Microbial Diagnosis

320 MIC

Prepared by

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(Practical Microbial Diagnosis)

Abstract

For

Microbial diagnosis

LAB#1

Introduction to Microbial Diagnosis and speciemens collection

Objective: To understand the: methods of diagnosis of bacteria, fungi and viruses and to understand types of specimens collection

***Microbial diagnosis:** is a study of microbial identification using methods:

1-Microscopic diagnosis: to study the shapes of microorganism use of:

A-Wet prep slide: add one drop of saline water (water contain concentrate of NaCl 1-3%) on slide and examine it under microscope).

B-Stains: to study the shapes of bacteria and fungus by using stain (simple stain use one type of stain or complex stain use two or more of stain).

Examples of simple stain (Lactophenol, safranin, methylene blue).

Examples of complex stain (Gram stain, Zeil Nilsson stain).

2-Cultural diagnosis: to study the morphology of colonies on culture media and use biochemical reaction to differentiate between bacteria and fungus.

3- serological diagnosis: to study the interaction between antigen and antibody to diagnostic viral infection or bacterial infection.

4-Moleculer diagnosis: to study and detect the bacterial infection or viral infection by use molecules diagnosis (example: Polymerase reaction technique(PCR)).

Specimen collection and saving and transport and processing

Microorganisms you can see in clinical microbiology lab:

1-Bacteria 2-Fungi (yeast or mold). 3-Viruses 4-Parasites.

Specimen excepts to see in microbiology lab receiving:

***Label the container of all specimens with name of patient and date of birth and time and date of sample collection on and the doctor test ordered.**

1-Urine: Urine has a long history as a specimen for analysis in clinical laboratories.

After blood, urine is the most commonly used specimen for diagnostic testing, monitoring of disease status and detection of drugs.

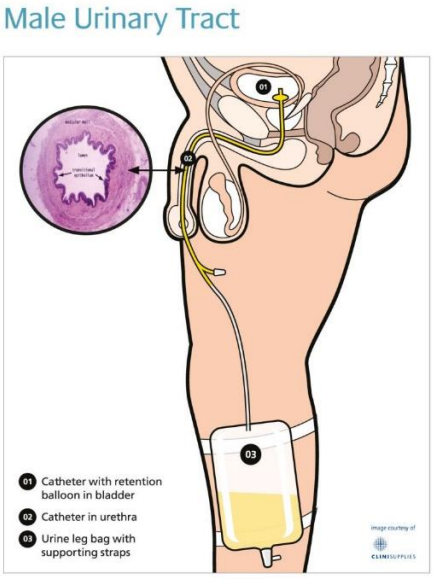
***Types of urine specimen:**

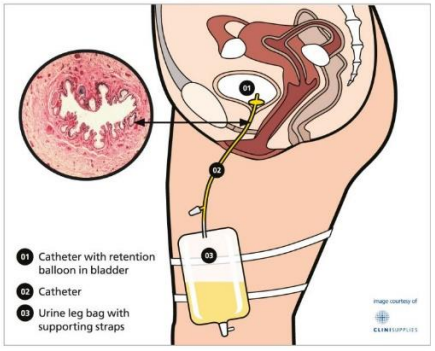
1-Random urine: collect form urine pass and put it in clean container and no time to determine to collect.

2-Mid-stream urine: collect from urine middle pass and put it on clean container.

3-Clean catch urine: collect urine you will use a special kit to collect the urine. It will most likely have a cup with a lid and wipes.

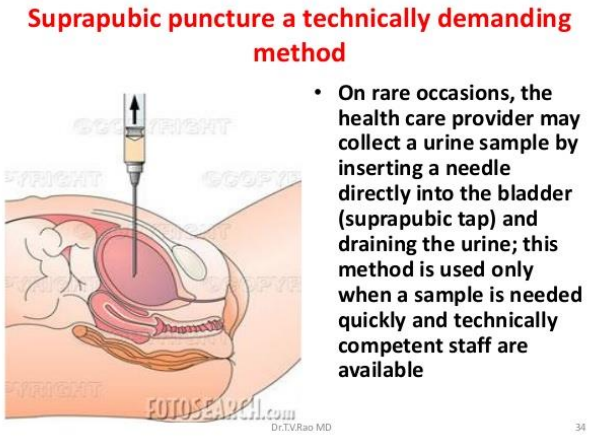
4-Urine catheter: **(**Example: Foley catheter) use a syringe followed by transfer to a specimen tube or cup alternatively, urine can be drawn directly from the catheter to an evacuated tube using an appropriate adaptor.





5-Urine suprapubic:

May be necessary when a non-ambulatory patient cannot be catheterized or where there are concerns about obtaining a sterile specimen by conventional means.



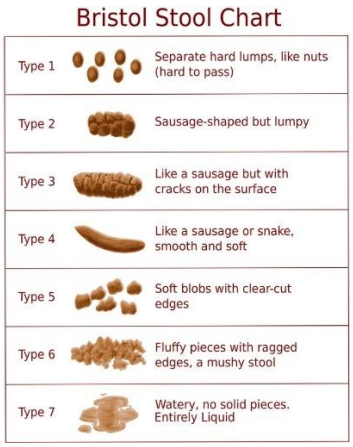
Urine saving:

Don't keep it in room temperature more than two hours

Save it in fridge for 72 hours after that, through it.

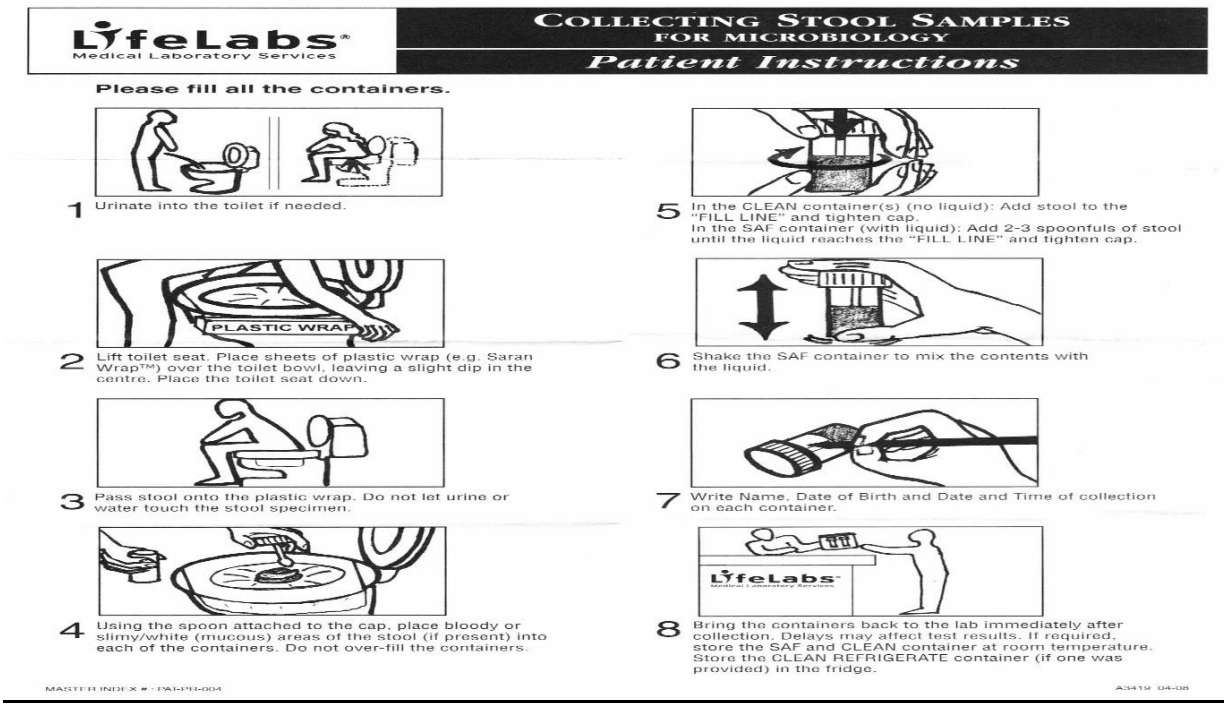
2-Stool(Feaces) specimen: are a specimen use to detect bacterial infection or parasite infection if a patient has symptoms.

Collect specimen to clean container (pass it in toilet in clean site and take by sterile spoon).



Shapes of stool specimen

Stool collection

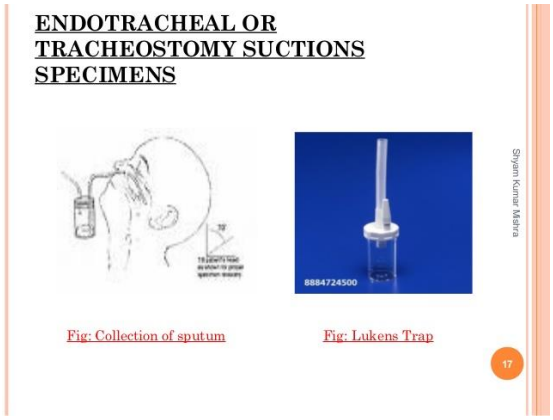


Stool saving: don't keep it in room temperature more than two hours.

Save it in fridge for 73 hours.

4-Respiratory samples: a specimen collection from respiratory tract (sputum, Endotracheal aspiration, tracheal aspiration, Nasopharyngeal aspiration, BAL).

Collect samples to clean container.



Save it in fridge for one week.

5- Cultural Swab:

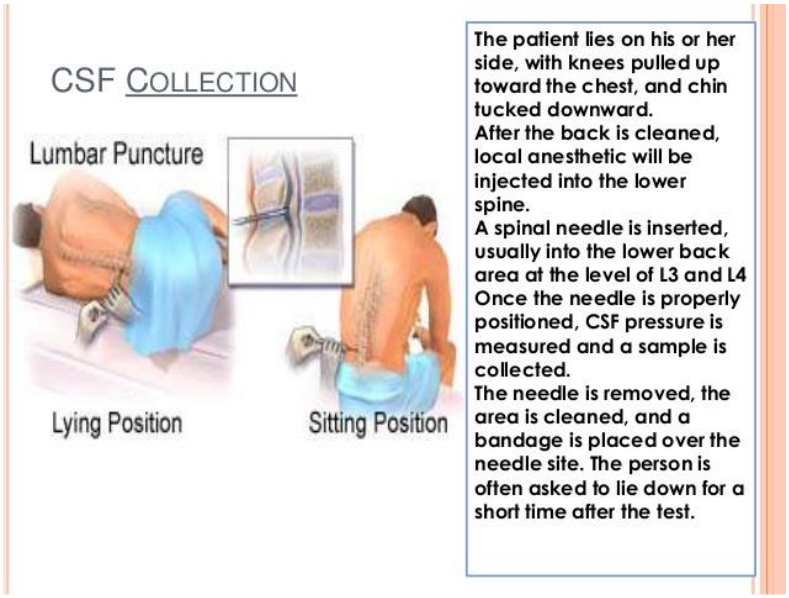
Use with gel to enhance and save microorganisms.

Examples: wound swab (Abscess or pus or boil etc..), Throat culture, Ear culture, Eye culture, Nasal culture.

Collect from body site in Aseptic tec. And send it to lab and save it in fridge for one week.

6-Body fluids:

are a fluid collect it from entire body (Cerebrospinal fluid(CSF) , Synovial fluid(from knee) , Peritoneal fluid(from abdominal) , Abdominal fluid , Bile fluid (from pancreatic) , Ascitic fluid (from abdomen site).



7-Blood specimen:

Most important clinical samples if the patient have fever, nausea we should to take blood samples to check it for microbiological examination.

Collect blood specimen with aseptic tec.

Save it for 20 days to 30 days.

LAB#2

Microbiological culture media

Objective: To understand the classification of culture media, preparation and processing of microbiological growth media

Microbiological culture media: Use to cultivating of microorganisms contains substances necessary to support microorganism.

Agar: is a solidifying agent use to solidify media and it is a polysaccharide extract from red algae, it melts at 84 c and solidify at 38 c.

Peptones: used as a source of nitrogen.

Meat and plant extracts: used as source of nutrients and contains of vitamins, amino acids, peptides, carbohydrates minerals.

Growth factor: Many organisms have specific growth factor requirement that must be included to the media for successful cultivation.

Example: Blood and yeast extracts

X factor (heme) and V(NAD) factor

Selective components: That inhibits the growth of non-target organisms.

Types of media:

1-General media: used to isolate pathogen and nonpathogenic microorganism (examples, Nutrient agar, potatoes dextrose agar).

2-Enriched media: Enriched media contain the nutrients required to support the growth of a wide variety of organisms, including some fastidious ones (Examples, Blood agar, Chocolate agar).

3-Enrichement media: promotes the growth of a particular organism by providing it with the essential nutrients and rarely contains certain inhibitory substance to prevent the growth of normal competitors (example, Selenite F broth).

4-Selective media: used for the growth of only selected microorganisms (example, EMB (Eosin Methylene agar for *E. coli*, MSA for *staph* spp.)

5-Differintial media: Differential or indicator media distinguish one microorganism type from another growing on the same medium. (Example, Mannitol salt agar, MacConkey agar).

***By the way some of media have both selective and differential in same time**

1-Nutrient agar: general-purpose, nutrient medium used for the cultivation of bacteria consists of digestive products of proteins (called peptones) and beef extract.

2-Blood agar: Non-selective Enriched Medium Enriched with 5% sheep blood, suitable for Hemolysis determination and provides X-factor Widely used for the growth of Pathogenic and Non-pathogenic Organisms Contains meat, peptones, beef extract, yeast extract and cornstarch.

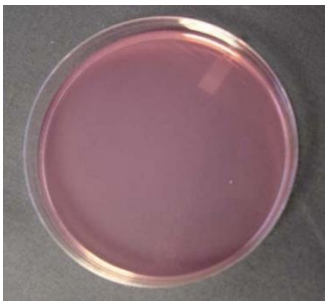
3-Chocolte agar: General purpose medium use for the isolation of many organisms including fastidious organisms like *Neisseria* and *Haemophilus*

Contain X(Hemin) and V(NAD) factors.

4-MaCckonkey agar: Selective medium and differential

Inhibit gram positive organisms by adding crystal violet

Differentiate between lactose fermenters (coliforms) and Non-lactose fermenters When an organism ferments lactose, pH drops, this causes the colony to become pink due to presence of Neutral red.



5-Sabroud dextrose agar: General clinical media for the isolation of Yeast and Fungi.

6-Blood culture : Used to determine presence of pathogens (Bacteria-Fungi) in blood stream a minimum of 10 ml of blood is taken through venipuncture and injected into two or more "blood bottles" (from patient and send it to lab) with specific media for aerobic and anaerobic organisms. A common medium used for anaerobes (is thioglycolate). And aerobic used Tryptic soya broth.

