Culturing blood

Blood is cultured to detect and identify bacteria or fungi. The presence of such organisms in the blood is called bacteraemia or fungaemia, and is usually pathological, because the blood is sterile. Transient bacteraemia occurs shortly after a tooth extraction or bronchoscopy, or urethral catheterization. Transient bacteraemia is generally due to commensal bacteria and usually resolves spontaneously through phagocytosis of the bacteria in the liver and spleen. Septicaemia is a clinical term used to describe bacteraemia with clinical manifestations of a severe infection, including chills, fever, malaise, toxicity, and hypotension, the extreme form being shock. Shock can be caused by toxins produced by Gram-negative rods or Gram-positive cocci.

Possible pathogens isolated from blood cultures

BACTERIA

Gram positive	***Gram negative***
Staphylococcus aureus	*Salmonella* Typhi
Viridans streptococci	Other *Salmonella* serovars
Streptococcus pneumoniae	*Brucella* species
Streptococcus pyogenes	*Haemophilus influenzae*
Enterococcus faecalis	*Pseudomonas aeruginosa*
Clostridium perfringens	*Klebsiella* strains
Anaerobic streptococci	*Escherichia coli*
	Proteus species
	Bacteroides fragilis
	Neisseria meningitidis
	Yersinia pestis

Also *Mycobacterium tuberculosis* (HIV-associated tuberculosis), *Leptospira* species, *Borrelia* species, rickettsiae, and *Bartonella bacilliformis*.

Commensals

Blood does not have a normal microbial flora. Common skin contaminants include coagulase negative staphylococci , viridans streptococci , micrococci , and *Corynebacterium* species .

1- Collect blood and inoculate culture media

Timing of blood collection

- blood should be taken before antibiotics are administered.
- The best time is when the patient is expected to have chills or a temperature spike.
- It is recommended that two or preferably three blood cultures be obtained, separated by intervals of approximately 1 hour (or less if treatment cannot be delayed). The advantages of repeated cultures are as follows:

— the chance of missing a transient bacteraemia is reduced;

— the pathogenic role of saprophytic isolates (e.g. *Staphylococcus epidermidis*) is confirmed if they are recovered from multiple vine punctures.

- A *strict aseptic technique must be used to collect the blood* .
- Blood volume:
  10 ml for adults; 2–5 ml for children, for infants and neonates, 1–2 ml

Two tubes should be used the first for optimal recovery of strictly aerobic microorganisms, the second for anaerobic culture.

Choice of culture media

Media selected for the culture of blood should be capable of providing the fastest growth and isolation of as wide a range of pathogens as possible. The following media are recommended:

The blood-culture broth like tryptic soy broth (TSB), Columbia agar and Columbia broth diphasic medium with added SPS (sodium polyanethol sulphonate). SPS prevents the blood from clotting, neutralizes complement and other antibacterial substances in fresh blood, and has some neutralizing effect on polymyxin B, streptomycin, and gentamicin should be present in the blood and Thioglycollate

broth medium is recommended to isolate strict anaerobes. The blood should be mixed with 10 times its volume of broth (5 ml of blood in 50 ml of broth) to dilute any antibiotic present and to reduce the bactericidal effect of human serum. Blood-culture bottles (125 ml) with a pre-perforated screw-cap and a rubber diaphragm must be used. Without delay, mix the blood with the broth and mix the blood in the EDTA container. incubation at 35–37 C and routinely inspected twice a day (at least for the first 3 days) for signs of microbial growth.

***Important*:** The blood must not be allowed to clot in the culture media because any bacteria will become trapped in the clot.

Asterile culture usually shows a layer of sedimented red blood covered by a pale yellow transparent broth. Growth is evidenced by:

— a floccular deposit on top of the blood layer
— uniform or subsurface turbidity
— haemolysis
— coagulation of the broth
— a surface pellicle
— production of gas
— white grains on the surface or deep in the blood layer.

