320 MBIO
Microbial Diagnosis
Lab 4

Aljawharah F. Alabbad
Noorah A. Alkubaisi
2017

Blood Culture

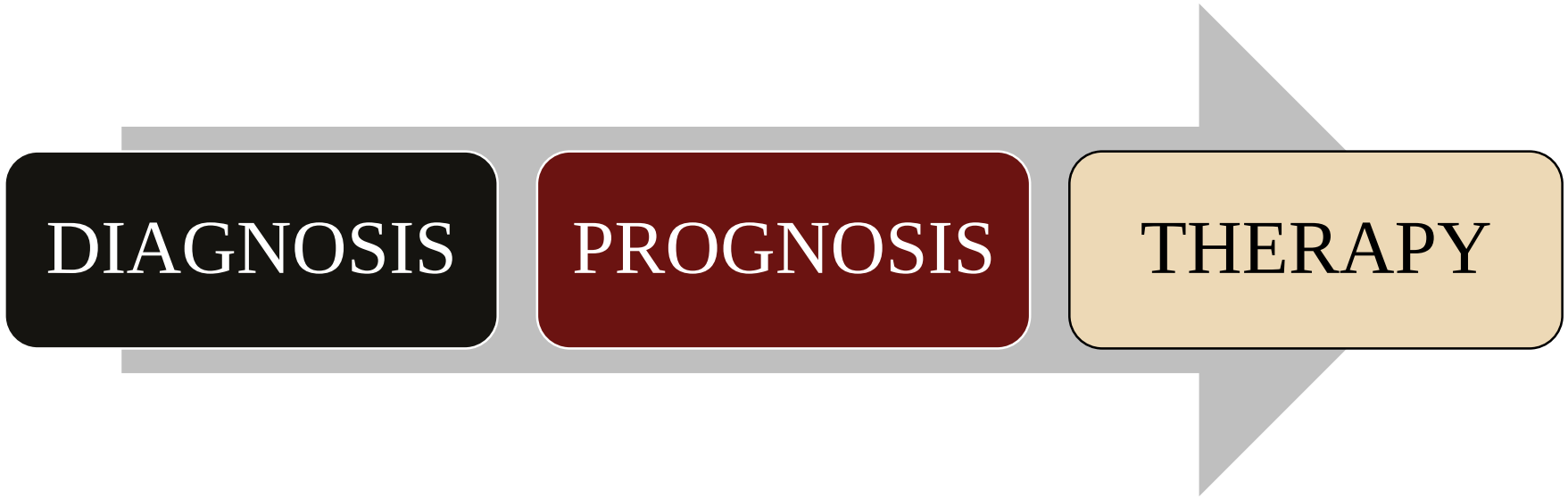
❖What is a blood culture ?

- 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.

❖Need for Blood Culture ?

- No microbiological test is more essential to the doctor than the blood culture. The finding of pathogenic microorganisms in a patient's bloodstream is of great importance in terms of diagnosis, prognosis, and therapy.

- The blood does not normally have a normal flora. A blood culture can show what microorganisms can be in the blood.



PURPOSE OF BLOOD CULTURE

❖Aim of the test

- An etiological diagnosis of blood by aerobic and anaerobic cultivation, with identification and susceptibility test of the isolated microorganism(s).

- Blood culture should be made for cases with suspected septicemia, endocarditis, and bacteremia secondary to localized infections (pneumonia, intra abdominal abscesses, pyelonephritis, epiglottitis, meningitis).
- In this case the blood culture may provide an etiological diagnosis of the localized infection.

