

51° CONGRESSO NAZIONALE AMCLI

8-11 MARZO 2024
PALACONGRESSI RIMINI

Corso Precongressuale A

DIAGNOSTICA DELLE INFEZIONI DEL TORRENTE CIRCOLATORIO
E INFEZIONI DI DEVICES ENDOVASCOLARI:
PERCORSI, BUONE PRATICHE ED INDICATORI

La corretta gestione della fase pre-analitica: una sola strategia?

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Bloodstream infections



Bloodstream infections (BSI) are defined by the presence of microorganisms in the bloodstream which can often be demonstrated through the positivity of one or more blood cultures.

BSI diagnosis represents one of the critical functions for the microbiology laboratory, as well as one of the most important.

PERCORSO DIAGNOSTICO

INFEZIONI DEL TORRENTE CIRCOLATORIO

Revisione Marzo 2023

Stages of the laboratory testing process	Related activities
PRE-ANALYTICAL	<ul style="list-style-type: none">- Request for diagnostic investigation- Sample handling- Sample transport
ANALYTICAL	<ul style="list-style-type: none">- Test execution- Test validation
POST-ANALYTICAL	<ul style="list-style-type: none">- Results analysis- Reporting

Approximately, **95% of the errors** in the laboratory diagnostic process occur in the **pre-analytical phase**.

BLOOD CULTURE

1) WHY?

2) WHEN?

3) HOW?

WHY?

Blood culture, the gold standard



Advantages

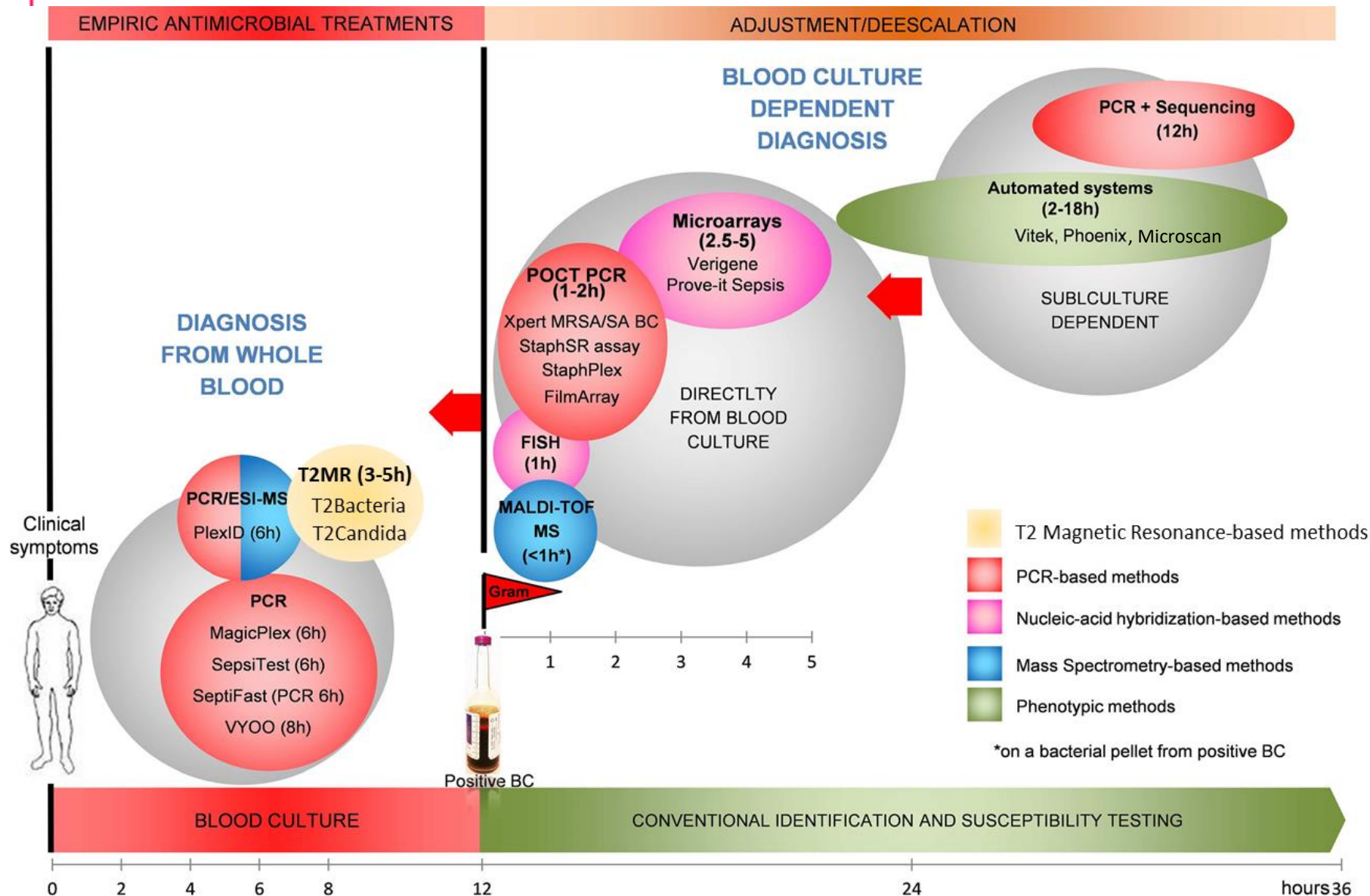
- 1 – Reduced costs
- 2 – Confirmation of diagnostic suspicion
- 3 – Microbial etiology identification
- 4 – Useful indications for targeted antibiotic therapy

Disadvantages

- 1 – Inability to identify slow-growing or fastidious microorganisms
- 2 – Affected by the empirical therapy in progress
- 3 – Long turnaround times
- 4 – High percentage of contamination by resident flora
- 5 – Sensitivity is related to the collection of an adequate blood volume



What technologies are available today for the rapid diagnosis of sepsis?



