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The 12th International & 17th National Congress on Quality Improvement in Clinical Laboratories



April 18-21, 2019 Tehran-Iran

THE ROLE OF THE MICROBIOLOGIST IN BLOOD STREAM INFECTION: NEW DIAGNOSTIC TECHNIQUES

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FONDAZIONE IRCCS POLICLINICO SAN MATTEO, PAVIA, ITALY

MICROBIOLOGY AND VIROLOGY DEPARTMENT

Sepsis

Sepsis is a **life-threatening condition** caused by the body's response to infection, which can lead to **tissue damage, organ failure, amputations** and **death**.



In the United States, in one year, more than

1.7 million people

had sepsis.¹ That's one person every twenty seconds.



Sepsis is the

3rd leading cause of death

in the United States after heart disease and cancer, killing more than **270,000 people** each year.¹ That's one person every two minutes.

As many as **87%** of sepsis cases

start in the community,

not in the hospital as is widely believed.¹



42%

of Americans have not heard of sepsis.²

<https://www.sepsis.org>

Sepsis

Consensus Definitions for Sepsis and Septic Shock



18.743 visualizzazioni

JAMA The Journal of the American Medical Association

Home Current Issue All Issues Online First Collections CME Multimedia

February 23, 2016, Vol 315, No. 8 >

< Previous Article Next Article >

Special Communication | February 23, 2016

CARING FOR THE CRITICALLY ILL PATIENT

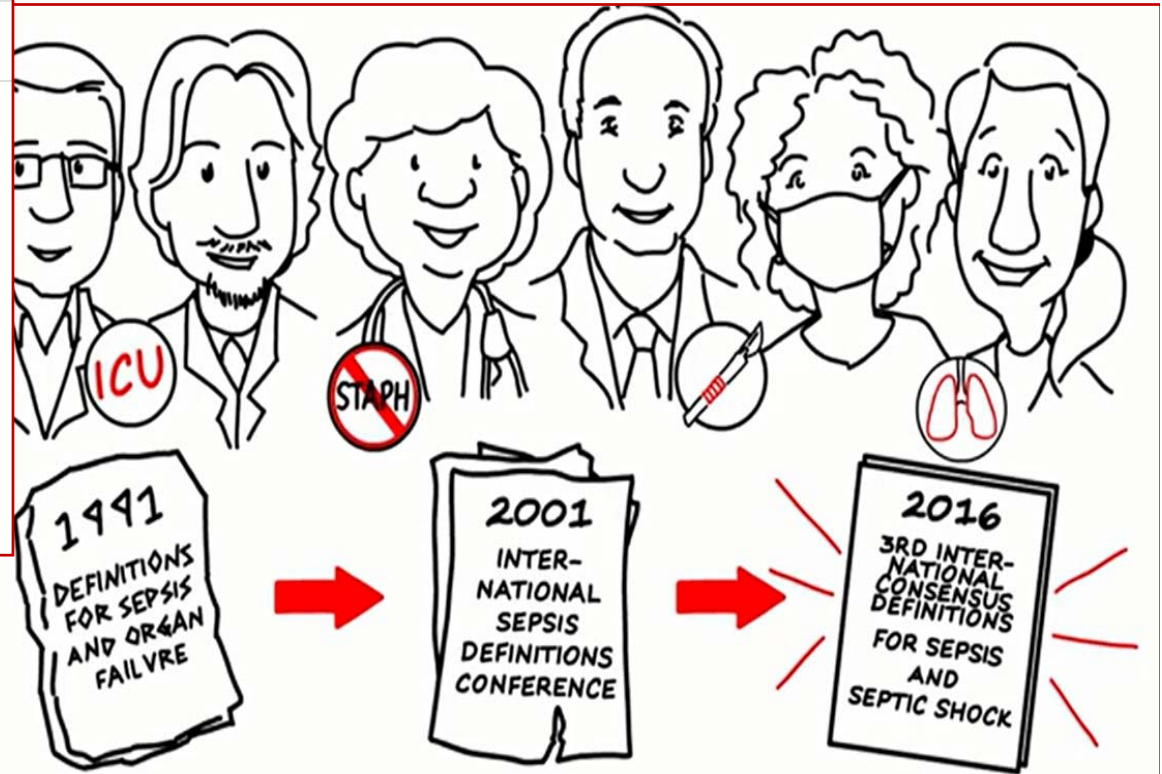
The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) FREE

Mervyn Singer, MD, FRCP¹; Clifford S. Deutschman, MD, MS²; Christopher Warren Seymour, MD, MSc³; Manu Shankar-Hari, MSc, MD, FFICM⁴; Djillali Annane, MD, PhD⁵; Michael Bauer, MD⁶; Rinaldo Bellomo, MD⁷; Gordon R. Bernard, MD⁸; Jean-Daniel Chiche, MD, PhD⁹; Craig M. Coopersmith, MD¹⁰; Richard S. Hotchkiss, MD¹¹; Mitchell M. Levy, MD¹²; John C. Marshall, MD¹³; Greg S. Martin, MD, MSc¹⁴; Steven M. Opal, MD¹⁵; Gordon D. Rubenfeld, MD, MS¹⁶; Tom van der Poll, MD, PhD¹⁷; Jean-Louis Vincent, MD, PhD¹⁸; Derek C. Angus, MD, MPH^{19,20}

[+] Author Affiliations

JAMA. 2016;315(8):801-810. doi:10.1001/jama.2016.0287.