Majors of blood serum infection BSI

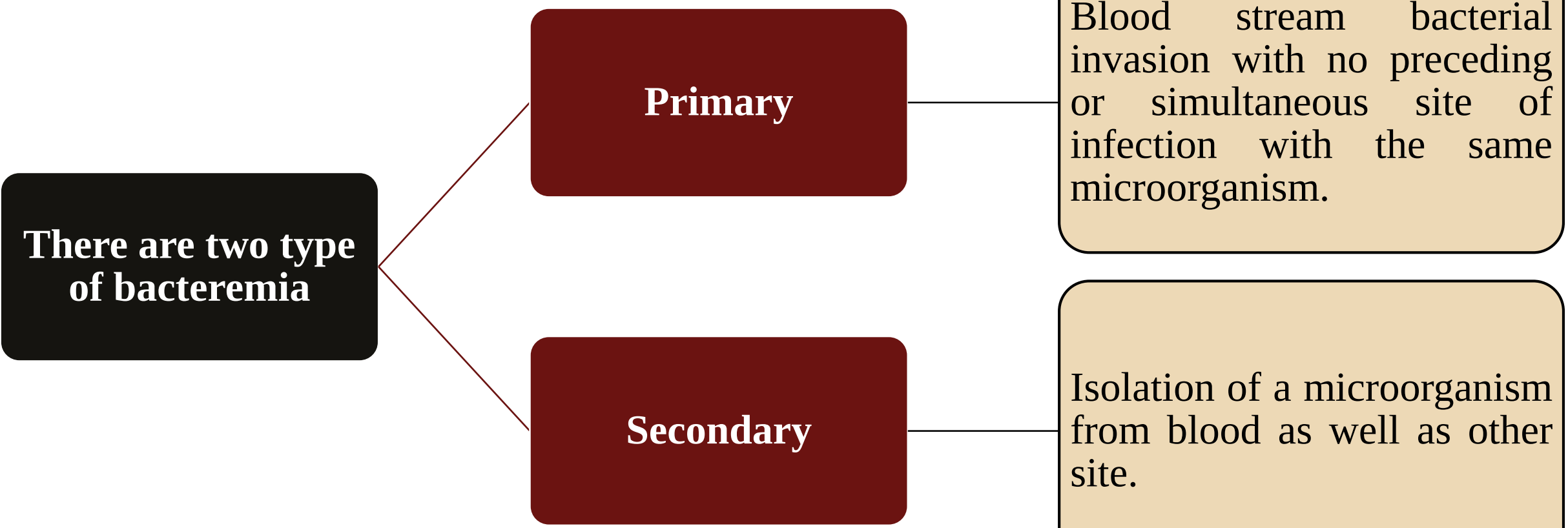
- intravascular those that originate within the cardiovascular system
- extravascular those that originate from bacteria entering the blood circulation through the lymphatic system from another site of infection.

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 (ex. Dental procedures).
- **Intermittent**
  - Can occur from abscesses at some body site that is “seeding” the blood.
- **Continuous**
  - Cardinal feature of endo vascular infections most notably acute, sub cute.


There are two type of bacteremia
Primary
Blood stream bacterial invasion with no preceding or simultaneous site of infection with the same microorganism.
Secondary
Isolation of a microorganism from blood as well as other site.

