Bacteremia and Sepsis: Clinical Microbiological Approach

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Objectives of Today's Class

- Define the following bloodstream infections: bacteremia and septicemia
- Define the classification of bacteremia
- List some of the most common causes of bloodstream infections
- Describe how to take blood for microbiological culture and how to transfer to the laboratory

Objectives of Today's Class

- Outline the guidelines used to determine whether agents isolated from blood cultures are true pathogens or probable contaminants

- Explain the importance of collection parameters associated with blood cultures for suspected cases of bloodstream infections, including collection time, the number of cultures, and the volume of blood required

- Briefly describe the interpretation of blood culture results

Learning Outcomes of Today's Class

- Definitions
- Indication of blood culture
- How to collect blood culture
- Various aspects that affect blood culture results
- Manual and automated systems

Key Points

- Collection and inoculation of blood into culture medium with the aim of growing pathogenic microorganism for diagnostic purposes especially in the cases of **bacteremia** or **septicemia**

What is bacteremia and septicemia?

- **Bacteremia is the presence of viable bacteria in the blood**
  - Bacteremia may be transient, continuous or intermittent

- **Sepsis** or **septicemia** is bacteremia plus clinical signs and symptoms of bacterial invasion and toxin production
  - **Sepsis is a clinical syndrome**

- It is characterized by the cardinal signs of inflammation
  - vasodilation
  - leukocyte accumulation
  - increased microvascular permeability

Classification of bacteremia by its origin

Primer

- Endovascular originated; infective endocarditis, endovascular catheter related infections etc.

Seconder

- Originating from focuses like pneumonia, urinary tract infection, abscesses etc.

Classification of bacteremia by duration

- Transient
  - Comes and goes
  - Usually occurs after a procedural manipulation
    - Dental procedures, endoscopy, diabetic foot ulcers etc.
- Intermittent
  - Can occur from abscesses at some body site that is "seeding" the blood
    - Undrained abscesses, infected catheters etc.
- Continuous
  - Organisms from an intravascular source that are consistently present in bloodstream
    - Infective endocarditis

Classification of bacteremia by microbiological agents

Aerobic	Anaerobic	Polymicrobial

• Bacteria
  • Gram-positive
  • Gram-negative
• Fungi
	• %1-17
	• *Bacteroides* spp.
	• *Fusobacterium* spp.
	• *Peptostreptococcus* spp.
	• *Clostridium* spp.
		• <%5
		• Presence of more than one microbiological agent

Classification of bacteremia by sources

Community acquired

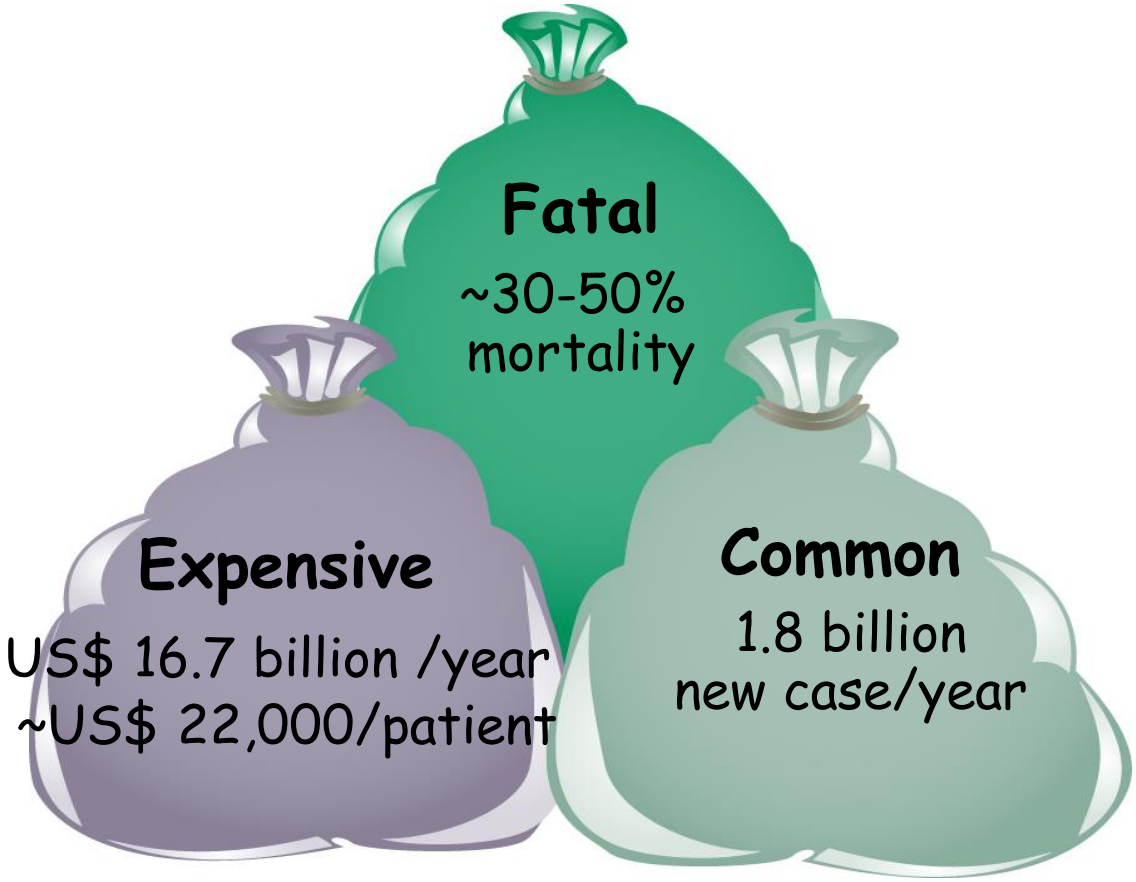
- *Escherichia coli*
- *Streptococcus pneumoniae*
- *Staphylococcus aureus*
- Other *Enterobacteriaceae* members
- *Neisseria meningitidis*
- Beta-hemolytic streptococci
- Enteric fever
- Brucellosis

Hospital acquired

- Coagulase negative staphylococcus (CoNS)
- *Escherichia coli*
- *Staphylococcus aureus*
- Other *Enterobacteriaceae* members
- *Pseudomonas aeruginosa*
- Enterococci
- Anaerobes
- *Streptococcus pneumoniae*
- Fungi

