U.O.C. PATOLOGIA CLINICA E MICROBIOLOGIA
P.O. CIVILE-OMPA RAGUSA
DIRETTORE Rosa M. Giordano

I Percorsi diagnostici in Microbiologia clinica

Ragusa, 2-3 Ottobre 2015 Sala AVIS
Via della Solidarieta' , 1

Nuove tecniche diagnostiche per la diagnosi microbiologica di sepsi: vantaggi e criticita'

Università degli Studi di Roma Tor Vergata

C.Fontana

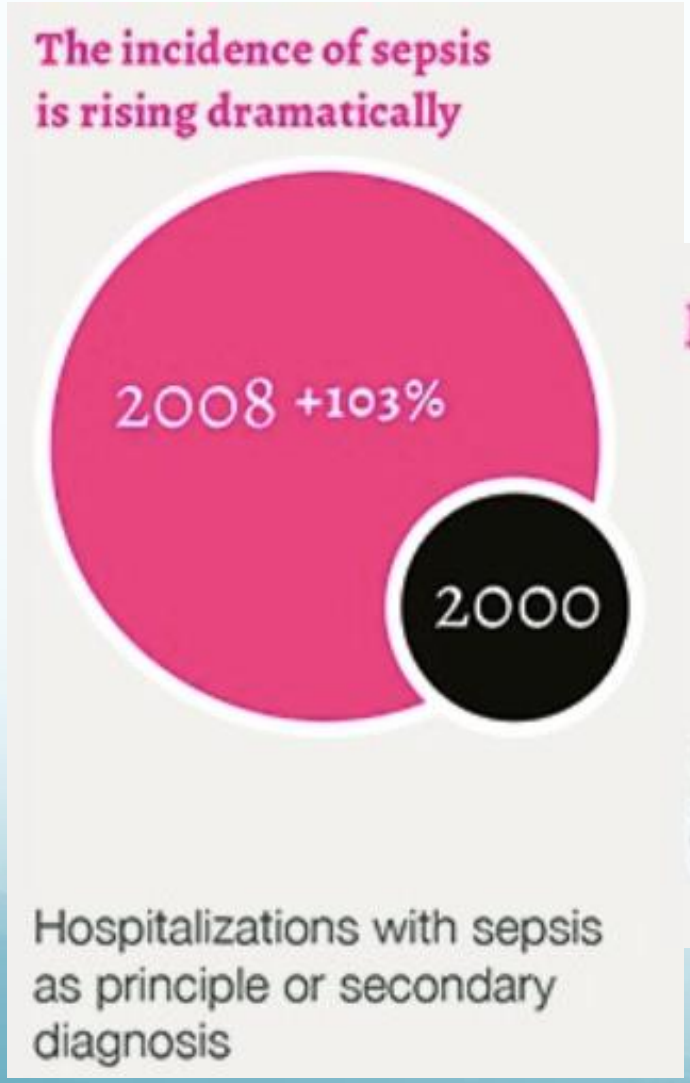
PTV
FONDAZIONE PTV
POLICLINICO TOR VERGATA

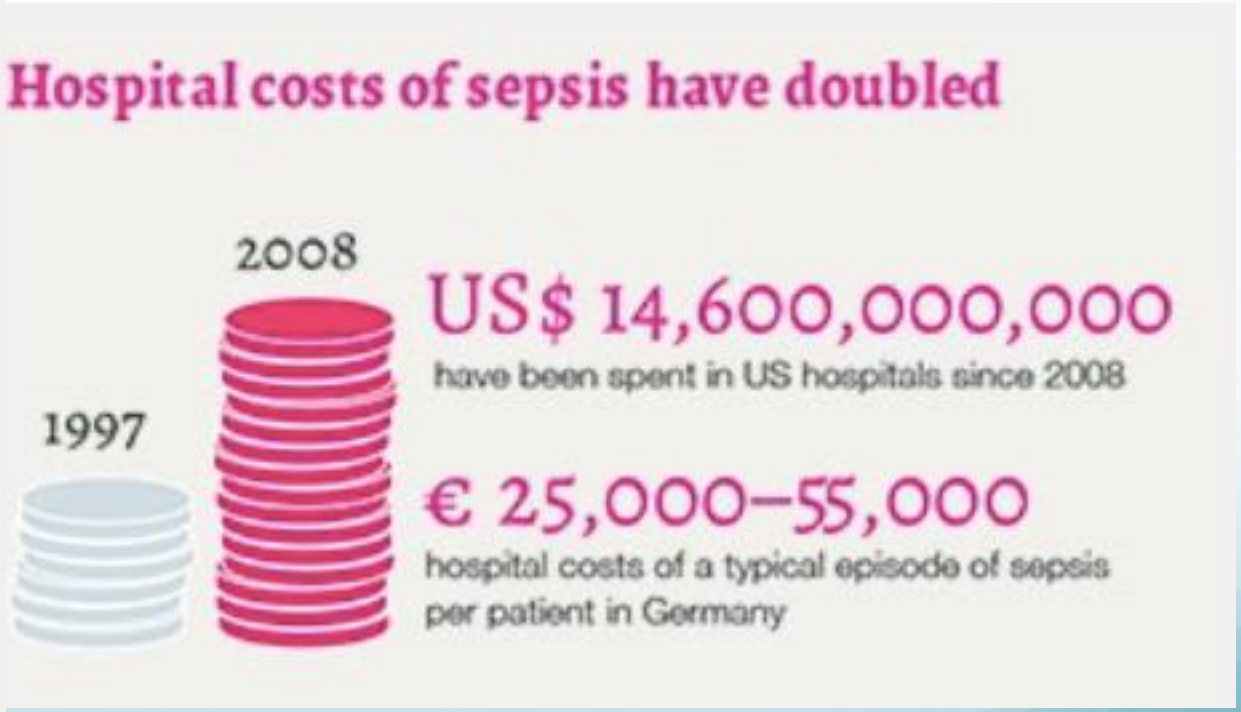
Solo alcuni numeri..

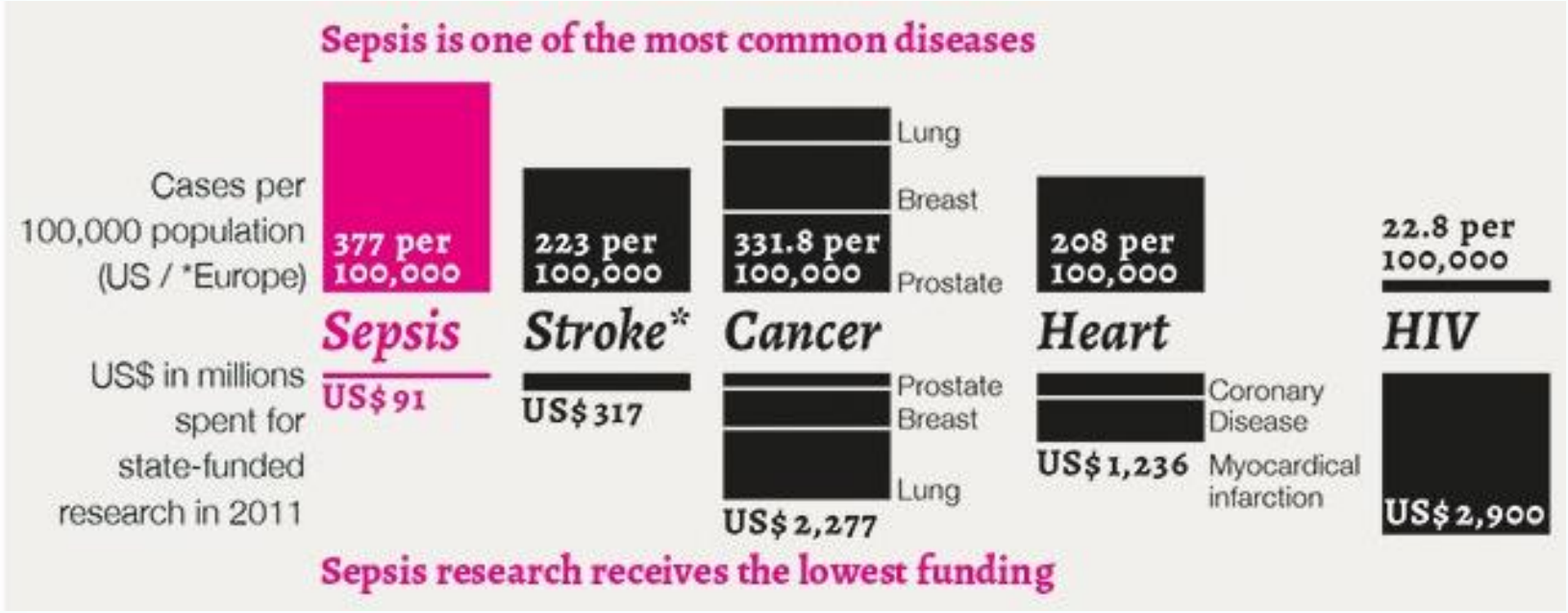
- La sepsi ha una mortalità 5 volte più alta dell'ictus e 10 volte superiore a quella dell'infarto.
- Si stima che nel mondo ogni TRE secondi un paziente muore per colpa di questa sindrome, che colpisce 27 -30 milioni di persone ogni anno.
- Nell'Unione Europea l'incidenza è elevatissima: 377 casi ogni 100mila abitanti e la sua frequenza è in aumento sia a causa dell'invecchiamento progressivo della popolazione sia a causa della tipologia di pazienti presenti nei nosocomi caratterizzati da patologie complesse e complicate da comorbilità.

4 milioni di bambini muoiono ogni anno (con incidenze maggiori nei paesi in via di sviluppo)

Ed il fenomeno è in aumento







We're working to reduce that number by 20% by 2020.

GSA
GLOBAL SEPSIS ALLIANCE

I propositi..

- Il problema è così sentito che lo scorso che tutti gli il 13 settembre si celebra "World Sepsis Day" con lo slogan "**Stop Sepsis Saves Lifes**" con il proposito di aumentare la consapevolezza della popolazione, ma stimolare anche gli addetti al miglioramento della gestione dei pazienti.
- Il proposito è ambizioso e include la riduzione del fenomeno del 20% entro il 2020.

Lotta integrata

- L’aggressione al fenomeno “sepsi” rappresenta uno degli esempi più eloquenti di lotta integrata che necessita la partecipazione attiva di varie figure del sistema sanitario a partire dall’intensivista, includendo necessariamente Microbiologo Clinico e Infettivologo ed il clinico.
- Tuttavia per conoscere il problema occorre anche misurarlo senza tralasciare nessun aspetto. Perché la conoscenza consente anche di individuare un percorso diagnostico virtuoso che ne possa contenere/ridurre gli effetti dannosi.

Quale il piano d'azione

Measure serum lactate

Obtain blood cultures prior to antibiotic administration

From the time of presentation, broad-spectrum antibiotics to be given within 1 h

Source of infection to be identified and drained within 6 h

In the event of hypotension and/or lactate >4 mmol/L (36 mg/dL):

- deliver an initial minimum of 20 mL/kg of crystalloid (or colloid equivalent)
- give vasopressors for hypotension not responding to initial fluid resuscitation to maintain mean arterial pressure ≥65 mmHg

In the event of persistent arterial hypotension despite volume resuscitation (septic shock) and/or initial lactate >4 mmol/L (36 mg/dL):

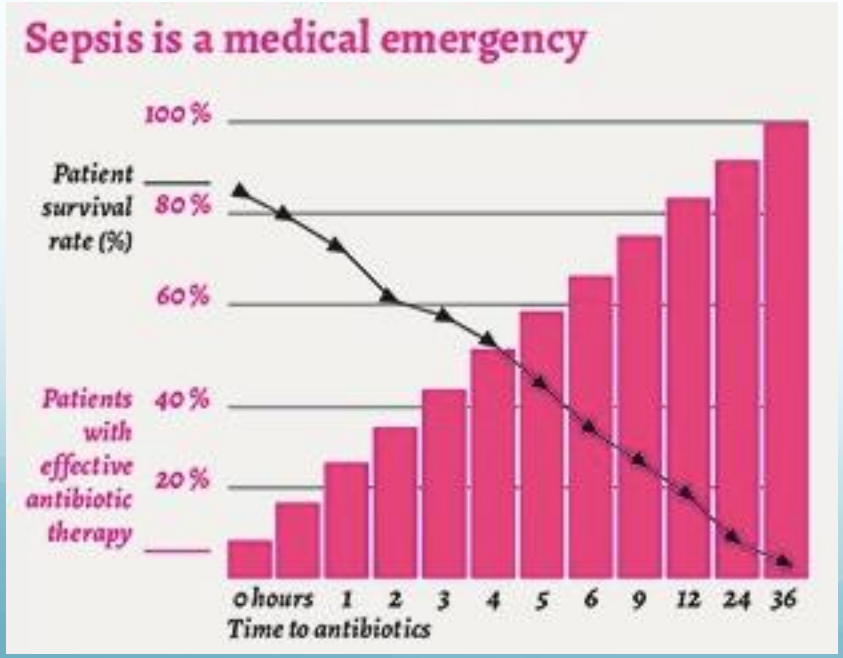
- achieve central venous pressure of ≥8 mmHg
- achieve central venous oxygen saturation of ≥70%

Intensive Care Med (2010) 36:222–231
DOI 10.1007/s00134-009-1738-3

The Surviving Sepsis Campaign Resuscitation Bundle. Source: www.survivingsepsis.org.

Cosa accade nella realtà

- Vari studi dimostrano che il tempo medio di intervento terapeutico è 119 min (79-192 min)
- Che l'approccio è empirico
- Se inadeguato mortality 61.9% versus 28.4%, P 0.001



Mortalità aumenta del 7.6%per ogni ora di ritardo nella somministrazione di antibiotici

Daniels, J Antimicrob Chemother 2011; 66

David F. Gaieski Crit Care Med 2010 Vol. 38, No. 3 1

La lotta contro il tempo: Speeding up clinical microbiology

- Il paziente settico lotta contro il tempo e con lui il clinico ed il laboratorio
- Purtroppo i tempi lunghi della coltura non ci aiutano
- Il risultato del Gram ha un tempo medio di 12 h a partire dalla raccolta del campione
- L'identificazione/ABG del patogeno 24-48 h

Quale il punto nodale…

- Disegnare un flusso di lavoro che consenta la riduzione della **latenza fra l'inizio dell'evento settico e il risultato microbiologico clinicamente utile**
- **Questo attraverso l'integrazione di tecnologie è possibile**

Integration of Technology Into Clinical Practice

Christopher D. Doern, PhD

Clin Lab Med. 2013 Sep;33(3):705-29. doi: 10.1016/j.cll.2013.03.004.

