2° CONGRESSO NEWMICRO

I laboratori di Microbiologia e la Clinical Governance

# Aspetti organizzativi e gestionali del laboratorio di Microbiologia

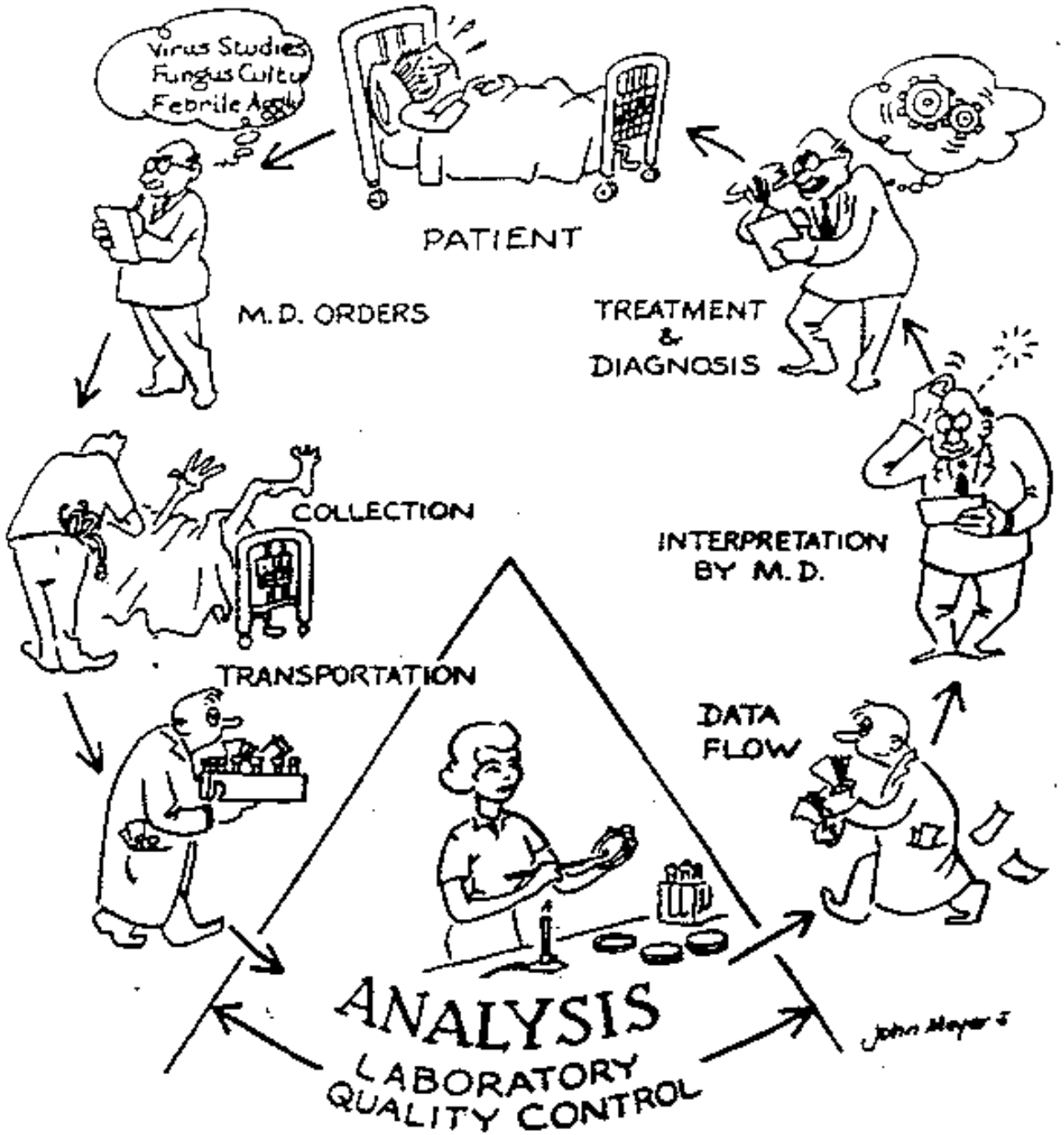
**Giovanni P. Gesu**

**S.C. Microbiologia e Virologia**

**Ospedale Niguarda Ca' Granda**

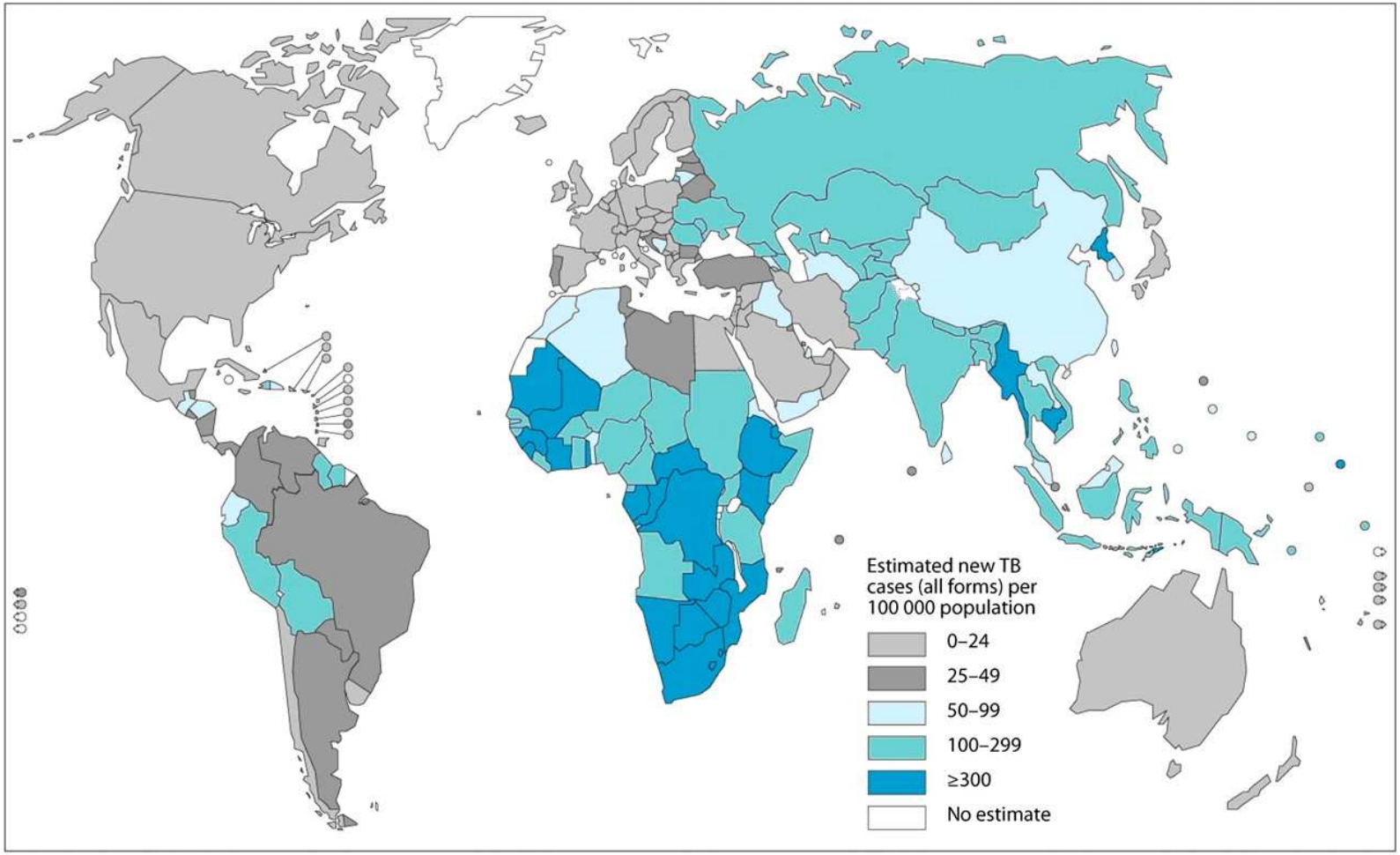
**Milano**

**Lazise 14 - 16 marzo 2012**


Virus Studies
Fungus Cultu
Febrile Ag
PATIENT
M.D. ORDERS
TREATMENT
&
DIAGNOSIS
COLLECTION
INTERPRETATION
BY M.D.
TRANSPORTATION
DATA
FLOW
ANALYSIS
LABORATORY
QUALITY CONTROL

The Journal of Infectious Diseases

# Estimated TB incidence by country, 2009.




The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Source: *Global Tuberculosis Control 2010.* WHO, 2010.

World Health Organization

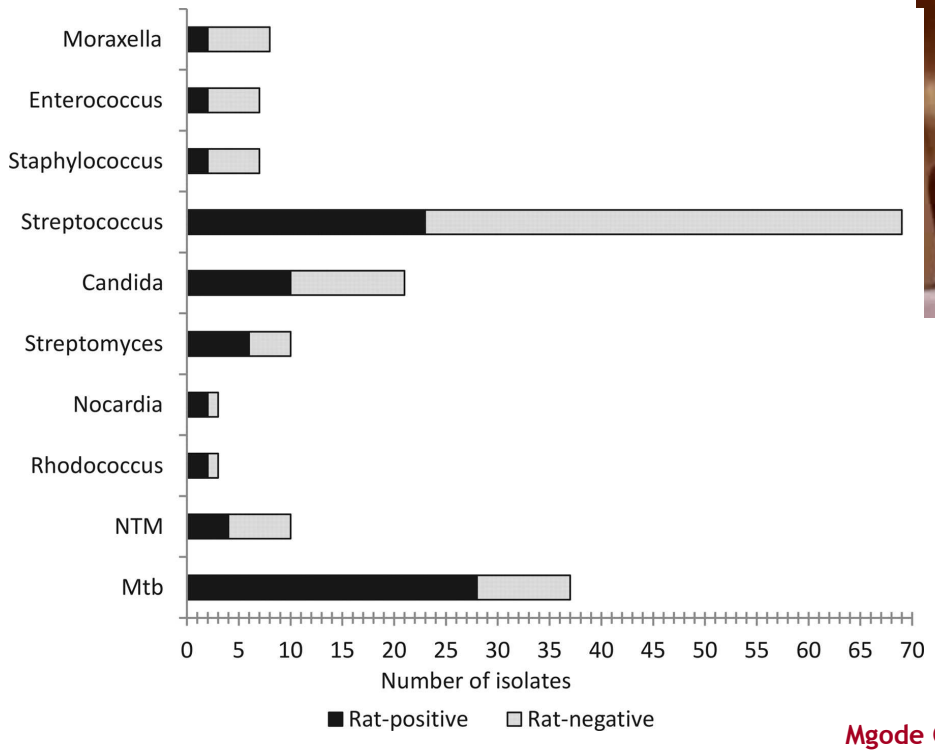


Weyer K et al *Journal of Infectious Diseases* 2011;204:S1196-S1202

Journal of Clinical Microbiology

# Diagnosis of Tuberculosis by Trained African Giant Pouched Rats and Confounding Impact of Pathogens and Microflora of the Respiratory Tract

