MALDI TOF VS. BC-GP



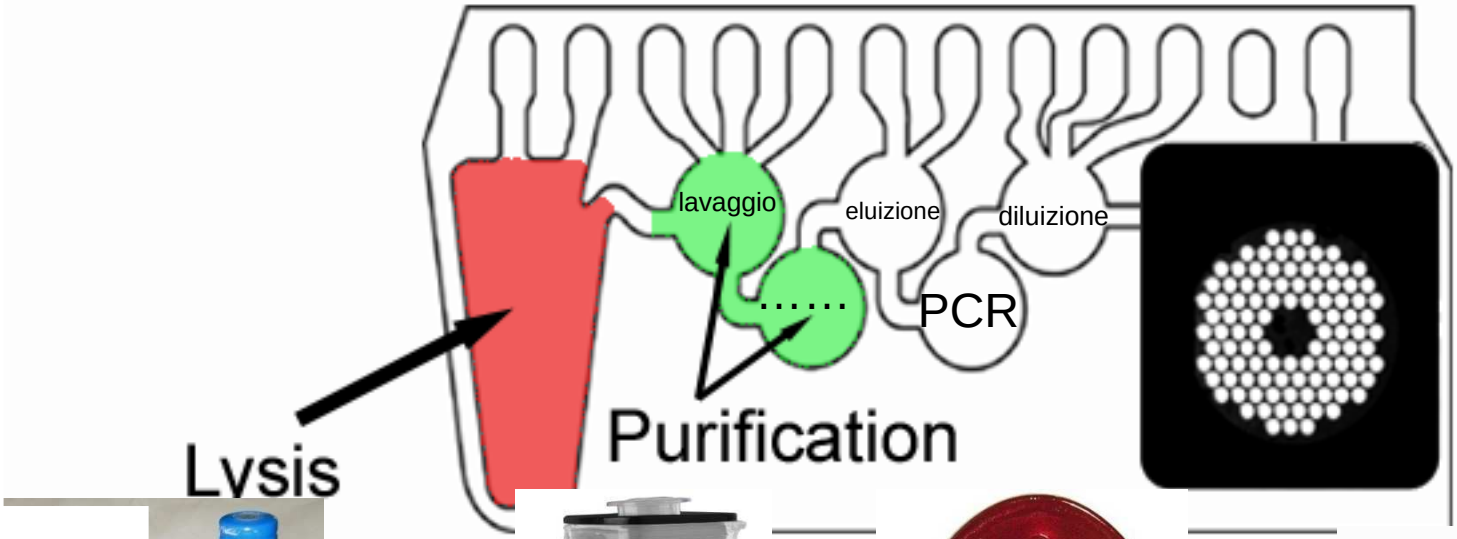
MALDI data adapted from: Kok et al., 2011; Loonen et al., 2011; Buchan et al., 2012; Klein et al., 2012; Martiny et al., 2012; Vlek et al., 2012

- J Clin Microbiol. 2013 Jan 30.
- **Evaluation of a Microarray Based Assay for Rapid Identification of Gram-Positive Organisms and Resistance Markers in Positive Blood Cultures.**
- Samuel LP, Tibbetts RJ, Agotesku A, Fey M, Hensley R, Meier FA.

We evaluated the performance of the Verigene Gram- Positive Blood Culture (BC-GP) Assay(®) (Nanosphere Inc., Northbrook, IL)…….

Concordance for detection of major pathogens such Staphylococcus aureus (n=45) and Enterococci (n=19) were 98% and 95% respectively.
Agreement for detection of resistance markers such as mecA and vanA/Bwere 92% and 100% respectively. The BC-GP assay is capable of providing rapid identification of gram-positive cocci as well as detection of resistance markers directly from positive blood cultures at least 24-48h earlier than conventional methods.

