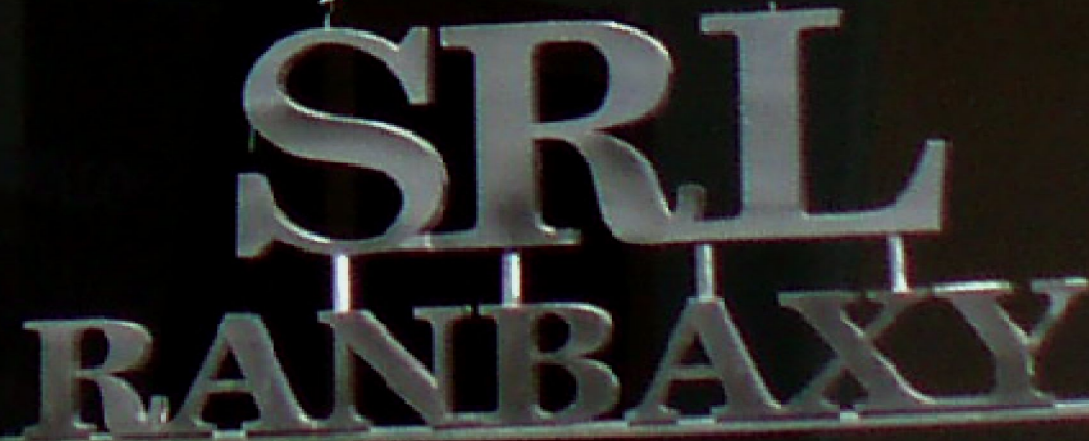


Diagnosis of sepsis: blood culture practices in India

The logo for SRL RANIBAXY is displayed in a metallic, three-dimensional font. The letters 'SRL' are positioned above 'RANIBAXY'. The logo is mounted on a dark, rectangular plaque which is suspended by two thin chains from a light-colored, textured wall. The background wall has a mottled, stone-like appearance with some shadows cast across it.

SRL
RANIBAXY

Dr. Ashok Rattan
Director

Regional Reference Laboratory, Gurgaon and NCR Laboratories

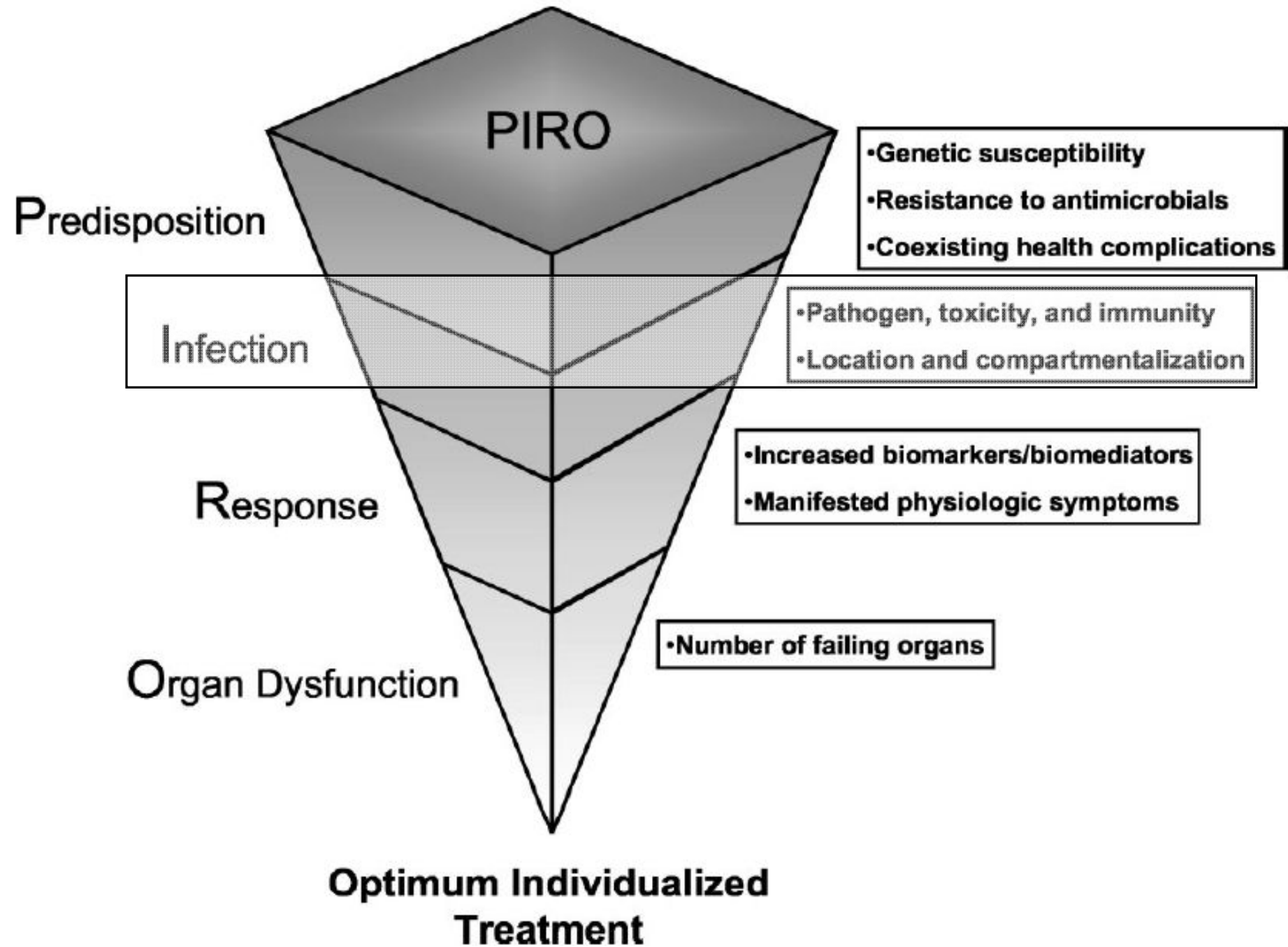


Sepsis

Sepsis is a complex syndrome caused by an uncontrolled systemic inflammatory response to a stimulus of **INFECTIOUS** origin, characterized by multiple manifestations which can result in dysfunction or failure of one or more organs and even death.

*Our arsenals for fighting off bacteria are so powerful and involve so many different defense mechanism, that we are more in danger from them than from the **INVADERS**. We like in the midst of explosive devices, we are mined !*

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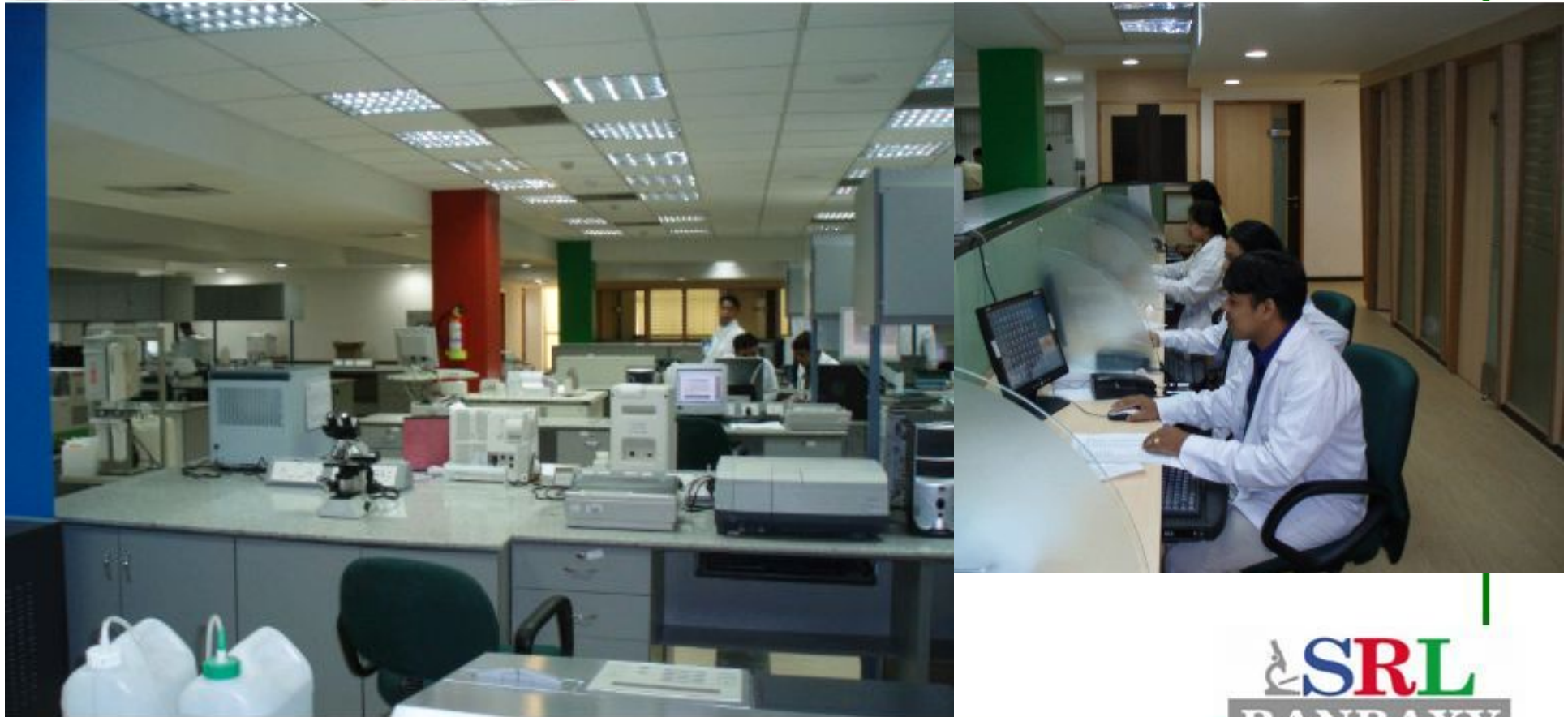
Blood cultures practices in India

