Le tecniche molecolari nella diagnosi di sepsi

1° Congresso NEWMICRO - Trento, 20 gennaio 2010

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SOC Microbiologia e Virologia
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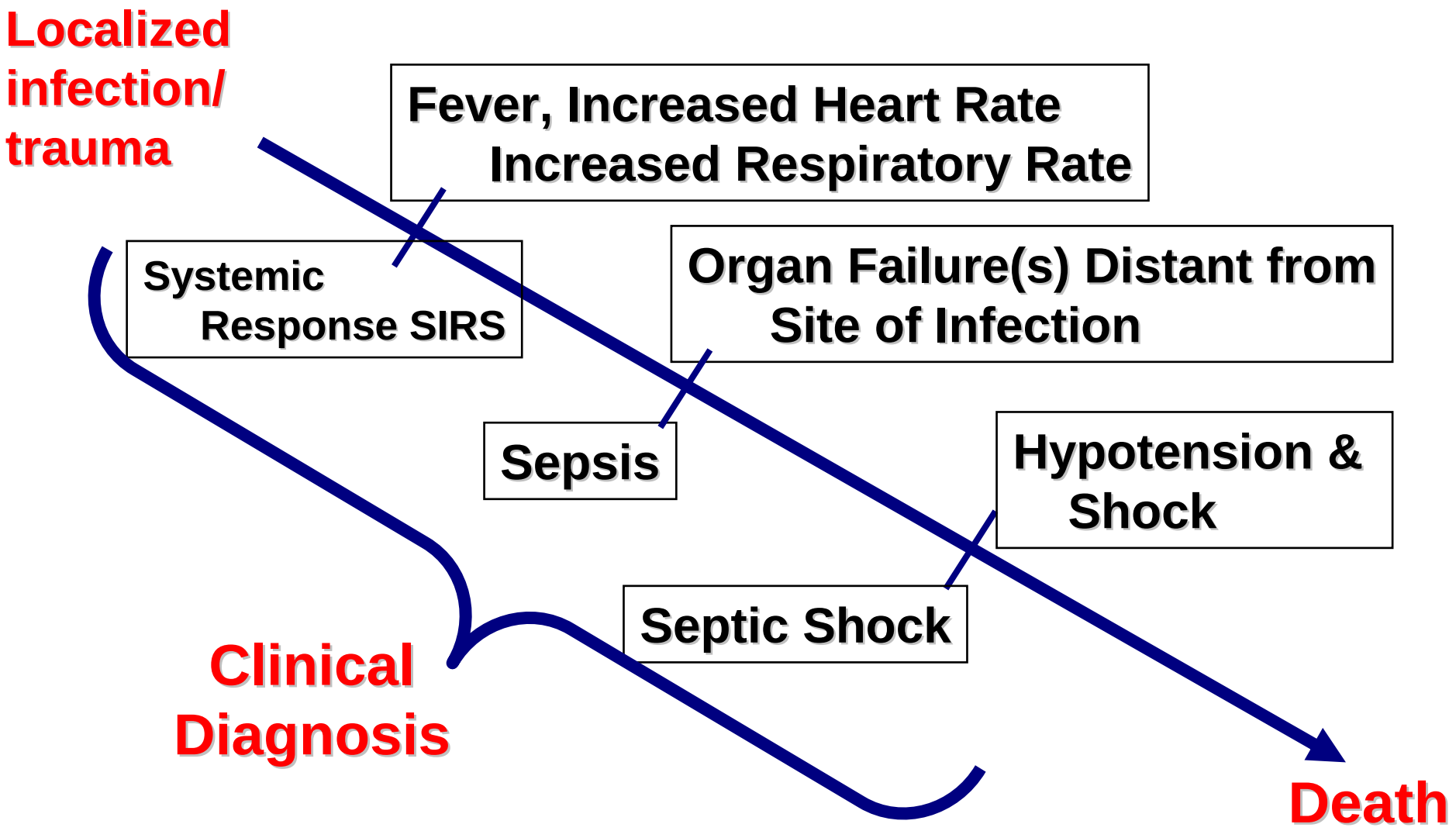
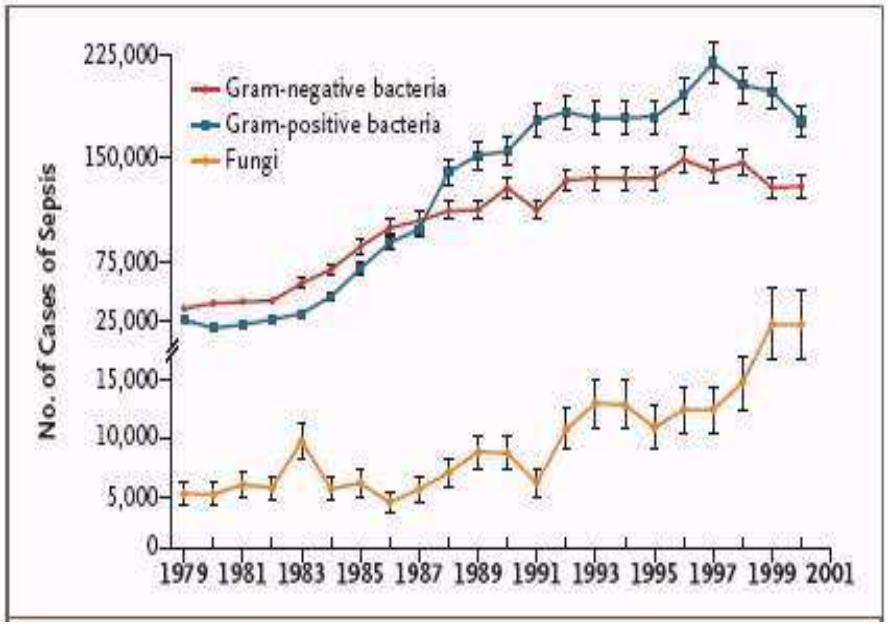
Sepsis: the continuum

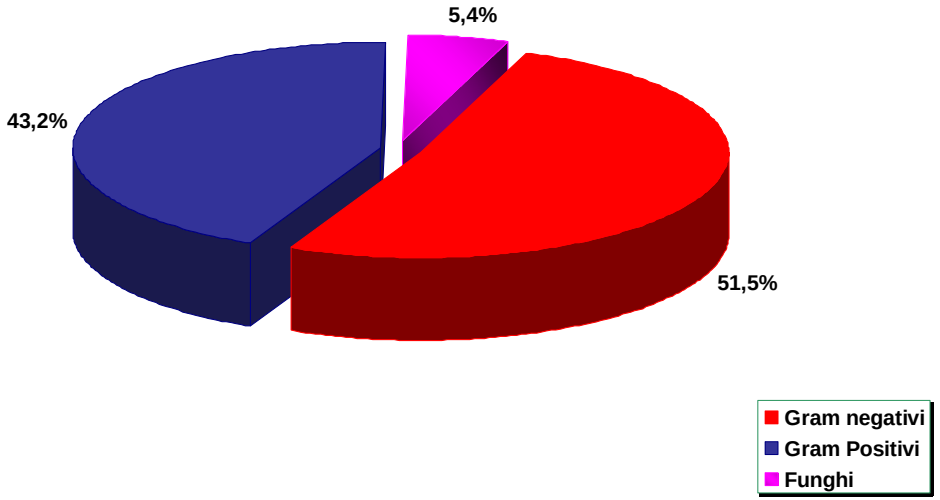


Aspetti eziologici



Martin GS et al. N Engl J Med 2003

Numero e tipologia microrganismi causa di sepsi in USA dal 1979-2000



Rita De Rosa SOC Microbiologia e Virologia Azienda Ospedaliera S.Maria degli Angeli – Pordenone

Tipologia e frequenza isolati a PN 2007- ‘08- ’09 (n = 1435)

Attualmente, il metodo di riferimento per diagnosticare gli agenti responsabili di sepsi è l'emocoltura

Puo' essere falsamente negativa per:

- **terapia antibiotica in atto**
- **volume di sangue insufficiente**
- **intermittenza dell'agente patogeno in circolo**
- **microrganismi non coltivabili**

Time-table of Blood Culture

Blood culture system

	Blood culture		Subculture	ID + DR Test	
	1 days	2 days	3 days	4 days	
Fungi	< 48 hrs		1 day	1 day	96 hrs
Gram (+) bacteria	< 24 hrs	1 day	1 day	72 hrs	Treatment start
Gram (-) bacteria	< 24 hrs	1 day	1 day	72 hrs	

*1: Benjamin J et al. (2005) J.Clin.Microbiol. 43(1):433-435
2: Horvath et al.(2003) J.Clin.Microbiol. 41(10):4714-4717
3: Murray et al.(1998)J.Clin.Microbiol. 26(6):1601-1603.
4: Sang Hyuk Park et al. (2010) Korean J Med . 30:276-83

Early Diagnosis in Sepsis

1 Accurate test can reduce the mortality caused by sepsis.

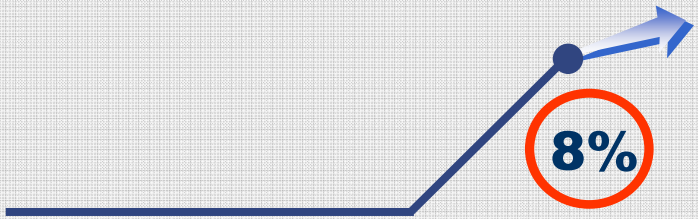
60~70% of patients with sepsis

70~90% of patients with severe sepsis

	Sepsis	Severe sepsis	Septic shock	Total number of deaths
Number of Patients	100	60	42	
Number of Deaths	10 (Mortality 10~30%)	18 (Mortality 30~50%)	21 (Mortality 50~60%)	49 (High Mortality: almost 50 %)

2 High-speed testing is essential for the rapid treatment

Administration of antibiotics 1 hr delay



Increase of mortality rate

<Medical Information of Eulji General Hospital Pharmacy Department July, 2008>

TECNICHE MOLECOLARI

Come guadagnare ore utili alla diagnosi

Identificazione dei patogeni da coltura

direttamente da campione di sangue

24/36h

Sangue

Crescita batterica su terreno

30/60h

AMPLIFICAZIONE

Identificazioni con tecniche molecolari

PCR patogeno specifica

PCR ad ampio range

Multiplex PCR Real Time

AMPLIFICAZIONE

PCR patogeno specif.
PCR ad ampio range,
Multiplex PCR

Ibridazione
FISH

Dna Strip Technology (ARNIKA)

Tecnologia con elevata sensibilità e specificità. Il DNA viene isolato, amplificato e rilevato attraverso una ibridazione e la reazione della fosfatasi alcalina su una striscia di membrana.

PCR-and Microarray-based (BMC Microbiology)

In 3 h è possibile eseguire: estrazione DNA, multiplex PCR, rilevazione ed identificazione di 14 e più diversi patogeni con *microarray probes*

Microarray

o matrice ad alta densità

Tecnica recente che sfrutta il rilevamento di specifiche sequenze nucleotidiche utilizzando una sonda di DNA (probe) costituita da un piccolo frammento di acido nucleico marcato con una sostanza fluorescente. Il metodo consente un elevato livello di sensibilità, specificità e un'ampia capacità di analisi.

La scelta di utilizzare tecniche molecolari su coltura, non può essere comparata in termini di TAT, ai metodi di biologia molecolare diretti

TECNICHE MOLECOLARI

Come guadagnare ore utili alla diagnosi

Test molecolari diretti

1 — ***SeptiFast Test® Roche***

2 — ***Magicplex Sepsis Real-time Test Seegene***

5 – 6 ore

- **Nella diagnostica della sepsi la“variabile tempo” è un valore di assoluta rilevanza in termini di outcome clinico**
- **In caso di sepsi grave la probabilità di sopravvivenza si esprime in termini di ore**
- **Ogni giorno perso per la diagnosi aumenta fino a due volte la probabilità di decesso del paziente**

Bouza E. e altri Clin Infect Dis 2004

N Engl J Med 2006;355:1699-713.

Management of Sepsis

James A. Russell, M.D.

A BETTER UNDERSTANDING OF THE INFLAMMATORY, PROCOAGULANT, AND immunosuppressive aspects of sepsis has contributed to rational therapeutic plans from which several important themes emerge.[1] First, rapid diagnosis (within the first 6 hours) and expeditious treatment are critical, since early, goal-directed therapy can be very effective.[2] Second, multiple approaches are necessary in the treatment of sepsis.[1] Third, it is important to select patients for each given therapy with great care, because the efficacy of treatment — as well as the likelihood and type of adverse results — will vary, depending on the patient.

LightCycler ® SeptiFast

Med Microbiol Immunol
DOI 10.1007/s00430-007-0063-0

ORIGINAL INVESTIGATION

A multiplex real-time PCR assay for rapid detection and differentiation of 25 bacterial and fungal pathogens from whole blood samples

Lutz Eric Lehmann · Klaus-Peter Hunfeld · Thomas Emrich · Gerd Haberhausen · Heimo Wissing · Andreas Hoeft · Frank Stüber

Received: 29 June 2007

SeptiFast Test validation

Diagnosi molecolare di sepsi con SeptiFast ® Roche Diagnostics

- **A partire da 3 ml di sangue intero in provette contenenti EDTA permette la diagnosi eziologica di sepsi in circa 5/6 ore**

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

LightCycler ® SeptiFast

Sangue intero
3 mL

Purificazione del DNA

PCR *real-time*

Gram (-)	Gram (+)	Funghi
Escherichia coli	*Staphylococcus aureus*	*Candida albicans*
Klebsiella (pneumoniae/oxytoca)	CoNS[1]	*Candida tropicalis*
Serratia marcescens	*Streptococcus pneumoniae*	*Candida parapsilosis*
Enterobacter (cloacae/aerogenes)	*Streptococcus spp.*[2]	*Candida glabrata*
Proteus mirabilis	*Enterococcus faecium*	*Candida krusei*
Pseudomonas aeruginosa	*Enterococcus faecalis*	*Aspergillus fumigatus*
Acinetobacter baumannii[3]		
Stenotrophomonas maltophilia		
	Staphylococcus epidermidis1	
	Staphylococcus haemolyticus1	
	Streptococcus agalactiae2	
	Streptococcus pyogenes2	
	Streptococcus mitis2	

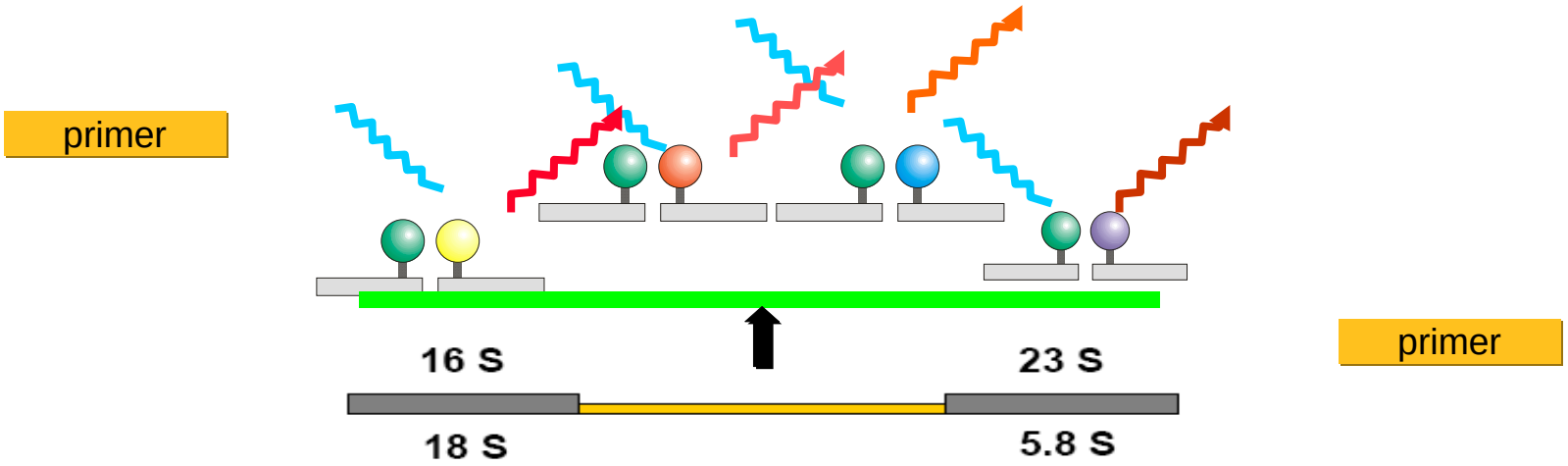
MRSA (mecA)