KEYWORDS

- MALDI-TOF MS • Walk away PCR • Clinical outcome(s) • Respiratory virus panel
- Immunocompromised • Decision support • Electronic medical record
- Polymerase chain reaction

KEY POINTS

- The first step in optimizing the clinical impact of new technology begins with selecting the right product for the laboratory environment.
- New technology can improve laboratory work flow but may not have the desired clinical impact if not properly implemented.
- MALDI-TOF MS promises to revolutionize the diagnosis of bacterial and fungal disease by identifying organisms faster, cheaper, and more accurately than conventional methods.
- New technological developments in molecular biology will allow laboratories without experienced molecular biologists to perform testing they previously could not.
- Passive reporting using an electronic medical record has greatly improved the transmission of laboratory information to physicians but also represents a barrier to optimal uptake of new technology.

E allora?

- Metodi molecolari (MALDI TOF based, PCR based, microarray, sequencing)
- Antibiogramma clinico

The changing role of laboratory medicine in clinical decision support during management of septicaemia. Timothy J J Inglis 2013

Mezzi alla portata di tutti

- Esistono varie strategie tutte efficaci, e per tutte le tasche

Accellerare ID e AST uso combinato di tecnologie di alto livello

Accellerare ID e AST

Accellerare la coltura e

Sub-coltura delle Emocolture Positive

Talvolta la subcoltura tradizionale fallisce…

© Med Sci Monit, 2009; 15(2): BR55-60
PMID: 19179962

www.MEDSCIMONIT.COM
Basic Research

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Accepted: 2008.06.03
Published: 2009.02.01

A novel culturing system for fluid samples

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Italy

Source of support: De

Authors' Contribution:
- **A** Study Design
- **B** Data Collection
- **C** Statistical Analysis
- **D** Data Interpretation
- **E** Manuscript Preparation
- **F** Literature Search
- **G** Funds Collection

Table 1. Comparative yields of clinically significant isolates of bacteria and yeast.

Specimens	No. of specimens (%)	No. of positive samples by routine culture	No. of negative samples by routine culture	No. of positive samples by URO-QUICK™	No. of negative samples by URO-QUICK™	*p* for URO-QUICK™ vs. routine method
ASB	106 (19.4%)	96	10	96	10	0.99
BAL	63 (11%)	58	5	58	5	0.99
Sputum	139 (25%)	134	5	136	3	0.28
Blood	47 (8%)	0	47	29	18	<0.0000005
PLEF	105 (19%)	18	87	24	81	0.30
CSF	26 (4.7%)	0	26	2	24	0.49
PERF	41 (7.5%)	14	27	16	25	0.49
Other Fluid	19 (3.4%)	3	16	6	13	0.26
Total	**546**	**323**	**223**	**367**	**179**	**0.007**

ASB – endothracheal aspirates; BAL – bronchoalveoar lavage; PLEF – pleural fluid; PERF – peritoneal fluid; CSF – cerebrospinal fluid.

Isolati ottenuti solo con HB&L non da coltura (nei 44 campioni)

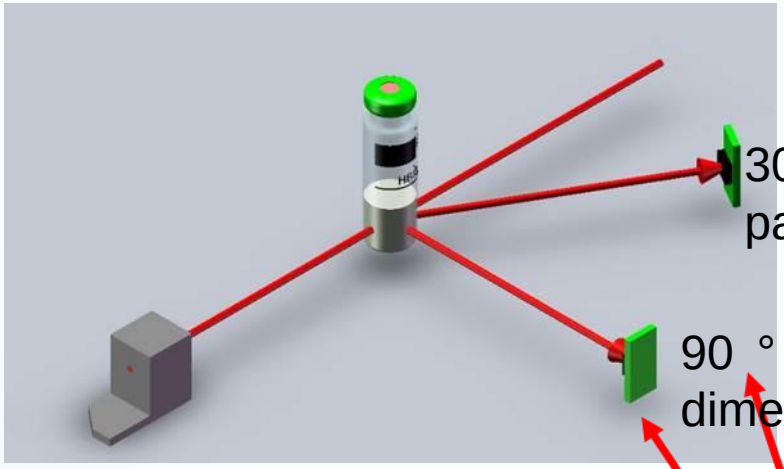
Table 2. Bacterial isolates uniquely obtained using URO-QUICK™.

Specimens	Total number of specimens (%)	Specimens uniquely positive by URO-QUICK™ (%)	Isolates (n)
ASB	106 (19.4%)	–	
BAL	63 (11%)	–	
Sputum	139 (25%)	2 (1.4%)	*Streptococcus pneumoniae, Pseudomonas aeruginosa*
Blood	47 (8%)	29 (61%)	*Sphingomonas paucimobilis, Serratia marcesens, Staphylococcus hominis, Staphylococcus epidermidis SCV* (n=5)*, *Staphylococcus aureus* (n=2), *P. aeruginosa* (n=2), *Oligella ureolyticum, Micrococcus luteus, Moraxella lacunata, Enterococcus faecalis* (n=2), *Escherichia coli* (n=3), *Corynebacterium propinquum, Corynebacterium jeikeium* (n=2), *Clostridium tyrobutiricum, Candida albicans* (n=2), *Campylobacter jejunii, Acinetobacter haemolyticus*
PLEF	105 (19%)	6 (5.7%)	*S. aureus, Rhodotorula glutinis, Bacillus pumilis, Burkholderia cepacia* (n=2), *Burkholderia gladioli*
CSF	26 (4.7%)	2 (7.6%)	*E. coli, S. aureus*
PERF	41 (7.5%)	2 (7.8%)	*Gemella morbillorum, Enterococcus avium*
Other Fluids**	19 (3.4%)	3 (15.8%)	*Acinetobacter baumanni-Klebsiella pneumoniae, Pasteurella multocida, S. aureus*
Total	546	**44 (8.0%)**	

ASB – endothracheal aspirates; BAL – bronchoalveolar lavage; PLEF – pleural fluid; PERF – peritoneal fluid; CSF – cerebrospinal fluid.
* Small colony variant (SCV); ** Other fluids (19 in total) included synovial fluid (n=5), ascitic fluid (n=9), drainage of infected central venous catheters (n=3), abdominal drainage (n=1), and cholecystic fluid (n=1).

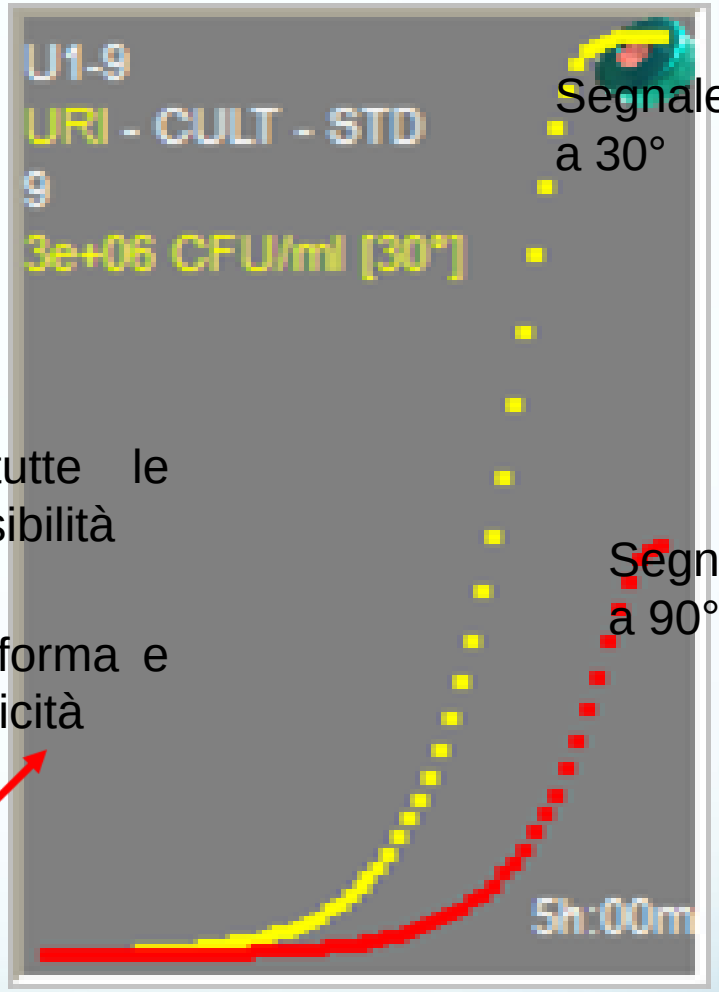
Ma il sistema del light scattering in tutte le sue versioni….

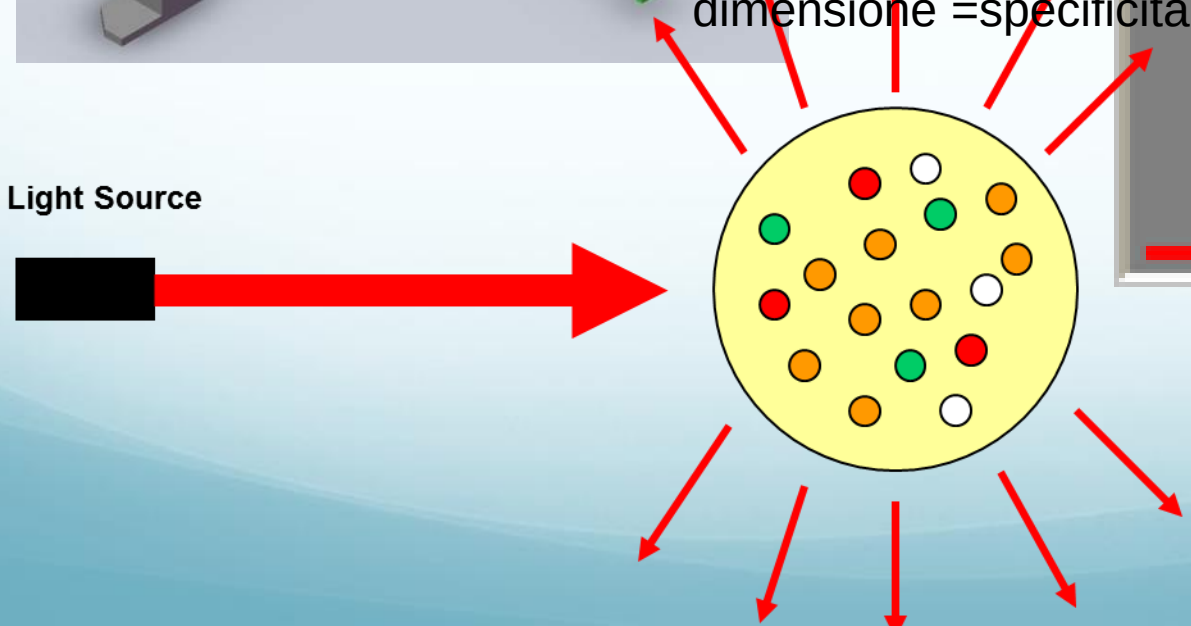
Aiuta anche nell’accellerare e aumentare la carica microbica utile a partire dai nostri brodi di coltura ci pone nella condizione d’eseguire in 1-2h un ID tradizionale (o un ID MALDI TOF based) o meglio ancora un antibiogramma sia esso tradizionale o accellerato



30 ° rileva tutte le particelle = Sensibilità

90 ° selettivo per forma e dimensione =specificità






HB&L


autoflex speed

ANTIBIOGRAMMA ALIFAX SU CAMPIONI POSITIVI DI EMOCOLTURE

1. SELEZIONE CAMPIONE POSITIVO ED ESAME AL MISCROSCOPIO

2. CENTRIFUGAZONE DI 2 ml DI EMOCOLTURA

5. RISULTATI IN 3 ORE

DI ANTIBIOTICI PERSONALIZZATO IN BASE A:

- Indicazione Gram
- Dati sull' antibiotico somministrato
- Dati riguardo il protocollo terapeutico e linee guida

