Blood Culture & Catheter-related bloodstream infection

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Infections of the Blood

✓ Blood Culture

✓ Culture of intravascular / Catheter tip
  - Intravascular Catheter-Related Infections

✓ Culture of Blood Bank Products
Infections of the Blood

✓ The suffix "-emia" refers to the circulatory system
✓ Bacteremia
✓ Fungemia
✓ Viremia

✓ Signs and symptoms may be present, but are not invariable

✓ If the patient is not aware of the circulating microbes, the condition is termed silent or subclinical
Infections of the Blood

✓ Septicemia / Sepsis is a clinical syndrome characterized by

✓ Fever (> 36°C) or hypothermia (< 36°C)
✓ Chills
✓ Malaise
✓ Tachycardia
✓ Hyperventilation
✓ Low or raised blood pressure
✓ Toxicity
✓ Failure of multiple organs is an important component of fatal sepsis
Sepsis

✓ Sepsis is a complex clinical syndrome that is defined as the systemic inflammatory response of the body to an infection

✓ It is the most common underlying cause of death in non-coronary ICUs where the mortality rate can be as high as 32% or 54% in case of severe sepsis or septic shock, respectively
Infections of the Blood

- Septicemia results when circulating bacteria multiply at a rate that exceeds their removal by phagocytes.

- The symptoms are produced by microbial toxins and/or cytokines produced by inflammatory cells.

- Septic shock is traditionally associated with gram-negative bacteria, which contain endotoxin.

- Gram-positive bacteria can also cause the sepsis syndrome.
Bacteremia / Transient

- Organisms, often members of the normal flora
- Brushing Teeth
- Chewing food
- Straining during bowel movements
Bacteremia / Transient

- Manipulation of infected Tissues
- Instrumentation of contaminated mucosal surface /mouth & urogenital & GI tract
- Surgery involving nonsterile
It is possible that a transient bacteremia was not efficiently cleared by host defense mechanisms – usually cleared from the blood within 30 to 45 minutes.

The liver and spleen play the primary role in clearing bacteria.

Intravascular neutrophils play only a minor role.

Encapsulated bacteria are more difficult to eliminate.
**Bacteremia / Intermittent**

**Most Infection**

- Intermittent bacteremia occurs when bacteria from an infected site are periodically released into the blood from
  - Extravascular Abscesses & spreading Cellulitis
  - Infections of body cavities, such as Empyema, Peritonitis
  - Meningitis
  - Pneumonia
  - Septic arthritis & Osteomyelitis
Bacteremia / Continuous

Usually infection is intravascular

- Septic Shock

- Bacterial Endocarditis
  - (infected endothelium)

- Endovascular infections
  - infected hardware

- Early Stages of
  - Typhoid
  - Brucellosis
Bloodstream Infection
Bacteria & Fungi

✓ Intravascular; Originate within cardio-vascular system

     Extremely Serious

✓ Endocarditis; The most common intravascular

✓ Suppurative Thrombophlebitis

✓ Catheter–Associated Bacteremia

✓ Extravascular; Bacteria entering blood circulation through the lymphatic system from another site of infection

     Most cases of clinically significant bacteremia
Bacteremia

✓ The source of organisms may not be determined in up to one third of bacteremias

✓ *Staphylococcus aureus* that colonizing bacteria in the nose may be the source of the systemic infection

✓ *Bacteremic Pneumococcal Pneumonia*: the severity of the infection is often increased and the prognosis for the patient worsened
# Mortality Rates and Risk Factors Associated With Bacteremia

<table>
<thead>
<tr>
<th>Age of Patient</th>
<th>MORTALITY %</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>13.8</td>
</tr>
<tr>
<td>21-40</td>
<td>32.8</td>
</tr>
<tr>
<td>41-50</td>
<td>42.9</td>
</tr>
<tr>
<td>&gt;50</td>
<td>49.8</td>
</tr>
</tbody>
</table>
Mortality Rates and Risk Factors Associated With Bacteremia

<table>
<thead>
<tr>
<th>Predisposing Conditions</th>
<th>MORTALITY %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery</td>
<td>16.3</td>
</tr>
<tr>
<td>Trauma</td>
<td>27.3</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>30.0</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>33.3</td>
</tr>
<tr>
<td>Renal failure</td>
<td>37.5</td>
</tr>
<tr>
<td>Neoplasm</td>
<td>42.1</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>71.5</td>
</tr>
</tbody>
</table>
Infections of the Blood

✓ Over the past several decades there has been a clear shift in the nature of the infecting flora

✓ The number of anaerobic isolates has decreased over that time,

✓ Whereas the number of isolates of Yeast and clinically significant coagulase-negative Staphylococci has increased
Mortality Rates and Risk Factors Associated With Bacteremia

<table>
<thead>
<tr>
<th>Type of Organism</th>
<th>MORTALITY %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pneumoniae</td>
<td>22.0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>27.7</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>32.7</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>35.5</td>
</tr>
<tr>
<td>Enterococci</td>
<td>45.5</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>48.0</td>
</tr>
<tr>
<td>Fungi</td>
<td>67.0</td>
</tr>
<tr>
<td>Unimicrobial bacteremia</td>
<td>37.7</td>
</tr>
<tr>
<td>Polymicrobial bacteremia</td>
<td>63.0</td>
</tr>
</tbody>
</table>
Blood culture

- The laboratory perform blood cultures correctly and report accurate results as soon as possible.

- Patients with positive blood cultures were 12 times more likely to die during hospitalization than those with negative blood cultures.
Blood culture

Successful factors

✓ 1- Possible type of bacteremia

✓ 2- Collection methods

✓ 3- Blood volume

✓ 4- Number & Timing of Blood Cultures
Blood culture

- **Adult**: < 1-30 CFU /ml, commonly found in patients with clinically significant bacteremia

- **Children & Infants**: 5 - 10 to 1000 CFU /ml

- **In septicemia**, it has been shown that very few bacteria are present in the blood
Blood culture

**Streptococcal endocarditis:**

- **1 to 30 bacteria/ml in 54% of samples**
- **31 to 100 bacteria/ml in 29% of samples**
- **More than 100 bacteria/ml in 17% of samples**
- **In 54% of cases, a 10 ml blood sample will only contain 10 to 300 bacteria**
Specimen Collection