***Cricetomys gambianus***

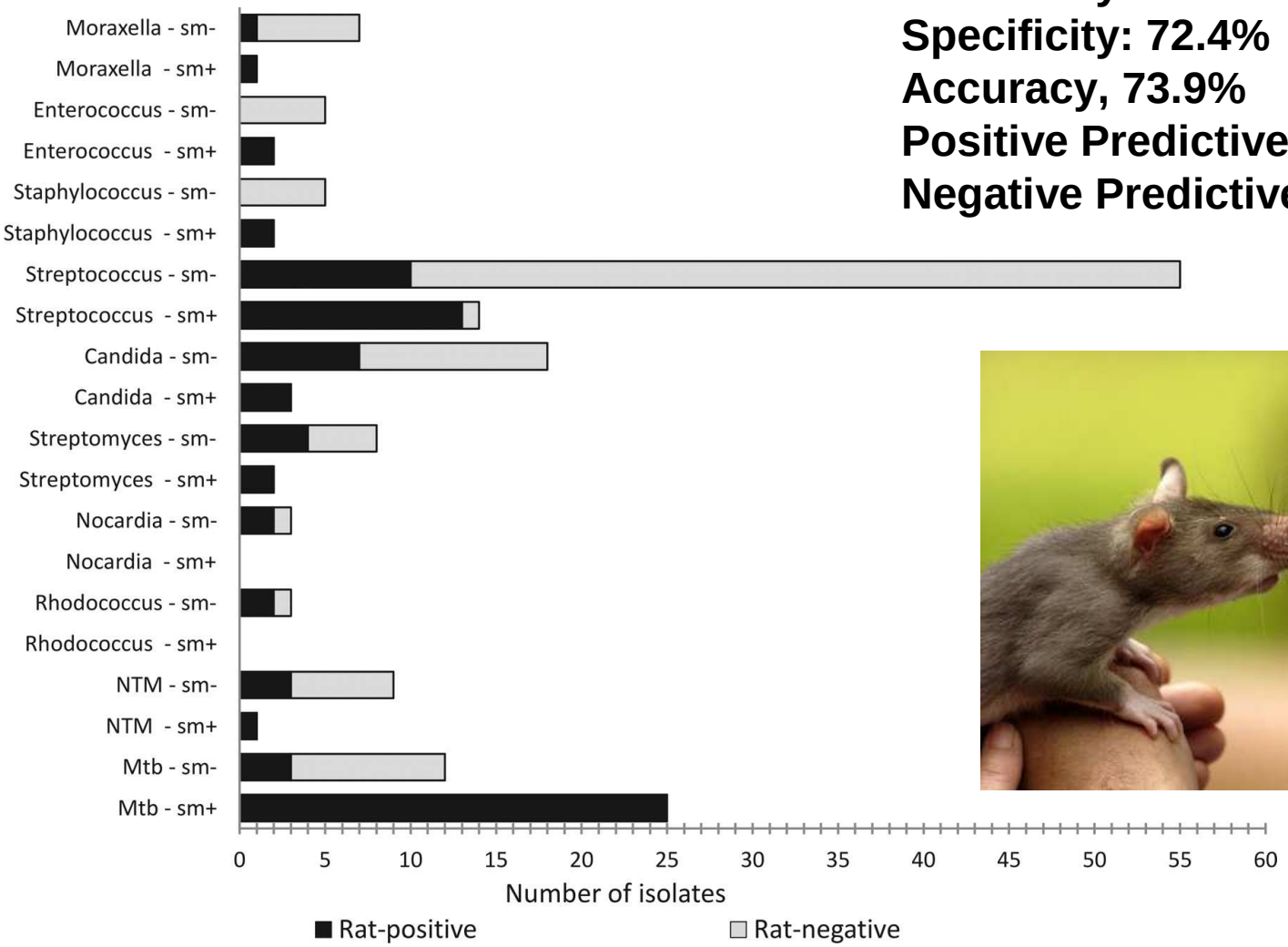



70 campioni esaminati da 2 ratti in 32 minuti

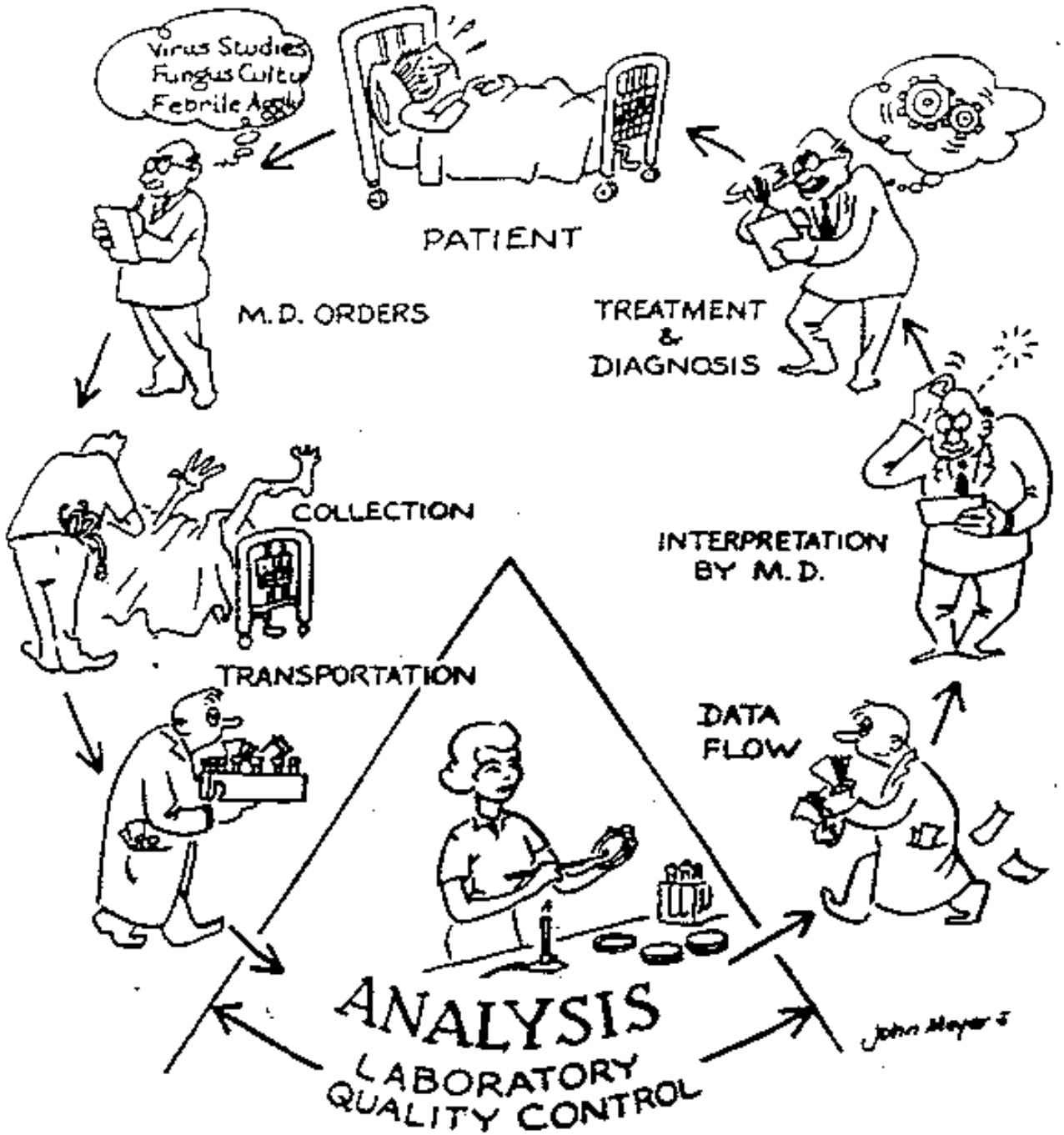
Mgode G F et al. Journal of Clinical Microbiology Febbruary 2012; 50:274-280

**Patterns of rat positive and rat-negative in smear-positive (sm+) and smear negative (sm−) sputum samples with Mycobacterium and nonmycobacterial microorganisms.**

**Sensitivity: 80.4%**
**Specificity: 72.4%**
**Accuracy, 73.9%**
**Positive Predictive Value: 41.7%**
**Negative Predictive Value: 93.8%**




Mgode G F et al. Journal of Clinical Microbiology Febbruary 2012; 50:274-280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# SIRS

## Sindrome da Risposta Infiammatoria Sistemica

Temperatura > 38°C o < 36°C

Frequenza cardiaca > 90 bpm

Frequenza respiratoria > 20/m o $Pa_{Co2}$ < 32

Leucociti > 12.000 o < 4.000/$mm^3$ o > 10% *band forms*

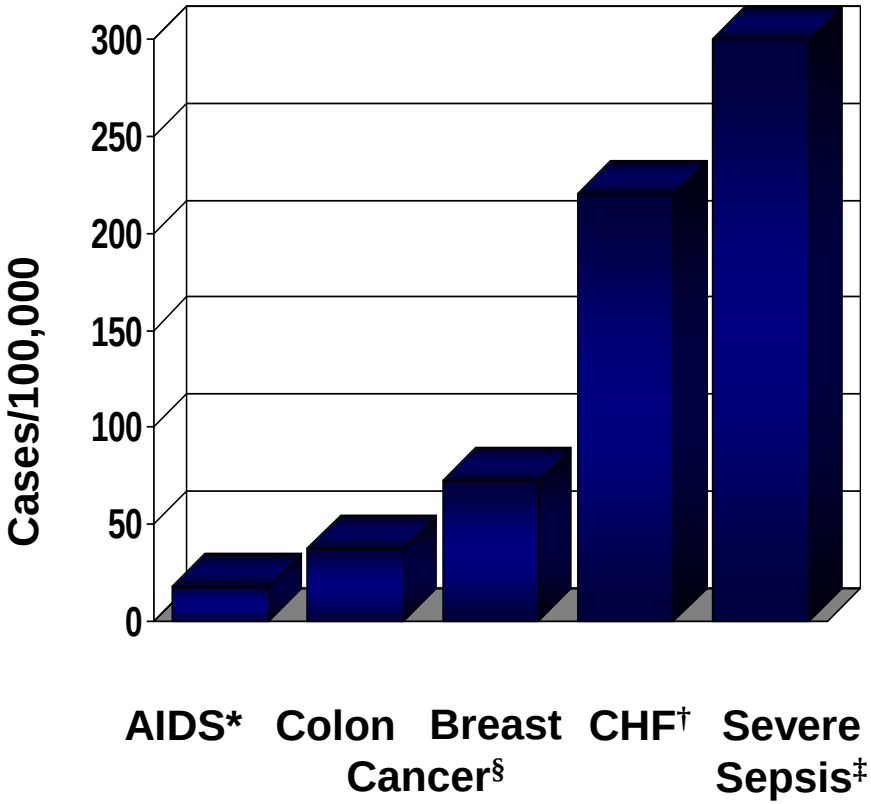
\+ Infezione → SEPSI

\+ Danno d'organo → SEPSI GRAVE

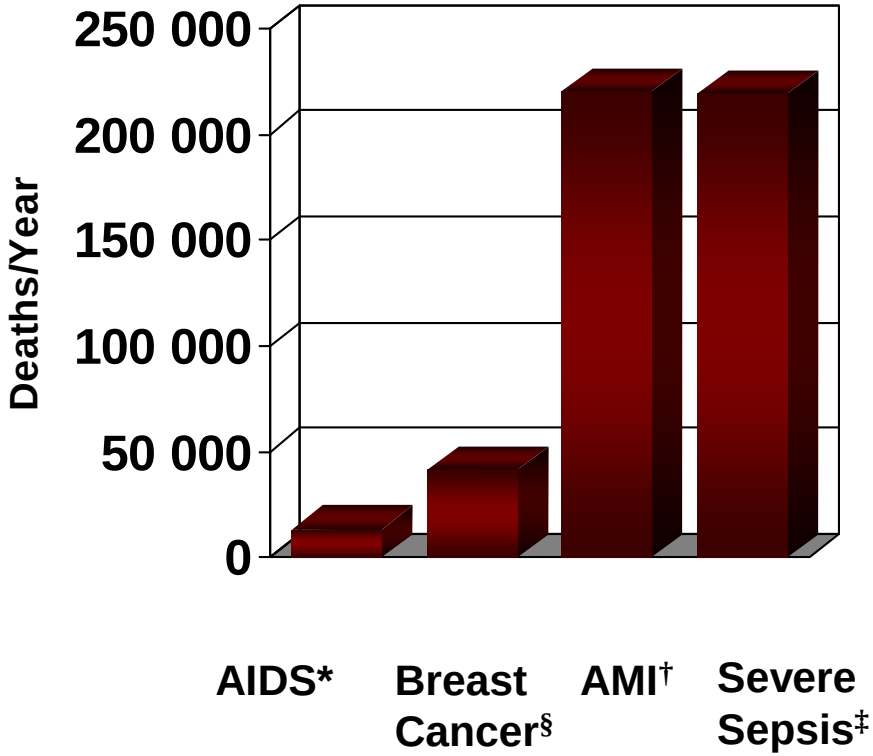
\+ Ipotensione non controllabile → SHOCK SETTICO