Arrivare prima con la farmaco

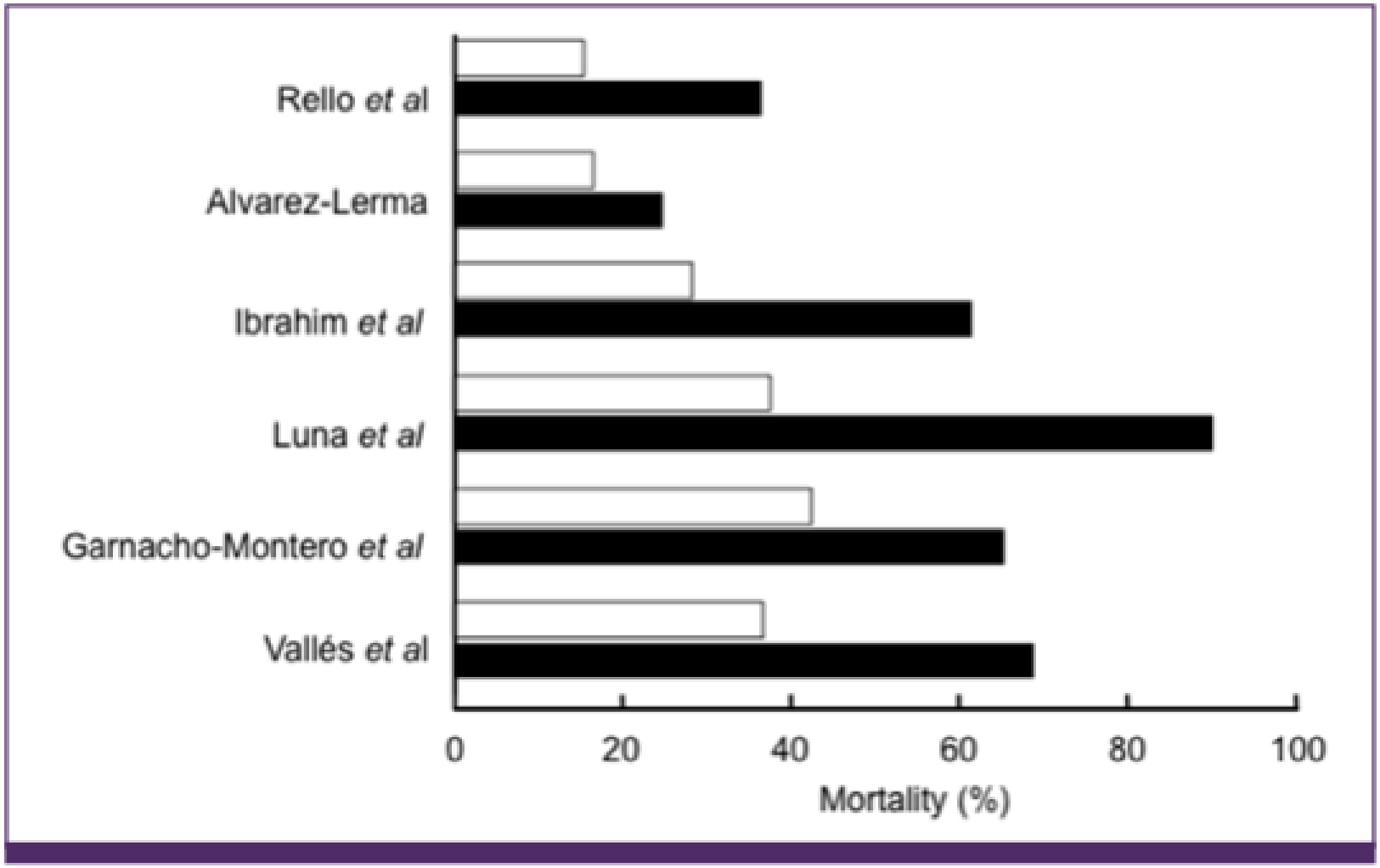
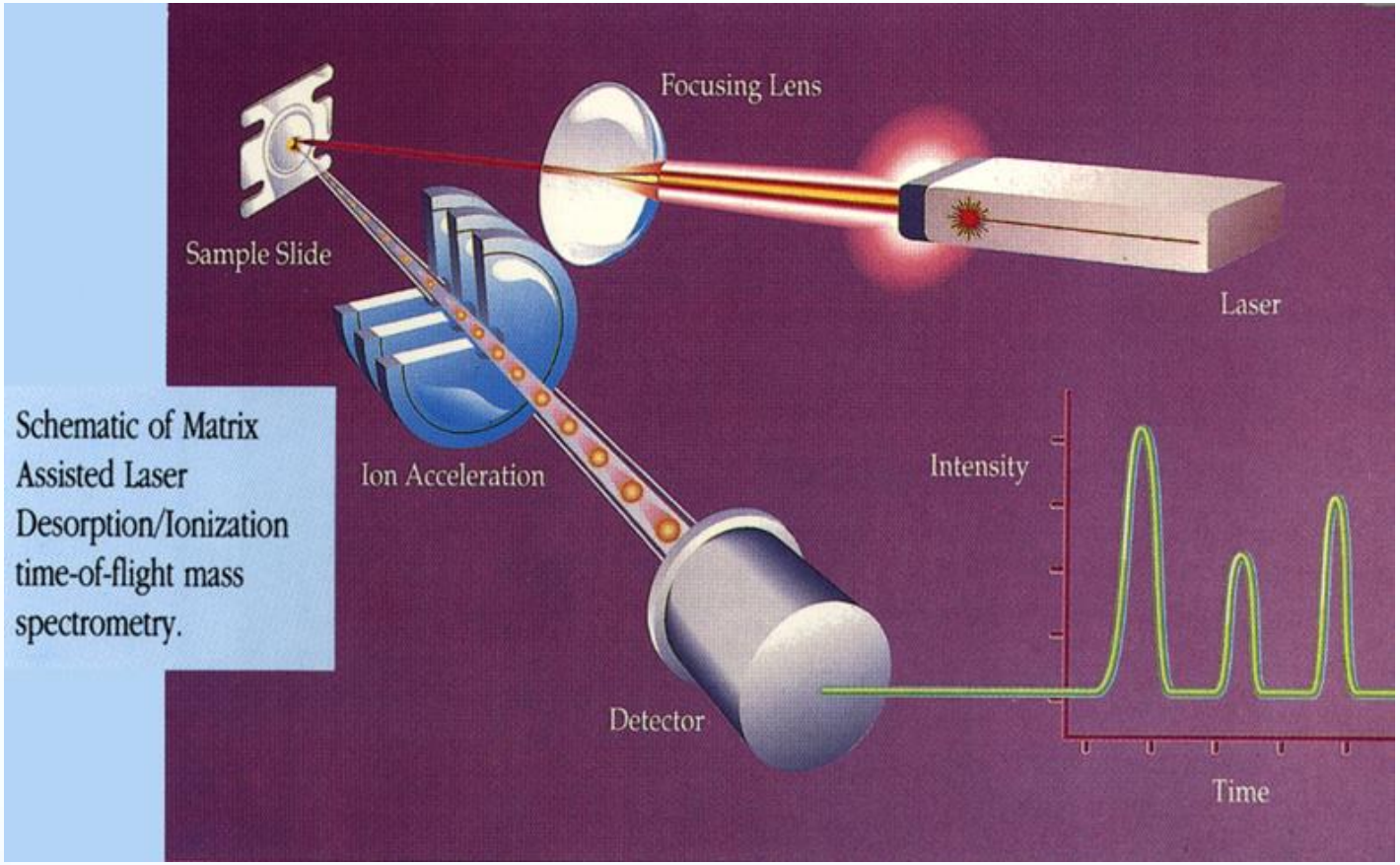


Figure 1. Outcomes in severe bacterial infection in relation to appropriateness of empirical therapy.

Open bars, appropriate therapy; black, inappropriate. Data are from references [11-16].

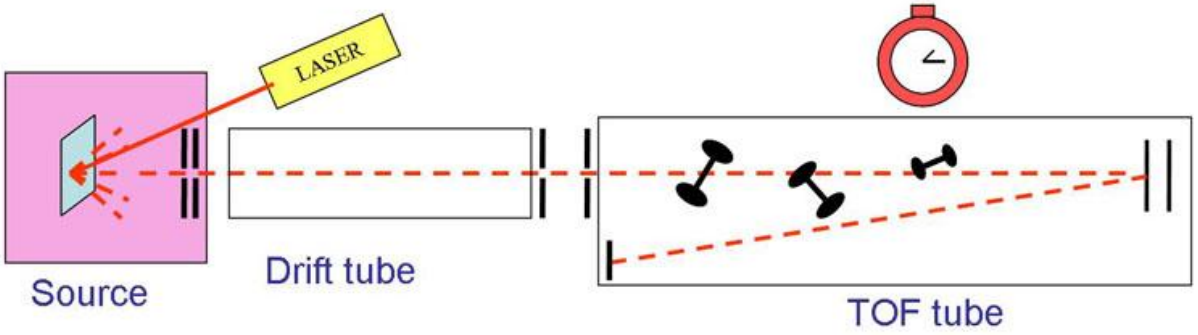
Novità introdotte

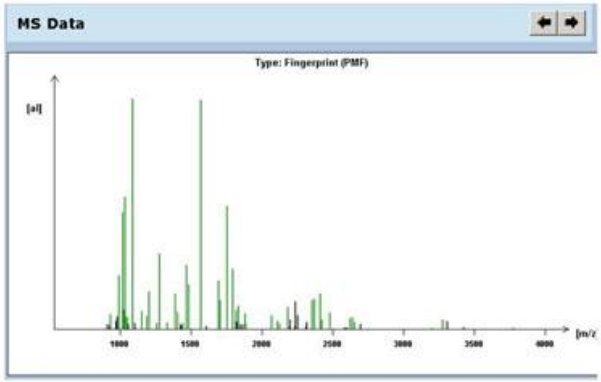
- Maldi TOF



Il principio su cui si basa la spettrometria di massa è la possibilità di separare una miscela di ioni in funzione del loro rapporto massa/carica generalmente tramite campi magnetici statici o oscillanti. Tale miscela è ottenuta ***ionizzando*** *le molecole del campione, principalmente* ***facendo loro attraversare un fascio di elettroni*** *ad energia nota. Le molecole così ionizzate sono instabili e* ***si frammentano in ioni più leggeri secondo schemi tipici in funzione della loro struttura chimica****.*

MALDI – TOF


LASER
Source
Drift tube
TOF tube


MS Data
Type: Fingerprint (PMF)
[a]
[m/z]
1000
1500
2000
2500
3000
3500
4000

Il suo impatto

OPEN ACCESS Freely available online

PLOS ONE

Gram-Stain Plus MALDI-TOF MS (Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry) for a Rapid Diagnosis of Urinary Tract Infection

Almudena Burillo[1,2*], Belén Rodríguez-Sánchez[1,4], Ana Ramiro[1], Emilia Cercenado[1,2,3,4], Marta Rodríguez-Créixems[1,3,4], Emilio Bouza[1,2,3,4]

1 Department of Clinical Microbiology & Infectious Diseases, Hospital General Universitario Gregorio Marañón - Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Madrid, Spain, 2 Facultad de Medicina, Universidad Complutense de Madrid (UCM), Madrid, Madrid, Spain, 3 Red Española de Investigación en Patología Infecciosa (REIPI RD06/0008/1025), Sevilla, Sevilla, Spain, 4 CIBER de Enfermedades Respiratorias (CIBERES CB06/06/0058), Palma de Mallorca, Islas Baleares, Spain

Abstract

Microbiological confirmation of a urinary tract infection (UTI) takes 24–48 h. In the meantime, patients are usually given empirical antibiotics, sometimes inappropriately. We assessed the feasibility of sequentially performing a Gram stain and MALDI-TOF MS mass spectrometry (MS) on urine samples to anticipate clinically useful information. In May-June 2012, we randomly selected 1000 urine samples from patients with suspected UTI. All were Gram stained and those yielding bacteria of a single morphotype were processed for MALDI-TOF MS. Our sequential algorithm was correlated with the standard semiquantitative urine culture result as follows: Match, the information provided was anticipative of culture result; Minor error, the information provided was partially anticipative of culture result; Major error, the information provided was incorrect, potentially leading to inappropriate changes in antimicrobial therapy. A positive culture was obtained in 242/1000 samples. The Gram stain revealed a single morphotype in 207 samples, which were subjected to MALDI-TOF MS. The diagnostic performance of the Gram stain was: sensitivity (Se) 81.3%, specificity (Sp) 93.2%, positive predictive value (PPV) 81.3%, negative predictive value (NPV) 93.2%, positive likelihood ratio (+LR) 11.91, negative likelihood ratio (−LR) 0.20 and accuracy 90.0% while that of MALDI-TOF MS was: Se 79.2%, Sp 73.5, +LR 2.99, −LR 0.28 and accuracy 78.3%. The use of both techniques provided information anticipative of the culture result in 82.7% of cases, information with minor errors in 13.4% and information with major errors in 3.9%. Results were available within 1 h. Our serial algorithm provided information that was consistent or showed minor errors for 96.1% of urine samples from patients with suspected UTI. The clinical impacts of this rapid UTI diagnosis strategy need to be assessed through indicators of adequacy of treatment such as a reduced time to appropriate empirical treatment or earlier withdrawal of unnecessary antibiotics.

Impact of Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry on the Clinical Management of Patients With Gram-negative Bacteremia: A Prospective Observational Study

CID 2013:56 (15 April)

Olivier Clerc,[1] Guy Prod'hom,[2] Christelle Vogne,[2] Alain Bizzini,[2] Thierry Calandra,[1] and Gilbert Greub[1,2]

[1]Infectious Diseases Service and [2]Institute of Microbiology, Lausanne University Hospital Center and University of Lausanne, Switzerland

Conclusions. In a low prevalence area for extended spectrum betalactamases (ESBL) and multiresistant gram-negative bacteria, MALDI-TOF performed on blood culture pellets had an impact on the clinical management of 35.1% of all gram-negative bacteremia cases, demonstrating a greater impact than Gram stain reporting. Thus, MALDI-TOF could become a vital second step beside Gram stain in guiding the empirical treatment of patients with bloodstream infection.

Quanto impatta un risultato sulla modifica/assestamento della terapia e quindi outcome del paziente.
Tanto più precoce e tanto più specifico = migliore terapia

Table 3. Impact of Sequential Gram Stain and MALDI-TOF Reporting

Impact of the Sequential Reporting	N = 202
Gram stain	42 (20.8)
Streamlining	16 (7.9)
Spectrum broadening	16 (7.9)
Introduction of empirical antibiotic therapy	10 (5.0)
MALDI-TOF MS	71 (35.1)
Streamlining	22 (10.9)
Spectrum broadening	31 (15.3)
Introduction of focused empirical antibiotic therapy	18 (8.9)

Percentuale di impatto

Can Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF) Enhance Antimicrobial Stewardship Efforts in the Acute Care Setting?

Pranita D. Tamma, MD, MHS;[1] Kennard Tan, MD;[2]
Veronique R. Nussenblatt, MD, MHS;[3]
Alison E. Turnbull, DVM, MPH;[4] Karen C. Carroll, MD;[5]
Sara E. Cosgrove, MD, MS[3]

Infect Control Hosp Epidemiol 2013;34(9):990-995

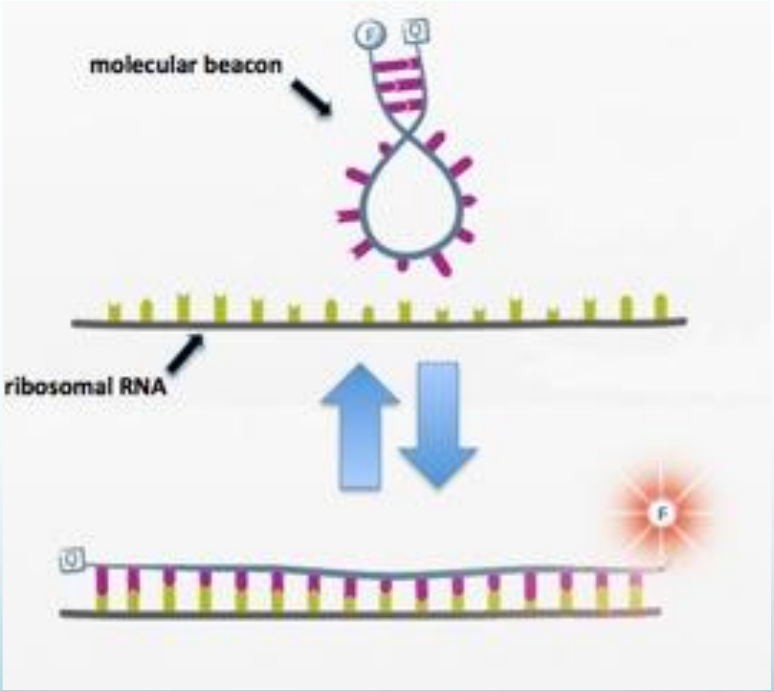
In summary, not only is MALDI-TOF fast, reliable, and cost-effective,[1] but it has the potential to impact time to appropriate therapy for a significant proportion of hospitalized patients with culture-proven infections. MALDI-TOF may replace conventional organism identification techniques in

Ma a metà della scala…

- FISH technology

Di cosa si tratta

- bbFISH è l'acronimo di beacon based FISH (***Fluorescent in situ hybridization***)



Il target

- Porzioni conservate 16S rDNA
- Si esegue direttamente su campione di emocoltura positiva
- Tempo di processazione circa 30-45 min
- Grande vantaggio, rispetto alla FISH tradizionale i tempi/temperatura di annealing e le caratteristiche insite nei beacon che **non annealati si richiudono** su se stessi e evitano i problemi di interferenza aspecifica nella fluorescenza (**mancano i lavaggi**)

Esperienza bbFISH

- Abbiamo condotto, sempre nell'ottica di contrarre i tempi di refertazione delle emocolture uno studio, in parallelo fra il nostro centro ed un altro ospedale del Nord Italia
- Si trattava di provare e di calare nelle realtà dei due ospedali un innovativo sistema basato sulla tecnologia bbFISH

