Blood Culture

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Culture:

A microbiological culture is a method of multiplying microbial organisms by letting them reproduce in predetermined culture medium under controlled laboratory conditions.

What is Blood Culture?

Blood culture is the laboratory procedure to determine the presence of microorganism in blood.

Purpose of Blood Culture:

- Diagnostic
- Therapeutic

Importance of Blood Culture

- Blood culture is a critical component and can either positively affect the patient's outcome by providing the diagnosis with accuracy or adversely affect the outcome by prolonging antimicrobial therapy and the length of hospital stay.

Indications of Blood Culture

- Bacteremia /Septicemia
- Meningitis
- Enteric fever
- Infective endocarditis
- Brucellosis
- Severe pneumonia
- Patients with PUO

Factors Influencing Blood Culture (BC)

Results of blood culture is quite variable. Sensitivity and specificity are influenced by:

- Culture System and Media
- Concentrations of microbes
- Volume of Blood
- Time of Blood Collection
- Host immune responses
- Intracellular existence of the bacteria

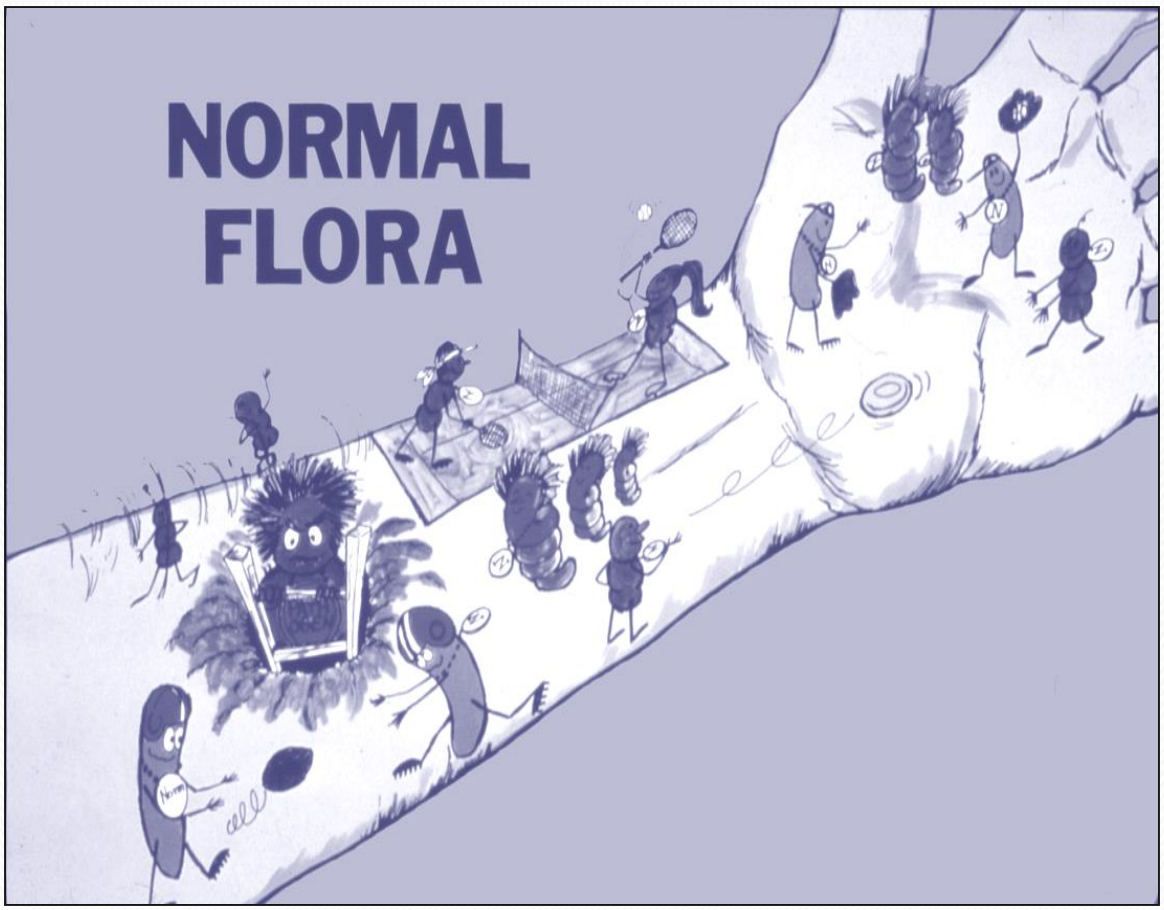
Types of Bacteremia

- Transient bacteremia usually follows mechanical or surgical manipulation of infected tissue, dental procedures etc.
- Intermittent bacteremia where bacteria are periodically released into the blood e.g. abscess
- Continuous bacteremia points to an intravascular infection e.g. infective endocarditic.