1 to 5 days

Culture Dependent Methods

**Overall Sensitivity & TAT
Limited by Blood Culture**



3-5 hours

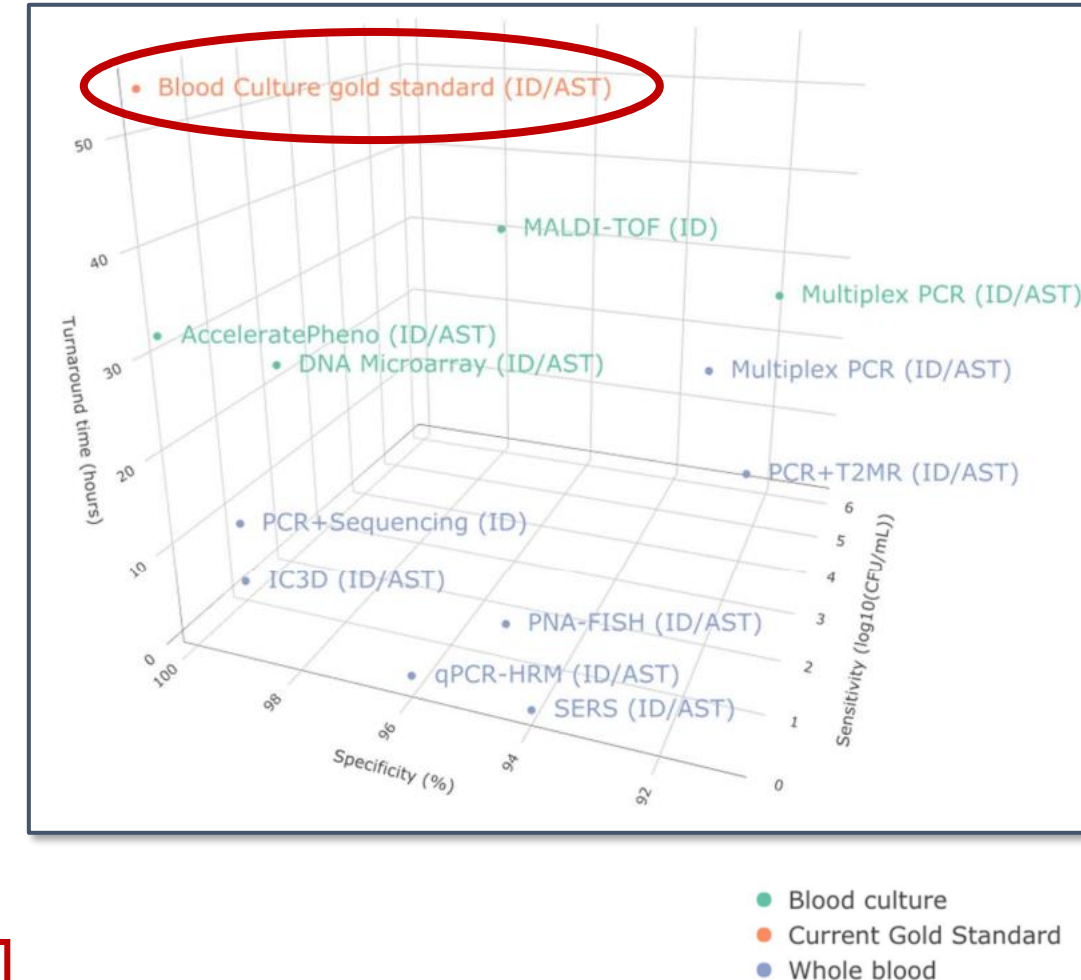
Culture Independent Methods

**Species/Target-Specific Results
Enables Targeted Therapy**

Comparison of Existing and Emerging BSI Diagnosis Technologies

Technologies	Sample	Company	ID				AST	
			Sens. (CFU/mL)	Spec.	Breadth	TAT	Output	TAT
EMERGING								
qPCR-HRM	WB *	Non-commercial	1	100%	37 bacteria (expandable)	8 h (with AST)	MIC	8 h (with ID)
SERS	WB	Spectral Platforms	1	94%	>30 pathogens	20 min	S/R (enzyme-based)	unspecified
ddPCR/ IC3D	WB	Velox Bio	10	100%	unspecified	1–4 h (with AMR)	resistance marker	1–4 h (with ID)
Flow Cytometry	BC (+)	FASTinov	N/A	N/A	N/A	N/A	MIC	<26 h
PNA-FISH	WB	HelixBind	<10	95%	21 pathogens	2.5 h (with AMR)	resistance marker	2.5 h (with ID)
EXISTING								
PCR+T2MR	WB	T2 Biosystems	1–10	91%	5 candida species, ESKAPE organisms >90 pathogens	27–29 h (with AMR)	resistance marker	27–29 h (with ID)
Multiplex PCR	WB	MagicPlex (SeeGene)	30	66–92%	with 27 pathogens at species level	27–30 h (with AMR)	resistance marker	27–30 h (with ID)
Real-time PCR+Sequencing	WB	SepsiTest (Molzzy)	10–40	86–100%	>1350 pathogens	30–31 h	N/A	N/A
Multiplex PCR	BC (+)	BioFire (FilmArray)	10 ⁶ to 10 ⁸	82–92%	8 Gram+ /11 Gram– /5 fungi	25 h (with AMR)	resistance marker	25 h (with ID)
DNA Microarray	BC (+)	Luminex (Verigene)	10–100	84–99%	8 Gram+ /5 Gram–	26.5 h (with AMR)	resistance marker	26.5 h (with ID)
MALDI-TOF + AST cards	BC (+)	Biomerieux (VITEK 2)	10 ⁶	61–98	1316 pathogens	30–36 h (with AST)	MIC	30–36 h (with ID)
PNA FISH + morphokinetic cellular analysis	BC (+)	Accelerate Diagnostics (Accelerate Pheno)	0.8 to 1.7	86–100	7 Gram+ /8 Gram– /2 fungi	32 h (with AST)	MIC	32 h (with ID)
Traditional Blood Culture	WB	BD (BACTEC)	1	100%	Broad	30 h	MIC	54 h (with ID)

* WB: direct from whole blood, BC (+): from positive blood culture; Sens.: sensitivity; Spec.: specificity; TAT: Turnaround time.





WHEN?

"As soon as possible"

**Blood culture must be collected as soon as possible,
upon clinical suspicion of BSI**

- ✓ It is not advisable to wait for the onset of chills and/or fever spike to perform the sampling (bacteremia often does not correlate with specific signs and symptoms)