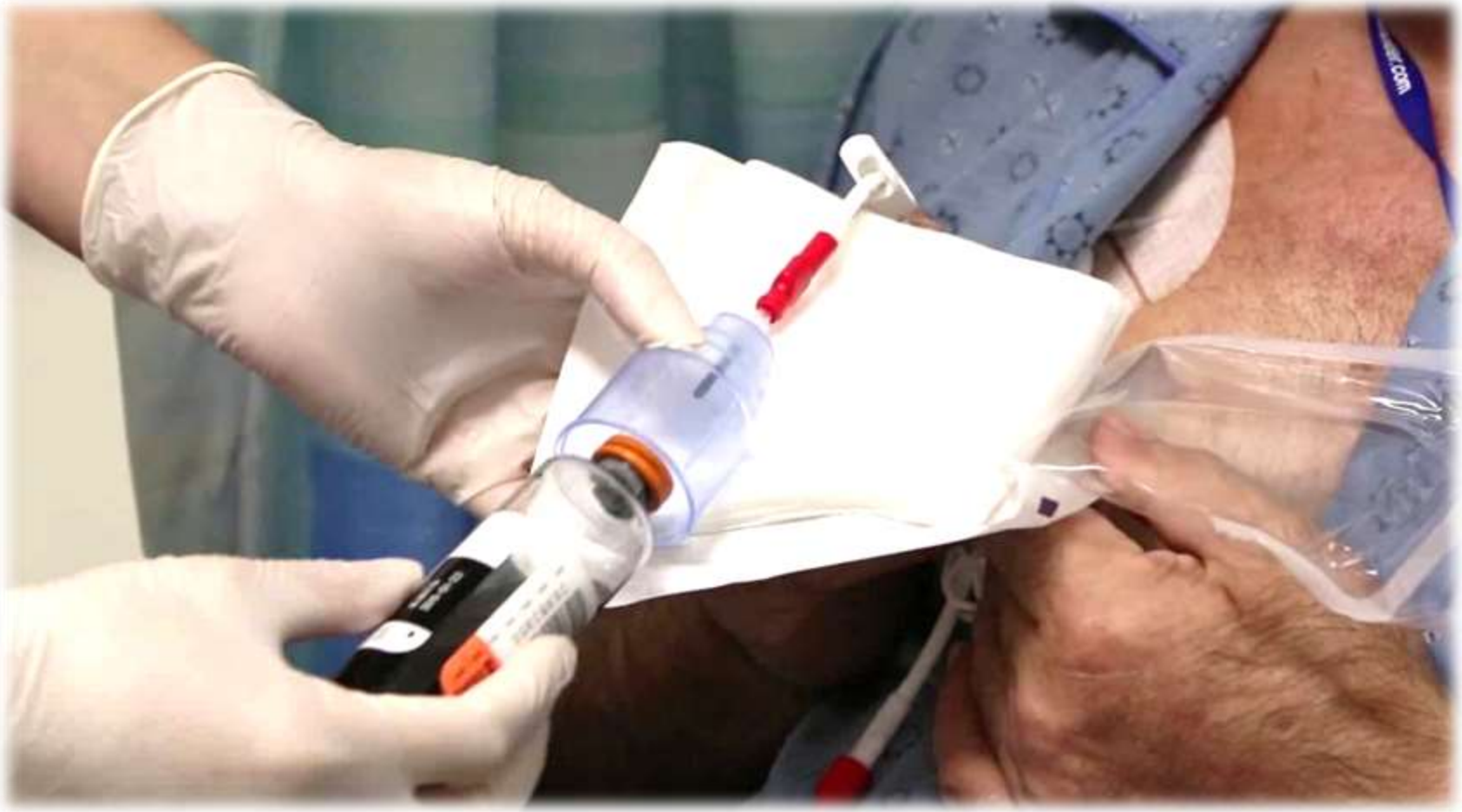
❖ Bacteremia Complications

1. **Warm shock:** fever, increased pulse, hyperventilation, and warm, dry flushed skin.
2. **Cold shock:** decrease blood pressure, increased pulse, and rapid, shallow respirations.
3. **Septic chock:** hemodynamic changes, decreased tissue perfusion and compromised organ & tissue function. Mortality 40 – 50 %

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

❖Types of Specimen

- **Whole blood**



❖ Standards 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.

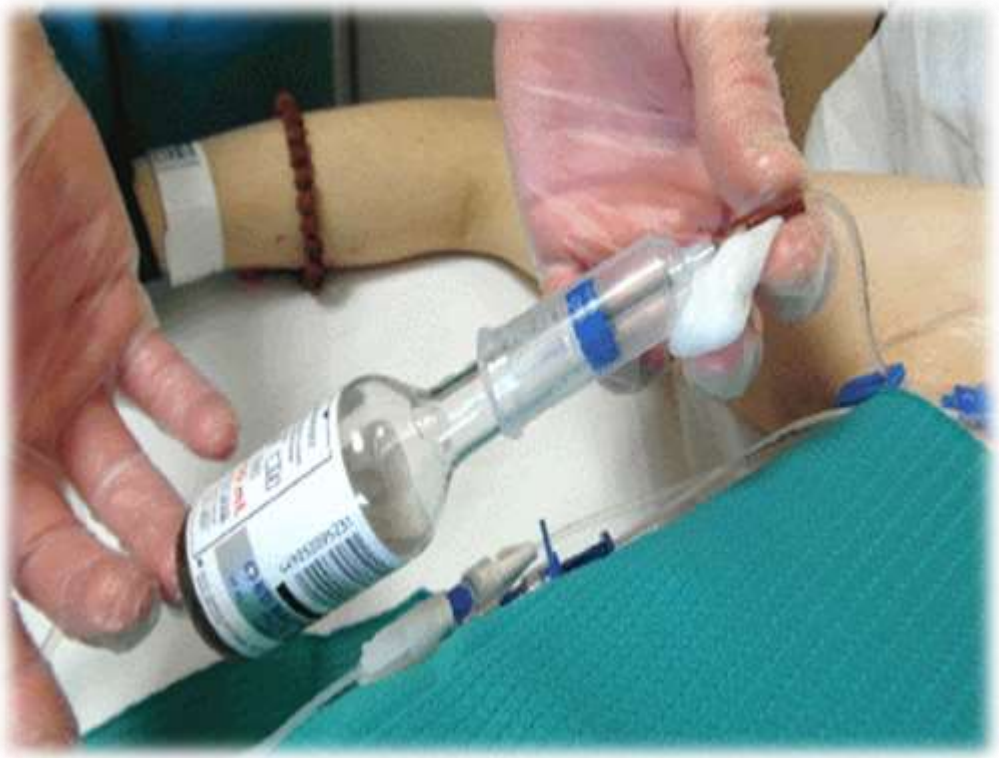
❖Specimen Collection

- 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 of mucous membrane in the mouth or by local irritation caused by scratching of the skin.