~ 90% dei microrganismi isolati

LightCycler® Septi*Fast* Test

Regione target – Internal Transcribed Spacer

Si trova tra i geni per rRNA 16S e 23S dei batteri e 18S e 5.8S dei funghi



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

LightCycler® SeptiFast Test *workflow del test*

Estrazione del campione (3 mL sangue-EDTA)
lisi meccanica
Purificazione

1

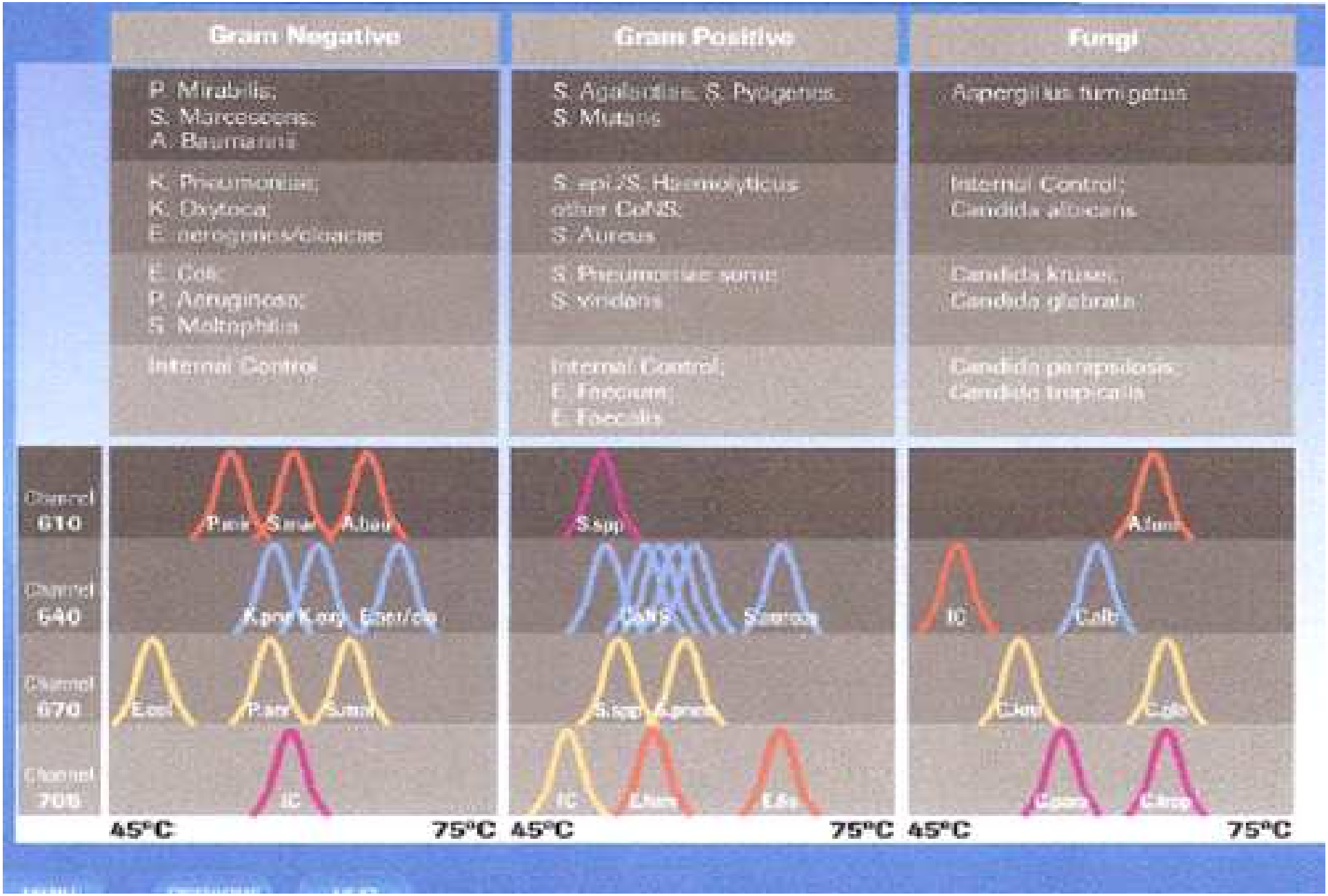
PCR Real-time in 3 parallele multiplex (1 canale per gram +, 1 per gram - , 1 per funghi)
Durante la PCR i prodotti specifici vengono rilevati mediante misura della fluorescenza delle sonde FRET (Fluorescence Resonance Energy Transfer)"
Analisi delle temperature di melting

2

Interpretazione automatica dei risultati

3

Identificazione di genere o specie
Sepsti*Fast* Identification Software (SIS)


Gram Negative
Gram Positive
Fungi
P. Mirabilis;
S. Marcescens;
A. Baumannii
K. Pneumoniae;
K. Oxytoca;
E. aerogenes/Velicicola
E. Coli;
P. Aeruginosa;
S. Maltophilia
Internal Control
S. Agalactiae; S. Pyogenes;
S. Mutans
S. epi/S. Haemolyticus
altri CoNS;
S. Aureus
S. Pneumoniae sorm;
S. viridans
Internal Control;
E. Faecium;
E. Faecalis
Aspergillus fumigatus
Internal Control;
Candida albicans
Candida krusei;
Candida glabrata
Candida parapsilosis;
Candida tropicalis
Channel 610
Channel 640
Channel 670
Channel 705
45°C
75°C
45°C
75°C
45°C
75°C

