Microbiology

BY:

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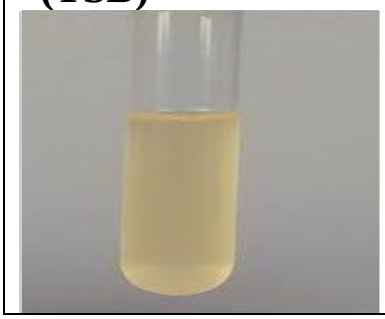
Culture: we have different kinds of media

Media	The medium contains	Selective/Enrichment/Biochemical of Media
Mac Conky (MAC) 	• peptone • lactose (to • bile salts • crystal violet • NaCl • neutral red • agar • H_2o	**Selective Media** Gram negative, Different between (**LF &NLF**) **Sugar:** lactose
Xylose lysine deoxy (XLD) 	• yeast extract • xylose • lysine • lactose • sucrose • sodium deoxycholate • sodium thiosulfate	**Selective Media** Different between (***Salmonella* & *Shigella***) **Sugar:** lactose, Xylose, Sucrose
Campylobacter (Camp) 	• Nutrient Broth • Casein hydolysate • Ferrous sulphate • Sodium pyruvate • Sodium desoxycholate • Agar • Bacteriological charcoal	**Selective Media** For *C.jejun* &*Brucella*

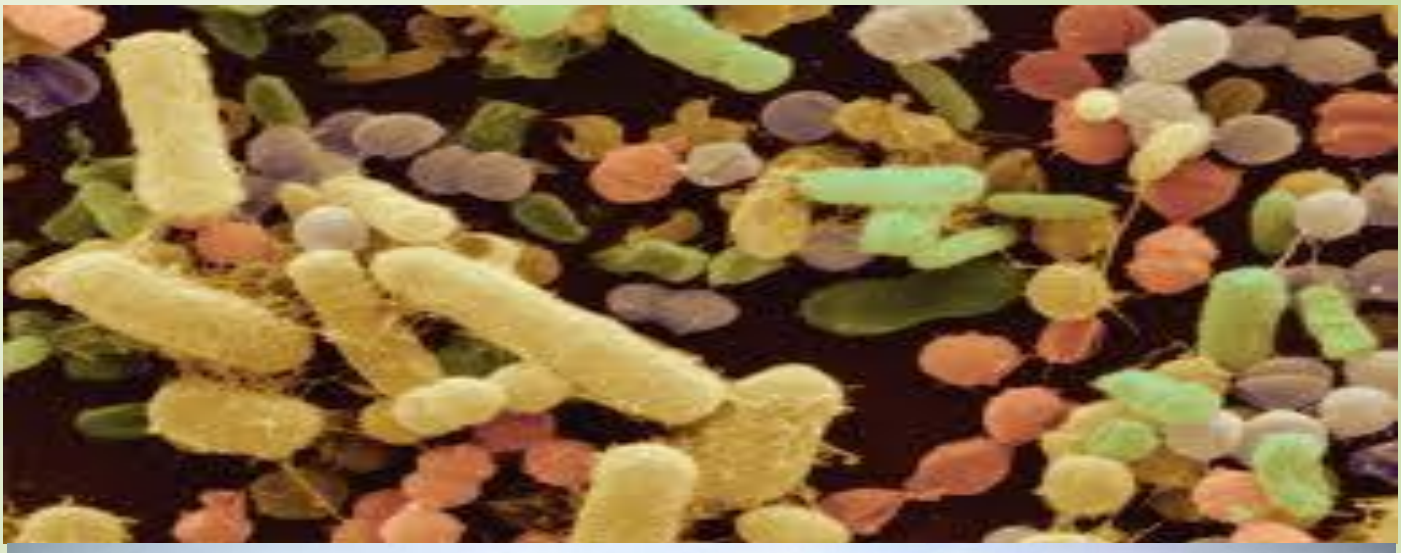
Manital salt agar (MSA) 	• enzymatic digest of • enzymatic digest of • beef extract • D-mannitol • sodium chloride • phenol red • agar	**Selective Media** For *Staph aureus* PH:7
Sabouroud media 	• dextrose • peptone • agar • pH 5.6	**Selective Media** For *Candida sp*
Selent F broth 	• Lactose • Sodium Selenite • Sodium Phosphate • H_2O	**Enrichment Media** For ***Salmonella* & *Shigella*** **Salt:** Sodium Biselenite
Blood agar 	• Meat extract • Tryptone • Sodium chloride • Agar	**Enrichment Media** Enriched and Differential media for gram (+/-)

Chocolate agar 	• 10% fresh sheep blood • beef extract (contains nutrients) • peptone • NaCl • agar (gelifying agent) • H_2O	**Enrichment Media** For Gram (+/-),H.Influenza
Thayer martin media (TM) 	• Tryptone • Soytone - • Sodium Chloride • Agar	**Selective Media** For ***Neisseria gonorrhia*** **Chocolate agar**+3 antibiotic (VCN) (Vancomycin for gram(+) ,Colistin for gram (-), Nystain for antifungal)
Lowenstein Jensen (LJ) 	• Magnesium • Sulfate Sodium • Citrate • L-Asparagine • Potato Flour • Malachite Green • Glycerol • Whole Egg	**Selective Media** For *Mycobacterium* TB
Trypticase soy broth (TSB) 	• Tryptone • Soytone • Sodium Chloride • Agar	**Enrichment Media** For any organism

Tri sugar iron (TSI) 	• agar • phenol red •lactose • sucrose •glucose •sodium thiosulfate •ferrous sulfate or ferrous •ammonium sulfate	**Biochemical media** Different between (***Salmonella* & *Shigella***) **Sugar:** lactose,Glucose,Sucrose
Ureas media 	•Peptone •Dextrose •Sodium chloride •Potassium phosphate, •monobasic Urea •Phenol red •Agar	**Biochemical media** Different between (***Salmonella* & *Proteus***) 1 g 1 g 2 g 20 g 0.012 g
Citrate media 	•citrate •ammonium ions, •bromothymol blue •inorganic ions	**Biochemical media** Different between (***E.Coli* (-) & *Klebsiella* (+)**)
Hektoen Agar 	•Proteose peptone • Yeast extract •Lactose • Sucrose • Salicin • Bile salts • Sodium chloride • Sodium thiosulphate • Ammonium ferric citrate • Acid fuchsin • Bromothymol blue • Agar	Selective and differential **Purpose:** Detects lactose fermentation, H_2S production, inhibits non-enterics used to recover *Salmonella* and *Shigella* from patient specimens.

Eosin-methylene Blue Agar (EMB) 	• peptone • lactose • sucrose • dipotassium phosphate • agar • eosin Y • methylene blue	**Selective Media** Differentiates lactose fermenters (*E. coli*) from non-fermenters (*Salmonella*, *Shigella*)
Cysteine Lactose Electrolyte Deficient (CLED) agar 	•Peptone •'Lab Lemco' powder •Tryptone •Lactose •L-Cysteine •Bromothymol blue •Agar	**Selective Media** differentiate urinary tract bacteria, since it inhibits *Proteus* species swarming and can differentiate between lactose fermenters and non-fermenters.

Bacteriology



Bacteriology

Bacteria are microscopic organisms whose single cells have neither a membrane-enclosed nucleus nor other membrane-enclosed organelles like mitochondria and chloroplasts.

Bacteriology laboratory :

1- General .
2- Blood.
3- Stool.
4- Urine .
5- TB .

All staffs of microbiology depend on two steps when introducing of organism :

- Gram stain .
- Culture .

- **Procedure of gram stain :**
  1. Prepare smear from specimen, air dry, heat fix.
  2. Add crystal violet (purple), wait 30 seconds. Rinse with water.
  3. Add Iodine (Iodine is a mordant; Mordant allows the crystal violet to stick to the cell wall of a gram positive bacteria. Wait a minute.
  4. Decolorize with alcohol until slide runs clear. Rinse with water.
  5. Counter stain with Safranin (red) and wait 30 seconds.Rinse with water.
  6. Blot, dry, examine
- **Culture :**

  There are two types of media :

  1- Solid .
  2- Liquid

1- Urine bench

Sample receiving urine:

1-Chemical analysis
2- Microscopy
3- Culture.

Chemical analysis:

1- pH 2- Specific Gravity (sp gr) 3- Protein
4- Glucose 5- Ketones 6- Nitrite
7- Leukocyte Esterase

Urine Microscopy:

We are looking for:

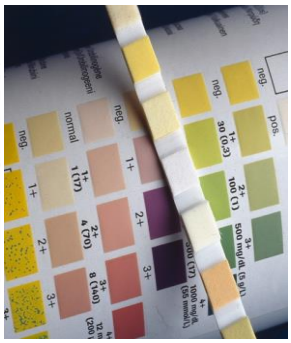
*Bacteria
*RBC(Red Blood Cells)
*WBC(White Blood Cells)
*Yeast
*Cast (Hyaline,Waxy.Cellular and Granular)
*Crystals (Cystine.Cholesterol.Sulphonamidem.Triplphosphate and Calcium Oxidate)
*Epithelial Cell

When WBC more 10 :We DO Culture the urine(whether we find bacteria or not)

When WBC lees 10 : We DON'T Culture the urine(but if the sample contains bacteria we have to culture)

When WBC lees 10 (for children less than 12 years ,in Patient& Oncology : We DO Culture the urine

When WBC OR RBC is high we write (Too Numerous To Count **TNTC**)



Urine culture:

we culture the sample on:

- ***Blood agar**
- ***MacConky agar**
- ***CLEDagar**
- ***Sabouroud**

→ Bacteria

→ yeast

Reading the culture

culture

Sabouroud	Blood	MacConky	CLED
grow by yeasts and Candida sp	**Hemolysis Patterns** Beta hemolysis Alpha hemolysis Gamma hemolysis	LF &NLF **LF → pink** **NLF → yellow**	LF &NLF LF → yellow NLF → colorless

1- MacConky:

A- LF → pink:

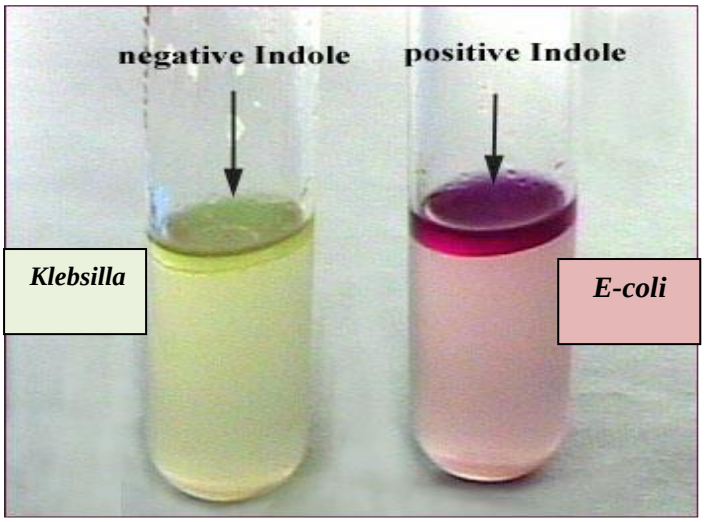
E-coli, Klebsilla, Pseudomonas, Acinto bacter, Aeruginosa.

B- NLF → yellow:

Shigella,Salmonella,Proteus.

