Lab 4.

Blood Culture (Media)

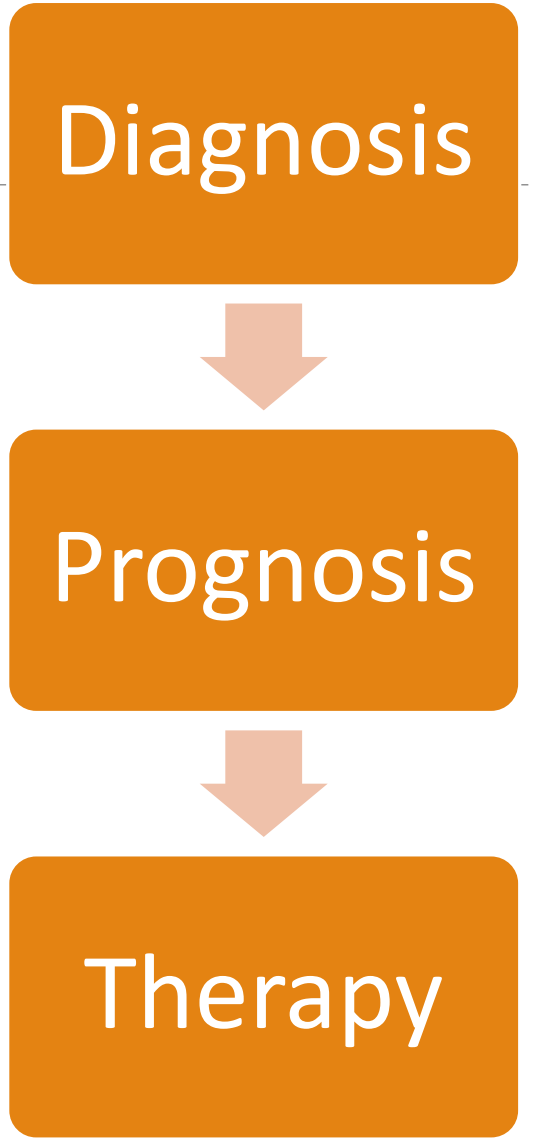
Blood Culture

❖What is a blood culture ?

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

❖Usage of Blood Culture

- It is an essential test to the doctor. The blood does not normally have a normal flora. A blood culture can show what microorganisms can be in the blood.
- The finding of pathogenic microorganisms in a patient's bloodstream is of great importance in terms of diagnosis, prognosis, and therapy.



Aim:

- To apply an etiological diagnosis of blood by aerobic and anaerobic cultivation, with identification and susceptibility test of the isolated microorganism(s).
- For cases of suspected septicaemia, endocarditis, and bacteraemia secondary to localized infections (pneumonia, intra-abdominal abscesses, yelonephritis, epiglottitis, meningitis).

Types of blood serum infection (BSI)

- Intravascular; originate within the cardiovascular system.
- Extravascular; originate from bacteria entering the blood circulation through the lymphatic system from another site of infection.