# Comparison With Other Major Diseases

## Incidence of Severe Sepsis

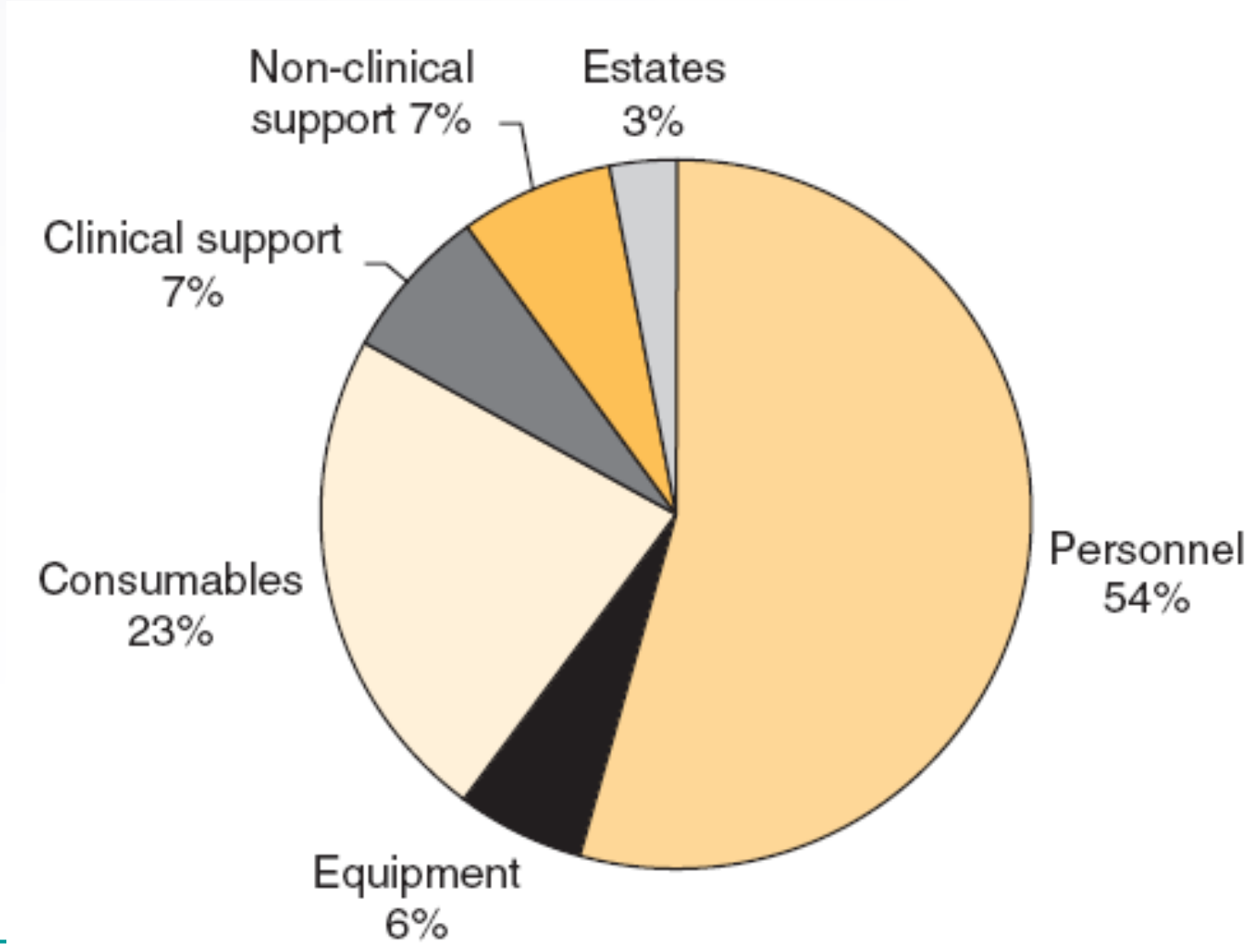



## Mortality of Severe Sepsis



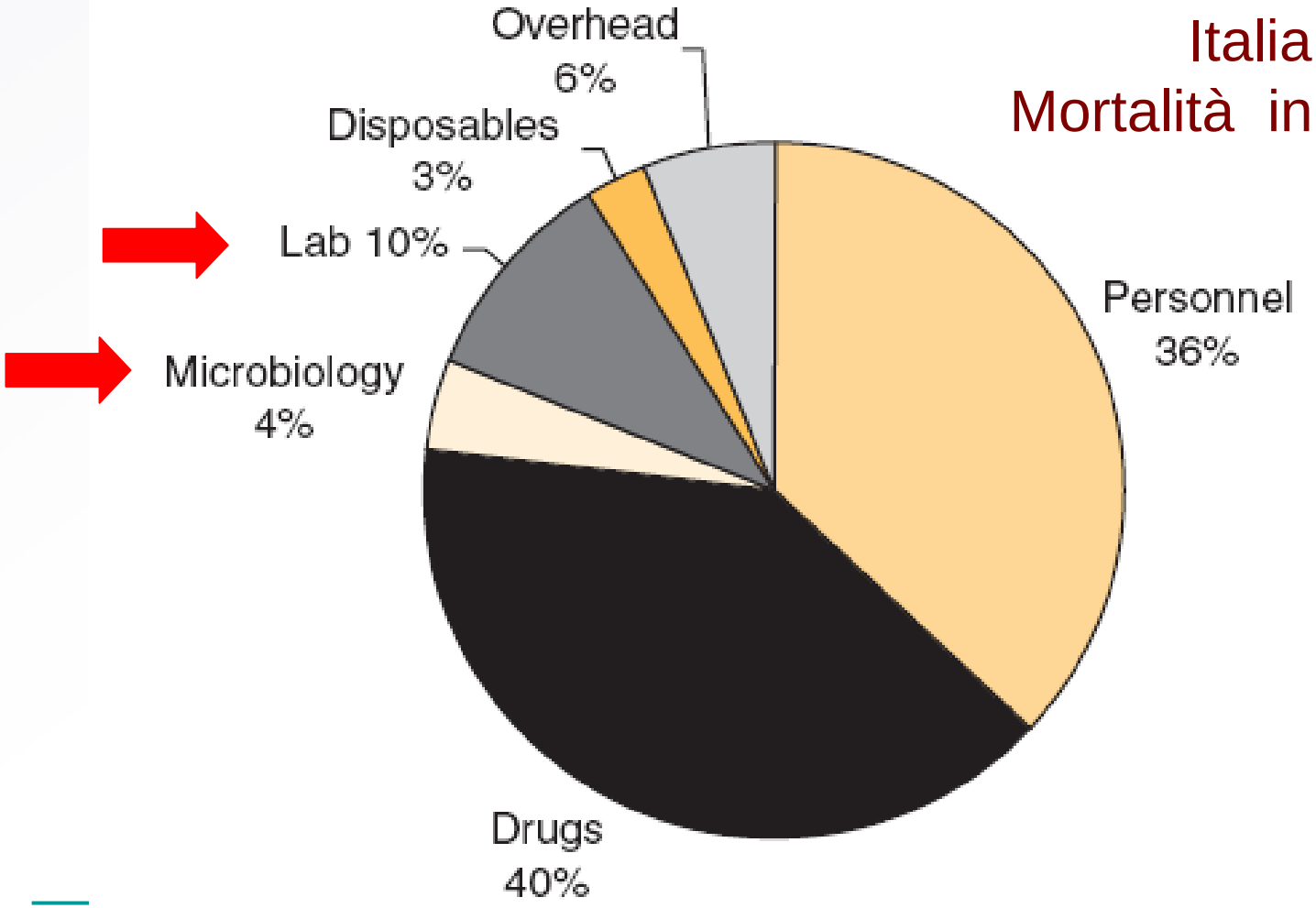

†National Center for Health Statistics, 2001. §American Cancer Society, 2001. *American Heart Association. 2000.
‡Angus DC et al. *Crit Care Med*. 2001;29(7):1303-1310.

# Proportion of different cost blocks within the total cost of Intensive Care Units




Burchardi H and Schneider H *Pharmacoeconomics* 2004; 22:793-813

# Distribution of costs for Intensive Care Unit (ICU) treatment of severe sepsis

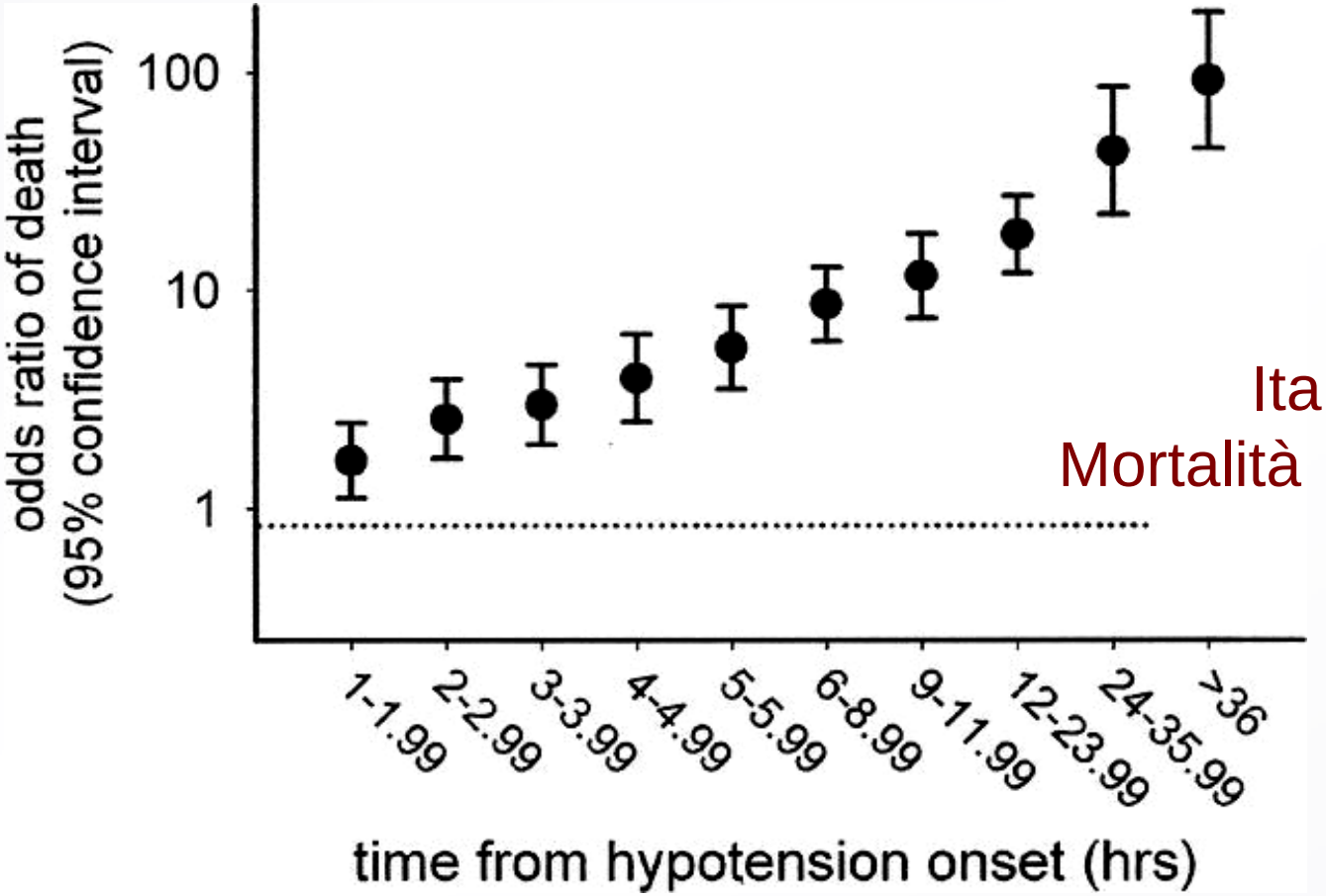



Italia 2009
Mortalità in ICU → 41%



Burchardi H and Schneider H *Pharmacoeconomics* 2004; 22:793-813

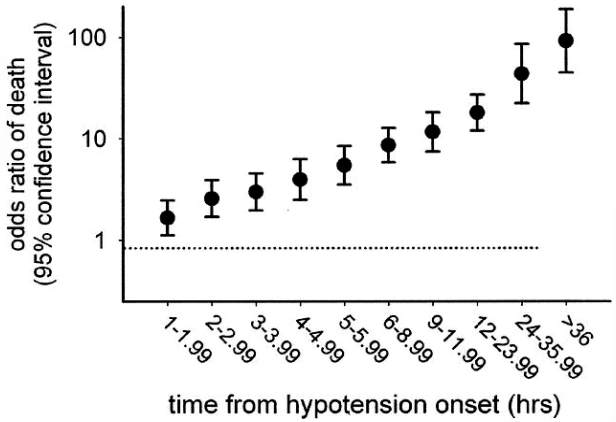
# Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock




Italia 2009
Mortalità in ICU → 73%

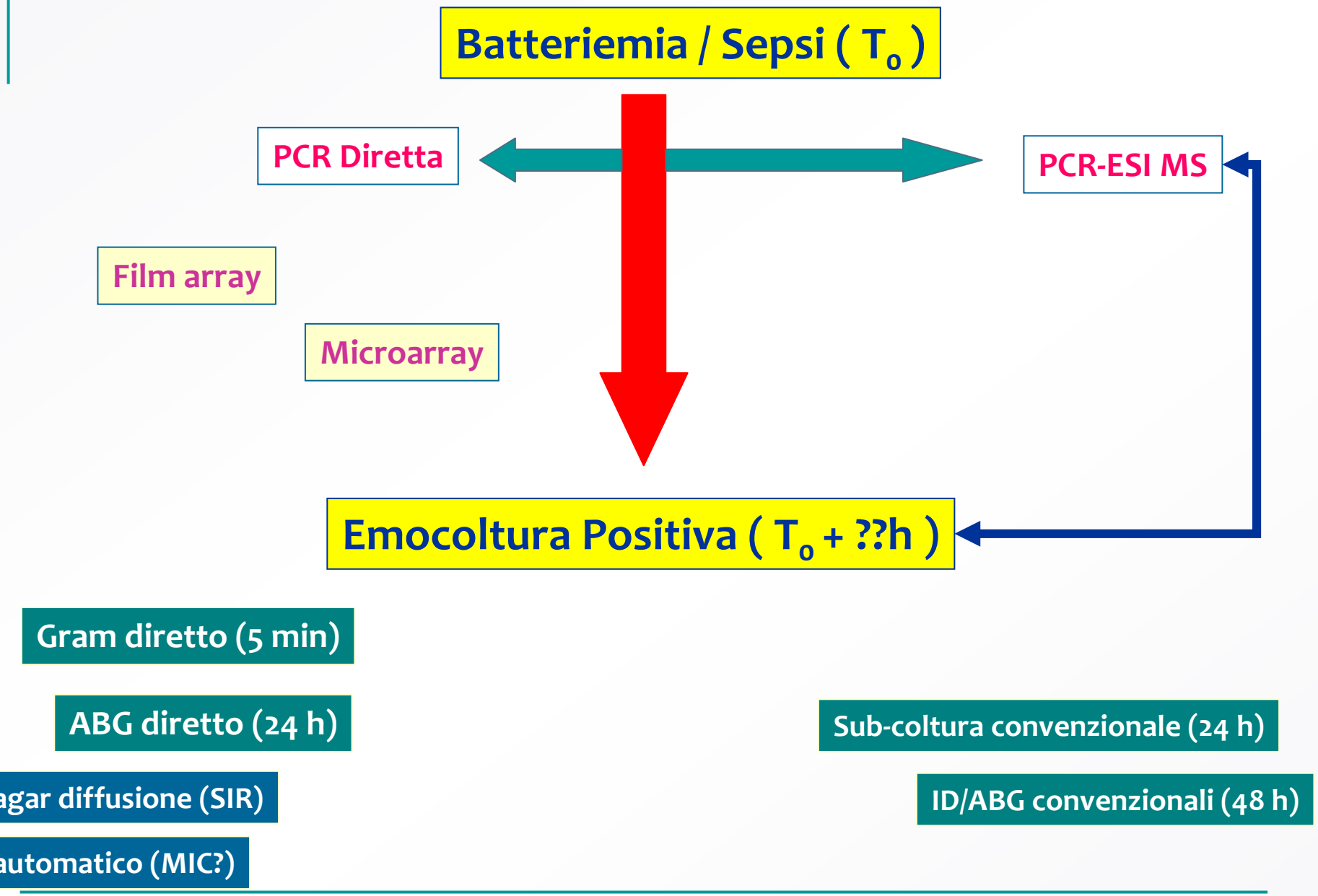
Kumar A et al *Critical Care Medicine* 2006; 34:1589-1596