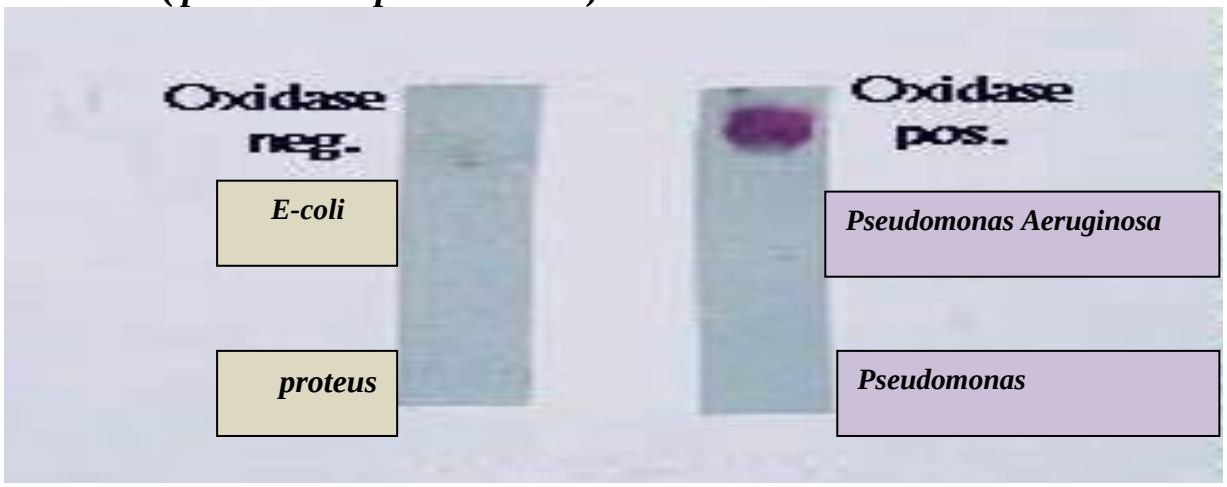
chemical reactions:

1-indole test (*E-coli and Klebsilla)*

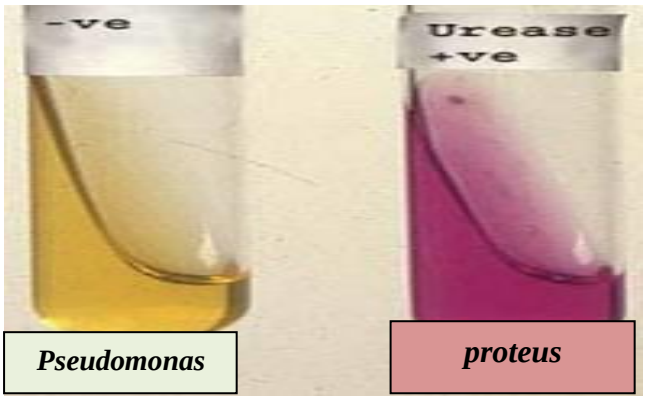


2- Oxidase test

A-*(E-coli* and *Pseudomonas Aeruginosa)*
B-(*proteus* and *pseudomonas*)

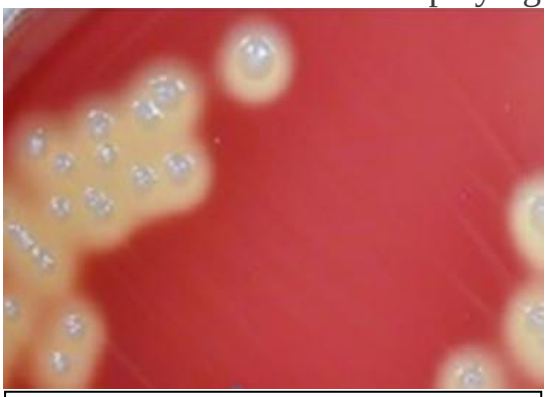


3-Urease test(*proteus* and *pseudomonas*)



2-Blood agar:

A- Beta hemolysis means that the bacteria's hemolytic exotoxins completely beak down the blood cells. The β-hemolysis pattern results in the media displaying clear halos around bacterial colonies.



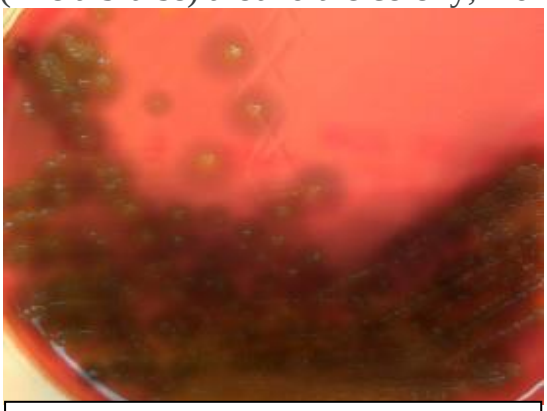
beta-hemolysis

Example:

Sterpt. Pyogenes

Sterpt. Agalactiae

B- Alpha hemolysis (α-hemolysis) means that the bacteria generate chemicals that only partially break down the blood cells. This results in the media showing a yellowish/greenish/brownish discoloration (like a bruise) around the colony, indicating incomplete hemolysis.



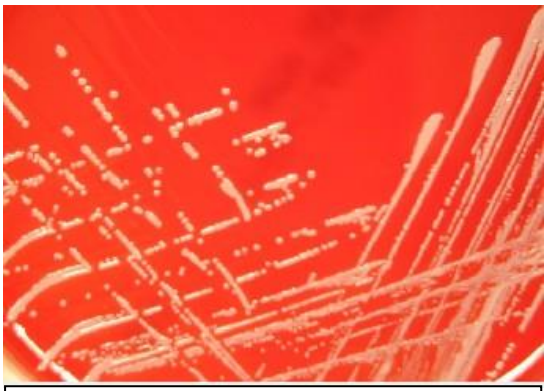
alpha-hemolysis

Example:

Sterpt. Pneumonia

Sterpt. Viridans

C- Gamma hemolysis is essentially no hemolysis at all. The bacteria have no effect on the red blood cells, and there is no change to the color of the medium.



gamma-hemolysis

Example:

Sterpt. Bovis

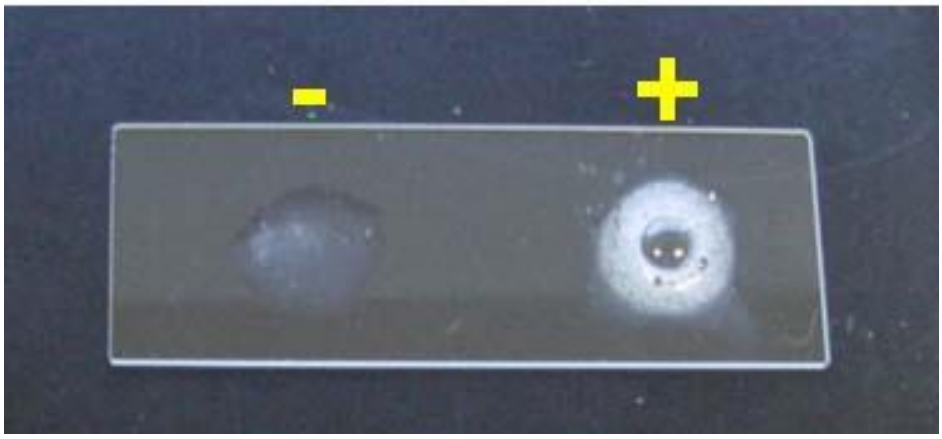
Sterpt. Faecalis

chemical reactions:

A-catalase test (*Staph.Aureuse, Sterpt. Pyogenes*)

Staph.Aureuse ⟶ + (bubbles)

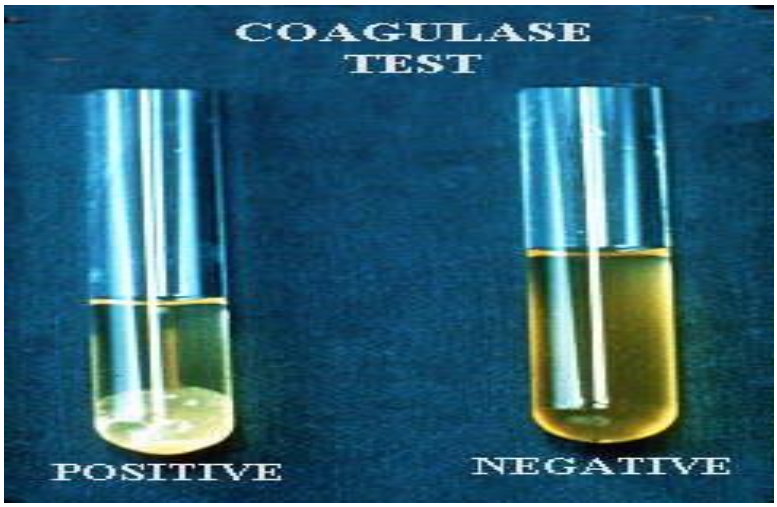
Sterpt. Pyogenes ⟶ _ (no bubbles)

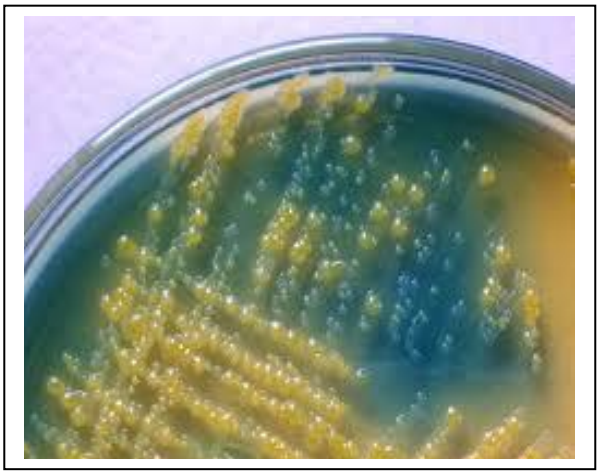


B-coagulase test(*Staph.Aureuse, another Staph*) :

Staph aureus ⟶ + (clumps)

another Staph ⟶ _ (no clumps)





3- Cled gar:

The same function MacConky

A- LF ⟶ yellow:

E-coli.

B- NLF ⟶ colorless:

Acinto bacter

***If the back of growth in the cled agar, blood agar and McConky**
It was the color of the bacteria in the :
cled ⟶ Yellow
blood agar⟶ haemolysis and large size
McConkie ⟶ pink
This is a high probability 85% (*E-Coli*).

***If the back of growth in the cled agar and gave me blue**
(Salmonella Or Shigella Or Stsphylo cocci)

***If growth does not appear in the cled agar and McConkie**
But the back of the growth of bacteria in the blood agar and small
This is kind of the Strept.

***If the back of growth in the cled agar, blood agar and McConkie , including bacteria was great :**
This is a high probability of *Candida*.

4- Sabouroud:

grow by *yeasts* and *Candida* sp

2- Blood bench

1- After sterilize the area very well, pull sample from the patient.
2- After that we should put the blood sample inside closed (Bactec bottles), it is including damper for antibiotic, we use tow Bactec bottles. One of the bottles will be incubate under aerobic condition and the other one will be under anaerobic condition. The goal from previous step is for the development of aerobic and anaerobic bacteria.
3- All the samples will insert in Bactec device (this device is using for discovering the Bactria from the sample), the sample should incubate for five days (sometimes it is take longer than five days, it is based on Doctor instructions).
4- After we incubated the samples for five days, if the result is negative, we should get rid of the sample, **but if the result is positive we will use the following steps:-**
A- Remove the sample from Bactec device and we should after that make gram stain slide and examine it under a microscope.
B- Positive sample will cultivate in three different media , and it will incubate for 24 hours and after that we will examine it.

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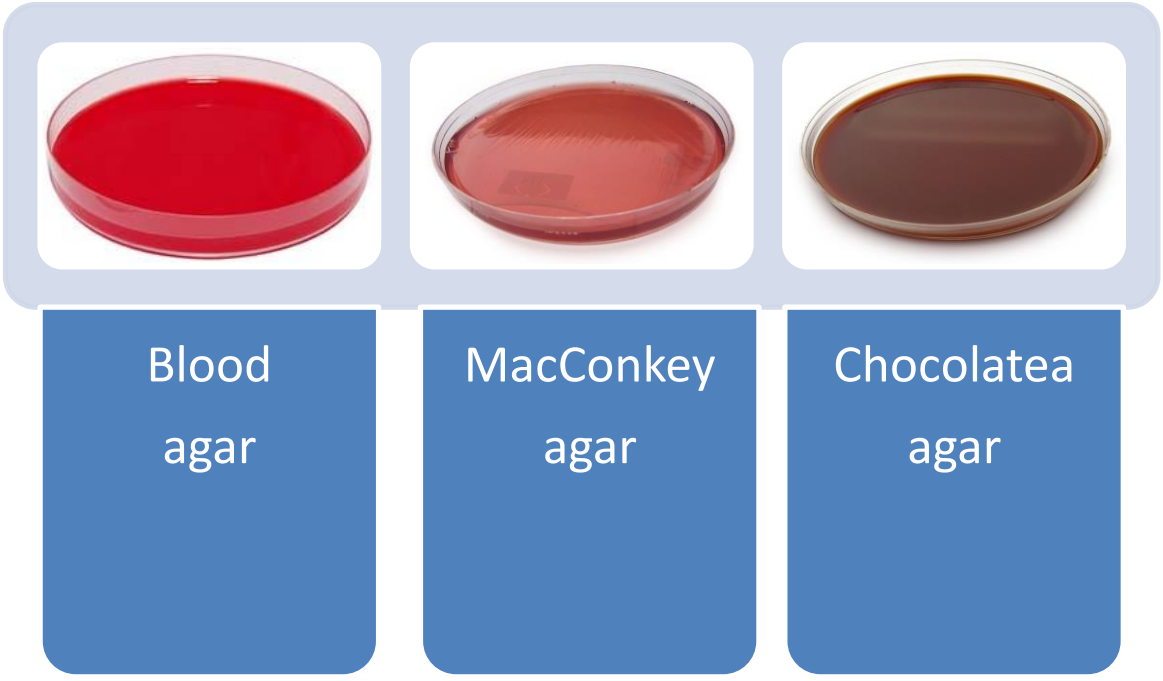
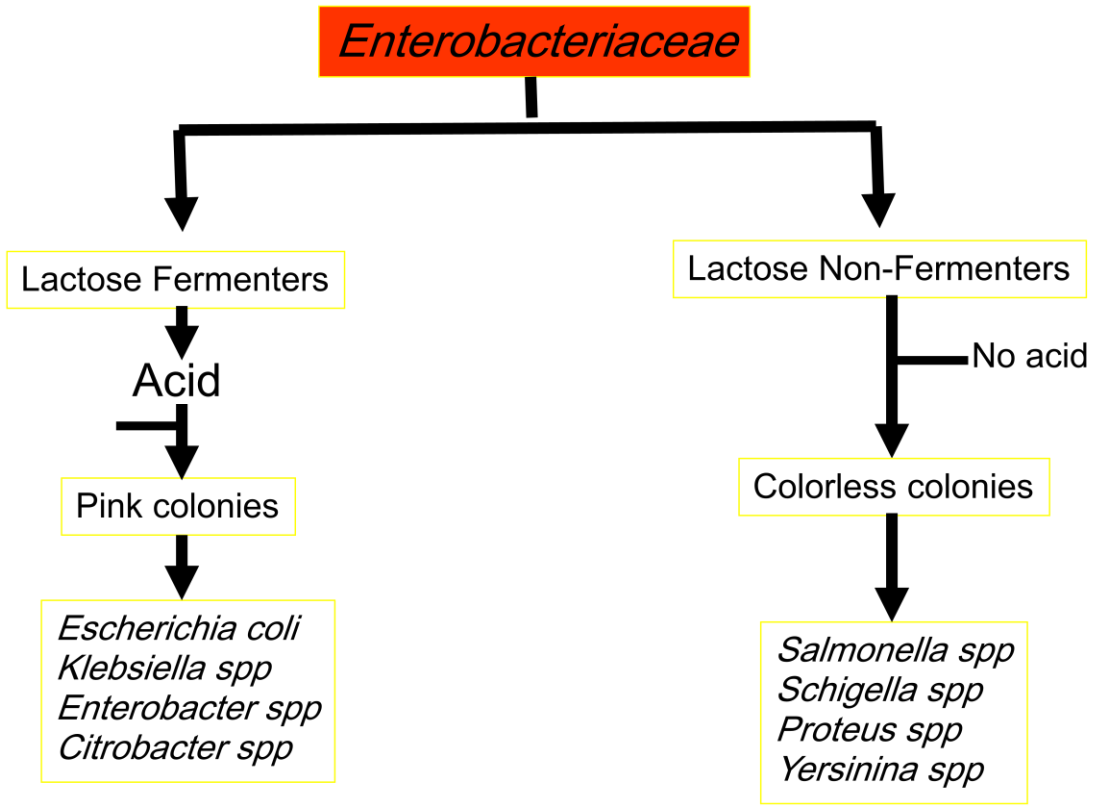
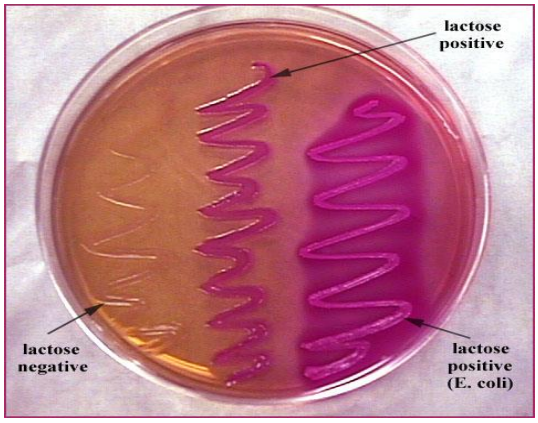