Definitions

Bacteremia → presence of bacteria in blood stream

Septicemia → presence of bacteria in CSF

Fungemia → presence of fungi in blood stream

Candidemia → presence of candida in blood stream

Clinical pattern of BSI

Transient

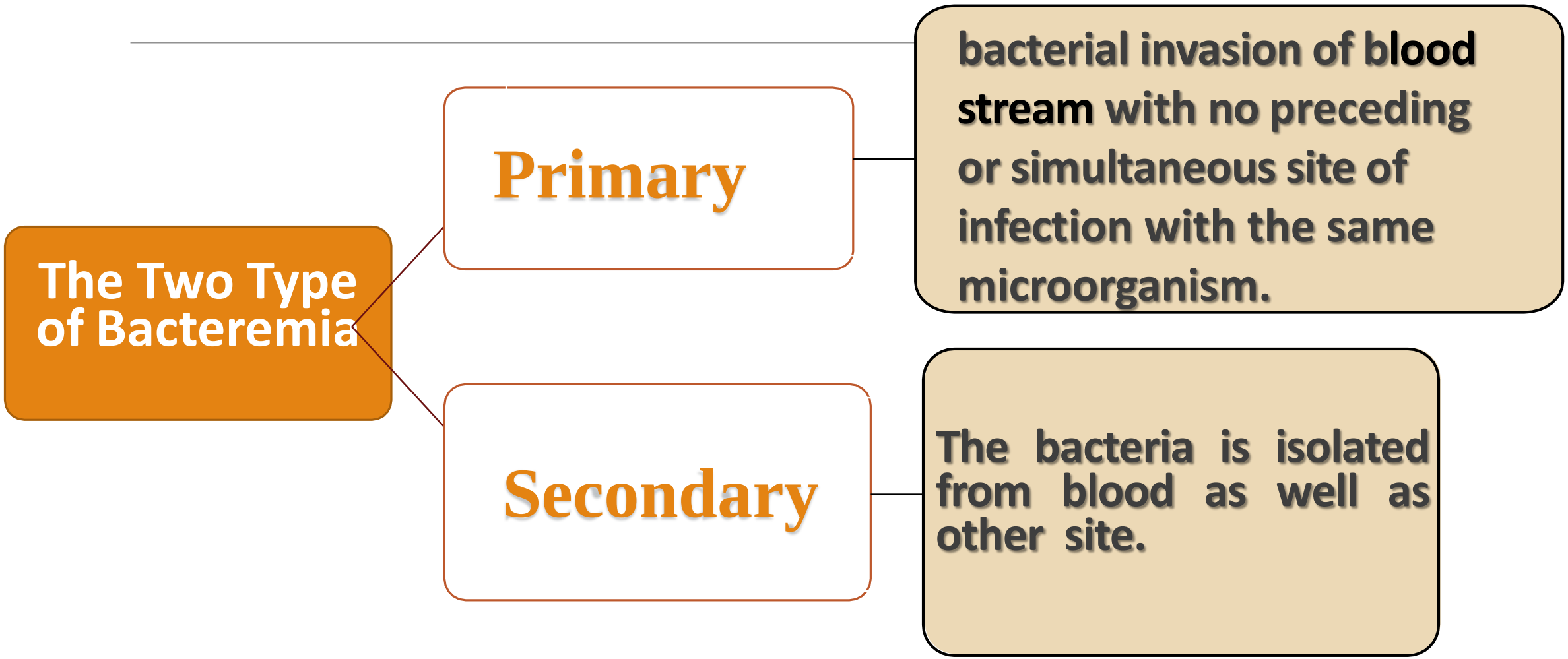
- Comes and goes.
- Usually occurs after a procedural manipulation (e.g. Dental procedures).

Intermittent

- Can occur from abscesses at some body site that is "seeding" the blood.

Continuous

Cardinal feature of endovascular infections most notably acute, sub-acute.


The Two Type of Bacteremia
Primary
bacterial invasion of blood stream with no preceding or simultaneous site of infection with the same microorganism.
Secondary
The bacteria is isolated from blood as well as other site.

Common pathogens

Bacteria	*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*
Parasite	Parasite can be found as transiently in the blood stream for example tachyzoites of *Toxoplasma gondii*	
Viruses	*Epstein barr virus (EBV)*	*HIV virus*
	Cytomegalovirus (CMV)	*Other human Retroviruses*
Fungi	*Candida albicans*	*Cryptococcus neoformans*
	Other candida spp	*Coccidoides immitis*

Blood Culture Medium

Aerobic	Anaerobic
Trytic soy broth (TSB)	Fluid thioglychollate medium (FTM)
• Pancreeatic digest of casein. • Enzymatic soy digest • Sodium chloride • Dipotassium phosphate • Dextrose • Sodium polyanethol sulphonate (SPS)	• Pancreeatic digest of casein. • Enzymatic soy digest • Sodium chloride • Dipotassium phosphate • Dextros • Sodium thioglychollate • Sodium polyanethol sulphonate (SPS) • Agar

❖Sodium polyanethol sulphonate (SPS)