I saggi

- In totale nei due centri sono state processate of 558 emocolture (377 positive e 181 negative)

positiva

negativa

Ma quale il goal dello studio

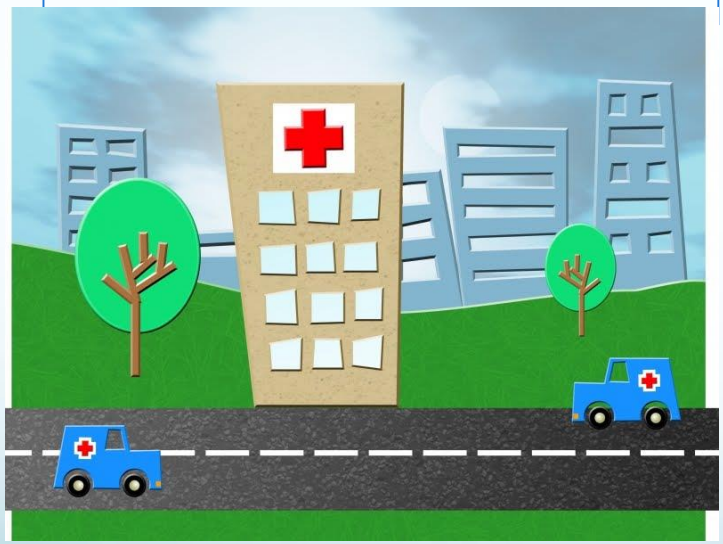
- Oltre a valutare le performances del sistema
- Vedere come la sua introduzione agisse sul TAT di centri con caratteristiche diverse

I due centri

Uno aperto H 12 (7.30-20.00)

7 giorni su 7

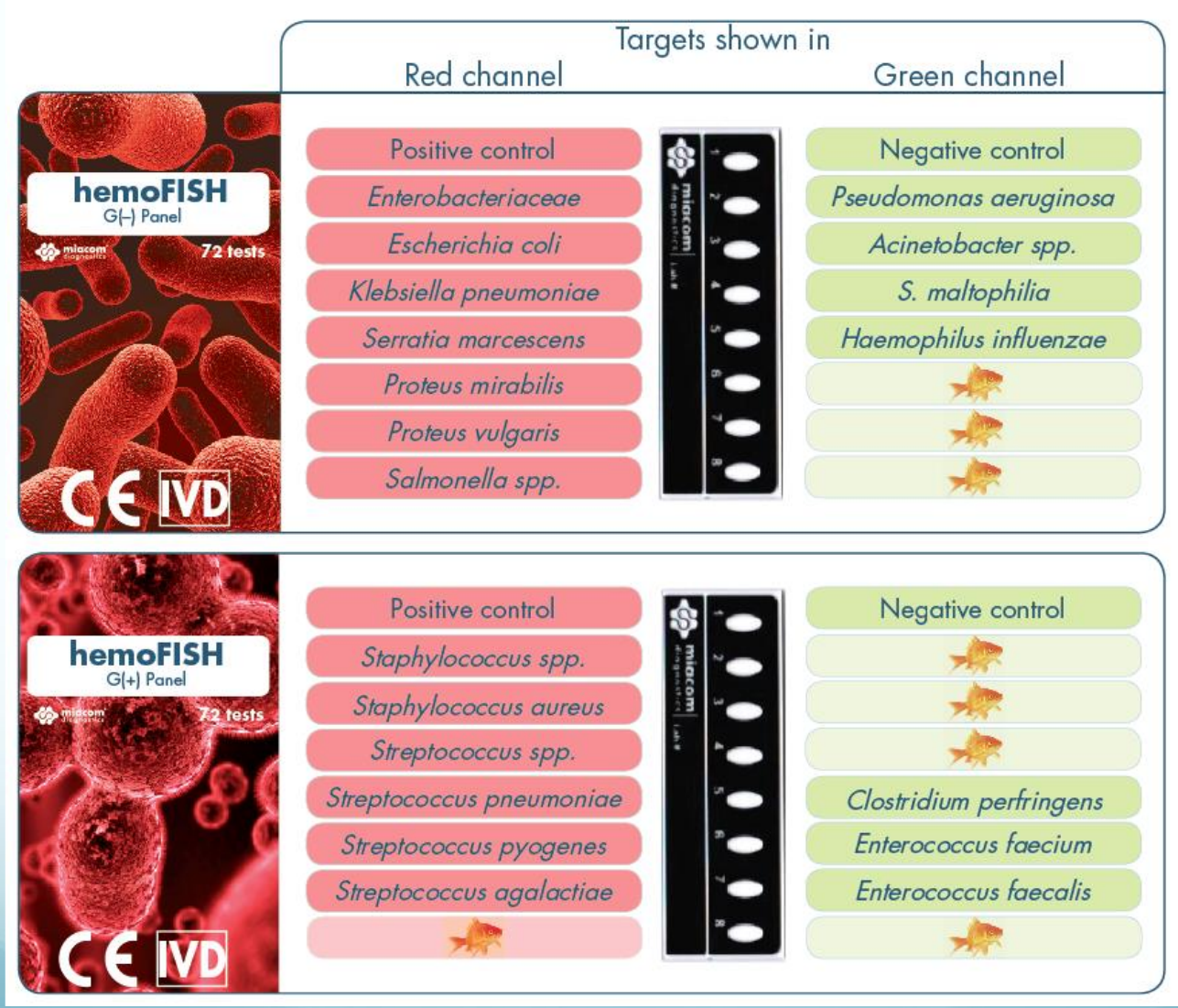
Ma laureato assente la domenica



Uno aperto 8.00-15.00 dal lunedì al venerdì

BMC Microbiol. 2014 Apr 22;14(1):99. doi: 10.1186/1471-2180-14-99.
Beacon-based (bbFISH®) technology for rapid pathogens identification in blood cultures.
Sakarikou C, Parisato M, Cascio GL, Fontana C1.

Il metodo


Targets shown in
Red channel
Green channel
hemoFISH
G(–) Panel
72 tests
CE IVD
Positive control
Enterobacteriaceae
Escherichia coli
Klebsiella pneumoniae
Serratia marcescens
Proteus mirabilis
Proteus vulgaris
Salmonella spp.
Negative control
Pseudomonas aeruginosa
Acinetobacter spp.
S. maltophilia
Haemophilus influenzae
hemoFISH
G(+) Panel
72 tests
CE IVD
Positive control
Staphylococcus spp.
Staphylococcus aureus
Streptococcus spp.
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus agalactiae
Negative control
Clostridium perfringens
Enterococcus faecium
Enterococcus faecalis

Workflow

Per evidenziare le criticità dell'assenza del microbiologo dal laboratorio

- Il primo centro ha arruolato tutte le emocolture consecutivamente positive (incluse quelle positivizzate la domenica)
- Il secondo centro ha incluso solo quelle positivizzate durante la presenza del laureato
- Questa differenza che poteva sembrare un errore ci ha in realtà consentito di valutare il reale impatto del metodo

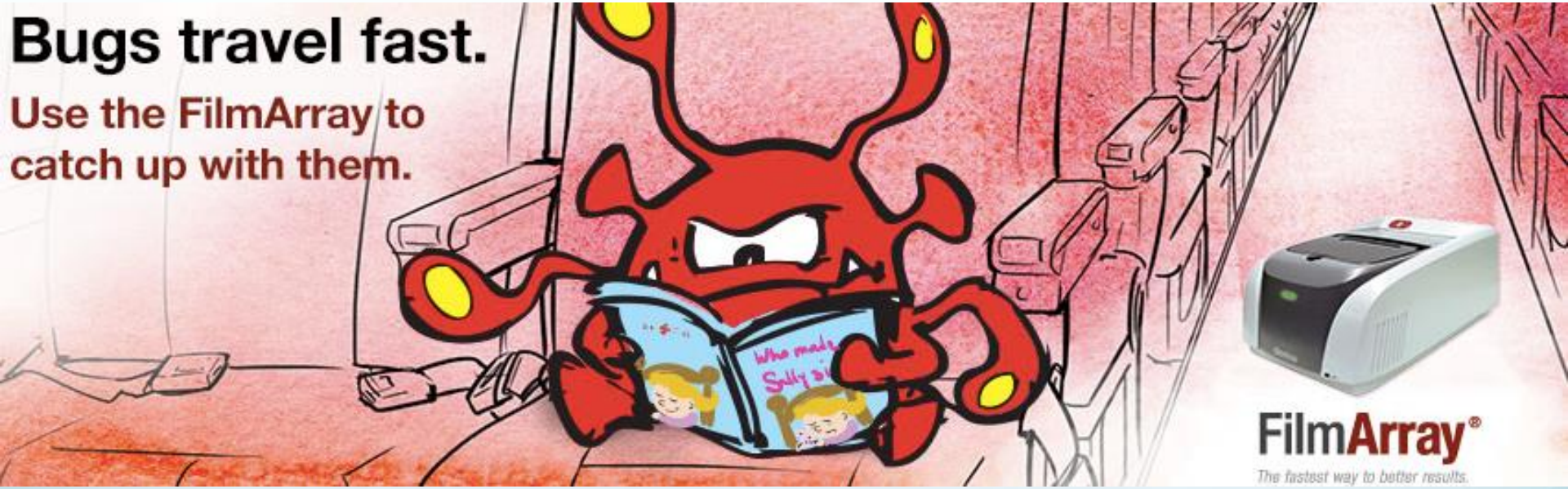
I risultati

Turn Around TimeExpressed in hours	Hospital of Rome	Hospital of Verona	Mean value between the hospitals
Average **TAT** bbFish® (h)	8.9	1.5	5.2
Average **TAT** of traditional culture method (h)	38.8	48.5	43.65
Two tailed p-value	0.0001		
Δ (earlier diagnosis) (h)	**29.9**	**47.0**	**38.45**

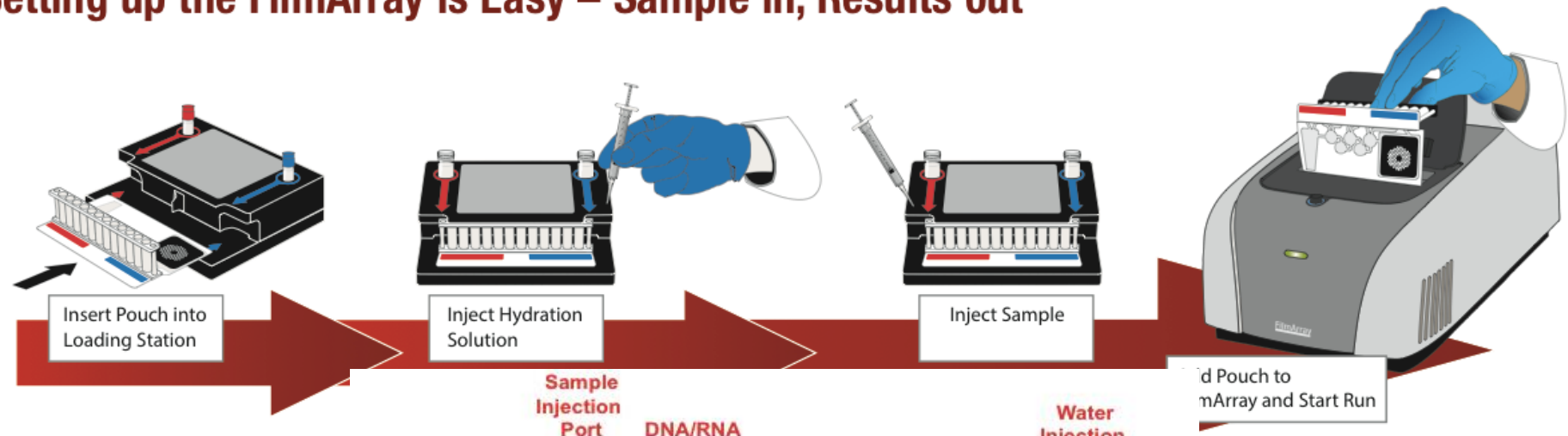
Sakarikou C, Parisato M Lo Cascio G, Fontana C.
BBFISH technology for rapid pathogens identication in Blood Culture. BMC Microbiology. 2014

Sul penultimo gradino

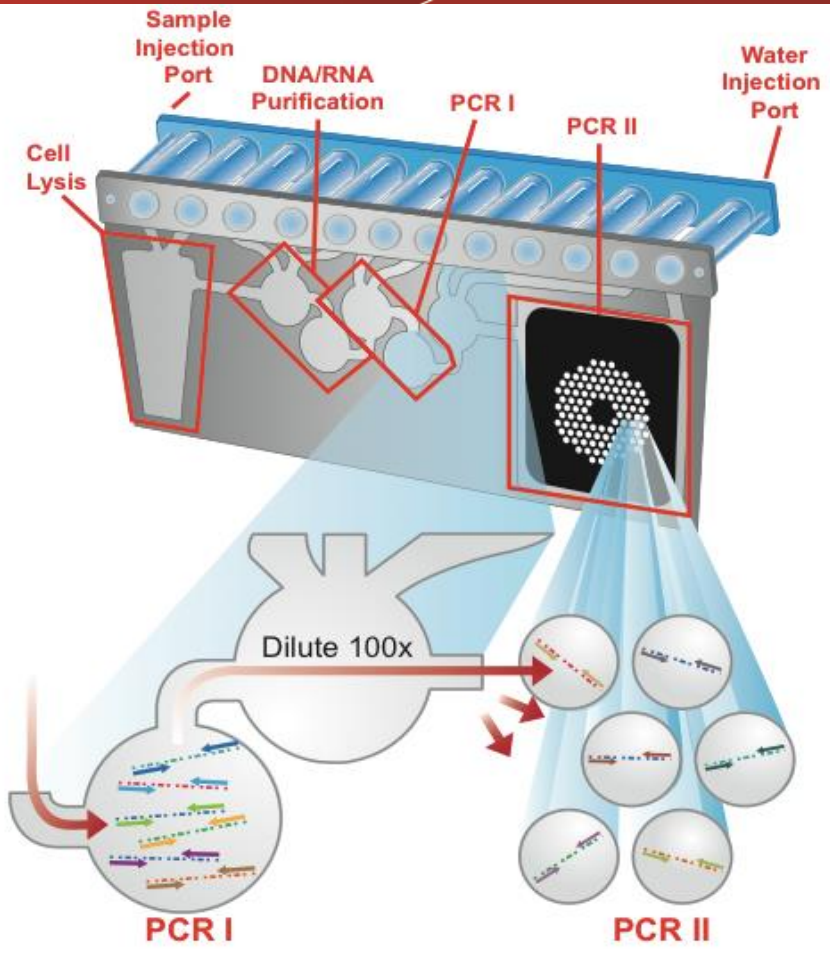
- Film Array



Setting up the FilmArray is Easy – Sample in, Results out



The FilmArray Pouch



Gram-positive bacteria

Enterococcus
Listeria monocytogenes
Staphylococcus
Staphylococcus aureus
Streptococcus
Streptococcus agalactiae
Streptococcus pneumoniae
Streptococcus pyogenes

Gram-negative bacteria

Acinetobacter baumannii
Haemophilus influenzae
Neisseria meningitidis
Pseudomonas aeruginosa
Enterobacteriacea
Enterobacter cloa
Escherichia coli
Klebsiella oxytoca
Klebsiella pneumo
Proteus
Serratia marcesce