Green venipuncture for aerobic and brown venipuncture for anaerobic and yellow venipuncture for pediatric and incubate it in BAcT/ALERT machine.

Lab#3

Isolation of bacteria and fungi from different parts of the human body

Objective: to learn suitable techniques of bacteria and fungi culturing to isolate and obtain a pure culture

Isolating a single bacterium species is the first step in identifying the bacteria possibly responsible for a disease process**.** The first requirement for physically isolating a bacterium is that it can be cultured in the laboratory. This requires knowledge of optimal temperature for growth, optimal oxygen requirements, and optimal nutritional needs. We work with a very limited number of bacteria in this course. The bacteria we work with are also very easy to culture in the lab. Most bacteria are not this agreeable!

There are two main ways to isolate organisms.

Streaking for isolation on an agar plate

The pour plate method

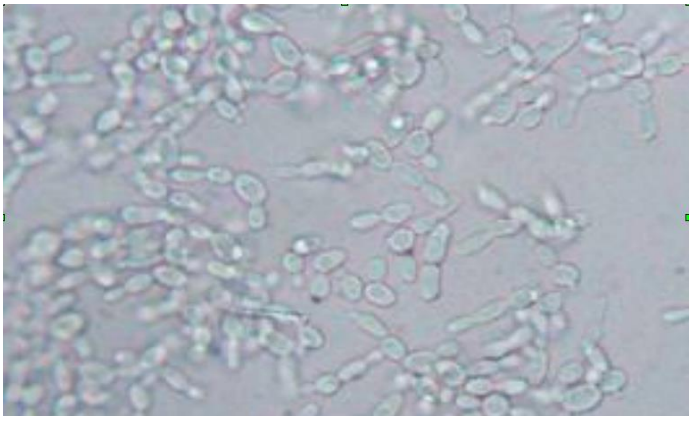
Lab#4

Microbiological Microscopic Diagnosis

Objective: To understand the: value, procedures, preparation, fixation and processing of bacterial smears as well as Staining types and methods.

1-Wet prep slide: use water saline on slide and mix it with microorganisms.

Lactobacillus wet prep.



Yeast wet prep

-Diagnostic by stain:

A-gram stain:

Is a differential stain use to divide two large group of bacteria Based on cellular content, Gram stain divides almost all bacteria into two main groups: gram positive- bacteria (Purple) and gram-negative bacteria(Red).

***Procedure of gram stain practical:**

***Materials:**

1-Crystal violet (primary stain). 2-Gram Iodine solution (mordant or fixation step).

3-Acetone-alcohol(decolorizer). 4-Safranine (Counter stain). 5-New glass slides

6-Fresh bacterial culture.7-Loop.8-Bensin flame.

***methods:**

1-Make a smear of bacterial colonies (take one colony and mix it with saline) and fix it under bensin flame till dry.

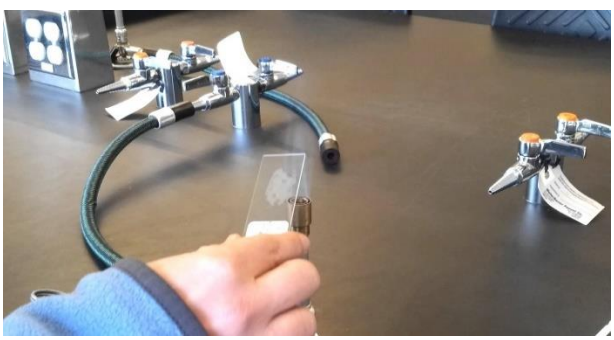
2-Flood (cover completely) the entire slide with crystal violet for 1 min and wash it by water.

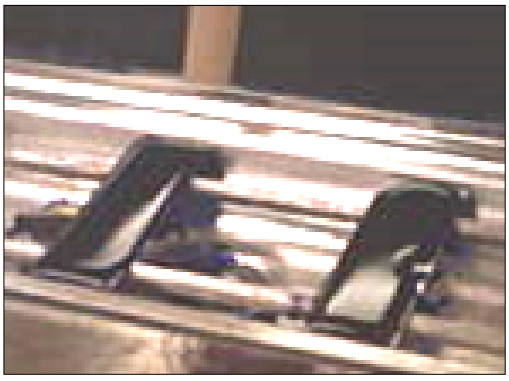
3-Flood the entire slide with Iodine solution for 1 min and wash it by water.

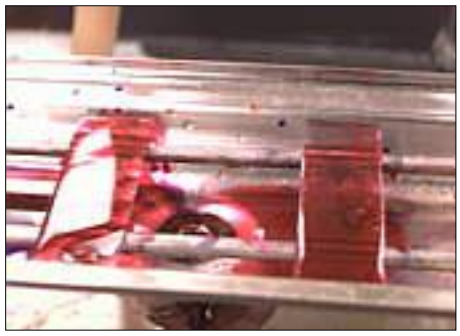
4-This step is best performed while holding the slide at a slant with forceps, rather than while sitting on a slide rack, add decolorizer(Acetone-alcohol) dropwise and allow it run off. Rinse with running tap water. Repeat this step until the blue dye no longer runs off the slide with the decolorizer. Rinse with running tap water and shake off the excess is somewhat tricky because using too much decolorizer could result in a false Gram (-) result. Likewise, not using enough decolorizer may yield a false Gram (+) result.

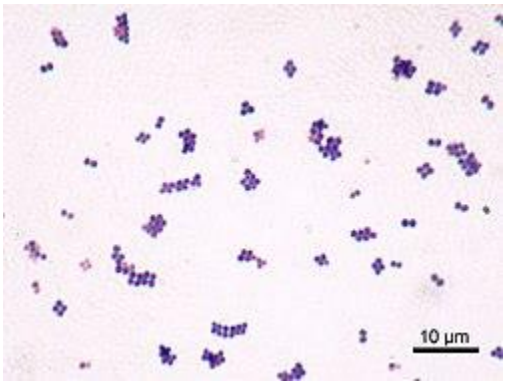
5-Flood the entire slide with Safranin for 1 min and wash it by water.

6-allow the slide to air dry before viewing under the microscope and add drop of xylene oil (immersion oil) and examine it under 100x eye lens.

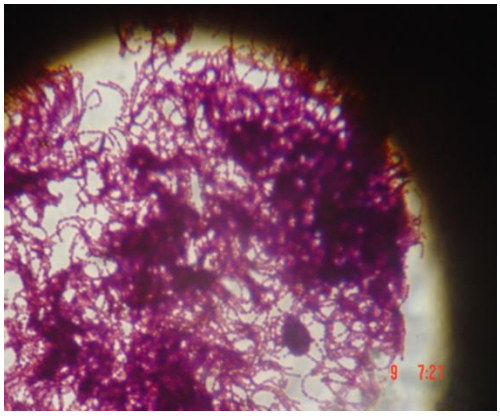




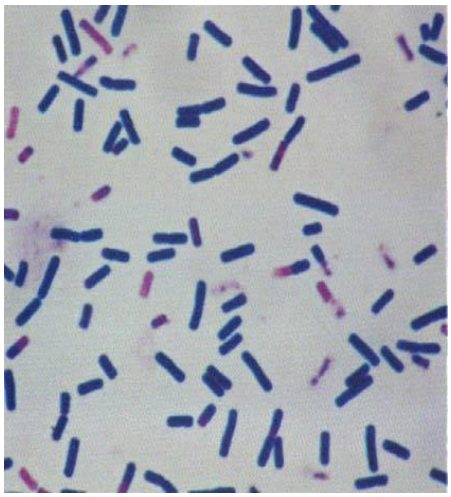




Gram positive cocci in clusters



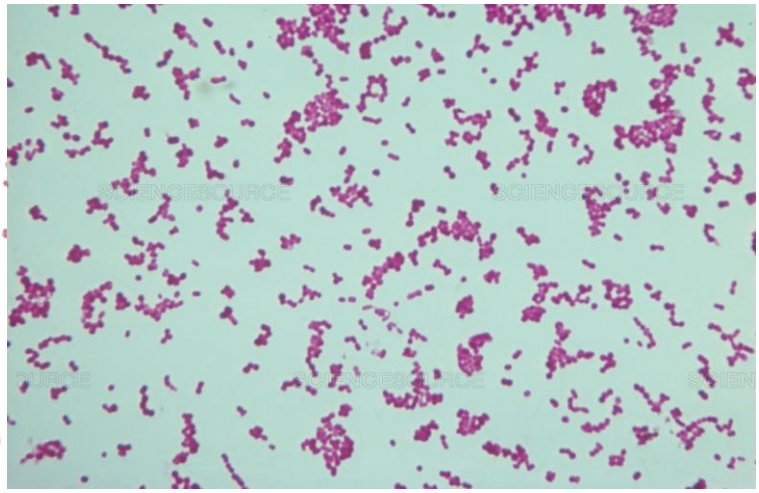
gram positive cocci in chains



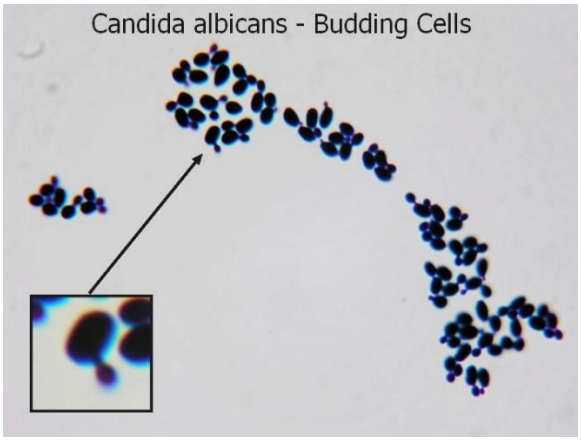
Gram positive rods

Gram negative bacilli

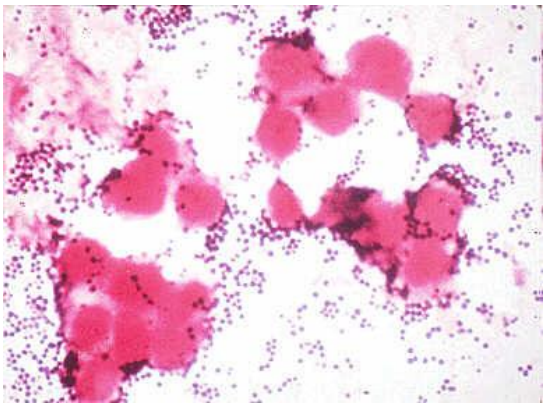
Gram negative coccobacilli



Gram negative cocci



Candida albicans gram positive

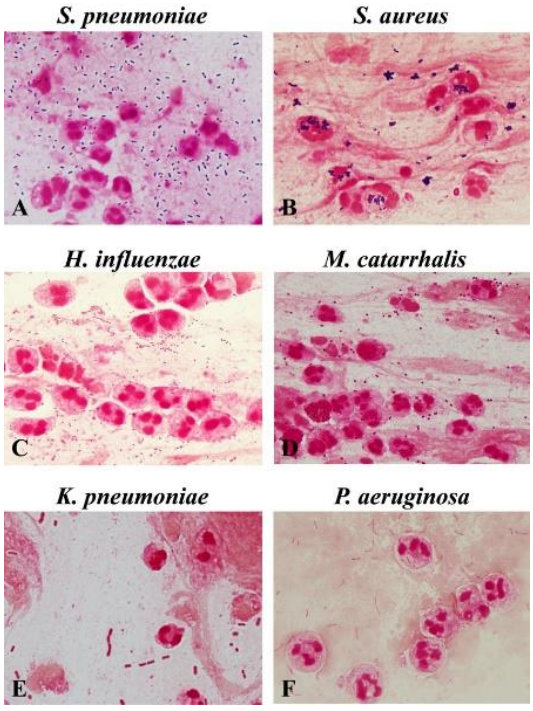


Wound specimen(exaudted)

Staphylococcus aureus (gram stain of specimen) .

Sputum gram stain

Look at pictures



B-Ziel -Nilsson stain: also known as the acid-fast stain, It is a special bacteriological stain used to identify acid-fast organisms, mainly Mycobacteria. *Mycobacterium tuberculosis* is the most important of this group because it is responsible for tuberculosis (TB) Acid-fast bacilli will be bright red after staining.

Materials and methods:

1-Sputum specimen 2-fresh culture for TB control 3-Glass slides

4-Carbol-fuchsin 5-acid alcohol 6-methylene blue7-Immersion oil.

Methods:

1-Flood fixed smear with carbolfuchsin for 6 minutes

2-Wash gently

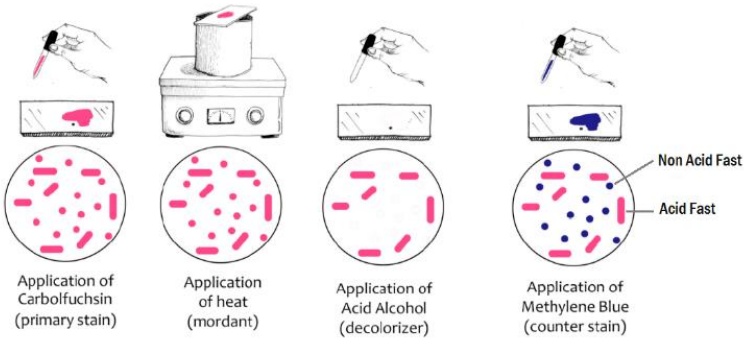
3-Decolorize with 3% acid alcohol for 2 minutes

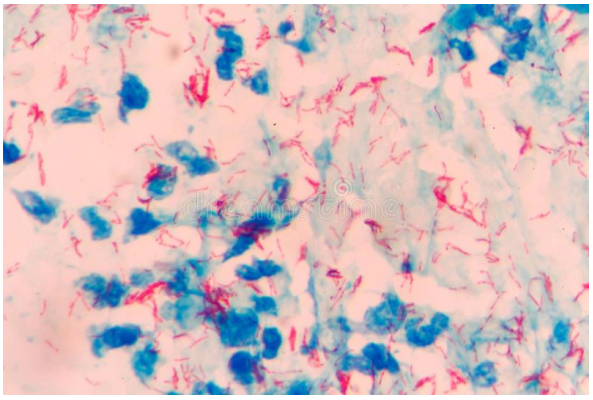
4-Wash gently

5-Counterstain with methylene blue for 2 minutes

6-Wash gently

7-Examine smear using oil immersion.





LAB#5

Gram positive Bacteria identification

Objective: To diagnosis of some dominant gram positive bacteria (Staphlococcus spp. and Streptococcus spp.)