SeptiFast Identification Software
Interpretazione automatica dei dati

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

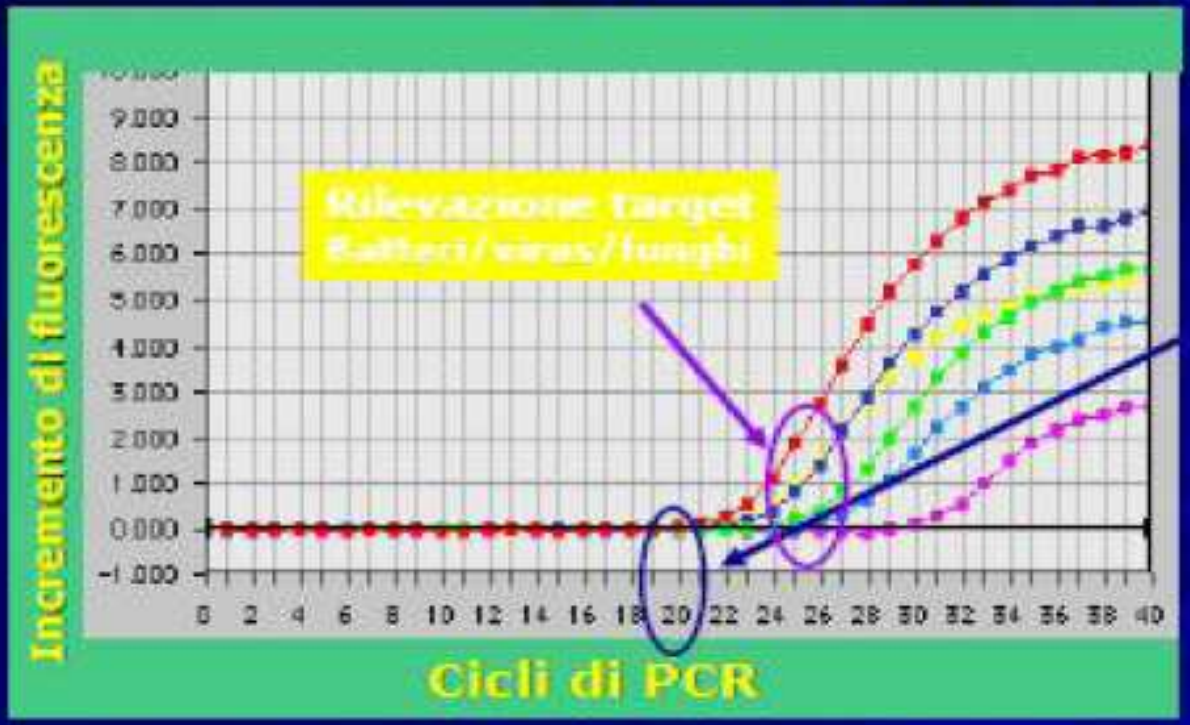
Assay Flags

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

A volte l'interpretazione clinica del dato ottenuto con l'emocoltura, è resa difficile dal fatto che i germi isolati possano appartenere alla normale flora saprofitica cutanea

Real-time PCR



Cut-off per CONS e Streptococchi

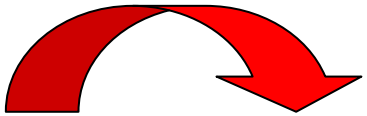
Costruito in modo da rilevare meno 1% delle contaminazioni con BC

One color = one target
Multiple colors = multiple targets

Le basse concentrazioni di CoNS e di streptococchi che riflettono contaminazioni non vengono refertate

Il tempo necessario per refertare un risultato negativo con l'emocoltura è stabilito dagli standard CLSI (Clinical and Laboratori Standard Institute, 2007) in 7 giorni.

Test molecolare diretto:



risposta negativa in 5/6 h

TURNAROUND TIME DI EMOCOLTURA E SEPTIFAST A CONFRONTO RAPPORTATO AI MICRORGANISMI ISOLATI

Microrganismo	Rilevazione molecolare (range)	Colorazione di GRAM	Identificazione biochimica
FUNGHI	14 (5/29)	36 ±12	96 ±12
BACILLI GRAM -	14 (5/29)	36 ±12	84±12
COCCHI GRAM +	14 (5/29)	36 ±12	84±12

Diamante et al RIMeL / IJLaM 2010

Multiplex Real-time PCR

Magicplex™ Sepsis Real-time Test screens for more than 90 pathogens which cover over 90%*[1,2,3] of sepsis-causing pathogens as well as 3 drug resistance markers (*mecA*, *vanA* and *vanB*) from whole blood sample.
This test is able to further identify the pathogens which were detected in the previous screening step with an additional 30 min.

1. Biedenbach *et al.* (2004) Diagn Microbiol Infect Dis 50(1):59-69.
2. Wisplinghoff *et al.* (2004) Clin Infect Dis 39(3) :309-317.
3. Koh *et al.* (2007) Korean J Lab Med 7(4):265-275.

Features

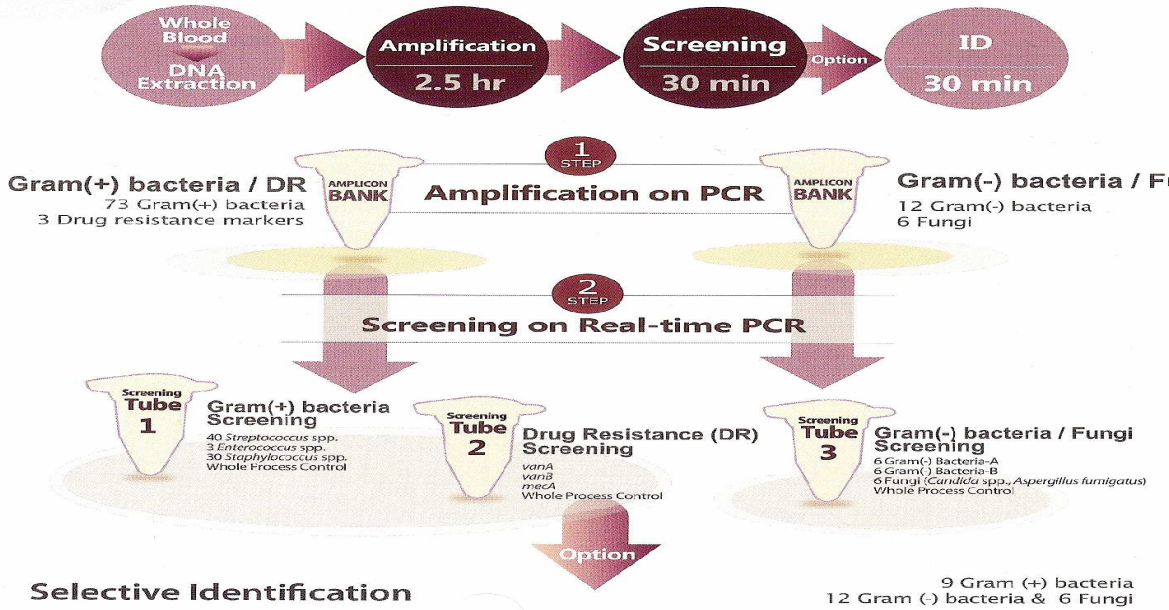
A. Faster and similar sensitivity as commercial blood culture method
B. Direct test from whole blood
C. Screening for more than 90 pathogens (>90% of sepsis causative pathogens) as well as 3 drug-resistance markers within 3 hrs after extraction
D. Further identification of pathogen detect ed within 30 min

Specimen

Whole Blood

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

Process of Magicplex™ Sepsis Real-time Test



Selective Identification

9 Gram (+) bacteria
12 Gram (-) bacteria & 6 Fungi

Streptococcus (ID 1)	*Enterococcus* (ID 2)	*Staphylococcus* (ID 3)	*Fungi* (ID 8, ID 9)	
S. agalactiae	*E. faecalis*	*S. epidermidis*	*C. albicans*	*C. glabrata*
S. pyogenes	*E. gallinarum*	*S. haemolyticus*	*C. tropicalis*	*C. krusei*
S. pneumoniae	*E. faecium*	*S. aureus*	*C. parapsilosis*	*A. fumigatus*

Gram(-) Bacteria A (ID 4, ID 5)		Gram(-) Bacteria B (ID 6, ID 7)	
P. aeruginosa	*S. marcescens*	*K. pneumoniae*	*E. coli*
A. baumannii	*B. fragilis*	*K. oxytoca*	*E. cloacae*
S. maltophilia	*S. typhi*	*P. mirabilis*	*E. aerogenes*

Conventional Multiplex Real-time PCR Method

1 ml blood sample

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

27 pathogens

- *mecA*
- *vanA*
- *vanB*
- Internal control

- *S. aureus*
- *S. epidermidis*
- *S. haemolyticus*
- Internal control

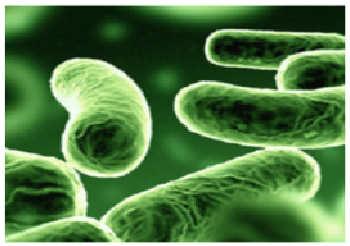
- *S. pneumoniae*
- *S. agalactiae*
- *S. pyogenes*
- Internal control