Blood volume

✓ 10 ml of blood per culture

✓ Increasing total volume cultured from 20 to 40 ml increased the yield by 19%

✓ Increasing the volume from 40 to 60 ml increased the yield by an additional 10%
<10 years : 1ml of blood for each year of life

Neonates to 1 year ( < 4 kg ) : 0.5 to 1.5 ml / tube

Children 1 to 6 years old : 1 ml per year of age, divided between two blood cultures
✓ It is safe to obtain as much as 4% to 4.5% of a patient’s total blood volume for culture
<table>
<thead>
<tr>
<th>Weight of patient (kg)</th>
<th>Total blood volume (ml)</th>
<th>Culture No.1</th>
<th>Culture No.2</th>
<th>Total volume for culture (ml)</th>
<th>% of total blood volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1-12.7</td>
<td>&gt;200</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>12.8-36.3</td>
<td>&gt;800</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>2.5</td>
</tr>
</tbody>
</table>
## Children & Infants

<table>
<thead>
<tr>
<th>Weight of patient (kg)</th>
<th>Patient's total blood volume (ml)</th>
<th>Recommended volume of blood for culture (ml)</th>
<th>Total volume for culture (ml)</th>
<th>% of patient's total blood volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 1</td>
<td>≤ 2.2</td>
<td>50-99</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1.1-2</td>
<td>2.2-4.4</td>
<td>100-200</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2.1-12.7</td>
<td>4.5-27</td>
<td>&gt; 200</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>12.8-36.3</td>
<td>28-80</td>
<td>&gt; 800</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>&gt; 36.3</td>
<td>&gt; 80</td>
<td>&gt; 2,200</td>
<td>20-30</td>
<td>20-30</td>
</tr>
</tbody>
</table>
Number of Blood Cultures

- Acute sepsis & Meningitis, Osteomyelitis, Arthritis, Pneumonia, Pyelonephritis draw 2 blood culture from 2 separate sites

- Fever of unknown origin /FUO & Subacute bacterial Endocarditis and other continuous bacteremia draw 3 blood culture
Timing of Collection

**Volume is more important than timing**

- Drawing blood cultures before (45 minute) or during fever spike
- **Intermittent**: 2-3 blood cultures spaced an hour apart
- For patients expected to seed bacteria intermittently into the blood, 80% of these are detected with the first culture and 99% within three cultures
The incidence of single blood culture

- Between **20%** and **30%** of the single blood cultures they reviewed were not clinically indicated.

- **Education reduced unnecessary collections from 38% to 12.5%**.

- Most of the others were ordered by physicians who were unaware that **one culture was not sufficient**.

- **Education reduced single blood cultures from 40% to 24%**.
Transport

- Do not refrigerate blood culture

- Hold at room temperature until processed, for maximum 4 hrs.
Antisepsis & Transportation

✓ 10% Povidone-Iodine (2 minutes)

✓ 2% Chlorhexidine gluconate in 70% isopropyl alcohol (1 minute)

✓ For pediatric, omit iodine and use 2 pads saturated with 70% isopropyl alcohol
Detection of Bacteremia

Conventional Methods

Manual Systems
Blood Culture /Media

✓ TSB : Trypticase Soy Broth

✓ BHI : Brain-Heart Infusion

✓ BHI may be equivalent or even superior for the recovery of yeasts and some bacteria

✓ Middelbrook 7H9 broth /Mycobacteria
Blood Culture /Media

Diphasic / Castaneda bottles

Diphasic system enables subculture, without risk of contamination, Easy observation of colonies & colonies isolated on the agar can be used directly for ID/AST

✓ Liquid phase: TSB or BHI

✓ Solid phase: TSA or BHI

Best medium for Brucella & Fungi
Blood Culture / Media

Growth factors

✓ Factors X and V, essential for *H. influenzae* & *Cardiobacterium*, *Actinobacillus*

✓ Vitamin **B6** for certain Streptococci
Blood culture

Because of the decline over the past 15 years in the proportion of positive blood culture that yield anaerobic bacteria laboratories discard processing all blood samples aerobically & anaerobically

Anaerobic bottle be limited to situations in which anaerobes might be expected (e.g., in patients with abdominal disease processes)
Blood culture

✓ **Bone Marrow Culture** should be reserved for culture for specific pathogens such as *Brucella*, *Salmonella*, *Listeria*, *Fungi*, and *Mycobacteria*

✓ Blood cultures for *Mycobacteria* and *Fungi* should be considered in appropriate clinical situations, such as when patients who are infected with HIV

✓ Most *Candida spp.* were recovered effectively in systems designed for bacteria
Anticoagulants

✓ If bacteria become entrapped within a clot, their presence may go undetected.

✓ **Heparin**, EDTA, Citrate inhibit numerous organisms

✓ **Sodium citrate (0.5-1%)** is inhibitory to some G+Cocci

✓ **Sodium Polyanethol Sulfonate (SPS)** 0.025% - 0.05% is best anticoagulant
Sodium Polyanethol Sulfonate  SPS

✓ Anticomplementary
  – Precipitates fibrinogen & serum complement
✓ Antiphagocytic : Inactivates neutrophils
✓ Anti aminoglycosides

✓ SPS prevents the killing of bacteria by innate cellular and humoral factors

✓ Higher concentrations of SPS have enhanced the growth of gram positive cocci
Sodium Polyanethol Sulfonate  SPS

✓ May inhibit the growth of a few microorganism such as some strains of Neisseria meningitidis, Moraxella catarrhalis, Gardnerella…

✓ Addition of 1.2 % gelatin has been shown to counteract this inhibitory, Difficult to inspect visually with gelatin
Blood / Broth ratio

✓ Ratio of about 1:15 is required to remove the antibacterial effects of normal human blood, but this may be reduced to between 1:3 and 1:10 by the addition of SPS.

✓ A 1:5 ratio of blood to medium has been found to be adequate in conventional blood culture.
Conventional blood culture

Atmosphere
Venting blood culture bottles

- To encourage the growth of obligate (strict) aerobe, such as Yeast & Pseudomonas aeruginosa transient venting may be necessary.

- Cleans the septum with 70% alcohol (Air dry 30 – 60 s)

- In BSC insert venting device through septum to release vacuum

- Carefully remove venting device
Repositionable cap

Extractable stopper

Large, transparent agar surface: even small colonies are easily visible

CO₂ atmosphere in direct contact with broth and agar

Agar + broth supplemented with growth factors (NAD, hemin, Vit. B6, purines and pyrimidines...)