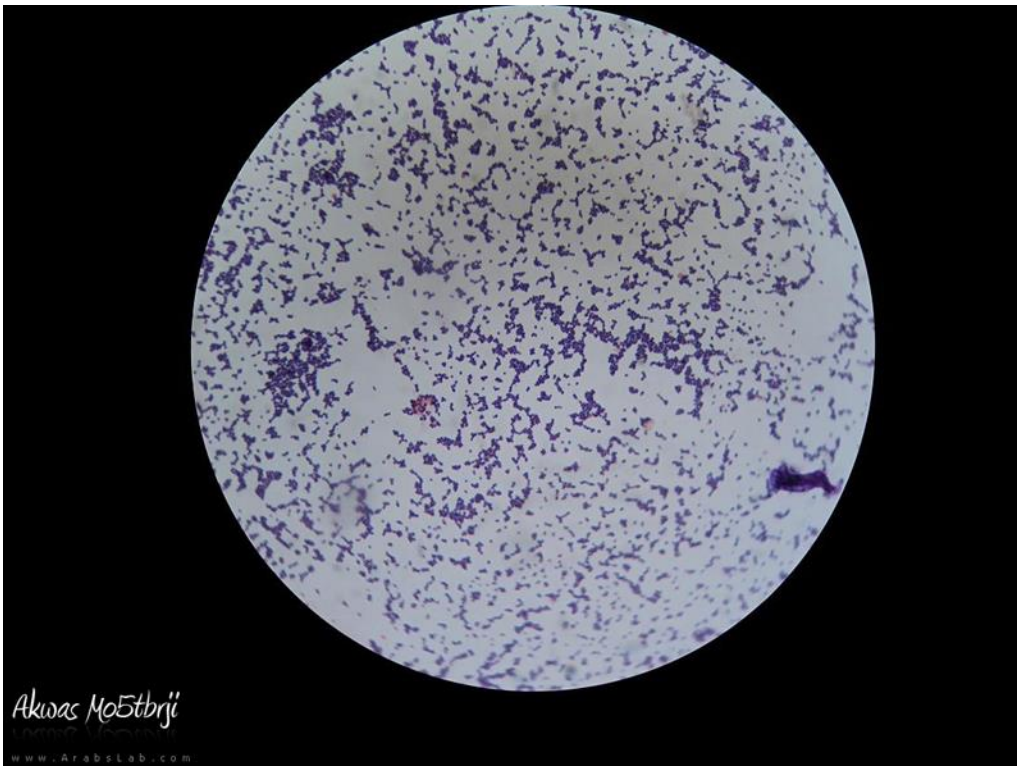
1-Gram positive cocci in clusters:

A-staphylococcus aureus B-Staphylococcus epidermidis C-Staphylococcus Saprophyticus.

2-Gram positive cocci in chains:

A-*Streptococcus pyogenes B-Streptococcus viridans C-Enterococcus faecium.*

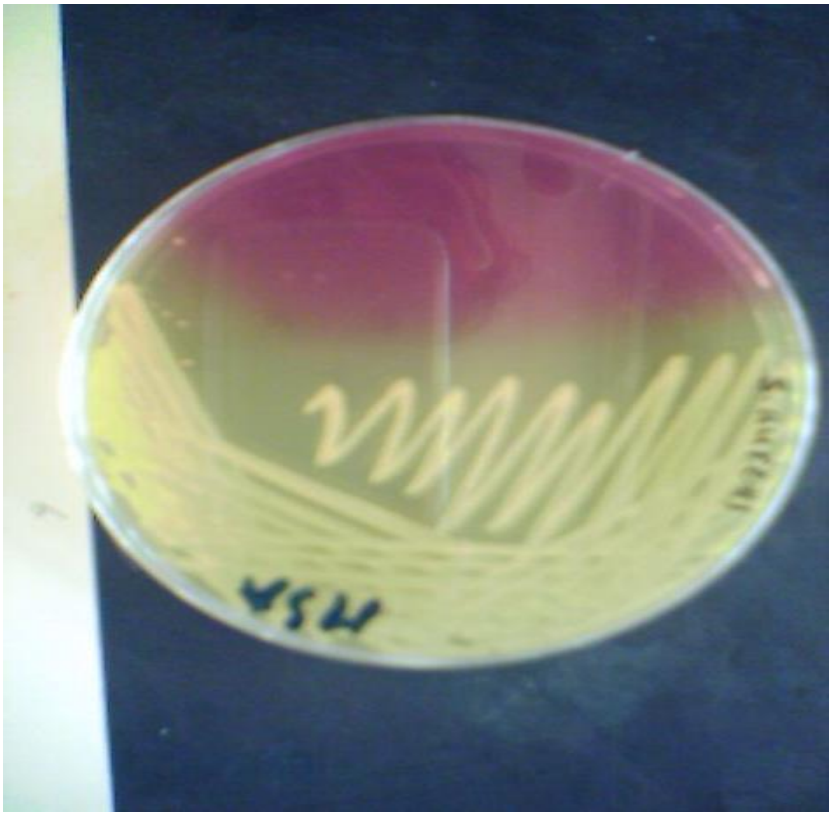
1- **Staphylococci : It is gram positive cocci arranged in clusters**



Staphylococcus aureus growing on M.S.A

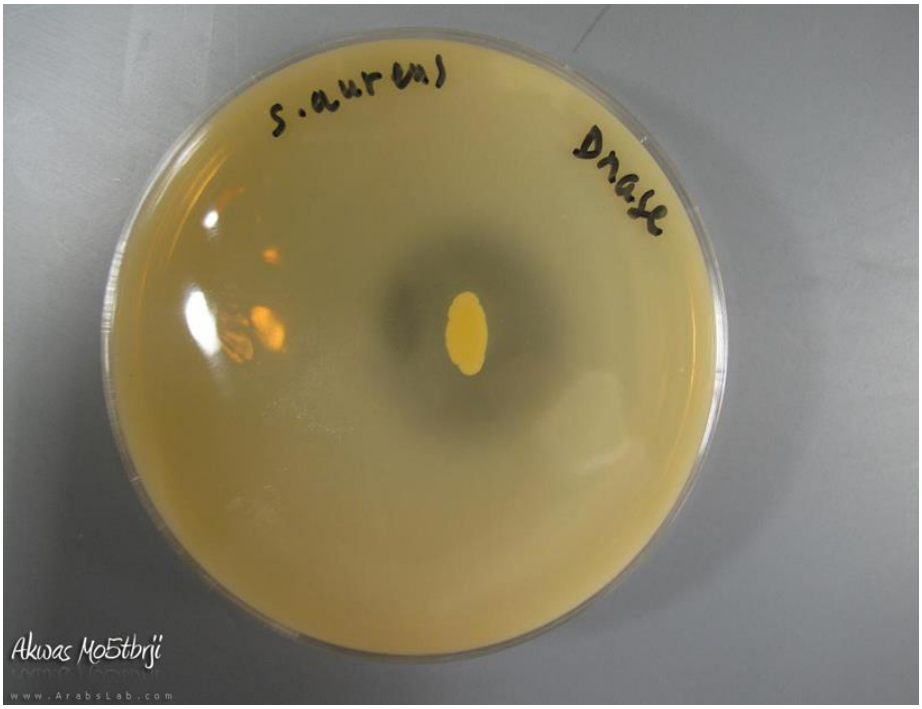
Manitol salt agar: is a selective and differential media and have a high concentration of salt, selective for staphylococcus species and inhibited growth of gram positive bacteria and gram negative bacilli and contain of mannitol sugar and phenol red indicator, differential between

mannitol fermenter(Staphylococcus aureus) and non-fermenter mannitol (coagulase negative staph.).



Staphylococcus aureus on Dnase medium

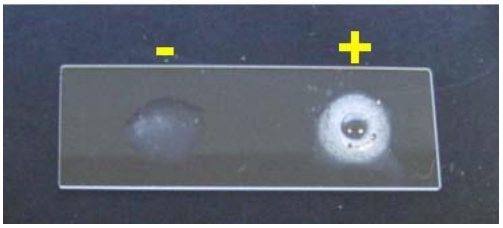
S. aureus is Dnase +ve and Note the clearance around the spot after adding 1N HCL



***<u>Biochemical test</u>:**

<u>1-Catalase</u>: is a common enzyme found in nearly all living organisms exposed to oxygen (such as bacteria)It catalyzes the decomposition of hydrogen peroxide to water and oxygen use to differentiate between *staph.sp. and strep sp.*

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

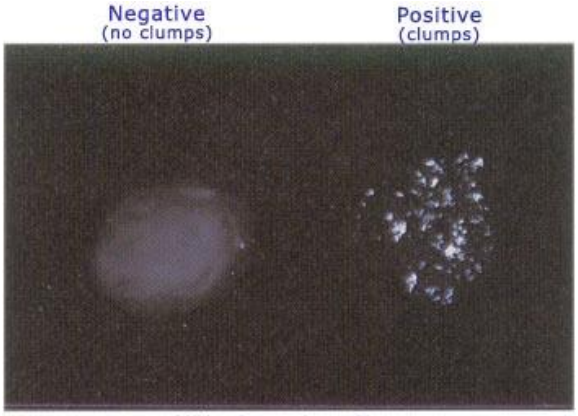


<u>2-Coagulase test</u>:

Is a protein enzyme produced by several *microorganisms* that enable the conversion of fibrinogen to fibrin. In the laboratory, it is used to distinguish between different types of *Staphylococcus* isolates. Importantly, *S. aureus* is generally coagulase-positive, meaning that a positive coagulase test would indicate the presence of *S. aureus*. A negative coagulase test would instead show the presence of coagulase negative organisms such as *S. epidermidis* or *S. saprophyticus*. However, it is now known that not all *S. aureus* are coagulase-positive.

A-Coagulase tube: the tube test uses rabbit plasma that has been inoculated with a staphylococcal colony (gram-positive cocci which are catalase positive). The tube is then incubated at 37 °C for 1.5 hours. If negative, then incubation is continued up to 18 hours.

B-Coagulase slide: test is run with a negative control to rule out autoagglutination. Two drops of saline are put onto the slide labeled with sample number, test and control. The two saline drops are emulsified with the test organism using a wire loop, straight wire, or wooden stick, a drop of plasma (rabbit plasma anticoagulated with EDTA is recommended) is placed on the inoculated saline drop corresponding to test, and mixed well, then the slide is rocked gently for about 10 seconds.

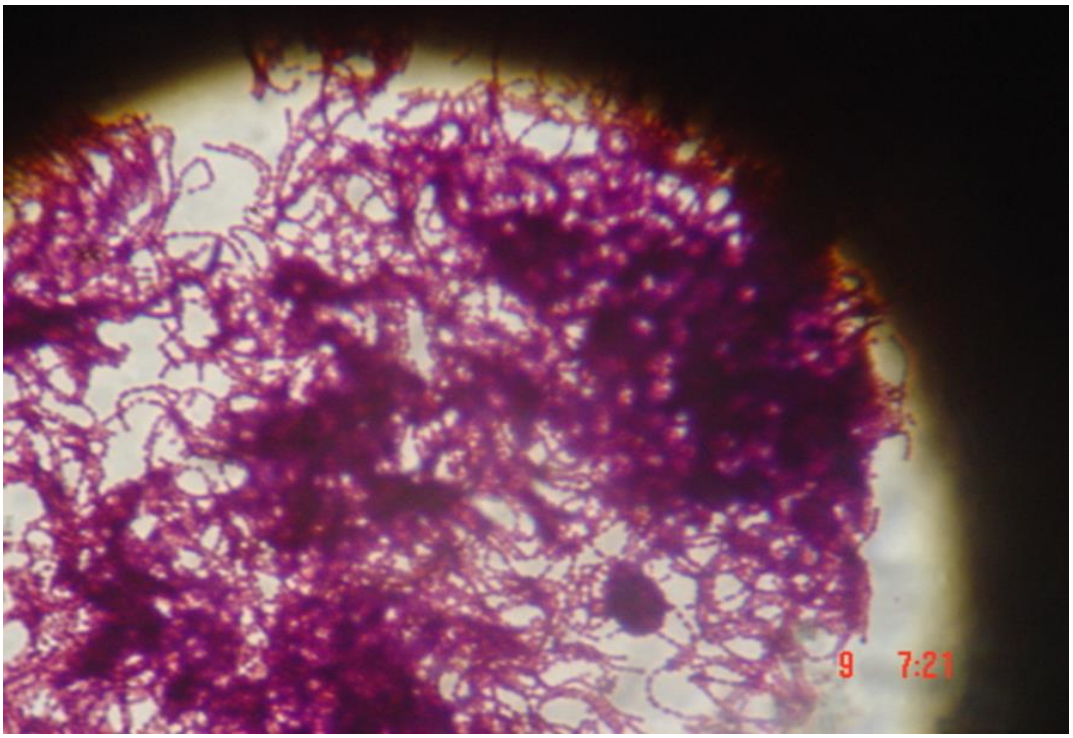


Slide Coagulase Test

Staphylococcus aureus is coagulase +ve

See the fibrin clot

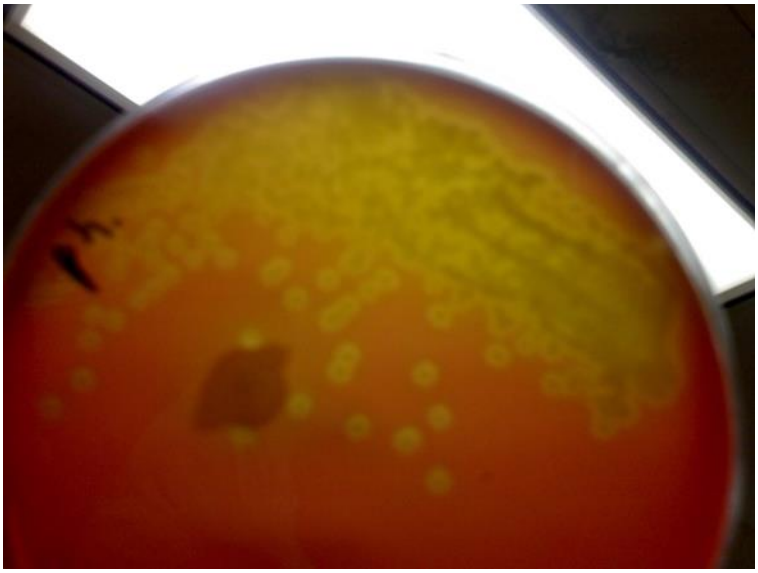
2- **Streptococci: It is Gram +ve cocci arranged in chains.**



-Blood agar differential between Streptococcus species:

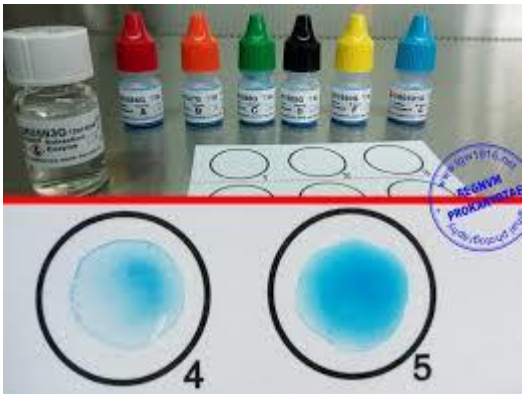
<u>A-BETA hemolysis:</u> Complete hemolysis. *(Streptococcus pyogenes and agalactiae)*

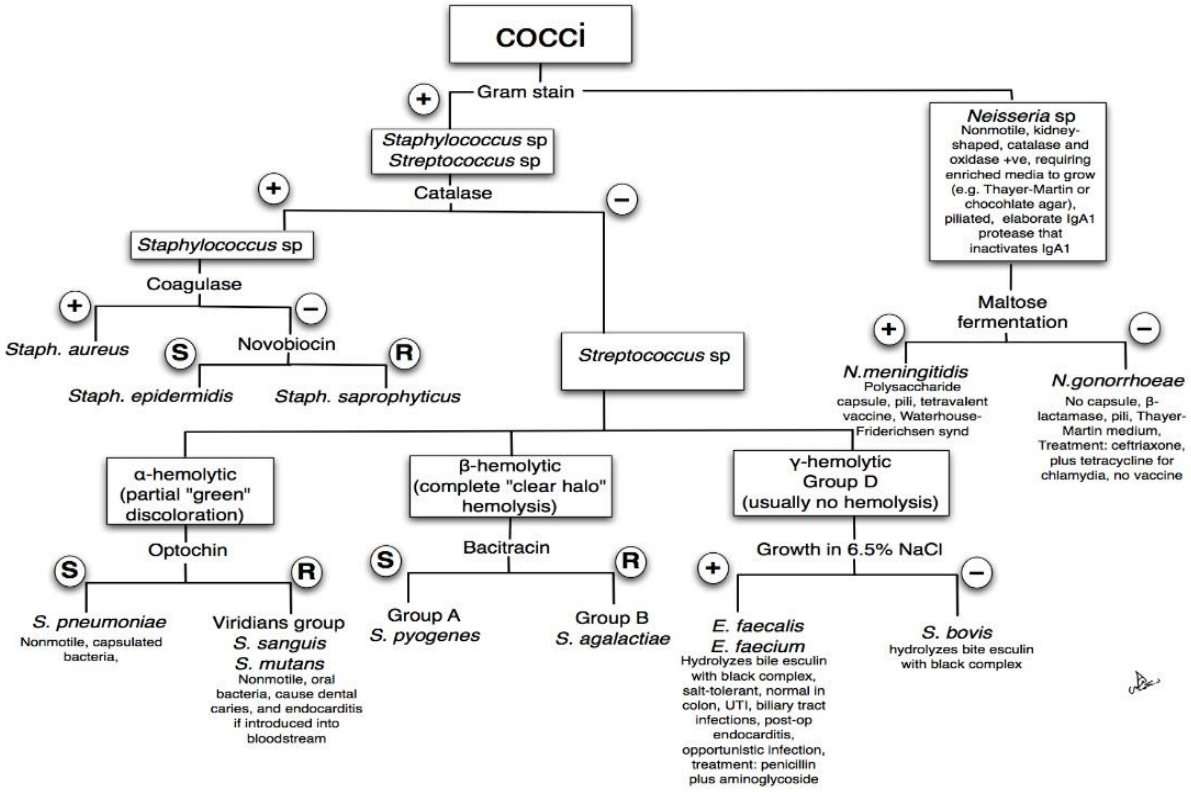
B-Alpha hemolysis: partial hemolysis this is sometimes called green hemolysis because of the color change in the agar. Other synonymous terms are incomplete hemolysis and partial hemolysis. Alpha hemolysis is caused by hydrogen peroxide produced by the bacterium, oxidizing hemoglobin to green biliverdin(*Streptococcus pneumonia and viridins).*



C-Gamma hemolysis(non-hemolysis):no hemolysis around colonies *(Enterococcus faecium and faecalis).*

-***Lancfield grouping:*** is grouping catalase-negative, coagulase-negative bacteria based on the carbohydrate composition of bacterial antigens found on their cell walls, the system, created by Rebecca Lancefield, was historically used to organize the various members of the family *Streptococcaceae*.



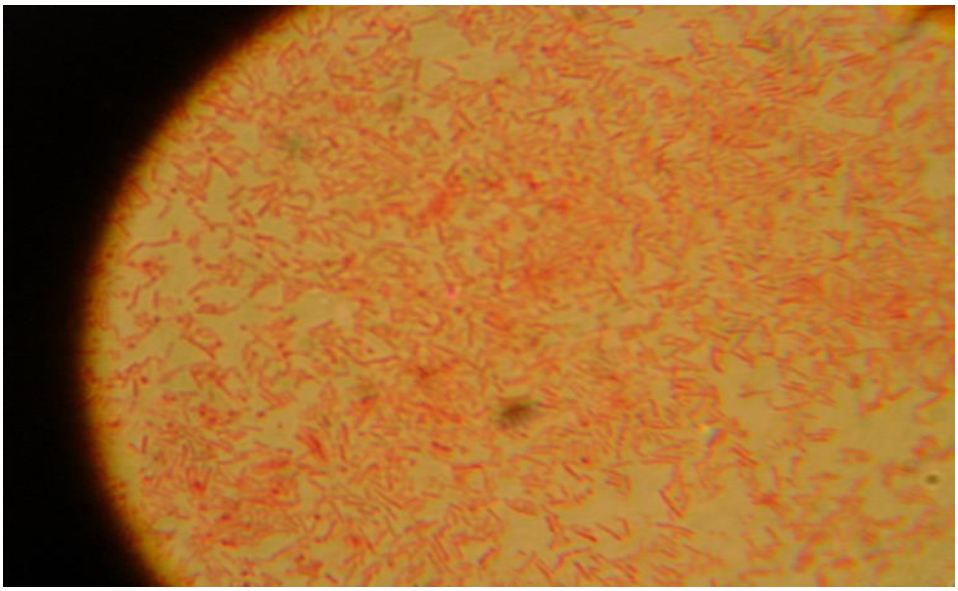

cocci
Gram stain
+
Staphylococcus sp
Streptococcus sp
Catalase
+
−
Staphylococcus sp
Coagulase
+
−
Staph. aureus
Novobiocin
S
R
Staph. epidermidis
Staph. saprophyticus
Streptococcus sp
α-hemolytic (partial "green" discoloration)
Optochin
S
R
S. pneumoniae
Nonmotile, capsulated bacteria,
Viridians group
S. sanguis
S. mutans
Nonmotile, oral bacteria, cause dental caries, and endocarditis if introduced into bloodstream
β-hemolytic (complete "clear halo" hemolysis)
Bacitracin
S
R
Group A
S. pyogenes
Group B
S. agalactiae
γ-hemolytic Group D (usually no hemolysis)
Growth in 6.5% NaCl
+
−
E. faecalis
E. faecium
Hydrolyzes bile esculin with black complex, salt-tolerant, normal in colon, UTI, biliary tract infections, post-op endocarditis, opportunistic infection, treatment: penicillin plus aminoglycoside
S. bovis
hydrolyzes bite esculin with black complex
Neisseria sp
Nonmotile, kidney-shaped, catalase and oxidase +ve, requiring enriched media to grow (e.g. Thayer-Martin or chocohlate agar), piliated, elaborate IgA1 protease that inactivates IgA1
Maltose fermentation
+
−
N.meningitidis
Polysaccharide capsule, pili, tetravalent vaccine, Waterhouse-Friderichsen synd
N.gonorrhoeae
No capsule, β-lactamase, pili, Thayer-Martin medium, Treatment: ceftriaxone, plus tetracycline for chlamydia, no vaccine

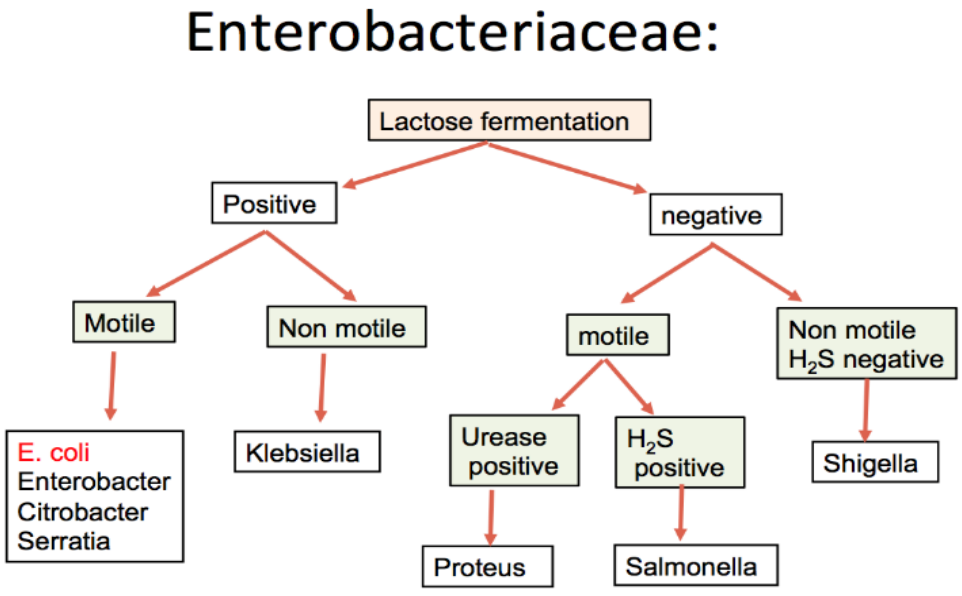
Lab#6

Diagnosis of some dominant gram negative bacteria by Microscopic, culture media and

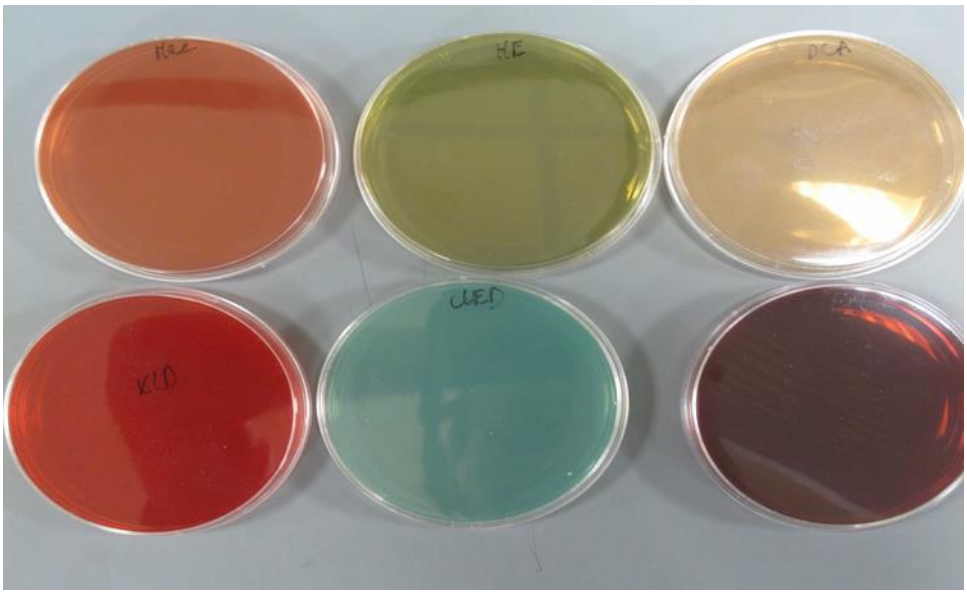
Objective: o diagnosis of some gram negative bacteria (Enterobacteriacae) by selective and differential media

1-Enterobacteriaceae: gram negative bacilli, lives in gastro tract (colon) called (coliform) some of them pathogens and another not pathogen.





Media used for culturing Enterobactriace



Name :	**Indicator :**
Macconkey agar (Mac) :	Neutral red
Deoxycholate citrate agar (DCA) :	Neutral red
Xylose lysine deoxycholate agar (XLD) :	Phenol red
Eosin methylene blue agar (EMB) :	Methylene blue
Hekton Enteric (HE) :	Acid Fuchsine+ Thymol blue Cystine lactose electrolyte deficient
Medium (CLED) :	Bromothymol blue

MacConkey Agar is a selective medium for gram-negative and differential medium for lactose fermenters. It uses to isolate and differentiate members of the Enterobacteriaceae

Principle

MacConkey Agar is a selective medium because it containing Bile salts and crystal violet inhibit growth of Gram-positive bacteria

MacConkey Agar is differential medium because it containing Lactose and neutral red

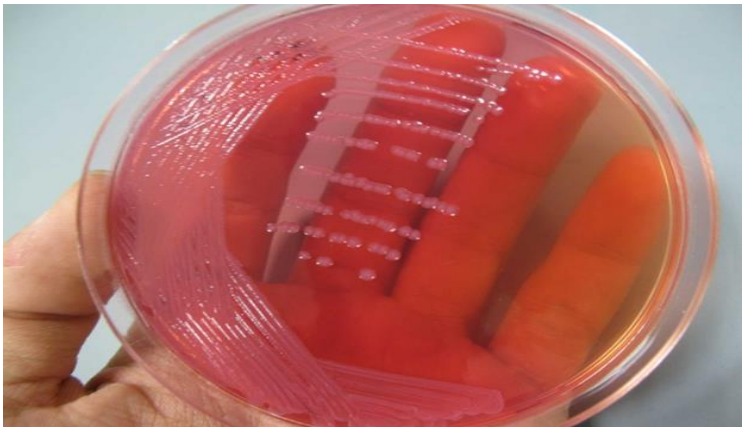
Eosin-methylene blue agar is selective for gram-negative bacteria against gram-positive bacteria. Escherichia coli colonies grow with a metallic sheen with a dark center

Principle

EMB agar contains peptone, lactose, sucrose, and the dyes eosin Y and methylene blue. The dye methylene blue in the medium inhibits the growth of gram-positive bacteria; small amounts of this dye effectively inhibit the growth of most gram-positive bacteria.