- *E. faecium*
- *E. faecalis*
- *E. gallinarum*
- Internal control

- *S. marcescens*
- *E. cloacae*
- *E. aerogenes*
- Internal control

- *K. pneumoniae*
- *K. oxytoca*
- *P. mirabilis*
- Internal control

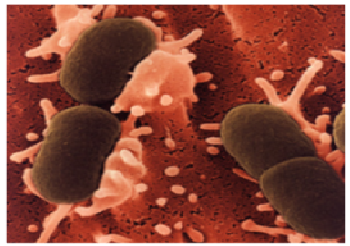
- *P. aeruginosa*
- *A. baumannii*
- *S. maltophilia*
- Internal control



- *E. coli*
- *B. fragilis*
- *S. typhi*
- Internal control

- *C. albicans*
- *C. tropicalis*
- *C. parapsilosis*
- Internal control

- *C. glabrata*
- *C. krusei*
- *A. fumigatus*
- Internal control



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

Workflow of Magicplex™

Amplicon Bank

- 73 G(+) bacteria
- 12 G(-) bacteria
- 3 Drug resistance marker
- 6 Fungi
- Internal control

30 min

Detection

More than 90 pathogens

Drug resistance : mecA, vanA, vanB

30 min

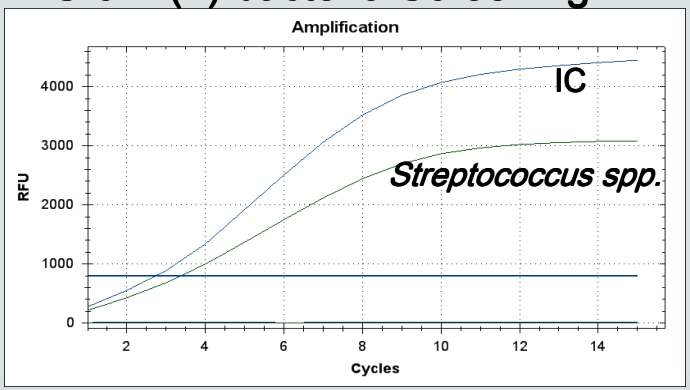
Selective Identification

27 pathogens

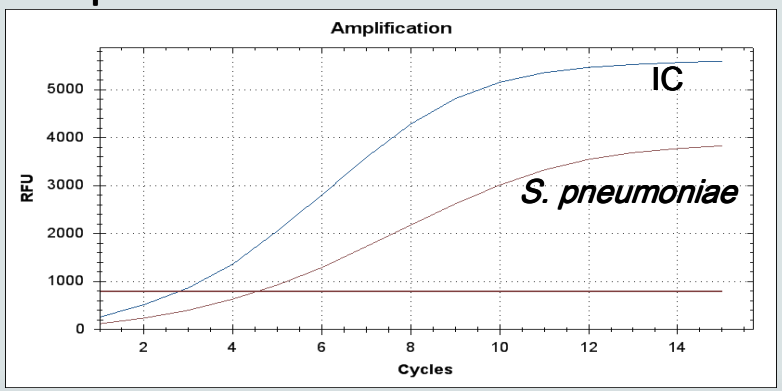
Gram (+) bacteria		***Gram (-) & Fungi bacteria***		
ID 1 *S. pneumoniae* *S. agalactiae* *S. pyogenes*	ID 2 *E. faecium* *E. faecalis* *E. gallinarum*	ID 4 *P. aeruginosa* *A. baumannii* *S. maltophilia*	ID 5 *S. marcescens* *B. fragilis* *S. typhi*	ID 6 *K. pneumoniae* *K. oxytoca* *P. mirabilis*
ID 3 *S. aureus* *S. epidermidis* *S. haemolyticus*		ID 7 *E. coli* *E. cloacae* *E. aerogenes*	ID 8 *C. albicans* *C. tropicalis* *C. parapsilosis*	ID 9 *C. glabrata* *C. krusei* *A. fumigatus*

In case of *Streptococcus pneumoniae* infection

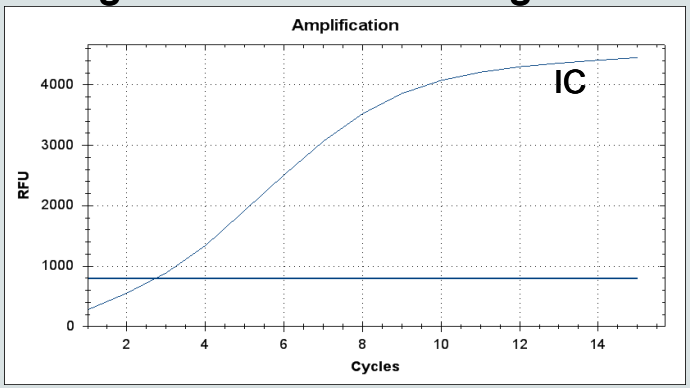
Gram (+) bacteria Screening



Streptococcus ID



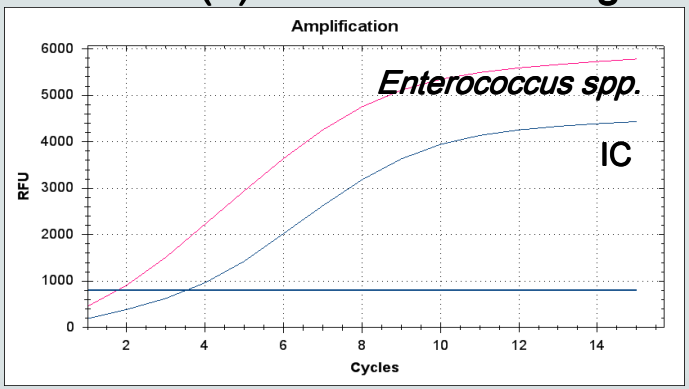
Drug resistance Screening



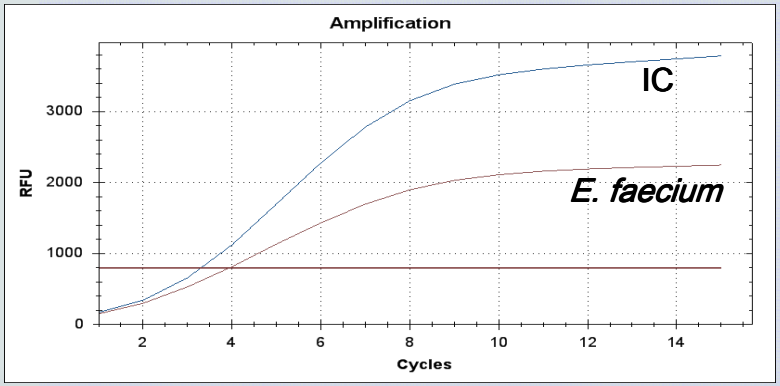
	Gram (+) bacteria Screening	Drug resistance	ID 1 (*Streptococcus* ID)
FAM	Internal control	Internal control	Internal control
HEX	***Streptococcus spp.***	VanA	*S. agalactiae*
Cal Red 610	*Enterococcus spp.*	VanB	*S. pyogenes*
Quasar 670	*Staphylococcus spp.*	MecA	***S. pnuemoniae***

In case of Vancomycin-resistant *Enterococcus faecium* infection

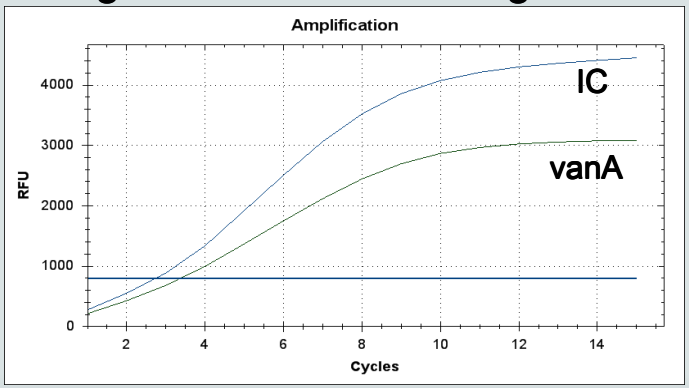
Gram (+) bacteria Screening



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*

Emocoltura vs PCR

pro e contro

- **L'emocoltura rimane il gold standard per l'isolamento dei batteri responsabili di sepsi**
- **Necessaria per eseguire i test di sensibilità e per scopi epidemiologici**

- **La risposta dell'emocoltura spesso eccede la rapidità di evoluzione clinica della sepsi**

PCR vs EMOCOLTURA

pro e contro

- **Notevole miglioramento dell'efficienza ed efficacia diagnostica con particolare riferimento al VPN e VPP, a cui si aggiunge la marcata riduzione del TAT rispetto all'esame colturale**

- **Non influenzata dalla terapia antibiotica e/o antimicotica**

- **Non consente (perché non sono disponibili i rispettivi *target*) di identificare alcuni microrganismi.**
- **Consente di ottenere solo una identificazione di specie e non un test di sensibilità.**
- **Il costo che sembra renderne l'utilizzo improponibile**

When one considers that the critical window for the appropriate management of an infection is (conservatively) <6 h, the wellbeing of a patient, instead of financial figures, should be the prime concern of healthcare administrations.