# Batteriemia / Sepsi ( $T_0$ )

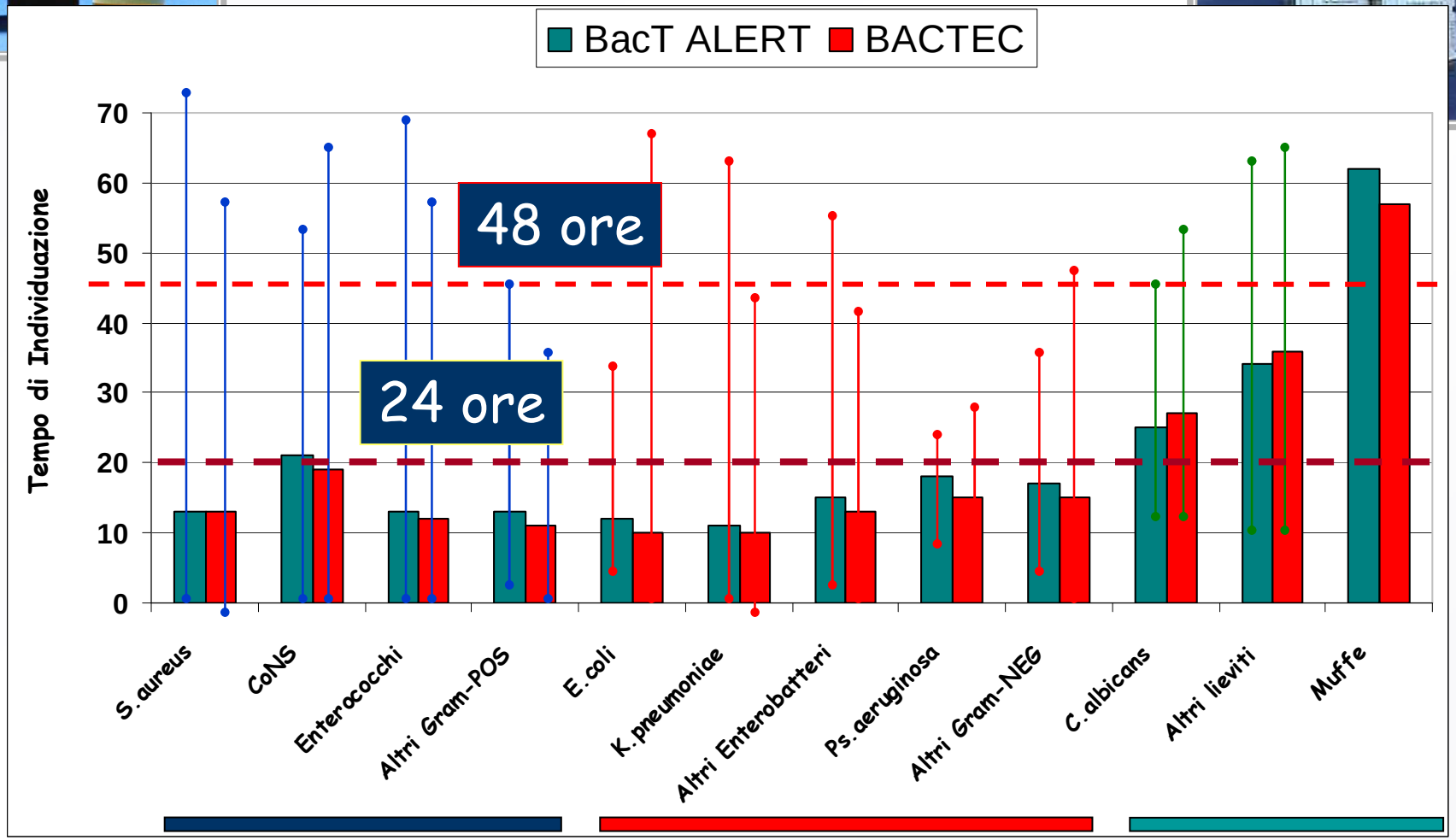




# Emocoltura Positiva ( $T_0$ + ??h )


Batteriemia / Sepsi ( $T_0$ )
PCR Diretta
PCR-ESI MS
Film array
Microarray
Emocoltura Positiva ( $T_0$ + ??h )
Gram diretto (5 min)
ABG diretto (24 h)
agar diffusione (SIR)
automatico (MIC?)
Sub-coltura convenzionale (24 h)
ID/ABG convenzionali (48 h)

# Emocolture a Monitoraggio Continuo:
# Tempi medi di Individuazione






Mirrett S et al *Journal of Clinical Microbiology* 2003; 41:2391-2394

# Septifast : Analytical Concept

**Sample**

**whole blood**
(1 ml)

**Sample Preparation**

Bacterial Lysis &
DNA Extraction

**PCR Analysis**

## Gram (-)
- *Escherichia coli*
- *Klebsiella* (pneumoniae, oxyt.)
- *Serratia marcescens*
- *Enterobacter* (cloacae, aerog.)
- *Proteus mirabilis*
- *Pseudomonas aeruginosa*
- *Acinetobacter baumanii*
- *Stenotrophomonas maltophilia*

## Gram (+)
- *Staphylococcus aureus*
- CoNS
- *Streptococcus pneumoniae*
- *Streptococcus* spp.
- *Enterococcus faecium*
- *Enterococcus faecalis*

## Fungi
- *Candida* (albicans, tropicalis parapsilosis)
- *Candida* (krusei, glabrata)
- *Aspergillus* (fumigatus)

**MRSA** (mecA)

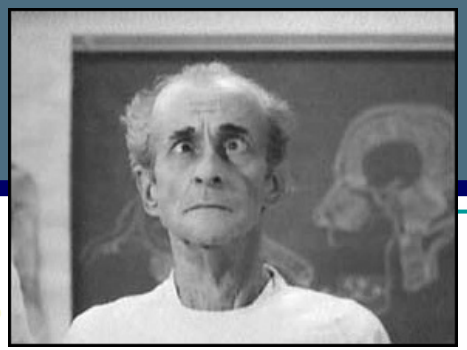
**VRE** (van A, B)

# MOLECULAR DIAGNOSIS OF SEPSIS USING SEPTIFAST
## work process and resources organization

| DAY 1 | | | DAY 2 |
|---|---|---|---|
| 10.00AM | 3.30PM | 7.00PM | 8.30AM |
| 1st RUN (SAMPLES COLLECTED DURING NIGHT AND FROM 9.00AM TO 10.00AM) | RESULTS ☎ | | |
| | 2nd RUN (SAMPLES COLLECTED FROM 10.00AM TO 4.00PM) | *Overnight* REACTION | RESULTS ☎ |

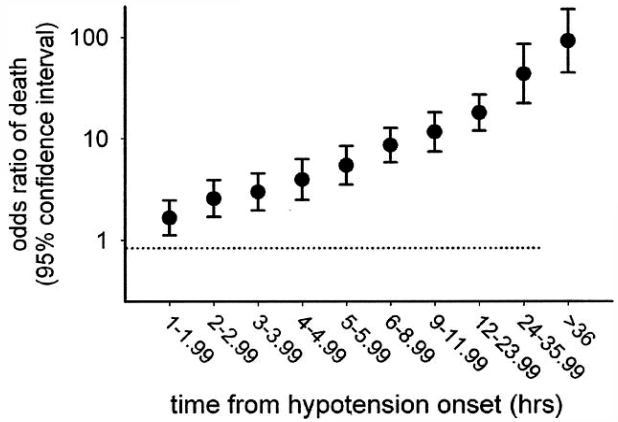
**TAT (ore) 6 – 11**

**TAT (ore) 16 - 22**



Courtesy of Prof. Massimo Clementi

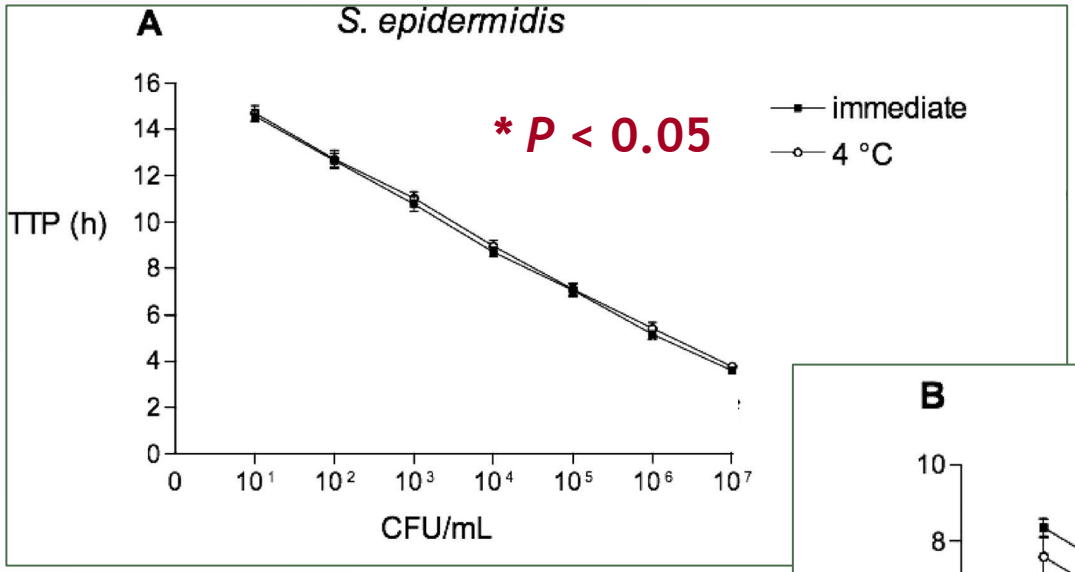
# Batteriemia / Sepsi ( $T_0$ )