- ✓ Blood culture should be performed before the start of empirical antibiotic therapy

Contextual evaluation of laboratory markers:

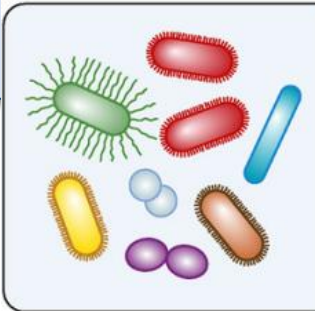
- C-reactive protein (PCR)
- Procalcitonin (PCT)
- White blood cell count

Lamy B *et al.*, Front. Microbiol. 2016

Rhodes A *et al.*, Intensive Care Med. 2017

De Plato F *et al.*, Clin Chem Lab Med. 2020

Common scenarios when initial blood cultures have high and low diagnostic utility for immunocompetent hosts




1) Is there an infection that requires blood cultures?

- Yes for severe sepsis/septic shock and syndromes with high or moderate risk of bacteremia
- If the above not present and the triggering event is fever; what are the other clinical findings? What other tests/cultures could be more useful?

“...Bacteremia detection was not enhanced by obtaining blood cultures closer to the time of the temperature spike, regardless of patient age and the pathogen causing bacteremia.”

“...there are currently no pediatric studies available on this subject.”



Low diagnostic value

Fever ± leukocytosis in stable patients without suspicion for endovascular infection

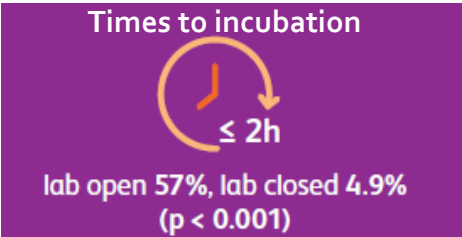
Postoperative fever within 48 h

Diagnostic value of initial blood cultures	Exception
High diagnostic value	
Severe sepsis/septic shock	NA
Infections associated with high or intermediate risk of bacteremia	NA
Low diagnostic value	
Fever ± leukocytosis in stable patients without suspicion for endovascular infection	Patients with splenectomy
Postoperative fever within 48 h	Presence of severe sepsis/ septic shock
Infections with low risk of bacteremia (e.g., cystitis, prostatitis, cellulitis, non-severe pneumonia, prosthetic joint infection)	Endovascular infection suspected
	Presence of severe sepsis/ septic shock
Persistent febrile neutropenia in hemodynamically stable patients with 2 negative sets	NA

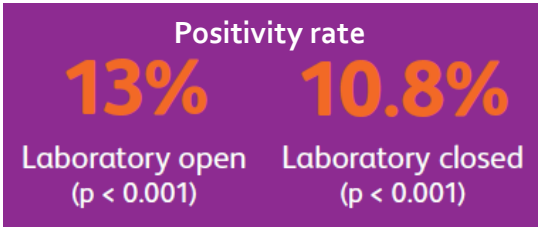
Impact of Pre-Analytical Time on the Recovery of Pathogens from Blood Cultures: Results from a Large Retrospective Survey

2. WHEN

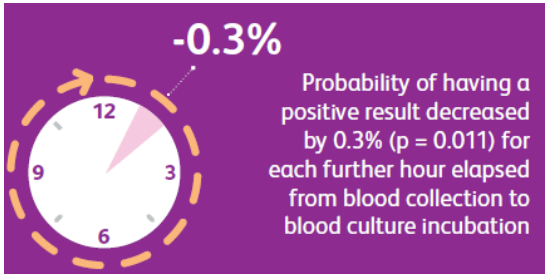
"As soon as possible"



When the laboratory was open, 57% of cultures were processed within 2 h. When the laboratory was closed, 4.9% of cultures were processed within 2 h (P<0.001).



