BLOOD CULTURE COLLECTION

DIYALA UNIVERSITY / MEDICINE COLLEGE
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AIM OF THE TEST

An etiological diagnosis of bacteremia by aerobic and anaerobic cultivation of the blood, with identification and susceptibility test of the isolated organism(s). Blood culture should be made for cases with suspected septicemia, endocarditis, and bacteremia secondary to localized infections (pneumonia, intraabdominal abscesses, pyelonephritis, epiglottitis, meningitis). In this case the blood culture may provide an etiological diagnosis of the localized infection.

TYPES OF SPECIMEN

Whole blood

CRITERIA OF SPECIMEN REJECTION

Blood collected in tubes or bottles other than aerobic and anaerobic blood culture bottles. If the information on the label does not match that of the request form. Specimens for anaerobic blood culture received in aerobic bottles or vice versa.

Pathogens ; *Blood is a sterile body fluid and normally contains commensals*

Gram-positive	Cocci	Staphylococcus spp., Staphylococcus aureus,
		Micrococcus spp
	Cocci	Streptococcus spp.
		Enterococcus spp.
	Cocci	Streptococcus pneumonia
	Bacilli//rods	Listeria spp.
		Corynebacterium spp.
		Clostridium spp. (anaerobe)
	Large, budding	Yeasts, for example, Candida spp.
Gram-negative	Cocci	Neisseria spp., Neisseria meningitidis
	Coccobacilli	Haemophilus spp., Haemophilus influenzae
		Escherichia coli
		Other coliforms, Klebsiella, Enterobacter spp.
		Pseudomonas spp.
		Bacteroides spp. (anaerobe)
Ziehl–Neelsen	Bacilli(acid-fast)	Mycobacterium spp.

PRE SPECIMEN PROCESSING

- **Patient preparing**
- The major difficulty in interpretation of blood cultures is potential contamination by skin flora. This difficulty can be markedly reduced by careful attention to the details of skin preparation and antisepsis prior to collection of the specimen.

SKIN PREPARATION

First cleanse the vein puncture site with isopropanol. Then use tincture of iodine or povidone iodine to disinfect the site using progressively larger concentric circles. Iodine should remain in contact with skin for about 1 minute or until dry to ensure disinfection. The vein puncture site must not be palpated after preparation. Blood is then drawn. Following vein puncture, alcohol is used to remove the iodine from the site.

SPECIMEN COLLECTION

Blood cultures should be drawn prior to initiation of antimicrobial therapy. If more than one culture is ordered, the specimens should be drawn separately at no less than 30 minutes apart to rule out the possibility of transient bacteremia by self-manipulation by the patient of mucous membranes in the mouth caused by brushing teeth, etc or by local irritations caused by scratching of the skin. The time of collection must be indicated. Strict aseptic technique is essential. If present remove the plastic cap from the blood culture bottles, swab the stoppers with tincture of iodine or povidone iodine and allow to dry. Collect 20mL blood in a sterile plastic syringe and inoculate at least 10 mL blood (as indicated on bottle) into each bottle or use Vacutainer and butterfly

QUANTITY OF SPECIMEN

Volume inoculated in sets of culture bottles for aerobic and anaerobic cultivation

Children below 2 years	**I mL of venous blood in 2 bottles**
Children *2-5* years	**2 mL of venous blood in 4 bottles**
Children 6-10 years	**3 mL of venous blood in 4 bottles**
Children 11-15 years	***5* mL of venous blood in 4 bottles**
Children above 15 years and adults	**5 mL venous blood in three sets of bottles (6 bottles).**

STORAGE ; **Pre-incubate or maintain specimen at room temperature. Do not refrigerate**