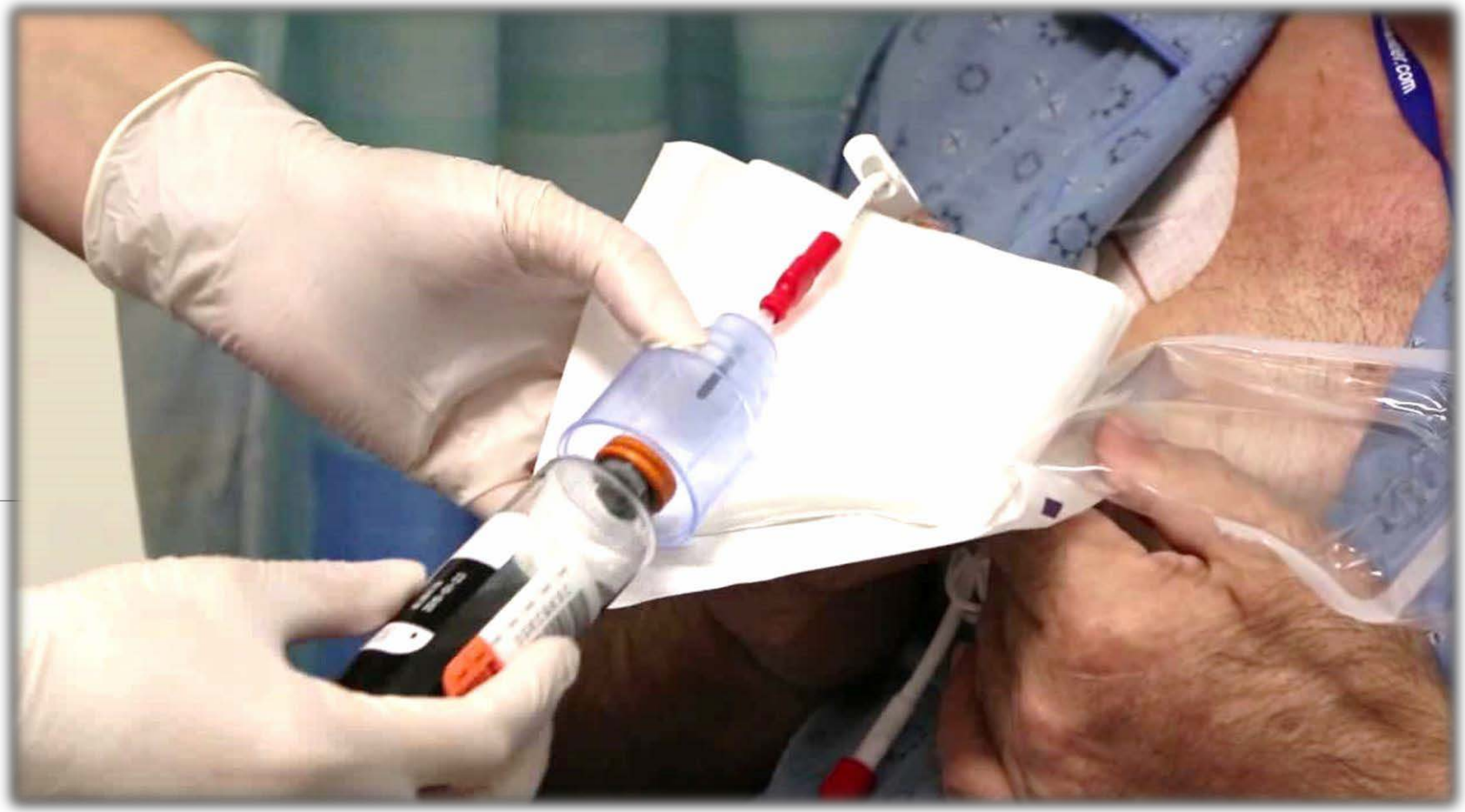
- The anticoagulant in blood culture medium must not harm the bacteria and must prevent clotting of the blood, since the clot would entrap bacteria and prevent their detection .

- The most commonly used preparation in blood media is 0.025% to 0.05% SPS.

➢ In addition to it's anticoagulant properties, SPS is: Anticomplementary, Antiphagocytic, and Interferes with the activity of some antimicrobial agents.