Tecnologia FilmArray DID



Estrazione e purificazione a.nucleici con biglie magnetiche

Amplificazione con nested multiplex PCR

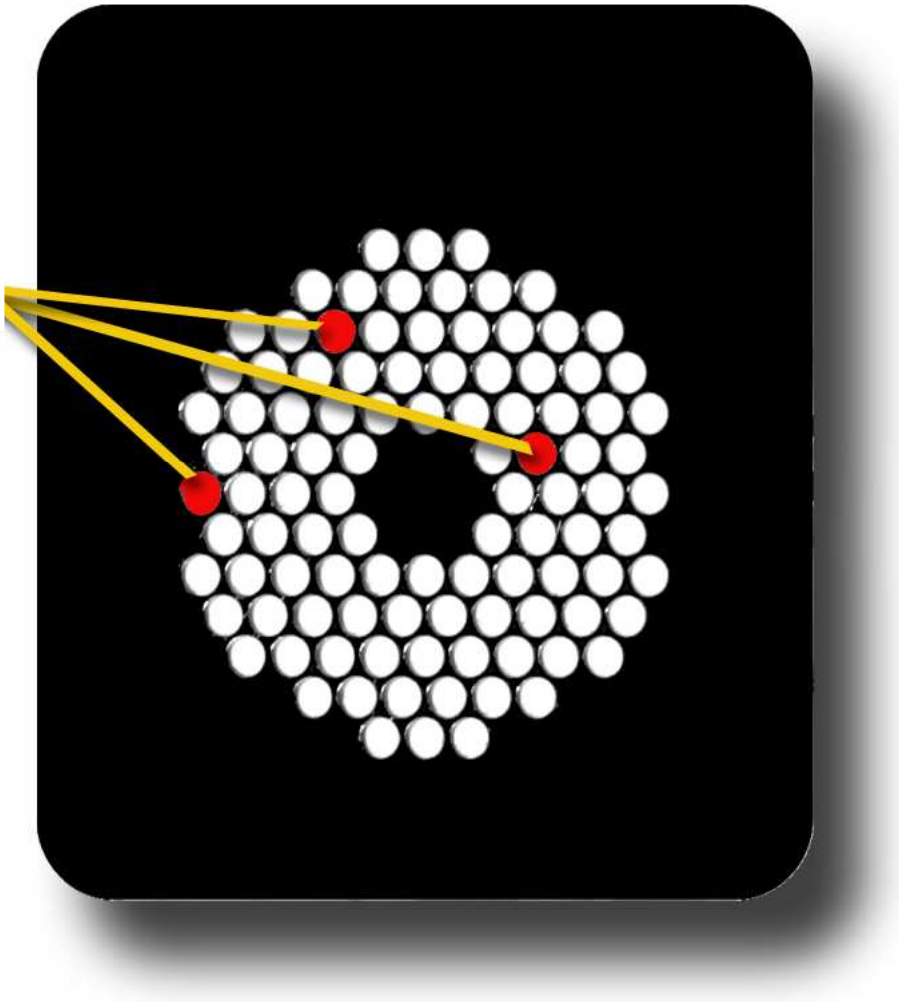
Rilevazione mediante ibridazione su array