Bissonnette L and Bergeron MG. CMI 2010.

Le tecniche molecolari nella diagnosi di sepsi

Dall'esperienza clinica sperimentale alla condivisione di un percorso diagnostico nei pazienti candidati ad impianto di endoprotesi aortica.

SHOCK, Vol. 34, No. 1, pp. 27–30, 2010

MOLECULAR IDENTIFICATION OF BLOODSTREAM PATHOGENS IN PATIENTS PRESENTING TO THE EMERGENCY DEPARTMENT WITH SUSPECTED SEPSIS

Manuela Avolio, Paola Diamante, Silvio Zamparo, Maria Luisa Modolo, Shamanta Grosso, Paola Zigante, Nilla Tosoni, Rita De Rosa, Paola Stano, and Alessandro Camporese

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ABSTRACT—The rapid detection of pathogens in blood is critical for a favorable outcome of patients with suspected sepsis. Although blood culture (BC) is considered the criterion standard for diagnosis of bloodstream infection, it often takes several days to detect the causative organism. In this study, we compared BC with a commercially available multiplex real-time polymerase chain reaction (PCR) assay to detect bacteria and fungi in blood samples from 144 patients admitted to the emergency department with suspected sepsis. Of 144 blood samples examined, 91 (63%) were negative by both methods and 53 (37%) were positive by at least one of the two methods. In 30 among all positive cases (56.6%), both methods identified the same organisms, in 13 cases (24.5%), BC identified organisms not detected by real-time PCR, and in 10 cases (18.9%), Septi*Fast* PCR assay gave positive results, whereas the BC was negative. In this study, we wished to compare Septi*Fast* results obtained by standard procedures, but future clinical studies are necessary to define Septi*Fast* PCR as support for BC in the early diagnosis of severe bloodstream infections.

TABLE 3. **Comparison of Septi*Fast* and BactAlert results**

Microorganisms	Total	Positive only with Septi*Fast*	Positive only with BactAlert	Positive with both methods (% concordance)
*B. capillosus**	1	0	1*	0
C. albicans	1	0	0	1 (100)
CoNS	6	0	6†	0
E. coli	20	4	1	15 (70)
E. faecalis	1	1	0	0
*E. fergusonii**	1	0	1*	0
Enterobacter cloacae	1	0	0	1 (100)
K. pneumoniae	3	1	0	2 (67)
*M. morganii**	1	0	1*	0
P. aeruginosa	1	0	1	0
Proteus mirabilis	1	0	0	1 (100)
S. aureus	5	1	1	3 (60)
Stenotrophomonas maltophilia	1	0	0	1 (100)
Streptococcus species	3	0	0	3 (100)
S. pneumoniae	6	3	0	3 (50)
*P. stuartii**	1	0	1*	0
Total	53	10	13	30

*Not detectable by Septi*Fast*.
†Sample contamination.

In fact, despite its limitations, Septi*Fast* could be useful as an adjunct to traditional culture methods to facilitate detection of BSIs (22), especially in cases where BC is negative but BSI is strongly suggested. For these clinical conditions, we wish to further investigate the use of Septi*Fast*.

Avolio et al. *Shock. 2010*

TABLE 5. **TAT (h) regarding Septi*Fast*/BC results calculated from the arrival of samples to our laboratory**

	Septi*Fast*+/BC+	Septi*Fast*+/BC−	Septi*Fast*−/BC−	Septi*Fast*−/BC+
Septi*Fast* result	15 (6 > 30)	15 (6 > 30)	15 (6 > 30)	15 (6 > 30)
BC-time to Gram stain	36 ± 12	—	—	36 ± 12
BC-estimated species identification (based on agar growth)	60 (48 – 72)	—	—	60 (48 – 72)
BC-definitive biochemical species identification	84 (72 – 96)	—	—	84 (72 – 96)
BC-definitive negative result	—	5 days	5 days	—

TABLE 2. **Comparison of BC and Septi*Fast* results**

	Septi*Fast* positive, %	Septi*Fast* negative, %	Total, %
BC positive, %	30 (21)	13 (9)	43 (30)
BC negative, %	10 (7)	91 (63)	101 (70)
Total, %	40 (28)	104 (72)	144 (100)

	Septi*Fast vs* BC
Sensitivity	**90,9%**
Specificity	**90,1%**
PPV	**75,0%**
NPV	**96,8%**

Avolio et al. *Shock. 2010*

TABLE 4. **Microorganisms and their probable diagnosis**

DNA detected (no. Septi*Fast*+/BactAlert− cases)	Clinically suggestive of
E. coli (4)	Urosepsis (2); pneumonia (1); necrotizing fasciitis (1)
S. aureus (1)	Aortic prosthesis infection
S. pneumoniae (3)	Pneumonia (1); meningitis (2)
E. faecalis (1)	Urosepsis
K. pneumoniae (1)	Urosepsis

Avolio et al. *Shock. 2010*

CASO CLINICO

B.F.: 62 anni; ipertensione, coronaropatia, portatore di *pacemaker*.

Nel 2004 AAA sottorenale trattato con EVAR.

Nel 2009 durante *follow-up* rilievo di *endoleak evolutivo* tipo II.

Trattamento dell'*endoleak* con puntura diretta TC guidata ed embolizzazione.

Dopo 9 gg, ricovero per iperpiressia.

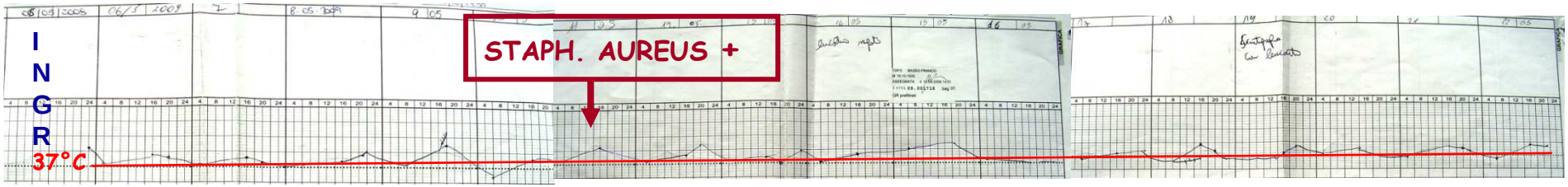
- All'ingresso prelievo di un primo set di emocolture ed immediato inizio di copertura antibiotica.

- Le condizioni cliniche non migliorano permanendo febbre con andamento setticemico. Emocolture negative.