- 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

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



- Obtaining the blood culture one half hour before a temperature increase is ideal because the **highest** concentration of organisms are circulating at that time, because the temperature increase is usually un predictable an educated guess must suffice in most cases when timing 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

- During blood culture collection all percussion should be taken to minimize the percentage of contaminated blood culture, to reduce the chance of contaminating organisms from the skin the vein puncture site should ideally be prepared as follows :

❖ 1st : Prepare area

Wash 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, if the site again be palpated after the iodine – alcohol preparation the finger must be disinfected or sterile gloves worn.

❖ 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.

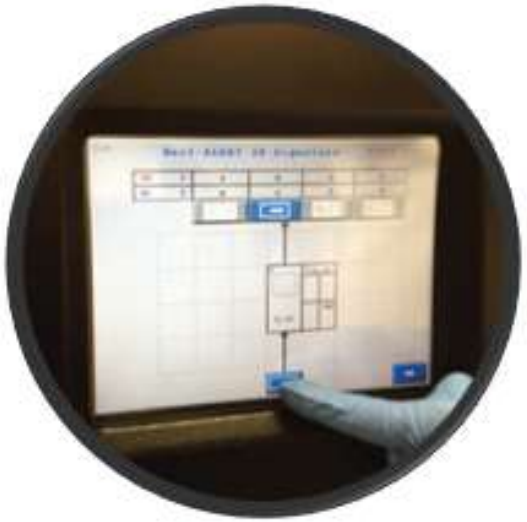
Transfer the recommended amount of blood into the culture bottles using aseptic technique if desired. First fill the aerobic bottle. Do not overfill the bottles! Any remaining blood may be used for additional tests.

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.