102 pozzetti

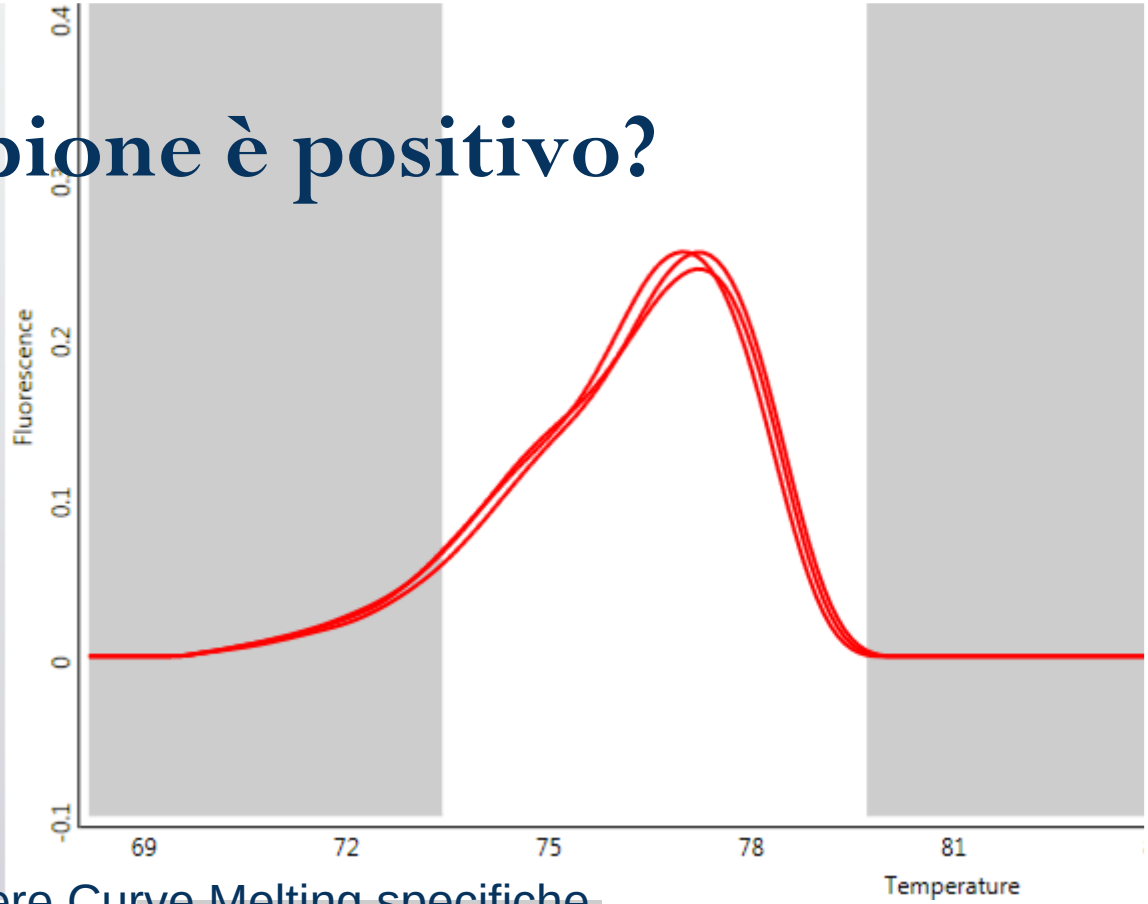
In ogni pozzetto primer specie specifici