- Internationally: Blood cultures are Positive in only 30 – 40% of sepsis cases
- Maybe positive in pts without sepsis
 - May be due to colonization, contamination
- Slow & expensive
- Gold standard
- Other cultures
 - CSF
 - Urine
 - Lines

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SRL Regional Reference Laboratory: The lab that never sleeps



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**Think Globally
Act locally**

Mandate for GGN

**1. Stand alone
lab for North & East**

**2. Support lab for
Hospitals in NCR**

8+3



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Blood stream infections in India

Author	Year	Place	% positive	common isolates
Khanal	2002	Pondicherry	44	Staph 25%, GNB 15%
Sharma	2002	Rohtak	33.9	Kleb, Sal, Ps, S.a
Chaudhry	1999	Tirupati	11.8	Ps, S.a 36.8% each
Kuruvilla	1999	Vellore	0.7/1000 births	GBS
Mitra	1997	Chandigarh	5	Anaerobic
Roy	1993	Calcutta	16.4	Candidemia
Dhawan	1993	Chandigarh	43.2	Viridans, S. aureus



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S. Bhattacharya: Indian J Med Micro 2005

FHVK 2007 blood isolates

Data thanks to Dr Balvinder Mohan Singh

Isolate	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Acinetobacter	10	2	1			2	1	4
Citrobacter				1				1
Enterobacter			2				1	
Enterococcus	1		2		1			
E. coli	1	7	5	1	2	3	4	3
K. pneum	3		3	3				1
Pseudomonas	2	2	1	1		2	2	1
S. para A			1	1				
S. typhi	3	1		1	4	4	5	1
S. aureus	2		3		2	1	3	1
CONS		2	2		2	3	7	2
S. pneum					2			
Yeast				2				1
Total	22	14	20	10	13	15	23	15

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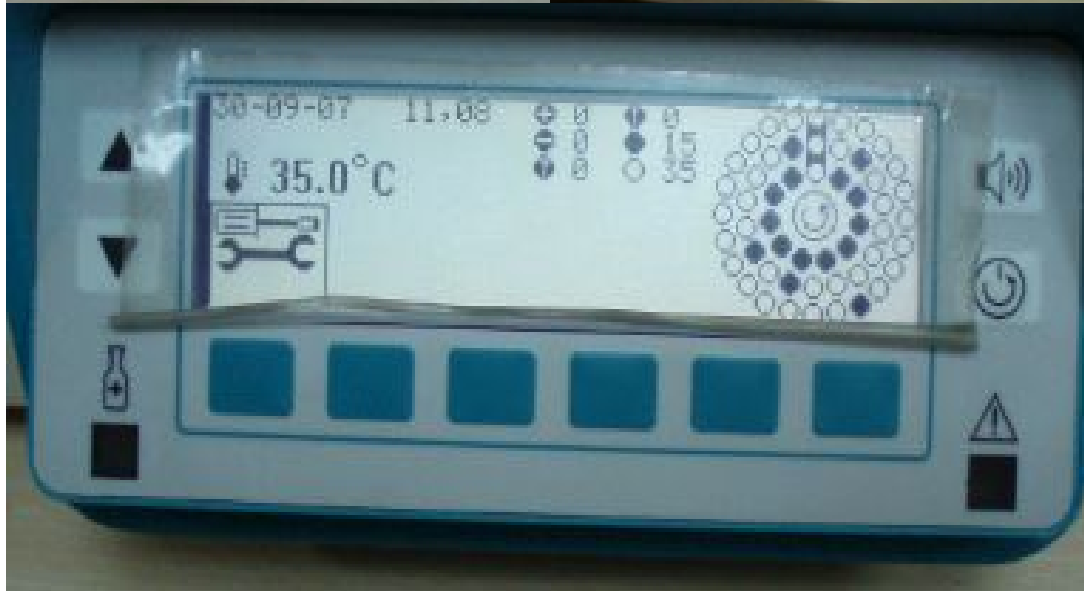
Manual Blood Culture



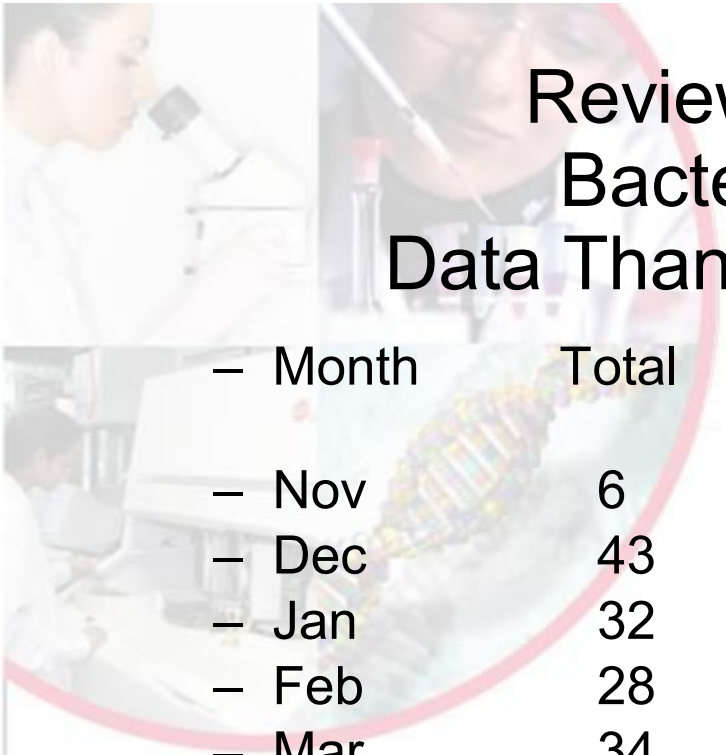
Manual blood culture with subculture on BA & Macconkey agar after 48 hours
5 days and 10 days before being reported as "Sterile"



Bactec culture with
Continuous monitoring,
No subculture required
Incubate for 5 days







Review of in house results Bactec Nov 06 – July 07 Data Thanks to Dr Bansidhar Terai

– Month	Total	Positive		
		48 hr	96 hrs	10 days
– Nov	6	1	0	0
– Dec	43	7	2	0
– Jan	32	7	3	0
– Feb	28	3	3	0
– Mar	34	1	1	0
– Apr	46	7	6	0
– May	59	10	3	0
– Jun	44	5	1	0
– Jul	63	5	4	0
– TOTAL	355	46	23	69

(19.43%)



IBIS



Data collected in 1993
Published in Lancet 1999
Coordinated by CMC, Vellore
Thanks to Dr M.K. Lalitha & Dr M. Steinhoff

Disc-diffusion test result

	Susceptible	Non-susceptible
Co-trimoxazole	134	173 (56.3%)
Chloramphenicol	256	51 (16.6%)
Erythromycin	294	13 (4.2%)
Oxacillin	303	4 (1.3%)*
Cefotaxime	307	0

*Intermediate resistance to penicillin by MIC testing (MIC, 0.25 $\mu\text{g}/\text{mL}$ for three, 0.50 $\mu\text{g}/\text{mL}$ for one isolate). Non-susceptible isolates had zone sizes in the resistant and intermediate category.