* After passing 24 hours, if there is no progress in the petri dishes, we will incubate it 24 hours as an addition, if it is also failed, we should get rid of the sample.
5- After incubated petri dishes we will read the results as follows:

MacConkey agar:



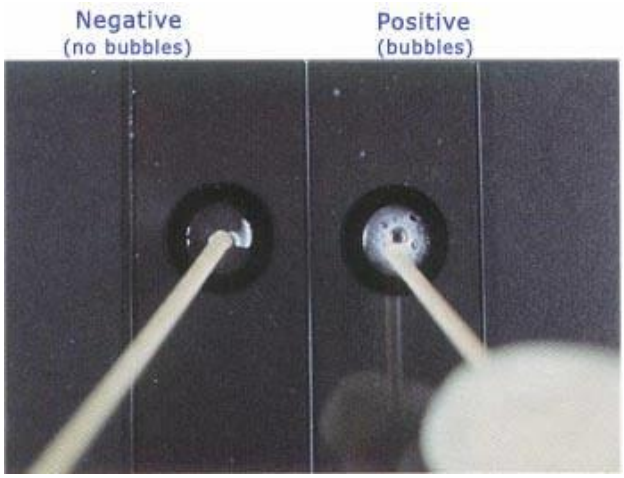


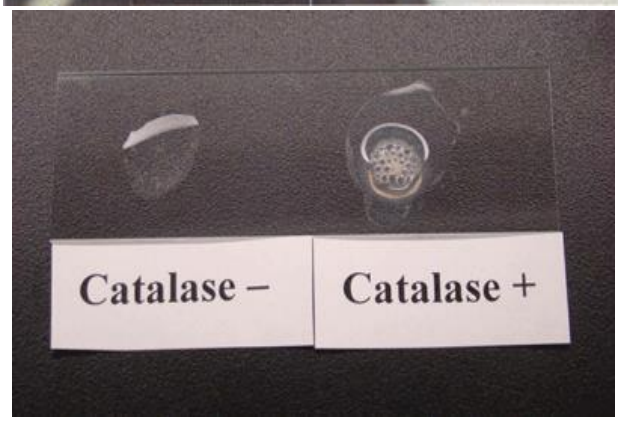
Blood agar: **There are 3 types of hemolysis :**

Catalase test: **it is a basic test to differentiate between Staphylococci and Streptococci**

Staph ⟶ + (bubbles)

Strept ⟶ _ (no bubbles)

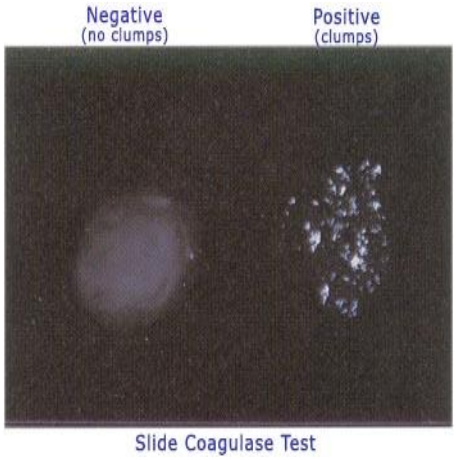




Coagulase test: it is used to differentiate types of *Staphylococcus* isolates.

Staph aureus ⟶ + (clumps)

another Staph ⟶ _ (no clumps)

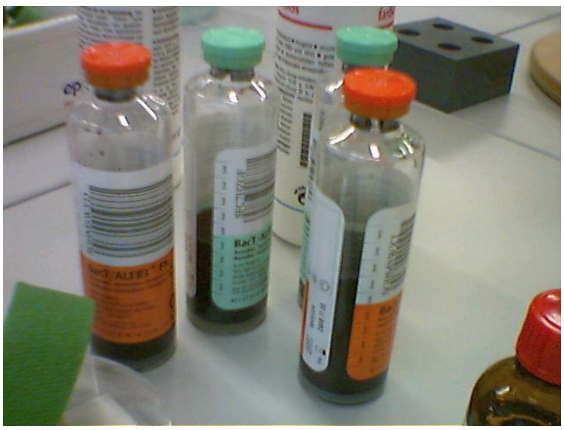


Chocolate agar: . Chocolate agar is used for growing fastidious respiratory bacteria, such as *Haemophilus influenzae* and *Neisseria meningitidis*.

PRINCIPLE:

When microorganisms are present in culture vials, they metabolize nutrients in the culture medium, releasing carbon dioxide into the medium. A dye in the sensor at the bottom of the vial reacts with CO2. This modulates the amount of light that is absorbed by a fluorescent material in the sensor. A photo detector at each station measures the level of fluorescence, which corresponds to the amount of CO2 released by organisms. Then the measurement is interpreted

by the system according to pre-programmed positivity parameters. At system startup, the onboard computer performs self-diagnostics and downloads operating instructions to the drawer rows. Then the instrument (s) automatically begin testing. Light Emitting Diodes (LEDs) behind the vials illuminate the rows, activating the vials' fluorescent sensors. After a warm -up period, the instrument's photo detectors then take the readings. A test cycle of all rows is completed every ten minutes. Positive cultures are immediately flagged by an indicator light on the front of the instrument, an audible alarm, and are displayed on the LCD display. When positive vials are identified, the lab technologist pulls them from the instrument for confirmation of results, and for isolation and identification of the organism.



3- General bench

The general bench receive all samples , except for the blood, feces, urine .

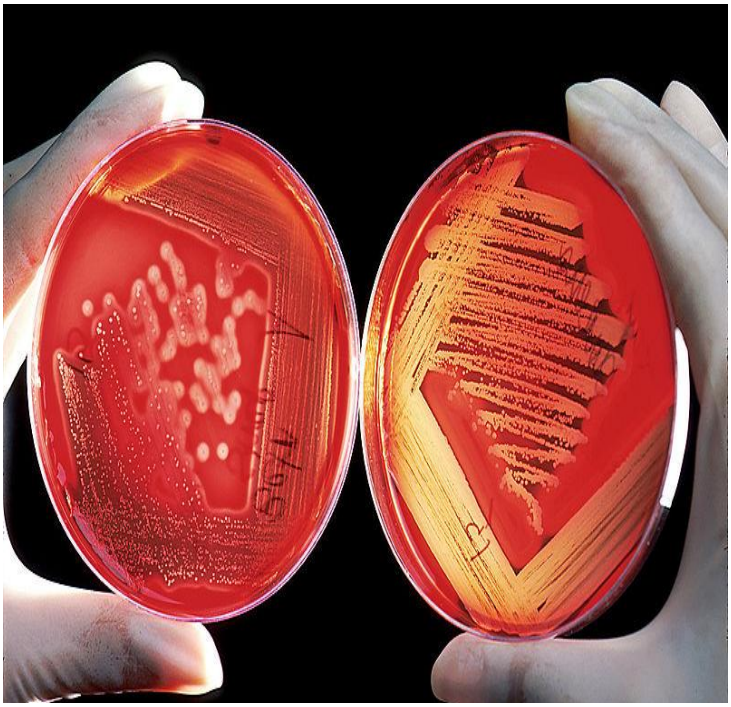
Samples :

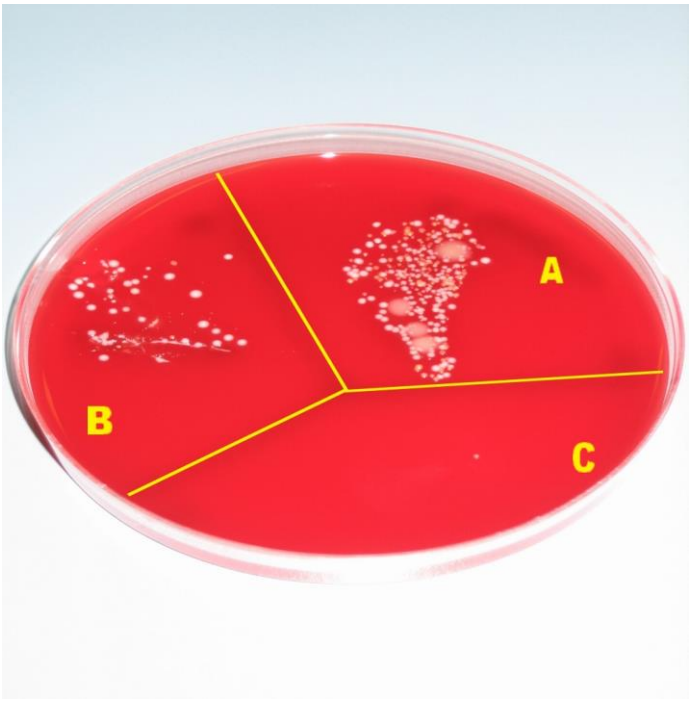
1- Swap : is all swaps from anywhere in the body , such as skin and nose .
2 – Cerebrospinal Fluid (CSF) .
3 – all Tissue and bone

Media used :