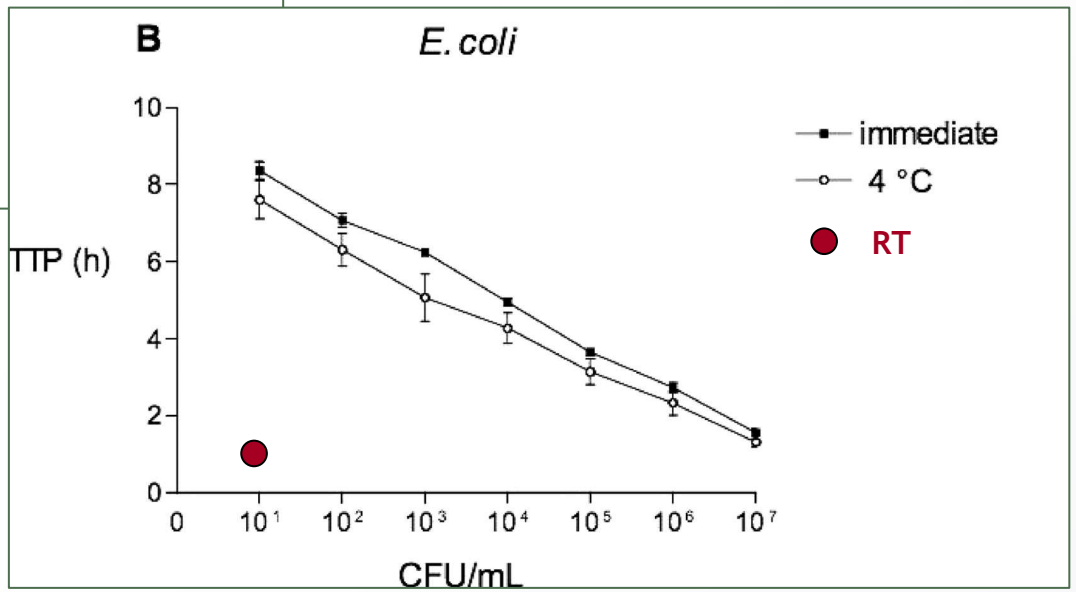


# Emocoltura Positiva ( $T_0$ + ??h )

# Influenza della Temperatura di Conservazione delle Emocolture sul Tempo di Positivizzazione



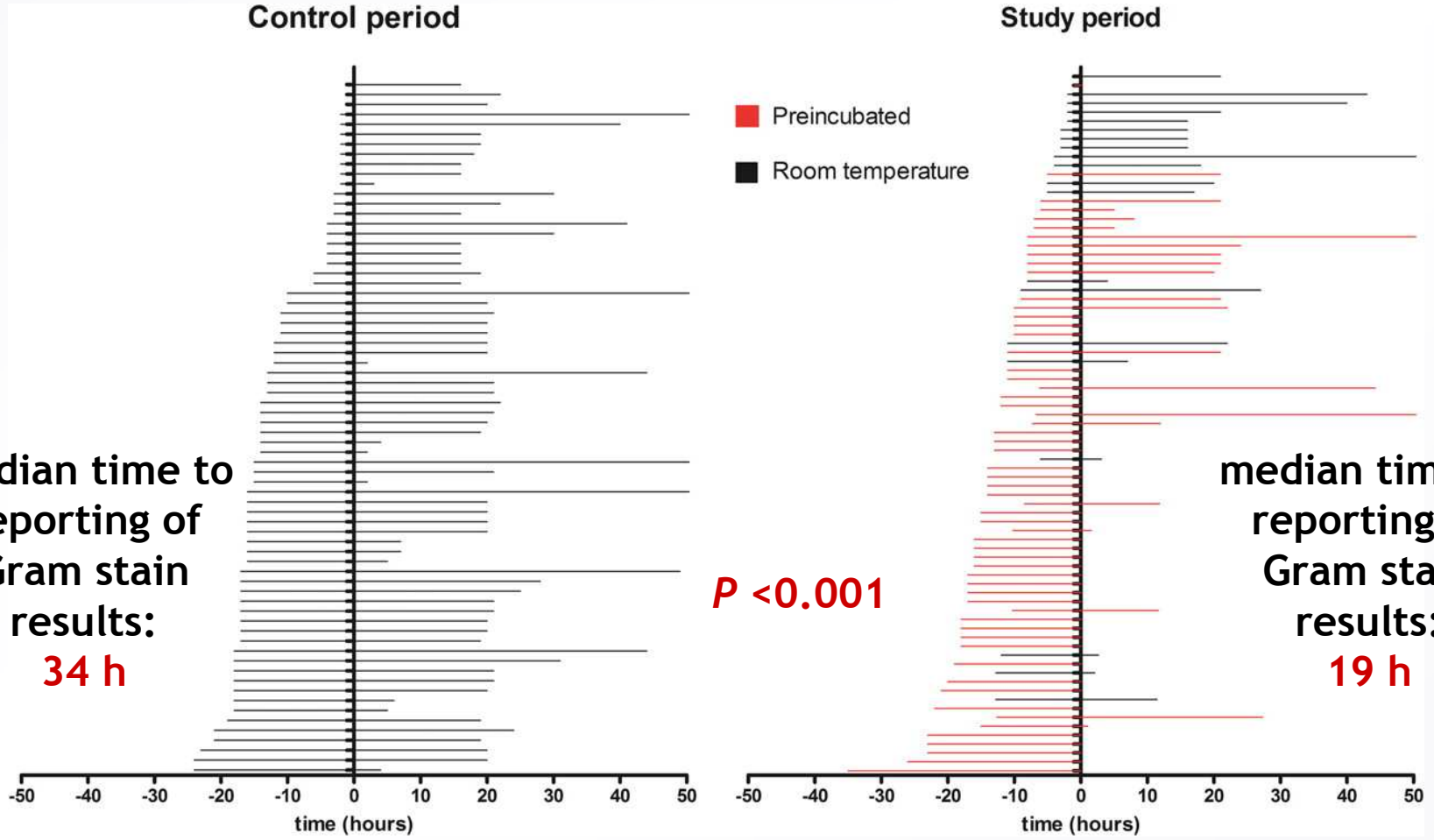







Schwetz I et al. *Journal of Clinical Microbiology* August 2007 45:2691-2694

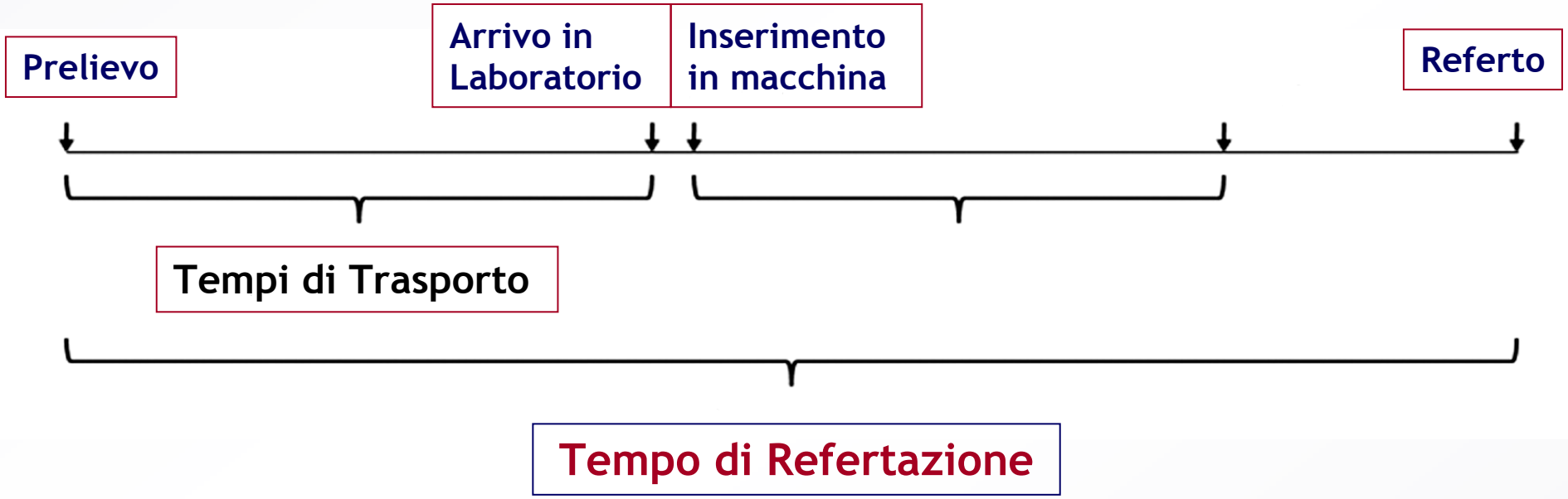
# Clinical Impact of Preincubation of Blood Cultures at 37°C




van der Velden LB et al *Journal of Clinical Microbiology*, January 2011; 49:275-280

# Emocolture

## Timeline e Impatto organizzativo




Trasporti automatici?
Posta pneumatica?
Strumenti in Reparto?

Strumento accessibile h24?

Strumento in Lab Urgenze

Azienda Ospedaliera
Ospedale Niguarda Ca' Granda

# Emocolture inviate dal Pronto Soccorso al Laboratorio Urgenze
(ore 20.00-08.00)

Periodo: 28.04.2010 – 15.10.2010

| | | |
|---|---|---|
| Pazienti | 156 | (6 in età pediatrica) |
| Prelievi | 291 | |
| Prelievi singoli | 27 | (17.31%) |

Pazienti con emocoltura positiva 51 (**32.69%**)

- Flacone aerobio 43
- Flacone anaerobio 7
- Flacone pediatrico 1

Azienda Ospedaliera
Ospedale Niguarda Ca' Granda

# Tempi di Trasporto e Tempi di positivizzazione (TTP)

## Emocolture inviate dal Pronto Soccorso al Laboratorio Urgenze

(ore 20.00-08.00)

Valutabile per 47 emocolture positive

| | |
|---|---|
| **Tempo medio di positivizzazione (TDP)** | **21.38 h** |
| Stafilococchi coagulasi negativi (10) | 26.06 h |
| *Escherichia coli* (1) | 73.09 h |
| *Actinomyces meyeri* (1) | 77.31 h |
| *Enterococcus faecium* (1) | 100.19 h |
| **TDP su 34 emocolture** | **14.49 h** |

# Immediate Incubation of Blood Cultures Outside Routine Laboratory Hours of Operation Accelerates Antibiotic Switching

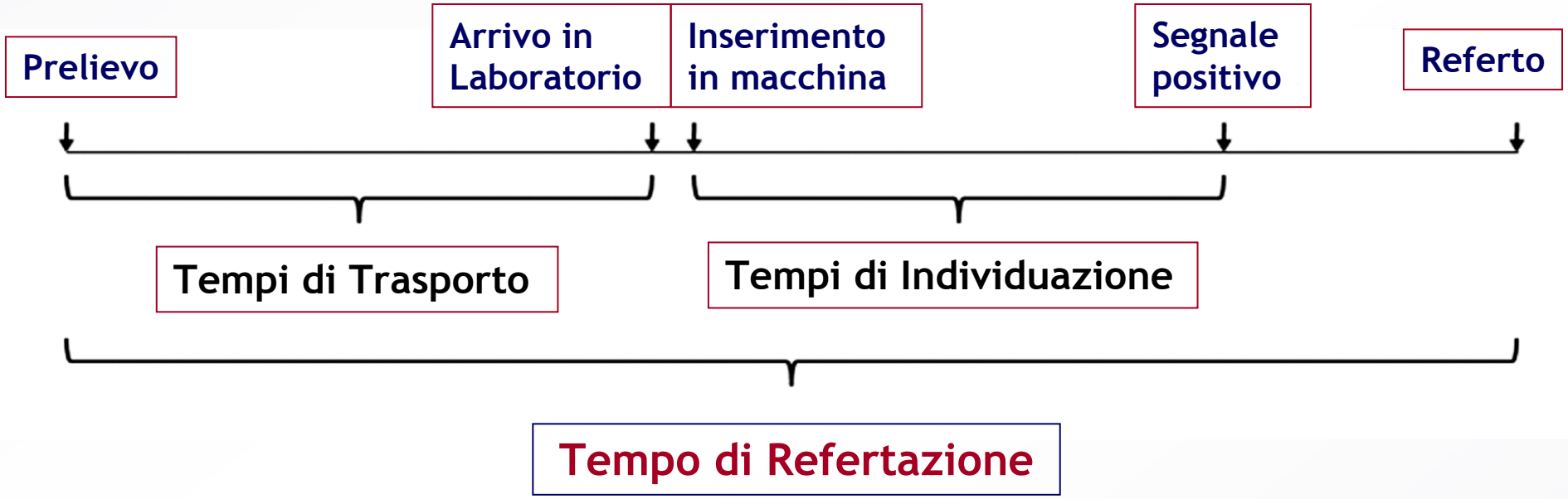
| Flaconi incubati | Subito | Mattina successiva | |
|---|---|---|---|
| | 1325 (139 pos) | 1334 (143 pos) | |
| Positivi dopo | 29 h | 39 h | $P < 0.001$ |
| Identificazione in | 44 h | 63 h | $P < 0.001$ |
| Test di sensibilità in | 62 h | 70 h | $P = 0.009$ |
| Terapia corretta dopo | 43 h | 64 h | $P = 0.024$ |



Kerremans JJ et al *Journal of Clinical Microbiology* November 2009; 47:3520-3523

# Emocolture

## Timeline e Impatto organizzativo

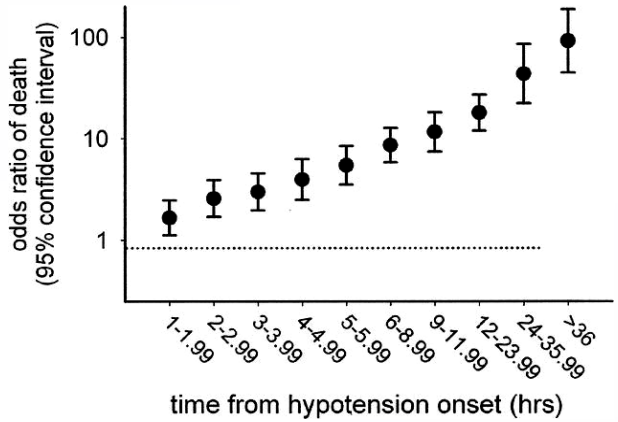



Trasporti automatici?
Posta pneumatica?
Strumenti in Reparto?

Strumento accessibile h24?