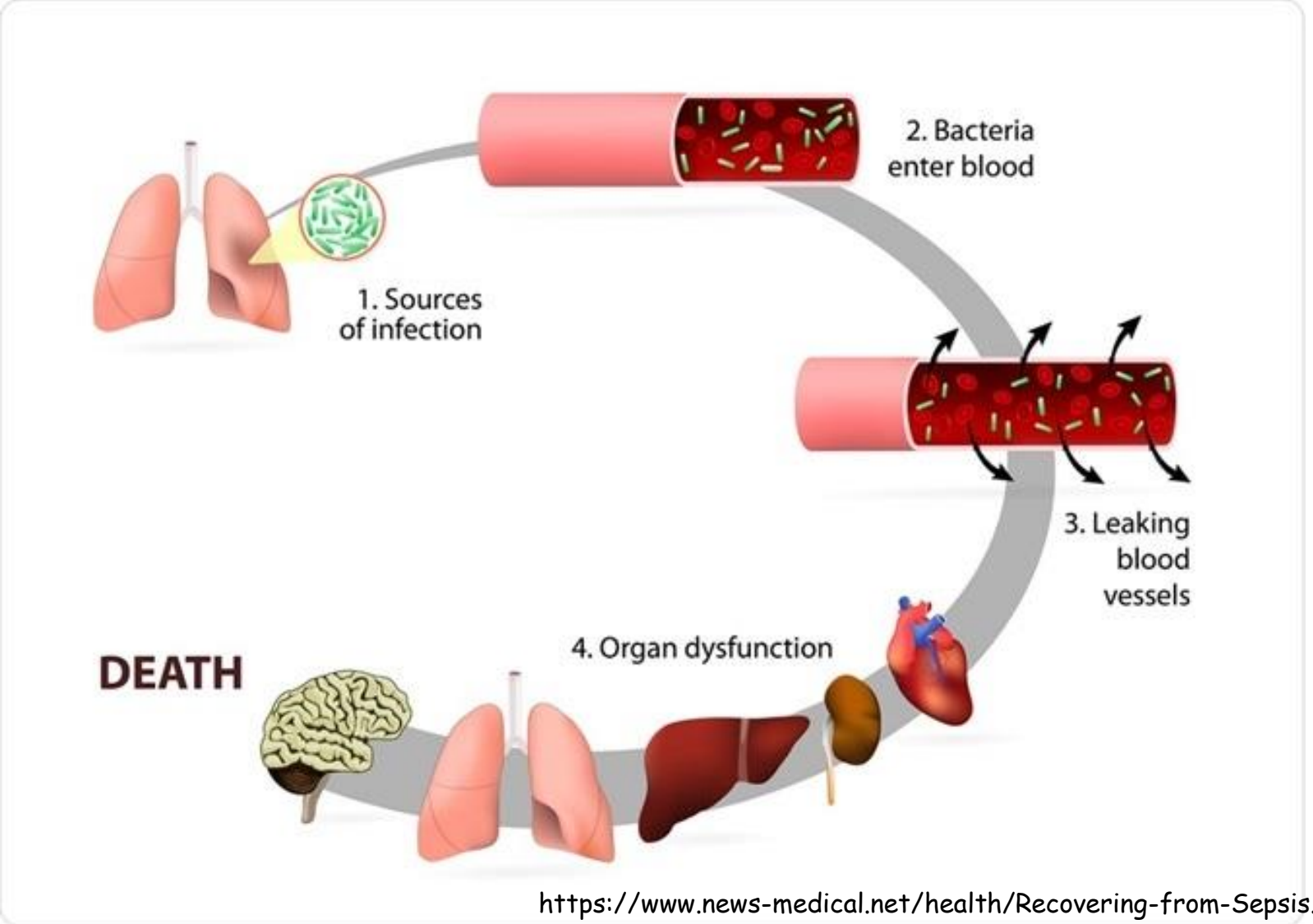
Sepsis: A global problem

An important burden on health expenditures



Rivers et al (2001). *N Eng J Med* 345(19):1368-1377,
Slade et al (2003). *Crit Care* 7(1):1–2

Sepsis Stages



https://www.news-medical.net/health/Recovering-from-Sepsis.aspx

Bacteremia	Septicemia / Sepsis
Simple presence of bacteria in the blood	It is the presence and active multiplication of bacteria in blood
Not as dangerous as septicemia	Potentially a life threatening infection
Less amount of bacteria are present in blood	Large amount of bacteria are present in blood
May result through a wound infection, surgical procedure, injection	Can arise from infections throughout the body, including infections in lungs, abdomen, urinary tract
Toxins are not produced	Toxins are produced
No symptoms/mild fever	Fever, chills, fast respiration, increase heart rate
Can resolve without treatment	Quickly leads to death if untreated
Rapidly removed from blood stream by immune system	Antibiotics are needed

Clinical Definition of Sepsis

- Temperature >38.3°C or <36°C
  - Normal range 36.5° – 37.5°
- Tachycardia
  - Heart rate >90 beats/min
- Tachypnea
  - Respiratory rate >20 breaths/min or $PaCO_2$ <32 mmHg
- Raised or very low white blood cell count
  - WBC >12,000 cells/mm^3, <4000 cells/mm^3, or >10 percent immature (band) forms

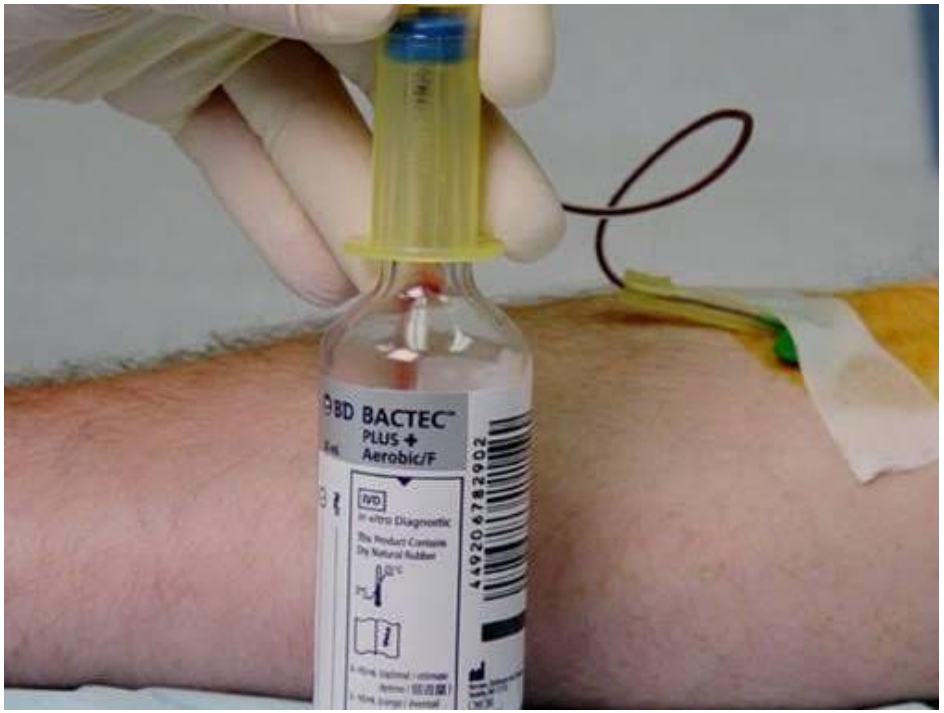
It exists if two or more of the above abnormalities are present, along with either a culture-proven or visually identified infection!

Sepsis

- Since sepsis is frequently a life-threatening infection, its early detection is essential

Blood Culture

- Blood culture is the single most important procedure to detect systemic infection due to bacteria



Organisms commonly isolated from blood cultures

- *Staphylococcus aureus*
- *Escherichia coli*
- CoNS
- *Enterococcus* spp.
- *Candida albicans*
- *Pseudomonas aeruginosa*
- *Klebsiella pneumoniae*
- Viridans streptococci
- *Streptococcus pneumoniae*
- *Enterobacter cloacae*
- *Proteus* spp.
- Beta-hemolytic streptococci
- *Anaerobic bacteria: Bacteroides* and *Clostridium* spp.

Bloodstream infections

Bloodstream infections are classified as;

- **Health care–associated infections (HAIs),** including;
  - **Device-associated infections (DAIs)** such as **central line–associated bloodstream infections (CLA-BSIs),**