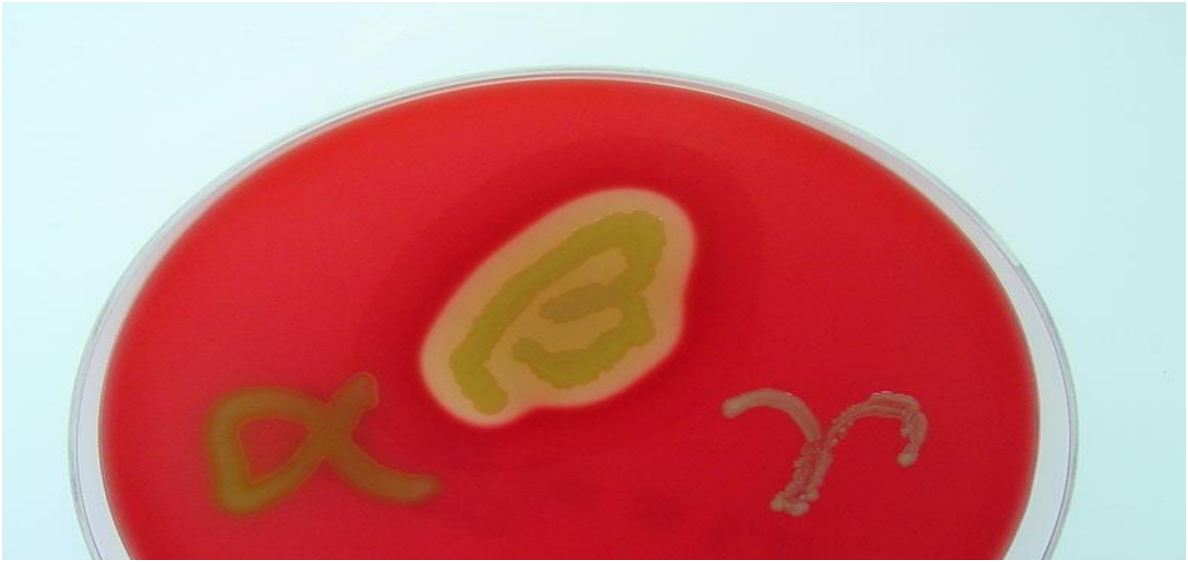
1-Blood Agar

Enrichment media and differential





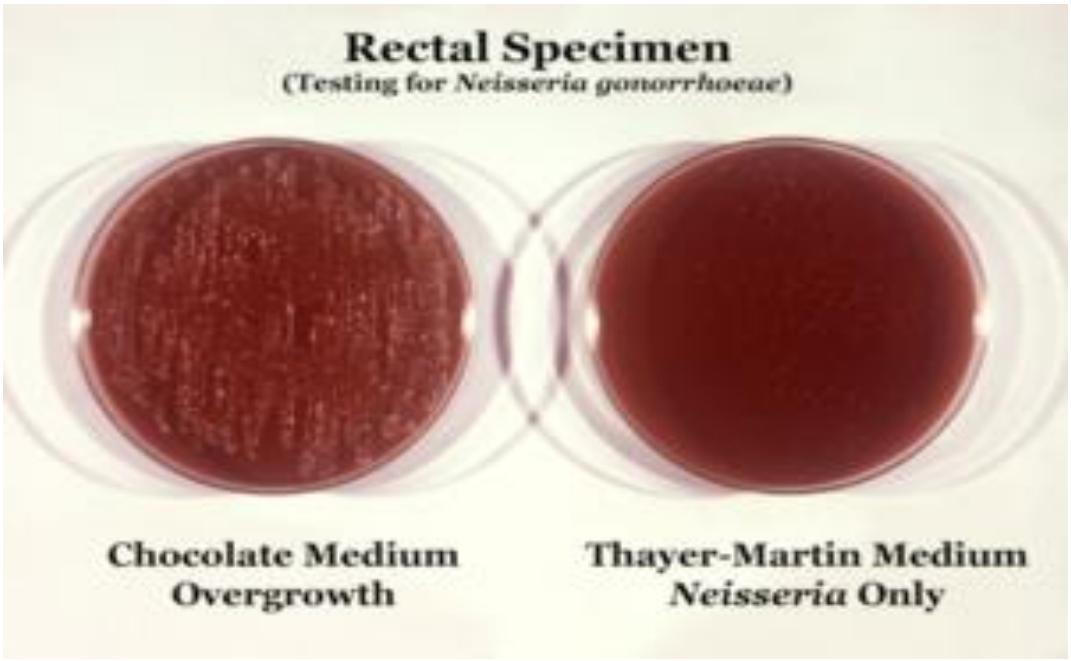
Red blood cells on an agar plate are used to diagnose infection. On the left is a positive Staphylococcus infection, on the right a positive Streptococcus culture.



Hemolysis of Streptococcus spp. (left) α-hemolysis (S. mitis); (middle) β-hemolysis (S. pyogenes); (right) γ-hemolysis (= nonhemolytic, S. salivarius)

2-Chocolate Agar

Chocolate agar (CHOC) is a type of blood agar plate in which the blood cells have been lysed by heating the cells to 56 °C. Chocolate agar is used for growing fastidious (fussy) respiratory bacteria, such as *Haemophilus influenzae*. No chocolate is actually contained in the plate; it is named for the coloration only.



3-MacConkey Agar

MacConkey agar is a culture medium designed to grow Gram-negative bacteria and differentiate them for lactose fermentation.

4-Cock meat

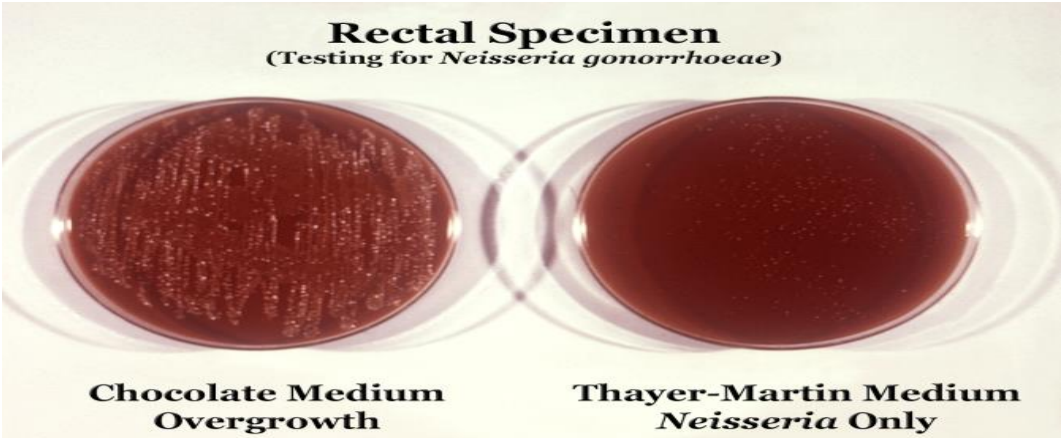
for cultivation of anaerobic microorganisms.

5-Sabouraud agar

Selective media , grow by yeasts and Candida sp

6_ Thayer martine agar:

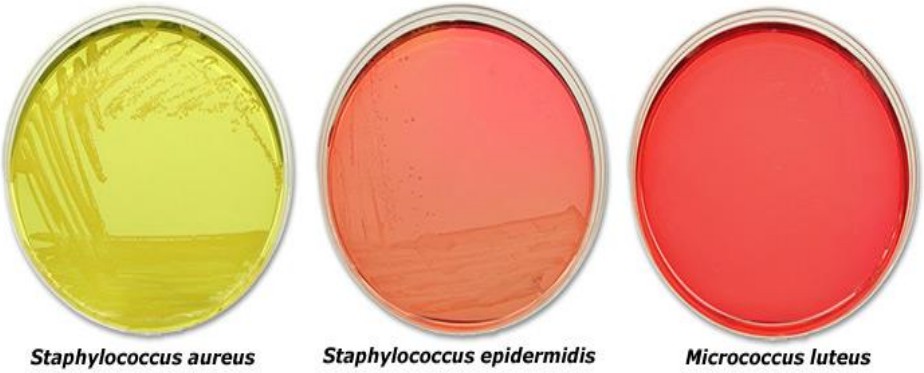
Thayer-Martin agar (or Thayer-Martin medium) is a Mueller-Hinton agar with 5% chocolate sheep blood and antibiotics. It is used for culturing and primarily isolating pathogenic *Neisseria bacteria*, including *Neisseria gonorrhoeae* and *Neisseria meningitidis*, as the medium inhibits the growth of most other microorganisms. When growing Neisseria meningitidis, one usually starts with a normally steril body fluid (blood or CSF), so a plain chocolate agar is used.



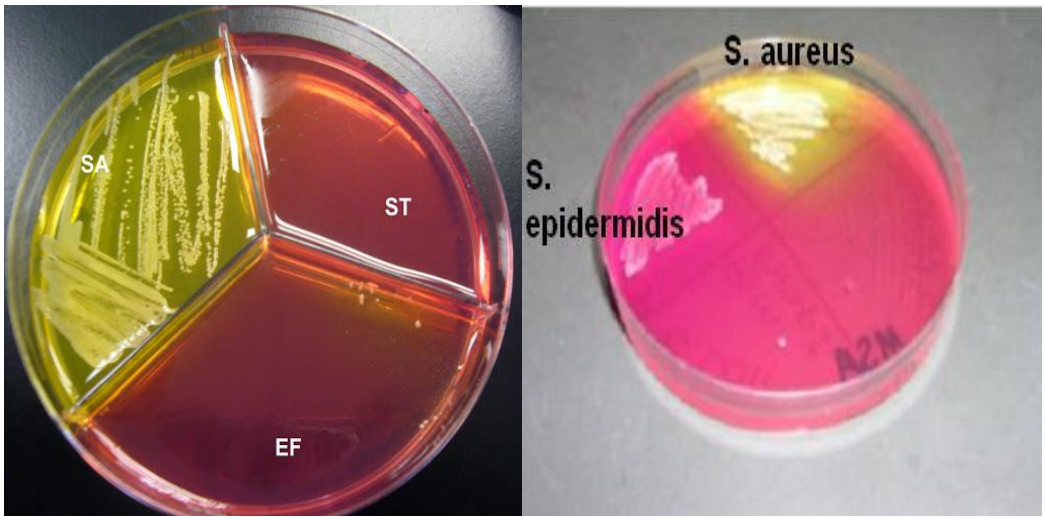
7 - Mannitol Salt Agar

is a commonly used growth medium in microbiology. It encourages the growth of a group of certain bacteria while inhibiting the growth of others. selective for gram positive bacterium Staphylococci since this level of NaCl is inhibitory to most other bacteria. Staphylococci aureus produce yellow colonies with yellow zones, whereas other Staphylococci produce small pink or red colonies with no color change to the medium.

for Halophilic bacteria especially staphylococcus species (staph. Aureus give yellow color , other staph. Give pink color)

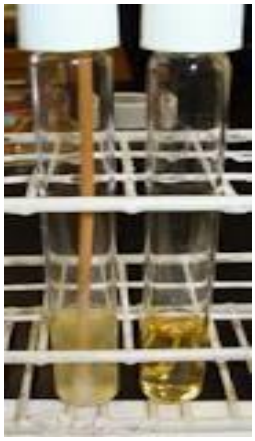


MSA (Mannitol Salt Agar)

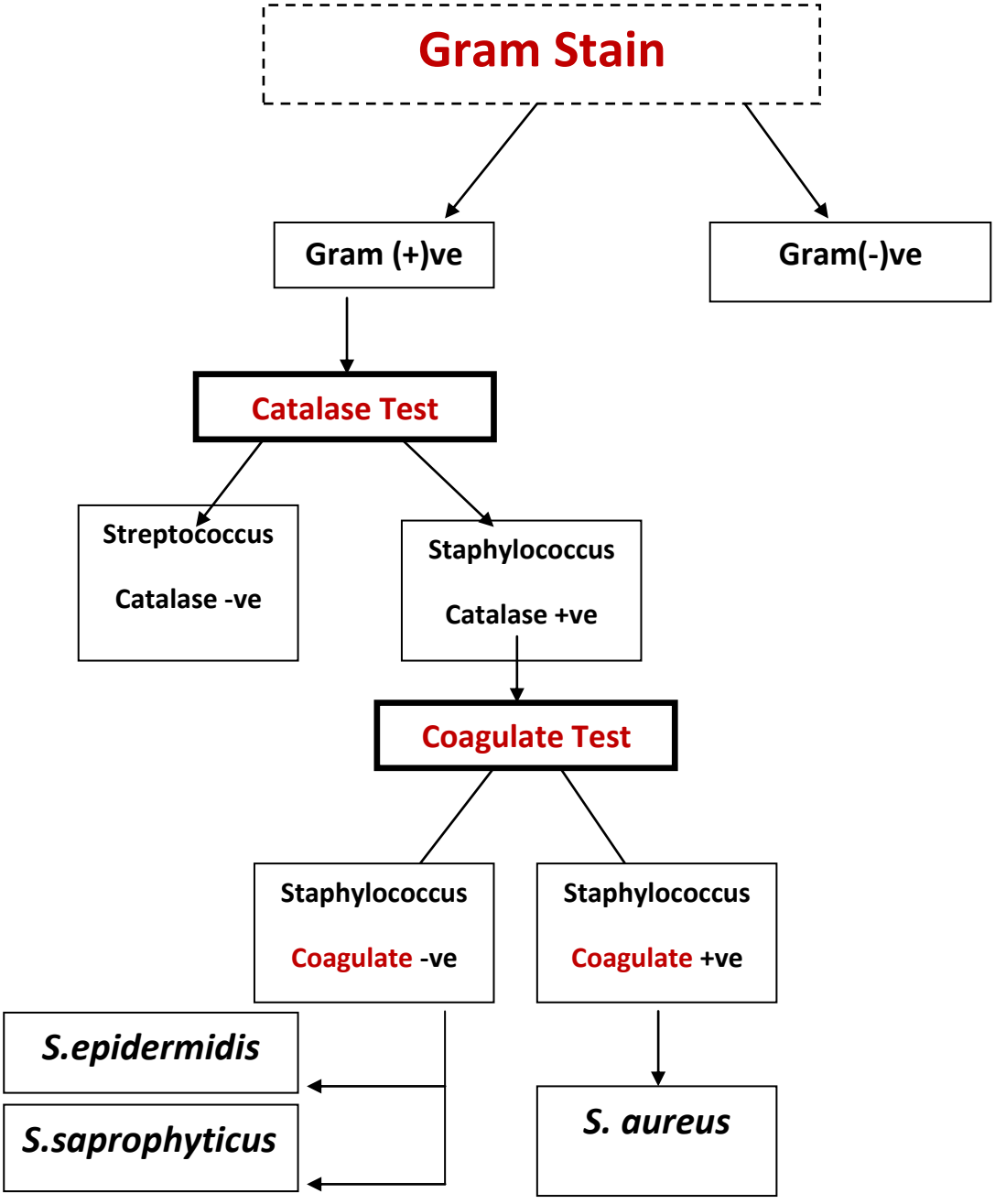


8- Todd-Hewitt broth:

Todd Hewitt Broth primarily is used for the growth of beta-hemolytic streptococci for use in serological testing. special media for CSF sample especially haemophilus influenzae ،is used for the cultivation of streptococcus and other fastidious microorganism



Reading and Test



3- Stool bench

Stool cultures for determine *Salmonella,Shigella* and *Campylobacter*
We have different kinds of media :
In the Stool lab working on some of them ..
Inoculation on :

• **Selenite-F broth :** Used as a selective enrichment for the cultivation of Salmonella spp. that may be present in small numbers and competing with intestinal flora.	
• **MacConky agar (MAC) :** specialized bacterial growth medium that is selective for Gram-negative bacteria and can differentiate those Gram- bacteria that are able to ferment lactose .	
• **Xylose lysine deoxy-cholate agar (XLD):** Selective and differential media .(different between *shigella* and *salmonella*) Salmonellae metabolise thiosulfate to produce hydrogen sulfide, which leads to the formation of colonies with black centers and allows them to be differentiated from the similarly coloured Shigella colonies	

• **Campylobacter agar (CAMPY):** **Used for the selective isolation of Campylobacter jejuni.** incubate at 42^{O}C in microaerophilic Campy Gas Pak Jar	
• **Hektoen agar (HE):** selective and differential agar , contains indicators of lactose fermentation and hydrogen sulfide H2S production; as well as inhibitors to prevent the growth of gram + bacteria.	

Oxidase test :

test used in microbiology to determine if a bacterium produces certain cytochrome c oxidases .

1. Place 1 or 2 drops of oxidase reagent on filter membrane.
2. Use a loop and pick a well-isolated colony from a fresh (18- to 24-hour culture) bacterial plate and rub onto a small piece of filter paper .
3. Observe for color changes.
4. Microorganisms are oxidase positive when the color changes to dark purple within 5 to 10 seconds so So is pseudomonas. Microorganisms are delayed oxidase positive when the color changes to purple within 60 to 90 seconds. Microorganisms are oxidase negative if the color does not change or it takes longer than 2 minutes.

Urease test :

Urease broth is a differential medium that tests the ability of an organism to produce an exoenzyme, called urease, that hydrolyzes urea to ammonia and carbon dioxide. The broth contains two pH buffers, urea, a very small amount of nutrients for the bacteria, and the pH indicator phenol red. Phenol red turns yellow in an acidic environment and fuchsia in an alkaline environment. Take 24-48 hours .

(a) (b) (c) (d)

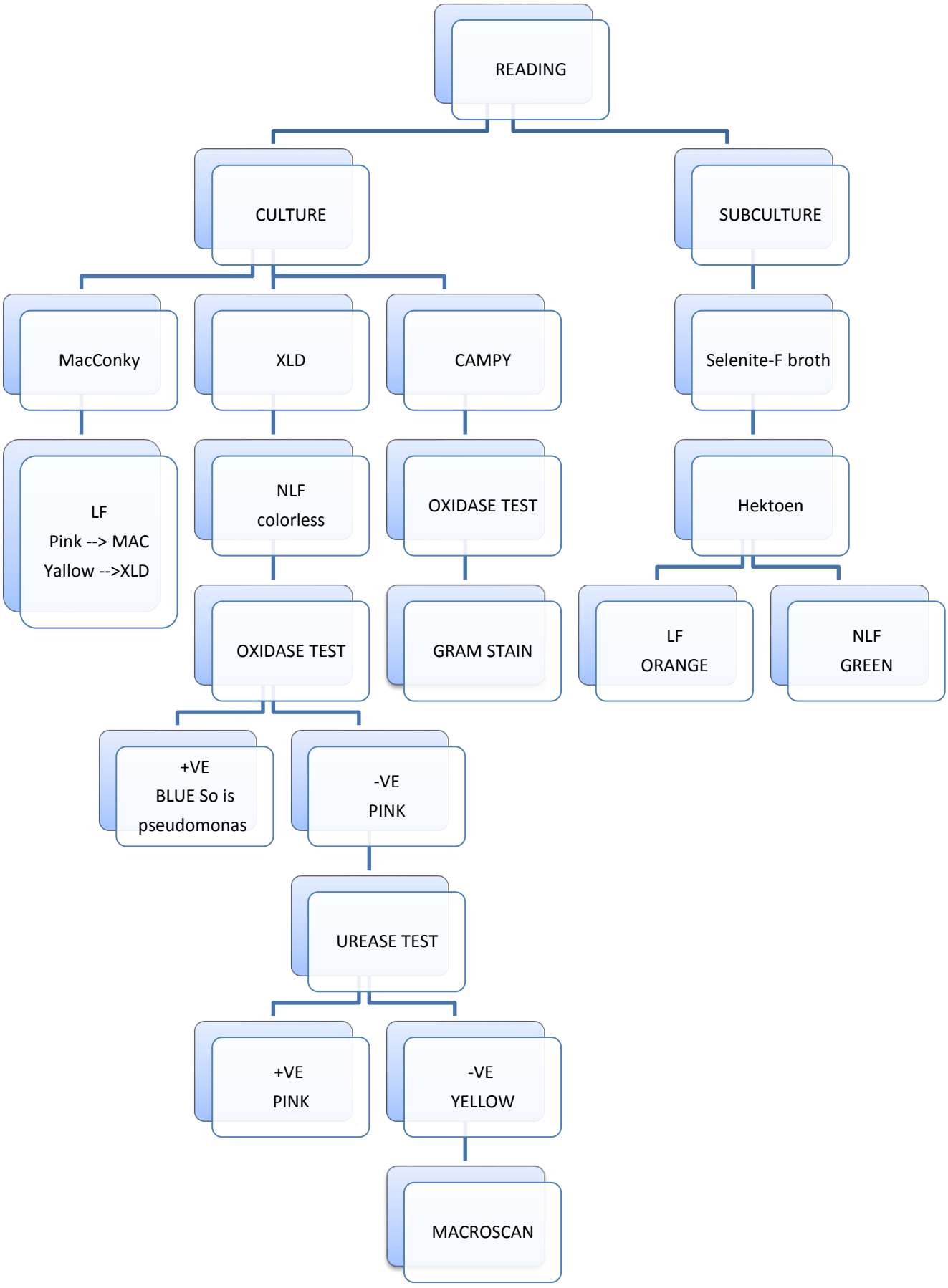
Urea agar test results. Urea agar slants were inoculated as follows:

(a) uninoculated,
(b) *Proteus mirabilis* (rapidly urease positive),
(c) *Klebsiella pneumoniae* (delayed urease positive),
(d) *Escherichia coli* (urease negative).

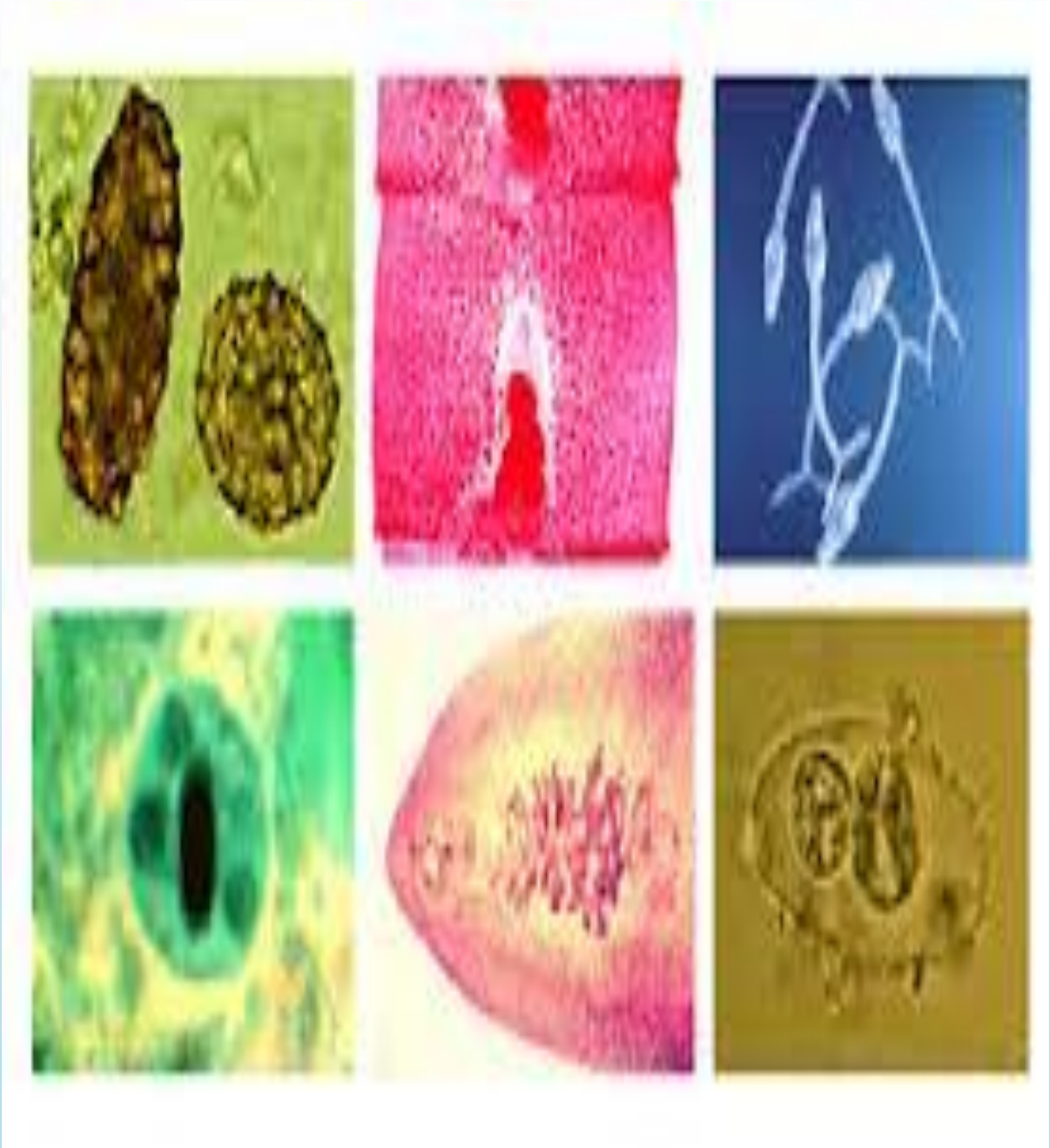
Note Any LF colonies on the MacConkey are not identified unless the specimen is from a child under 2 yrs. of age. Then a maximum of 5 isolated colonies morphologically resembling E. coli are replated to a nutrient agar for serology the following day.

Subculture the selenite F to HE and incubate overnight at 35-37°C For 24 hr.

Campylobacter plates are examined after 48 hrs. of incubation at 42^{O}C in the CAMPY microaerophilic jar. Perform an oxidase test on any colonies suspicious of Campylobacter. If positive, confirm with gram stain for the characteristic small gram negative comma shaped bacilli (S shaped).