Collection of blood

- Blood must be taken during continuous bacteremia and before initiation of antibiotic therapy.
- Bed side collection is preferable.
- Inoculation in culture media should be made immediately.
- Optimal time - “as early as possible”.

Careful attention to details of skin preparation prior to collection of specimen



Aseptic Measures

- Hand wash.
- Disinfection of the venipuncture site with 70% ethanol and 2% tincture of iodine.
- Blood should be collected using a sterile syringe and needle.
- Decontamination of blood culture bottle cap.
- Bottles are sent to the laboratory after proper labeling.

Right way to take Aseptic Measures

Volume of blood to be collected

- “The higher the volume of blood cultured the higher the yield of positive blood cultures” has been a well-accepted dictum.
- This rule has not been questioned in the era of highly automated blood culture machines, nor has it been correlated with clinical variables.

Volume of blood according to age

Adult:

- 10 ml per bottle (two bottles)

Pediatric:

- Neonates: 1- 2 ml per bottle
- Infants and children: 2 - 5 ml per bottle

“Laboratories should routinely monitor the volume of blood cultured as a quality assurance activity…….”

Standard Blood-Broth ratio is -1 : 10 for dilution of natural bactericidal substances in blood (e.g, complement, phagocytic cells).

Concentration of Organisms in Bacteremia

In Adults:

- Gram Negative Bacteremia <1 to 10 organism/ml
- Gram Positive Bacteremia 1 to 300 organism/ml

In Children:

- 75% of children > 100 organism/ml

Possible pathogens

- Microorganisms that almost always represent true infection:
  - Staphylococcus aureus
  - Escherichia coli
  - Salmonella typhi / paratyphi
  - Streptococcus agalctiae
  - Pseudomas aeruginosa

Possible Contaminant

- If blood cultures are collected properly, no more than 2-3 % of all blood cultures are contaminated.
- Isolates that rarely represent true infection:
    - Corynebacterium spp.
    - Propionibacterium acnes
    - Bacillus spp.

Blood Culture System

- **Manual/Conventional Culture System-** Evidence of growth is detected by visible mass or colony formation.
- **Automated/Continuous-monitoring Culture System-** Evidence of growth is detected by radiometric, colorimetric and by sensor methods.

Manual Culture System

- **Advantages**
  - Technically simple
  - Cost effective
  - Useful in small laboratories with minimum workload
- **Disadvantages**
  - More false positive
  - Labor intensive
  - Need visual inspection for growth e. g, turbidity, pellicle formation, hemolysis and gas production.

Automated Culture System

- **Advantages**
  - Rapid result with higher sensitivity
  - Expandable to accommodate larger volume
  - Reduce the technician manipulation time
  - More reliable due to reduced contamination

- **Disadvantages**
  - Expensive
  - Need regular maintenance
  - Continuous power supply is mandatory

Manual Culture System

I. Conventional Method

- Castaneda Method (Biphasic media)
- Broth Culture
- Roche Septi-chek biphasic media

II. Lysis-Centrifugation Method

Blood Culture Media

- Trypticase soy broth
- Brain-Heart infusion broth
- Columbia broth
- Roche Septi-chek biphasic media
- Thioglycolate broth (anaerobic bacteria)

“No one medium or system is capable of detecting all microorganisms.”(Monica Cheegeburg).

Antimicrobial Neutralization Media

- Antimicrobial removal device (ARD)
- Fastidious Antibiotic Neutralizer (FAN) media
- BACTEC resin-containing media

ARD

FAN Bottles

Broth Culture

- Blood is put into liquid media e,g. Brain Heart Infusion Broth or Trypticase Soy Broth.
- Then subcultures are made in suitable solid media.
- BC bottles are incubated about about for 7 days.
- Extended incubation (14-21 days) may be needed for more fastidious organism e.g, HACEK group (*Haemophilus, Actinobacillus, Cardiobacterium, Eikenella* and *Kingella* species).

Supplement used in Broth

- Sodium Polyanethol Sulfonate (SPS)
- Functions:
  - Anticoagulant
  - Anti-phagocytic and anticomplementary
  - It also inhibits streptomycin, Kanamycin, gentamycin, and Polymixin B
- PABA to nutrarlize certain drugs e,g. Sulfonamide.

Bottle containing Broth

Biphasic Method

- Castaneda Method: In this bottle there is both solid and liquid phase of media of choice e,g, TSB/TSA.
- Upper two-third of agar is inoculated by simply tilting the vial and flooding the surface.
- Colonies grow on the agar are easily visible.
- This method minimizes the opening of culture bottle and limit the contamination and autoinfection of Lab. personnel e,g. Brucellosis.