Volume of blood: up to 10 ml

40 ml of broth + SPS anticoagulant (0.025%)

Extractable stopper

Partial vacuum (-700 mbar) and CO₂ enriched atmosphere

Volume of blood: up to 10 ml

100 ml of modified Wilkins Chalgren broth + SPS anticoagulant (0.025%)

Clear broth for easy reading

Pre-reduced medium (cysteine) Redox potential: -200 mV
Incubation

✓ At 35–37°C for minimum 7 days

✓ Constant agitation of the bottles during the first 24 hrs of incubation also enhances the growth of most aerobic bacteria

✓ After 6 to 18 (24) hrs blind subculture 0.25ml onto a Chocolate blood agar plate & should be held for 5 days in 5-10% CO2 in a humidified atmosphere
Incubation

✓ **After 48 – 72 hrs of incubation of blood cultures, a second blind subculture may be performed.**

✓ **Subcultures at 7 days**
In a study of 20,155 blood culture bottles only 42 bottles (0.3%) turned positive after 7 days of incubation.

Holding manual blood cultures beyond 7 days is unnecessary?!?!
Incubation

- Hold blood culture bottles at least for **2 weeks** if bacterial endocarditis, suspected & **patient on Antimicrobial therapy**

- **Fungemia > 2 weeks**
  - Venting blood culture
  - Incubation at 35 & 25

- Incubation for up to **28 days (4-6 weeks)** if the diagnosis of **brucellosis** is suspected
Incubation

✓ For *biphasic blood culture*, gently invert bottle, allowing liquid to come into contact with the agar slide surface for a few time

✓ Perform this step *twice daily for first 2 days & then 24-48 for remaining days*, *(48-72 for brucella)*
Gram stain

- During the **first 24 hrs** of incubation
- Gram stain (methanol fixation)
- Gram stain positive: Bacteria about $10^5$ CFU/ml
Gram stain

✓ The routine microscopic examination of macroscopically negative blood culture bottles after 24 hours of incubation is probably not indicated for routine detecting growth.

✓ Because the number of organisms that can be detected by Gram's stain (about $10^5$ CFU) is not appreciably less than the $10^6$ to $10^7$ CFUs required to produce visible turbidity of the broth.
Detecting Growth

- Visually inspect each bottle daily for visible signs of growth,

- **Turbidity**: Aerobic gram negative bacilli, Staphylococci

- **Hemolysis**: Streptococci, Staphylococci, Listeria, Bacillus

- **Gas production**: Aerobic gram negative bacilli (e.g.: E.coli)

- **Pellicle formation**: Pseudomonas spp., Yeast, Bacillus

- **Clotting**: Staphylococci aureus

- **Puffballs**: Staphylococci, Streptococci
Detecting Growth

✓ Some organisms such as, Brucella spp, Francisella, Haemophilus & N.meningitidis and may be present in blood culture media without showing visible signs of growth.
Brucella
Comments

✓ All isolates from blood cultures should be stored for an indefinite period, preferably for at least 1 year.

✓ Freezing at –70°C in skim milk

✓ Storing an agar slant of the isolate under sterile mineral oil at room temperature is a good alternative to freezing.
Detection of Bacteremia

Instrumented Systems
Automated Blood Culture
Automated Blood Culture

✓ **1st generation** 14*CO2 –labeled substrate (palmitic acid) metabolized by mycobacteria.

✓ **New generation** Continuous-Monitoring blood culture...CO2
Automated Blood Culture

✓ Continuous-Monitoring Blood Culture Systems CMBCSs

✓ Culture vials are monitored individually at intervals 10 to 15 minute for evidence of microbial growth
Automated Blood Culture

✓ The headspace *Atmosphere* of aerobic bottles usually contains air with various concentrations of CO2

✓ Depending on the system, the headspace of *anaerobic* bottles usually contains combinations of CO2 and nitrogen
Automated Blood Culture

✓ Automated Blood Culture alerts the microbiologist that a culture is positive, after which the relevant bottles can be removed for Gram's stain and subculture.

✓ The media selected for subculture can be chosen based on the gram reaction and morphology of the microbes.
Automated Blood Culture

✓ If organisms are not visualized, a blind subculture should be performed and the bottle returned to the instrument for continued incubation

✓ Multiple studies have demonstrated that bottles need be incubated only for 5 days
Automated BC / Comparative Studies

✓ The advantages of continuous-monitoring blood culture systems include a decrease in laboratory workload
  – Processing only the positive cultures

✓ Decrease in the number of false-positive results and pseudobacteremia (because of decreased handling and sampling of the bottles)

✓ Significant increase in the speed of detection and in the rate of microbial recovery
Bactec 9050
BacT/Alert 3D/ bioMérieux
As the first continuous-monitoring blood culture system
BacT/ALERT 3D/ bioMérieux

In the presence of CO2, the color of the sensor turns from green to yellow
BacT/ALERT 3D

✓ Recovery of a wide range of organisms
✓ >95% recovery within 24 hours
✓ >98% within 72 hours

✓***Fewer False Positive***
**BacT/ALERT® Blood Culture Collection Procedure**

**1. Skin Preparation**
- **Preparation Solution:**
  - After locating the vein, vigorously scrub the venipuncture site with PDI® Chlorhexidine Swabsticks for 30 seconds.
  - Allow the site to air dry before venipuncture.
  - Do not palpate the vein.

**2. Bottle Preparation**
- Inspect each blood culture bottle before use to ensure integrity of bottle and sensor on bottom of bottle is intact. The sensor is normally a uniform grayish-green color and a yellow color would indicate contamination of the broth. Discard any bottle found to be damaged or with a sensor that is yellow.
- Remove protective flip top overcap.
- **NOTE:** The septum is not sterile and must be disinfected.
- Disinfect the septum with 70% alcohol.
- **NOTE:** The bottle has been premixed with 5mL increments. Mark the desired fill volume level on the bottle for 10mL.