Yeast

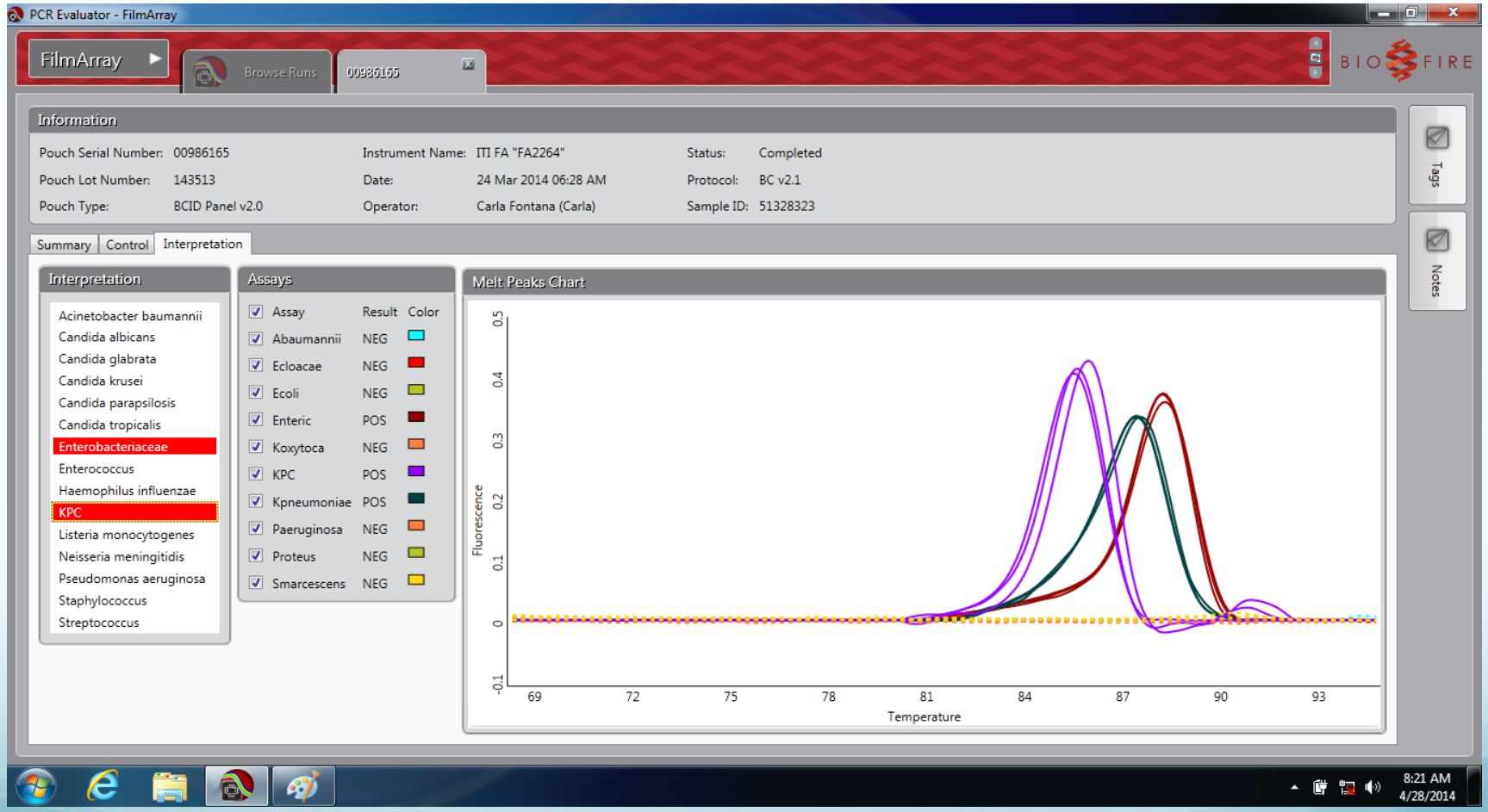
Candida albicans
Candida glabrata
Candida krusei
Candida parapsilosis
Candida tropicalis

Antimicrobial resistance genes

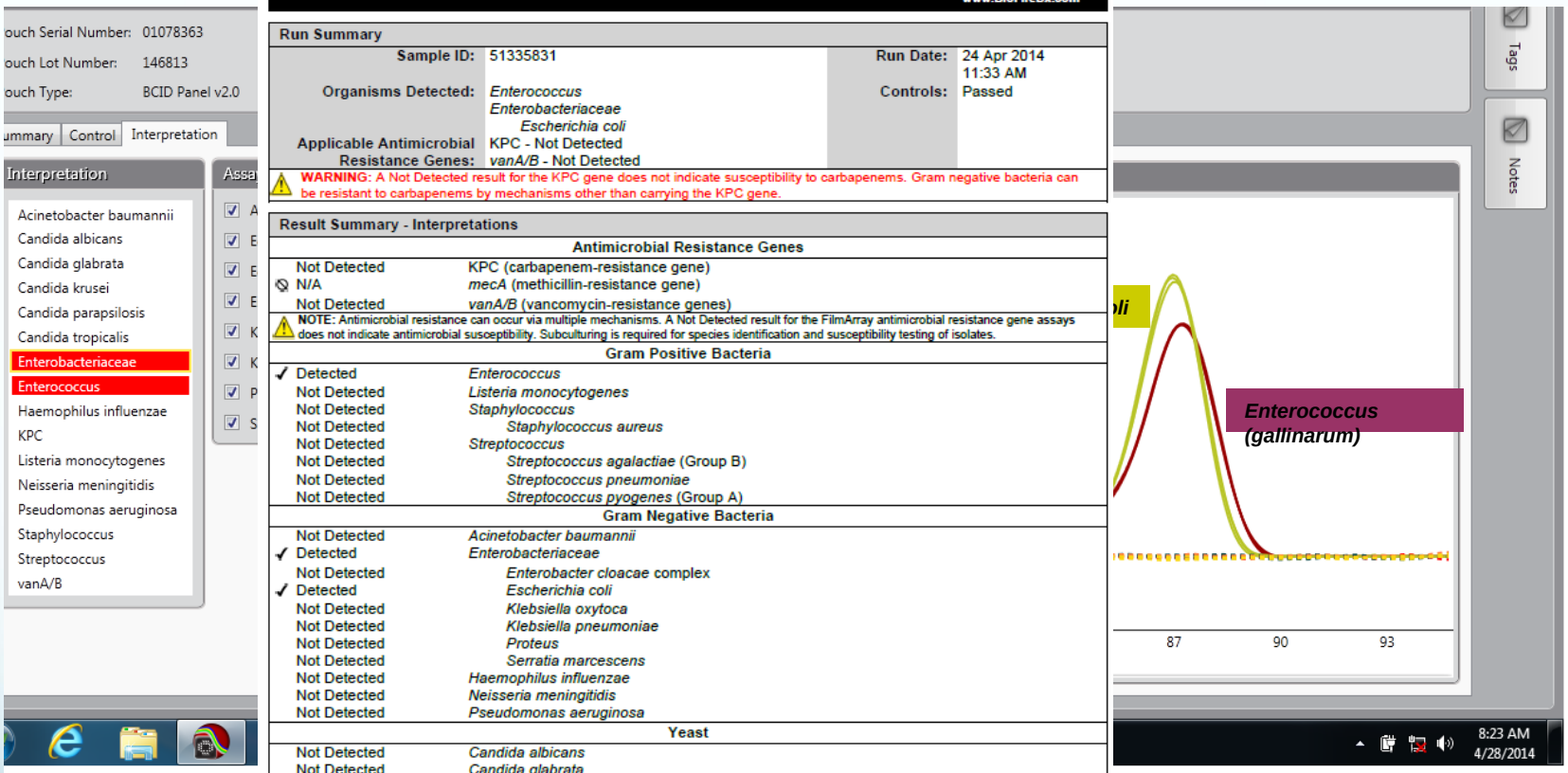
mecA - methicillin resistance
vanA/B - vancomycin resistance
KPC - carbapenem resistance

Sapere che in nostro germe si chiama *K.pneumoniae* e che KPC produttore in un'ora dalla positività del flacone impatta sulla terapia in maniera importante!

Indicazioni rapide su AST



Emocoltura mista



FilmArray® BCID Panel

BIOFIRE

www.BioFireDx.com

Run Summary

Sample ID:	51335831	**Run Date:**	24 Apr 2014 11:33 AM
Organisms Detected:	*Enterococcus* *Enterobacteriaceae* *Escherichia coli*	**Controls:**	Passed
Applicable Antimicrobial Resistance Genes:	KPC - Not Detected *vanA/B* - Not Detected		

⚠ **WARNING**: A Not Detected result for the KPC gene does not indicate susceptibility to carbapenems. Gram negative bacteria can be resistant to carbapenems by mechanisms other than carrying the KPC gene.

Result Summary - Interpretations

Antimicrobial Resistance Genes	
Not Detected	KPC (carbapenem-resistance gene)
⦸ N/A	*mecA* (methicillin-resistance gene)
Not Detected	*vanA/B* (vancomycin-resistance genes)
⚠ **NOTE**: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for the FilmArray antimicrobial resistance gene assays does not indicate antimicrobial susoeptibility. Subculturing is required for species identification and susceptibility testing of isolates.	
Gram Positive Bacteria	
✓ Detected	*Enterococcus*
Not Detected	*Listeria monocytogenes*
Not Detected	*Staphylococcus*
Not Detected	*Staphylococcus aureus*
Not Detected	*Streptococcus*
Not Detected	*Streptococcus agalactiae* (Group B)
Not Detected	*Streptococcus pneumoniae*
Not Detected	*Streptococcus pyogenes* (Group A)
Gram Negative Bacteria	
Not Detected	*Acinetobacter baumannii*
✓ Detected	*Enterobacteriaceae*
Not Detected	*Enterobacter cloacae* complex
✓ Detected	*Escherichia coli*
Not Detected	*Klebsiella oxytoca*
Not Detected	*Klebsiella pneumoniae*
Not Detected	*Proteus*
Not Detected	*Serratia marcescens*
Not Detected	*Haemophilus influenzae*
Not Detected	*Neisseria meningitidis*
Not Detected	*Pseudomonas aeruginosa*
Yeast	
Not Detected	*Candida albicans*
Not Detected	*Candida glabrata*
Not Detected	*Candida krusei*
Not Detected	*Candida parapsilosis*
Not Detected	*Candida tropicalis*

Run Details

Pouch:	BCID Panel v2.0	**Protocol:**	BC v2.1
Run Status:	Completed	**Operator:**	Carla Fontana (Carla)
Serial No.:	01078363	**Instrument:**	ITI FA "FA2264"
Lot No.:	146813		

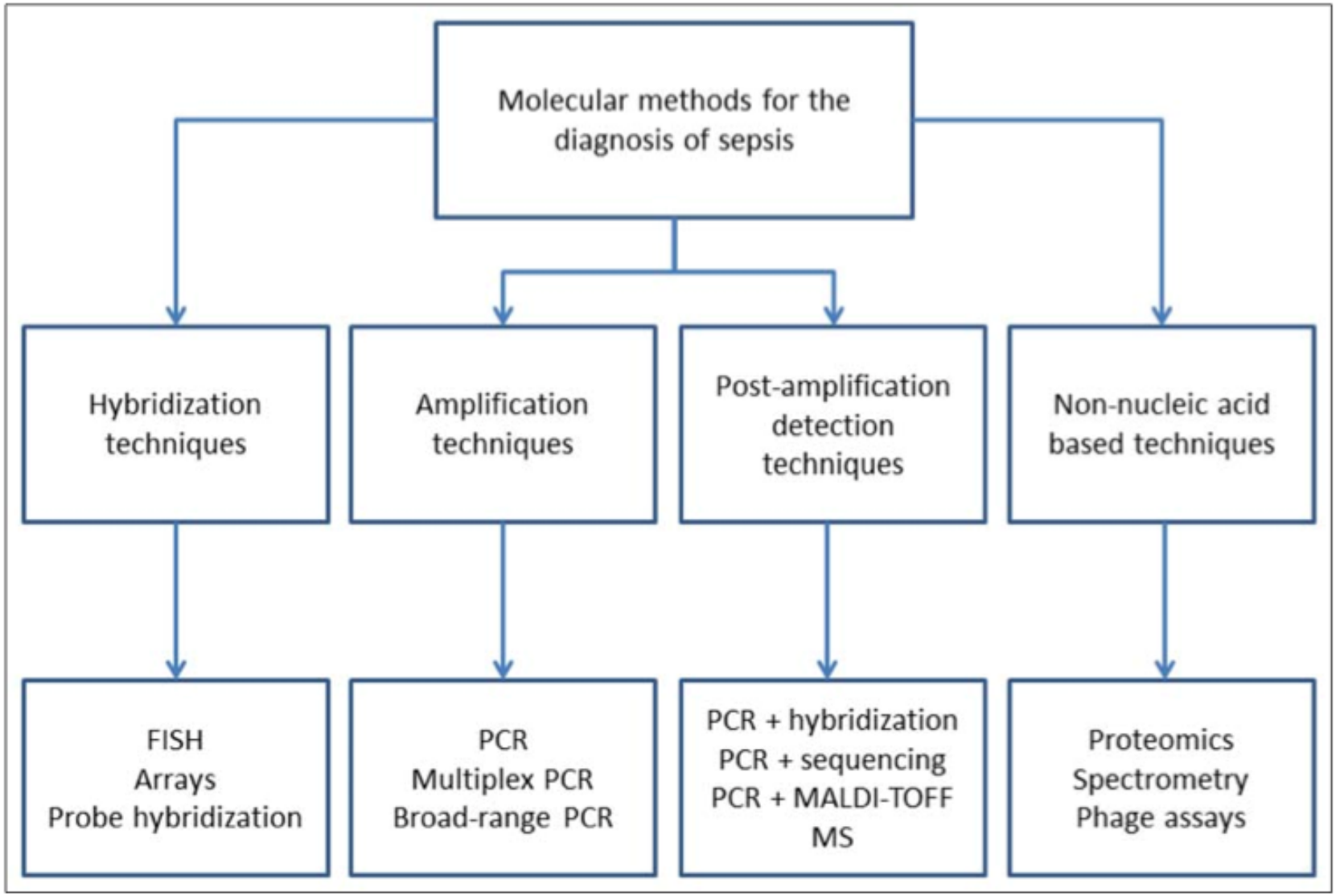


Fig. 1. Diagnostic techniques for the diagnosis of sepsis (modified after [21])

A partire da BC o sangue

Table 1. Commercially available molecular assays for the diagnosis of sepsis using positive blood cultures or whole blood as sample type

		ria, resistance genes			
B. Using whole blood					
Xpert MRSA/SA	Real-time PCR	2	75–100	98.4–99.4	[64, 170]
SeptiFast	Multiplex real time PCR assay for bacterial and fungal pathogens	25, plus mecA as reflex test	60–95	74–99	See Table 2
VYOO	Multiplex PCR with gel electrophoresis	34plus mec A, vanA/ B/C, SHV, CTX-M	30–51	n.d.	[85]
SepsiTest	Broad-range PCR with sequencing	>300 pathogens	61–88.5	83.5–85.8	[57, 91, 92]