Types of Specimen

- **Whole blood**



❖ Standards of specimen rejection

- If the blood collected inside 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.

❖Specimen Collection (1/2)

- Blood cultures should be drown 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; either of mucous membrane in the mouth or by local irritation caused by scratching of the skin.

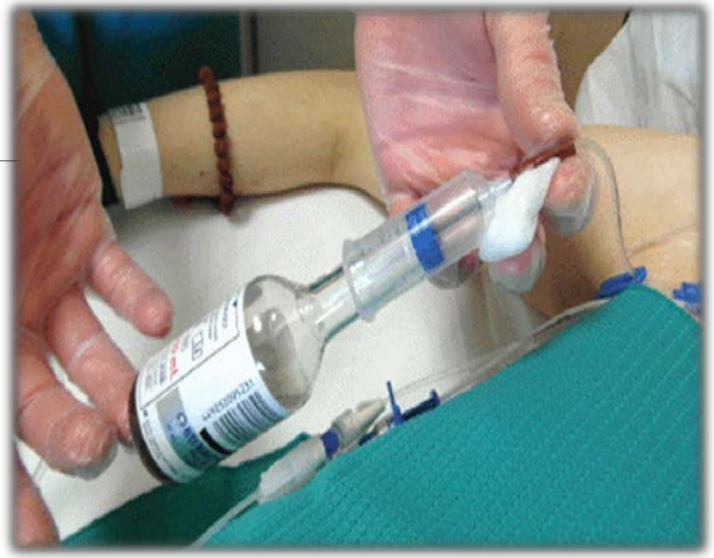
❖Specimen Collection (2/2)

The numbers of bacteria are generally higher in the **acute (initial)** stage than at a later stage of the disease.

- **Small children** usually have higher numbers of bacteria in the blood than adults. The number is also higher when the fever rises than when it is falling.
- For patients expected to seed bacteria intermittently into the blood, **80%** of these are detected with the first culture and **99%** within the three cultures.

❖Collection Time

1- Before starting antibiotics therapy, its generally recommended that the first two sets of blood cultures be taken one hour apart and the third set after 3-6 hours.



2- Half hour before a temperature increase is ideal. Since the temperature increase is usually un predictable, an educated guess must be done to determine the timing of blood cultures.

Volume of Blood Culture Collected According To Age of Patients

Age of patient	No. of blood bottle
Children below 2 years	1 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 3 sets of bottles (6 bottles).

❖Collection Procedure

- All precautions should be taken to minimize the percentage of contaminated blood culture
  - For example: To reduce the chance of contaminating organisms from the skin, the vein puncture site should ideally be prepared as follows :

1st : Prepare area

Wash the skin with soap, rinse with sterile water or saline.

Apply 1-2 % tincture of iodine or povidone–iodine and allow drying for 1-2 minutes.

Remove the iodine with 70 % alcohol wash

Wipe the hand with alcohol preparation to disinfect or wear sterile gloves.

2nd : Collection of Blood

Remove Flip Caps from the tops of the selected culture bottles. Disinfect the septa of the bottles with alcohol or iodine preparation and allow to dry.

Perform venipuncture with syringe and collect the desired amount of blood. If the vein is missed a new needle should be used.

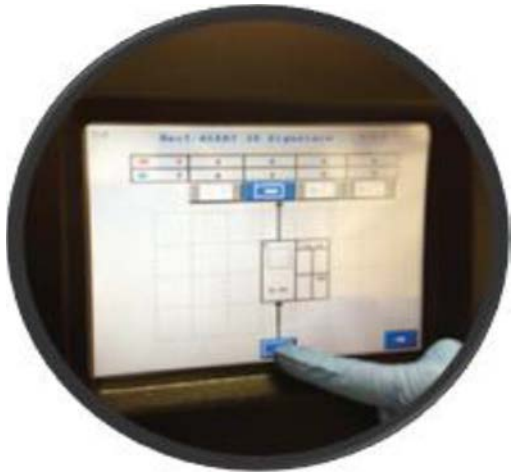
Transfer the recommended amount of blood into the culture bottles using aseptic technique if desired. First fill the aerobic bottle. Do not overfill the bottles! Any remaining blood may be used for additional tests.