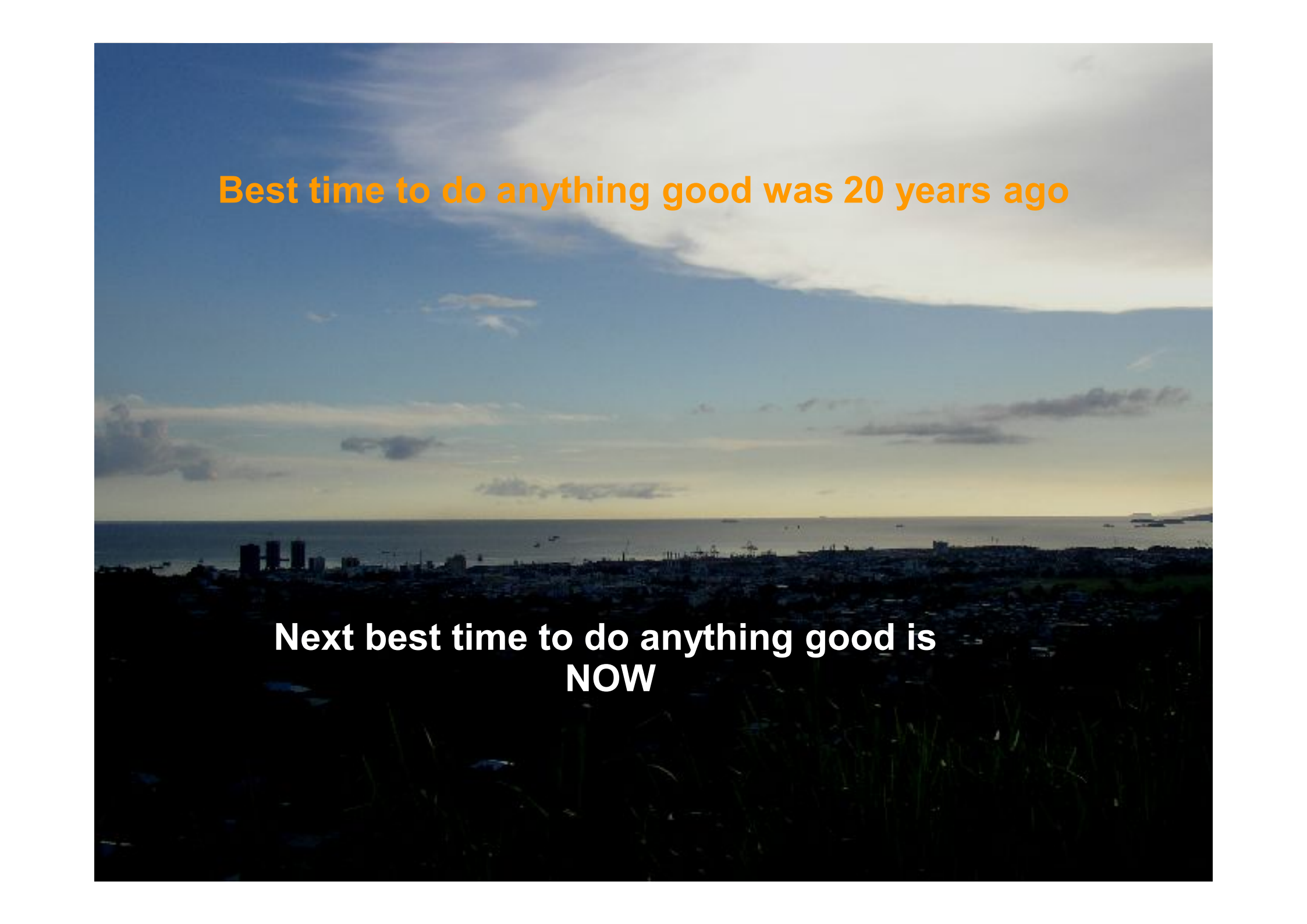


1990 – 1993
10,000 blood cultures / year
One *S.pneumoniae* isolate

“RUBBISH”

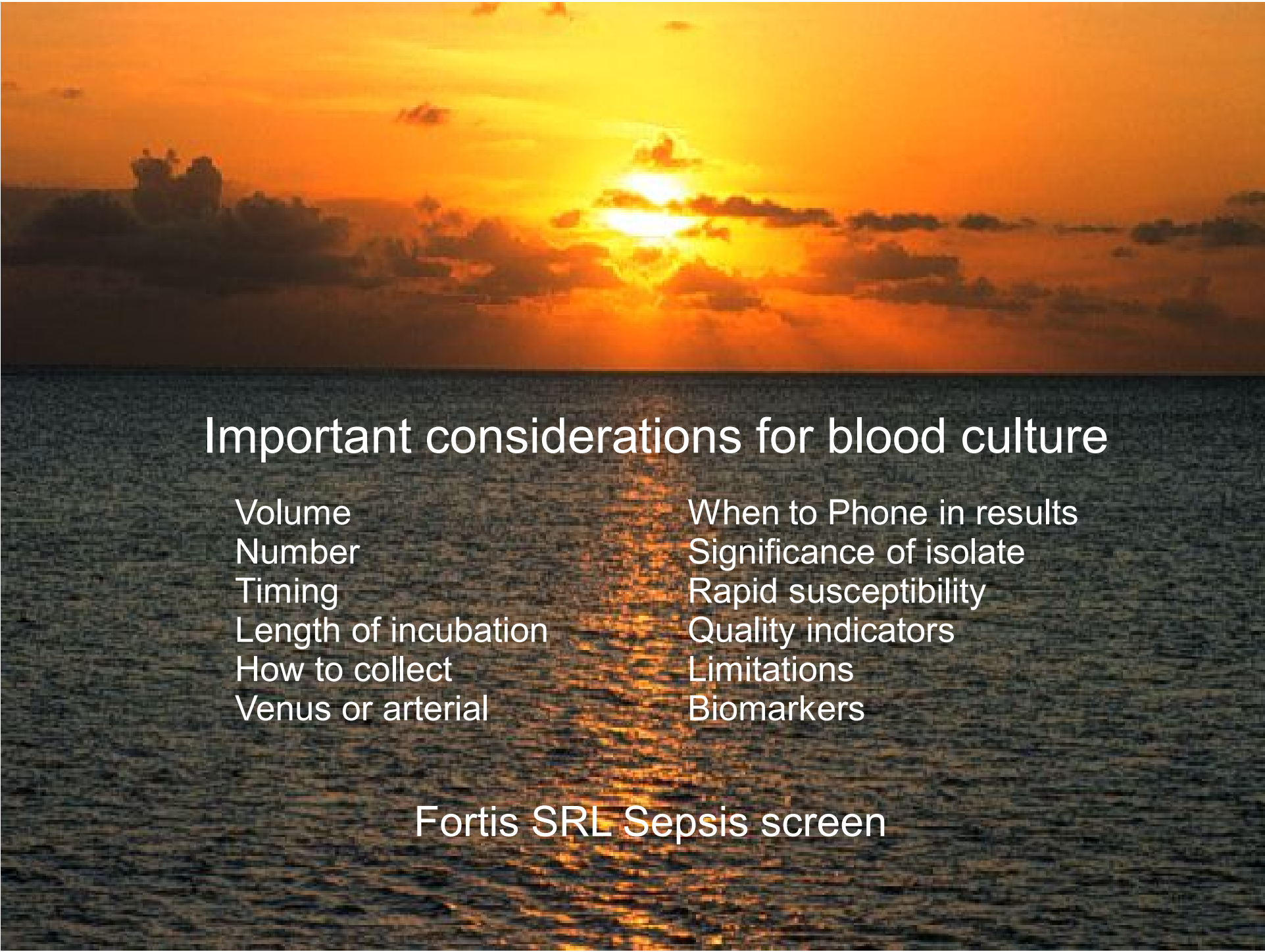
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Best time to do anything good was 20 years ago

Next best time to do anything good is
NOW

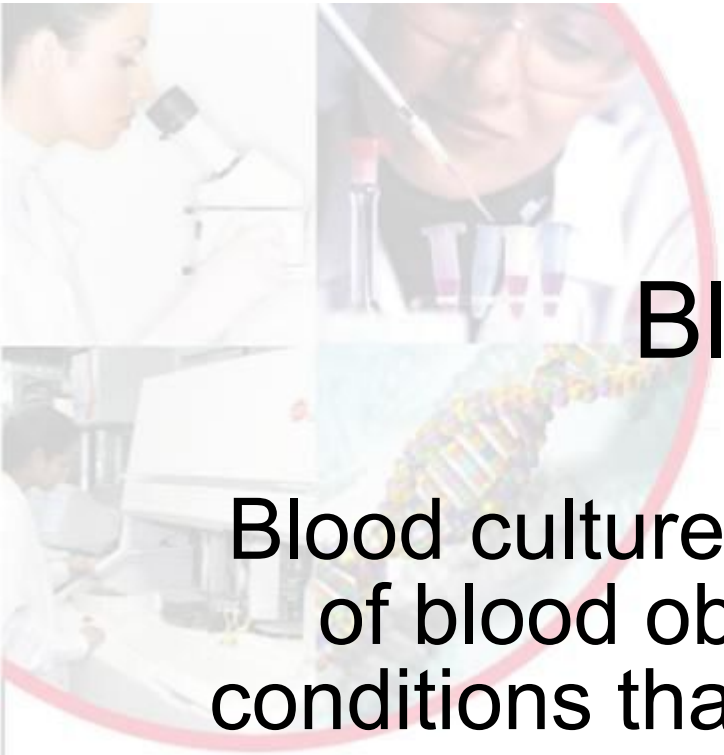


Important considerations for blood culture

Volume
Number
Timing
Length of incubation
How to collect
Venus or arterial

When to Phone in results
Significance of isolate
Rapid susceptibility
Quality indicators
Limitations
Biomarkers

Fortis SRL Sepsis screen



Blood culture

Blood culture is defined as a volume of blood obtained under aseptic conditions that is inoculated to one or more bottles.

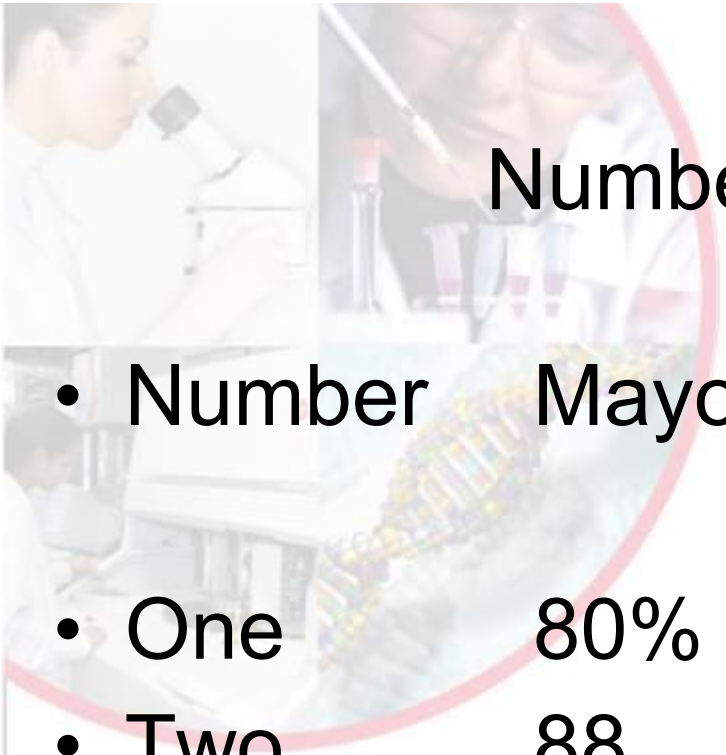
22 – 29% mortality

Volume of blood

- Single most important variable in recovering organism
- Direct relationship between diagnostic yield & blood volume
- Volume sampled yield increase
- 10 ml → 20 ml 30%
- → 30 ml 47
- → 40 ml 7
- Optimum volume from adult 20 to 30 ml