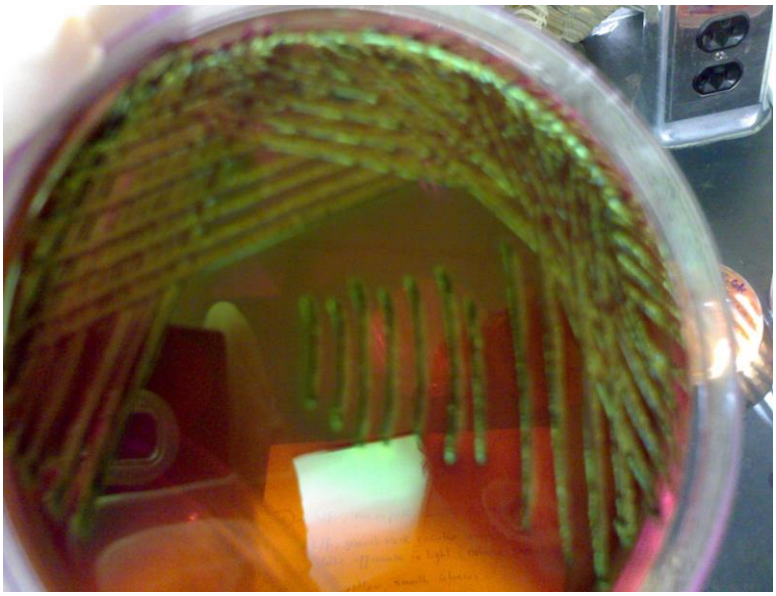
Eosin is a dye that responds to changes in pH, going from colorless to black under acidic conditions.

E. Coli on Mac. Medium



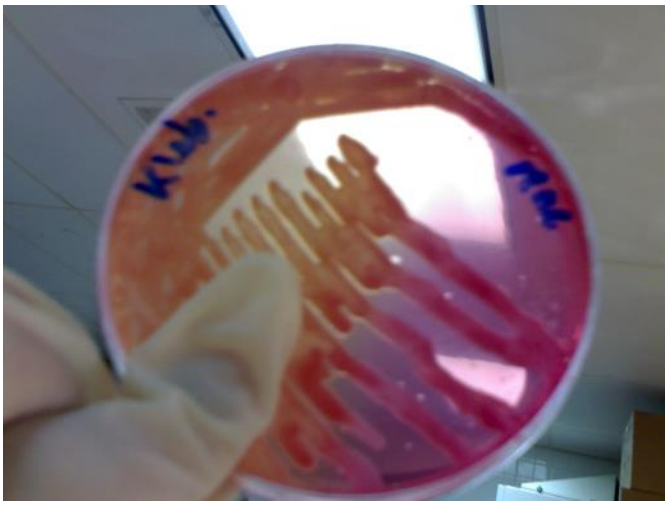
Lactose fermenting, red or pink ,smooth colonies .

E. Coli on EMB medium



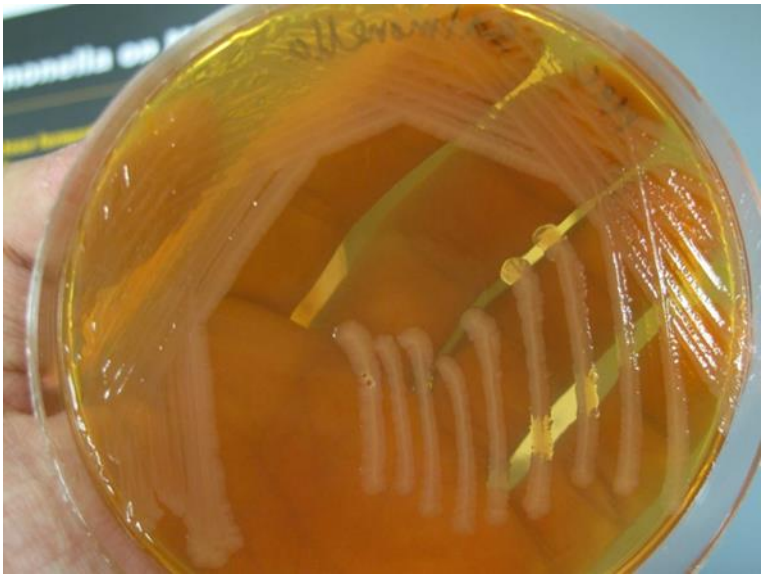
Lactose fermenting , greenish black smooth colonies with metallic appearance (metallic sheen

Klebsiella on MAC. Medium



Lactose fermenting , large , red , and mucoid colonies

Salmonella on MAC medium

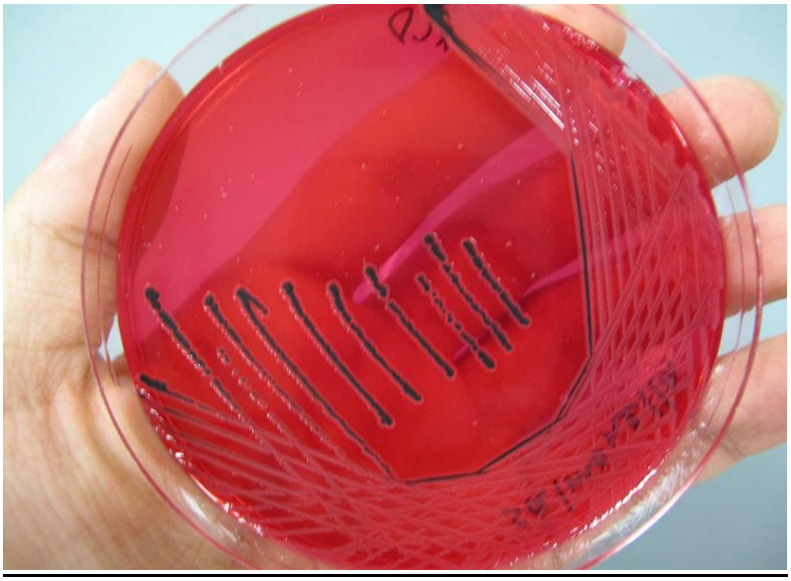


Non lactose fermenting, colorless, and smooth colonies

Salmonella on HE medium

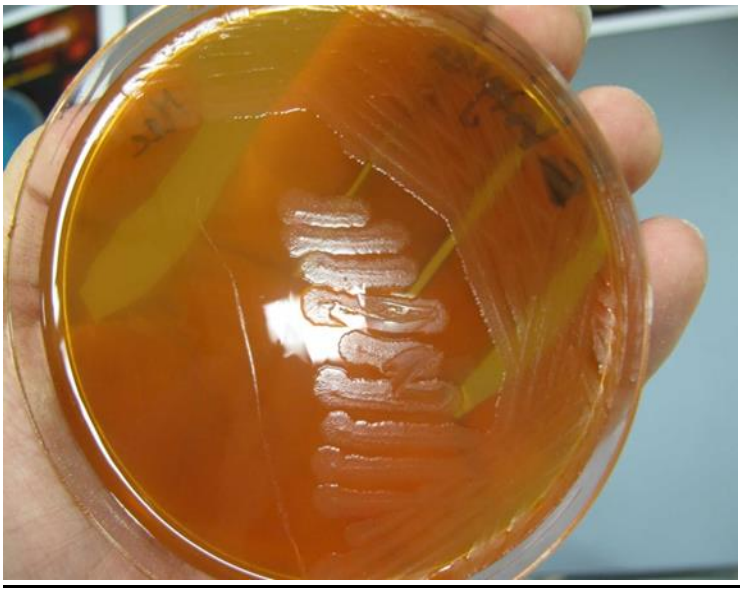
Non lactose fermenting , colorless , smooth colonies , with or without black center

Salmonella on XLD medium



Non lactose fermenting , colorless , smooth colonies with or without black center

Shigella on MAC medium



Non lactose fermenting , colorless ,smooth colonies

Proteus on Nutrient agar

Swarming (starting as waves from the point of inoculation) with fishy smell

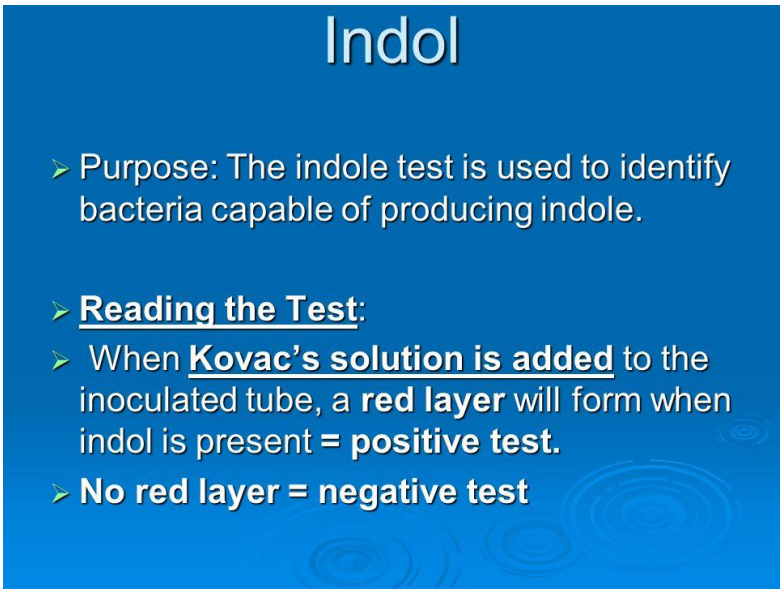
Lab#7

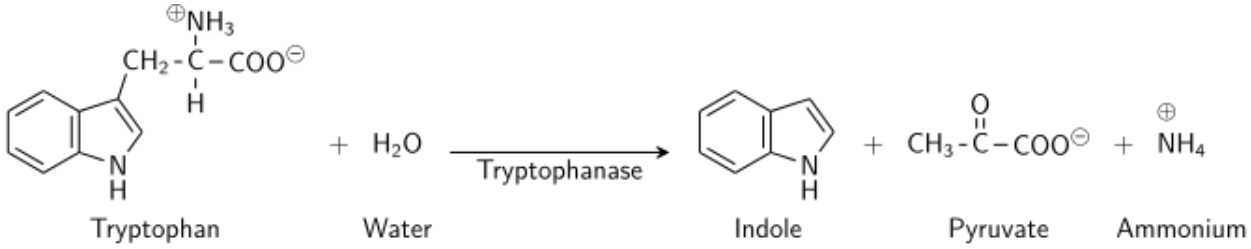
Diagnosis of some dominant gram negative bacteria by biochemical reactions

Objective: To diagnosis of some gram negative bacteria (Enterobacteriace) by biochemical reactions reagents (indol, urase, citrate Citrate utilization , Vogues proskauer, Methyl red, Oxidase and Decarboxylase)

*Biochemical test:

1-Indole test:





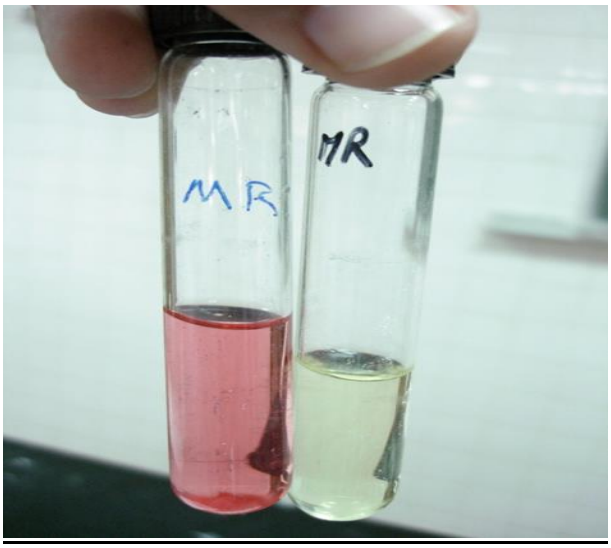
Positive: Red ring EX. E.col

Negative: Yellow ring Ex. Salmonella

2- Methyl red test

Medium: Glucose phosphate peptone water

Reagent : Methyl red reagent



Positive: Bright red color Ex. Kleb

Negative No change Ex. E.col

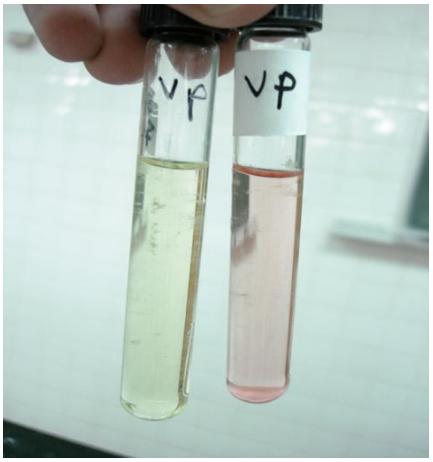
3- **Vogues proskauer test**

Medium : Glucose phosphate peptone water

Reagents : potassium hydroxide + alpha naphthol

Positive: Bight red color Ex. Kleb

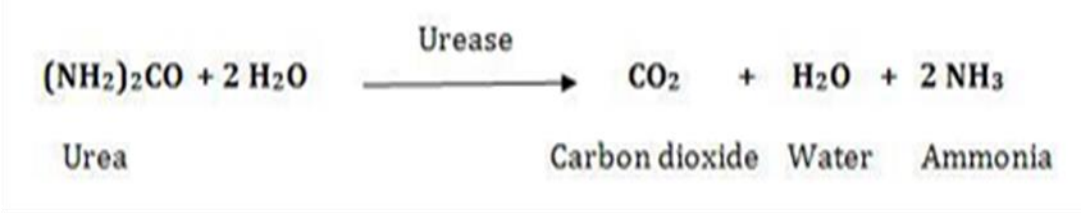
Negative: No change Ex. E.coli



4- **Urease test**

Medium : urease medium (urea agar base + urea 40%) .