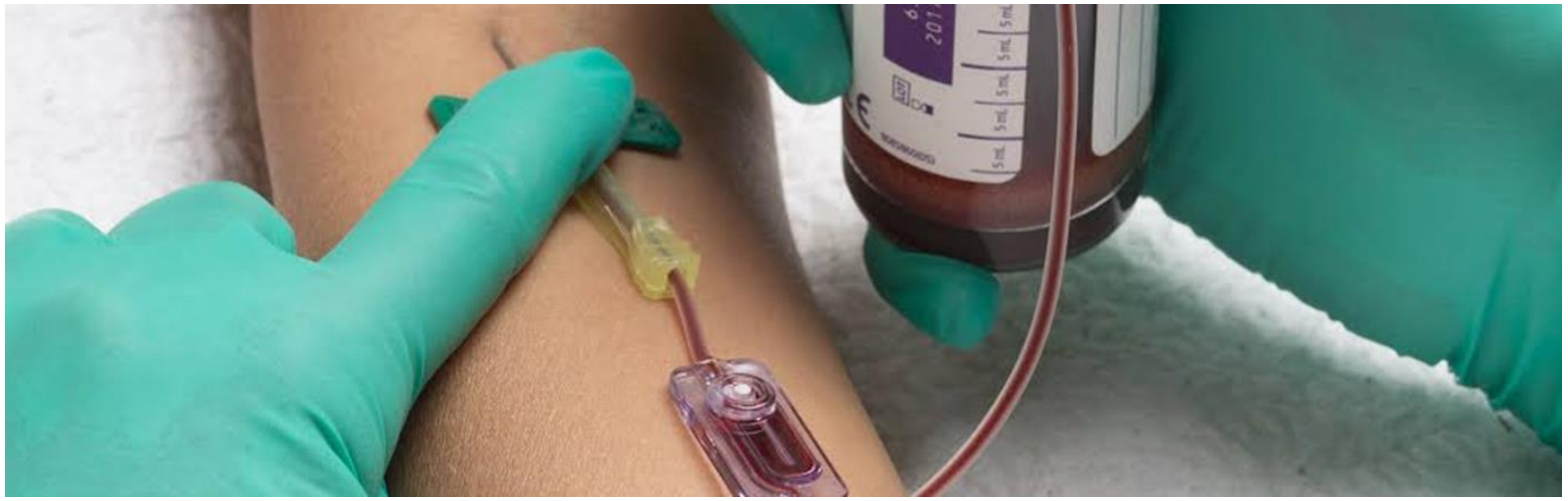
and

- **Community-acquired bloodstream infections (CA-BSIs)**

The time when the bloodstream infection is detected is critical when separating HAIs from CA-BSIs

Detection of Bacteremia

- To detect bloodstream infections, a patient's blood must be obtained **by aseptic venipuncture and then incubated in culture media**
- Bacterial growth can be detected using techniques ranging from manual to automated methods



Detection of Bacteremia

- After sufficient growth, the organism's phenotypic (biochemical profile), nucleic acid or protein spectrum is identified as a particular organism;

- If it is considered pathogenic or if a treatment is necessary for the patient, the organism is then tested for susceptibility to various antimicrobial agents

What is a blood culture?

- A blood culture is a laboratory test in which blood is injected into bottles containing culture media to determine whether microorganisms have invaded the patient's bloodstream

Blood Culture

- Taking blood for culture is an important procedure
  - As blood cultures are used to detect the cause of an infection leading to bloodstream infection

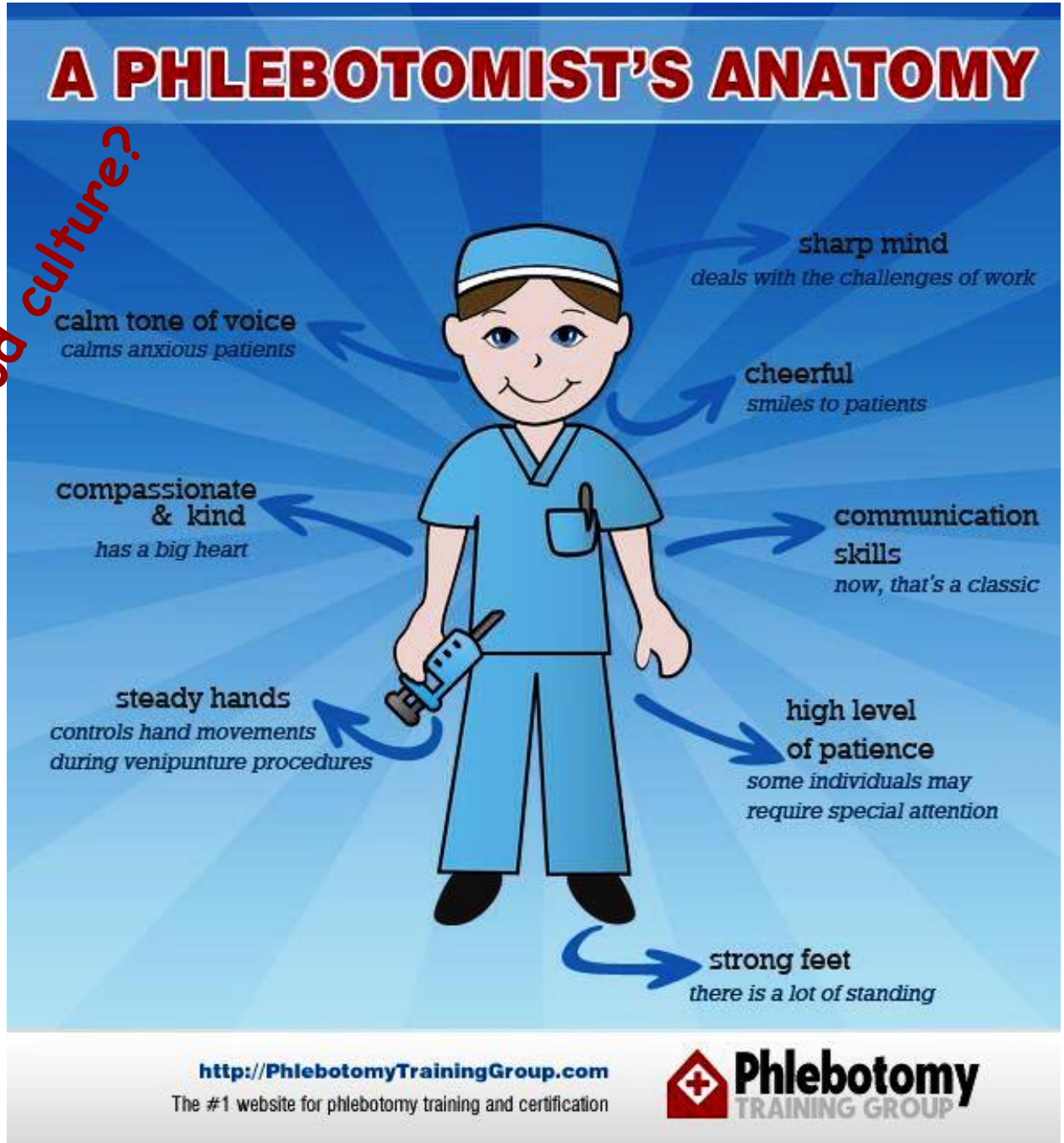
- The results are important because they help guide appropriate treatment
  - However, microorganisms are present on the skin surface of patients, staff, and the patient environment which can result in contamination of blood cultures

Blood Culture

- Contamination can cause confusion and potentially, inappropriate treatment
  - Because it is sometimes difficult to determine if a positive blood culture is due to genuine bacteremia or if it is a false positive result caused by contamination

- **In healthy persons, properly obtained blood specimens are sterile**

How to take a blood culture?



Blood Culture

Blood cultures should be taken:

1. Only when there is an appropriate indication
2. At the correct time
3. Using the correct technique in order to prevent contamination of the sample and to minimize risk to patients and staff

Specimen Collection

- The vein from which the blood is to be drawn must be chosen before the skin is disinfected

Specimen Collection

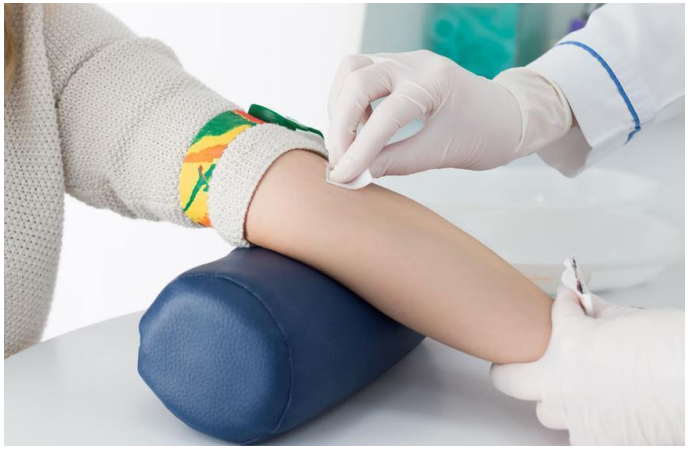
- Careful skin preparation before collecting the blood sample is of paramount **importance** to reduce the risk of introducing contaminants into blood culture media