*Concordance with blood culture-dependent assays; n.d., not determined

StaphPlex	Multiplex PCR plus microarray	1	100	95.5–100	[61]
Staph SR	Multiplex PCR assay	1 plus mecA	50–100	86.8–98.4	[62, 63]
MALDI-TOF	Matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry	Hundreds	76–80	96–100	[20, 75]
Prove-it sepsis	Multiplex PCR and microarray	50 plus mec A gene			
Verigene	Nucleic-acid-based microarray	Gram-positive or -negative bacteria, resistance genes	92–96	n.d.	[86–88]
Filmarray	PCR	Gram-positive or Gram-negative bacte-ria, resistance genes	91	n.d.	[85]

Con dive

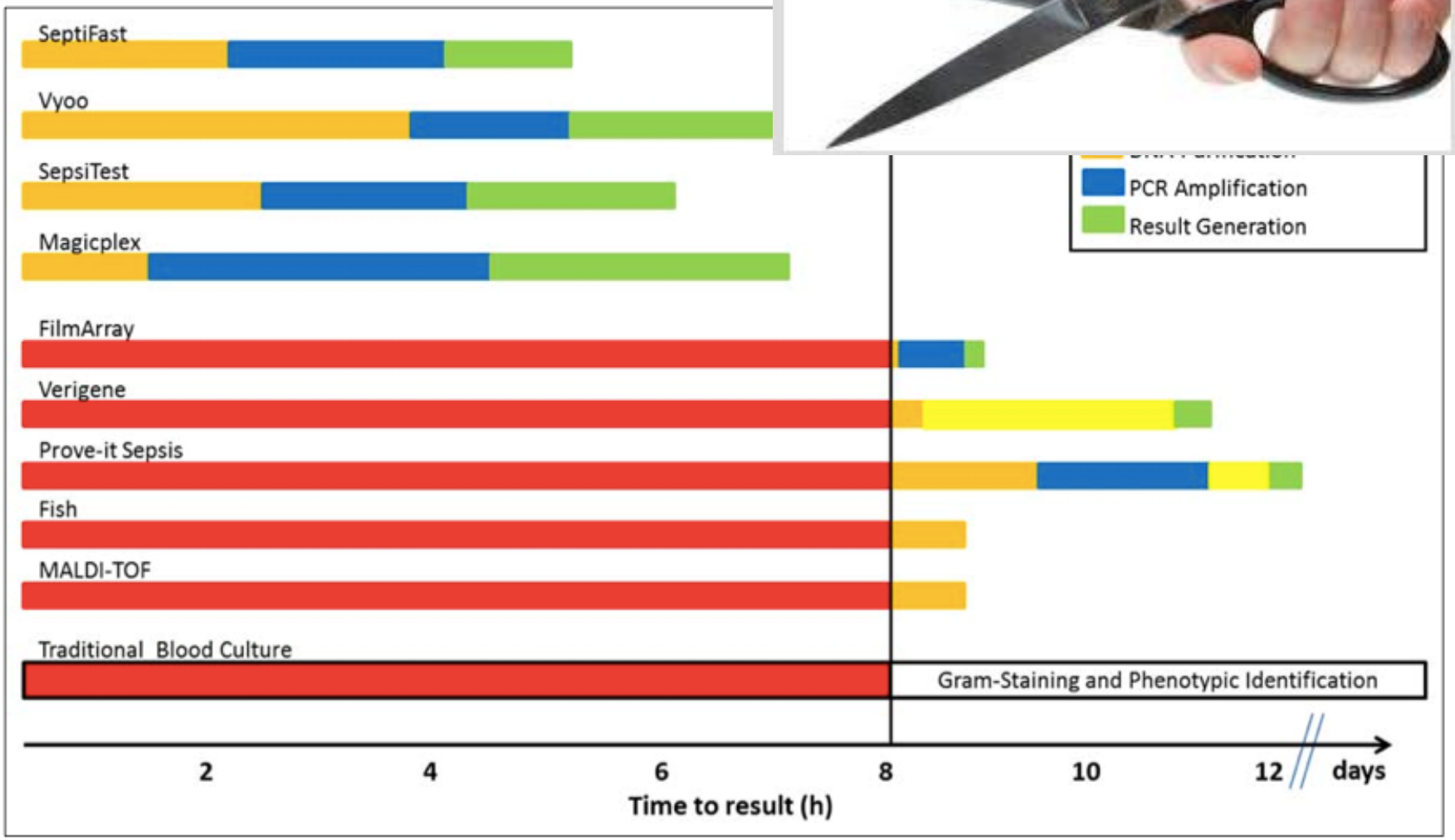


Fig. 2. Time to result of selected blood culture-dependent and blood culture-independent technologies for the diagnosis of sepsis; the vertical line indicates the duration of blood culture (fixed at 8 h or longer for this figure)

Che impattano su vari aspetti

Table 3. Patient outcome and hospital costs for patients with bloodstream infection treated using routine medical management with or without the SeptiFast test*

	Routine management (Mean ± *SD*)	Routine management plus SeptiFast (Mean ± *SD*)	*p* value
28-day mortality	13 (27%)	14 (26%)	n.s.
6-month mortality	20 (37%)	20 (41.6%)	n.s.
Stay in ICU	31.0 ± 19.4	22.9 ± 29.9	<0.05
Stay in hospital	21.3 ± 23.4	18.3 ± 21.4	<0.05
Stay in ICU survivors	24.1 ± 21.9	18.3 ± 11.4	<0.05
Number of antibiotics used per patient	5.1 ± 3.1	4.2 ± 2.2	<0.05
Antibiotic treatment cost per patient	3576 €	2812 €	<0.05
Cost of ICU stay	32798 €	24246 €	<0.05
Cost of ward stay	5824 €	4988 €	<0.05
Total cost	42198 €	32228 €	<0.05

n.s., not significant
*Modified after ref. [17]

European Journal of Microbiology and Immunology 4 (2014) 1

Metodi molecolari

- Facendo un distinguo importante:
  - **Quelli da flacone positivo** (FISH, MALDI TOF, MICROARRAY ecc)
  - **Quelli diretti da sangue** (Real Time PCR e derivati)

Salto nel futuro

Da emocoltura

- Oltre al film array
- GenMark (e-sensor)
- Master diagnostica

Bioelectric Detection of DNA and the Automation of Molecular Diagnostics

By Daniel H. Farkas, Ph.D., HCLD

Clinical Micro Sensors, Inc., Pasadena, California

Clinical molecular diagnostics is revolutionizing how laboratory medicine and clinical diagnostics are done. At the dawn of the new millennium, however, molecular diagnostics represents only a small fraction of the total volume of hospital-based and reference laboratory-based diagnostic tests and none of the volume of testing done in the physician office laboratory (POL), emergency room, blood collection center, family planning clinic and sexually transmitted diseases (STD) clinic. There are several reasons for these relatively low volumes. *In vitro* nucleic acid amplification technologies that define the "gold standard" of molecular diagnostics, *e.g.*, polymerase chain reaction (PCR) and ligase chain reaction (LCR), are complex (often requiring dedicated space), time-consuming (taking hours to complete), expensive relative to more conventional clinical laboratory techniques, and demand the most highly trained, specialized medical technologists. Furthermore, some enzyme-based amplification methods are inhibited by specimen components. Relatively complicated and expensive specimen preparation technologies must often be used to prepare patient specimens for molecular diagnostics tests. There are, of course, certain "state of the art" tests whose clinical value transcends these problems and are widely used. A prime example is the FDA-approved Human Immunodeficiency Virus (HIV) viral load monitoring test done by quantitative reverse-transcriptase PCR (RT-PCR).

Molecular diagnostics is the fastest-growing segment of laboratory medicine principally because of the exquisite sensitivity and specificity inherent in nucleic acid biochemistry; in other words, disease management is enhanced. The wide use of molecular diagnostics beyond the venues of the clinical and reference laboratory will continue to be inhibited by the logistical and economic reasons stated above. A low-cost, automatable solution is essential for

Principio

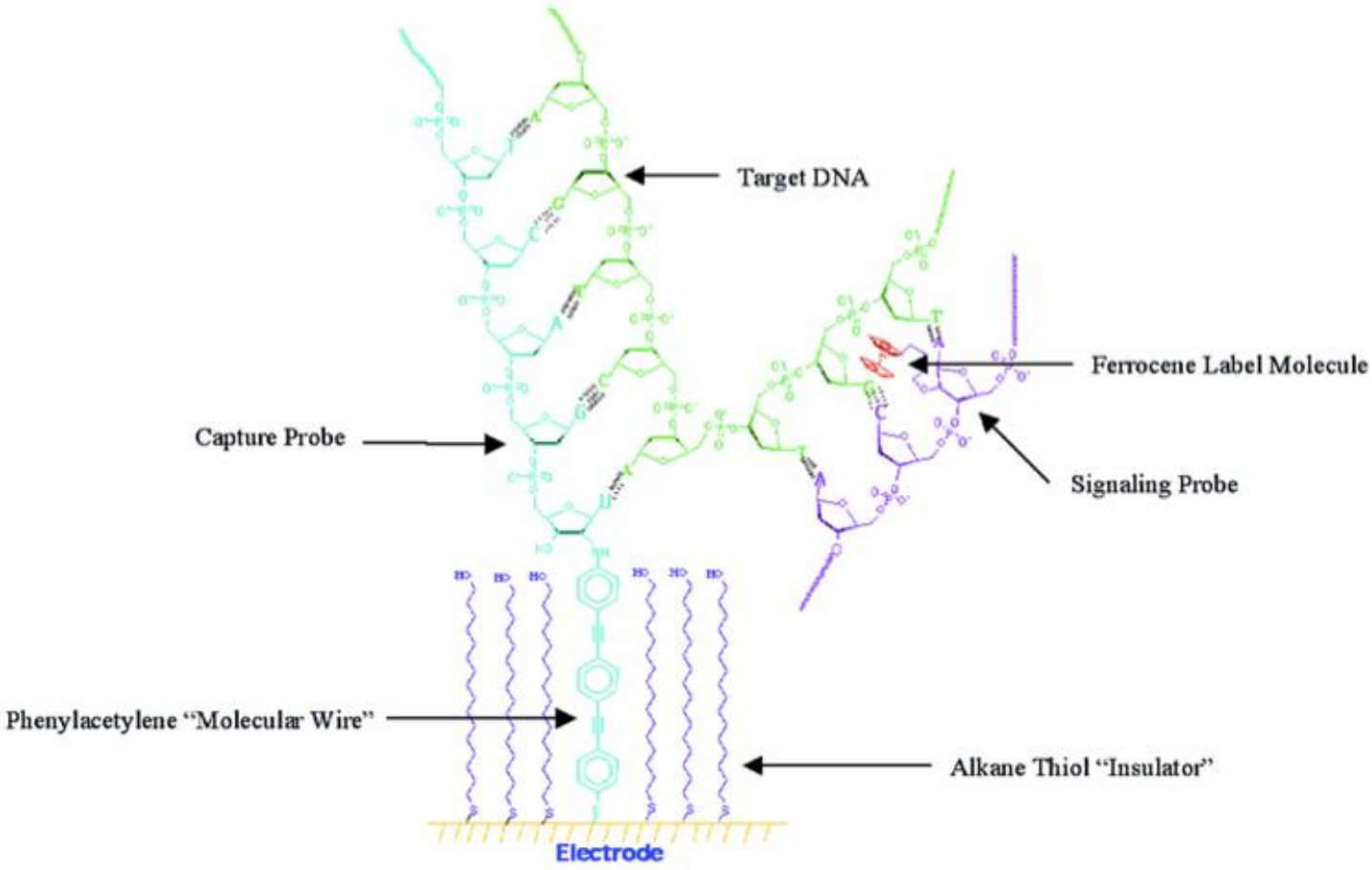
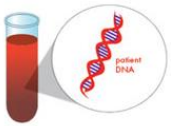
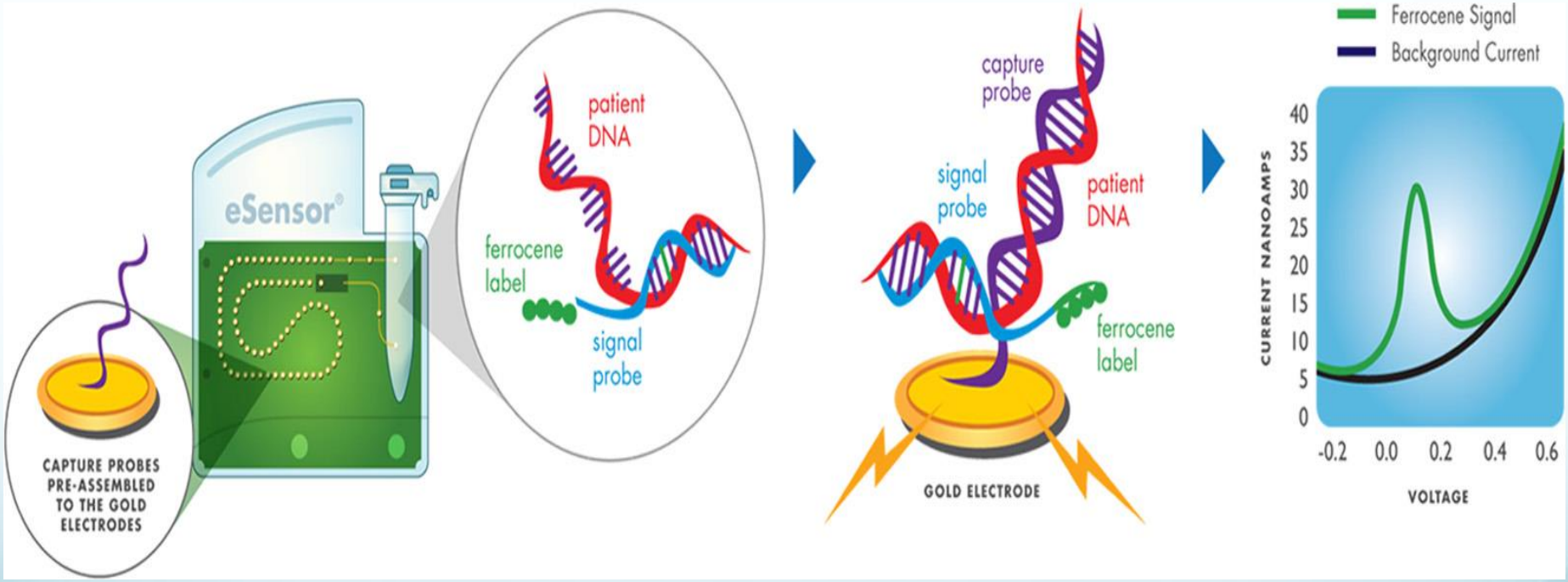


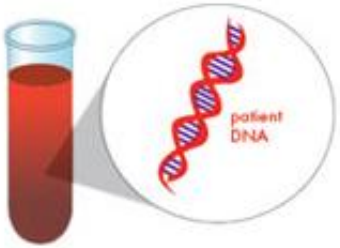
Figure 3.
Chemical Surface of CMS Electrode: Ferrocene label (shown in red) covalently attached to a signaling probe (purple). The capture probe (aqua) is attached to the gold microelectrode through phenylacetylene "molecular wires" (aqua) that maintain the desired electrical contact between the probe and the electrode (gold) surface. The electrode surface is electrically insulated with a monolayer coating of alkane thiols (blue) to prevent unwanted redox species that may be present in the specimen from interfering with the measurements of the test system.