Tutti I target sono testati in 3 pozzetti diversi

Per ogni pozzetto vengono generate curve di Melting

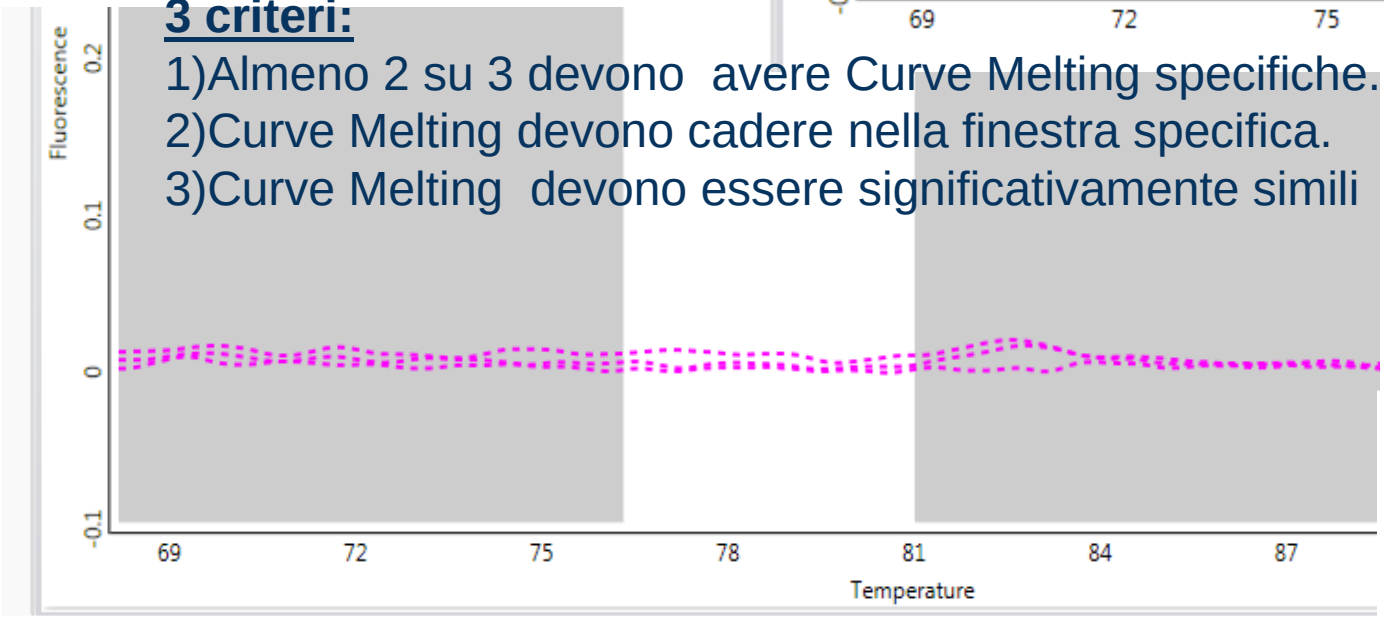


Quando un campione è positivo?



3 criteri:

1)Almeno 2 su 3 devono avere Curve Melting specifiche.
2)Curve Melting devono cadere nella finestra specifica.
3)Curve Melting devono essere significativamente simili



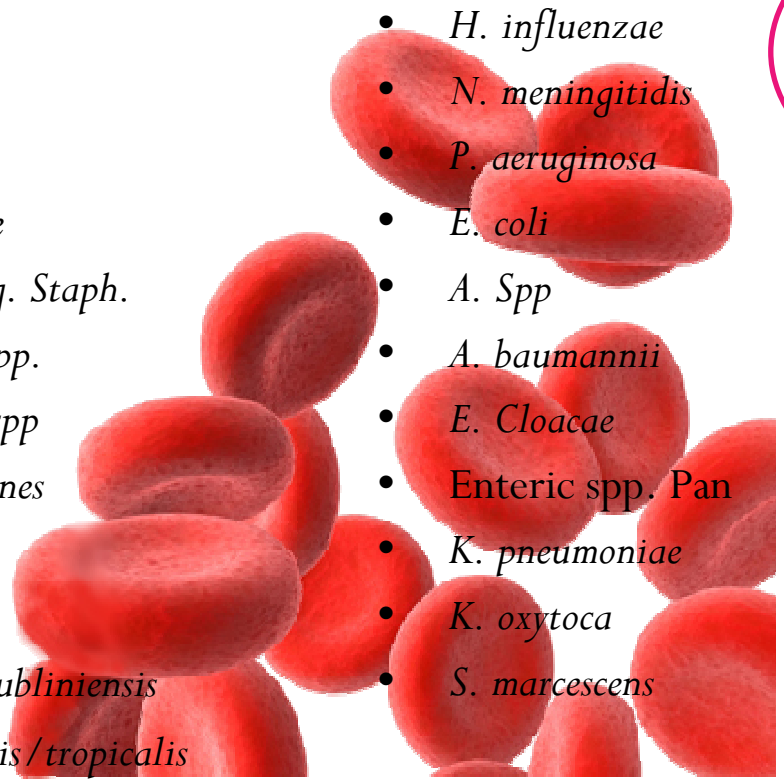
Pannello per emocoltura

Gram + :

- *S. aureus*
- *S. pyogenes*
- *S. agalactiae*
- *S. pneumoniae*
- *Coagulase Neg. Staph.*
- *Enterococcus spp.*
- *Streptococcus spp*
- *L. monocytogenes*

Funghi:

- *C. albicans/dubliniensis*
- *C. parapsiolosis/tropicalis*
- *C. glabrata*
- *C. krusei*



Gram - :