https://www.verywellhealth.com/tips-for-making-a-blood-draw-easier-3156931

Preparation of the Site

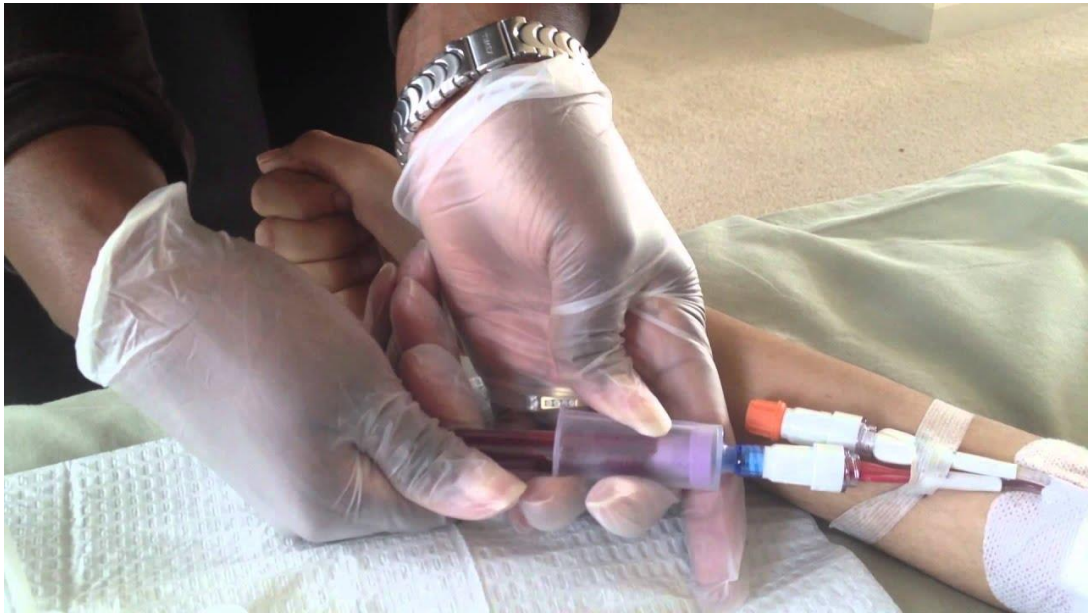
Antisepsis

- Once a vein is selected, the skin site is defatted (fat removal) with 70% isopropyl alcohol, and an antiseptic is applied to kill surface and subsurface bacteria
  - Iodine tincture (iodine in alcohol) and chlorhexidine are equivalent for skin preparation before drawing blood cultures



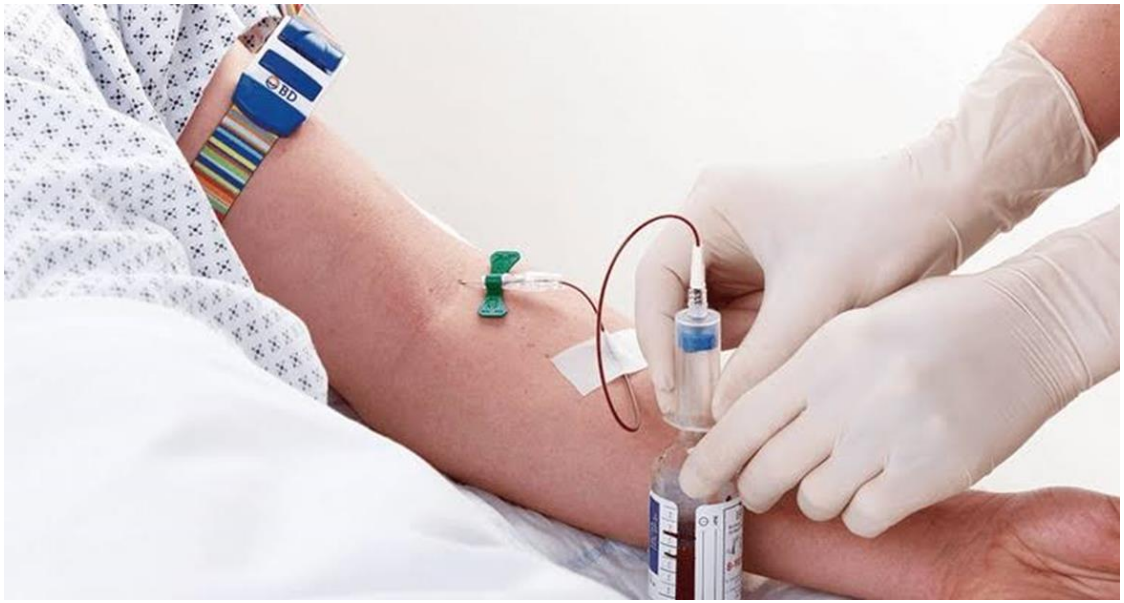
Specimen Collection

- If a patient has an existing IV line, the blood should be drawn below the existing line
  - Blood drawn above the line will be diluted due to the fluid being infused

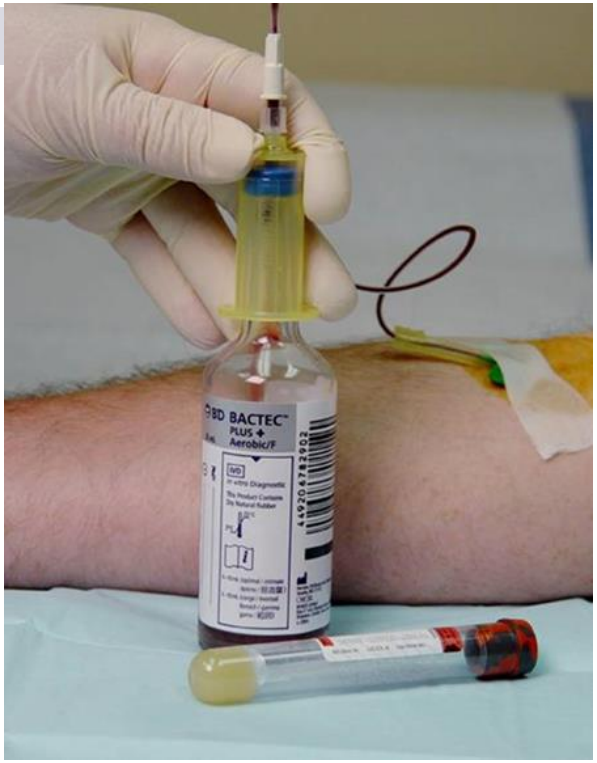


Specimen Collection

- It is less desirable to draw blood through a vascular shunt or catheter
  - Because these prosthetic devices are difficult to decontaminate completely and may be colonized with a microbial biofilm within the vasculature of the patient



Blood Culture



IMPORTANT!!!

- The volume of blood cultured
- The dilution of blood in the culture medium
- The use of both aerobic and anaerobic culture media
- The duration of incubation