Strumento in Lab Urgenze

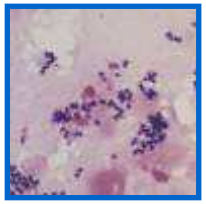
# Batteriemia / Sepsi ( $T_0$ )





## Emocoltura Positiva ( $T_0$ + ??h )

**Gram diretto (5 min)**

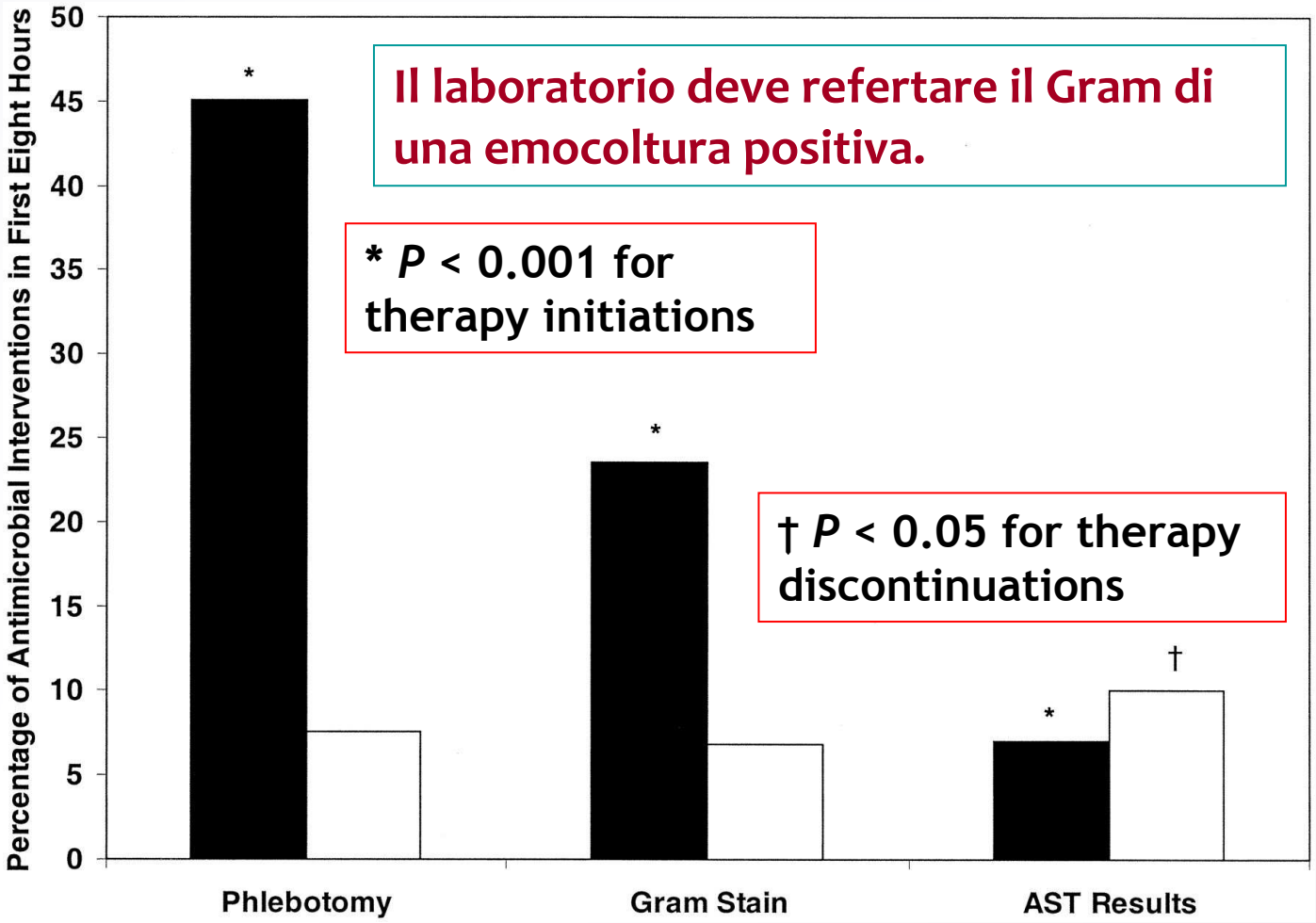


Cocchi Gram-positivi (stafilococchi)

Bacilli Gram-negativi

Miceti lievitiformi

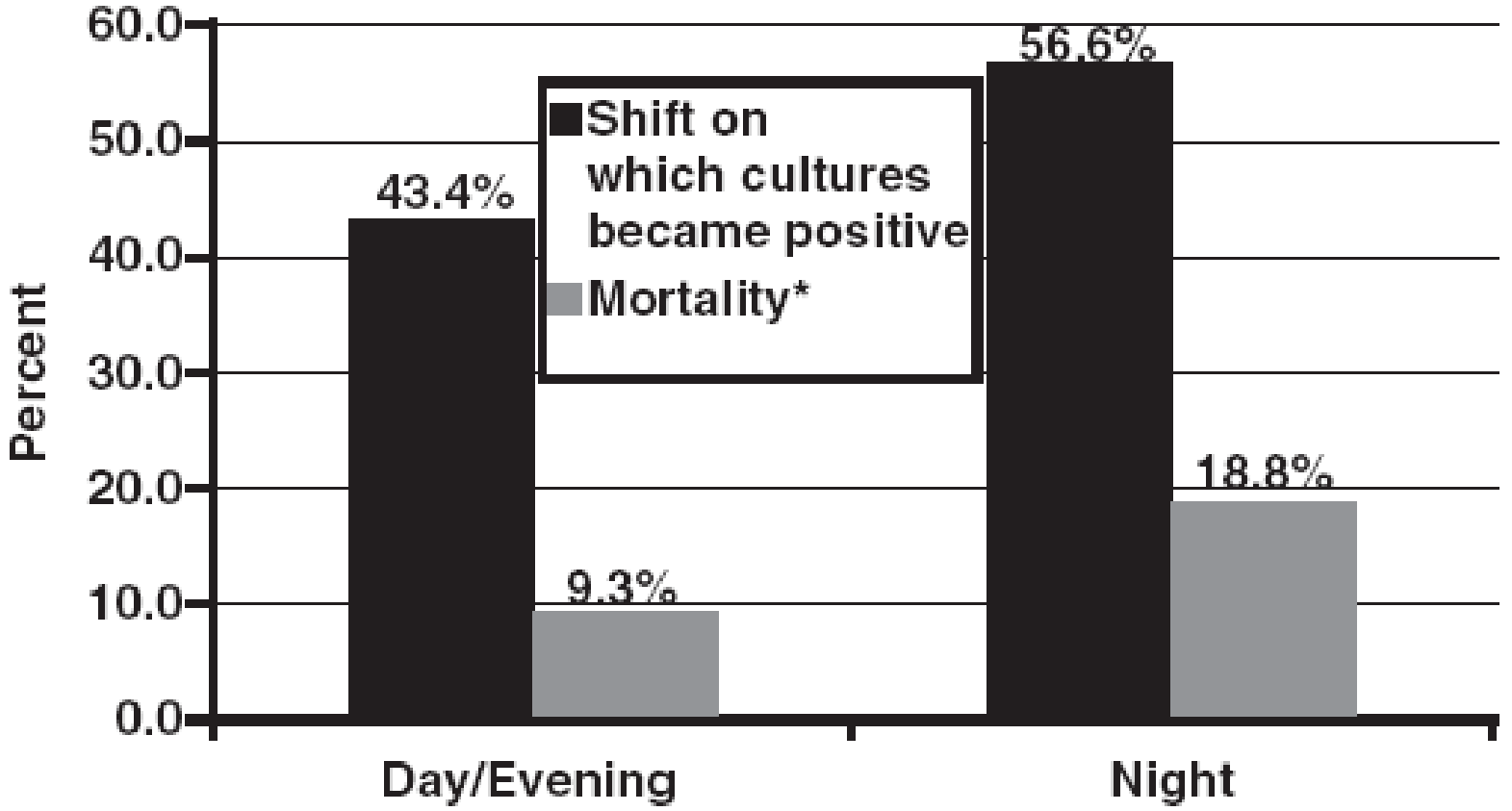
# Detection and Treatment of Bloodstream Infection: Laboratory Reporting and Antimicrobial Management






Munson EL et al *Journal of Clinical Microbiology*, January 2003, 41:495-497

# Decreased Mortality Associated With Prompt Gram Staining of Blood Cultures




**Average Gram TAT → 0.1 hour**

**Average Gram TAT → 3.3 hours**

***P* < 0.0001**

Barenfanger J et al *American Journal of Clinical Pathology* 2008; 130:870-876

# *C. albicans*/*C. glabrata* PNA FISH

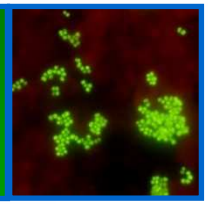

Positive Blood Culture

Gram Stain Performed

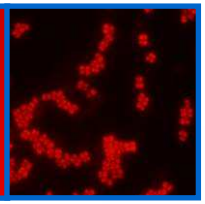
**Yeast**


**53%**[1] **16%**[1] **31%**[1]

***C. albicans*** **(Green Cells)**



***C. glabrata*** **(Red Cells)**

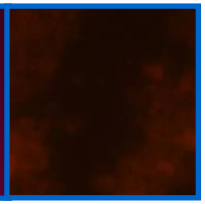


**Other yeast**

**e.g.**

**(*C. parapsilosis* 14%**

***C. tropicalis* 10%)**



**1% Fluconazole - R**

**28% Fluconazole - R**

**9% Fluconazole - R**

**Fluconazole w/o prior azole exposure**[2]

**Aggressive therapy. Consider non-azole Rx**[3,4]

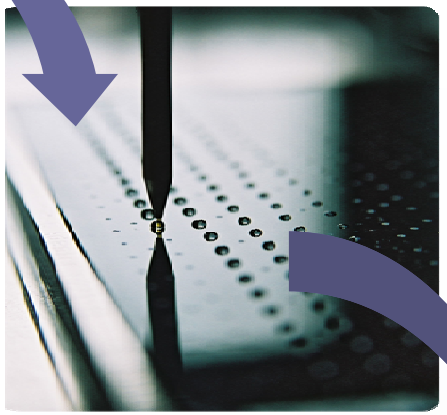
**Treat based on species distribution until culture identification is confirmed**


1. Pfaller et al. J Clinical Microbiology. 2002 Mar;40(3):852-856
2. Forrest et al. J. Clinical Microbiology. 2006 Sep;44(9):3381-3
3. Pappas et al. IDSA Guidelines. CID. 2004 Jan;38: 161-189
4. Spellberg et al. CID. 2006 Jan; 42:244-51