1 Patient sample is obtained & DNA extraction is performed.

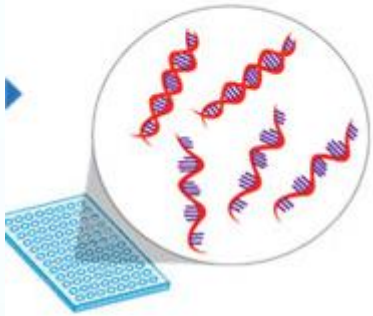


Rilevazione Bioelettrica in diagnostica molecolare



1 Patient sample is obtained & DNA extraction is performed.

2 Polymerase chain reaction (PCR) is performed to amplify patient DNA, referred to as target DNA.



3 An exonuclease reaction is performed to create single-stranded DNA.

4 Multiplex detection and result reporting are performed on the XT-8 system.

MASTER DIAGNOSTICA
Ditta produttrice strumento e kit: Master Diagnostica (Spagna-Granada) Ditta distributrice in Italia: ABANALITICA (Italia-Padova)

- sfrutta il sistema REVERSE DOT-BLOT: il DNA target amplificato, viene fatto ibridizzare con delle sonde complementari al gene target, fissate su un supporto detto appunto Chip

- Il kit permette di identificare almeno 36 specie di patogeni tra batteri, miceti e 20 geni per la resistenza agli antibiotici.

MICETI

- Ps
- Ac
- St
- Es
- Kl
- Ne
- Se
- Pr

- **Candida spp.:**
- **C. tropicalis**
- **C. glabrata**

- **Candida Albicans**

ae spp.:

a

ganii

ica

- Streptococcus agalactiae
- Lysteria monocytogenes

Le resistenze

Gram-positive bacteria:

- *mec*A: for *S. aureus* (MRSA)
- *van*A-*van*B: for *Enterococcus spp.* (VRE)

Gram- negative bacteria:

- Extended spectrum β-lactamases (ESBLs):
  - *bla*SHV
  - *bla*CTX-M

Carbapenemases:

- kpc
- sme
- nmc/imi
- ges
- vim
- gim
- spm
- ndm
- sim
- imp
- oxa23 like
- oxa24 like
- oxa 48 like
- oxa51like
- oxa58 like

I passaggi

- Si parte da un campione di emocoltura positiva
- Non è richiesta estrazione, infatti le alte temperature dei primi cicli di PCR sono sufficienti per lisare le cellule.
- E’ richiesto un comune termociclatore end-point per l’amplificazione del campione
- Mix 1:contiene i primers per i geni batterici ed i geni per la resistenza a: meticillina (mecA); vancomicina (vanA e vanB); beta-lattamasi (blaSHV, blaCTX-M);
- Mix 2: primers geni per la resistenza ai carbapenemici

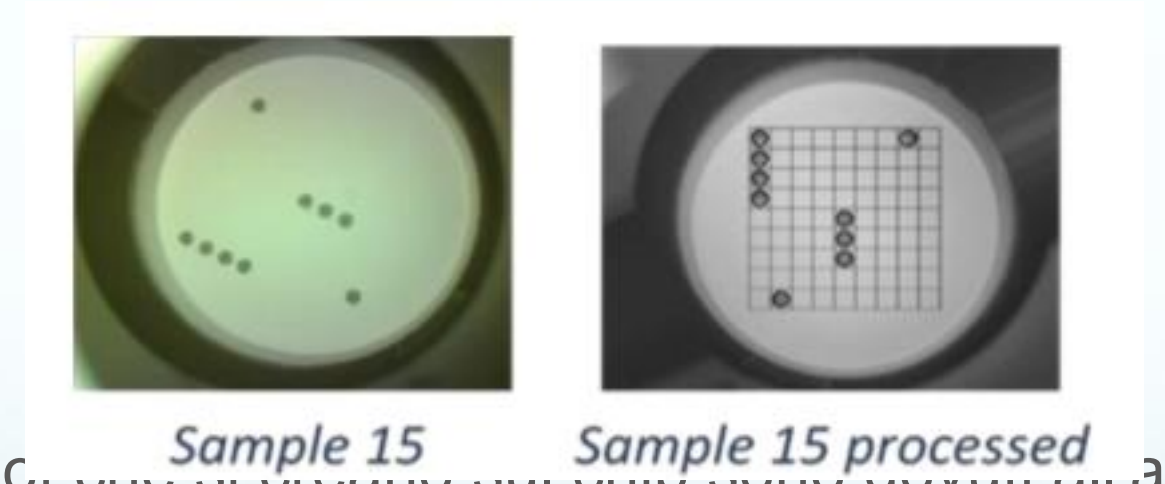
La strumentazione

- hybrispot 24 fino a 24 campioni
- Completamente automatico
- Non necessita di materiali di consumo
- Reagenti contrassegnati da codici a barre, letti dallo strumento
- Consente di effettuare 2 protocolli insieme
- Provvisto di lampada UV
- **Primo step: preleva la soluzione di ibridazione e l'amplificato**
- Durata protocollo ibridazione: circa 60 min per analizzare 12 campioni
- L'ago dispensatore viene lavato con un'apposita soluzione di lavaggio ogni volta che
- dispensa un campione
-


hybriSpot 24
vitro

I risultati

- Al termine del protocollo, hybrispot 24 acquisisce una foto di ogni chip ed il software elabora l'immagine.



- Gli spot che si creano sul chip sono dovuti all'avvenuta ibridazione tra le sonde fissate sul chip ed il DNA del campione amplificato.

RIEPILOGANDO:

Emocoltura

NO ESTRAZIONE!

Multiplex PCR

2h 30 min

Ibridazione

30-90 min

DURATA TOTALE DEL WORKFLOW: 4 H

I sistemi da sangue

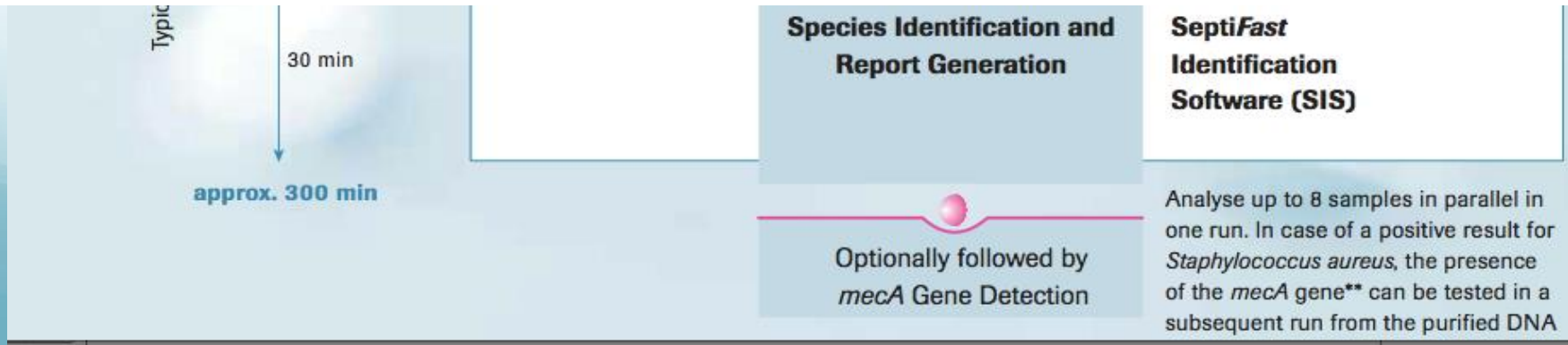
- Roche
- T2MR
- Seegene
- Iridica

Test diretti da sangue

Gram (-)	Gram (+)	Fungi
• *Escherichia coli*	• *Staphylococcus aureus*	• *Candida albicans*
• *Klebsiella (pneumoniae / oxytoca)*	• CoNS (Coagulase negative *Staphylococci)**	• *Candida tropicalis*
• *Serratia marcescens*	• *Streptococcus pneumoniae*	• *Candida parapsilosis*
• *Enterobacter (cloacae / aerogenes)*	• *Streptococcus spp.***	• *Candida krusei*
• *Proteus mirabilis*	• *Enterococcus faecium*	• *Candida glabrata*
• *Pseudomonas aeruginosa*	• *Enterococcus faecalis*	• *Aspergillus fumigatus*
• *Acinetobacter baumannii*		
• *Stenotrophomonas maltophilia*		

△ **Table 1**

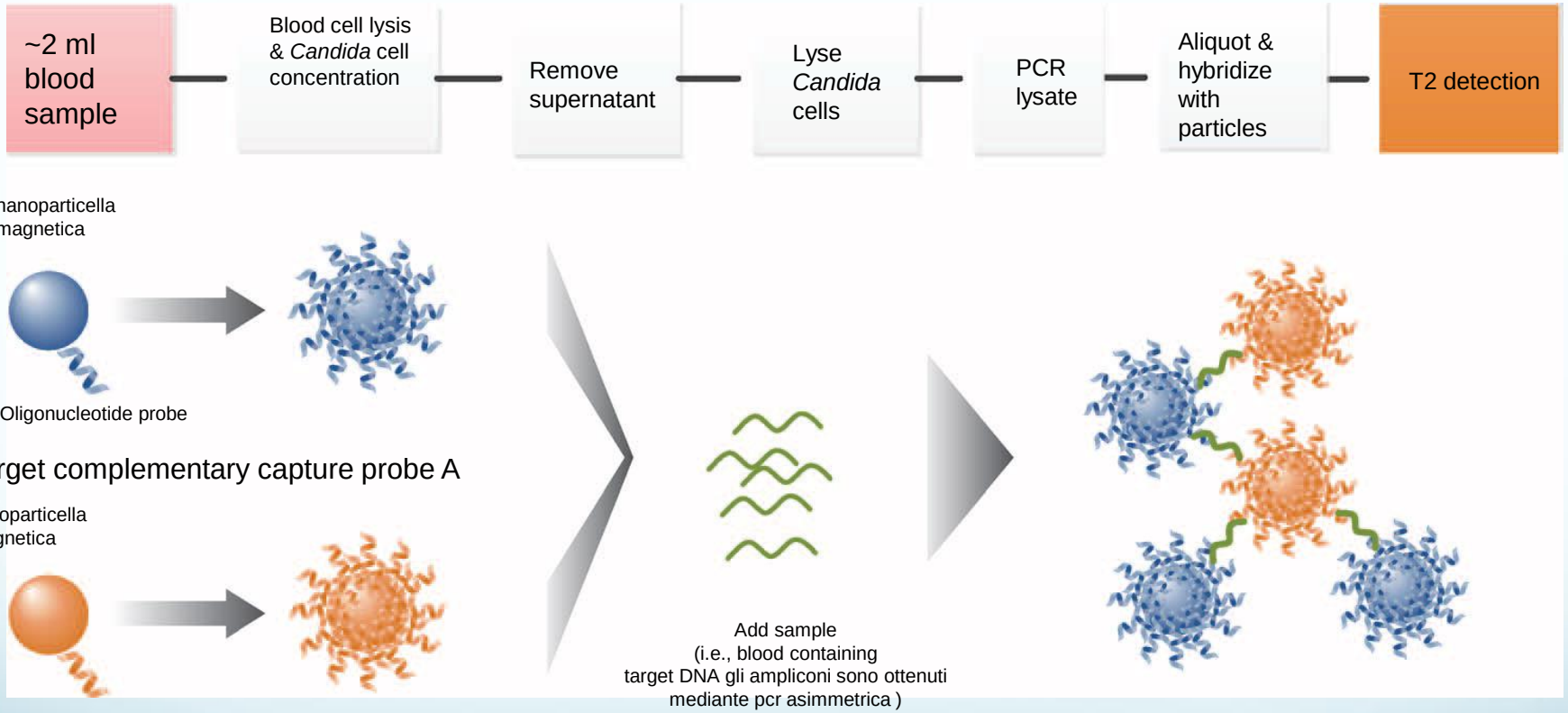
**S. epidermidis, S. haemolyticus* ***S. pyogenes, S. agalactiae, S. mitis*



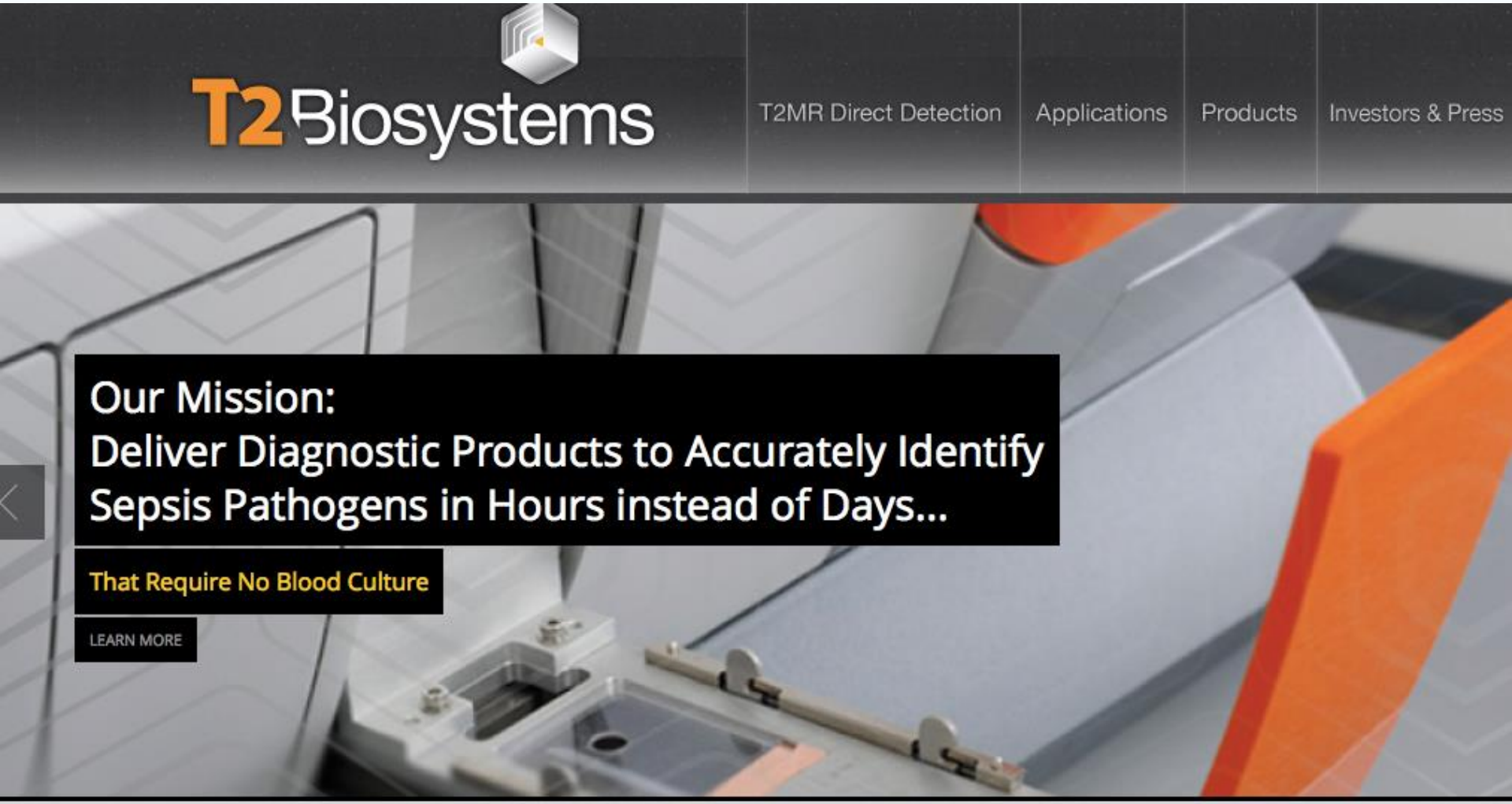
Septifast Roche

- E’ stato il primo metodo sul mercato
- È relativamente rapido (6h) ma non ha accesso random per cui il secondo paziente va in coda
- Relativamente costoso
- Continuerà la produzione?