The prevalence of positive cultures was significantly lower for samples collected when the laboratory was closed compared to open (11% vs 13%, P<0.001).



Delayed loading of vials into the instrument resulted in lower detection rates for different microorganisms.

Microbiological results of Blood cultures	Lab open ^a n (%)	Lab closed n (%)	OR ^b (95%CI)	p ^b
Negative	19927 (87.0%)	25011 (89.2%)	1	
Positive (all)	2978 (13.0%)	3039 (10.8%)	0.84 (0.80–0.89)	<0.001
Bacteria (all)	2751 (12.0%)	2819 (10.0%)	0.84 (0.80–0.89)	<0.001
Gram positive bacteria	1483 (6.9%)	1417 (5.4%)	0.80 (0.74–0.86)	<0.001
Gram negative bacteria	1194 (5.7%)	1331 (5.1%)	0.90 (0.83–0.98)	0.01
Yeasts	227 (1.0%)	220 (0.8%)	0.85 (0.70–1.03)	0.090

HOW?

What is the best strategy?



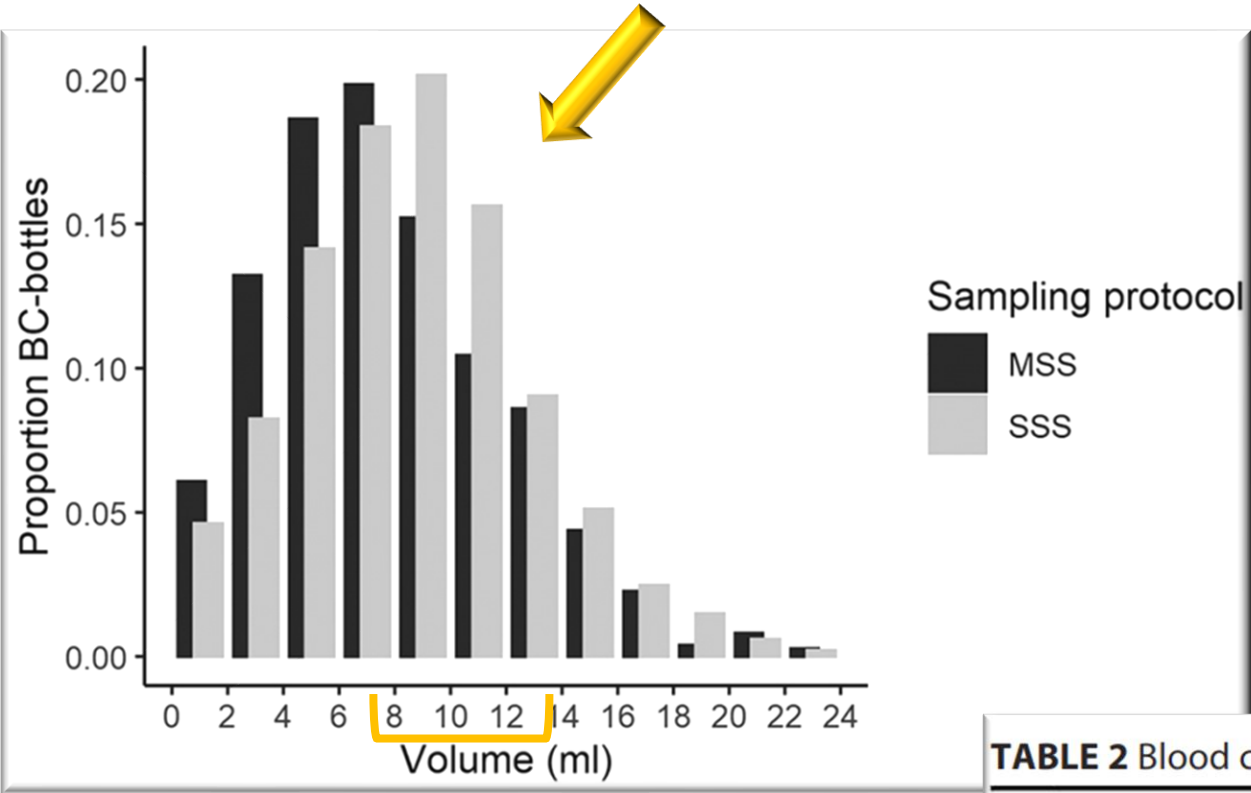
❖ Single sample strategy

- PICC team
- Single venipuncture
- Total blood withdrawal: **40 ml**

❖ Multi sample strategy

- What kind of patient?
- What kind of infection?

Single-Site Sampling versus Multisite Sampling for Blood Cultures: a Retrospective Clinical Study



BC bottles sampled with SSS had a significantly higher proportion of bottles with sample volumes in the ranges of 8–10 ml and 10– 12 ml compared with BC bottles sampled with MSS.