# MALDI BioTyper: Standard Operation Protocols (SOP)

Colonia

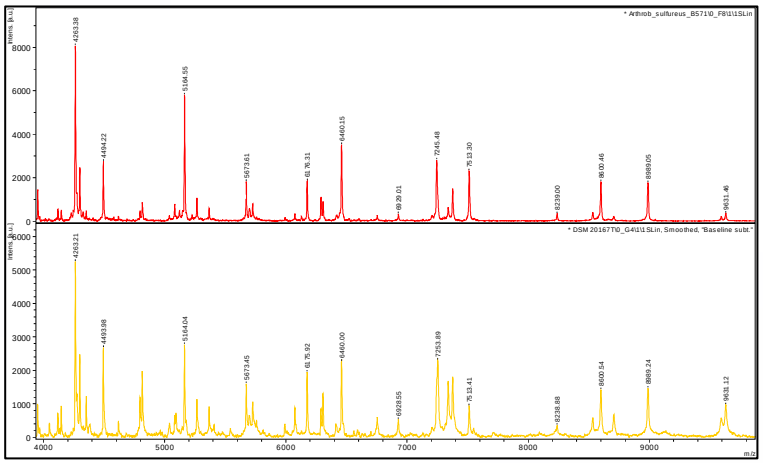
Spot: film sottile su piastra



1 µL di matrice

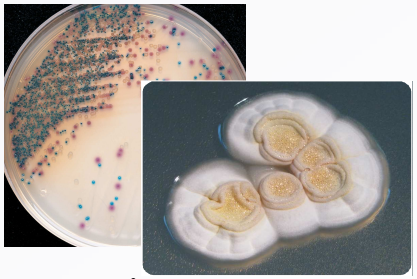

Inserimento

**10 minuti**



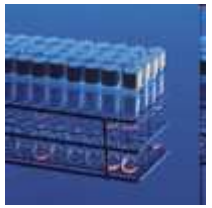
# Workflow

Seng *Clinical Infectious Diseases* 2009



Colonia

Emocolture positive



Terreni liquidi

Urine positive

**Identificazione**

Tempo 6 - 20 min

Costo 2.44 €

**Gram**

Tempo 5 min

Costo 0.60 €

**Test sensibilità**

Tempo 24-48 h

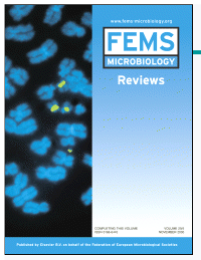
**Identificazione**

Tempo 5-48 h

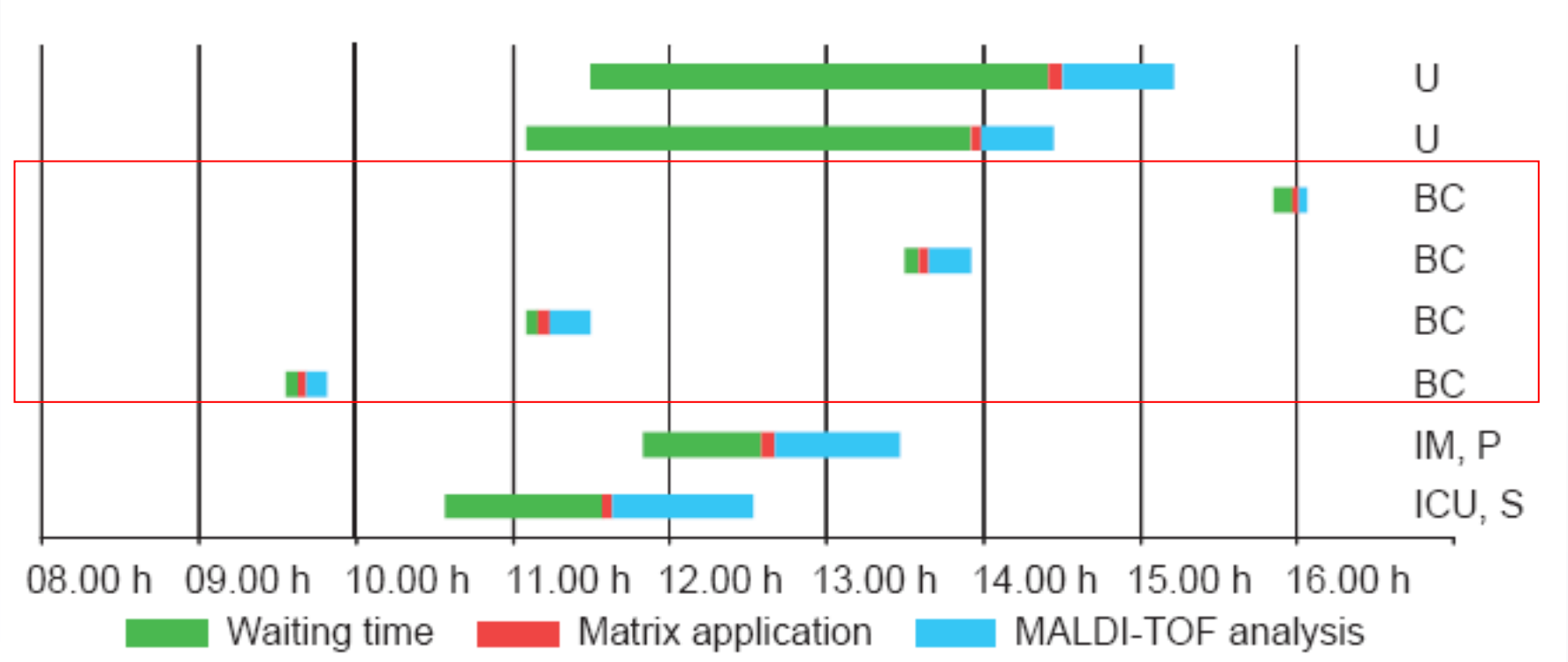
Costo 4.50- 13.85 €

**Test sensibilità**

Tempo 24-48 h



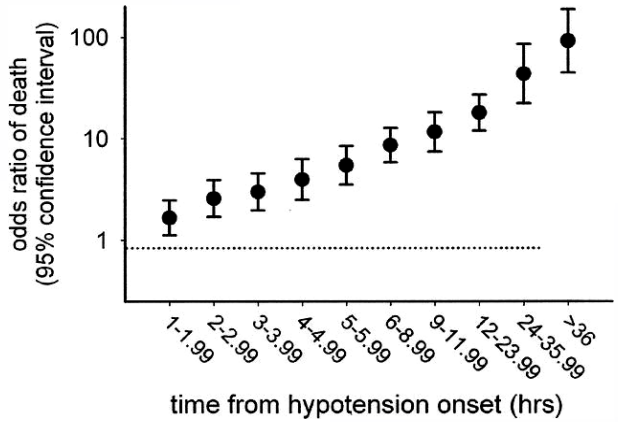

# Importance of the organization of a working day to optimize time to results






Croxatto A et al *FEMS Microbiology Reviews* March 2012; 36:380-407

# Batteriemia / Sepsi ( $T_0$ )





## Emocoltura Positiva ( $T_0$ + ??h )

Gram diretto (5 min)

Bacilli Gram-negativi

MALDI-TOF (20 min)

*Klebsiella pneumoniae*

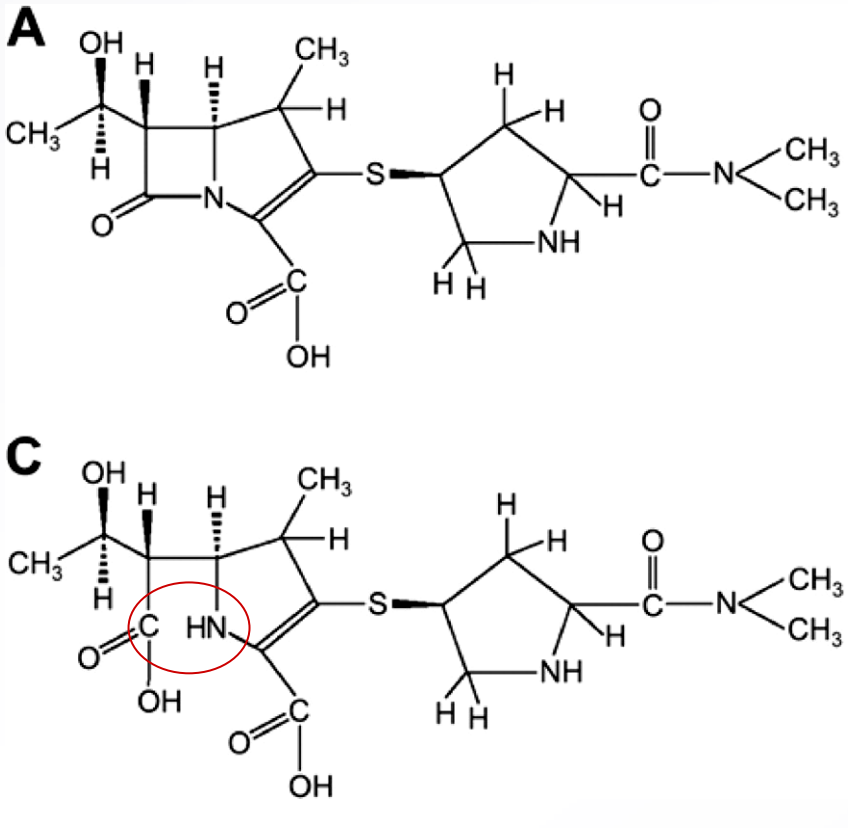
ESBL??

KPC??


# Carbapenemase Activity Detection by MALDI-TOF

Meropenem 383.464 Da

Meropenem sale disodico 427.422 Da




Meropenem degradato 401.483 Da

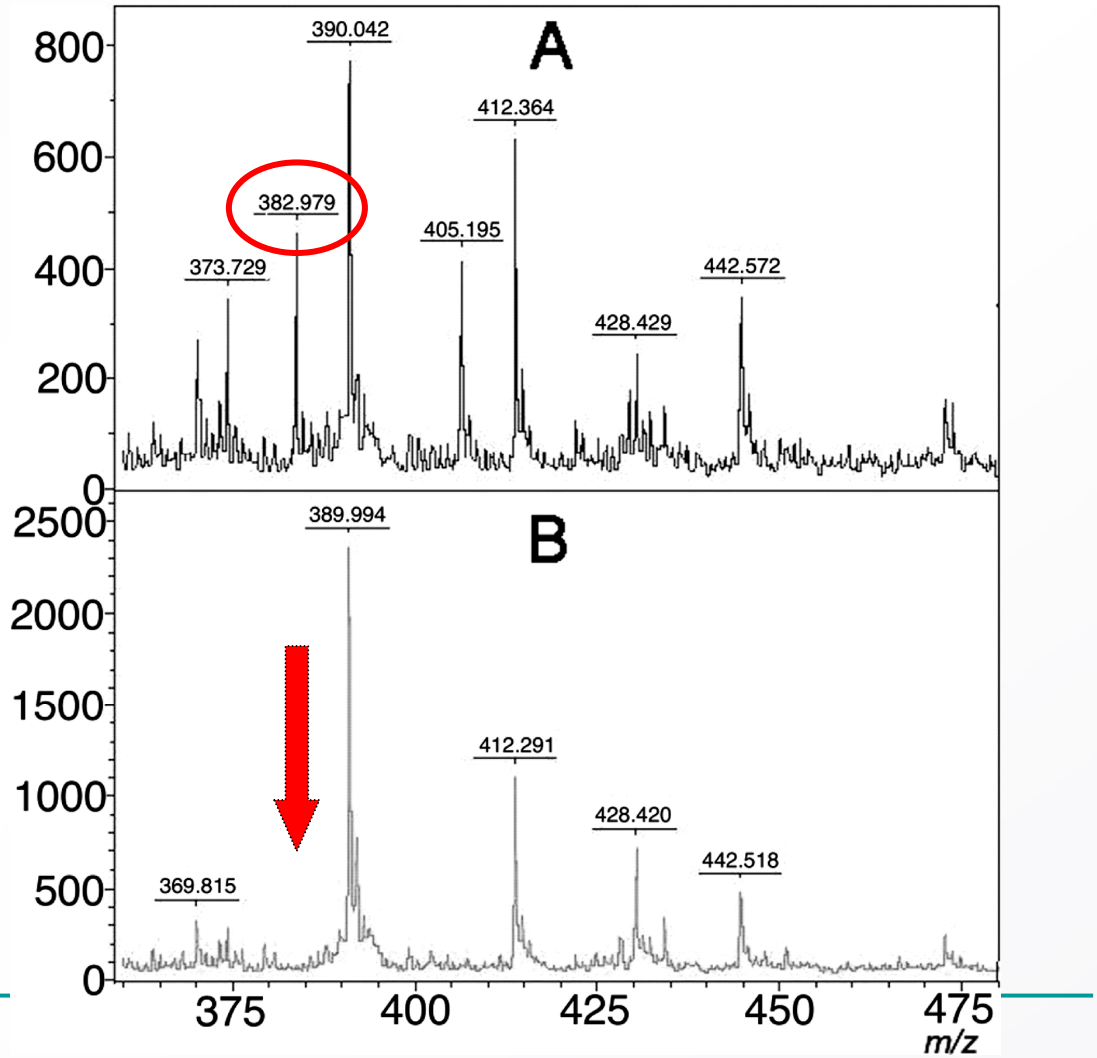
Meropenem sale trisodico 467.420 Da

Hrabák J et al *Journal of Clinical Microbiology*, September 2011; 49:3222-3227


# Spettri di massa nel test di idrolisi del Meropenem

**assenza di carbapenemasi**

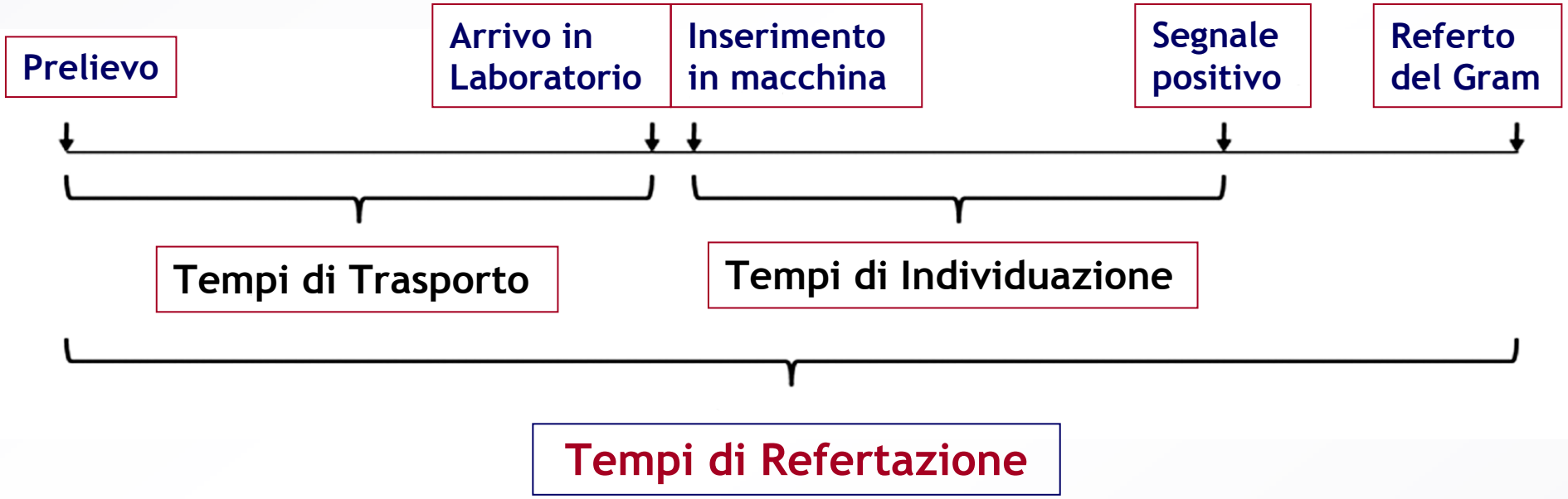
**produttore di carbapenemasi (KPC-2)**




Hrabák J et al*Journal of Clinical Microbiology*, September 2011; 49:3222-3227

Emocolture

# Timeline e Impatto organizzativo




Trasporti automatici?
Posta pneumatica?
Strumenti in Reparto?