Pediatrics

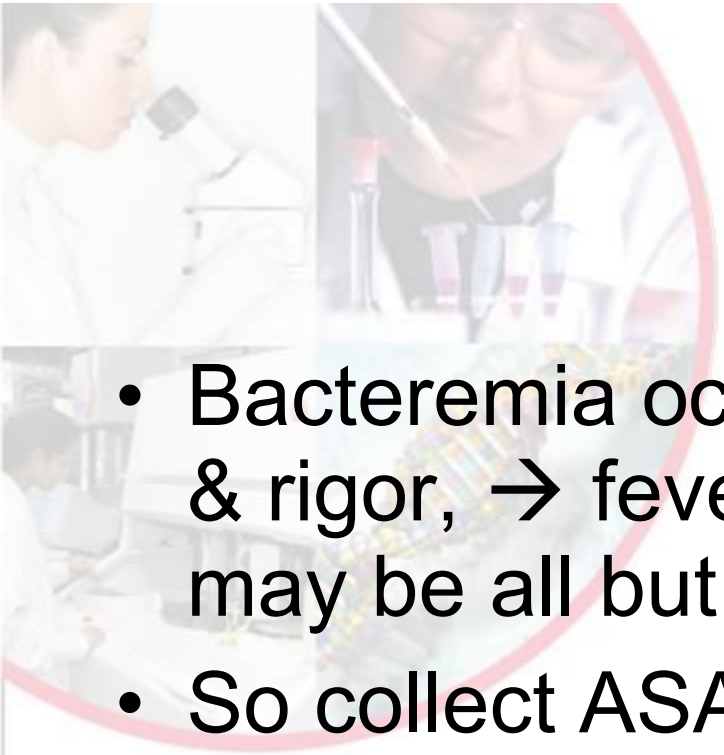
Weight of patient		Total Blood Volume (ml)	Recommended Volume of Blood for Culture (ml)		Total Volume for Culture (ml)	% of Total Blood Volume
Kg	Lb		Culture No. 1	Culture No. 2		
≤ 1	≤ 2.2	50-99	2	-	2	4
1.1-2	2.2-4.4	100-200	2	2	4	4
2.1-12.7	4.5-28	>200	4	2	6	3
12.8-36.3	28-82	>800	10	10	20	2.5
>36.3	>80	>2200	20-30	20-30	40-60	1.8-2.7



Number of blood cultures

• Number	Mayo	Weinstien	Cockerel
• One	80%	91	65
• Two	88	99	80
• Three	99		96

- ASM recommendations: Two sets within 24 hours; one set consists of two bottles



Timing

- Bacteremia occurs one hour before chills & rigor, → fever occurs after this, bacteria may be all but cleared at that time
- So collect ASAP
- Interval between 2 sets
 - Urgent cases within one hour
 - In less urgent cases → 1 to 2 hours apart



Length of incubation

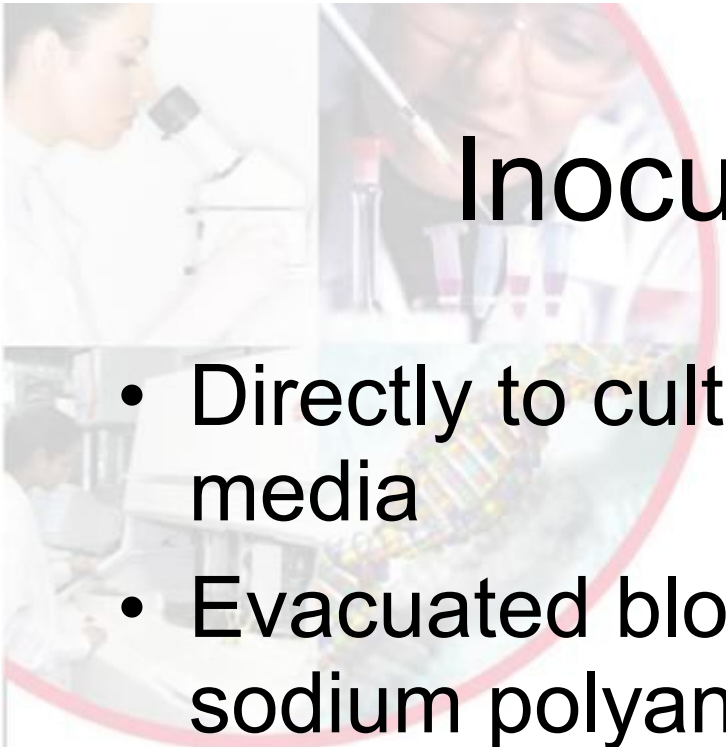
- 5 days
- ? Fastidious bacteria
 - Brucella,
 - Caprocytophaga,
 - Campylobacter
- HAECEK
 - Haemophilus
 - Actinobacillus
 - Cardiobacter
 - Eiknella
 - Kingnella
 - Special media rather than longer incubation



Bactec bottles

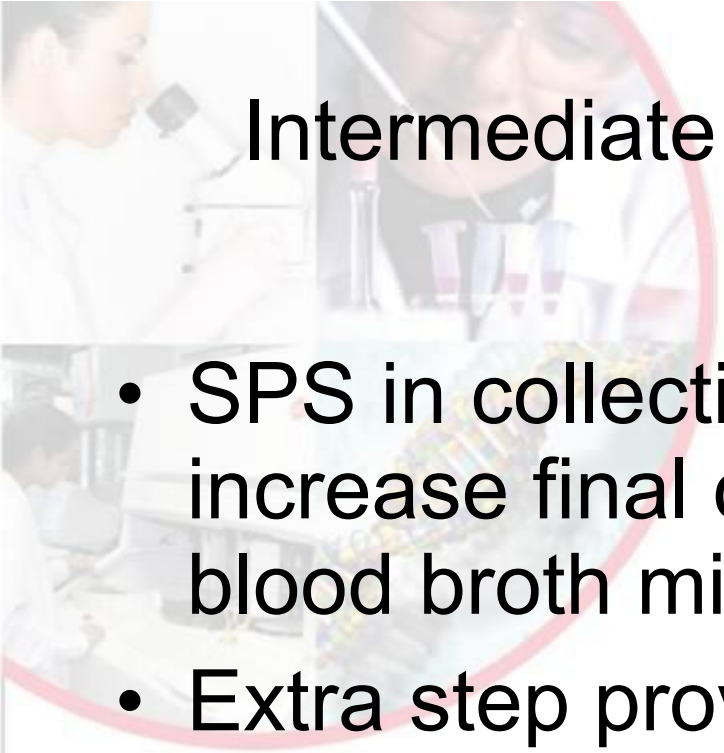
- Aerobic PLUS
- Anaerobic PLUS
- Peds PLUS
- Lytic media
- Myco F

- Set:
 - Aerobic + Anaerobic;
 - Aerobic + Fungal



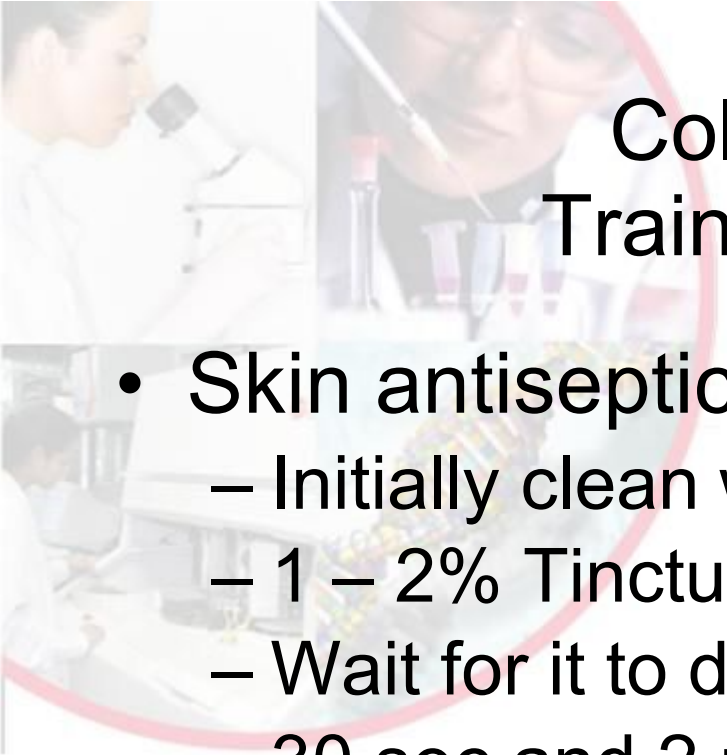
Inoculation of blood

- Directly to culture vials containing broth media
- Evacuated blood collection tube containing sodium polyanetholsulfonate (SPS)
 - SPS is preferred anticoagulant, since citrate, heparin, EDTA & oxalate may be toxic to some bacteria
 - SPS is toxic to *N. meningitides*.