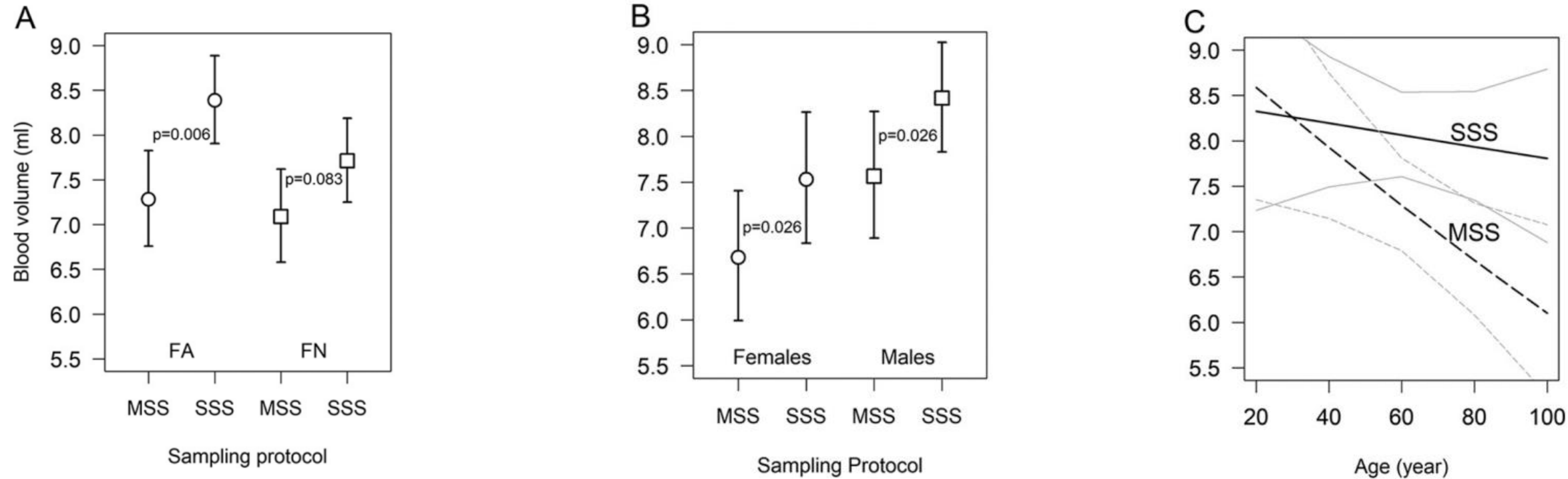
There was no significant difference in BC positivity between SSS (19.72%) and MSS (19.31%) sampling protocols ($P = 0.64$).

TABLE 2 Blood culture results for patients with two BC bottle sets sampled with MSS and SSS

BC result	MSS, <i>n</i> (%)	SSS, <i>n</i> (%)	<i>P</i> value
Total number of patients	3,744	5,133	
Positivity	723 (19.3)	1012 (19.7)	0.635
Bloodstream infection	578 (15.4)	814 (15.9)	0.595
Contaminant growth	178 (4.8)	244 (4.8)	0.999
Polymicrobial growth	57 (1.5)	91 (1.8)	0.363

BC, blood culture. SSS, single-site sampling. MSS, multisite sampling
FA = BacT/Alert FA Plus bottle. FN = BacT/Alert FN Plus bottle

Single-Site Sampling versus Multisite Sampling for Blood Cultures: a Retrospective Clinical Study



The mean blood sampling volume was higher for SSS compared with MSS in FA bottles ($P = 0.006$). Mean blood volume in FA bottles compared with FN bottles was higher in SSS but was similar in MSS ($P=0.001$ and $P=0.28$ respectively).

The SSS protocol resulted in significantly higher blood sample volumes compared with the MSS protocol for both male and female patients ($P = 0.026$). Nevertheless, blood sample volumes were significantly higher for male compared with female patients in both protocols.

Sample volumes were significantly lower in older patients. The association between age and sample volume was more prominent for MSS.

HOW?

Collecting blood culture in case of subacute endocarditis

The “**multi sample strategy**” is indicated for the diagnosis of bacteremia associated with **subacute infectious endocarditis**.

In this case, collect 3 sets of blood culture by venipuncture every 15-30' to document **continuous bacteremia**.

If the first two-three sets result negative, repeat blood sampling after 24 hours.



Miller *et al.*, Clinical Infectious Diseases. 2018

AMCLI ETS. Percorso Diagnostico INFEZIONI DEL TORRENTE CIRCOLATORIO - Rif. 2023-13, rev. 2023

HOW?

Collecting blood culture in case of catheter-related infection

In the patient with CVC, in whom catheter-related sepsis is suspected (or cannot be excluded), it is essential to collect samples:

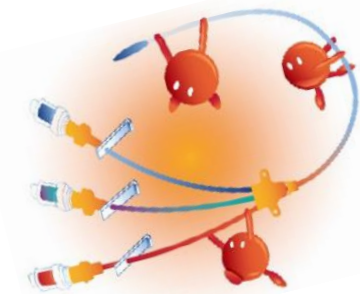
- ✓ From peripheral vein and from the catheter(s), at the same time
- ✓ Inoculate the same amount of blood to each bottle, this allows interpretation of results based on growth times.