Strumento accessibile h24?

Laboratorio operativo h24?

Strumento in Lab Urgenze

Personale Tecnico formato

# Laboratori (%) che effettuano test "diretti" sul brodo dell'emocoltura

| | Identificazione | Antibiogramma |
|---|---|---|
| Italia * | 9% | 25% |
| USA ** | 31% | 75% |
| UK *** | 51% | 96% |

*Goglio e al., Indagine Nazionale AMCLI, 1988

** Clinical Microbiology Newsletter, 1986

*** PHLS, 1989

# La realtà italiana

Effettuano:

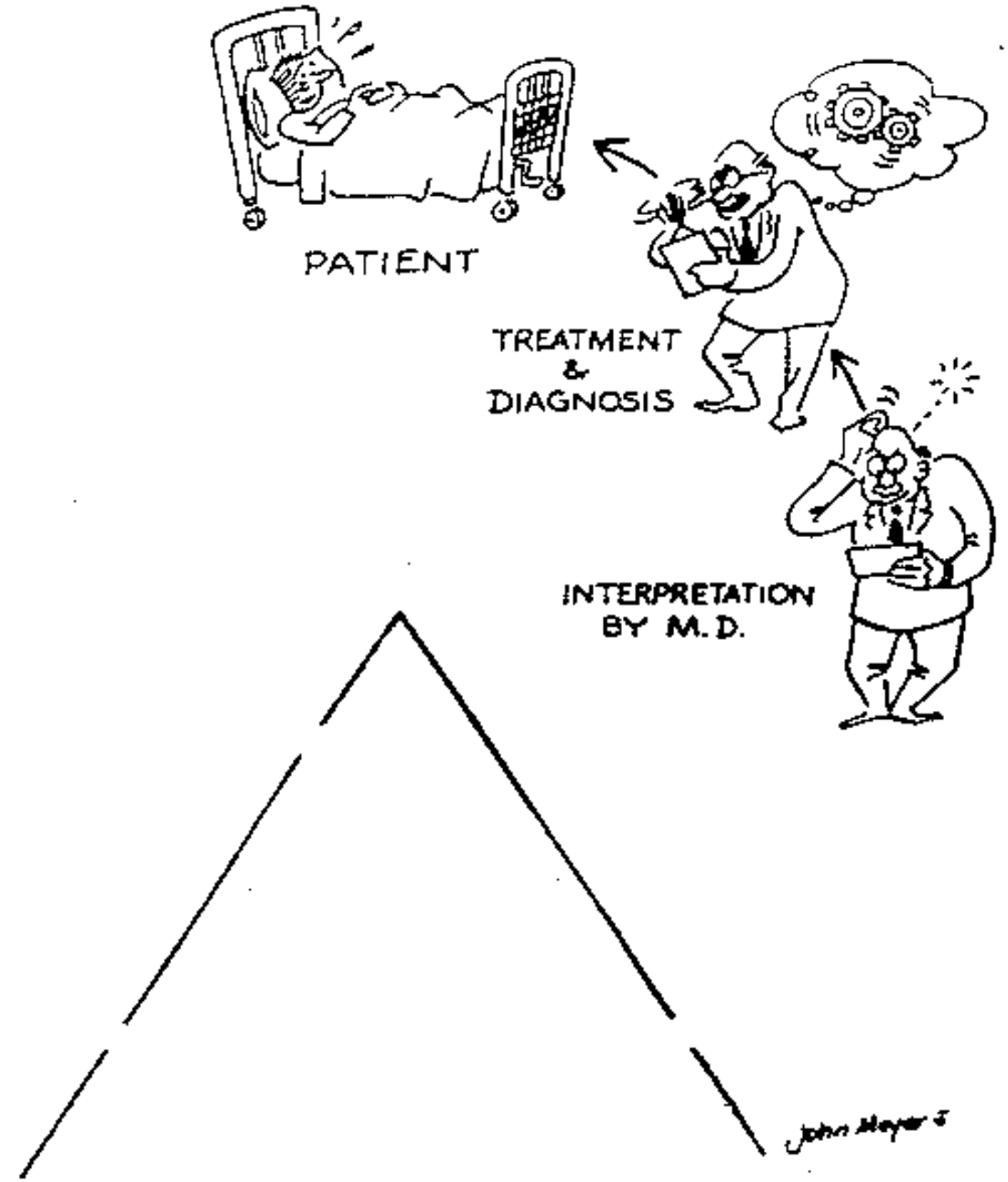
- Esame microscopico 76,9%
- Antibiogramma diretto 17,9%
- Identificazione diretta 9,3%

Su 94 lab, quelli che effettuano l'es. microscopico, l'antibiogramma diretto e sono aperti 7/7 giorni, sono:

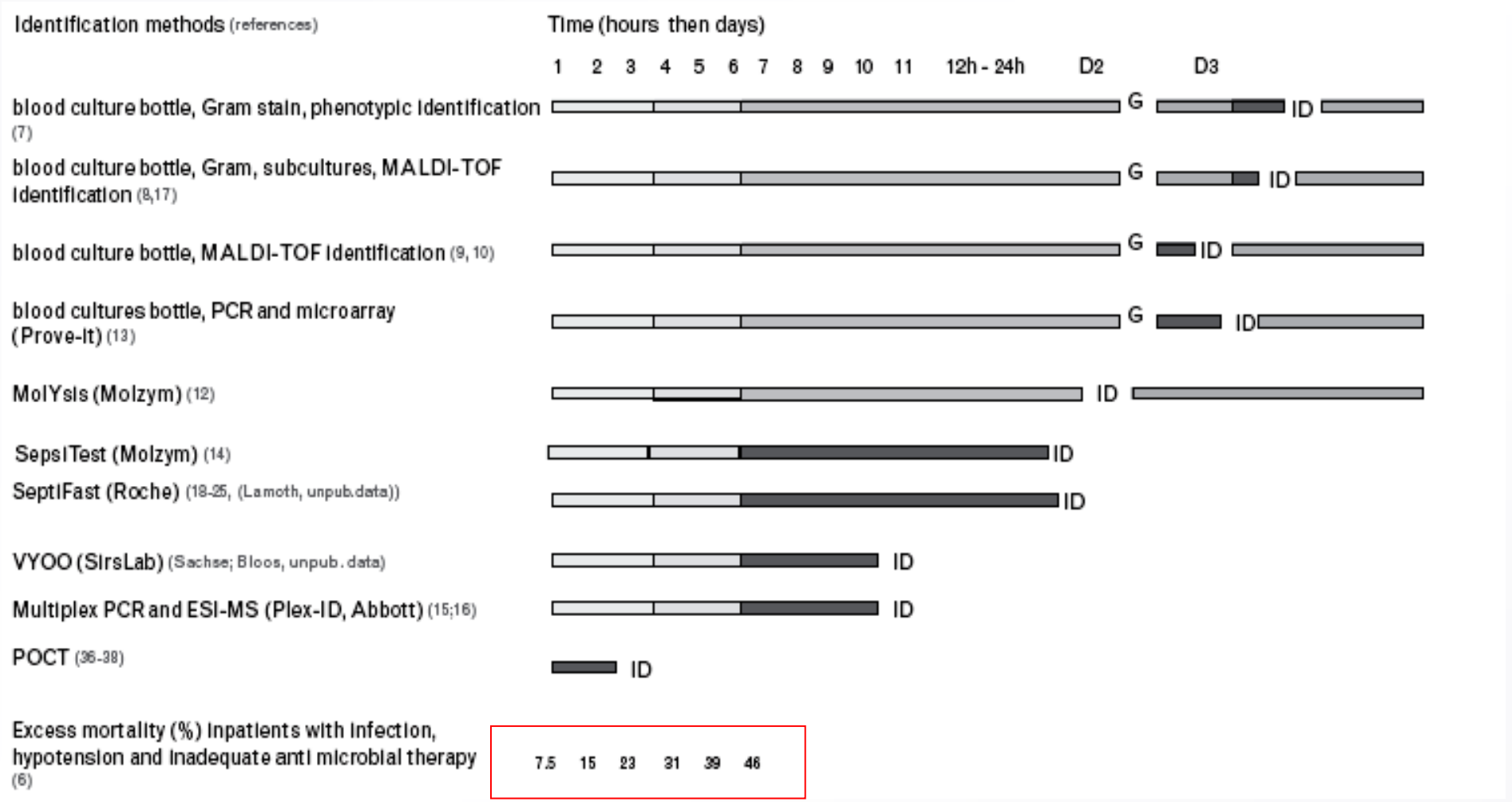
6

Indagine AMCLI-APSI sulle emocolture in Italia. P.L.Nicoletti, A.Goglio-2002

*Wah Ming Chang*



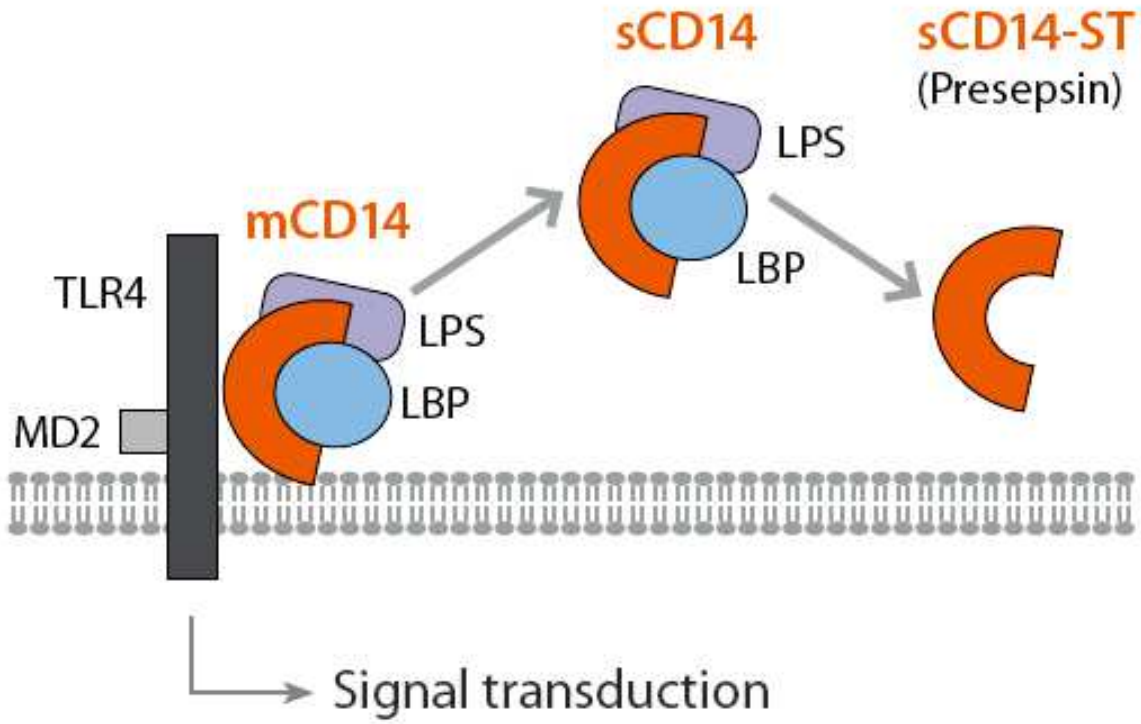
# Identification methods and time for reporting results



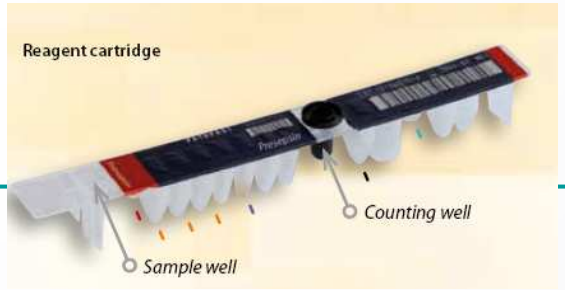



Leggieri N et al *Current Opinion in Infectious Diseases* 2010; 23:311-319

## Mechanism of Presepsin secretion




mCD14: membrane CD14; sCD14: soluble CD14; sCD14-ST: soluble CD14 subtype (=Presepsin); LPS: lipopolysaccharide; LBP: lipopolysaccharide binding protein, TLR4: toll-like receptor 4; MD2: Co-Protein of TLR4.

GRAZIE PER L'ATTENZIONE

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