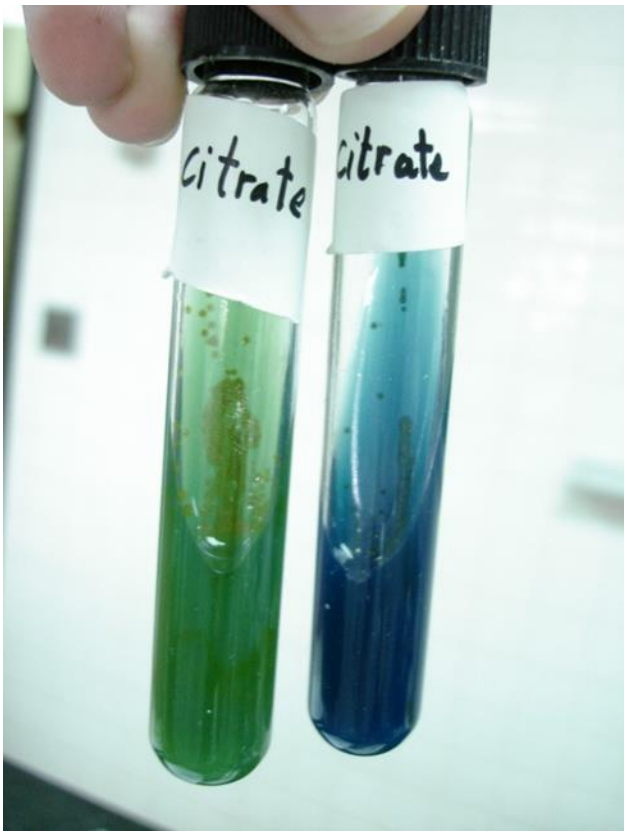
Indicator : Phenol red



Negative: No change Ex. Shigella, E.Coli, Salmonella

Positive: Red or pink Color Ex. Proteus, Brucella spp

5- Citrate utilization test



Positive: Turbid blue Ex. Kleb

Negative : Green Ex. E.coli

6- Triple sugar iron (TSI)

Medium: containing 3 types of sugars Glucose , Lactose , and sucrose

Indicator : Phenol red .

Slant : alkaline (K) : appears red in colour .

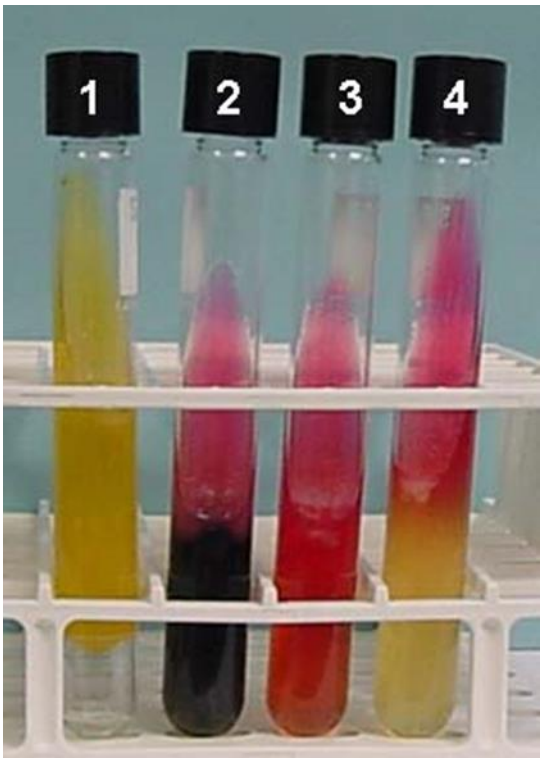
acidic (A) : appears yellow in colour .

Butt : alkaline (K) : appears red in colour **.**

acidic (A) : appears yellow in colour .

Gas : appears as air bubbles .

H2S : appears as black colour ex. protus and salmonella .



Tube 1: Acid slant/ Acid butt/ Gas/ no H2S

Tube 2: Alk slant/ Acid butt/ Gas/ H2S

Tube 3: Alk slant/ Alk butt/ no gas/ no H2S

Tube 4: Alk slant/ Acid butt/ no gas/ no H2S

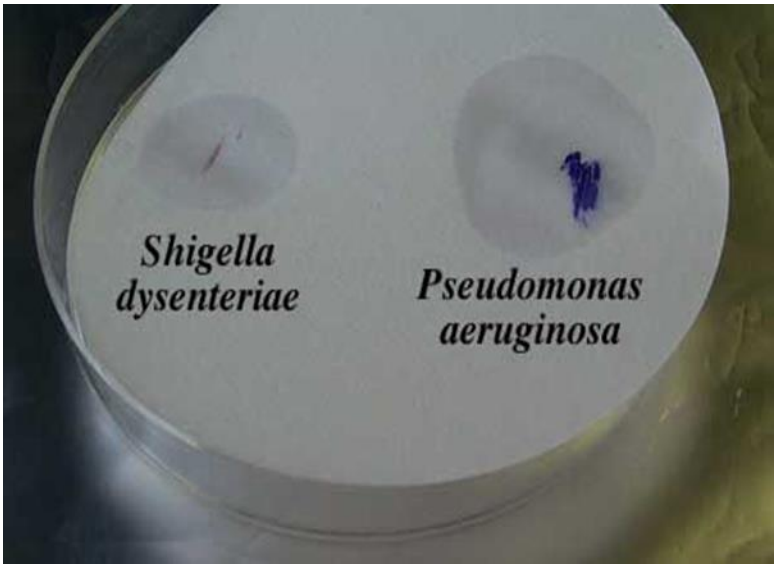
7- Oxidase test :

A piece of filter paper is soaked with oxidase reagent (Tetra methyl-P- phenylenediamine) + colony of the tested organism smeared on this filter paper .

The oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain. When present, the cytochrome c oxidase oxidizes the reagent (tetramethyl-p-phenylenediamine) to (indophenols) Blue color end product. When the enzyme is not present, the reagent remains reduced and is colorless.

Positive : blue purple colour appears within10 seconds Ex. Pseudomonas .

Negative : no change Ex. E. coli



Lab#8

Identification of bacteria by API system

Objective: To understand the: principle, procedures, preparation and reading the results

8- API 20E TEST

Identification system for *Enterobacteriaceae* and other non-fastidious Gram-negative rods.

SUMMARY AND EXPLANATION:

API 20 E is a standardized identification system for *Enterobacteriaceae* and other non-fastidious, gram negative rods which uses 21 miniaturized biochemical tests and a database, the complete list of those organisms that it is possible to identify with this system is given in the Identification Table at the end of this package insert.

PRINCIPLE

The API 20 E strip consists of 20 microtubes containing dehydrated substrates. These tests are inoculated with a bacterial suspension that reconstitutes the media, during incubation, metabolism produces color changes that are either spontaneous or revealed by the addition of reagents, the reactions are read according to the reading Table
and the identification is obtained by referring to the analytical profile Index or using the identification software.

CONTENT OF THE KIT

Kit for 25 tests (ref. 20 100)

- 25 API 20 E strips
- 25 incubation boxes

- 25 result sheets
- 1 clip seal
- 1 package insert

Kit for 100 tests (ref. 20 160)

- 100 API 20 E strips (4x25 strips)
- 100 incubation boxes
- 100 result sheets
- 1 clip seal
- 1 package insert

COMPOSITION OF THE STRIP:

The composition of the API 20 E strip is given in the reading table of this package insert.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

Reagents:

- API NaCl 0.85 % Medium, 5 ml (Ref. 20 230) or
API Suspension Medium, 5 ml (Ref. 20 150)
- API 20 E reagent kit (Ref. 20 120) or
individual reagents: TDA (Ref. 70 402)
JAMES (Ref. 70 542)
VP 1 + VP 2 (Ref. 70 422)
NIT 1 + NIT 2 (Ref. 70 442)
- Zn reagent (Ref. 70 380)
- Oxidase (Ref. 55 635*)

* reference not sold in certain countries: use an equivalent reagent.

- Mineral oil (Ref. 70 100)
- API 20 E Analytical Profile Index (Ref. 20 190) or
identification software (consult bioMérieux).

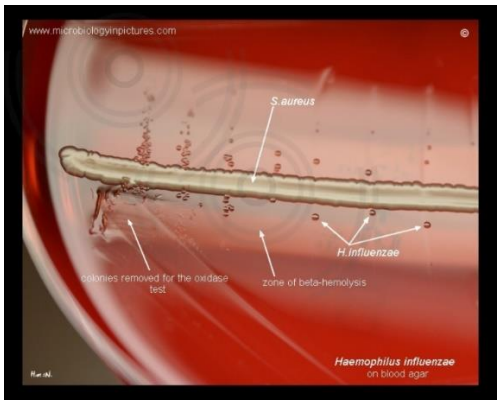
The API system

API 20 E after incubation…Positive results for all tests :

API 20 E after incubation…Negative results for all tests :

Another gram negative

1-*Haemophillus influenzae*: gram negative variable (coccobacilli or bacilli) fastidious organism require x and v factor and can grow in chocolate agar and blood agar around *staphylococcus aureus,* oxidase positive.

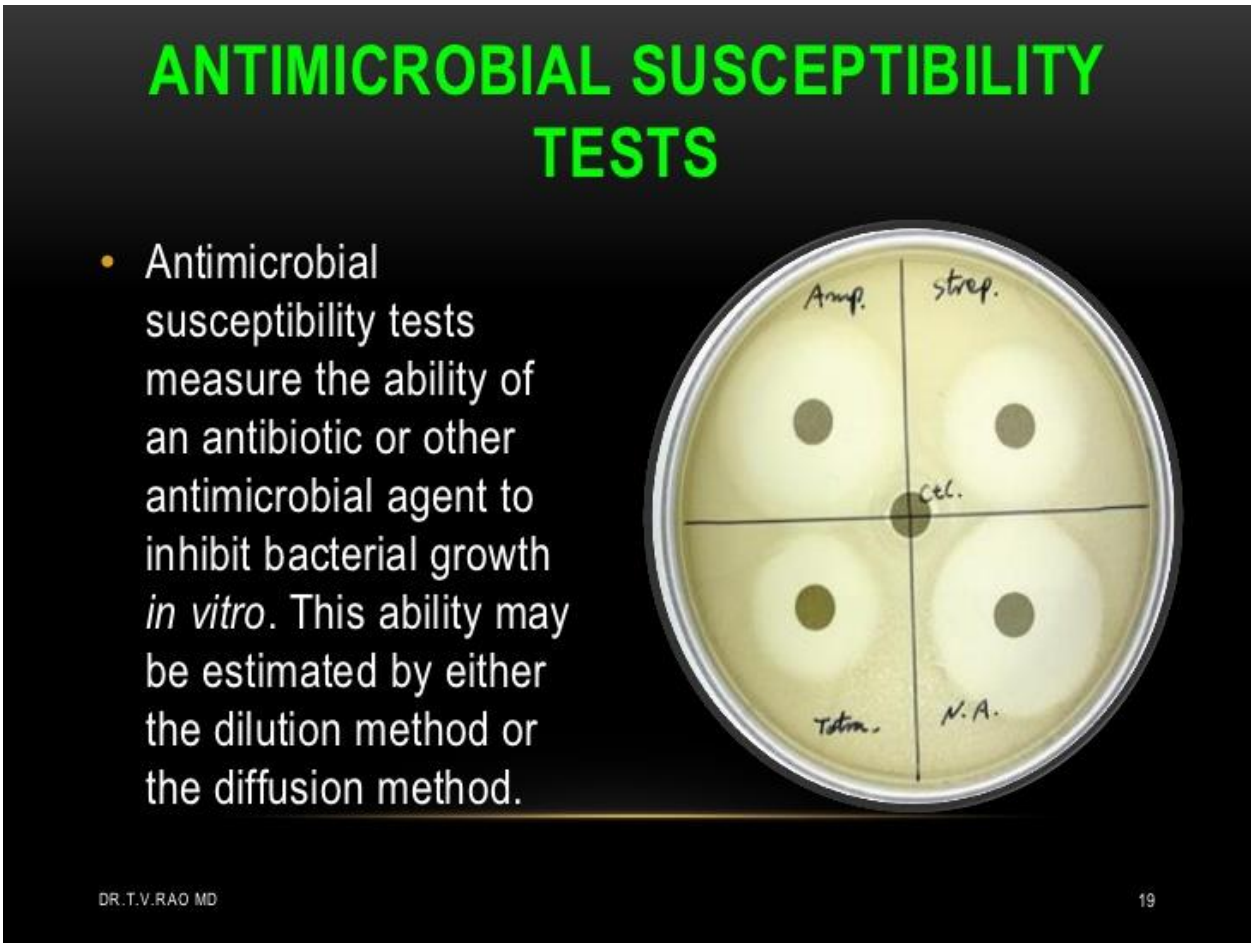


Neisseria gonorrhoeae: **gram negative cocci, oxidase positive, fastidious organism, can grow on Thayer martin agar (chocolate agar with substances to selective growth of Neisseria).**

LAB#9

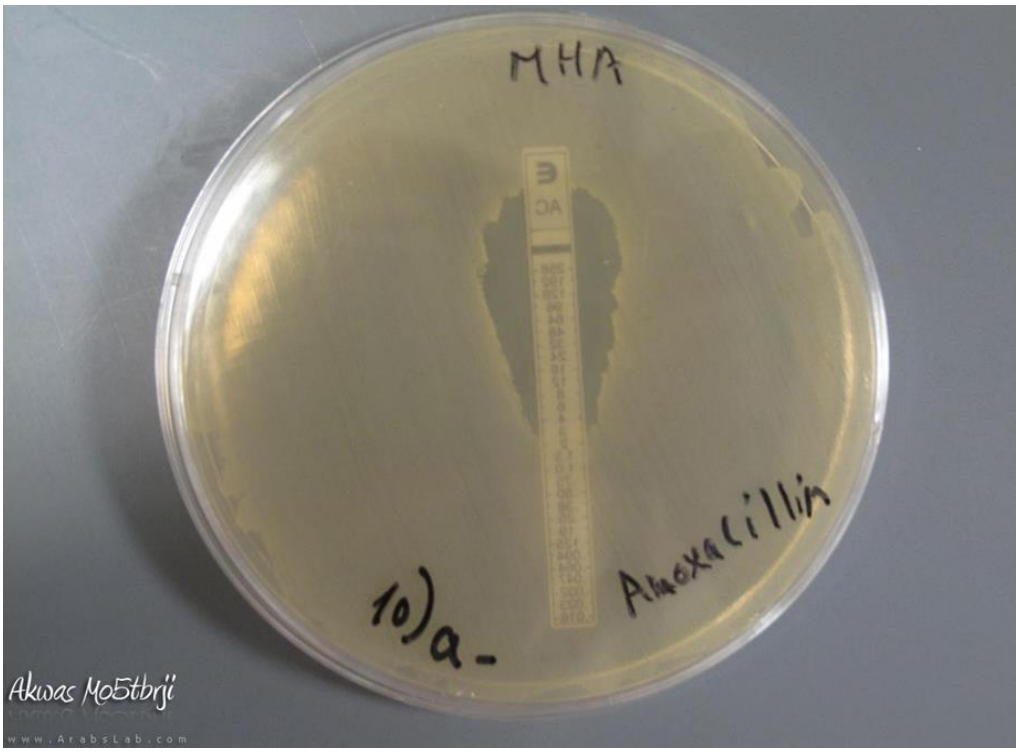
Antimicrobial susceptibility testing

Objective: To understand the: types and procedures of susceptibility testing techniques (E-test, MIC and MBC)