Conclusion:

If CR-BSI is suspected, blood culture collection from all catheter lumens should be taken in parallel with blood from peripheral veins. A differential TTP ≥ 120 minutes in blood cultures taken through the catheter lumen and peripheral veins is highly suggestive of catheter-related infection of bacterial etiology.

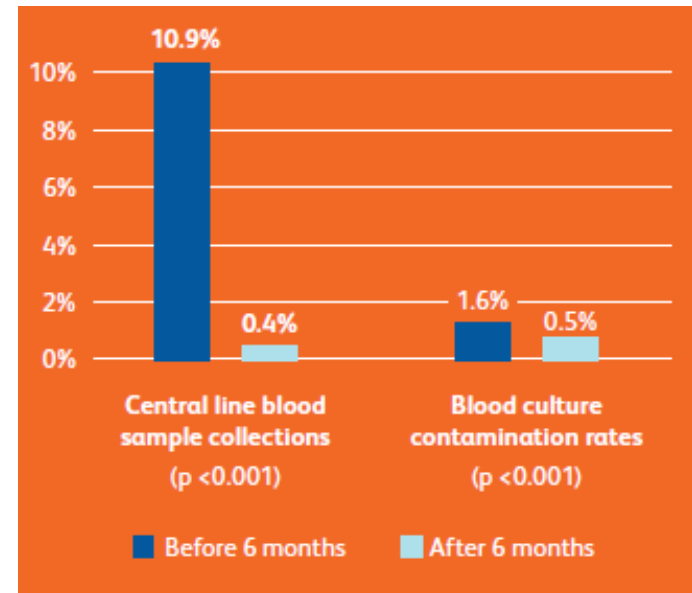
Obtaining Blood Cultures by Venipuncture versus from Central Lines: Impact on Blood Culture Contamination Rates and Potential Effect on Central Line–Associated Bloodstream Infection Reporting



3-year observational study (Jan 2010 – Dec 2012)

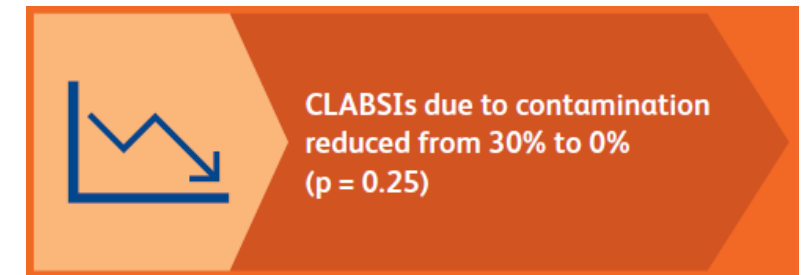
The nursing team has been re-educated on:

- ✓ the importance of applying an alcoholic chlorhexidine-based antiseptic
- ✓ how to take venous samples
- ✓ the procedure to reduce the risk of catheter-related contamination



The proportion of blood samples obtained for culture from central lines decreased from 10.9% during January-June 2010 to 0.4% during July-December 2012 ($P < 0.001$). The proportion of blood cultures that were contaminated decreased from 84 (1.6%) of 5,274 during January-June 2010 to 21 (0.5%) of 4,245 during January-June 2012 ($P < .001$).

Contamination and CLABSI rates were compared pre- and post-change



The reduction in blood culture contaminants yielded an estimated annualized savings of \$378,000 in 2012 when compared to 2010

HOW?