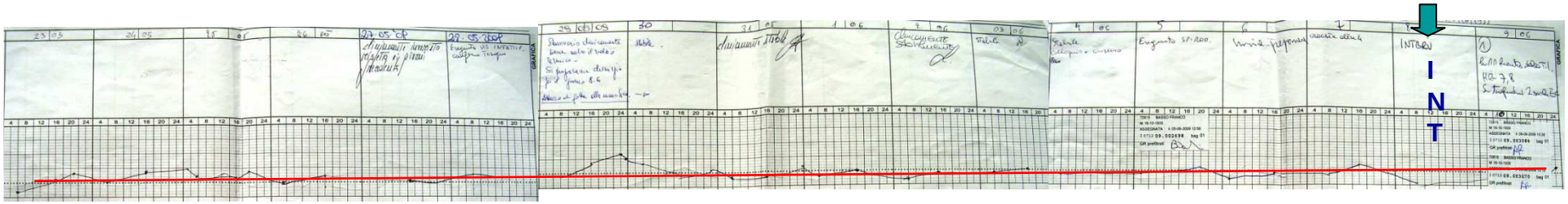
- Ipotesi infettivologica di infezione all'interno della sacca aneurismatica (esclusa dal circolo dall'endoprotesi) non supportata dal riscontro microbiologico.

- Suggerimento microbiologico di eseguire SeptiFast e contestuale esecuzione di un secondo set di emocolture.

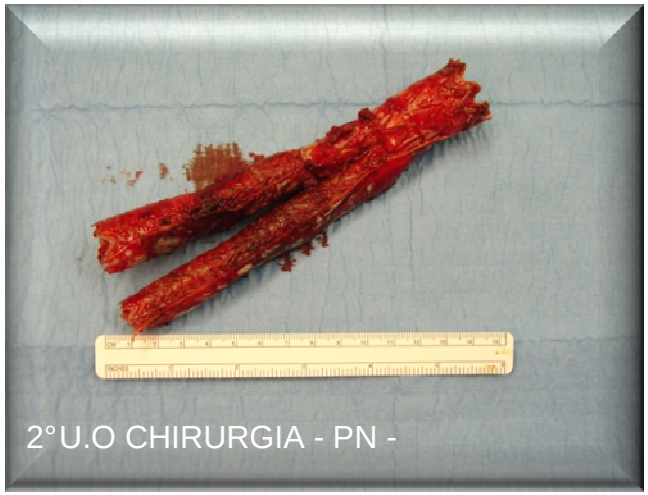
- La diagnosi di infezione protesica viene dunque confermata solo in 12^ giornata dal risultato di SeptiFast positivo per ***Staphylococcus aureus***.



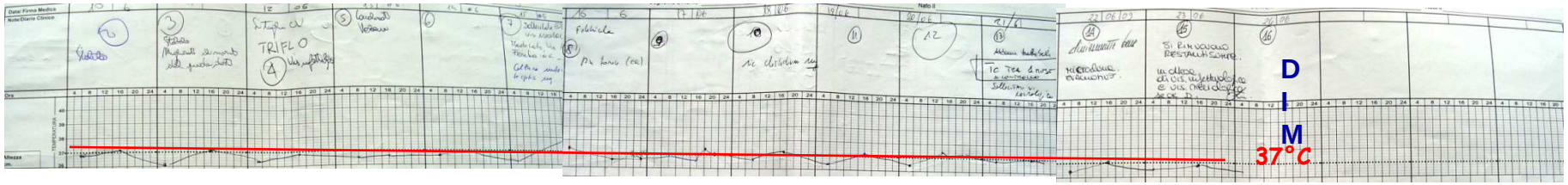
- anche il secondo set di emocolture si confermerà negativo.



- Il paziente viene avviato con successo, sotto copertura antibiotica specifica e senza ulteriori complicanze, ad un nuovo intervento chirurgico con rimozione dell'endoprotesi ed impianto di protesi omologa.

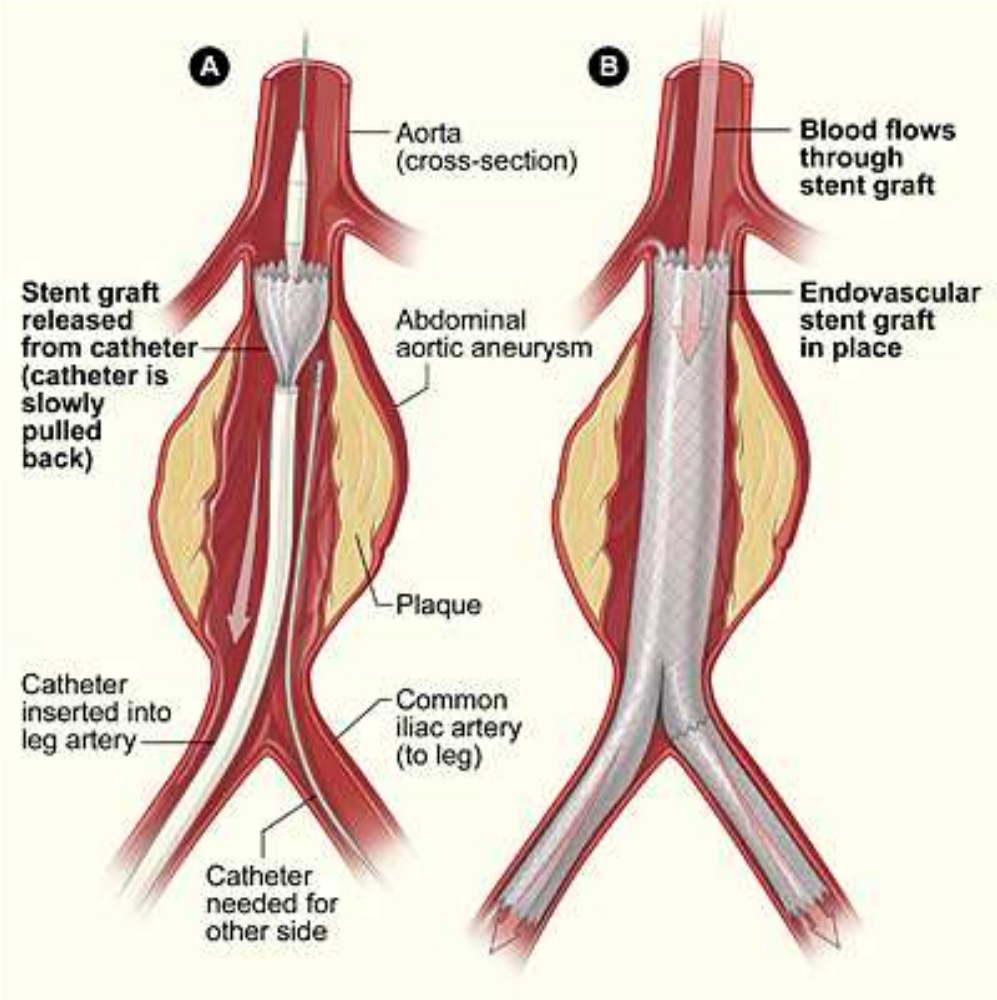


➢Dopo degenza di 51 giorni il paziente viene dimesso.



COSTI ?

SITO DEL GRAFT: SACCA ANEURISMATICA COME " NICCHIA ECOLOGICA "
PECULIARE NELLE ENDOPROTESI AORTICHE



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JAC

Diagnosis and treatment of prosthetic aortic graft infections: confusion and inconsistency in the absence of evidence or consensus

S. F. FitzGerald[1]*, C. Kelly[2] and H. Humphreys[1]

Prosthetic aortic grafts are used to treat abdominal aortic aneurysm and occlusive vascular disease. Graft insertion is complicated by infection in 0.5–2% of cases[1] and is associated with considerable morbidity and mortality. *Staphylococcus* species are the most commonly implicated causative organisms,[2] with *Staphylococcus aureus* more likely in early infection and coagulase-negative staphylococci such as *Staphylococcus epidermidis* more likely in late infections.[3,4] Gram-negative bacilli and *Enterococcus* species are regularly recovered from cultures as are anaerobes and fungi,[5] but these often represent colonization when isolated from superficial wound swabs. In addition, a sizable minority (14%) of infections are polymicrobial.[6] However, many suspected aortic graft infections are treated without knowing the identity or antimicrobial susceptibilities of the causative organism, because suitable specimens were not obtained or because antibiotic treatment was instituted before the collection of appropriate samples for culture.