**3. Venipuncture and Bottle Inoculation**
- Collect all necessary materials for a direct draw.

**Tips 'n' Hints**
- Recommended blood to broth ratio is 1:5 to 1:10. As the volume of blood drawn is increased, the yield of positive cultures increases. Optimal ratio of blood should be drawn from adults (10mL per bottle).
- When labeling the bottles, do not cover the peel-off section of the barcode labels or the lot numbers.
- Orient additional patient label vertically for scanning efficiency.
- For best volume control, mark fill level on side of bottle prior to collection.
- Do not overtight the bottle, as this may cause false positive readings.
- When using the Tet/Holder Blood Culture Device (Adapter Cap and Insert), be sure lead is connected firmly and the needle is straight when attaching and leaving the septum. Twisting or turning the bottle may increase the chance that the smear may not retract and seal.
- To avoid contamination of the blood culture, inoculate blood culture bottles first. Then fill additional blood collection tubes.

**Availability**
- **BacT/ALERT Blood Culture Collection Kit**
  - 21402, 21404
- **Tet/Holder Blood Culture Device (Adapter Cap and Insert)**
  - 21403, 21405

*BacT/ALERT* is a registered trademark. *Biomerieux* is a registered trademark.
3. Venipuncture and Bottle Inoculation

**Direct Draw with Blood Collection Set or WorkSafe™ Blood Culture Collection Kit**

- Collect all necessary materials for a direct draw:

1a. Gather Tourniquet, Bact/Alert® bottles, SaT Holder® Adapter, wing, Chlorascrub™ Swabstick, gauze, alcohol prep pads.

1b. Open the WorkSafe Blood Culture Collection Kit which contains all the necessary materials in a single, convenient package.

2. Perform venipuncture. When the needle is in the vein, secure it with tape or hold it in place.

3. Place Adapter cap on the aerobic (blue or green label) Bact/Alert® culture bottle septum and press down to penetrate and obtain blood flow. Hold the Adapter Cap down on the bottle.

4. Using the fill indicator lines on the label, obtain 8-10ml of blood. Move the Adapter Cap from the aerobic bottle to the anaerobic (purple or orange label) bottle (if required) and continue the collection.

5. If additional blood is required for other tests, place the Adapter Insert into the Adapter Cap and snap into place. This makes the cap compatible with vacuum collection tubes.

6. When final tube is filled, remove tourniquet. Terminate the venipuncture, activate the safety shield, grab the wing with one hand and slide the wings back until it locks in place.

7. Dispose of wing and adapter cap in a sharps container.

8. Label specimens and place in plastic biohazard zip lock bag. Bottles can now be safely transported via pneumatic tube system.

**Availability**

- WorkSafe Blood Culture Collection Kit
- SaT Holder® Blood Culture Device (Adapter Cap and Insert)

- 279102-279105*
- 100/case
- 96004**

*See bioMerieux catalogue
**See Smiths Medical catalogue
EPS System / Trek Diagnostic

ESP® 80A AEROBIC BROTH
For use in the ESP Culture System II
For In Vitro Diagnostic Use
Store at 15-30°C

ESP® 80N ANAEROBIC BROTH
For use in the ESP Culture System II
For In Vitro Diagnostic Use
Store at 15-30°C
Automated Blood Culture

✓ Pediatric Ratio: 1:5 to 1:10

✓ Supplemented: X & V for H. influenzae

✓ Reduced SPS for improved detection Neisseria spp.....

✓ Addition of 1.2% gelatin has been shown to counteract this inhibitory
Automated Blood Culture

✓ A Resin containing medium that inactivated most antibiotic non-selectively by adsorbing them to the surface of the Resin particles.

✓ A Resin containing media may enhance isolation of *Staphylococci*, particularly when patients are receiving bacteriostatic drugs.

✓ The use of resin media significantly improved the recovery of members of the family *Enterobacteriaceae*, *Enterococci*, *Streptococcus pneumoniae*, and *viridans streptococci*
Automated Blood Culture

- **Ratio of Blood –to- Broth**: 1 : 3

- **Charcoal containing medium for inactivated antibiotic**

- **Improved abilities to detect Staphylococci and Yeast**

- **Incubation time**: 5 days (3 days)
1. **BACTEC PLUS Aerobic/F Culture Vial**

Optimum blood volume for each vial is 8 to 10 mL; 3 to 10 mL of blood is acceptable.

a. Each vial contains:

- 25 mL Enriched Soybean-Casein Digest broth (TSB)
- 0.05% Sodium Polyanetholesulfonate (SPS)
- Cationic and Non-ionic Adsorbing Resins
- Carbon dioxide (CO₂)
- Oxygen (O₂)
- Sensor for the detection of fluorescence

b. Store at 2° to 25° C
4. **BACTEC MYCO/F LYTIC**

A non-selective culture medium for the recovery of yeast and fungi from blood and body fluids and mycobacteria from blood specimens. The optimum specimen volume is 3 to 5 mL; 1 mL to 5 mL is acceptable.

a. Each vial contains:

- 40 mL Processed Water
- Middlebrook 7H9 Broth Base
- Brain Heart Infusion
- Casein Hydrolysate
- Supplement H
- Inositol
- Glycerol
- SPS
- Tween 80
- Pyridoxal HCl
- Ferric Ammonium Citrate
- Potassium Phosphate
- Saponin
- Antifoam
- CO₂ and O₂
- Sensor for the detection of fluorescence.
Isolator

Wampole Laboratory
Benefits of Lysis Centrifugation

- Greater recovery of Intracellular bacteria (e.g.: brucella), yeast, filamentous fungi
- Colony for direct ID & AST
- Quantify CFU present in Blood
- Rapid detection of polymicrobial bacteremia
Lysis Centrifugation Isolator

- Tube containing Saponin to lysis blood cells, Polypropylene glycol to decrease foaming, SPS as anticoagulant, EDTA to chelate calcium ions & inhibit complement, ...

- 30 minute centrifugation at 3000 g

- Entire sediment plated to selected media in BSC.
Lysis Centrifugation Isolator

Inject blood into Isolator tube.

Gently invert the tube 4-5 times to mix the blood with the tube contents.

Inoculate plates with blood lysate in a line down the centre of each plate.
Significance of Positive Cultures
Significance of Positive Cultures

Polymicrobial Bacteremia / fungemia

✓ 3% of all positive blood cultures

✓ Higher Mortality

✓ Predisposing factors: self-injection, Burns, Gastrointestinal sources
Significance of Positive Cultures

✓ In most general hospital 8 - 14 % ( 6 - 12 % ) of blood culture will be Positive

✓ Half of all positive blood cultures represent contamination

✓ The value of obtaining more than a single blood culture is that it also assists in interpreting the clinical significance of positive results
Significance of Positive Cultures

If an institution has a baseline blood culture contamination rate of 3%, the probability of recovering the same organism in two culture sets from a patient, and of that organism being a contaminant, is less than 1 in 1,000 (0.03 x 0.03 = 0.0009)
Significance of Positive Cultures

☑ Laboratories that recover contaminants at rates greater than 3% should suspect improper phlebotomy technique
Significance of Positive Cultures

✓ **CoNS, diphtheroids, Micrococcus spp., Bacillus spp., and viridans group** are considered contaminants if certain criteria are met.