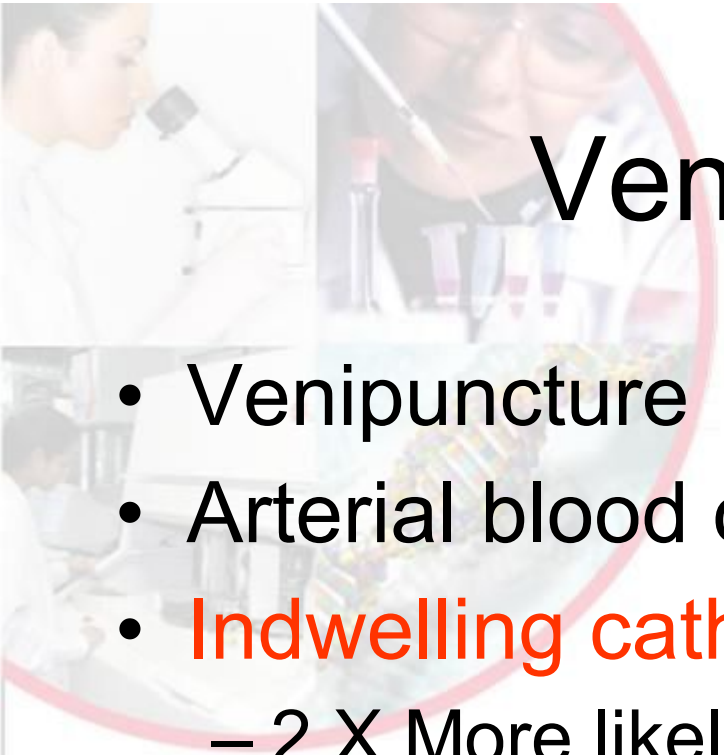
Intermediate collection tube should be discouraged

- SPS in collection tube will be added & increase final concentration of SPS in blood broth mix
- Extra step provides additional opportunity for contamination
- Increase risk of exposure of lab personnel to blood stream pathogens
- May compromise cultures that are delayed



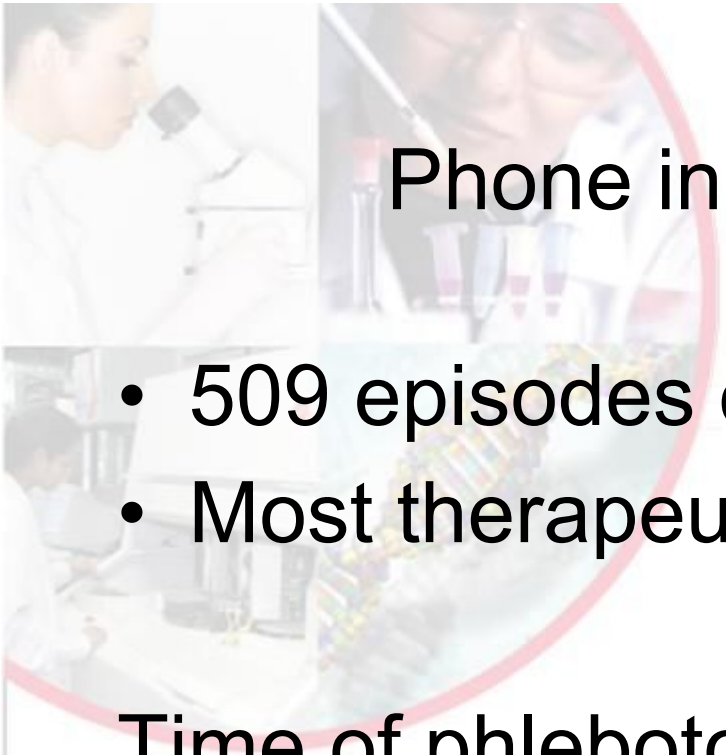
Collection of blood Trained phlebotomists

- Skin antiseptics
 - Initially clean with 70% alcohol
 - 1 – 2% Tincture Iodine or iodophor
 - Wait for it to dry
 - 30 sec and 2 minutes
 - Friction is essential
 - If wants to palpate vein, clean gloved finger similarly
- Now single needle transfer, in pre HIV era double needle transfer



Venus or arterial

- Venipuncture
- Arterial blood of no greater value
- **Indwelling catheter:**
 - 2 X More likely to be contaminated
 - To determine CV infection collect blood simultaneously from cath and a distance site, if time to positive is > 2 hours between peripheral blood and hub blood, then CV infection is confirmed



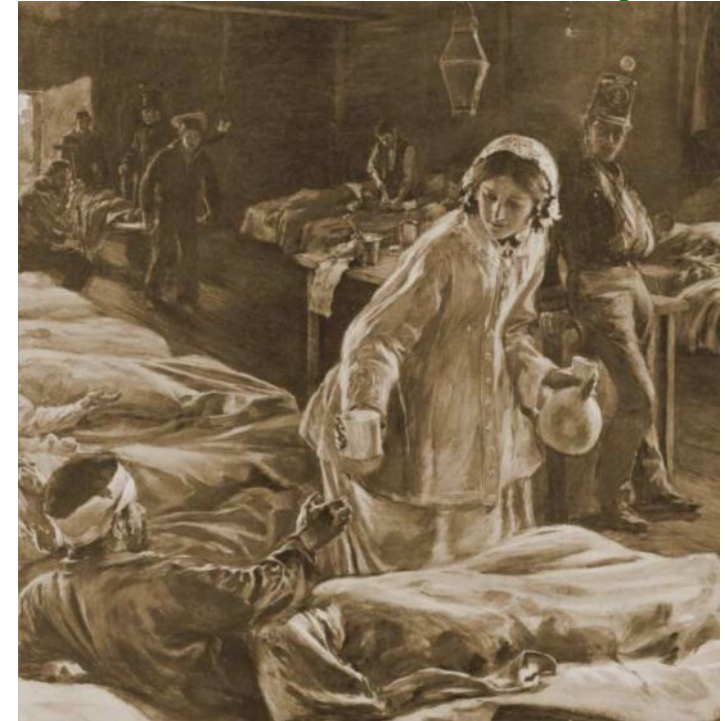
Phone in Blood Culture results

- 509 episodes of BSI
- Most therapeutic interventions

	Start	Stop
Time of phlebotomy	45%	7%
Phone in Gram stain		
Results from positive	23	5
Release of final AST		
& ID results	5	9

Significance of isolation / isolate

- Clinical signs & symptoms
- WBC count
- Number of bottles positive
- X ray findings
- Clinical course



- “Clinical” Microbiologists MUST go on ward rounds along with ICU consultants



Nearly always significant

Staphylococcus aureus

Streptococcus pneumoniae

Escherichia coli and other members of the family Enterobacteriaceae

Pseudomonas aeruginosa

Candida albicans

Sometimes significant

Enterococci (80%)

Viridans streptococci (40-60%)

Coagulase-negative staphylococci (20-40%)



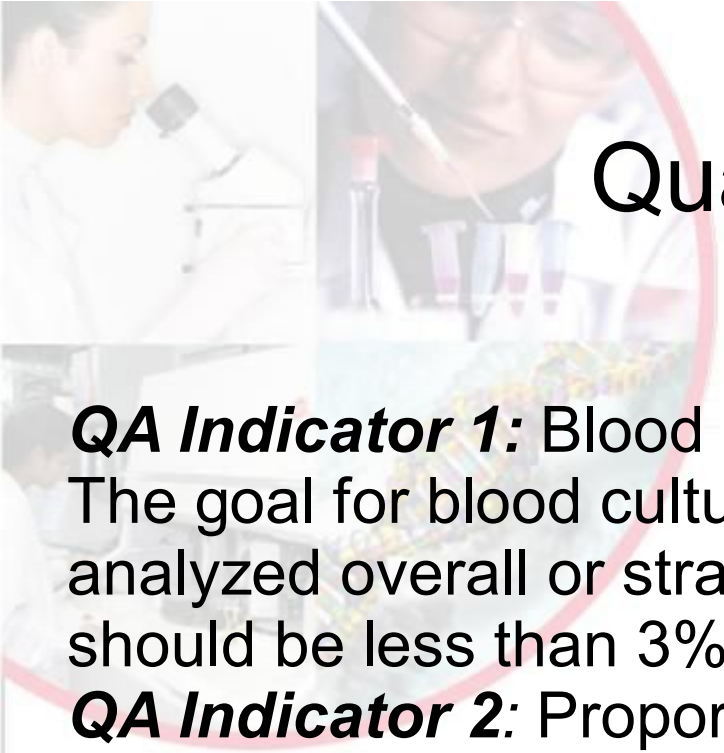
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~~Rapid susceptibility~~

- Positive bottle
- 5 ml
 - Centrifuged low x for 5 min to pellet RBC
 - Transfer supernatant and centrifuge to pellet bacteria
 - Adjust turbidity of pellet in sterile NS to 0.5 McFarland and perform AST



Quality Indicators

QA Indicator 1: Blood culture contamination rate.
The goal for blood culture contamination rate, whether analyzed overall or stratified by location, phlebotomist, etc., should be less than 3%.

QA Indicator 2: Proportion of blood culture bottles inoculated with more or less than the recommended volume of blood. For adults, each blood culture bottle should be inoculated with 10 mL of blood.