NOTE

- Label the bottles according to the routine procedure. When using a sticker do not cover the tear-off section of the barcode label.
- 1:5 to 1:10 blood/broth ratio is the appropriate ratio to achieved, this dilution minimizes the effects of microbial inhibitors present in blood and dilutes any antimicrobial agents.

❖ 3rd : Specimen Incubation

- Culture should be retained for atleast 6 – 8 weeks before being discarded as negative, at 35 °C.
- Sub culture 1st after 24 H, and then after every 48 H or if culture appears turbid.

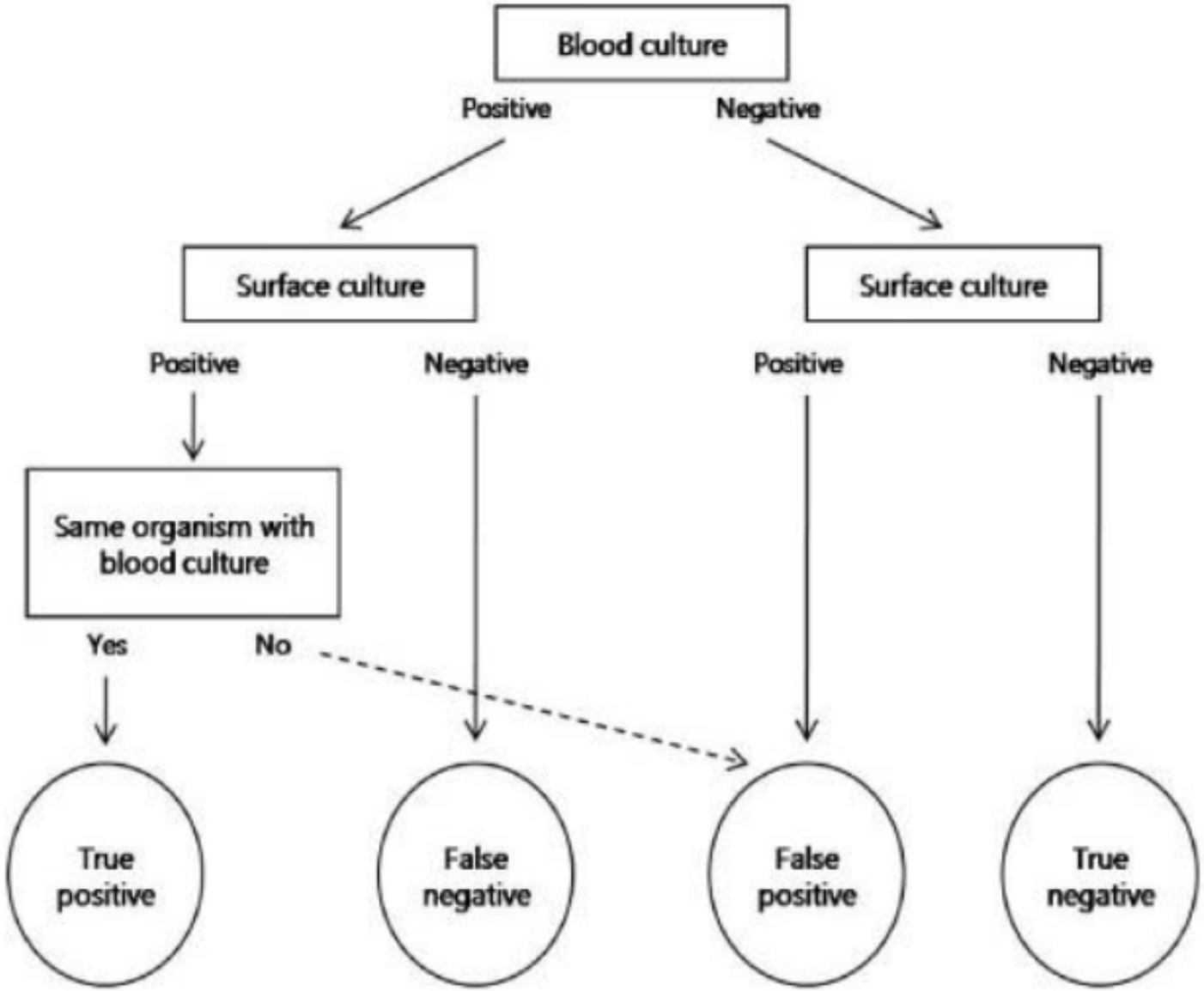


Touch

Scan

Load

❖4th : Result


Blood culture
Positive
Negative
Surface culture
Surface culture
Positive
Negative
Positive
Negative
Same organism with blood culture
Yes
No
True positive
False negative
False positive
True negative

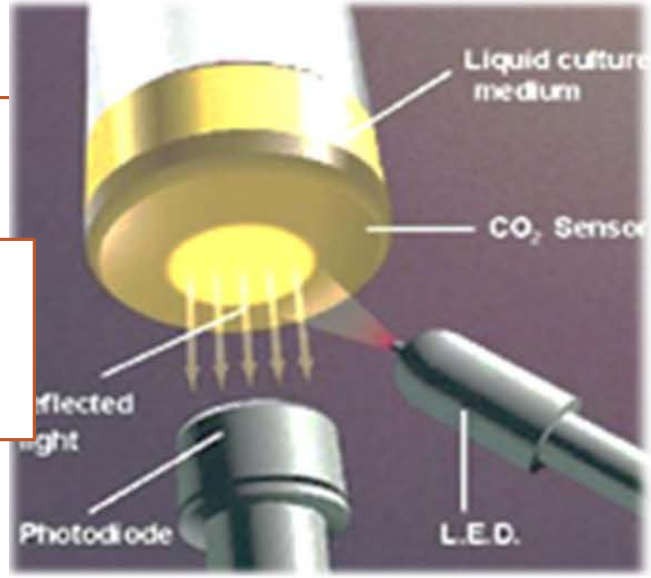
❖Reading the result

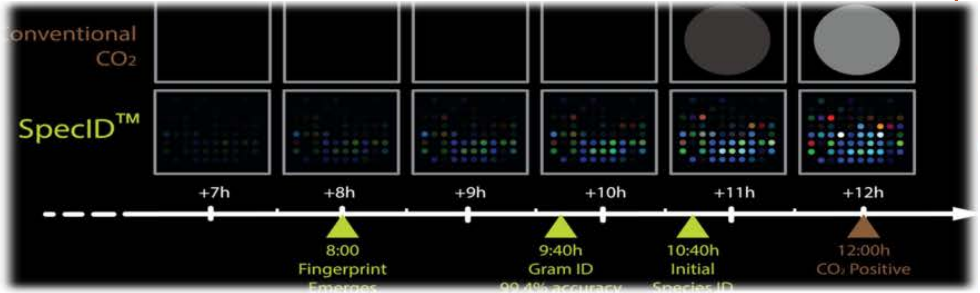
1. Microorganisms multiply in the media, generating CO_2.

- As CO_2 increases, the sensor in the bottle turns a lighter color.