READING
CULTURE
SUBCULTURE
MacConky
XLD
CAMPY
Selenite-F broth
LF
Pink --> MAC
Yallow -->XLD
NLF
colorless
OXIDASE TEST
Hektoen
OXIDASE TEST
GRAM STAIN
LF
ORANGE
NLF
GREEN
+VE
BLUE So is
pseudomonas
-VE
PINK
UREASE TEST
+VE
PINK
-VE
YELLOW
MACROSCAN

Barasitology

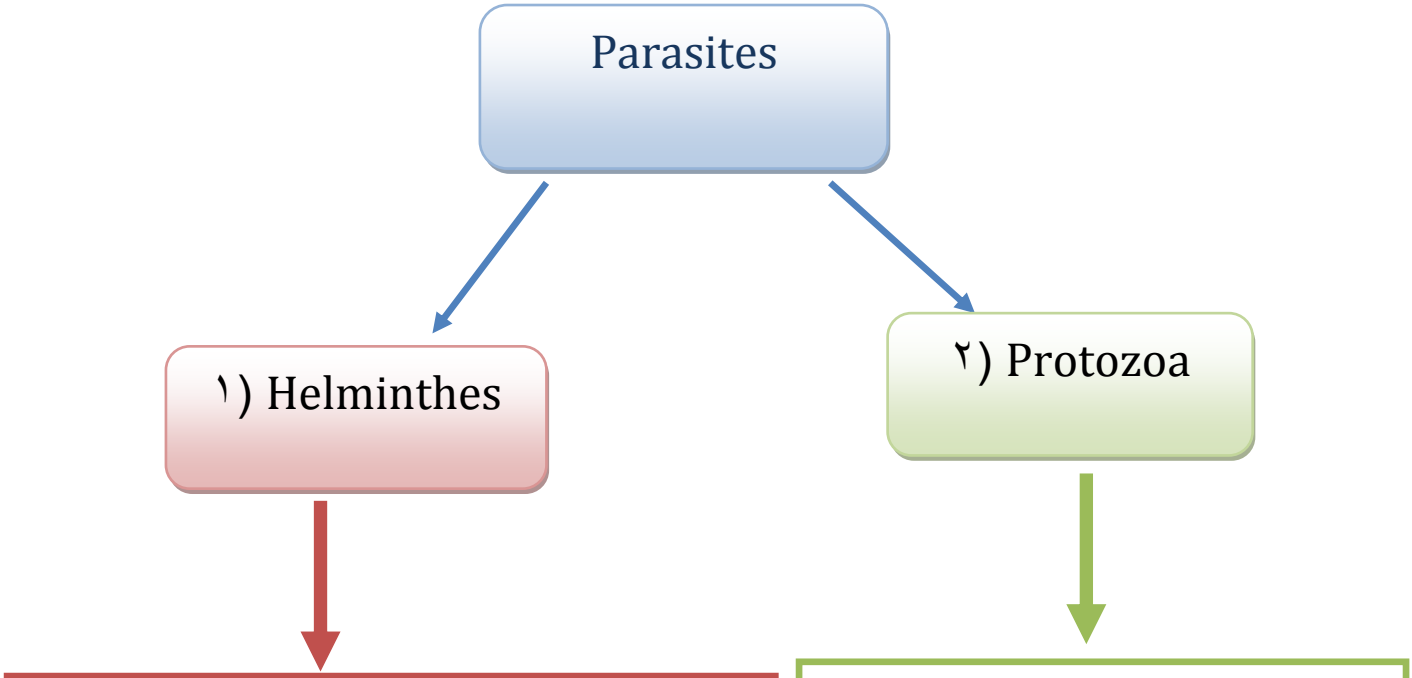


Parasite

There are two types :

Endoparasite : the parasite that lives inside the body .

Ectoparasite : the parasite that lives inside the body



A- Trematode:

-are small parasitic flatworms

- that use vertebrates as their definitive host

- includes the flukes

A-Rhizopoda:

e.g. amoeba

- move by means of pseudopods

-Rhizopods are heterotrophic

-The disease is spread by fecal contamination of drinking water, raw vegetables and careless food handlers.

B- Cestode

called tapeworms

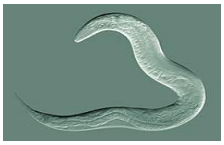
-An adult tapeworm consists of a head, neck and chain of segments called proglottids

B- Ciliata:

e.g. paramecia

- move by **cilia**

- common almost everywhere there is water — in lakes, ponds, oceans, rivers, and soils



C- Nematode

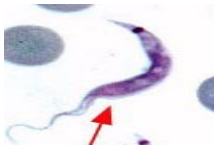
-called **roundworms**

-Nematodes are simple roundworms. Colorless, unsegmented, and lacking appendages.

C- Sporozoa: **e.g. malaria**

-Sporozoans are nonmotile, unicellular protists which are commonly parasitic on vertebrate animals.

D- Flagellates:



e.g. trypanosomes

- moves by flagella.

❍Culture and tests:

Types of tests:

1) Occult blood.

2) stool analysis

❍Material and method

1- Occult blood

Fecal occult blood (FOB) refers to blood in the feces that is not visibly apparent.

★object of test:

Detection if the sample contain blood

★Method:

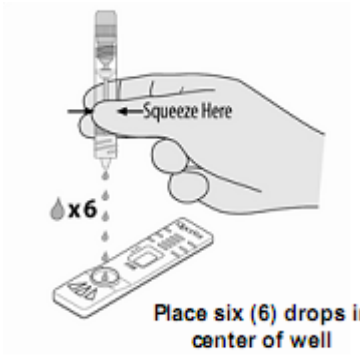
Holding the Collection Tube vertically, dispense

١- six (6) drops of

specimen solution from the Collection Tube into the center of the

.Sample Well

2- Read resultes at 5–10 MINUTES. Some positive results may be seen earlier



★Results:

- Positive Result:

If both a C-line and a T-line are present, the result is positive

-Negative Result:

If only the C-line develops in the control region .of the test strip, the result is negative

2- stool analysis

Stool analysis

Wet method | Concentration | Trichreme stain

(Paratest)

To detect if the stool sample contain parasite.

★Material:

-Container containing 4 ml Formalene

-filter

-Droper

★Method:

١-Add the mixture and mix

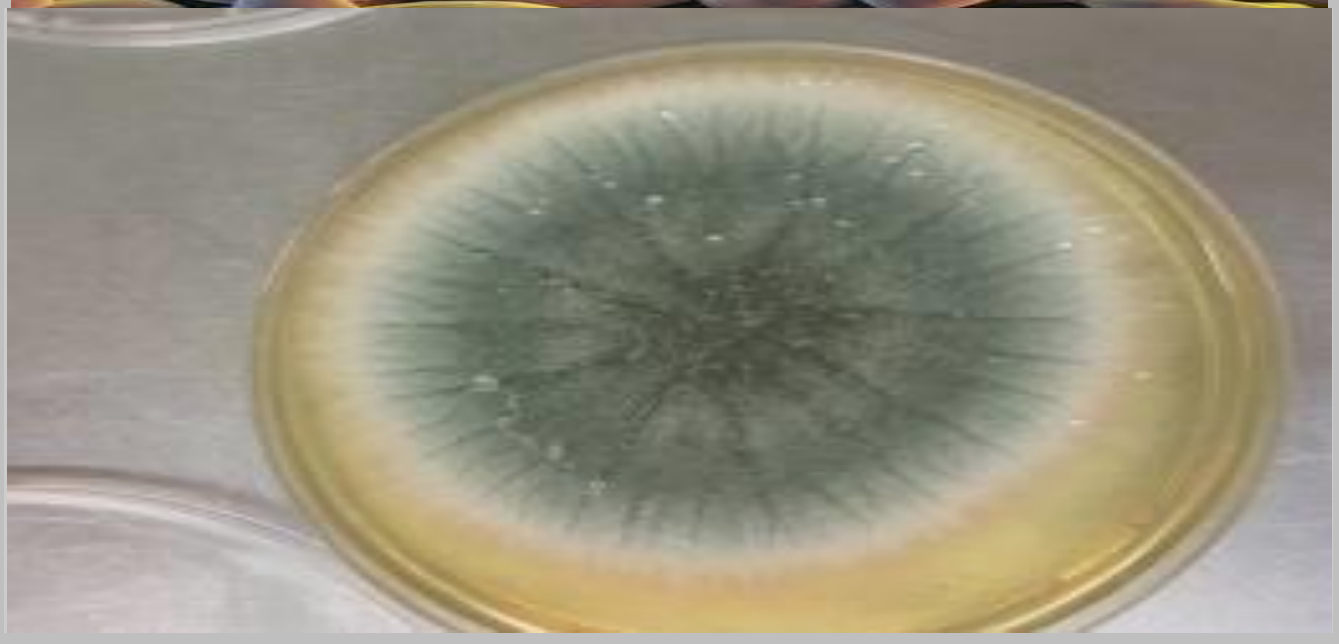
٢- upside down the sample for 15 minutes

3- use new slide and add 1 drop of mixture and 1 drop of iodine

(iodine for kill parasite)

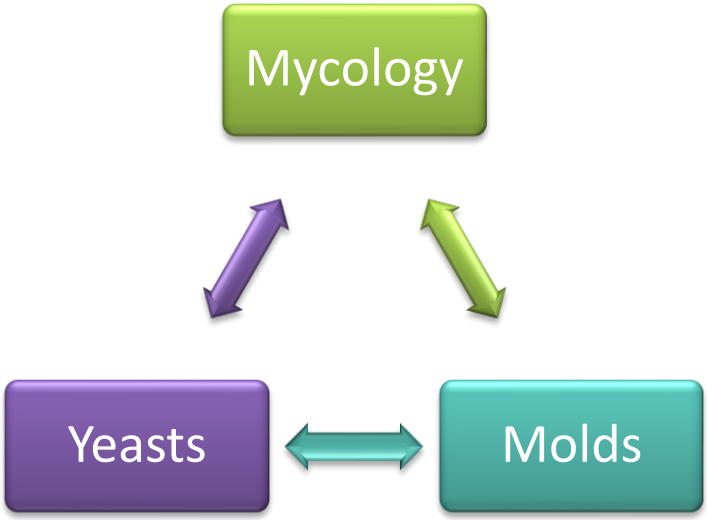
4- observe under microscope.

Mycology



Mycology laboratory:

The medical mycology laboratory is about demonstration , isolation and identification of pathogenic fungi occurring in body tissue , fluids and cutaneous materials.



Main lab:

1-Receiving
2-Labeling
3-Processing
4-Identifcation

The processing of samples consists of:

1-culture 2- staining

The routine fungal culture we use:

Sabouraud dextrose agr (SDA)

It may be different according to the addition of some inhibitory supplement or PH change.

1- Neutral Sabouraud dextrose agar (NSDA):

The is just sabouraud dextrose agar without any addition of supplement, will a neutral ph7, bacteria and fungus are growth on it.

2- Sabouraud dextrose agar with chloramphenicol (SDAC):

Allow the growth of fungus only inhibits bacteria and other common skin –hair.

3- Sabouraud dextrose agar with chloramphenicol and actidion (SDA A/C):

Allow growth of fungi inhibitor bacterial growth . Different of between fungal species.

According to specimen types 4-8 weeks are R.T

4 weeks: skin, nail, hair

6 weeks: body hniel , respiratory spasm, biopsy

8 weeks: Cerebrospinal fluid(CSF), bone marrow

Sabouraud dextrose agar

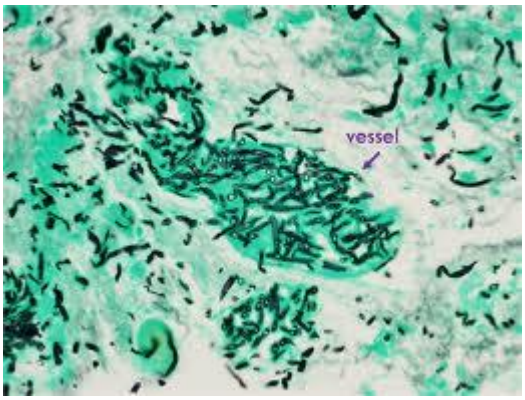
4-Brain-heart infusion (BHI):

Incubation period of 6 weeks at 37c.

Stain:

1- Grocott's methenamine silver stain (GMS):

For *Pneumocystis jirovecii*



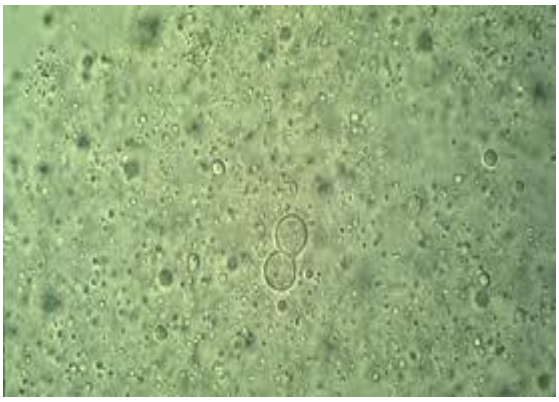
2- Diff-Quick stain:

Routine stain for all specimen except: nail ,hair ,skin

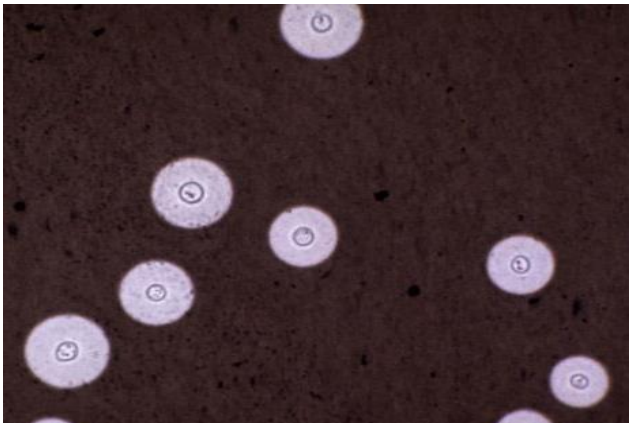
3- KOH (Potassium Hydroxide) preparation:

For specimen nail ,hair ,skin

Test for *Candida albicans*



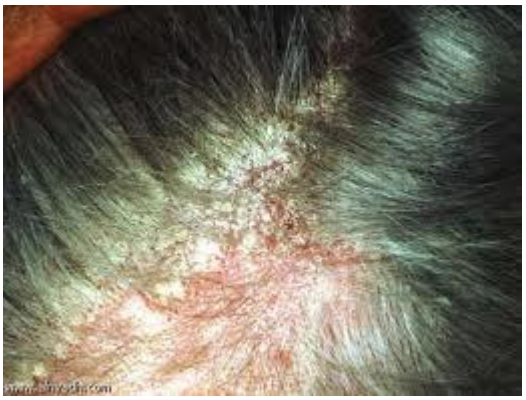
4-For CSF sample stain with INDIA INK for the detection of the capsule of *Cryptococcus neoformans.*