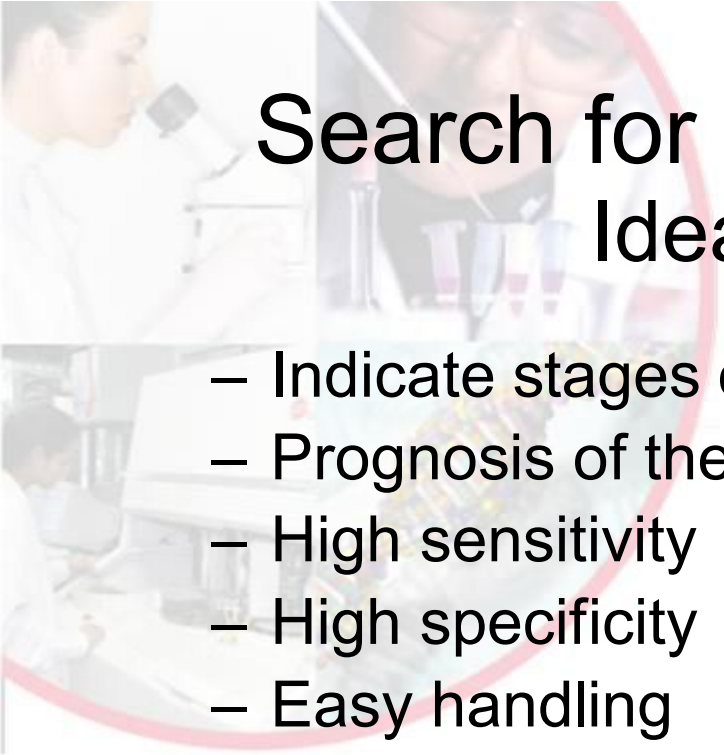
QA Indicator 3: Proportion of blood cultures submitted that include only a single inoculated bottle.

QA Indicator 4: Proportion of blood cultures submitted that must be rejected for any cause.

Blood cultures

- Positive in 30 – 40%
- Maybe positive in pts without sepsis
- May be due to colonization, contamination
- Slow & expensive
- Gold standard
- Other cultures
 - CSF
 - Urine

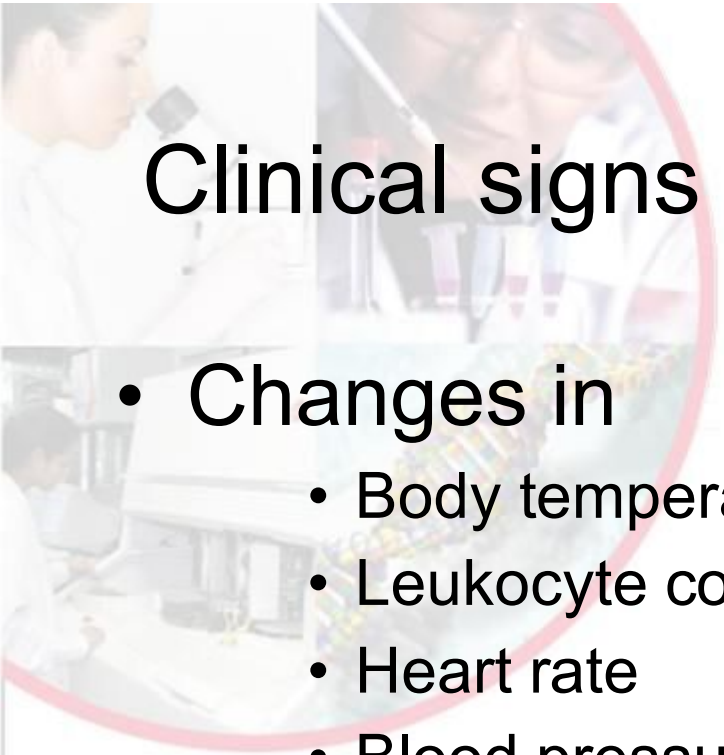




Search for a biomarker for sepsis

Ideal sepsis marker

- Indicate stages of disease
 - Prognosis of the patient
 - High sensitivity
 - High specificity
 - Easy handling
 - Low cost
 - Short TAT
- Sepsis results from an imbalance in the host regulation of pro-inflammatory SIRS and the compensatory anti-inflammatory response syndrome.



Clinical signs of systemic inflammation

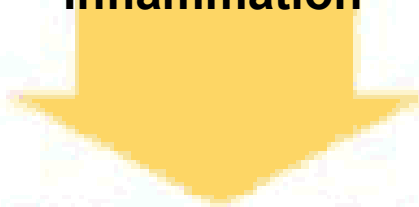
- Changes in
 - Body temperature
 - Leukocyte count
 - Heart rate
 - Blood pressure
 - Respiratory rate
 - Neither specific nor sensitive for sepsis
- **A sepsis marker must add value to physician's clinical judgment**



Demands that a sepsis marker must meet

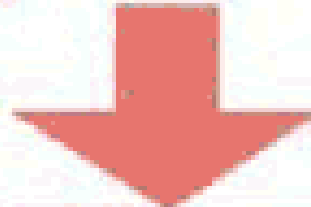
- Shorten time & improve diagnosis
- Facilitate differentiation between infectious & non infectious causes of inflammation
- Differentiate between viral & bacterial infections
- Reflect effectiveness of antibiotic treatment