Specimen Volume

the success of a blood culture → the volume of blood processed

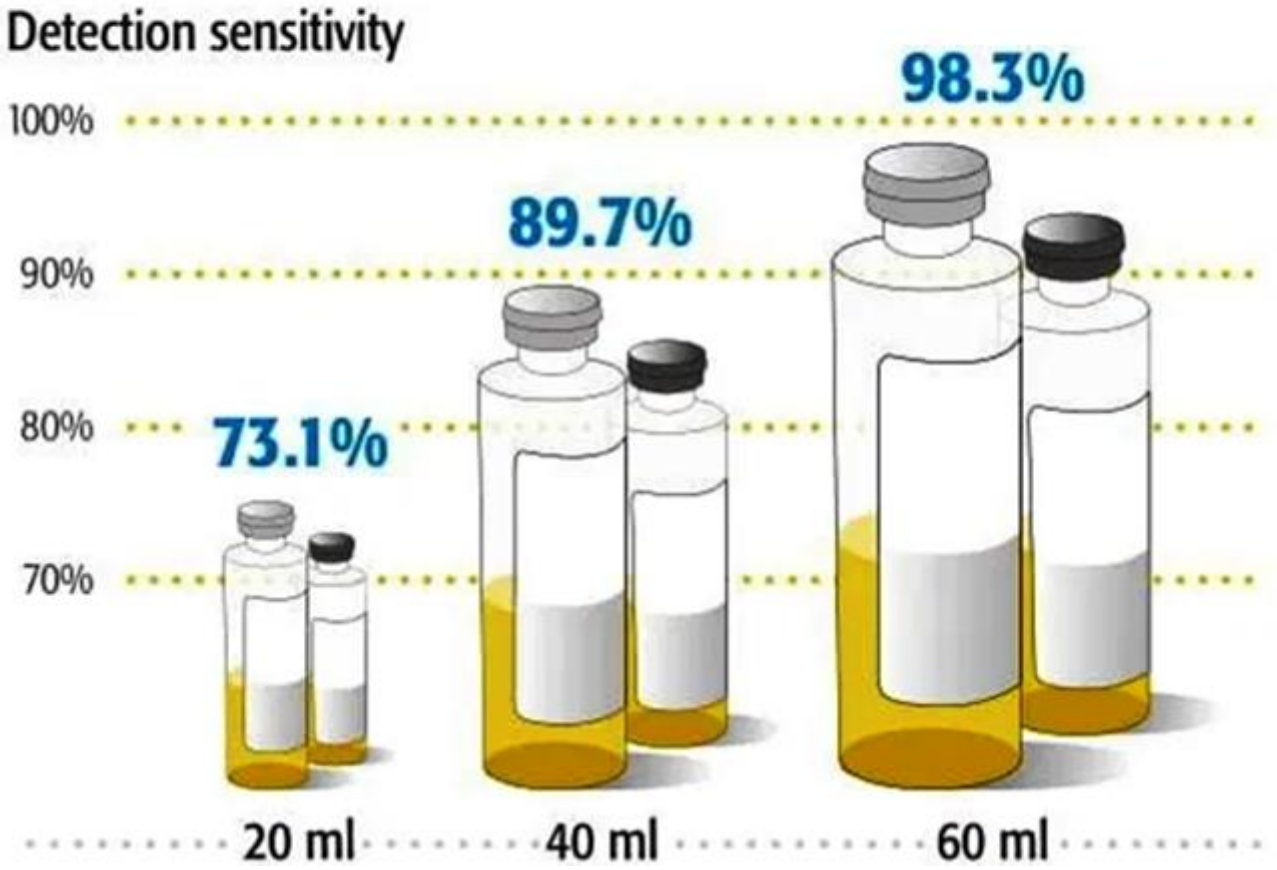
→ Important!!!

the volume of blood → isolation of the infecting organism

Specimen Volume

Cumulative sensitivity of blood culture sets

Adapted from Lee *et al*. Detection of Bloodstream Infections in Adults: How Many Blood Cultures Are Needed? J Clin Microbiol. 2007; 45:3546-3548



https://microbeonline.com/blood-culture-indications-timing-and-volume/

Specimen Volume

Adults' & Children's blood volume is different !

ADULTS

- Collection of two sets of cultures using 10 to 20 mL of blood per culture is strongly recommended for adults

CHILDREN

- Quantities less than 1 mL may not be adequate to detect pathogens

How

Many?

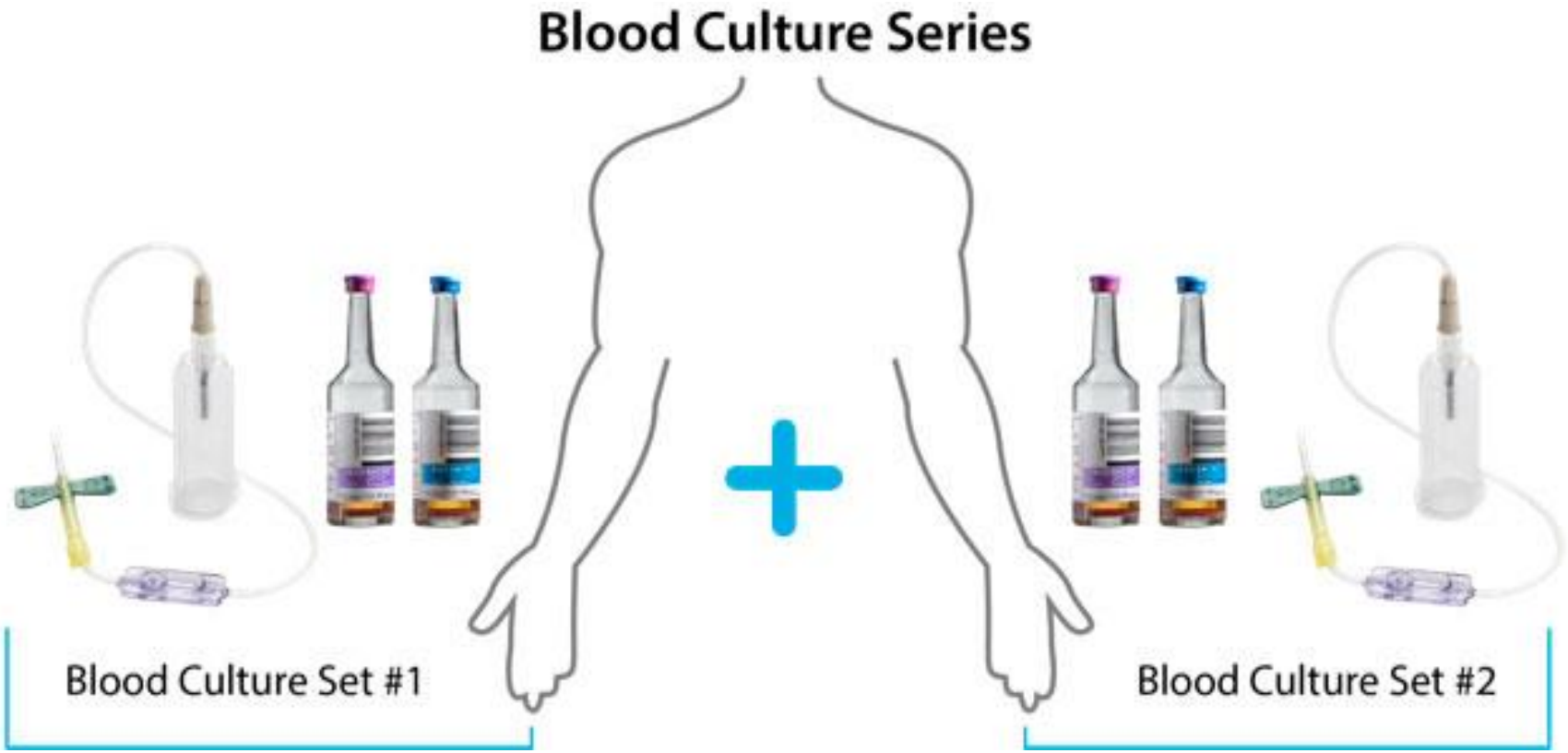
Blood volumes suggested for cultures from infants and children

Adapted from Kellogg *et al*. Frequency of low-level bacteremia in children from birth to fifteen years of age. J Clin Microbiol. 2000; 38:2181-2185.