The specimens take of:

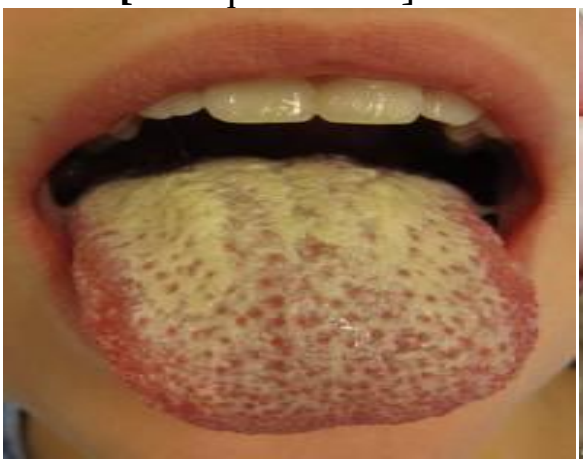
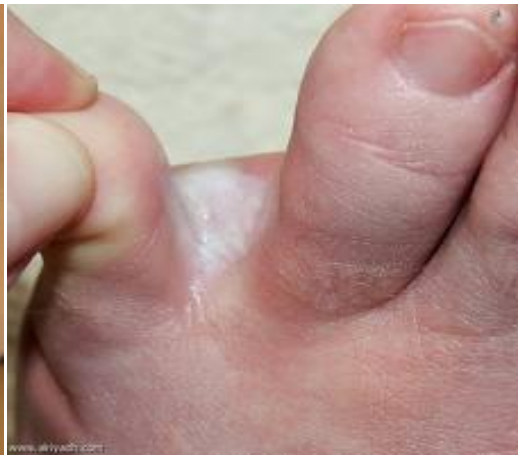
1-superficial:[skin, nail and hair]

Culture: [NSDA,SDAC and SDA-A/C]
Incubation period of 4 weeks at R.T
if no growth after **4weeks**,send at the final report as no growth after **4weeks** .
Stain: [KOHpreparation **or** KOH with calcofluor**].**

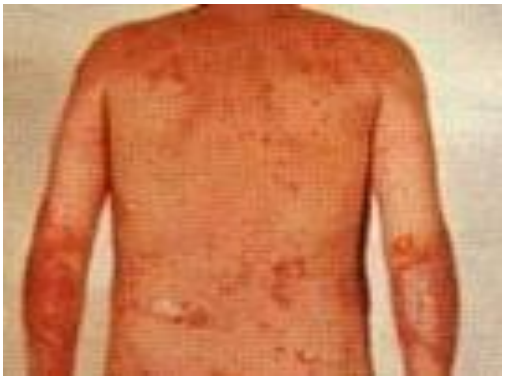
2- Swabs:

Culture: [NSDA,SDAC and SDA-A/C]
Incubation period of **4 weeks** of R.T and BHI at 37c.If no growth after 4weeks, send final report as no growth.
Stain: [Diffquick stain].

3-Tissues:

Culture: [NSDA,SDAC,SDA-A/C and BHI]
Incubation period of **6 weeks** at R.T and BHI at 37c.If no growth after 6weeks,send final report as no growth.
Stain [GMS, Diff-quick and KOHpreparation**].**




Identification:

If there is a growth on the media, take from the culture and put it on slide with 2drops of lacto phenol cotton blue[LPCB] and make a tease mount preparation. then subculture on potatoes dextrose agar[PDA] for sporulation, incubate at R.T ,after growth on PDA make LPCB mount for identification.

Yeast lap :

Processing samples:

1-Germ tube test(GTT)

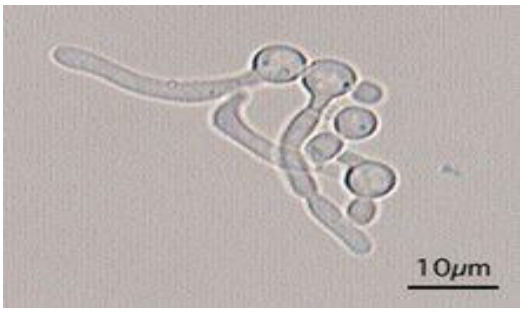
the important test for yeast

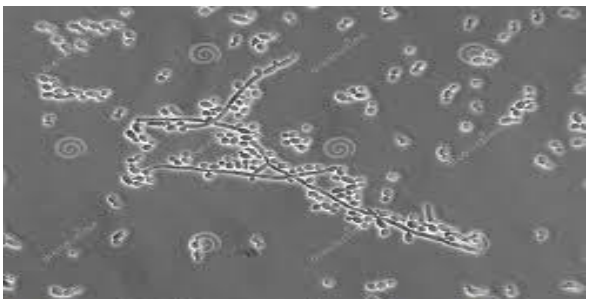
Negative:(AP120C)

In the AP120C test always the first well should be negative{-} (control) and the second should be positive {+} (control).

Positive: (SDA-A/C{45C})

SDA-A/C{37C}: **[Candida resistant]** at cycloheximide (Actidione)





2-Chlamydospores in CMA media(CMA)

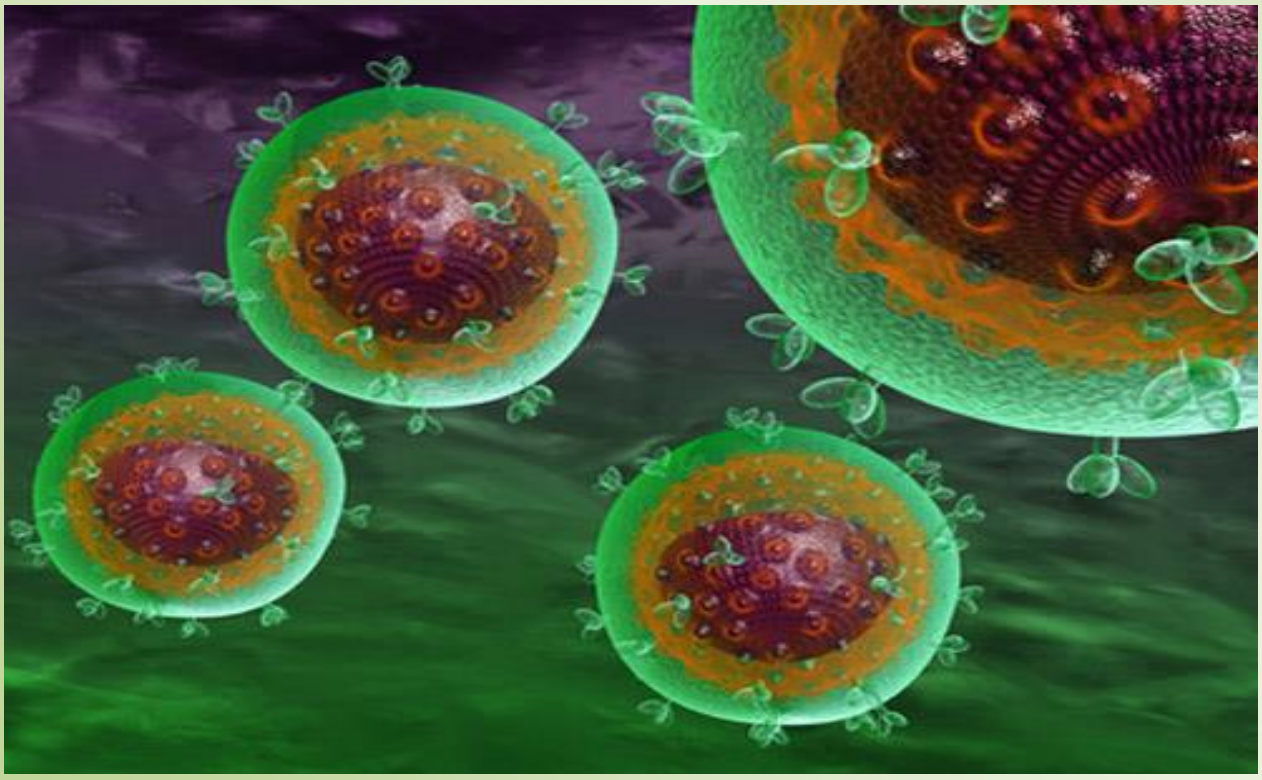
Room temperature.

3- Sensitivity:

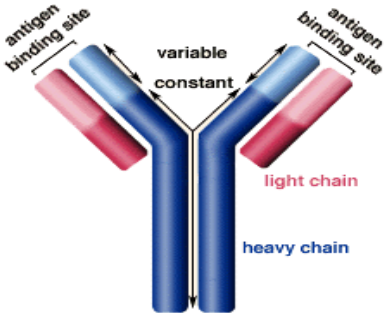
We do sensitivity for sample which taken from sterile sites ,specially blood and CSF

Do sensitive on Roswell Park Memorial Institute medium (RPMI) and incubate at 37c for 24-48h (E-test) or micro dilution in micro plates or yeast one micro dilution

Virology



Antibodies (also known as immunoglobulins abbreviated Ig) are gamma globulin proteins that are found in blood and are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses.



Antigens A substance that when introduced into the body stimulates the production of an antibody

Immunoassay

A laboratory technique that makes use of the binding between an antigen and its homologous antibodin

order to identify and quantify the specific antigen or antibody in a sample

Analyte The sample being analyzed and in immunoasssays the analyte is either Antibody or Antigen

Is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample.

ELISA technique

 An ELISA test uses components of the immune system and chemicals to detect immune responses in the body (for example, to infectious microbes).

The technique is divided into

1- Competitive ELISA

2- Sandwich ELISA (also called direct ELISA)

3- Indirect ELISA

Competitive ELISA

The labelled antigen competes for primary antibody binding sites with the sample antigen (unlabeled). The more antigen in the sample, the less labelled antigen is retained in the well and the weaker the signal).

Sandwich ELISA

The ELISA plate is coated with Antibody to detect specific antigen

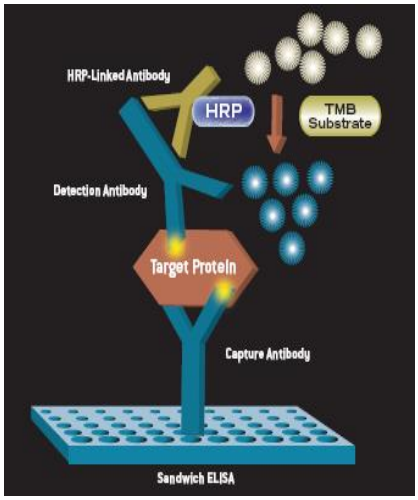
(1) Plate is coated with a capture antibody;

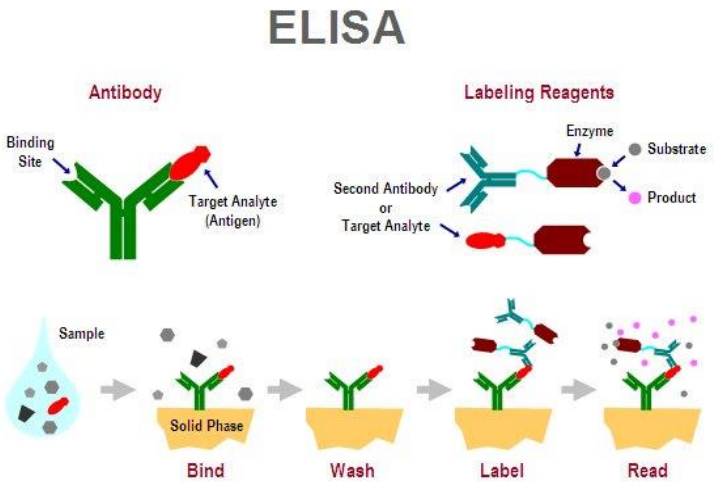
(2) sample is added, and any antigen present binds to capture antibody;

(3) detecting antibody is added, and binds to antigen;

(4) enzyme-linked secondary antibody is added, and binds to detecting antibody;

(5) substrate is added, and is converted by enzyme to detectable form.





Indirect ELISA

The protein antigen to be tested for is added to each well of ELISA plate, where it is given time to adhere to the plastic through charge interactions.

A solution of non-reacting protein is added to block any plastic surface in the well

Then the serum is added, which contains a mixture of the serum antibodies, of unknown concentration, some of which may bind specifically to the test antigen that is coating the well.

Afterwards, a secondary antibody is added, which will bind to the antibody bound to the test antigen in the well. This secondary antibody often has an enzyme attached to it

A substrate for this enzyme is then added.

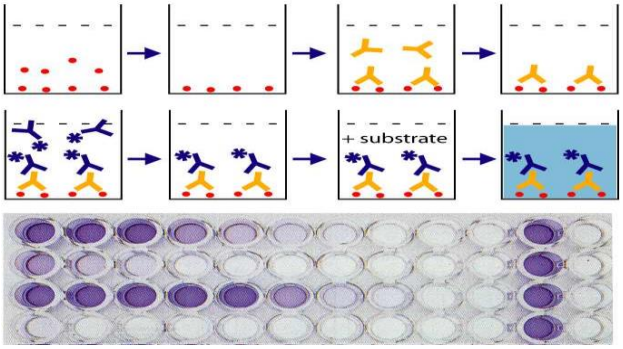
This substrate changes colour upon reaction with the enzyme.