Bottles containing Biphasic media

Automated Culture System

- BACTEC 9000 series - Radiometric CO_2 detection
- BacT-ALERT system - Colorimetric CO_2 detection
- Versa TREK system - CO_2 & O_2 detection
- Extra Sensing Power (ESP) - Detection of O_2, Co_2, H_2 & N_2.

BACTEC

- The system was introduced in early 1970s to detect CO_2 concentrations in the headspace of vials.
- Broth media contain substrates labeled with radioactive ^{14}C, which is released as CO_2 into the vial headspace during microbial metabolism.
- An aliquot of this head-space atmosphere of each bottle is drawn through a sterile needle and analyzed for radioactivity.

BACTEC 9000 Series

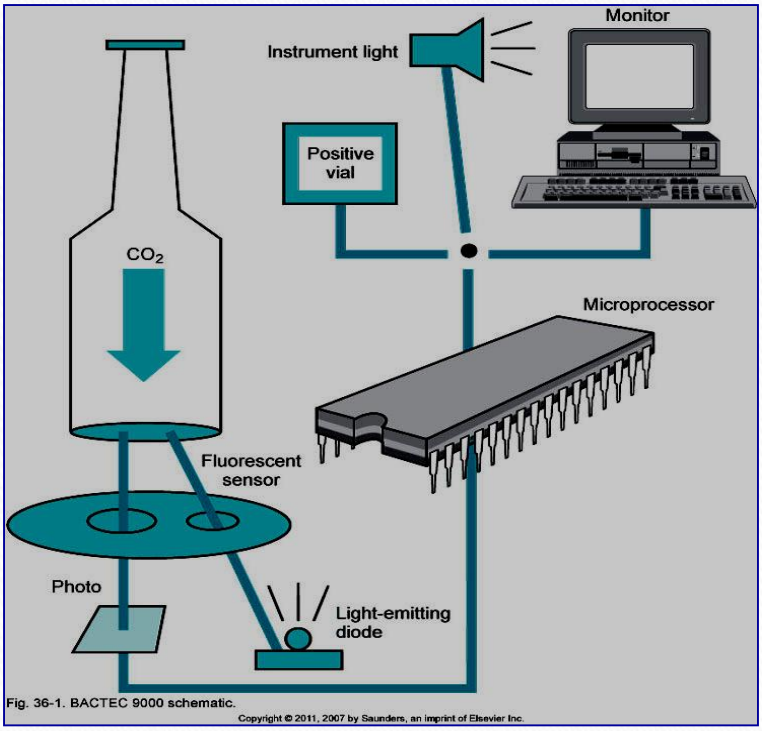


Fig. 36-1. BACTEC 9000 schematic.

Fluorescent light is used to detect changes in CO2 levels

BacT/Alert

- Another continuous monitoring blood culture system based on colorimetric detection of CO_2 by means of a sensor internally attached to the bottom of bottle.
- When CO_2 concentration increases in the medium; diffuses to the sensor through permeable membrane.

BacT/ALERT

BacT/ALERT system

- Carbon dioxide production results in low pH .
- Results in color change from **green to yellow**.

FAN

- The ecosorb (absorbent charcoal) supplemented BHI broth for the BacT/Alert FAN media significantly increased the yield over the standard media or ESP 80A media, especially for patients receiving antimicrobial therapy when blood cultures were performed.
- FAN media have been reported to be comparable to the BACTEC resin-containing media.

Resin

- Non-ionic and cationic exchangers that neutralize many different antibiotics which might be present in the patient due to prior treatment
- Help to lyse blood cells so that intra-cellular organisms are set free

Reasons of contamination

- Culture contamination represents false positive results and are not uncommon in microbiology laboratories with rates being reported as high as around 50% of all positive cultures.

- The increased use of intravascular devices and the practice of taking cultures through invasive lines are also important when considering contamination rates.
- American Society of Microbiologists published standards, that state the contamination rates must not exceed 3%, rates however will differ widely between institutions.

You can eliminate most, but not all contamination???

- Positive results depends on clinical presentation, how the culture was taken, the organisms grown and the time taken to be positive.
- A positive culture result does not necessarily indicate bacteremia; false-positive results occur due to contamination.
- A negative culture result does not necessarily rule out bacteremia; false-negative results may occur.

- The significance of positive blood culture results are challenging to both Clinicians and Microbiologists.
- There is urgent need to distinguish contaminants from pathogens.

- Blood cultures, as described herein, currently represent the "gold standard" for diagnosis of septicemia. Nonetheless, they have limitations.
- No one culture medium or system in use has been shown to be best suited to the detection of all potential bloodstream pathogens.
- Some microorganisms grow poorly, or not at all, in conventional blood culture media and systems.

- It can be inferred that the continuous monitoring systems are preferred platform.
- However various new methods are on the horizon that will aid or even replace these systems with much reproducibility in future!

Thank you!