CONTAINER

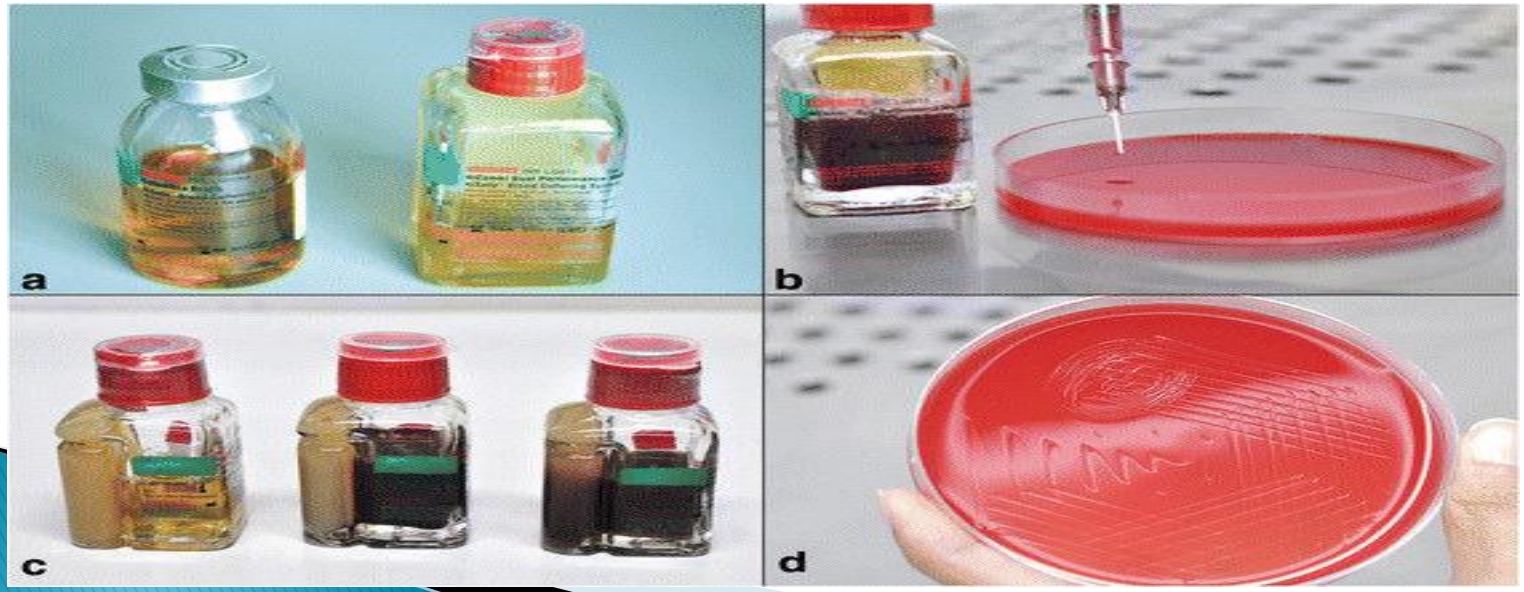
One aerobic and one anaerobic blood culture bottle. Do **not** vent.

SPECIMEN PROCESSING MEDIA

Aerobic/Anaerobic Blood Culture Bottles

METHOD

Blood is injected to both aerobic and anaerobic bottles and incubated for up to10 days at 37 Discard as negative after the 10 days incubation period is expired. During the incubation period, a gram stain and subculture onto appropriate media should be done.



CULTURE AND ISOLATION

** The test bottles should be visually examined within 24 h and at daily intervals thereafter. The bottles should be carefully removed from the incubator to avoid disturbing the sedimented blood and examined for any visual evidence of microbial growth, such as turbidity, hemolysis, gas production, or formation of discrete colonies.

** Since autolysis of some microorganisms may occur after prolonged incubation of inoculated blood culture bottles, subcultures should be taken at various incubation intervals. After 24- to 48-h incubation, a small quantity (0.1 to 0.5ml) of blood-broth mixture should be removed by sterile syringe and needle and subcultured to plates of enriched and selective media. This procedure should be repeated after an incubation period of 7 days if the culture appears negative, or earlier as growth appears.

** usefull information can be obtained by observing the cultures for typical appearances.If visible evidence of growth appears, the broth should be examined by the Gram stain and subcultured onto appropriate media for isolation and identification.

POST SPECIMEN PROCESSING

Interfering factors

Patient on antibiotic therapy

Result reporting

Any isolated organism will be reported. Antibiotic sensitivity will also be included with the report.

TURN AROUND TIME

Initial blood culture results will be reported as soon as it shows growth. Final results with sensitivity will be issued after 24-48 hours of the initial report. Negative results will be issued after 10 days of culture submission.

INTERPRETATION OF POSITIVE BLOOD CULTURES

- Virtually any organism, including normal flora, can cause bacteremia
- A negative culture result does not necessarily rule out bacteremia; false-negative results occur when pathogens fail to grow
- A positive culture result does not necessarily indicate bacteremia; false-positive results occur when contaminants grow.
- Gram-negative bacilli, anaerobes, and fungi should be considered pathogens until proven otherwise.
- The most difficult interpretation problem is to determine whether an organism that is usually considered normal skin flora is a true pathogen.

Limitations

- Three negative sets of blood cultures in the absence of antimicrobial therapy are usually sufficient to exclude the presence of bacteremia. One set is seldom ever sufficient.1 Prior antibiotic therapy may cause negative blood cultures or delayed growth.

POSITIVE BLOOD CULTURE

Positive blood culture is the gold standard for diagnosing bacteraemia and fungaemia. Processing a blood culture can take several days, and includes use of semi-automated incubation with growth detection and a broad range of laboratory techniques such as Gram staining, phenotypic or molecular identification and antimicrobial susceptibility testing on a cultured isolate. Sensitivity and specificity of a blood culture and time-to-positivity depend on a number of factors related to host/pathogen interaction, collection and transport of the specimen to the laboratory and methods employed to process the specimen.

Thank You for your Attention!!!!

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	Cocci	Streptococcus pneumoniae
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Common pathogens	
Streptococcus spp	*Bacteroides fragilis* and other anaerobic bacteria
Staphylococcus aureus	Coagulase negative staphylococci
Listeria monocytogenes	Enteric gram negative bacilli
Corynebacterium jeikeium	*Neisseria meningitides*
Haemophilus influenza	Non fermenter gram negative bacilli
Salmonella typhi	
Pseudomonas aeruginosa	
Fungi	
Candida albicans	*Cryptococcus neoformans*
Other *candida* spp	*Coccidoides immitis*
Histoplasma capsulatum	