✓ **If two or more blood cultures are obtained and only one is positive, the isolate is reported as a probable contaminant** and susceptibility testing is not done unless the physician calls the laboratory.
Reporting

✓ **Verbal report**: A report of gram positive cocci in chains is more helpful than a report of G + cocci alone.

✓ **Written report**: Positive at 4 hrs or 24 hrs or.....

✓ No growth after 7 Days

✓ No growth in 1 week
Interpretation

✓ Determining the Clinical Significance of an Isolate is Physician’s Responsibility
Procalcitonin (PCT) /Biomarkers for sepsis

✓ PCT is the prohormone of the hormone calcitonin

✓ PCT can be produced by several cell types and many organs in response to pro-inflammatory stimuli, in particular by bacterial products

✓ Early diagnosis and appropriate therapy of sepsis
## PCT reference ranges and interpretation

<table>
<thead>
<tr>
<th>Normal values: &lt; 0.05 ng/mL</th>
<th>Healthy individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PCT &lt; 0.5 ng/mL</strong></td>
<td>Minor or no significant systemic inflammatory response</td>
</tr>
<tr>
<td><strong>PCT ≥ 0.5 and &lt; 2.0 ng/mL</strong></td>
<td>Significant, but moderate systemic inflammatory response</td>
</tr>
<tr>
<td><strong>PCT ≥ 2 and &lt; 10 ng/mL</strong></td>
<td>Severe systemic inflammatory response, most likely due to in sepsis</td>
</tr>
<tr>
<td><strong>PCT ≥ 10 ng/mL</strong></td>
<td>Important almost exclusively due to severe bacterial sepsis or septic shock</td>
</tr>
</tbody>
</table>
Bacteria Commonly Isolated from Blood Cultures

- Coagulase-negative staphylococci
- Staphylococcus aureus
- Viridans streptococci
- Enterococcus spp.
- Beta-hemolytic streptococci
- Streptococcus pneumoniae
- Escherichia spp.
- Klebsiella spp.
- Pseudomonas spp.
- Enterobacter spp.
- Proteus spp.
- Anaerobic bacteria—Bacteroides and Clostridium spp.
Coagulase Negative Staphylococci

☑ Species of coagulase Negative Staphylococci other than *S. epidermidis*, *S. capitis*, and *S. haemolyticus* were almost always clinically insignificant,

☑ These three species accounted for 98% of the significant isolates and 89% of the insignificant isolates
For reasons that are unclear, bacteremias with nonfermenting gram-negative bacilli are more often polyclonal (more than one molecular type) than are bacteremias with other gram-negative bacilli.
Endocarditis
Endocarditis

✓ The most common intravascular

✓ Acute and subacute

✓ Most cases of endocarditis involve the left side of the heart, which is the high-pressure side of the system

✓ Heart valves especially those that previously damaged, present convenient surfaces for attachment of bacteria
Endocarditis

✓ Gram-positive bacteria are the most common etiologic agents
  – Viridans streptococci
  – Staphylococcus aureus
  – Enterococci
Agents of Infective Endocarditis

Viridans streptococci*
Nutritionally deficient streptococci
Enterococci*
*Streptococcus bovis
*Staphylococcus aureus*
Staphylococci (coagulase-negative)
*Enterobacteriaceae
Pseudomonas spp. (usually in drug abusers)
*Haemophilus spp., particularly H. aphrophilus
Unusual gram-negative bacilli (e.g., Actinobacillus, Cardiobacterium, Eikenella)
Yeast
Other (including polymicrobial infectious endocarditis)

*Most common organisms associated with native valve endocarditis in nondrug-abusing adults.
Endocarditis

✓ The Gram-negative bacteria of the HACEK group

✓ Haemophilus aphrophilus

✓ Actinobacillus actinomycetemcomitans

✓ Eikenella corrodens

✓ Kingella kingae
Endocarditis

Culture-negative Endocarditis

✓ Chlamydia pneumoniae

✓ Coxiella burnetii (Q fever)

✓ Bartonella spp

✓ Legionella spp.
Prosthetic-valve Endocarditis

✓ 3-6% of patients

✓ Early stage <60 days after surgery
  – Skin and Wound microbes predominate

✓ Late stage >60 days after surgery
  – Organisms that infect native valves
Endocarditis

- Fastidious microbes grow slowly

- Prolong incubation beyond the routine cutoff period

- Blind staining of bottles after incubation for 7 days and/or the total period of incubation
Endocarditis

- *Streptococcus sanguis* group
- *Streptococcus mutans* group

Are most frequently isolated in *Streptococcal* endocarditis
# Commonly Isolated Groups of Viridans Streptococci

<table>
<thead>
<tr>
<th></th>
<th>Mannitol</th>
<th>Sorbitol</th>
<th>Voges-Proskauer</th>
<th>Hydrolysis of Arginine</th>
<th>Hydrolysis of Esculin</th>
<th>Urease</th>
<th>Hemolytic Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anginosus group</td>
<td>−/v</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>α, β, Non</td>
</tr>
<tr>
<td>Mitis group</td>
<td>−</td>
<td>−/v</td>
<td>−</td>
<td>+/-/v</td>
<td>+/-/v</td>
<td>−</td>
<td>α</td>
</tr>
<tr>
<td>Mutans group</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>α, β, Non</td>
</tr>
<tr>
<td>Salivarius group</td>
<td>−</td>
<td>−</td>
<td>+/v</td>
<td>−</td>
<td>+/v</td>
<td>+/v</td>
<td>α</td>
</tr>
</tbody>
</table>

+ Positive test result; −, negative test result; v, variable test result; Non, nonhemolytic.
Streptococcus bovis

Group D antigen
✓ BEA + & NaCl 6.5% - & Van :s &LAP:+
✓ &PYR : - & Hemolysis : non-beta & 45 C+