Inflammation

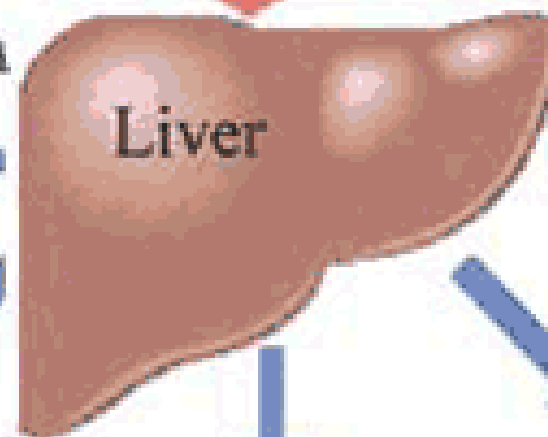


Cytokines

IL-1 IL-6 TNF

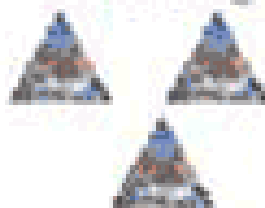


Positive Acute Phase Reactants

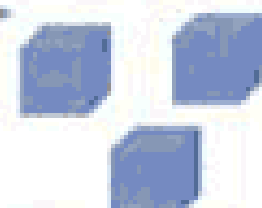


Liver

Fibrinogen



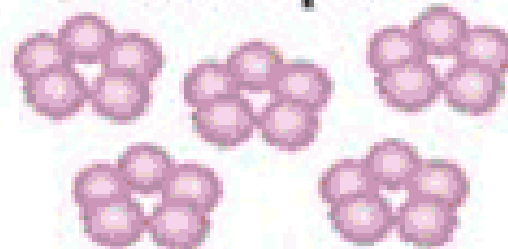
Haptoglobin



Serum amyloid A

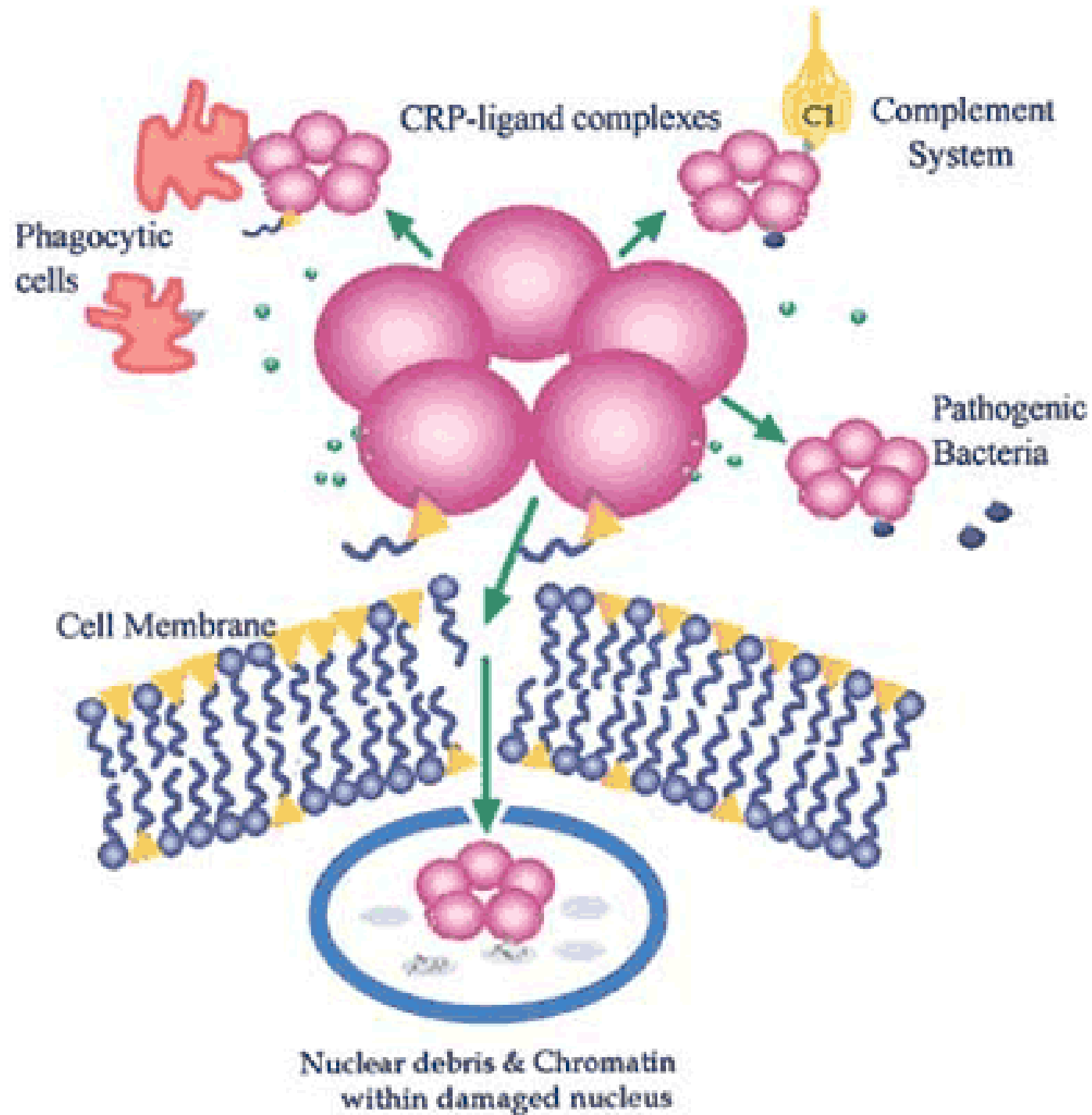


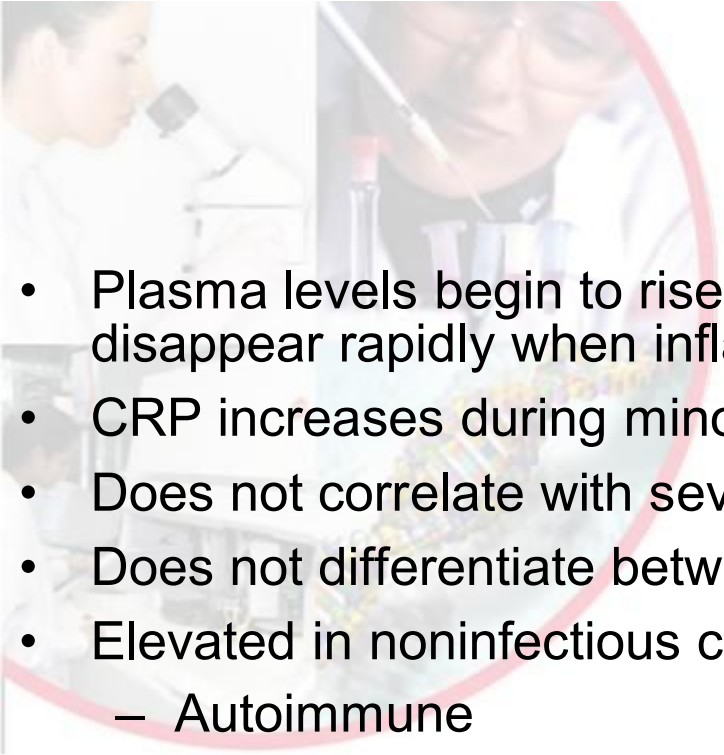
C-reactive protein



C3

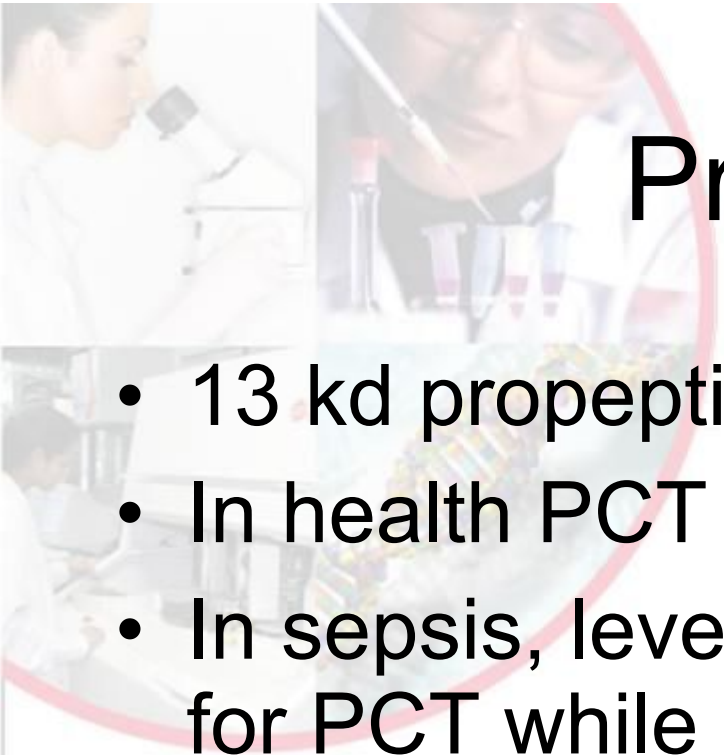






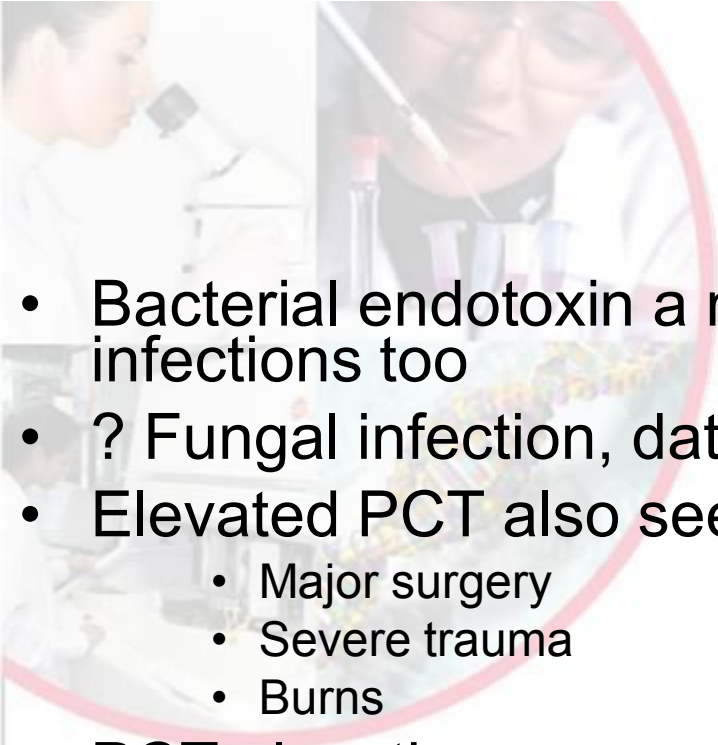
CRP

- Plasma levels begin to rise within 4 to 6 hours, peak in 24 to 48 hours & disappear rapidly when inflammatory process resolves
- CRP increases during minor infections
- Does not correlate with severity of host response
- Does not differentiate between survivors & non survivors of sepsis
- Elevated in noninfectious conditions
 - Autoimmune
 - Rheumatic disorders
 - Coronary syndromes
 - Malignant tumors
 - Surgery
- Elevated CRP has poor predictive value for sepsis
- **If 2 CRP reading (24 hours apart) are < 10mg/L, PPV for no sepsis or resolved infection is 99%**