Risonanza magnetica in laboratorio: **biosensori combinati con la nanotecnologia**



Il DNA target, se presente, forma degli ibridi con le nanoparticelle che interagiscono tra loro formando dei grappoli. **Aumentando di volume le nanoparticelle variano la loro carica magnetica**, Questa variazione agisce alterando il campo magnetico delle molecole di acqua presenti. La misura di questa variazione permette la rivelazione del target. La variazione del campo magnetico e' proporzionale alla concentrazione dell campione e viene misurato come una qulasiasi NMR.



T2 candida e T2 Bacteria The T2Candida Panel provides a species-specific result in 3 to 5 hours with no blood culture, enabling physicians to make timely treatment decisions to reduce adverse outcomes, patient mortality and costs.

Seegene


Specimen
Extraction
Amplification
M
Multiplex
Real-time
PCR
Result &
Data
Analysis
6 h

Identification	
ID 6 . Gram (-) bacteria- B	ID 7 . Gram (-) bacteria- B
K. pneumoniae *K. oxytoca* *P. mirabilis*	*E. coli* *E. cloacae* *E. aerogenes*
ID 8 . Fungi	ID 9 . Fungi
C. albicans *C. tropicalis* *C. parapsilosis*	*C. glabrata* *C. enusei* *A. fumigatus*
P. aeruginosa *A. baumannii* *S. maltophilia*	*S. marcescens* *B. fragilis* *S. typhi*

Product	Cat. No.	Size
Sepsis Amplification	SE8000Y	50 rxns
Sepsis Screening Real-time Detection *	SE8T01Y	50 rxns
Sepsis ID 1~9 Real-time Detection *	SE8301Y~SE8309Y	50 rxns

A. **High sensitivity**

B. **Direct test from whole blood**

C. **Screening for more than 90 pathogens (>90% of sepsis causative pathogens) as well as 3 drug-resistant markers within 3 hrs after extraction**

D. **Further identification of 27 pathogens detected within 30 min with no additional amplification.**

Sistema iridica

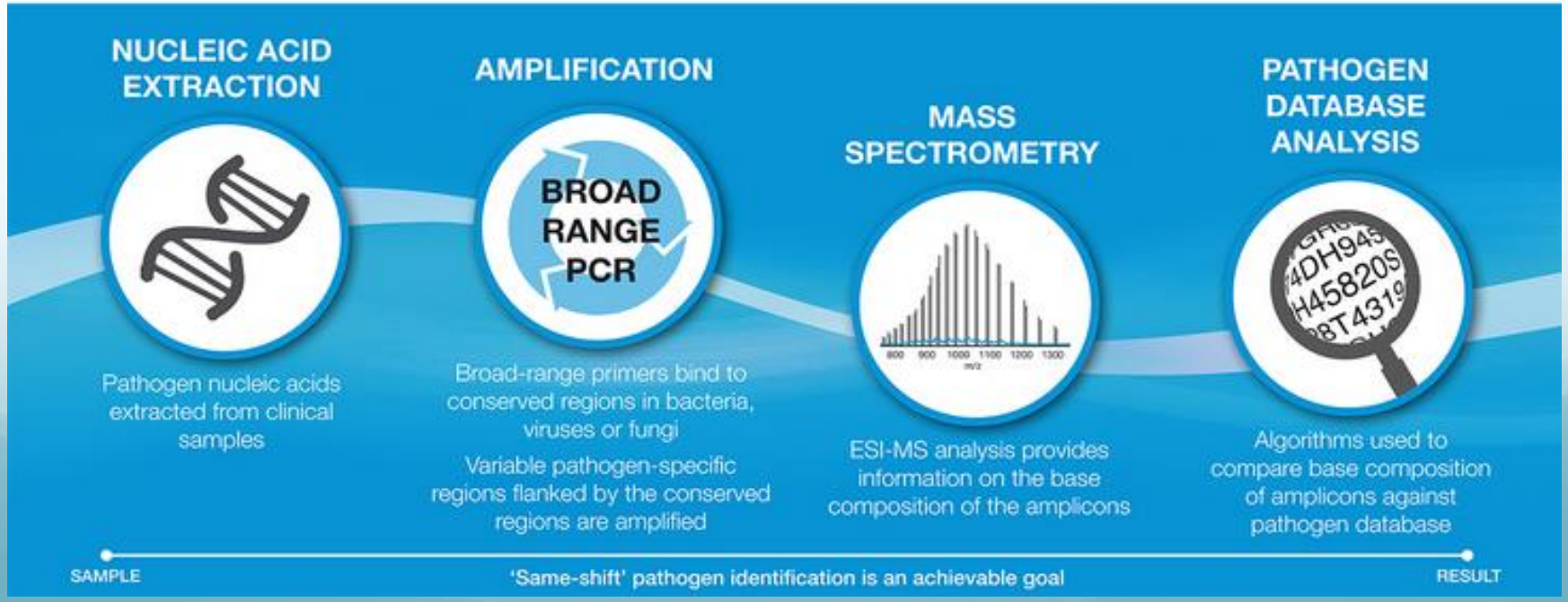
iridica. identify early . treat with confidence . transform care .

A NEW ERA IN MICROBIAL DIAGNOSTICS

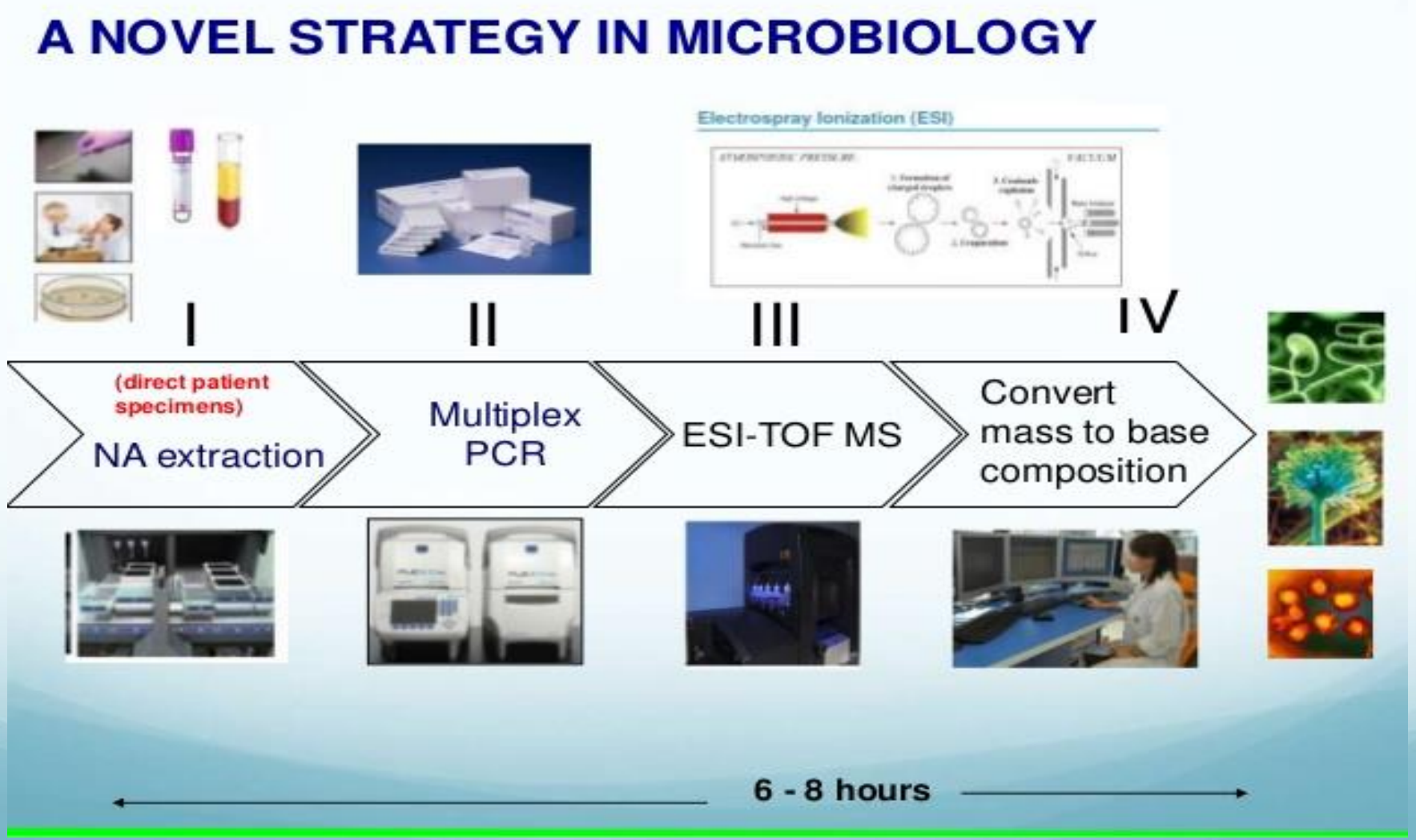
over 1,000 pathogens . 6 hours . directly from clinical samples .

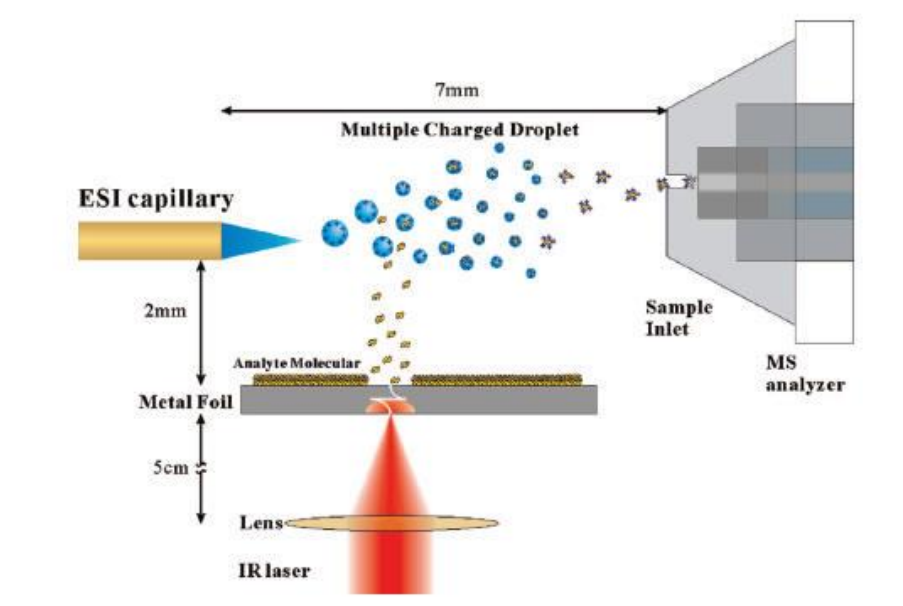
Abbott
A Promise for Life

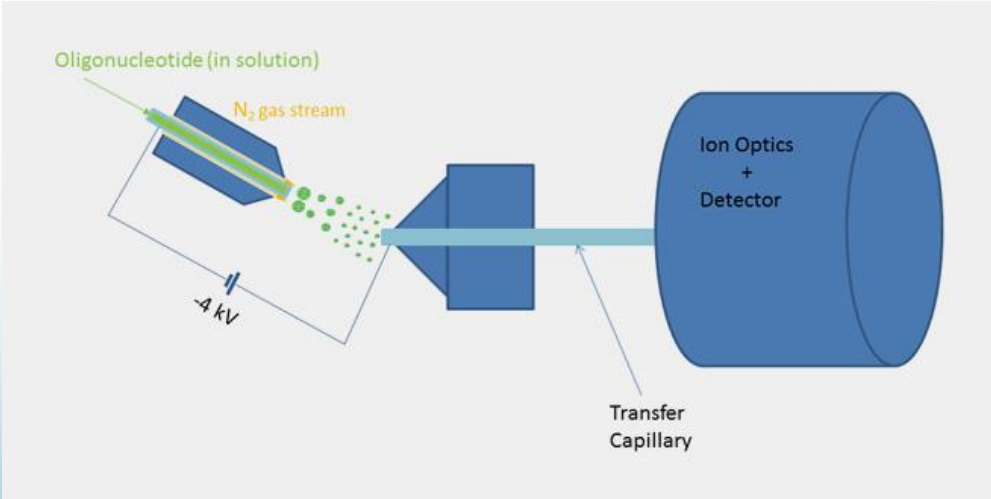
PCR/ESI-MS: Polymerase chain reaction/electrospray ionization-mass spectrometry



PCR coniugata con Electrospray Inonizaton Mass Spectrometry (PCR/ESI-MS Process)




7mm
Multiple Charged Droplet
ESI capillary
2mm
Analyte Molecular
Metal Foil
5cm
Lens
IR laser
Sample
Inlet
MS
analyzer


Oligonucleotide (in solution)
N_2 gas stream
-4 kV
Ion Optics
+
Detector
Transfer
Capillary


iridica
nowing
The Future is
See More, Act

Conclusioni

- Il fenomeno sepsi è in aumento
- Non ci dobbiamo far trovare impreparati
- Dobbiamo mettere in campo tutte le nostre risorse e competenze
- Ma dobbiamo farlo in modo intelligente spendendo e innovando (in continuo) **se, quando e dove** necessario
- Senza pensare che **il nuovo sia da utilizzare sempre e comunque**, ma va calato nella singola realtà, senza dimenticare che alcune volte semplici soluzioni sono altrettanto efficaci
- Questo è il “take home message” del nostro percorso rivisto