Weight of patient		Patient's total blood volume (ml)	Recommended volume of blood for culture (ml)		Total volume for culture (ml)	% of patient's 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	4.5-27	>200	4	2	6	3
12.8-36.3	28-80	>800	10	10	20	2.5
>36.3	>80	>2,200	20-30	20-30	40-60	1.8-2.7

https://microbeonline.com/blood-culture-indications-timing-and-volume/

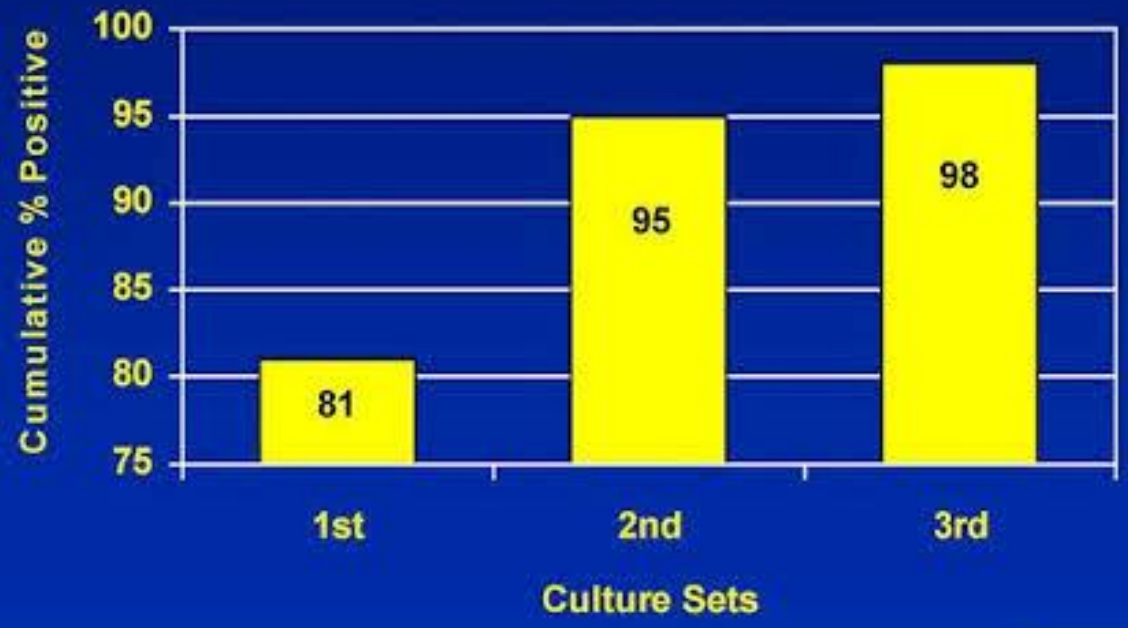
Number of Blood Culture



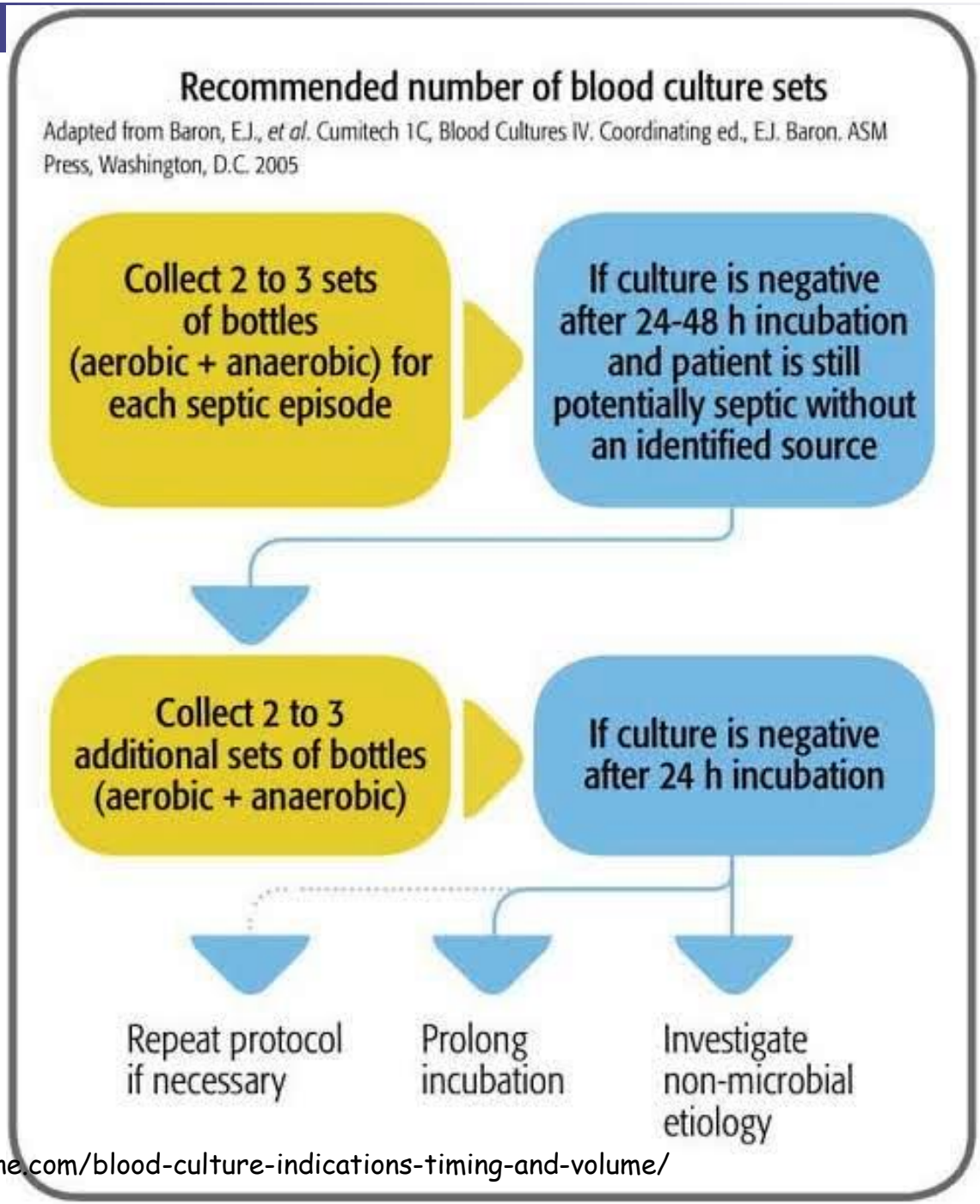
Number of Blood Cultures Sets

"With adequate volume of blood, 2 - 3 blood culture sets are sufficient to detect nearly all episodes of bacteremia and fungemia"

Dunne et al. CUMITECH Blood Cultures III; 1997, ASM Press



Rohner and Auckenthaler 1999 Clin Microbiol Infect; 5:513



https://microbeonline.com/blood-culture-indications-timing-and-volume/

35%-50% of positive blood cultures are actually **FALSE** positive

Timing of Blood Cultures

- Fever and chills occur appr. 1 h after the microbial invasion of the bloodstream
  - **Optimal time**: just before anticipated onset of the chill or fever, **but** difficult to predict
- Typically collected as soon as possible after the onset of fever or chills or whenever serious infection is suspected

Dunne et al. CUMITECH Blood Cultures III; 1997, ASM Press

Timing of Collection

- Before starting the antimicrobial therapy
- At the time of fever peak
- Minimum 30-60 minute interval between 2 samples except in critically ill septic patient
  - In continuous bacteremia, timing of blood culture is not important, but in intermittent bacteremia 2 or 3 culture should be spaced an hour apart

How long should it take to transport the blood culture bottles to the laboratory?

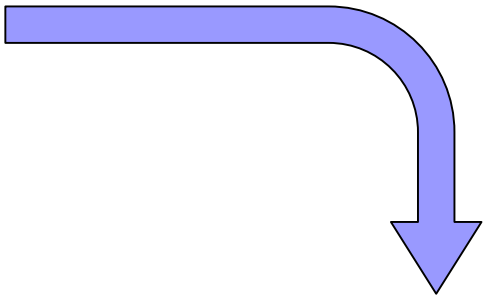
- As soon as possible
- Ideal transport time <30 min
- Transport time must not exceed 2 hours!!!

Miscellaneous Matters

Anticoagulation

- Blood drawn for culture must not be allowed to clot
  - If the infecting organism becomes entrapped within a clot, its presence may go undetected

- Heparin,
- EDTA, and
- Citrate



inhibit numerous organisms and are **not** recommended for use

Miscellaneous Matters
Anticoagulation

- The best anticoagulant available for blood cultures:
  - **Sodium polyanethol sulfonate** (SPS, Liquoid) in concentrations of 0.025% to 0.03%
- SPS, however, may inhibit the growth of a few microorganisms
  - Such as some strains of *Neisseria* spp., *Gardnerella vaginalis*, *Streptobacillus moniliformis*, and all strains of *Peptostreptococcus anaerobius*
  - Although the addition of 1.2% **gelatin** has been shown to counteract this inhibitory action of SPS, the recovery of other organisms decreases

Miscellaneous Matters
Dilution

- Blood / medium ratio is important !!!
  - Affects the time and the possibility of bacterial growth
- Excessive or little blood affects the sensitivity of the test
  - False negative result !!!