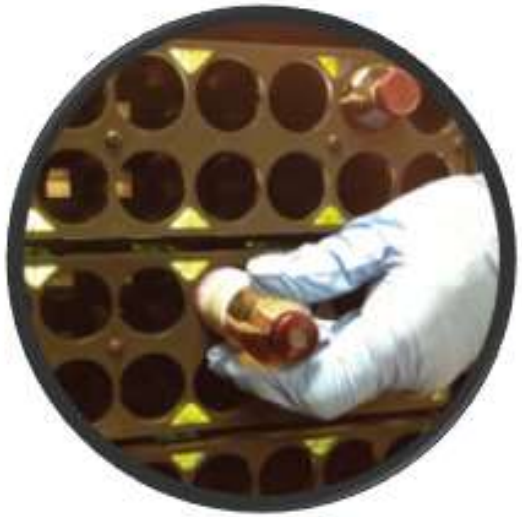

clinell
clinell
clinell

❖ 3rd : Specimen Incubation



Touch

Scan

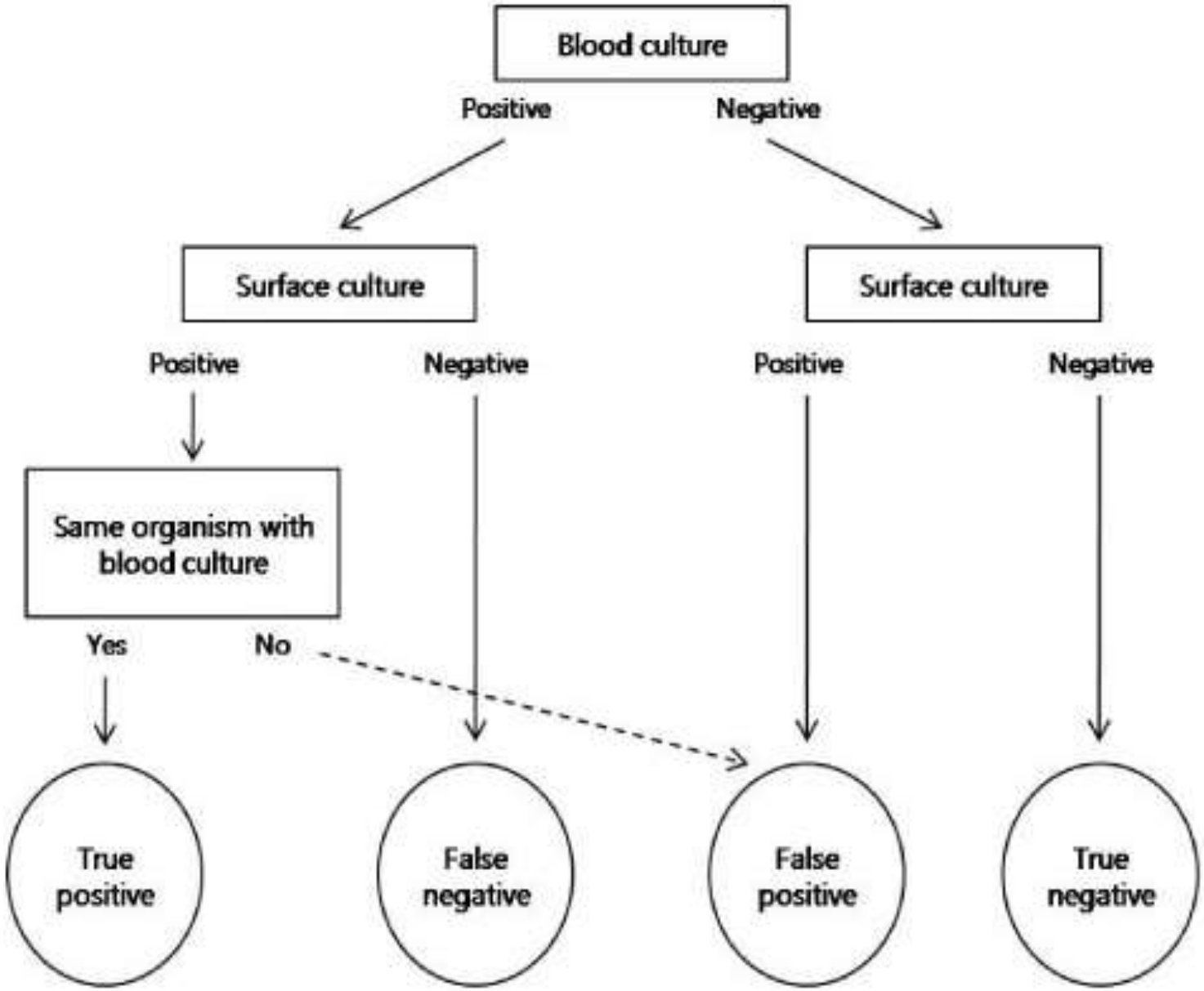


Load

❖Incubation

- Culture should be retained for at least 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.