- *H. influenzae*
- *N. meningitidis*
- *P. aeruginosa*
- *E. coli*
- *A. Spp*
- *A. baumannii*
- *E. Cloacae*
- Enteric spp. Pan
- *K. pneumoniae*
- *K. oxytoca*
- *S. marcescens*

Resistenze:

- mecA
- Van A/B
- KPC

Tempo necessario 1h

refertazione

FilmArray®
BCID Panel

FilmArray® Idaho Technology Inc.
www.idahotech.com

Run Summary

Sample ID:	Careggi Prova 1	**Run Date:**	05 Nov 2012 8:41 AM
Detected:	*Enterobacteriaceae* *Klebsiella pneumoniae* KPC [1]	**Controls:**	Passed
Equivocal:	None		

Annotations

[1] KPC – presumptive association with Enterobacteriaceae, Acinetobacter baumannii or Pseudomonas aeruginosa when also detected.

Result Summary

Not Detected	*Acinetobacter baumannii*
Not Detected	*Candida albicans*
Not Detected	*Candida glabrata*
Not Detected	*Candida krusei*
Not Detected	*Candida parapsilosis*
Not Detected	*Candida tropicalis*
✓ Detected	*Enterobacteriaceae*
Not Detected	*Enterobacter cloacae complex*
Not Detected	*Escherichia coli*
Not Detected	*Klebsiella oxytoca*
✓ Detected	*Klebsiella pneumoniae*
Not Detected	*Proteus*
Not Detected	*Serratia*
Not Detected	*Enterococcus*
Not Detected	*Haemophilus influenzae*
Not Detected	*Listeria monocytogenes*
Not Detected	*Neisseria meningitidis*
Not Detected	*Pseudomonas aeruginosa*
Not Detected	*Staphylococcus*
Not Detected	*Staphylococcus aureus*
Not Detected	*Streptococcus*
Not Detected	*Streptococcus agalactiae*
Not Detected	*Streptococcus pneumoniae*
Not Detected	*Streptococcus pyogenes*
✓ Detected	KPC
Not Detected	*mecA*
Not Detected	*vanA/B*

Run Details

Pouch:	BCID Panel v2.0	**Protocol:**	BC v2.1
Run Status:	Completed	**Operator:**	Marco Palumbo (Marco)
Serial No.:	00300468	**Instrument:**	ITI FA "FA2146"
Lot No.:	112812		

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journal homepage: www.elsevier.com/locate/diagmicrobio

Rapid identification of pathogens from positive blood cultures by multiplex polymerase chain reaction using the FilmArray system☆,☆☆,★

Anne J. Blaschke [a,*], Caroline Heyrend [a,b], Carrie L. Byington [a], Mark A. Fisher [c,d], Elizabeth Barker [b], Nicholas F. Garrone [b], Stephanie A. Thatcher [b], Andrew T. Pavia [a], Trenda Barney [e], Garrison D. Alger [e], Judy A. Daly [c,e], Kirk M. Ririe [b], Irene Ota [b], Mark A. Poritz [b]