Miscellaneous Matters
Dilution

- Traditionally, a **1:10** ratio of blood to medium was required for successful bacterial growth
  - Several new commercial media containing resins or other additives have demonstrated enhanced recovery with as low as a **1:5** ratio
  - All commercial blood culture systems specify the appropriate dilution

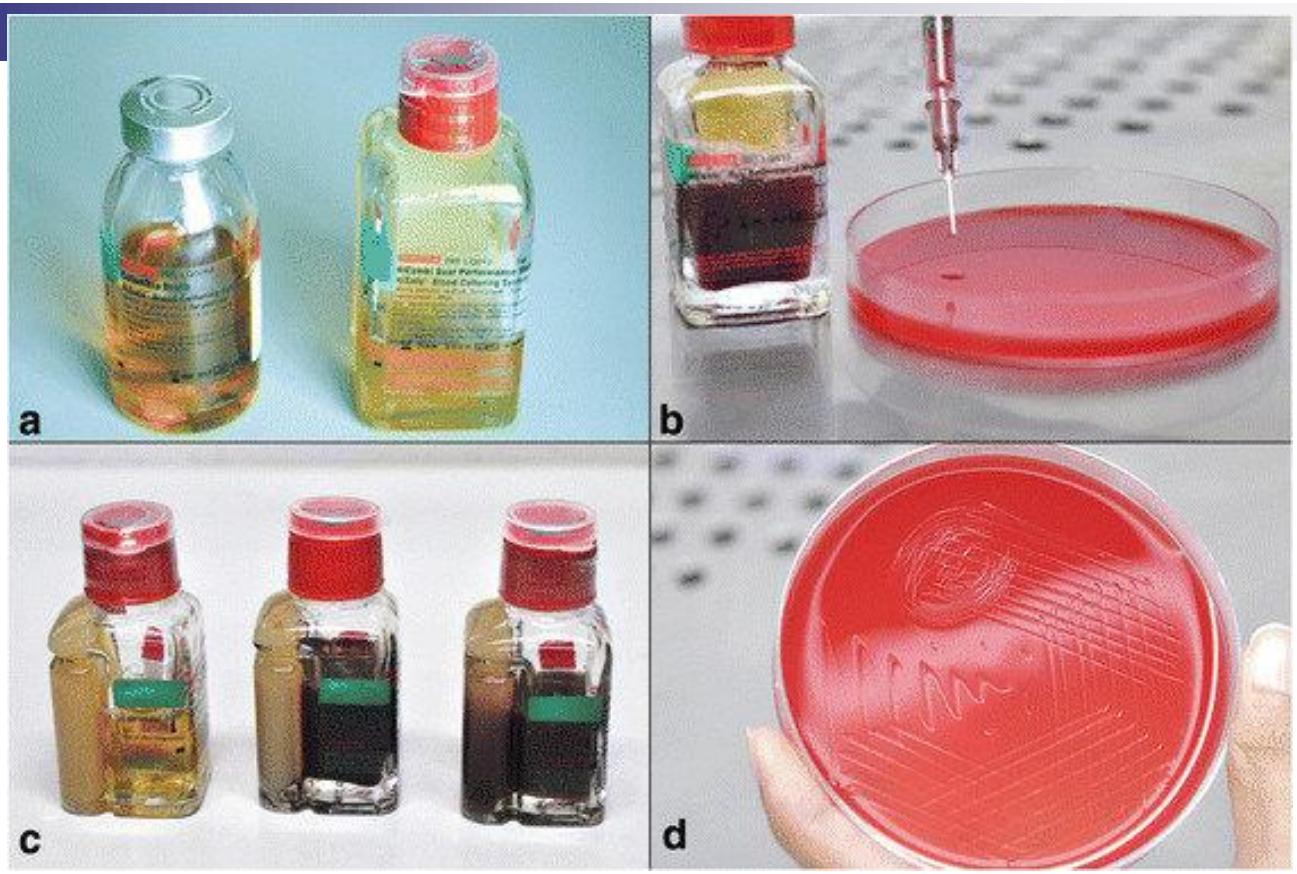
Blood Culture Media

- Blood culture media have been developed as enrichment broths to encourage the multiplication of as few as a single organism
- These media will enhance growth of contaminating organisms, including normal microbiota of human skin

Miscellaneous Matters
Blood Culture Media

- Basic blood culture media contain a **nutrient broth** and an **anticoagulant**
  - Most blood culture bottles available commercially contain **trypticase soy broth**, **brain-heart infusion broth**, supplemented with **peptone** or **thioglycolate broth**
  - More specialized broth bases include **Columbia** or **Brucella broth**

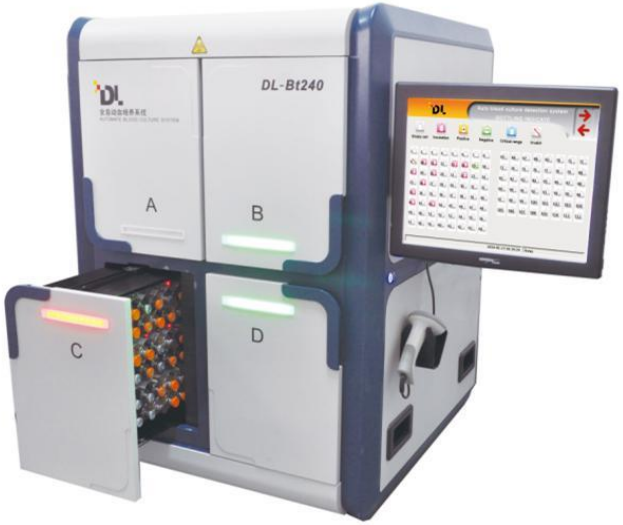
Manual blood culture systems



In manual systems, the blood culture bottles are examined two or three times a day for the first 2 days and daily thereafter for 1 week

In the manual method, blind subcultures of all the blood culture bottles on days 2 and 7 may be necessary

Automated blood culture systems



- Automated blood culture systems use a variety of methods to detect positive cultures
- These automated methods allow frequent monitoring of the cultures—as often as every few minutes—and earlier detection of positive ones

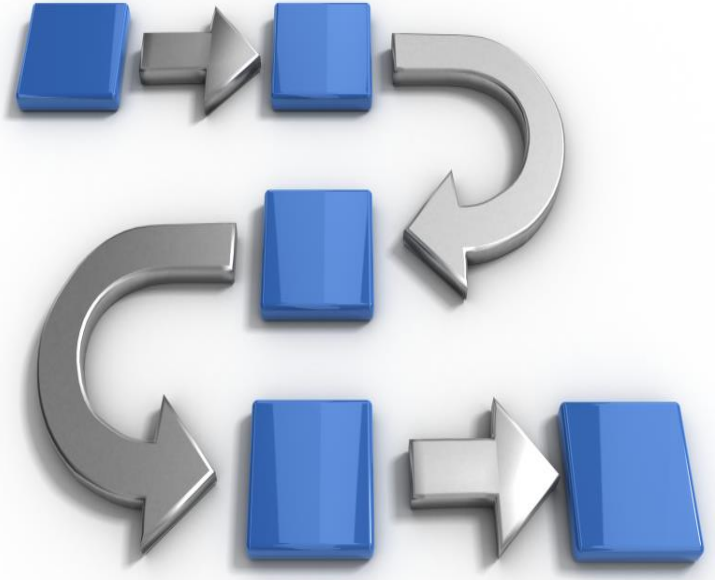
Standard incubation time: 5 days !!!

Attention !