Blood volume to be collected

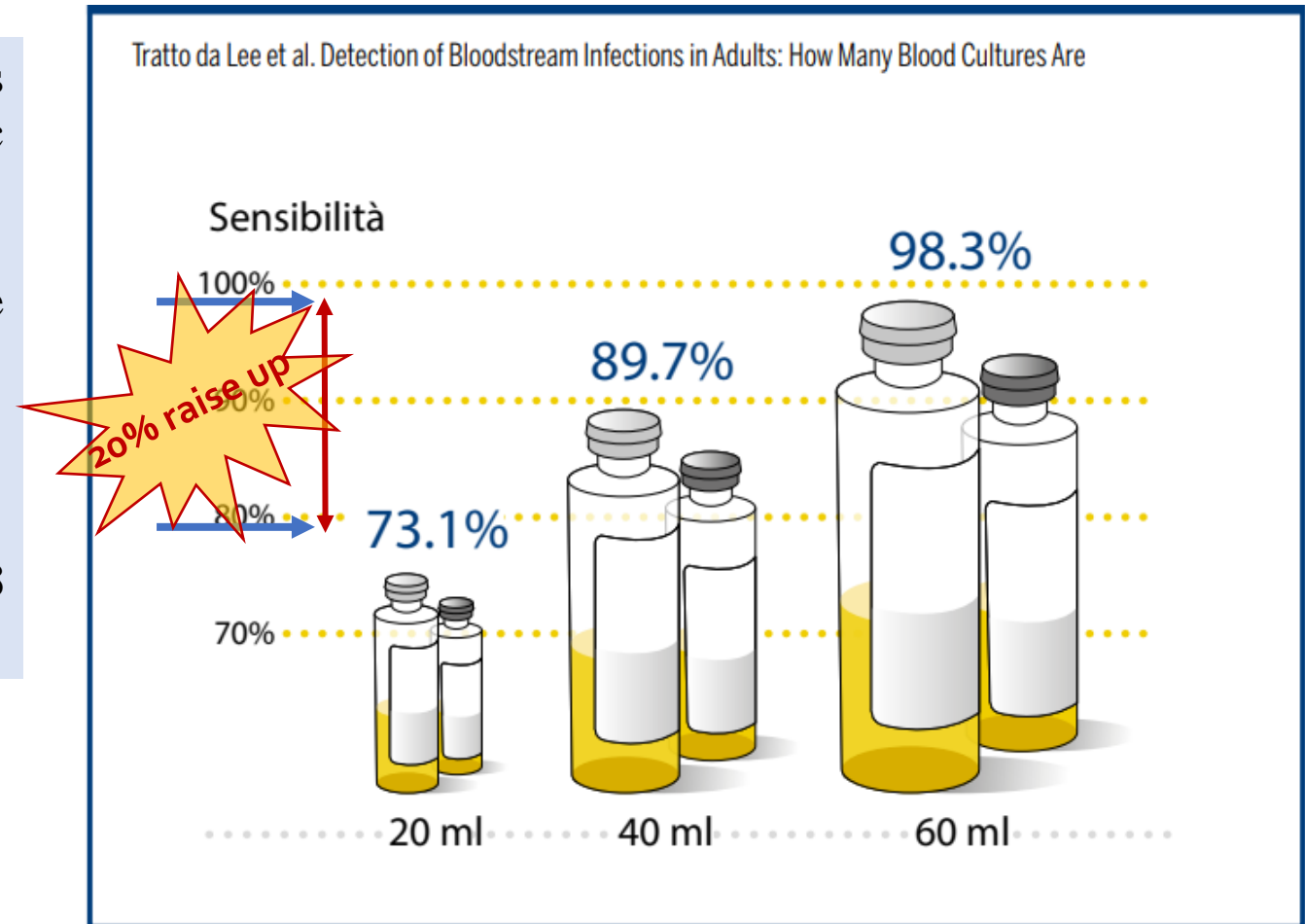
The **blood volume** taken for each set of blood cultures represents the most important variable for the diagnostic outcome.

According to the guidelines, the blood volume to be collected for each set must be equal to at least **30/40 mL**.






Collect at least **two blood culture sets**.

The recommended volume for each bottle is between **8 and 10 mL**

The probability of isolating the pathogen in the event of sepsis is **73.1% with a single sample** and increases to **89.7% and 98.3% with two or three blood culture sets**, respectively.



Blood volume in pediatric patients

WEIGHT (Kg)	Type of <u>BacT</u> /ALERT blood-culture bottle to use		Blood quantity to inoculate in the bottle	N. of bottle per set	N. of set
≤1	<u>BacT</u> /ALERTPF Plus Pediatric Bottle		2 ml	1	1
1.1-2	<u>BacT</u> /ALERTPF Plus Pediatric Bottle		2 ml	1	2
2.1-12.7	<u>BacT</u> /ALERTPF Plus Pediatric Bottle		4 ml	1	2
12.8-36.3	- <u>BacT</u> /ALERT FA Plus Aerobic Bottles (*) - <u>BacT</u> /ALERT FN Plus Anaerobic Bottles (*)		10 ml ^(*)	2 ^(*)	3
>36.3	- <u>BacT</u> /ALERT FA Plus Aerobic Bottles - <u>BacT</u> /ALERT FN Plus Anaerobic Bottles		20-30 ml	2	3

Blood volume to be inoculated into the pediatric bottle is **1 to 4 ml**

(*) If the indicated quantity of blood is not available for each bottle, inoculate only the PEDIATRIC bottle (yellow cap) for each set.

One vs. Two Blood Cultures in Children

Characteristics associated with taking two blood cultures per culturing episode

	1 Culture n (%)	2 Cultures n (%)	p^1
Full sample	160,964 (90.1)	17,738 (9.9)	<0.001
Ward			
Emergency department	85,213 (98.2)	1604 (1.8)	
Pediatric intensive care	7672 (70.0)	3281 (30.0)	
Neonatal intensive care	11,075 (86.5)	1727 (13.5)	
Other	57,004 (83.7)	11,126 (16.3)	<0.001
Age group			
<1 month	37,924 (88.5)	4925 (11.5)	
1–12 months	39,886 (93.4)	2833 (6.6)	
1–11 years	72,371 (90.6)	7535 (9.4)	
12–18 years	9983 (81.4)	2280 (18.6)	<0.001
Unknown	800 (82.9)	165 (17.1)	
Blood culture timing			
First 3 hospital days	109,374 (93.6)	7519 (6.4)	
After day 3	12,408 (64.3)	6885 (35.7)	
Unknown	39,182 (92.2)	3334 (7.8)	

¹ Chi-squared test.