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Sepsis Definition

LIFE-THREATENING ORGAN DYSFUNCTION



CAUSED BY



DYSREGULATED HOST RESPONSE TO INFECTION

SEPSIS CLINICAL CRITERIA

INFECTION



+

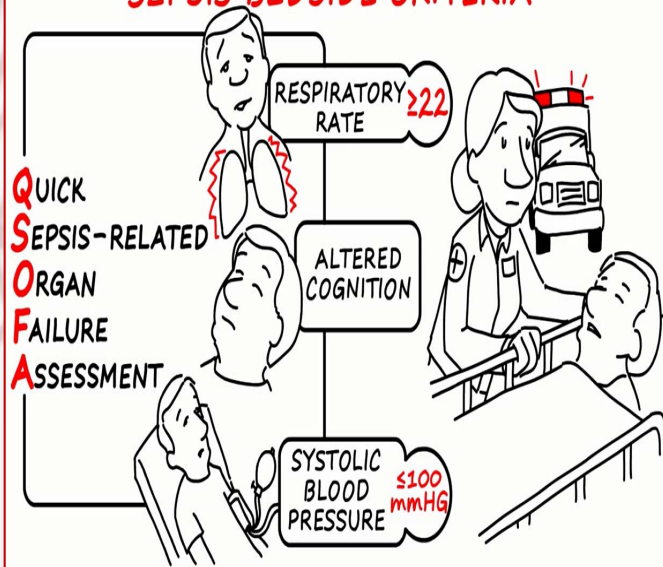
CHANGE IN:

SEPSIS-RELATED
ORGAN
FAILURE
ASSESSMENT

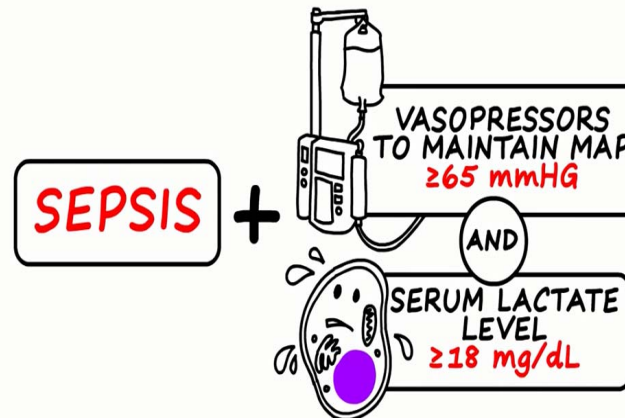
≥ 2



SEPSIS BEDSIDE CRITERIA



SEPTIC SHOCK



IN THE ABSENCE OF HYPOVOLEMIA

Delay Kills



1st WORLD SEPSIS CONGRESS
CHALLENGES AND OPPORTUNITIES IN THE FIGHT AGAINST SEPSIS
A FREE ONLINE CONGRESS

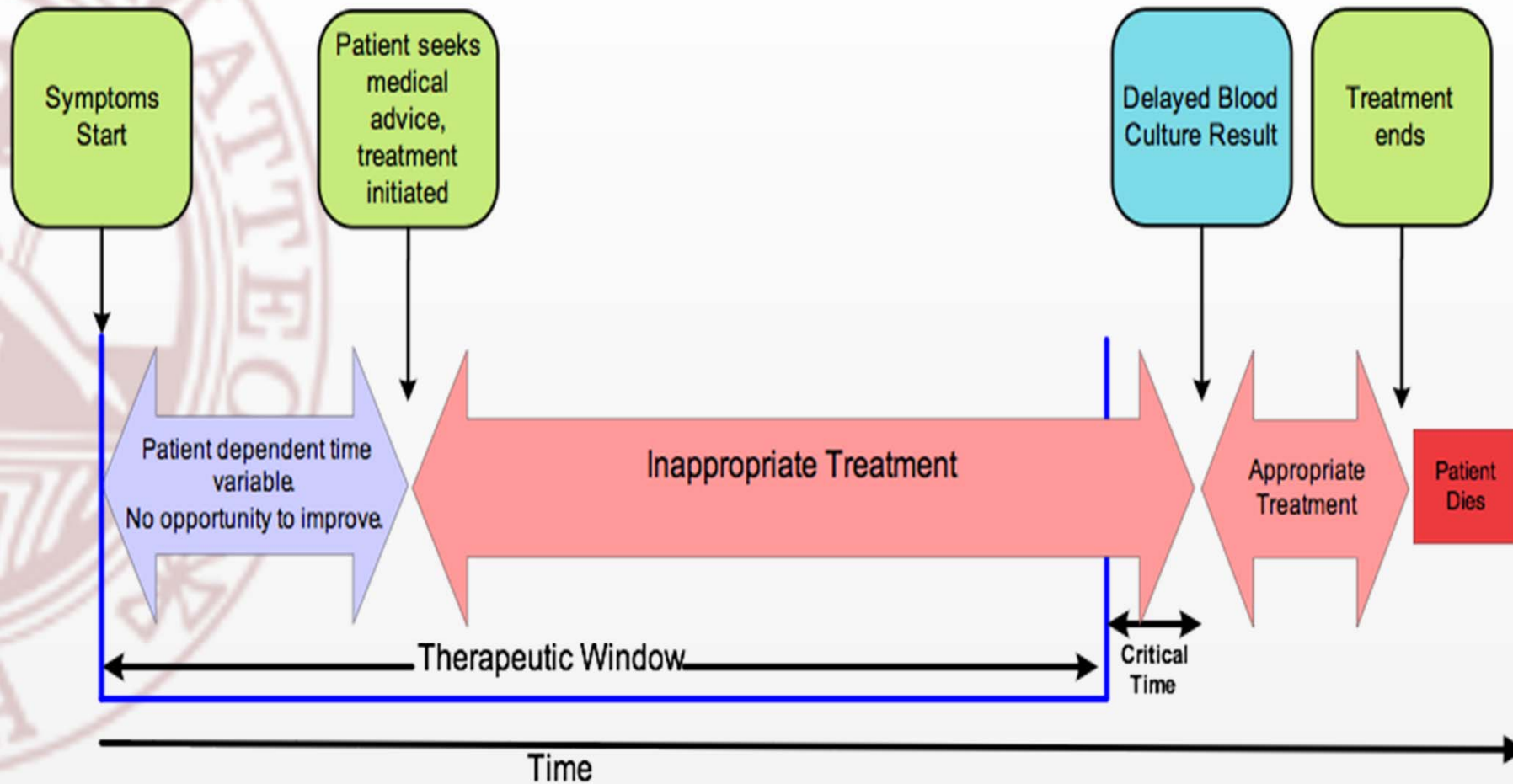
September 8th to 9th, 2016

#WSC16

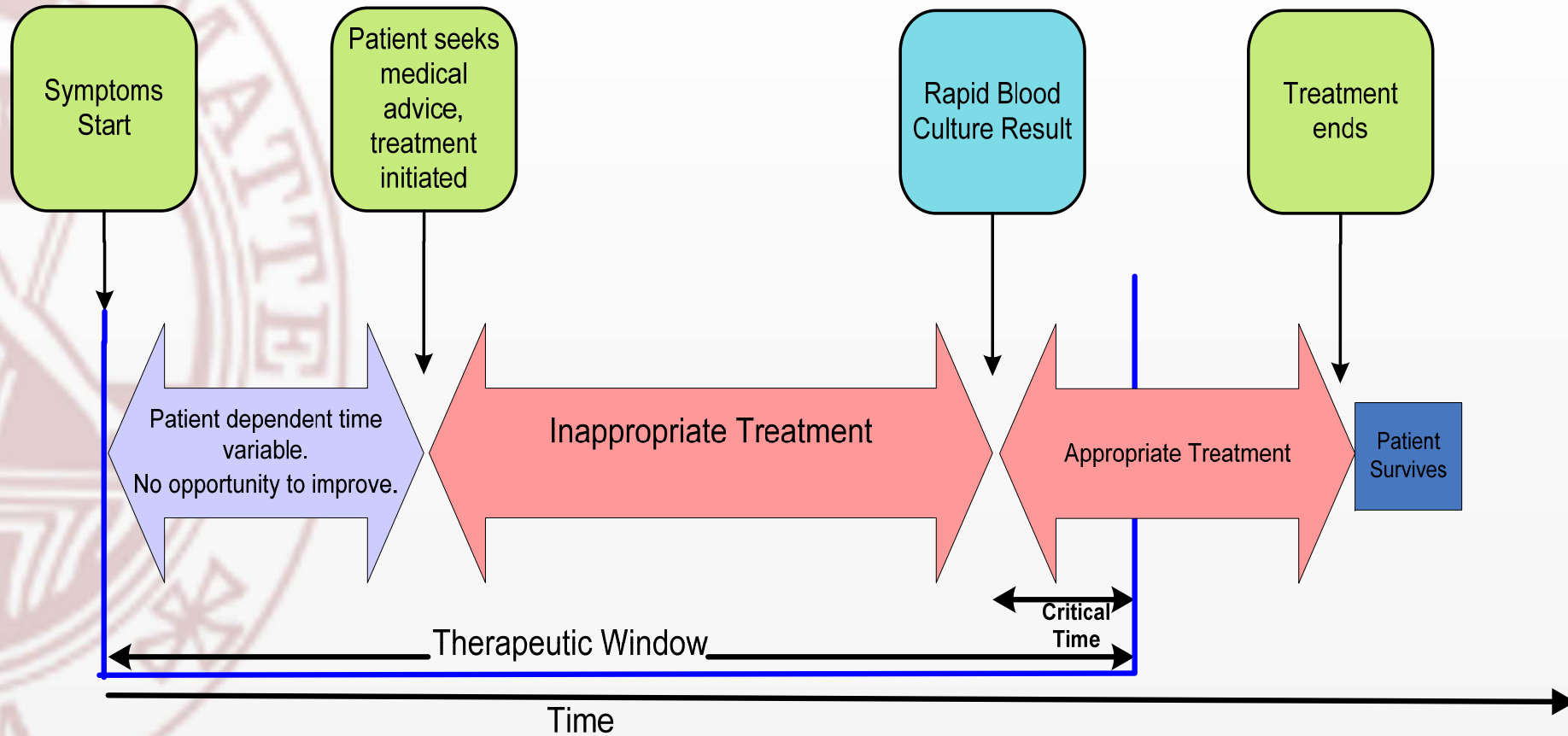
INFORMATION & REGISTRATION:
WORLDSEPSISCONGRESS.ORG

For each hour's delay in administering antibiotics in septic shock, mortality increases by 7.6%

Therapeutic window



Therapeutic window



Gold standard: BLOOD CULTURES

Blood culture is an essential diagnostic tool for the recognition of sepsis.

Preanalytical phase is essential for appropriate diagnosis:

- Sampling
- Volume
- Proper sample handling
- Timely incubation

Pre-Analytical Standards: sampling

- Before the beginning of antibiotic therapy or before a new administration of antibiotic (when the antibiotic concentration in the blood is minimum)
- Fever not necessary for sampling
- Blood cultures must be performed at the same time from different venous sites