✓ Streptococcus bovis is commonly associated with endocarditis

✓ Blood isolates associated with Gastrointestinal malignancy & may be an early indicator of Gastrointestinal cancer
Clostridium

- Clostridium septicum is frequently associated with neoplastic disease, particularly carcinoma of the colon

- Clostridium perfringens bacteremia result in sudden, dramatic hemolysis, which can be rapidly fatal
Vitamin B6-Dependent Streptococci

✓ Nutritionally deficient /variant /satelliting

✓ Abiotrophia & Granulicatella

✓ Subculturing onto 5% sheep blood agar plate & either overlaying a streak of staphylococcus aureus or dropping a pyridoxal disk

✓ Streptococci growing as tiny satellites next to the streak.
Brucella

✓ The spectrum of human brucellosis, a zoonosis, ranges from

✓ Subclinical infection to Acute (less than 2 months)

✓ Subacute (2 to 12 months)

✓ Chronic illness, often manifested by recurrent symptoms over many years
Brucella

✓ Evaluation of Conventional Castaneda and Lysis Centrifugation Blood Culture Techniques for Diagnosis of Human Brucellosis

✓ The lysis centrifugation technique has been found to be more sensitive in both acute (20% higher sensitivity) and chronic (40% higher sensitivity) forms of brucellosis
Brucella

✓ Bacteremia during first 3 weeks

✓ Blood culture positive 70%-90%

✓ Best media: Brucella broth & TSB

✓ The Bottles Should be continuously vented & incubated in 10% CO2 at 37C at least 4 weeks (vented or screw cap loosened)
Brucella

- Blind Subcultures at 4 days and weekly onto Choc. or BHI agar or brucella agar

- Incubated in 5-10% CO2 at 37C in a humidified atmosphere

- All subculture plate should be held for a minimum 5 days

- BACTEC 9000 the diagnosis of more than 95% in 7 Days
Brucella

✓ Safety : BSC

✓ Colonies after 48 hrs. : small ,raised , white to cream & glistening & brownish with age

✓ Very tiny & pale gram negative coccobacilli / sand -like

✓ Urea agar : positive / Rapid

✓ Most strain : oxidase positive
Brucella / safranin for 3 minutes
<table>
<thead>
<tr>
<th>Characteristic or test</th>
<th>Brucella</th>
<th>EO-2, EO-4 Psychrobacter immobilis</th>
<th>Psychrobacter phenylpyruvicus</th>
<th>Oligella ureolytica</th>
<th>Actinobacillus spp.</th>
<th>Bordetella bronchiseptica, Ralstonia paucula (IV c2)</th>
<th>Bordetella hinzii</th>
<th>Haemophilus spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain morphology</td>
<td>Tiny CCB, stains faintly</td>
<td>Small CCB, rods, EO-2 in packets</td>
<td>CCB</td>
<td>Tiny CCB</td>
<td>CCB, rods</td>
<td>CCB, rods</td>
<td>CCB</td>
<td>CCB</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>V</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Urea</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>14% Positive</td>
<td>+</td>
<td>V</td>
</tr>
<tr>
<td>Motility</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+, delayed</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>PDA</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>V</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Nitrate</td>
<td>+</td>
<td>V</td>
<td>68%</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>−</td>
<td>NA</td>
</tr>
<tr>
<td>Nitrite</td>
<td>−</td>
<td>V</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>NA</td>
</tr>
<tr>
<td>TSI</td>
<td>Alkaline</td>
<td>Alkaline</td>
<td>Alkaline</td>
<td>Alkaline</td>
<td>Acid/acid</td>
<td>Alkaline</td>
<td>Alkaline</td>
<td>No growth</td>
</tr>
<tr>
<td>MAC, 48 h</td>
<td>−, poor</td>
<td>−, poor</td>
<td>−, poor</td>
<td>−, poor</td>
<td>−, poor</td>
<td>−, poor</td>
<td>−, poor</td>
<td>−, poor</td>
</tr>
</tbody>
</table>

* Reactions extracted from references 7 and 9. NA, not applicable; V, variable; CCB, coccobacilli.

* O. ureolytica is primarily a uropathogen.

* A. actinomycetemcomitans is urea negative and rarely oxidase positive. Urea-positive Actinobacillus organisms are from animal sources.

* Grows only on CHOC, or on blood agar associated with staphylococcus colony.

* Use rapid urea test to increase sensitivity.

* TSI, triple sugar iron agar.
Phenotypic identification of Brucella species and biovars associated with human infections

<table>
<thead>
<tr>
<th>Species</th>
<th>Biovar</th>
<th>CO₂ required</th>
<th>H₂S production</th>
<th>Urease</th>
<th>Growth on dye media</th>
<th>Agglutination in monospecific serum</th>
<th>Acriflavin</th>
<th>Lysis by phage Tb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;5 min</td>
<td>&gt;5 min</td>
<td>Thionin 1:25 × 10³</td>
<td>1:50 × 10³</td>
<td>1:100 × 10³</td>
</tr>
<tr>
<td>B. melitensis</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>v</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>v</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>4</td>
<td>v</td>
<td>+</td>
<td>–</td>
<td>v</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>5</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>–</td>
<td>–</td>
<td>v</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>v</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. abortus</td>
<td>1</td>
<td>v</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>v</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>v</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>v</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. suis</td>
<td>1</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>h</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>h</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>h</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
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<td>4</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>h</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>h</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B. canis</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>h</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

- Reactions based on those obtained in the SBRL, CDC; those given in Laboratory Techniques in Brucellosis, 2nd ed., World Health Organization, Geneva, Switzerland, 1975.
- Heart infusion agar with lead acetate paper.
- Warm urea slant inoculated heavily.
- Agglutination and phage procedures given in Laboratory Techniques in Brucellosis, 2nd ed.
- Thilisi, a Brucella phage originally isolated in the former USSR, has been designated as the reference phage.
- Rare strains are urease negative.
- Formerly B. abortus biovar 9; biovars 7 and 8 were deleted by the International Committee on Bacterial Taxonomy, Subcommittee on Taxonomy of Brucella (reference 6 in the Brucella abortus description).
- Usually immediate or instant reaction.
- Some strains studied in SBRL, CDC, grew at this concentration.
- B. canis forms a stringy mass or "gel" of increased viscosity when suspended in phenolized saline and agglutinates in specific antiserum.
Nosocomial Bloodstream Infection