E-test

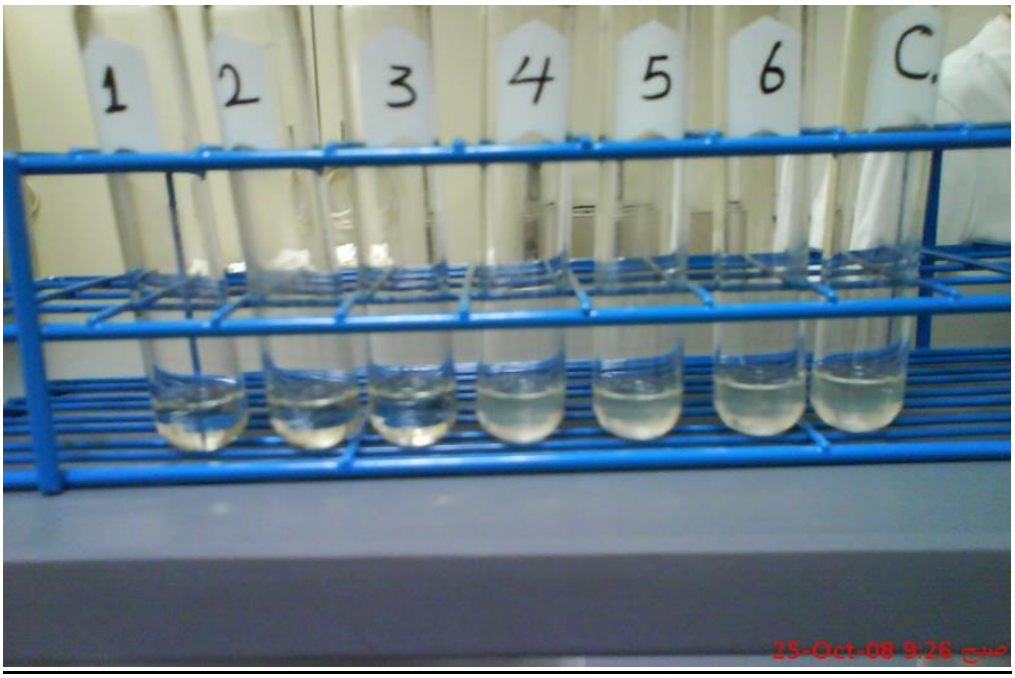

MHA
Cefepime
25-Oct-08 9:27 صبح


MHA
10)a-
Amoxacillin
Akwas Mo5tbrji
www.ArabsLab.com

M.I.C. [Minimum Inhibitory Concentration]

It is the lowest concentration of antimicrobial required to inhibit the growth of the bacteria.

- Check the last clear tube



LAB#10

Serological diagnosis for different pathogens

Objective: To understand the: Types, procedures and Principles of agglutination and ELISA techniques

Serology: is the study of serum and other body fluids. In practice, the term usually refers to the diagnostic identification of antibodies in the serum. Such antibodies are typically formed in response to an infection (against a given microorganism), against other foreign proteins (in response, for example, to a mismatched blood transfusion), or to one's own proteins (in instances of autoimmune disease).

Serological tests used to diagnosis viral or bacterial infection.

a. Agglutination

b. Precipitation

c. Complement-fixation

d. Enzyme-linked immunosorbant assay or ELISA (also known as Enzyme immunoassay.

e. Fluorescent antibody technique

Agglutination

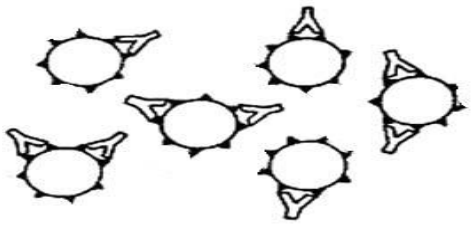
Agglutination is the interaction between antibody and a antigen results in visible clumping. It is usually used for soluble Antigens.

Phases of Agglutination:

Agglutination is a two-Phase reaction

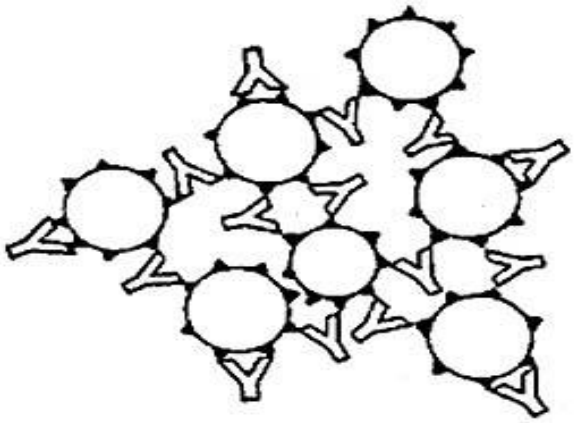
1- Primary Phase (Sensitization)

Antibody reacts with a single antigenic determinant (epitope) on the surface of Ag.



2- Secondary Phase (Lattice formation)

Antibody must be able to bridge the gap between particles so that at least one Fab portion is attached to an epitope on each of two adjacent particles



Types of Agglutination test

1) Direct Agglutination Test
2) Indirect (Passive) Agglutination Test
3) Reverse passive agglutination

1) **Direct Agglutination Test**

Direct agglutination reactions test patient serum for the presence of antibodies against Antigen found naturally on cells

Principle

Combination of an Antigen found naturally on cells (such as bacteria, fungus, and erythrocytes) reacts with its antibody to form antigen-antibody complex particles clump/agglutinate

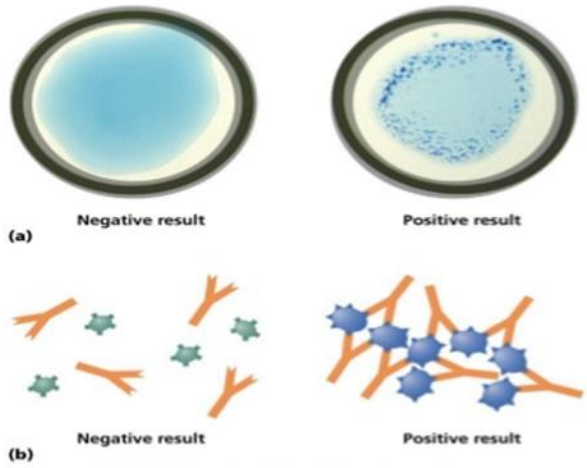
Examples

Blood grouping

Widal test (typhoid fever)

Agglutination tests can be carried out on:

1- Slide agglutination



2- Tube agglutination

For example, the tube agglutination uses to detect the presence of antibody against Salmonella typhi O (somatic antigen) and H (Flagella antigen) as well as S. paratyphi AH S. paratyphi BH and in the given serum sample.

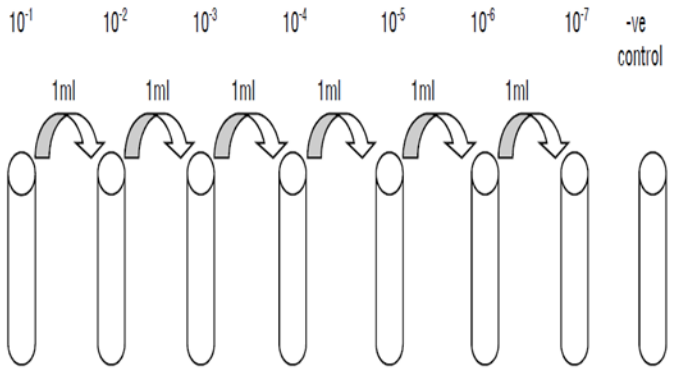
Procedure

1- Dilution of serum

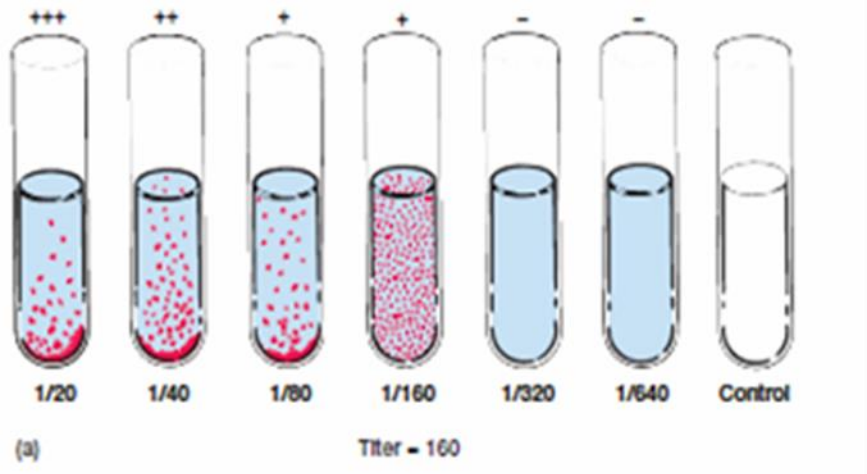
2- Addition of antigen solution

3- Incubation for 1 hour at 37° C

4- Read the Result



Tube no	1	2	3	4	5	6	7	8
Saline-	1.9 ml	1 ml	1ml	1ml	1ml	1ml	1ml	1ml
Test Serum-	+ 0.1ml							
	1:20	**1:40**	**1:80**	**1:160**	**1:320**	**1:640**	**1:1280**	



3- Microliter plates

2) **Passive agglutination (Indirect agglutination test)** it used to detect patient serum antibodies against soluble antigens

Principle

Using synthetic particles called latex beads (polystyrene material); they act as carrier for soluble antigen in agglutination tests change soluble Ag more visible and can be read rapidly (3-5 min) after of mixing the beads with the test sample

Example

1- Rheumatoid factor (RF) test

2- Antistreptolysin O (ASO) test

Anti-streptolysin O Test (ASO)

ASO testing is a procedure that detects the presence of antibodies against toxin streptolysin O of streptococcus pyogen, in human serum.

Principle

This method is based on an immunological reaction between streptococcal exoenzymes bound to biologically inert latex particles and streptococcal antibodies in the test sample, an visible agglutination reaction will take place within 2 minutes.

The reagent concentration is 200 IU/mL

Elevated levels may indicate:

Active streptococcal infection

Sore throat

Rheumatic fever

LAB#11

Serological diagnosis for different pathogens

***ELISA (The enzyme-linked immunosorbent assay**):

ELISA is the most common laboratory technique which is used to measure the concentration of an analytic (usually antibodies or antigens) in sample.

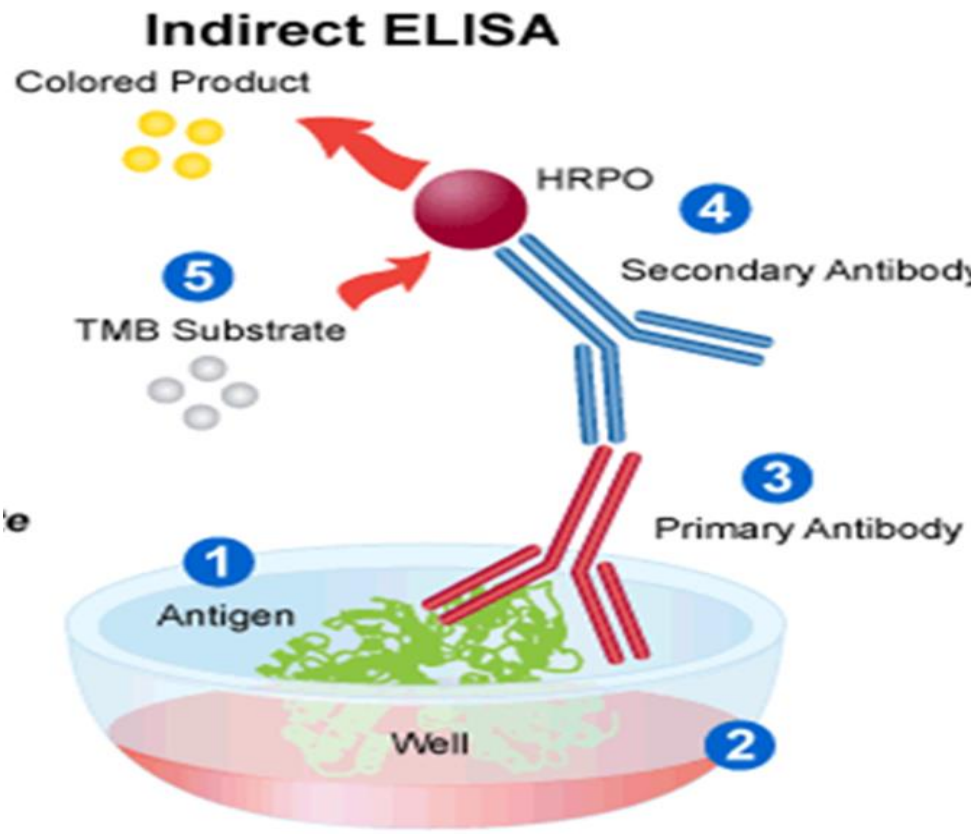
ELISA is a popular format of "wet-lab" type analytic biochemistry assay that uses a solid-phase enzyme immunoassay (EIA) to detect the presence of a substance, usually an antigen, in a liquid sample or wet sample

ELISA may be run in a qualitative or quantitative format

Types of ELISA

1) Indirect ELISA
2) Sandwich ELISA
3) Competitive ELISA

1-**Indirect ELISA:** is a two-step ELISA which involves two binding process of primary antibody (sample antibody) and labeled secondary antibody.