- Pay attention: label samples indicating draw site

Surviving sepsis campaign: International guidelines for management of severe sepsis and septic shock. @2013 Society of Critical Care Medicine, European Society of Intensive Care Medicine.

Guidelines for evaluation of new fever in critically ill adult patients: 2008 update from the American College of Critical Care Medicine and the Infectious Diseases Society of America . Naomi P. O'Grady, et al. Crit Care Med 2008 Vol. 36, No. 4

Pre-Analytical Standards: optimal blood volume

- Blood culture volume is the most significant factor affecting the detection of organisms in bloodstream infection
- For adult patients it is recommended that 40-60mL of blood be cultured (2-3 sets)
- The volume of blood drawn with the culture tube should be $\geq 10\text{mL}$

Surviving sepsis campaign: International guidelines for management of severe sepsis and septic shock.
2013 Society of Critical Care Medicine, European Society of Intensive Care Medicine.

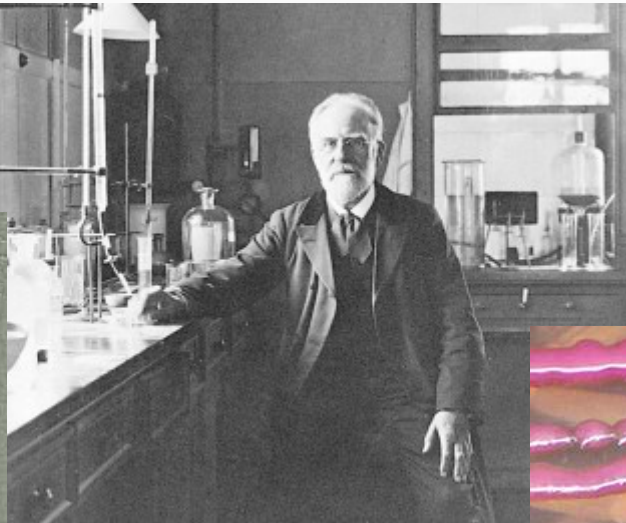
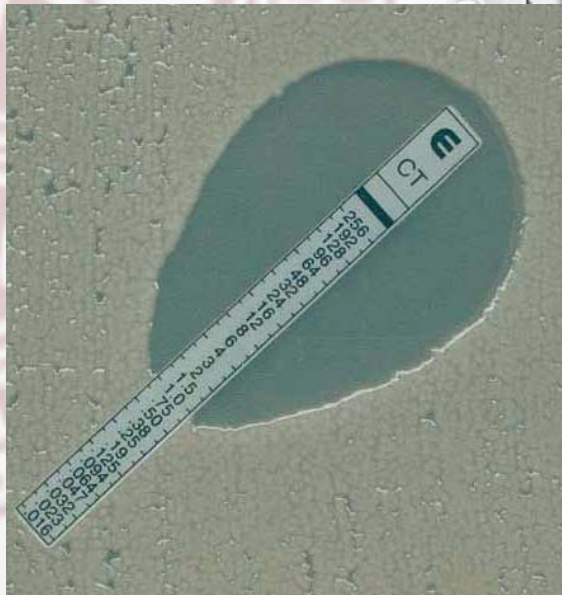
Pre-Analytical Standards: preincubation time