a glycopeptide antibiotic or linezolid. Blood cultures are often negative, particularly in late-onset infection. Various techniques such as broth culture and sonication of the graft may be used to enhance the recovery of biofilm-forming organisms[10] from graft or infected material, and, in the future, molecular methods such as PCR may contribute significantly. Greater efforts to confirm a

patients. However, as *S. aureus* is the organism most likely to be isolated in early infection, and as methicillin resistance is increasingly common, empirical treatment of early-onset infection should perhaps include a glycopeptide where MRSA is prevalent.[26] With regard to late-onset infections, the guidelines recommend that antibiotic treatment be deferred until the infective aetiology has been confirmed, except in the very ill patient. However, more specific and evidence-based recommendations are required for empirical therapy in this group of patients and in those in whom no pathogen is ever identified, as well as for pathogen-specific therapy and prophylaxis. Furthermore, these recommendations need to include the criteria for diagnosis, to clearly indicate when antibiotics should be commenced, as well as offering advice on the total duration of therapy, including if and when life-long suppressive treatment is indicated. A detailed treatment algorithm for the management of prosthetic joint infections, along with specific antimicrobial recommendations and a proposed infection score to assist in diagnosis has been devised and would be a useful template.[27]

FitzGerald et al, *J Ant Chem, 2005*

There are many unresolved questions concerning the optimal choice of antibiotic therapy for patients with aortic graft infections that need to be addressed. Do the BSAC recommendations for prosthetic vascular graft infections directly apply to aortic graft infections? Because tissue or pus may not be accessible for laboratory processing unlike with many other vascular infections, and because aortic rupture may be fatal, is a different approach to empirical therapy warranted? Should a glycopeptide be routinely used to empirically cover MRSA and methicillin-resistant coagulase-negative staphylococci? A retrospective review of the impact of MRSA in a vascular unit found that ~50% of vascular patients known to be colonized with MRSA developed clinical infection due to MRSA, and that the proportion of wound and graft infections caused by MRSA had increased from 4% to 63% over the 6 year study period.[28] Is the choice and duration of antibiotic therapy influenced by whether or not partial graft excision can be carried out, or when any form of surgical intervention is not possible? What is the appropriate duration of therapy for these differing categories of patients? There are a small number of cases reported in the

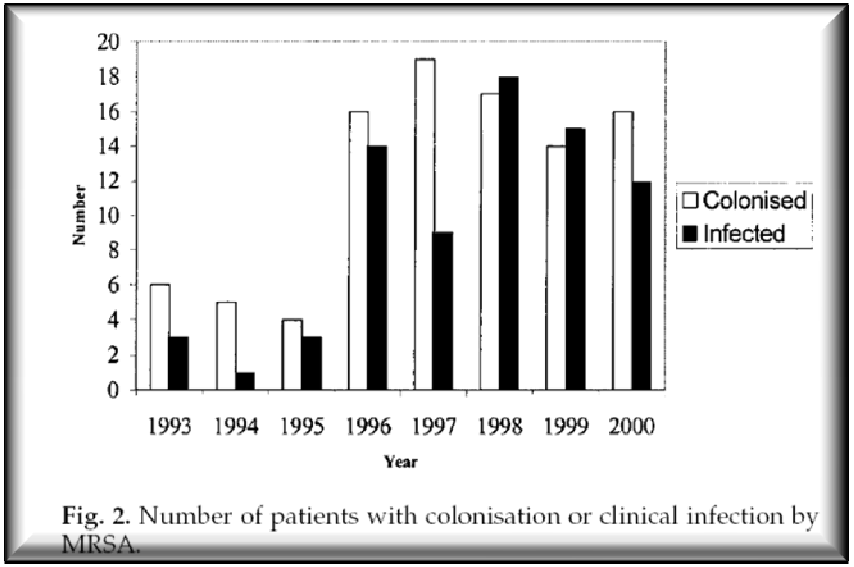

Fig. 2. Number of patients with colonisation or clinical infection by MRSA.

FitzGerald et al, *J Ant Chem, 2005;* ***Nasim et al,*** *Eur J Vasc Endovasc Sur, 2001*

✓*Should a glycopeptide be routinely used to empirically covered MRSA?*

Such therapy puts the patient at risk of adverse drug reaction and the aquisition of resistant organisms

In many clinical scenarios, there are no easy answer and decisions must be made following the input of all clinicians involved, i.e. vascular surgeon, microbiologist/infectious, interventional radiologist and others, taking due cognisance of the individual patient's conditions and state

FitzGerald et al, *J Ant Chem, 2005*

in contesti nei quali la diagnostica colturale standard non consente di avere delle risposte specifiche, i **test molecolari** di cui parliamo, se ben utilizzati sono sostenibili e permettono di ottenere risultati in grado di modificare efficacemente l'intervento terapeutico con un **decisivo impatto sull'outcome clinico del paziente**.

LINEE GUIDA PER LA PROFILASSI ANTIBIOTICA IN CHIRURGIA

REGIONE AUTONOMA FRIULI VENEZIA GIULIA

Agenzia Regionale della Sanità

CHIRURGIA VASCOLARE

Distinzione per tipo o gruppi di interventi	Antibiotico e posologia	Farmaco alternativo
• Chirurgia arteriosa interessante l'aorta addominale, una protesi o che comporta un'incisione inguinale • Procedure brachiocefaliche che coinvolgono protesi vascolari o patch-implantation (es. endarteriectomia carotidea)	Cefazolina 2 g	Vancomicina o Teicoplanina *

http://www.sanita.fvg.it/ars/specializza/progetti/allegati/tot-00.pdf

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PROTOCOLLO PER LA GESTIONE DEI PAZIENTI DA SOTTOPORRE AD INTERVENTI DI ENDOPROTESI AORTICA

GESTIONE PRE-OPERATORIA

Esecuzione programmata di tampone nasale per ricerca di Stafilococco aureo meticillino resistente (MRSA):

- Il tampone può essere eseguito in regime ambulatoriale non oltre una settimana prima dell'intervento presso gli ambulatori della SOC di Microbiologia e Virologia (senza appuntamento, tutti i giorni dalle ore 8.00 alle 10.00) con richiesta specifica (ricerca di MRSA su tampone nasale)
- Se il risultato del tampone è **negativo**, al momento dell'intervento si sottopone il paziente a profilassi con cefazolina
- Se il risultato del tampone è **positivo**, al momento dell'intervento si sottopone il paziente a profilassi con vancomicina

GESTIONE DI EVENTUALI EPISODI INFETTIVI POST-CHIRURGICI

In caso di picco febbrile dopo 24-35 ore dall'intervento:

- Eseguire un set di tre emocolture
- Eseguire un prelievo di sangue per la diagnostica molecolare di sepsi**
- Se necessario ripetere i prelievi a distanza di un giorno

Indicazioni per l'utilizzo del Septi*Fast*

<u>sepsi/sepsi grave/shock settico</u>
criteri SIRS **+** diagnosi accertata o sospetta di infezione
± disfunzione d'organo

- Sospetta endocardite* e/o infezioni da protesi endovascolari
- Pazienti con quadro clinico di polmonite
- Infezioni di cute e tessuti molli
- Pazienti settici immunodepressi**
- Sospetta infezione fungina invasiva
- Pazienti *non responder* alla terapia (anziché colturale dopo *wash-out*)

***Casalta et al.** *Eur J Clin Microbiol Infect Dis* **2009**
**** Varani et al.** *Journal of Infection* **2009**

Prospettive future

I test diagnostici molecolari nelle nostre mani, per la diagnosi di sepsi, test altamente preziosi ma egualmente impegnativi e costosi devono essere considerati come **TEST DI NICCHIA**, applicabili a popolazioni di pazienti selezionati sulla scorta di un'esperienza condivisa con i clinici.

In collaborazione con

Dr. M. Bonea, Chirurgia 2, AO S.Maria degli Angeli, Pordenone

GRAZIE PER L'ATTENZIONE

Manuela Avolio, Paola Diamante
Microbiologia e Virologia - Pordenone

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