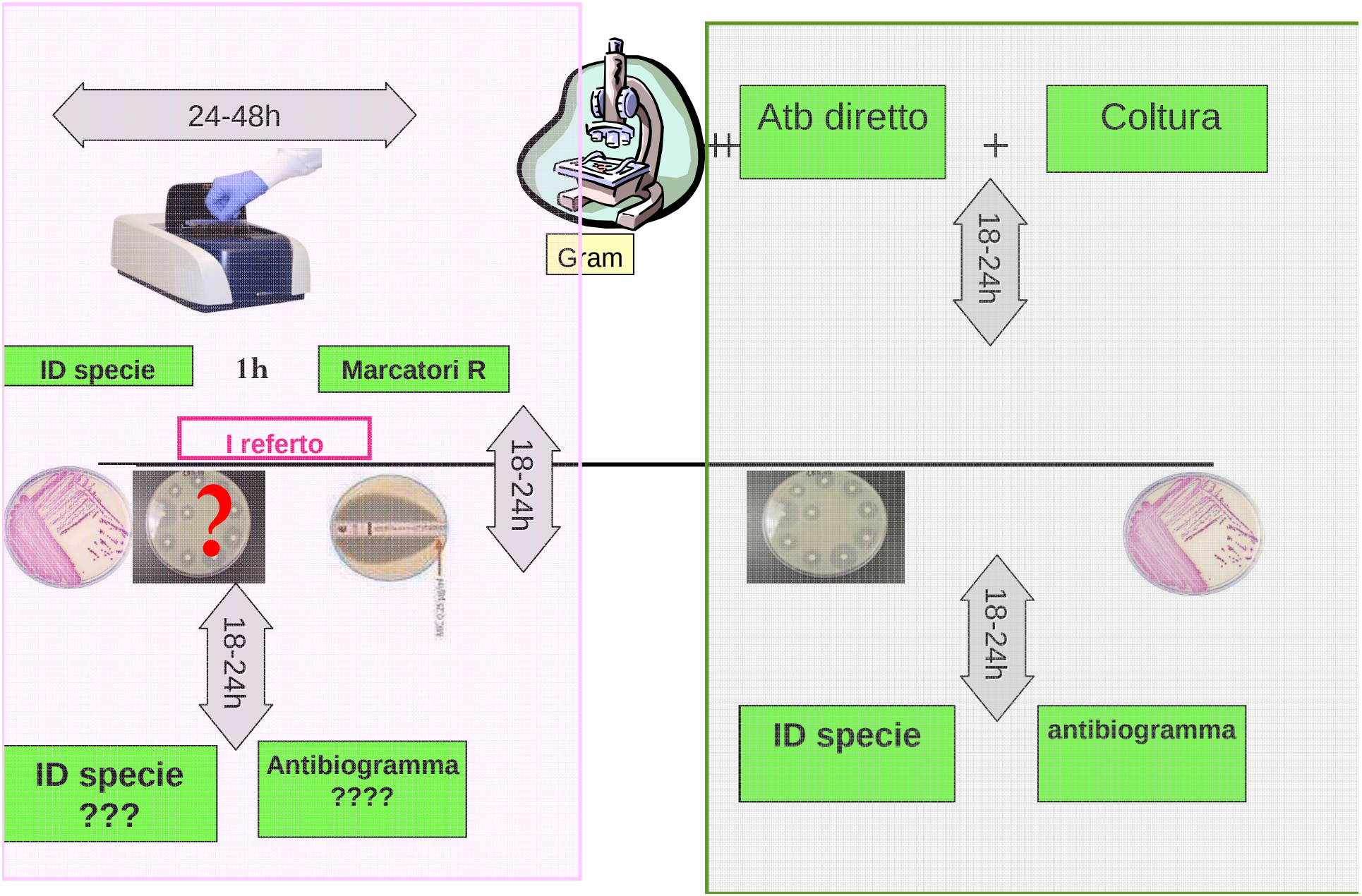
[a] Department of Pediatrics, University of Utah, Salt Lake City, UT, USA
[b] Idaho Technology, Inc., Salt Lake City, UT, USA
[c] Department of Pathology, University of Utah, Salt Lake City, UT, USA

Accuratezza identificazione patogeni 91%
Accuratezza identificazione MRSA 100%

In addition, the FA System performs all steps of the assay, from nucleic acid extraction to interpretation of amplification data in a closed system using a single pouch on a minimally processed clinical sample. The laboratory procedures required are not technologically complex and can be performed by persons who do not have training in molecular techniques (Poritz et al., 2011).

. The data presented here suggest that the FA BC system could be an important and useful tool for the management of bloodstream infection in the future.

Emocoltura


24-48h
Gram
Atb diretto
+
Coltura
18-24h
ID specie
1h
Marcatori R
I referto
18-24h
18-24h
18-24h
ID specie ???
Antibiogramma ????
ID specie
antibiogramma

Il loro costo impone l'uso solo su pz selezionati

Riduzione dei Costi

Aumentare la qualità del servizio

Riorganizzare per sopravvivere

Riduzione del TAT