- To optimise the clinical utility of blood culture results, the interval between collection of samples and the incubation should be kept to a minimum
- The samples must be kept at room temperature
- Inoculated bottles should be incubated as soon as possible, and within a maximum of 2-4 hours

Yesterday

- **Conventional practice**

- Identifications and sensitivities of microorganisms may not be available until 24-48 h post flagging positive



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Today

- **Rapid identification and sensitivity tests**
 - Molecular methods (Xpert, FilmArray)
 - MALDI-TOF mass spectroscopy
 - Antigen agglutination test
 - Coagulase test
 - Automated identification/sensitivity methods

Identification and sensitivity within 1-8 hours

POSITIVE BDs

Gram stain

Negative:
reincubation within
2 h

Positivo cocchi + (stafilococchi, streptococchi), bacilli -,
bacilli +, lieviti

Communication to the clinician and
reporting on the registe

Culture on plate

Cocci Gram pos in cluster

Cocci Gram pos in chain

Bacilli Gram neg

Polymicrobial

Yeasts

Direct coagulase

Lecture after 4 h

Rapid
MALDI-ID

Positive

Negative

Genexpert
MRSA

Informations as : urinary
antigen ? Pneumococcal
pneumoniae?
Endocarditis?

Rapid MALDI-ID

Rapid MALDI-ID

Genexpert
Carba-R

Rapid test

Film ARRAY

Film ARRAY

Antibiogram

POSITIVE BDs

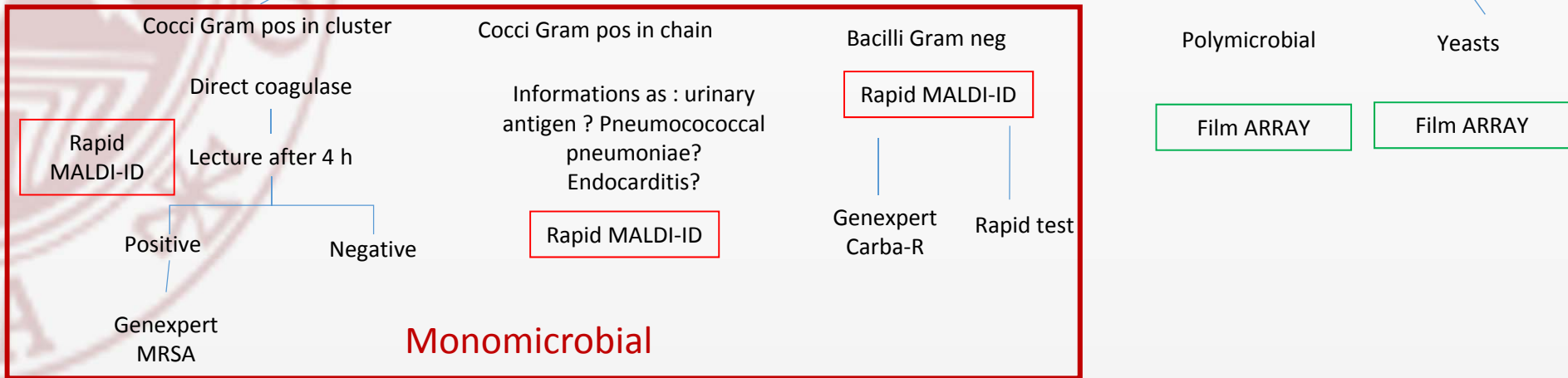
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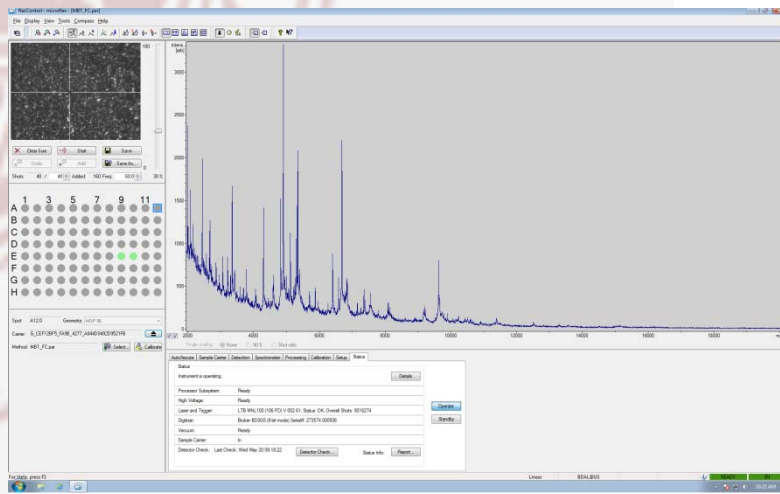
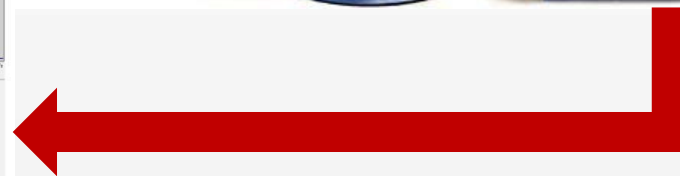
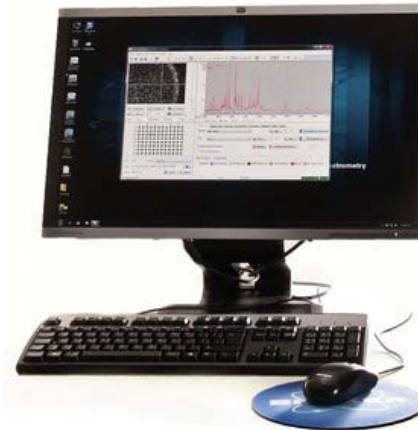
Communication to the clinician and
reporting on the registe