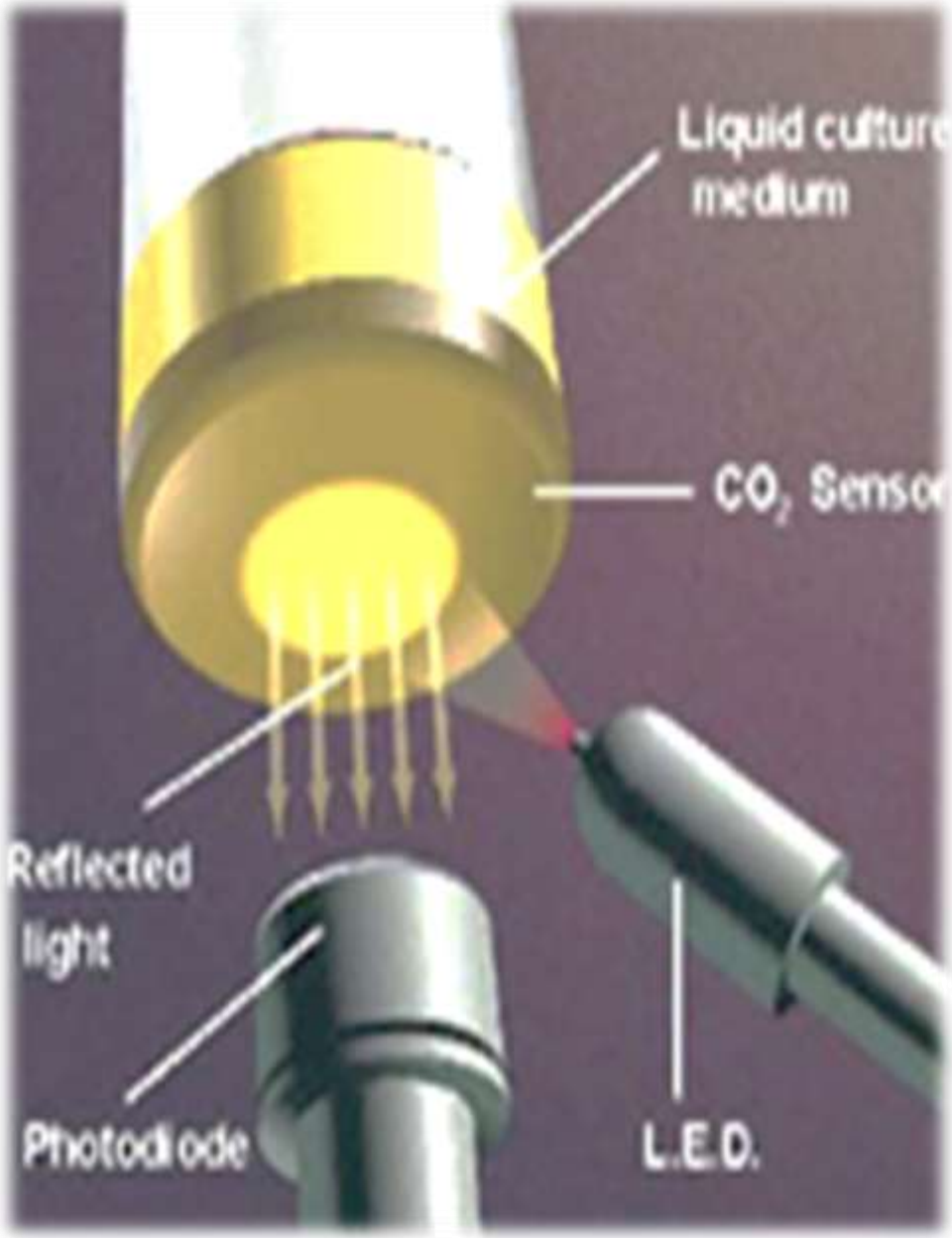
❖ 4th : Result

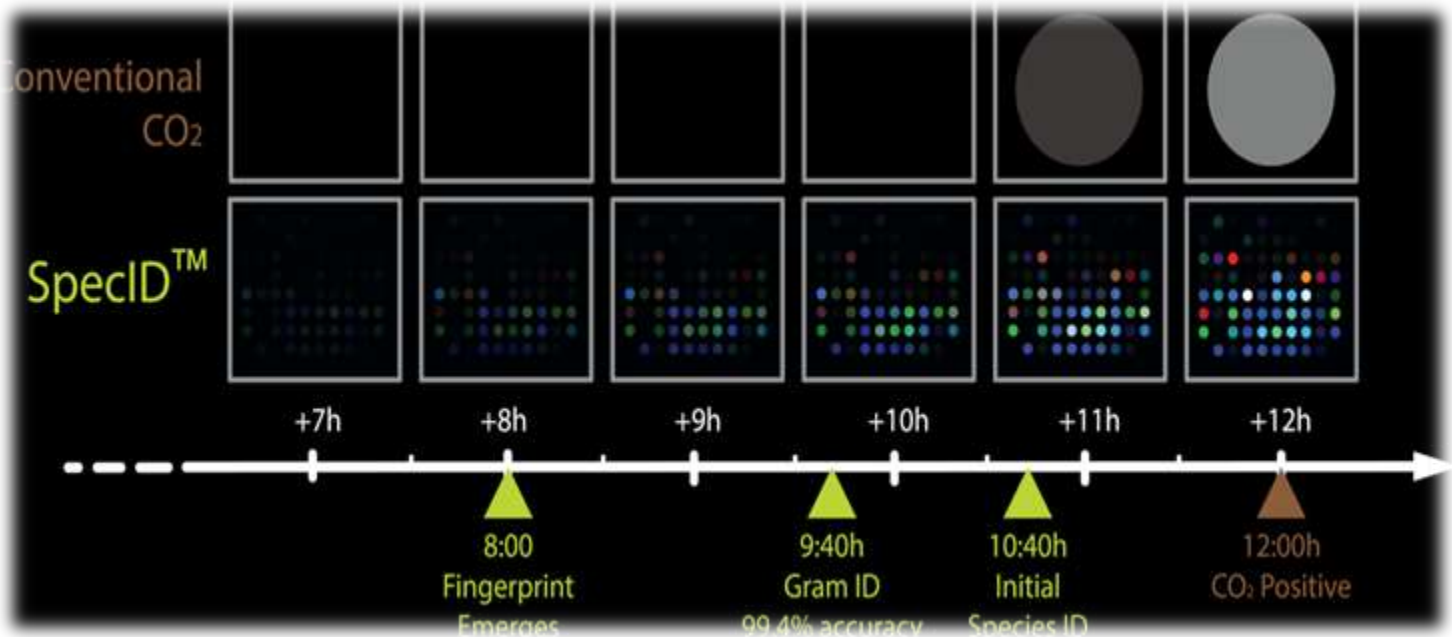

Blood culture
Positive
Negative
Surface culture
Surface culture
Positive
Negative
Positive
Negative
Same organism with blood culture
Yes
No
True positive
False negative
False positive
True negative

❖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. Measuring reflected light, the monitors and detects color changes in the sensor.

- **It's depending on the system machine.**

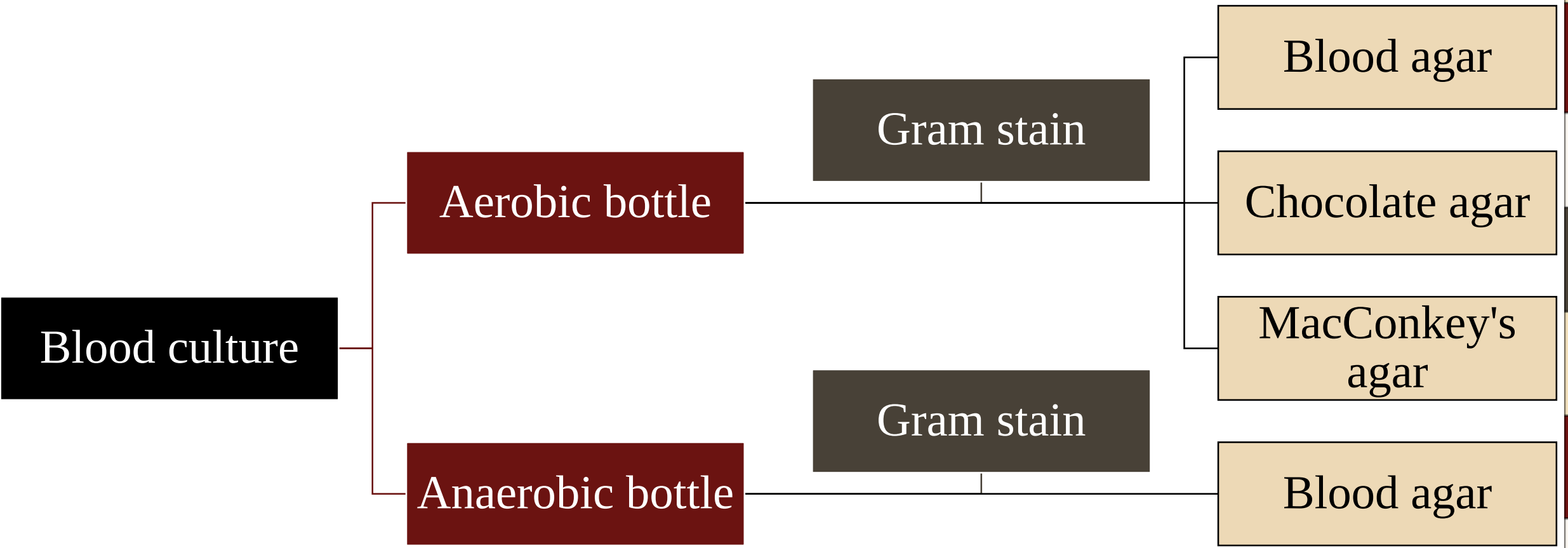

Liquid culture medium
CO_2 Sensor
Reflected light
Photodiode
L.E.D.


Conventional CO_2
SpecID™
+7h
+8h
+9h
+10h
+11h
+12h
8:00
Fingerprint
Emerges
9:40h
Gram ID
99.4% accuracy
10:40h
Initial
Species ID
12:00h
CO_2 Positive


Positive
Negative

❖ 5th : Specimen Processing

- The bottle incubated for 24 hour before plating to enhance the growth of bacteria, **aerobic bottle plate** on blood agar, MacConkey, and chocolate. In CO_2 incubator for 24 hour, **anaerobic** incubate anaerobically on blood agar for 48 hour, and the negative bottle should be re-incubated and tested after 10 days before discarded as negative culture.