Catheter-related bloodstream infection
Nosocomial Bloodstream Infection

Intravascular Device–Related Bloodstream Infection

✓ Central venous catheters are the most frequent cause of nosocomial bloodstream infection

✓ Estimated 250,000 to 500,000 episodes of IVD-related bloodstream infection occur in the U.S annually

✓ These episodes are associated with an attributable mortality rate of 12% to 25%
<table>
<thead>
<tr>
<th>Microbiology of Device-Associated Bacteremia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative staphylococci including <em>Staphylococcus epidermidis</em> *</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> *</td>
</tr>
<tr>
<td><em>Enterococcus spp.</em> *</td>
</tr>
<tr>
<td><em>Serratia marcescens</em> †</td>
</tr>
<tr>
<td><em>Candida albicans</em> ‡</td>
</tr>
<tr>
<td><em>Candida tropicalis</em> ‡</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> §</td>
</tr>
<tr>
<td><em>Klebsiella spp.</em> †</td>
</tr>
<tr>
<td><em>Enterobacter spp.</em> †</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em> †</td>
</tr>
<tr>
<td><em>Corynebacterium</em> (especially <em>C. jeikeium</em>)</td>
</tr>
<tr>
<td><em>Burkholderia cepacia</em> complex §</td>
</tr>
</tbody>
</table>

*Most common pathogen for long-term lines; also associated with lipid infusions in neonates.
†Frequently associated with contaminated infusate.
‡Most often associated with total parenteral nutrition; usually along the catheter path, but occasionally as a result of contaminated infusate.
§May arise from a water source (e.g., infusate) or may reflect cutaneous colonization.
||*C. jeikeium* bacteremia occurs almost exclusively in severely immunosuppressed patients who are or have been receiving broad-spectrum antibiotics and who have indwelling intravascular devices.
TABLE 3. Most common pathogens isolated from hospital acquired bloodstream infections

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>27</td>
<td>37</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Gram-negative rods</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Candida spp.</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Microorganism</td>
<td>No. (%) of isolates</td>
<td></td>
</tr>
<tr>
<td>----------------------------------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>11 (37.9)</td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>7 (24.1)</td>
<td></td>
</tr>
<tr>
<td>Yeast</td>
<td>6 (20.7)</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>3 (10.3)</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>2 (6.9)</td>
<td></td>
</tr>
</tbody>
</table>

---

*Includes 9 Staphylococcus epidermidis isolates (7 were methicillin-resistant strains) and 2 Staphylococcus species isolates (all were methicillin-resistant strains).

*Includes 5 Serratia marcescens isolates, 1 Enterobacter aerogenes isolate, and 1 Enterobacter cloacae isolate. There were no multidrug-resistant strains.

*Includes 3 Candida parapsilosis isolates, 2 Candida albicans isolates, and 1 Saccharomyces cerevisiae isolate (all yeasts were susceptible to amphotericin B, fluconazole, itraconazole, and voriconazole, except for the S. cerevisiae isolate, which was resistant to itraconazole).

*All isolates were methicillin resistant.

*Both isolates were ampicillin resistant.
Coagulase-negative staphylococci

- Coagulase-negative staphylococci are the **most frequent causes of catheter-related infections**

- They can produce extracellular slime that facilitates adherence and may limit the access of antibiotics, and may reduce the host's inflammatory response

- There may be **difficulty in interpretation of the significance** of these isolates as coagulase-negative staphylococci are commonly isolated from contaminated blood cultures
Scanning electron micrograph of a Staphylococcus biofilm
Yeasts

✓ 8% of all Nosocomial bloodstream infections

✓ Candida albicans and nonalbicans

✓ Malassezia furfur (in patients receiving intralipid infusions)
Catheter-related bloodstream infection

✓ **Peripheral venous catheter**: Usually inserted into the veins of the forearm or the hand; most commonly used short-term intravascular device; rarely associated with bloodstream infection

✓ **Nontunneled CVC (Central venous catheters)**: Most commonly used CVC; accounts for an estimated 90% of all catheter-related bloodstream infections; increased risk of infection with internal jugular vein site of insertion
Triple-Lumen Catheter
CVC
Skin organisms
Endogenous
Skin flora
Extrinsic
HCW hands
Contaminated disinfectant

Contaminated catheter hub
Endogenous
Skin flora
Extrinsic
HCW hands

Contaminated infusate
Extrinsic
Fluid
Medication
Intrinsic
Manufacturer

Fibrin sheath, thrombus

Hematogenous
from distant infection
Contamination during insertion of administration set spike or during container change

In-line filter may trap bacteria but shed endotoxin

Contamination may reach system through defects in containers

Contamination during manufacture

Contamination due to malfunctioning air inlet filter

Contamination may also enter the system through
1. Pressure measuring devices, transducers
2. Heparinized flush solutions
3. Stopcocks
4. I.V. piggyback
5. Y. junctions
6. Administration of blood products or medications
7. CVP manometers

Contamination may reach circulation at the catheter insertion site

Contamination may enter system at catheter/administration set junction
Collection from IV catheter

✓ Using 2 separate 70% alcohol preps, scrub catheter hub connection for 15 s. Air dry (30-60 s)

✓ Collect and discard blood; 3 ml for adult & 0.2 ml for pediatric

✓ Using new syringe, collect blood for culture
The catheter-tip sample was taken after scrubbing the skin surrounding the insertion site with 2% chlorhexidine and cutting off the tip (distal 5-cm segment) using sterile scissors.
The most widely used laboratory technique for the clinical diagnosis of catheter-related infection is the semiquantitative method, in which the catheter segment is rolled across the surface of an agar plate, *roll plate technique*.

*Semiquantitative (>15 cfu per catheter segment)*
Short-term catheters, including arterial catheters/ IDSA , 2009 / NEW

✓ For short-term catheter tip cultures, the roll plate technique is recommended for routine clinical microbiological analysis

✓ For suspected pulmonary artery catheter infection, culture the introducer tip
Semiquantitative method

✓ The terminal 4cm segment of the catheter tip is rolled over the entire surface of the blood agar plate (and optionally, either MAC or EMB), 5 times and incubate for 72-96 hrs at 35 C in candle jar then the number of colonies counted.