Culture on plate



Antibiogram

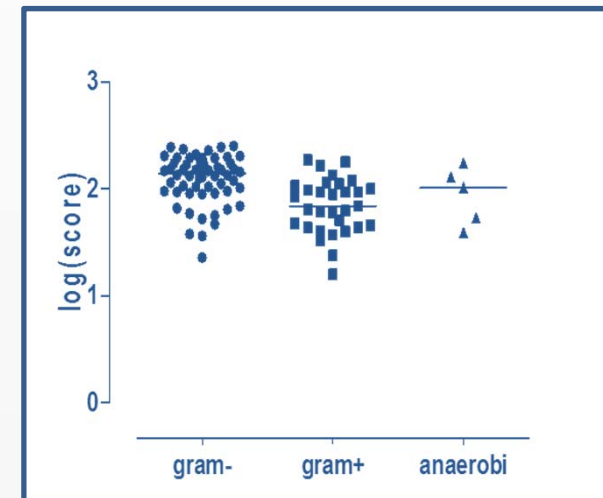
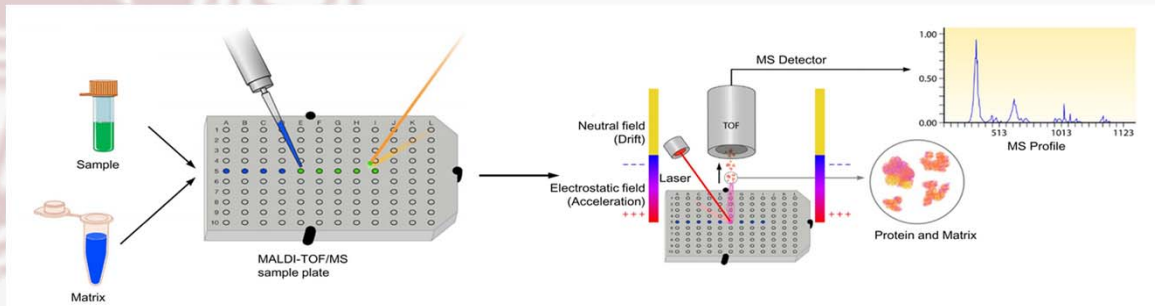
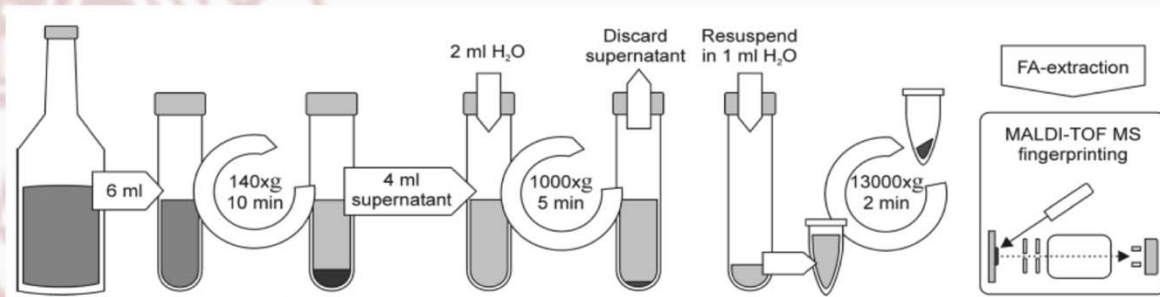
Matrix Assisted Laser Desorption Ionization- Time Of Flight



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MALDI-TOF – Rapid identification



Detection score in MALDI-TOF

Software elaboration provides a probabilistic estimate of **match reliability**

Meaning of Score Values

Range	Description	Symbols	Color
2.300 ... 3.000	highly probable species identification	(+++)	green
2.000 ... 2.299	secure genus identification, probable species identification	(++)	green
1.700 ... 1.999	probable genus identification	(+)	yellow
0.000 ... 1.699	not reliable identification	(-)	red

1.7 to 3 is adequately high score for diagnosis

Servizio: Laboratorio Di Microbiologia (MIC)

Dati del paziente

Paziente	
Data di nascita	
Sesso	
Tessera sanitaria	
Codice Fiscale	

Dati della richiesta

Id.Richiesta	
Data accettazione	
Reparto Richiedente	
Medico Richiedente	
Stato richiesta	X Aperta