Taking more than one BC was most common in the PICU (30.0%) and least common in the ED (1.8%). Taking more than one BC was also more common among children aged 12–18 (18.6%) and afterday 3 of hospitalization (35.7%)

True pathogen detection rate in one-culture vs. two-culture blood culturing episodes

1 Culture n (%) with True Pathogen	2 Cultures n (%) with True Pathogen
1687/160,964 (1.0)	1576/17,738 (8.9)
685/85,213 (0.8)	199/1604 (12.4)
175/7672 (2.3)	353/3281 (10.8)
121/11,075 (1.1)	155/1727 (9.0)
706/57,004 (1.2)	869/11,126 (7.8)
367/37,924 (1.0)	371/4925 (7.5)
427/39,886 (1.1)	363/2833 (12.8)
708/72,371 (1.0)	640/7535 (8.5)
176/9983 (1.8)	191/2280 (8.4)
9/800 (1.1)	11/165 (6.7)

Test of proportions $p < 0.001$ for full sample and for all wards and age groups.

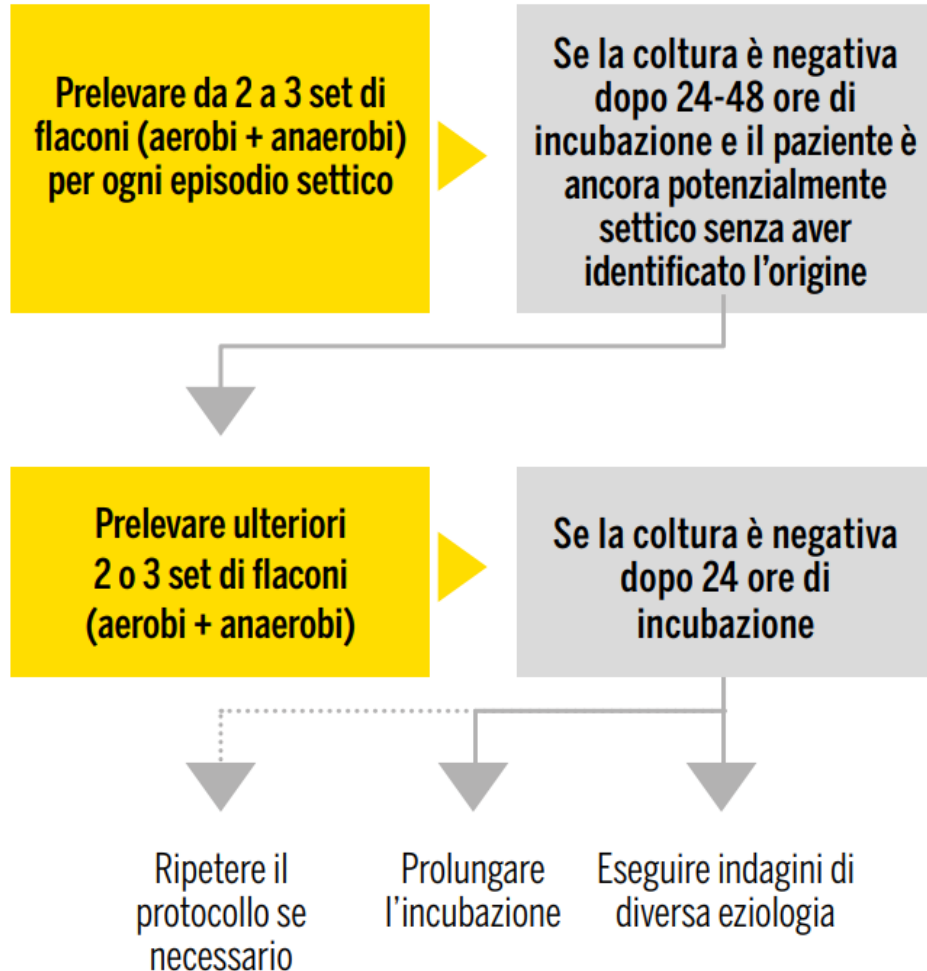
A true pathogen was detected in 1.0% (1687/160,964) of single-culture episodes, compared to 8.9% (1576/17,738) of two-culture episodes ($p < 0.001$).

The difference in detection rate was most pronounced in the ED.

N° of blood culture bottles

Number of recommended blood culture sets

Tratto da Baron, E.J., et al. Cumitech 1C, Blood Cultures IV. Coordinating ed., E.J. Baron. ASM Press, Washington, D.C. 2005



The total number of blood culture bottles must be between 2 and 3 pairs.

↳ necessary for the interpretation of contaminating microorganisms

Exception in selected cases

↳ a single blood culture in adults is possible in critically ill patients or in haematological patients.

In subacute endocarditis

↳ take the 3 sets at an interval of 15-30' to document continuous bacteremia)

Type of blood culture bottles

- A blood culture set is by definition composed of **two bottles** (1 for aerobes and 1 for anaerobes).

Anaerobic bottles allows the growth of strictly anaerobic bacteria, but it has been demonstrated that these bottles are also more effective in the recovery of staphylococci, enterococci and enterobacteria.

- The different bottles and sets **must be recognizable** by the Microbiology laboratory



Concluding remarks

- Sepsis management need a **multidisciplinary team** responsible for implementing antimicrobial and diagnostic stewardship.
- The combined use of **new technologies** allows a fast diagnostic approach which shows a positive impact on early targeted therapy
- The Microbiologist must participate in **educational training** activities for the correct execution of blood culture sampling.



Need a multidisciplinary approach to optimize workflow, communication of test results, and rapid intervention on results (diagnostic and antimicrobial stewardship)



Microbiology Staff OPBG

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*Thank you
for your attention*

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