Blood culture
Aerobic bottle
Anaerobic bottle
Gram stain
Gram stain
Blood agar
Chocolate agar
MacConkey's agar
Blood agar

- 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.

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)

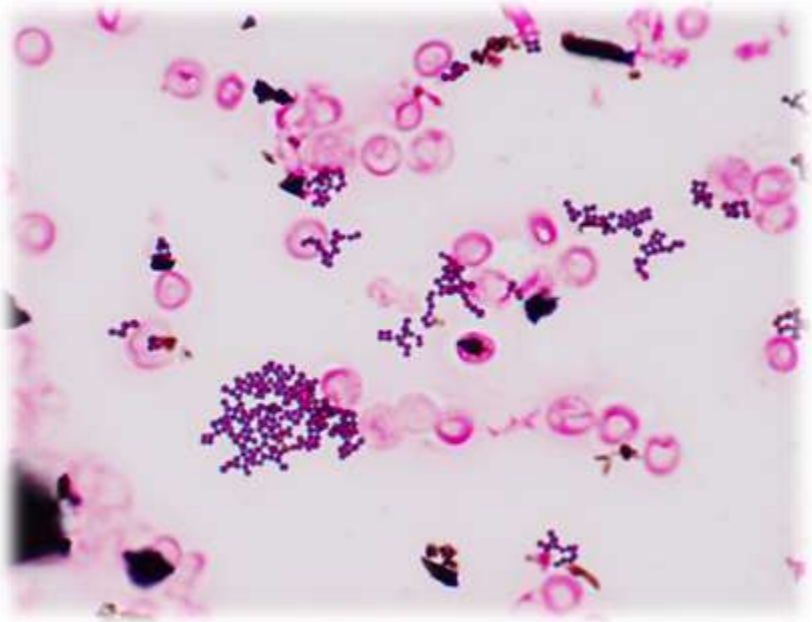
- The anticoagulant in blood culture medium must not harm the bacteria and must prevent clotting of the blood, which entrap bacteria and prevent their detection .
- The most commonly used preparation in blood media is 0.025% to 0.05% SPS.

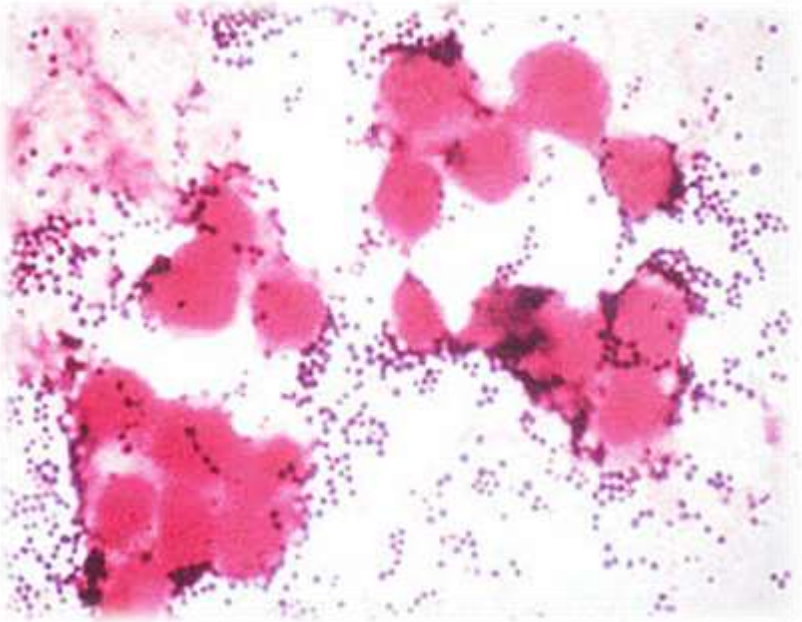
- In addition to it’s anticoagulant properties, SPS is :
  - **Anticomplementary**.
  - **Antiphagocytic**.
  - **Interferes** with the activity of some antimicrobial agents.

❖6th : Sub-Culturing

Blind Sub-Culturing syringe and drip methods

❖7th : Gram staining





Any Questions