Informazioni aggiuntive

1 record estratto	
Lista Analisi	<u>EMOAE/481 EMOAN/481</u>

Risultati

9 records estratti

Analisi	T.Prel	Esito	Risultato	Normalit	Flag	Un.misura
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Emocolture

Emocoltura per Aerobi su Sangue Cat. Venoso		Esito coltura :	Positiva			
		Tempo di crescita	11.08			
		Esame microscopico diretto (colorazione di Gram) :	Presenza di Diplococchi Gram positivi.			
		In corso :	Identificazione ed antibiogramma			
		Identificazione preliminare maldi:	Enterococcus faecalis			
Emocoltura per Anaerobi su Sangue Cat. Venoso		Esito coltura :	Positiva			
		Tempo di crescita	10.48			
		Esame microscopico diretto (colorazione di Gram) :	Presenza di Diplococchi Gram positivi.			
		In corso :	Identificazione ed antibiogramma			

Uscita

Trov

1

2

3

/risconsdetail.jsp

Commu

Rete WHP

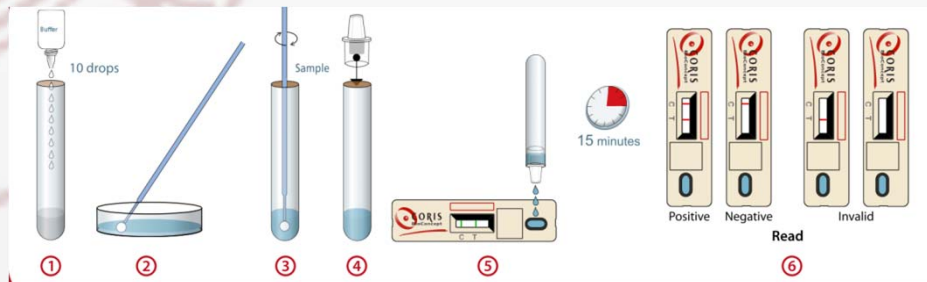
Spaccio CR

mlDAP.jsp

Rapid test for resistance detection



20 minutes



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Xpert® MRSA/SA BC

Accurate Detection of MRSA and SA in Positive Blood Culture Specimens in About an Hour

1 hour



Xpert® Carba-R

Xpert® Carba-R can detect and differentiate the most prevalent carbapenemase gene families in isolates expressing resistance in just 48 minutes.

> Xpert Carba-R differentiates between KPC, NDM, VIM, IMP, and OXA-48 gene families, representing more than 90 different genes responsible for carbapenemase production

Rapid ESBL test 2 hours



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POSITIVE BDs

Gram stain

Negative:
reincubation within
2 h

Positivo cocchi + (stafilococchi, streptococchi), bacilli -,
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Negative

Genexpert
MRSA

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antigen ? Pneumococcal
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Endocarditis?