2- Examine the specimen microscopically

Centrifuge a sample of EDTA anticoagulated venous blood or heparinized capillary blood and make smears of the buffy coat layers. Stain as follows:

_ ***Gram smear*:** To detect Gram positive and Gram negative bacteria, particularly when the patient is an infant or young child.

_ ***Ziehl-Neelsen smear*:** To detect AFB when the patient has AIDS or suspected HIV disease.

_ ***Giemsa or rapid Field's smear*:** To detect borreliae, or parasites such as trypanosomes, malaria parasites, and microfilariae.

Subculturing a blood culture broth

A strict aseptic technique must be used to avoid contaminating the culture.

1 -Using an ethanol-ether swab, cleanse the top of the bottle. Using a sterile needle and small syringe, insert the needle through the rubber liner in the cap, and withdraw about 1 ml of the broth culture.

2 -Inoculate the broth on:

– Blood agar

– Chocolate (heated blood) agar

– MacConkey agar

Incubate the blood agar plate anaerobically for up to 48 hours, the chocolate agar plate in a carbon dioxide atmosphere for up to 48 hours, and the MacConkey agar plate erobically overnight.

3- Swab the top of the culture bottle and re incubate.

Notes: Some microorganisms may grow without producing turbidity or visible alteration of the broth. Other organisms, e.g. pneumococci, tend to undergo autolysis and die very rapidly. For this reason, some laboratories perform routine subcultures on chocolate agar after 18–24 hours of incubation. A blind subculture may be made at the end of 7 days of incubation, by transferring several drops of the well-mixed blood culture (using a sterile Pasteur pipette) into a tube of thioglycollate broth, which in turn is incubated and observed for 3 days.

Contamination of blood cultures can be avoided by meticulous skin preparation. However, even in ideal conditions, 3–5% of blood cultures grow ―contaminants‖ originating from the skin (*S. epidermidis, Clostridium* spp., diphtheroids) or from the environment (*Acinetobacter* spp., *Bacillus* spp.). Such organisms may behave as pathogens and cause endocarditis. A true infection should be suspected in the following situations:

— if the same organism grows in two bottles of the same blood specimen;

— if the same organism grows in cultures from more than one specimen;

— if growth is rapid (within 48 hours);

— if different isolates of one species show the same biotypes and antimicrobial-susceptibility profiles.

All culture results should be reported to the clinician, including the contaminants. The identification of two or more agents may indicate polymicrobial bacteraemia, which can occur in debilitated patients, but may also be due to contamination.

Summary of Blood Culture Procedures

Day 1

			ADDITIONAL
1	**Collect Blood Inoculate culture media**	*Using an aseptic technique, dispense:* ■ 10–12 ml blood into Columbia agar diphasic medium and mix. Incubate up to 7 days (4 weeks when brucellosis is suspected). ■ When anaerobic infection is suspected, dispense 5 ml blood into thioglycollate broth and mix. Incubate up to 14 days. ■ 2 ml into EDTA and mix.	***When S.* Typhi *is suspected*:** Ox-gall broth is recommended ***When brucellosis is suspected*:** Tryptic soya diphasic medium is recommended
2	**Examine Microscopically**	*Prepare buffy coat smears from EDTA blood*: ■ Gram smear ■ Giemsa smear	■ ***Zn smear*:** From a patient with AIDS. Look for AFB.

Day 2 and Onwards

3	**Examine and Report Cultures**	*After overnight incubation*: ■ Examine diphasic culture. Subculture (even when no growth is seen): – Blood agar and MacConkey agar. Incubate aerobically – Chocolate agar. Incubate in CO_2. ■ Examine toluidine blue smear (diphasic culture). ■ Examine thioglycollate culture. Subculture and examine microscopically. Incubate subculture anaerobically. *Note*: When there is no growth, wash slope of diphasic culture. Reincubate cultures. Subculture as indicated. ***Examine subcultures for likely pathogens*** (See beginning of subunit 7.14) Identify organisms	***Perform antimicrobial susceptibility tests as indicated***