The colour change shows that secondary antibody has bound to primary antibody,

which strongly implies that the donor has had an immune reaction to the test antigen.

The higher the concentration of the primary antibody that was present in the serum, the stronger the colour change.

spectrometer is used to give quantitative values for colour strength



An example of an ELISA experiment

Before starting the work read kit instruction carefully

1- The 96 well plate is labeled carefully and the first wells are used to draw the standard curve

The sample is added to plate in duplicate or triplicate and then the mean result is calculated

The quality control sample which is provided with the kit is treated as the test samples

Results

After reading the results the standard curve is drawn were the concentration is blotted on the X-axis and the absorbance on the Y-axis

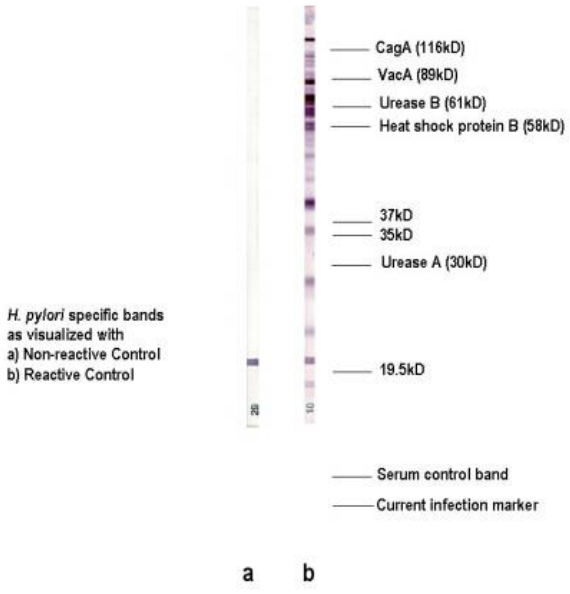
(ELISA) **(Architect i2000 sr)**

Architect i2000 sr :

This device use it to examine samples, it is have a same functions for ELISA device.

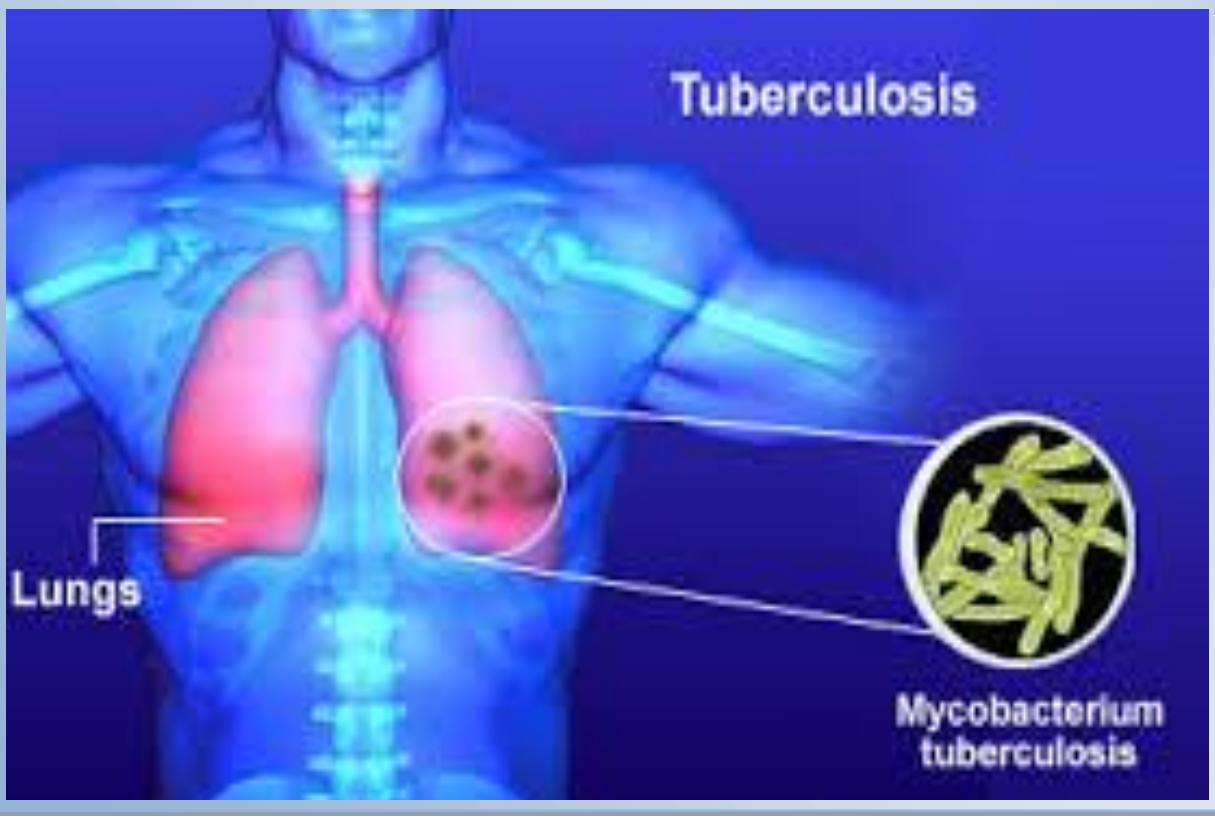
confirmatory test :

FIGURE 1



H. pylori specific bands as visualized with
a) Non-reactive Control
b) Reactive Control

TUBERCULOSIS (TB)



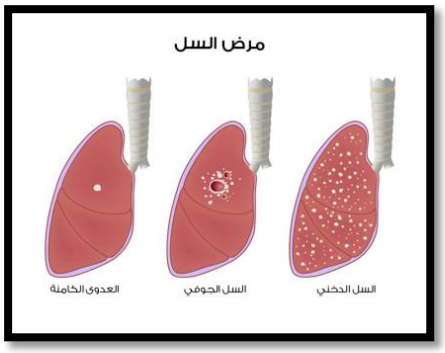
T.B TUBERCULOSIS

Tuberculosis (TB) is an infectious disease caused by bacteria whose scientific name is *Mycobacterium tuberculosis*.

It was first isolated in 1882 by a German physician named **Robert Koch** who received the Nobel Prize for this discovery.

TB most commonly affects the lungs but also can involve almost any organ of the body.

Many years ago, this disease was referred to as "consumption" because without effective treatment, these patients often would waste away. Today, of course, tuberculosis usually can be treated successfully with antibiotics.



There is also a group of organisms referred to as atypical tuberculosis.

These involve other types of bacteria that are in the *Mycobacterium* family. Often, these organisms do not cause disease and are referred to as "colonizers" because they simply live alongside other bacteria in our bodies without causing damage. At times, these bacteria can cause an infection that is sometimes clinically like typical tuberculosis. When these atypical mycobacteria cause infection, they are often very difficult to cure. Often, drug therapy for these organisms must be administered for one and a half to two years and requires multiple medications.

A person can become infected with tuberculosis bacteria when he or she inhales minute particles of infected sputum from the air.

The bacteria get into the air when someone who has a tuberculosis lung infection coughs, sneezes, shouts, or spits (which is common in some cultures).

People who are nearby can then possibly breathe the bacteria into their lungs.

You don't get TB by just touching the clothes or shaking the hands of someone who is infected.

Tuberculosis is spread (transmitted) primarily from person to person by breathing infected air during close contact.

There is a form of atypical tuberculosis, however, that is transmitted by drinking unpasteurized milk. Related bacteria, called *Mycobacterium bovis*, cause this form of TB. Previously, this type of bacteria was a major cause of TB in children, but it rarely causes TB now since most milk is pasteurized (undergoes a heating process that kills the bacteria).

T.B Laboratory

Safety :

1- Double door
2- Biological Safety Cabinet (BSC).
3- Gloves
4- Mask (Type N99 ,N94)
5- Lab coat

Examination :

1- Routine bacteria culture.
2- T.B culture .

Specimen :

A. All contaminated specimens requiring decontamination include **sputum**, **bronchial wash**, **skin**, **soft tissue**, **gastric lavage**, and **urine**

Specimen collection

1. Collect 3 sputum specimens for acid-fast smears and culture in patients with clinical and chest x-ray findings compatible with tuberculosis.
2. These three samples should be collected at 8-24 hour intervals (24 hours when possible) and should include at least one first morning specimen.
3. Smear results should be available within 24 hours of collection

B. Sterile sites not requiring decontamination include **CSF**, **bone marrow**, **blood** and **biopsy sites.**

Body Fluids :

Pleural effusion : fluid between the two membranes that envelop the lungs.
Pericardial effusion : fluid around the heart.
Ascites : fluid in the abdominal cavity
CSF Cerebrospinal fluid : fluid that surrounds the brain and spinal cord

Procedure :

1- Specimens from sterile sites

The use of two types of media (for specimens other than blood), including one liquid medium (when possible) or a comparable culture method, is recommended for optimal isolation of mycobacteria. Inoculate each specimen on the following media i.e.

1. LJ Pyruvate Agar
2. LJ Glycerol Agar and
3. MGIT broth medium

Use stains

Acid Fast Stain (AFB):

1- Fluorescence stain :

recommended for the examination of clinical specimens because of their increased sensitivity and speed, since they may be examined at a lower magnification than the carbol fuchsin stain.

1. Flood the slide with the fluorochrome stain.
2. Stain for 15 min.
3. Rinse the slide with water; drain the excess water from the slide.
4. Flood with 0.5% acid-alcohol.
5. Decolorize for 2 min.
6. Rinse the slide with water; drain the excess water from the slide.
7. Flood the slide with the counter stain (potassium permanganate or acridine orange).
8. Counterstained for precisely 2 min (a longer period may quench the fluorescence of the AFB when using the permanganate as counter stain).
9. Rinse with water; drain the excess water from the slide.
10. Air dry; do not blot.
11. Examine the smear with a fluorescent microscope with a 25 X or 40 X objective
12. The morphology is confirmed under oil immersion.
13. Fluorochrome-stained slides may be directly restained with the carbol fuchsin stain after immersion oil is removed with xylene.

Result :

1. Positive: yellow to orange fluorescence (depending upon the filter system used) against a black background. Artifacts may fluoresce, so morphology must be carefully scrutinized.
2. Negative: no fluorescence

2- Ziehl–Neelsen stain :

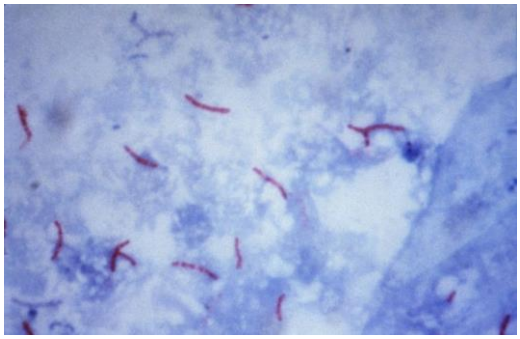
Best , accurate contain 3 parts :

1. carbol fuchsin.
2. acid alcohol.
3. methylene blue

1. Cover the heat-fixed slide with filter paper at 2 by 3 cm to minimize precipitation of crystals due to the heating and to keep the stain on the slide. Include QC information on reagent container and in QC records.
2. Flood the filter paper strip with the carbol fuchsin stain.
3. Heat the slide to steaming on an electric staining rack or with a Bunsen burner.
4. Let stand for 5 min. Add more stain if the smear dries, but do not reheat.
5. Remove filter paper strips with a forceps and discard into a container to be autoclaved later.
6. Wash slides with water.
7. Flood smear with acid-alcohol and allow destaining for 2 min.
8. Wash smear with water and drain.
9. Flood smear with the counter stain (methylene blue or brilliant green) for 1 to 2 min.
10. Rinse with water, drain, and then air dry.
11. Examine with a 100 X oil immersion objective using a light microscope.

Result :

1. Positive: red-stained rods against blue or green background, depending on the counter stain used
2. Negative: no red-stained rods observed.



2- Contaminated clinical specimens:

sputum , urine and other specimens from sites contaminated by normal flora.

For optimal recovery of mycobacteria, all clinical specimens known to be contaminated (e.g. Sputum) requires liquefying the organic debris (digestion) and eliminating contaminating organisms (decontamination). Decontaminating procedures are toxic to mycobacteria. Five methods available for digestion and decontamination of specimens for culture of acid-fast bacteria are discussed in this procedure. The most commonly used procedure in the United States is the N-acetyl-L-cysteine–sodium hydroxide (NALC-NaOH) method. Specimens (other than blood) are routinely inoculated on media that support optimal growth of the majority of clinically relevant mycobacterial species.

The use of two types of media (for specimens other than blood), including one liquid medium (when possible) or a comparable culture method, is recommended for optimal isolation of mycobacteria. Inoculate each specimen on the following media i.e.

1. LJ Pyruvate Agar
2. LJ Glycerol Agar and
3. MGIT broth medium