✓ Do not accept catheter tip in saline or transport medium.
Semiquantitative method

Roll Plate

1. Skin surface-catheter interface
2. Sterile scissors
   - Approximately 5 cm (2 in)
3. Dry, sterile, screw-capped tube or sterile urine cup
4. Sterile forceps
5. Blood agar plate
   - Roll 4 or 5 times across surface of agar

Exogenous catheter tip
Semiquantitative method

Identify to at least the genus level any of the following

✓ Each organism with count > 15 CFU

✓ For > 15 CFU of gram-positive rods perform only Gram stain and catalase & check hemolysis

✓ For < 15 CFU, identify only significant pathogens (e.g.: Candida albicans, Group A Streptococci, and Gram-negative rods)

✓ Do not perform AST on isolates unless the blood culture is positive and comparative results are desirable
Anti-infective catheter tip

IDSA, 2009 / NEW

✓ For cultures of an anti-infective catheter tip, use specific inhibitors in the culture media

✓ Chlorhexidine–Silver Sulfadiazine-Impregnated Central Venous Catheters

✓ The neutralizing medium consisted of sodium oleate, sodium thiosulfate, Tween 80, lecithin
Catheter-related bloodstream infection laboratory techniques

✓ Quantitative culture of the catheter segment requires either flushing the segment with broth, or vortexing, or sonicating it in broth, followed by serial dilutions and surface plating on blood agar.

✓ Quantitative / vortex or sonication methods (\(>10^2\) cfu per catheter segment)

✓ Sensitivities of the 3 methods are as follows: sonication, 80%; roll plate method, 60%; and flush culture, 40%–50%.
Catheter-related bloodstream infection laboratory techniques

- **Quantitative cultures of peripheral and CVC blood samples**

- This technique relies on quantitative culture of paired blood samples, one of which is obtained through the central catheter hub and the other from a peripheral venipuncture site - **Isolator**

- Simultaneous quantitative cultures of blood samples with a ratio of >5:1 (CVC vs. peripheral)

>3:1 (CVC vs. peripheral); **Significant IDSA**, 2009 / NEW
Catheter-related bloodstream infection laboratory techniques

- Differential time to positivity (positive result of culture from a CVC is obtained at least 2 h earlier than is a positive result of culture from peripheral blood) - Significant Automated Blood Culture

- Differential time to positivity for CVC versus peripheral blood cultures sensitivity was 91% and specificity was 94%
Table 3. Comparison of the validity values (95% CI) of 3 techniques for the detection of catheter-related bloodstream infection.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Semiquantitative superficial cultures&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>Differential quantitative blood cultures&lt;sup&gt;a,c&lt;/sup&gt;</th>
<th>Differential time to positivity&lt;sup&gt;b,c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>78.6 (59.0–91.7)</td>
<td>71.4 (51.3–86.8)</td>
<td>96.4 (81.7–99.9)</td>
</tr>
<tr>
<td>Specificity</td>
<td>92.0 (87.0–95.6)</td>
<td>97.7 (94.3–99.4)</td>
<td>90.3 (85.0–94.3)</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>61.1 (43.5–76.9)</td>
<td>83.3 (62.6–95.3)</td>
<td>61.4 (45.5–75.6)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>96.4 (92.4–98.7)</td>
<td>95.6 (91.4–98.1)</td>
<td>99.4 (96.6–99.9)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>90.2 (85.3–93.9)</td>
<td>94.1 (90.0–96.9)</td>
<td>91.2 (86.4–94.7)</td>
</tr>
</tbody>
</table>

**NOTE.**  
<sup>a</sup> P values for the comparison of validity values between semiquantitative superficial cultures and differential quantitative blood cultures were: sensitivity, .75; specificity, .01; positive predictive value, .09; negative predictive value, .79; and accuracy, .15.  
<sup>b</sup> P values for the comparison of validity values between semiquantitative superficial cultures and differential time to positivity were: sensitivity, .13; specificity, .61; positive predictive value, .99; negative predictive value, .12; and accuracy, .83.  
<sup>c</sup> P values for the comparison of validity values between differential quantitative blood cultures and differential time to positivity were: sensitivity, .04; specificity, <.001; positive predictive value, .10; negative predictive value, .04; and accuracy, .29.
Catheter-related bloodstream infection (CR-BSI) 2007

✓ Only 15%–25% of the central venous catheter tips that reach the microbiology laboratory turn out to be culture-positive, thereby confirming CR-BSI.

✓ Furthermore, not all CR-BSIs require the catheter to be withdrawn; therefore, diagnosis of CR-BSI by conservative methods (without catheter withdrawal) is highly convenient.
Infusate-related bloodstream infection

IRBSI
Infusate-related bloodstream infection

- Infusate-related bloodstream infection is uncommon and is defined as the isolation of the same organism from both infusate and separate percutaneous blood cultures, with no other source of infection.

- The sudden onset of symptoms of bloodstream infection soon after the initiation of an infusion resulting from the administration of contaminated iv fluid, is often diagnostic.
Infusate – Associated Bacteremia

Gram Negative Bacilli

✓ **Pseudomonas fluorescens** Bloodstream Infections Associated with a Heparin/Saline IV Flush *CDC, 2005*

✓ **P. fluorescens** is a member of the fluorescent pseudomonad, Optimal temperature range for growing the organism is 25°C–30°C

✓ **Serratia marcescens** blood stream infections associated with contaminated magnesium sulfate solutions
Culture of Blood Bank Products

CBBP
Culture of Blood Bank Products

✓ Bacterial Contamination of blood products is rare

✓ Predominance of **Gram-Negative bacilli**, ability to proliferate at 0 to 6 C and produce endotoxin

✓ Gram-positive bacteria (except Listeria) grow poorly in cold

✓ **Platelets stored at RT (22 C)** are now the **most common cause of transfusion-related** sepsis caused by **Staphylococci**, **Pseudomonas**, **Enterobacter cloacae**
Culture of Blood Bank Products

✓ Remove 20 ml of blood or blood product

✓ Inoculate 3-5 ml into 4 blood culture bottles aerobic & anaerobic

✓ Incubate at 35 C & RT for 7 days

✓ Continue as like as Blood culture procedure
Culture of Blood Bank Products

✓ Patient’s blood should be cultured simultaneously

✓ Positive culture of blood bank product and patient’s blood samples yield the same organism is significant but does not establish the source of contamination (blood collection, blood bank, water bath in which frozen blood product are thawed, ward, etc).