2. The monitors measuring reflected light, and detects color changes in the sensor.

It's depending on the machine system





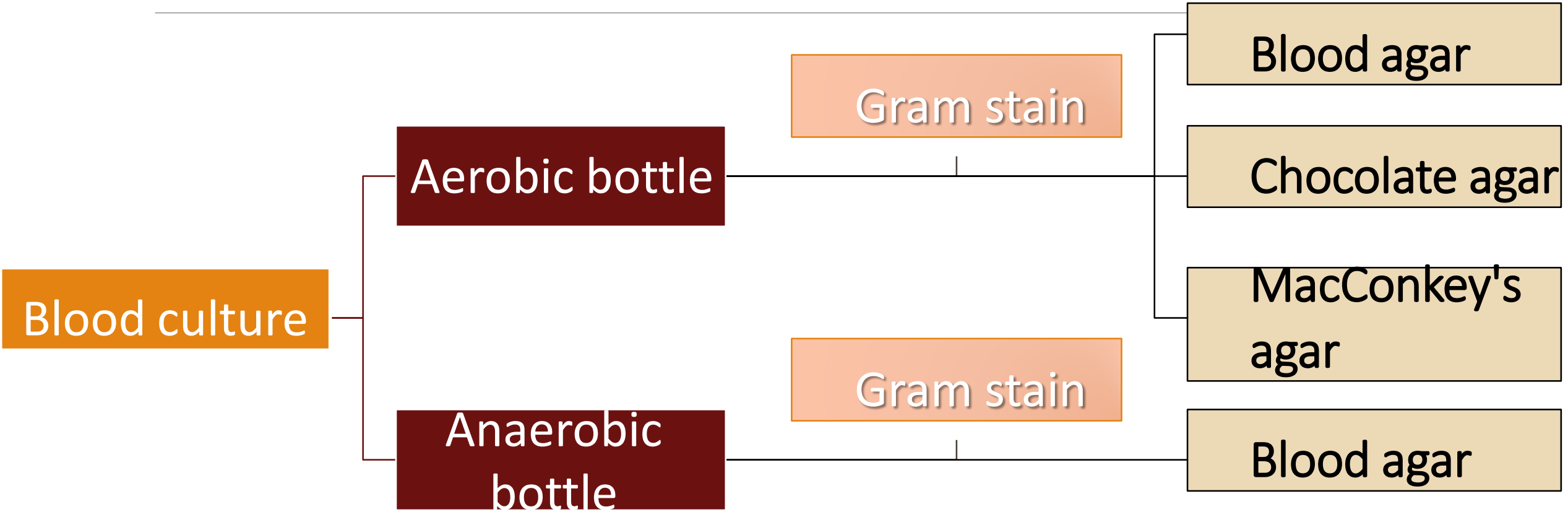
❖ 5th : Specimen Processing

The bottle incubated for 24 hours before plating to enhance the growth of bacteria:

A- **Aerobic bottle** plate on blood agar, MacConkey and chocolate Agar.
B- In CO_2 incubator for **anaerobic** incubate anaerobically on blood agar for 48 hour.

The negative bottle should be re-incubated and tested after 10 days before discarded as negative culture.

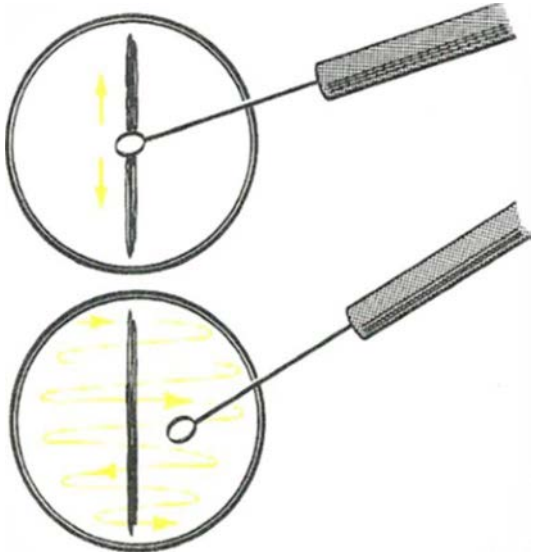
❖ 5th : Specimen Processing



❖ 5th : Specimen Processing

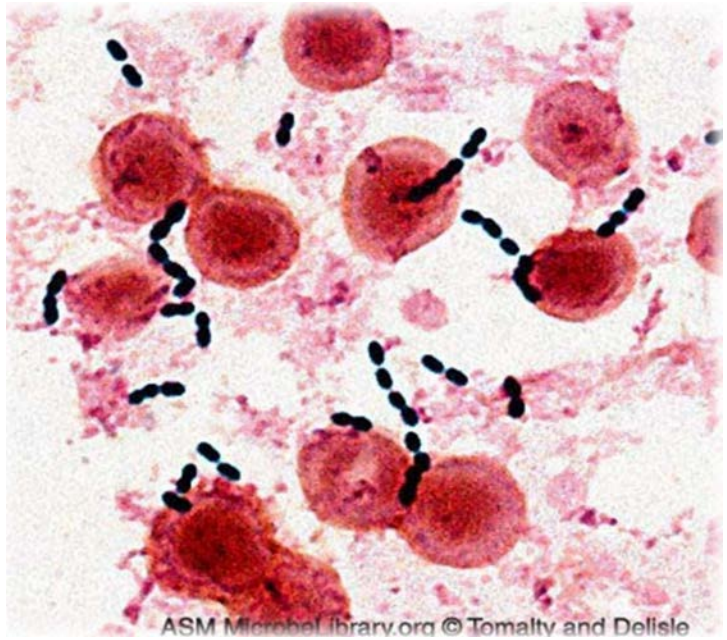
If **slow** growing organisms are suspected as ***Brucella*** **spp.**, its should be clearly indicated on the requisition form and the culture bottles should be further incubated for 2-4 weeks before being reported out as negative.