Rapid MALDI-ID

Bacilli Gram neg

Rapid MALDI-ID

Genexpert
Carba-R

Rapid test

Polymicrobial

Film ARRAY

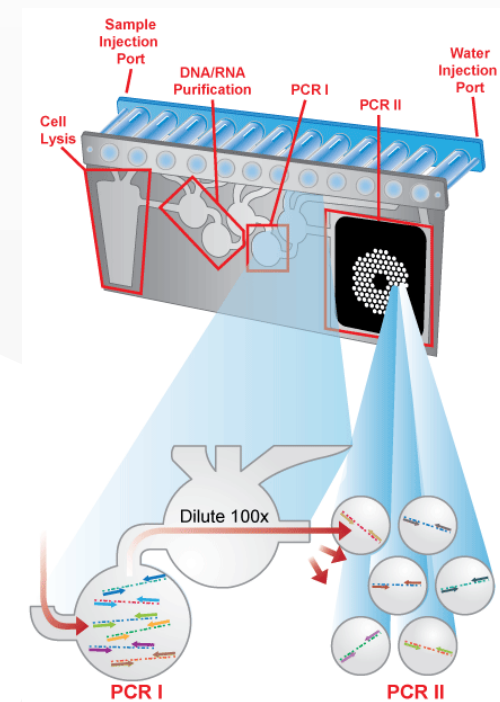
Yeasts

Film ARRAY

Antibiogram

FilmArray

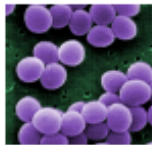
FilmArray[®] Instrument



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FilmArray



Gram-Positive Bacteria

Enterococcus

Listeria monocytogenes

Staphylococcus

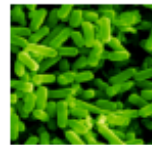
Staphylococcus aureus

Streptococcus

Streptococcus agalactiae

Streptococcus pneumoniae

Streptococcus pyogenes



Gram-Negative Bacteria

Acinetobacter baumannii

Haemophilus influenzae

Neisseria meningitidis

Pseudomonas aeruginosa

Enterobacteriaceae

Enterobacter cloacae complex

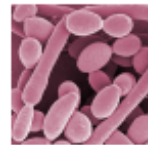
Escherichia coli

Klebsiella oxytoca

Klebsiella pneumoniae

Proteus

Serratia marcescens



Yeast

Candida albicans

Candida glabrata

Candida krusei

Candida parapsilosis

Candida tropicalis



Antibiotic Resistance Genes

mecA – methicillin resistant

vanA/B – vancomycin resistant

KPC – carbapenem resistant

FilmArray™

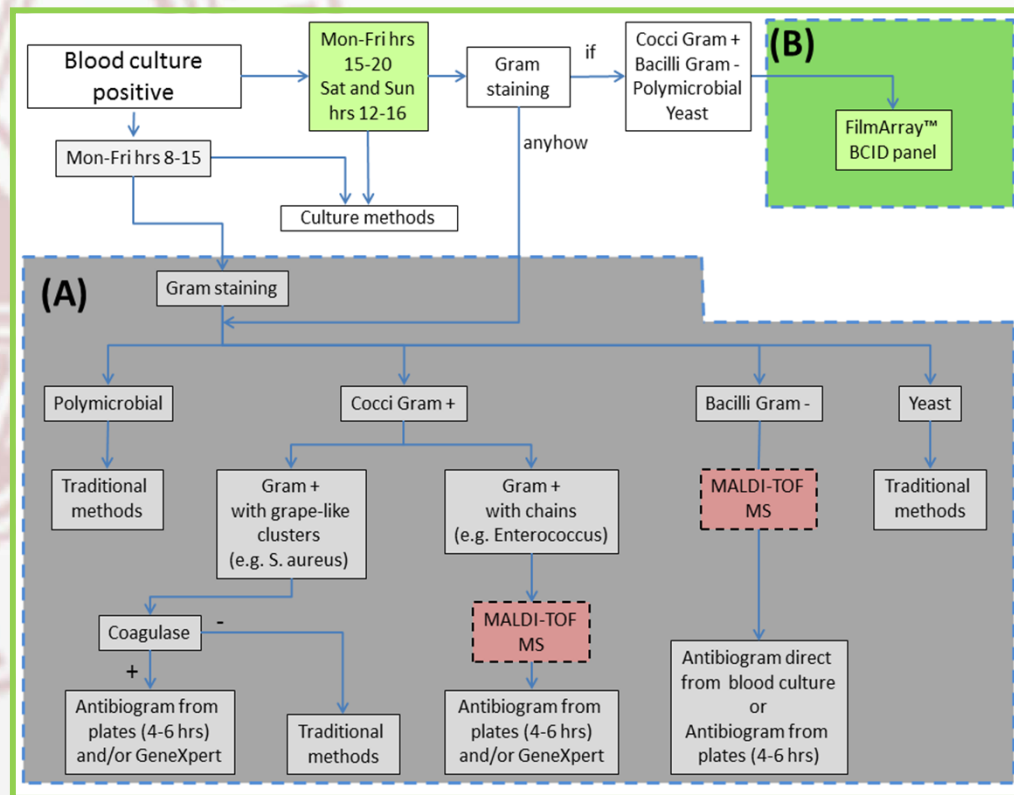
The fastest way to better results.



↑ FilmArray™ BCID Panel

FilmArray

- The study was conducted prospectively between March 2016 and February 2017 at the University Hospital Foundation IRCCS Policlinico San Matteo in Pavia
- BCs were submitted for conventional (culture-based ID) and/or rapid (MALDI-TOF MS; FA BCID panel) methodologies, according to the following BSI diagnostic algorithm:



- path A (rapid ID with MS, rapid resistance test)
- path B (FA BCID test)
- path C (path A without MS)

Appropriateness of therapy at 24 h

Antimicrobial Therapy	Path A with MS (n=100)	Path B with FA (n=100)	Path C without MS (n=100)
Appropriate therapy at t=0	35/100 (35%)	25/94 (26.6%)	36/100 (36%)
Appropriate therapy at 24 h	38/65 (58.5%)	54/69 (78.3%)	35/64 (54.7%)
Appropriate therapy at 48 h	59/65 (90.8%)	59/69 (85.5%)	55/64 (85.9%)
Appropriate therapy at >48 h	65/65 (100%)	69/69 (100%)	64/64 (100%)
Other		2 deaths 4 patients without therapy	

Conclusion

- Diagnosis of sepsis: as rapid as possible
- Microbiology lab: determinant role in timely diagnosis to reduce hospital stay, health costs and mortality
- MALDI-TOF rapid ID:
 - High sensitivity for gram negative bacilli
 - Rapid identification in 20-25 minutes
- FilmArray:
 - Polimicrobial bacteriemia
 - Yeasts
 - Markers of resistance
- The availability of various methods and instruments must be exploited to tailor the the diagnostic path according to the characteristics and the severity of the patient



THANK YOU



Dott. Piero Marone
Dott.ssa Patrizia Cambieri
Dott.ssa Bianca Mariani

Bacteriology Laboratory- Microbiology and Virology-

Fondazione IRCCS San Matteo, Pavia. Italy

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