- In most types of bacteremia, examination of direct blood smears is not useful

- Because few organisms are typically present in the blood of a septic patient, it is not worthwhile to perform a Gram stain of blood for microscopic analysis

Sample processing and workflow

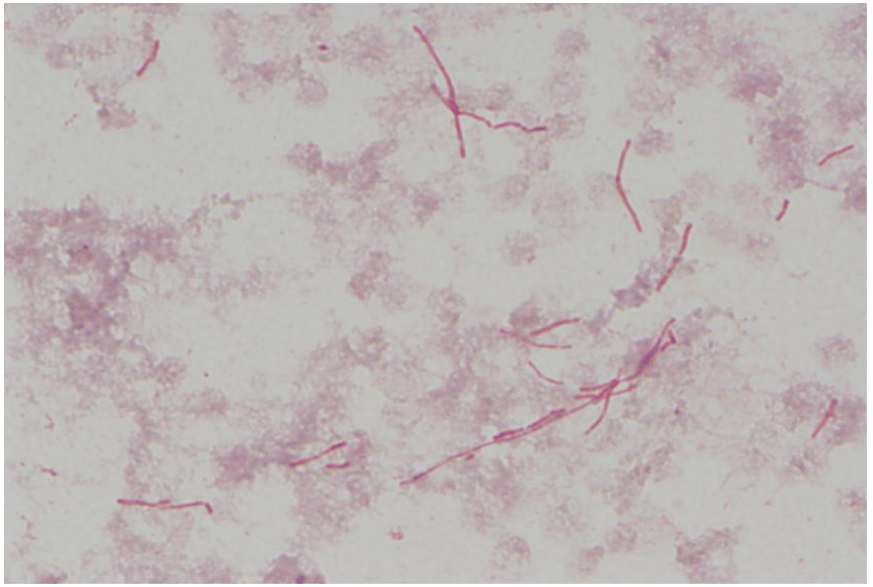


Handling Positive Blood Cultures

- Most laboratories use a broth-based automated blood culture method
  - Designed to maximize sensitivity, detection algorithms of automated blood culture instruments lead to a certain percentage of false positive results

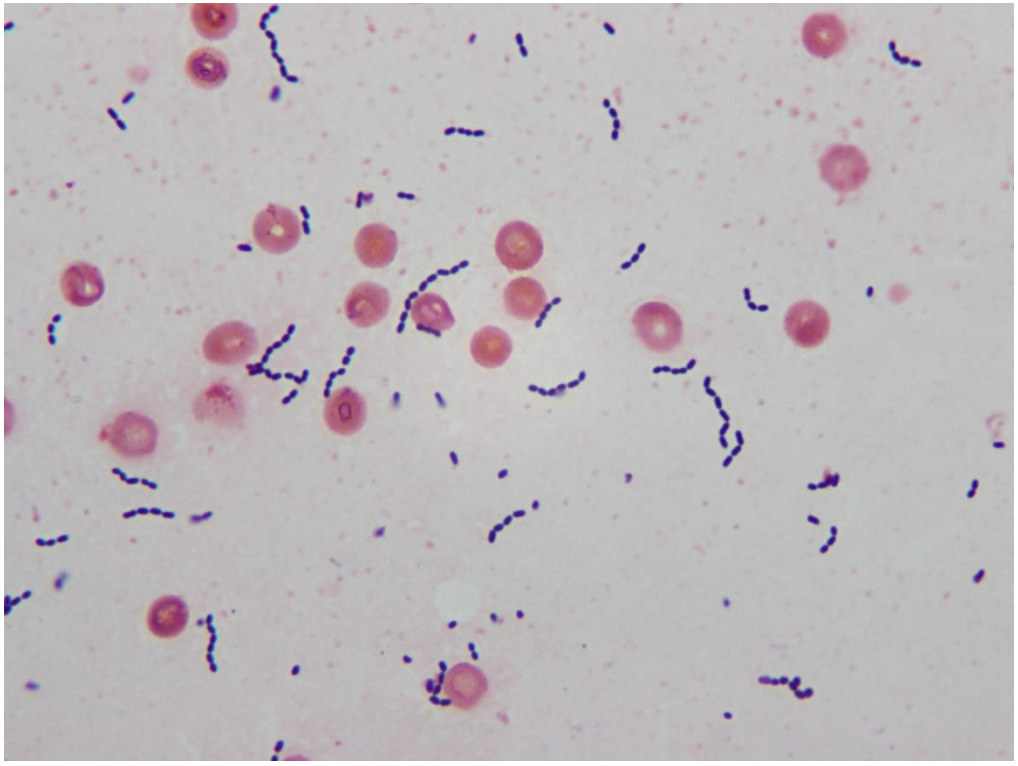
Handling Positive Blood Cultures

- **All blood culture procedures are done in the biosafety cabinet !!!**
- When a positive culture is indicated according to the automated detection system, a Gram-stained smear of an air-dried drop of medium should be performed



Handling Positive Blood Cultures

- As soon as a morphologic description can be tentatively assigned to an organism detected in blood, the physician should be contacted and given all available information



Handling Positive Blood Cultures

- If no organisms are seen on microscopic examination of a bottle that appears positive, subcultures should be performed

Handling Positive Blood Cultures

- The incidence of polymicrobial bacteremia or fungemia ranges from 3% to 20% of all positive blood cultures
  - For this reason, samples must be resubcultured for isolated colonies

Handling Positive Blood Cultures

POSITIVE SIGNAL

- There is microorganism in Gram stain, but no growth in subculture
- The appropriate media or incubation conditions for culture???
- Try alternative diagnostic methods (Serology, PCR…)

- There is a positive signal in culture bottles but no microorganism in Gram stain
- False-positive signal???
- Try another staining method

What is Contamination of Blood Culture?

«Growth of one or more skin flora microorganism in only one of the blood culture set of the patient

&

the absence of a clinical / microbiological evidence regarding an infection which is related to this organism in the patient»

If the same microorganism is growth in the both sets of blood cultures (even it is a contaminant microorganism):

Set 1		Set 2		RESULT
Aerobic	Anaerobic	Aerobic	Anaerobic	
				Negative
				Contamination
				Contamination
				Positive
				Positive
				Positive
				Positive
				Positive
				Positive
				Positive

White boxes: There is no microbiological growth
Blue boxes: There is microbiological growth

Interpretation of the results & Reporting

- Identify at genus level!
- Do not perform antimicrobial susceptibility tests!!!

CONTAMINATION

TRUE POSITIVITY

- Identify at species level!
- Perform antimicrobial susceptibility tests!!!

Interpretation of Blood Culture Results

- The following criteria may be helpful in differentiating "true positives" from contaminated specimens:

1. Growth of the same organism in repeated cultures obtained at different times from separate anatomic sites strongly suggests true bacteremia
2. Growth of different organisms in different culture bottles suggests contamination but occasionally may follow clinical problems such as wound sepsis or ruptured bowel

Interpretation of Blood Culture Results

3. Growth of normal skin microbiota, e.g., coagulase-negative staphylococci, diphtheroids (corynebacteria and propionibacteria) or anaerobic gram-positive cocci, in only one of several cultures suggests contamination

Growth of such organisms in more than one culture or from specimens from a high-risk patient, such as an immunocompromised bone marrow transplant recipient, enhances the likelihood that clinically significant bacteremia exists

Interpretation of Blood Culture Results

4. Organisms such as viridans streptococci or enterococci are likely to grow in blood cultures from patients suspected to have endocarditis, and gram-negative rods such as *Escherichia coli* are likely to grow in blood cultures from patients with clinical gram-negative sepsis

Therefore, when such "expected" organisms are found, they are more apt to be etiologically significant

"I have a blowup of your blood culture, and as you can see, the prognosis looks positive."

THE END

Thanks for having patience with me ☺

KEEP
CALM
AND
Love
Laboratory

References

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- Manual of Clinical Microbiology; Jorgensen JH, Pfaller MA, 11th Ed; ASM Press, 2015 p:15
- Koneman's Color Atlas and Textbook of Diagnostic Microbiology; Procop GW, 7th Ed, Wolters Kluwer, 2016