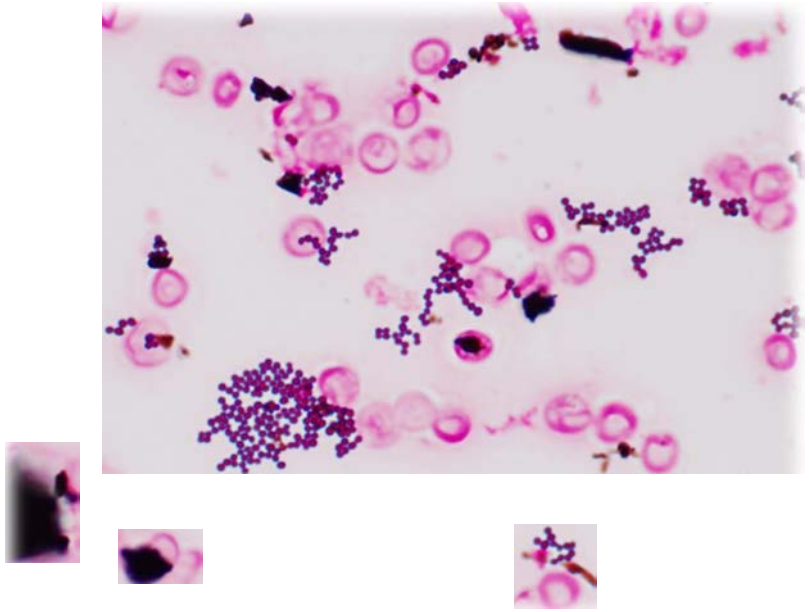
❖6th : Sub-Culturing

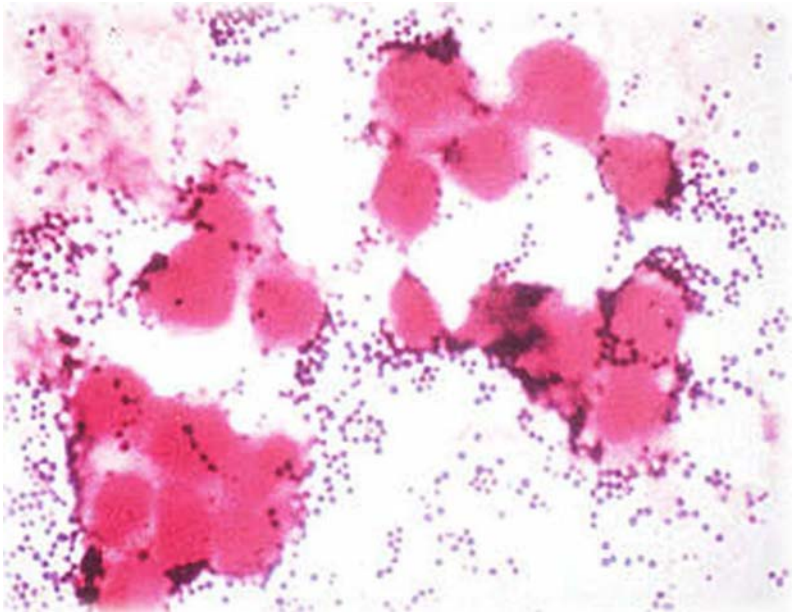


Blind Sub-Culturing syringe and drip methods

❖ 7th : Gram staining







Any Questions