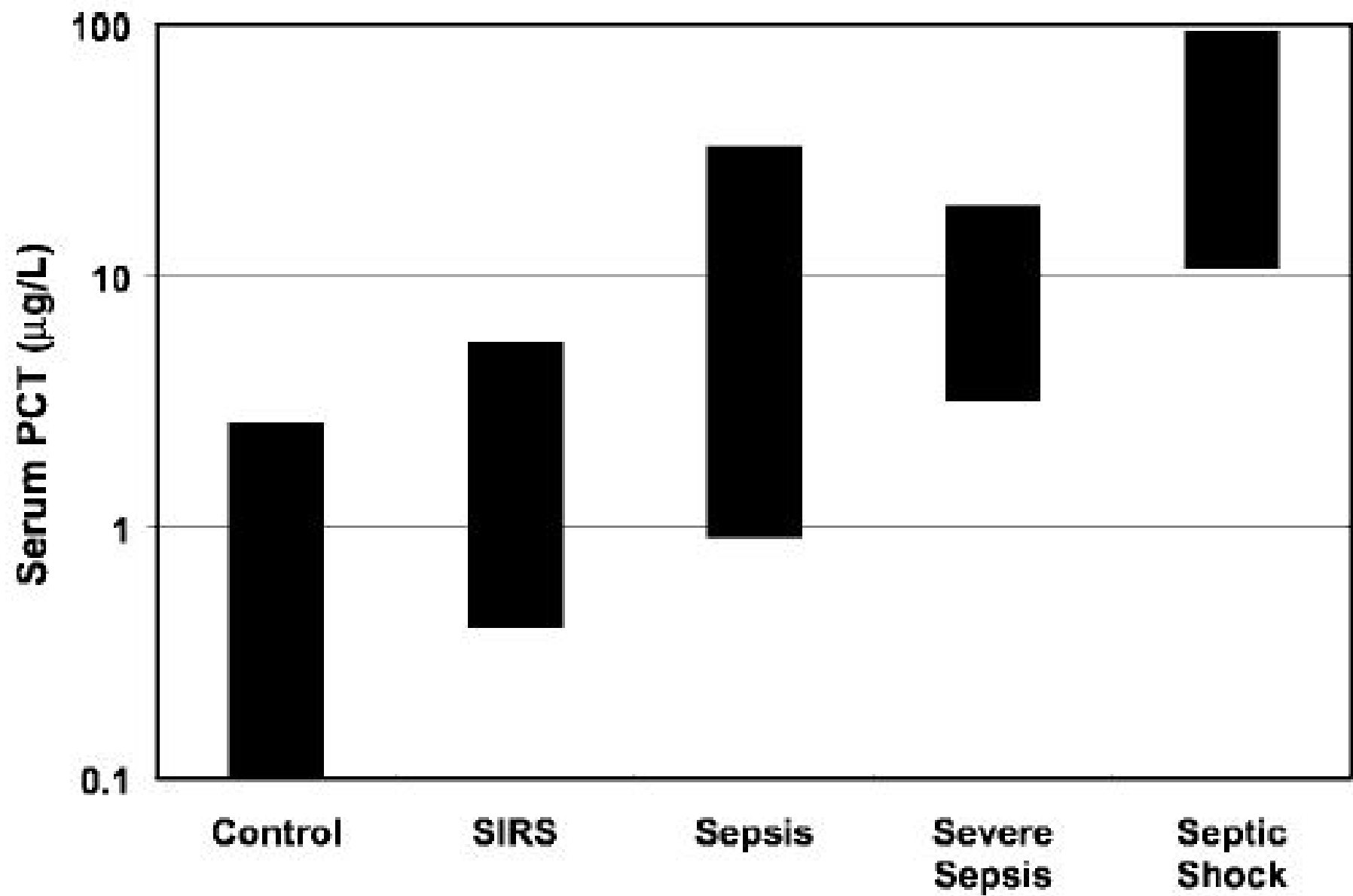
Procalcitonin

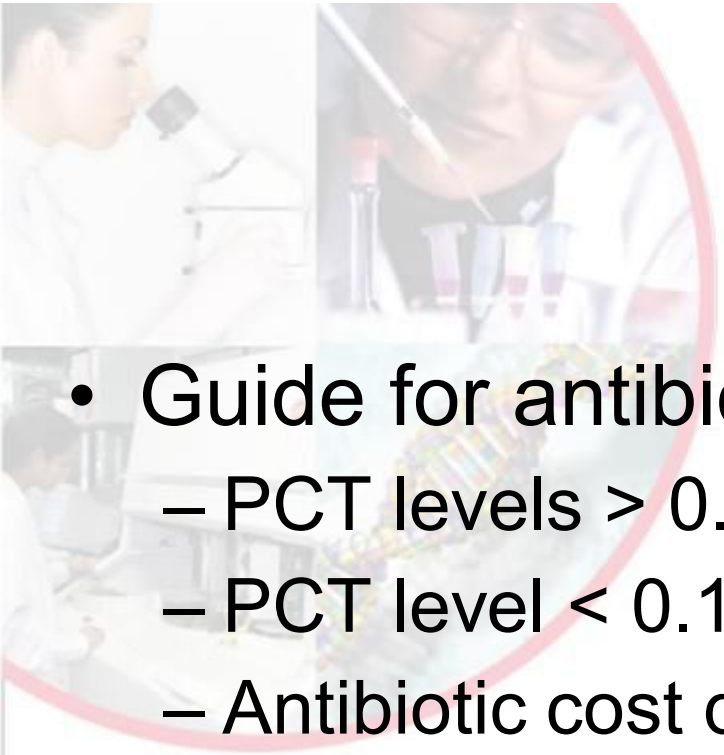
- 13 kd propeptide of calcitonin
- In health PCT < 0.1 ng/ml
- In sepsis, levels increase 5000 to 10000x for PCT while calcitonin levels are same
- Calcitonin half life 10 min, PCT 24 hours
- Physiological role of PCT & site of production not completely understood



PCT

- Bacterial endotoxin a major stimulus, but increased in GPC infections too
- ? Fungal infection, data conflicting
- Elevated PCT also seen in
 - Major surgery
 - Severe trauma
 - Burns
- PCT elevation occurs within 2 hours of endotoxemia, bacteremia
- PCT is a marker of severe infections & sepsis
- Pts with PCT
 - < 0.5 ng/ml are unlikely to have severe sepsis,
 - >2 ng/ml are at high risk,
 - 10 ng/ml occurs in pts with organ failure
- PCT has strong negative predictive value





PCT

- Guide for antibiotic therapy
 - PCT levels > 0.5 ng/ml $\rightarrow$ strongly encouraged
 - PCT level < 0.1 ng/ml $\rightarrow$ not encouraged
 - Antibiotic cost decreased by half
 - Prospective trial conducted in
 - CAP
 - ICU



PCT

- Correlates very well with onset of organ dysfunction
- Helps differentiate between infectious & noninfectious etiology of systemic inflammation
- Helps monitor progress of focus control
- Help monitor efficacy of antibiotic treatment



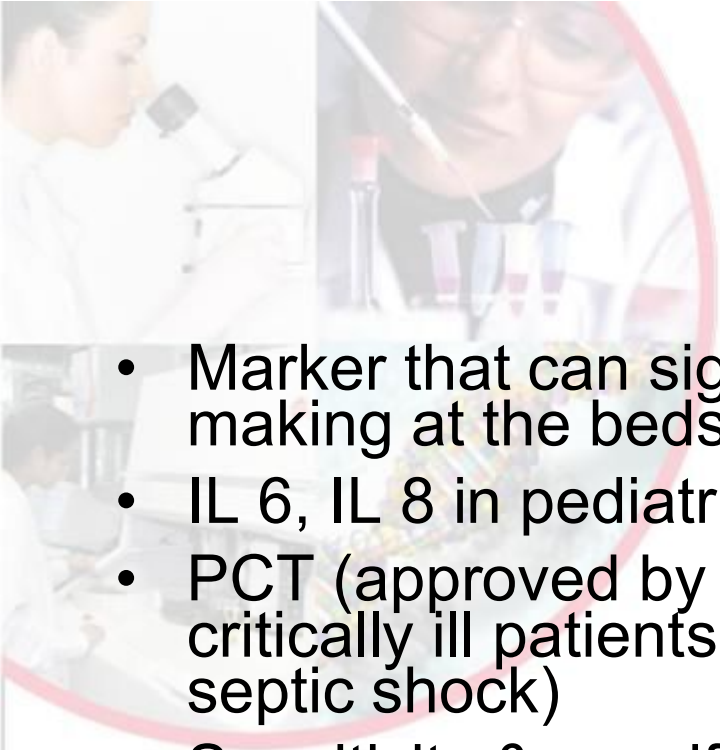
Available methods

- PCT – Q
- PCT sensitive LIA
- PCT LIA
- PCT LIA US
- LIAISON
- PCT VIDAS
- PCT sensitive Kryptor



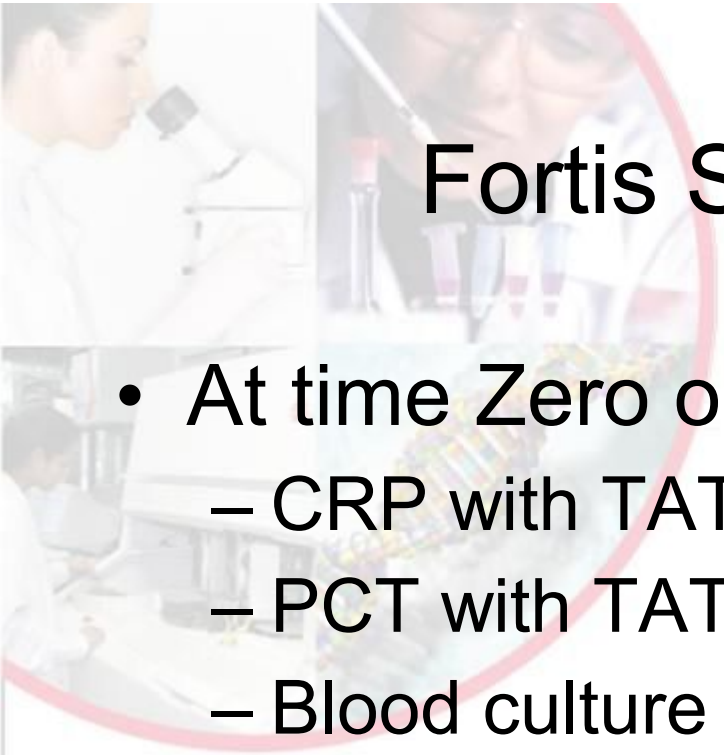
Other potential biomarkers

- Pro-inflammatory cytokines: TNF α , IL-6
- Anti-inflammatory cytokines: IL-8
- Endotoxin
- TREM-1
- Adrenomedullin
- LPS binding protein
- Protein C
- Endocan
- Atrial & Brain natriatic peptide



Summary

- Marker that can significantly alter clinical decision making at the bedside
- IL 6, IL 8 in pediatrics & neonatology
- PCT (approved by FDA as a tool for risk assessment in critically ill patients for progression to severe sepsis & septic shock)
- Sensitivity & specificity superior to CRP, IL 6, IL 8, TLC, Body temp
- PCT rises 2 to 4 hours after septic insult
- Differentiates between infectious & non infectious causes
- PCT levels considerably higher in bacterial infections
- PCT levels may guide antibiotic therapy



Fortis SRL Sepsis screen

- At time Zero on admission to ICU
 - CRP with TAT 30 min
 - PCT with TAT 3 hours
 - Blood culture with continuous monitoring
- Repeat after 48 hours and SOS
- Before initiation of antibiotic therapy
 - If CRP < 10 mg/L → **stop antibiotics**
 - PCT < 0.5 ng/ml → **withhold antibiotics**

Strategy for Antibiotic Resistance Impedance to Spread

FORTIS SRL SARIS

Right agent
Correct dose
Appropriate duration

Clinical cure

• Inhibition of non pathogenic bacteria
Selection of resistant mutants

• Toxicity / side effects

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SARIS

- Effective antimicrobial stewardship
 - Audit & feedback
 - Guidelines & algorithms
 - Antibiotic order form
 - Combination
 - De escalation
 - Dose optimization
 - Parenteral → oral
 - ~~Cycling~~



SARIS

- Comprehensive Infection control
 - Managing data and information
 - Policies & procedures
 - Regulatory requirements
 - Employee health
 - Prevent transmission, investigate outbreaks
 - Education & training
 - Mobilize resources: human & financial

striking back
at
antimicrobial
resistance



Antimicrobial Stewardship Program



**Thank You
for your attention**

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