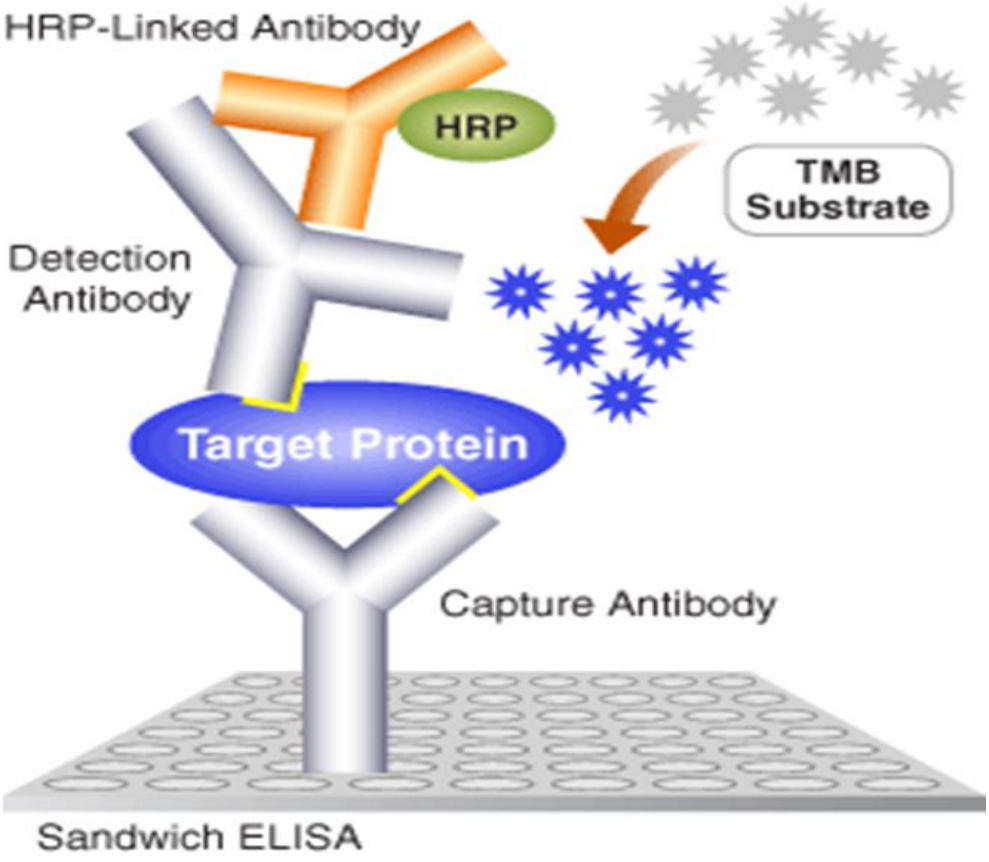
Component of Indirect ELISA

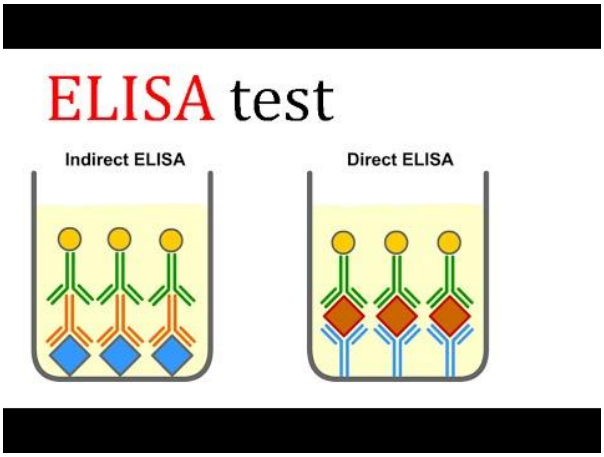
1) **Solid phase = 96 well microplate**
2) **sample contain primary antibody (sample antibody)**
3) **Blocking solution : 0.25% - 2% bovine serum albumin, 2% non-fat dried milk and 5 - 10% foetal calf serum**
4) **Washing buffer (phosphate buffer saline)**
5) **Conjugate (enzyme linked secondary antibody) : (for assays of (human) antibodies use anti-(human) IgG**
6) **Substrate e.g hydrogen peroxide**
7) **Chromogen**
8) **Stop solution (0.5 M NaOH or H2SO4)**

2-**Direct ELISA(Sandwich ELISA):**

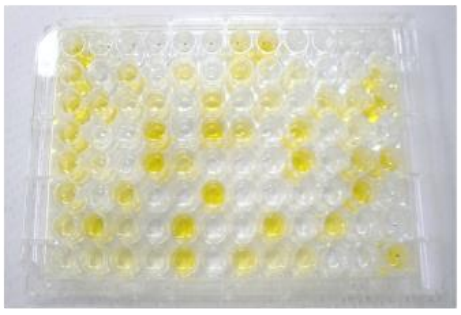
Sandwich ELISA is highly efficient in sample antigen detection. It is quantifying antigens between two layers of antibodies (i.e. capture and detection antibody).

The sandwich ELISA quantify antigens between two layers of antibodies (i.e. capture and detection antibody).

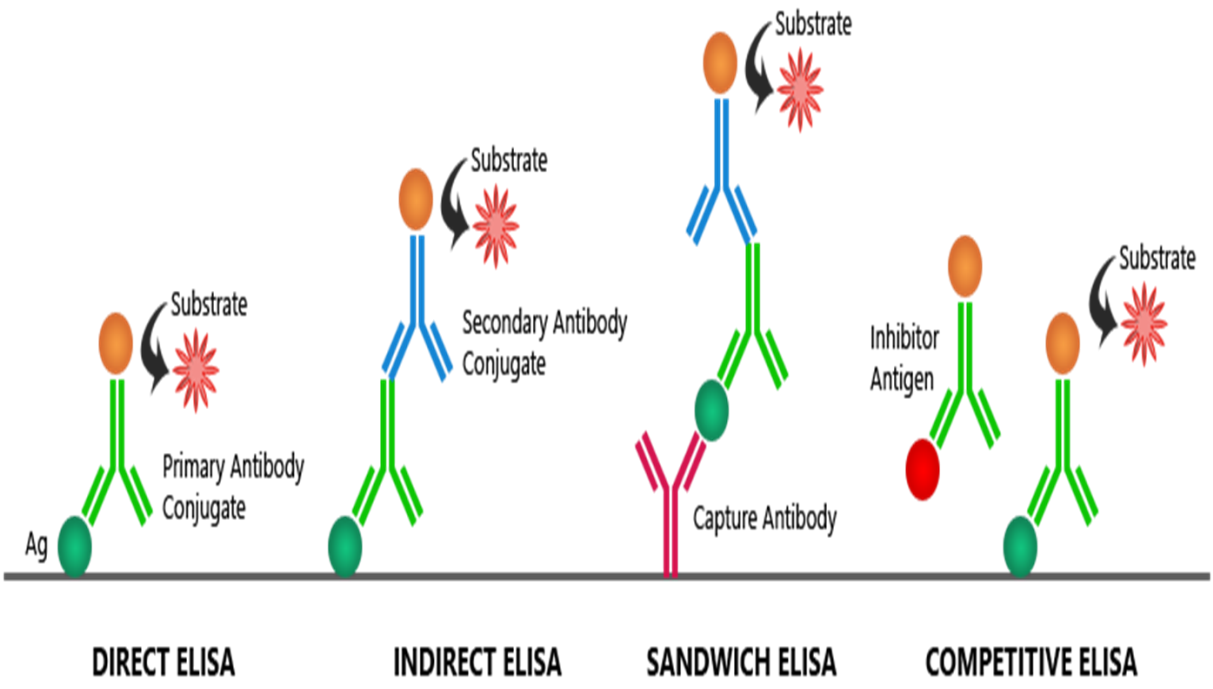




ELISA: An example of an assay using a 96-well plate.



The yellow color indicates that the target protein is present. The higher degree of the color, the higher concentration of the target protein.



Lab#12

Diagnosis of pathogens by molecular methods (PCR – technique)

Objective: To understand the: Types, procedures and Principles PCR

PCR (Polymerase reaction) is an in-vitro enzymatic reaction used for amplification of a specific DNA fragment that lies between two regions of known nucleotide sequence.

Use to diagnosis of viral infection and bacterial infection

PCR Requirements:

1) **Template DNA**
2) **RNA primers (Two primers forward primer and reveres primer)**
3) **DNA polymerase**
4) **dNTPs; dTTP, dCTP, dATP, dGTP**
5) **Buffer**

Three steps of PCR principles:

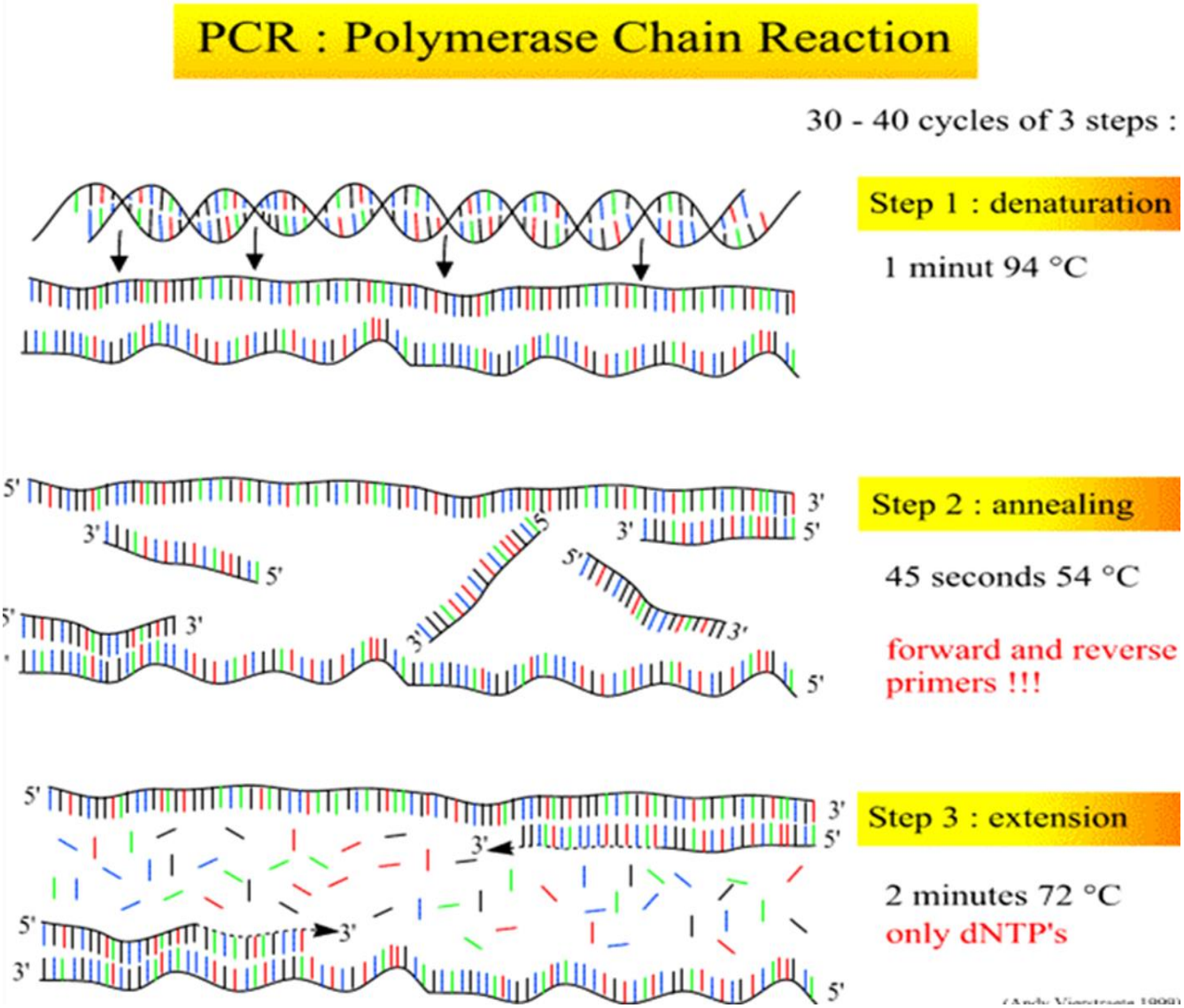
1-**Denaturation**: the two DNA stands are separated by breaking down the hydrogen bonds between complementary bases at **92-96°C.**

2-**Annealing**: Two specific primers are linked to sequences flanking the target region **(37-65°C).**

Primers serve as a starting unit for DNA polymerase

3-**Extension**: Two specific primers are linked to sequences flanking the target region **(72°C).**

Primers serve as a starting unit for DNA polymerase.



Types of PCR

PCR is of the following types

1-Real – Time PCR (RT-PCR):

In this type, the DNA amplification is detected in real time with the help of a fluorescent reporter. The signal strength of the fluorescent reporter is directly proportional to the number of amplified DNA molecules
This type is based on the same principle, but the only difference is that it is associated with a device to determine the real time to start the reaction and then the real quantity of copies of DNA. This depends on the presence of free radioactive nitrogen bases to determine this, which makes it easier for researchers to determine the presence of the gene and the amount of the gene without reaching the end of the specific thermocouples and its results chart.

2- Nested PCR

This was designed to improve sensitivity and specificity. They reduce the non-specific binding of products due to the amplification of unexpected primer binding sites.

3- Multiplex PCR

This is used for the amplification of multiple targets in a single PCR experiment. It amplifies many different DNA sequences simultaneously.

4- Quantitative PCR

It uses the DNA amplification linearity to detect, characterize and quantify a known sequence in a sample.

5- Arbitrary Primed PCR

It is a DNA fingerprinting technique based on PCR. It uses primers the DNA sequence of which is chosen arbitrarily.

GeneXpert:

The Gene pert test is a molecular test for Genes responsible for Multi drug resistance bacteria which diagnoses MRSA or TB or Carbapenem resistance **by detecting the presence of genes bacteria, as well as testing for resistance to the drug Methicillin.**

Lab#13

Yeast identification

1-Germ tube test:

Use to differentiate between Candida albicans and candida sp.

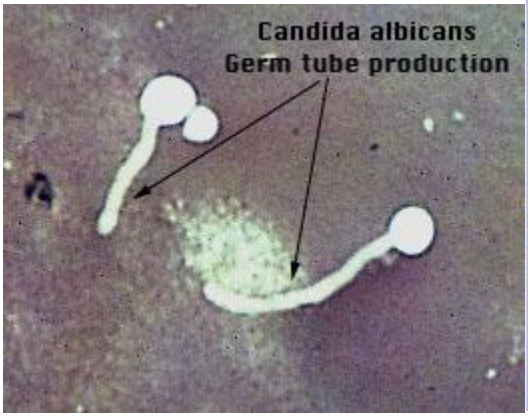
Tube contains of human plasma or horse plasma

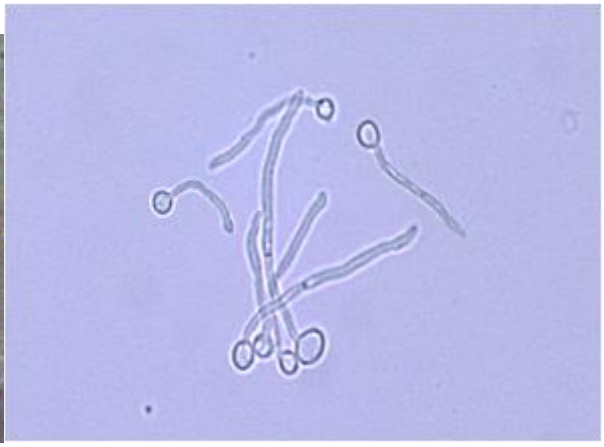
***Procedure:**

1-suspention one colony of yeast on plasma.

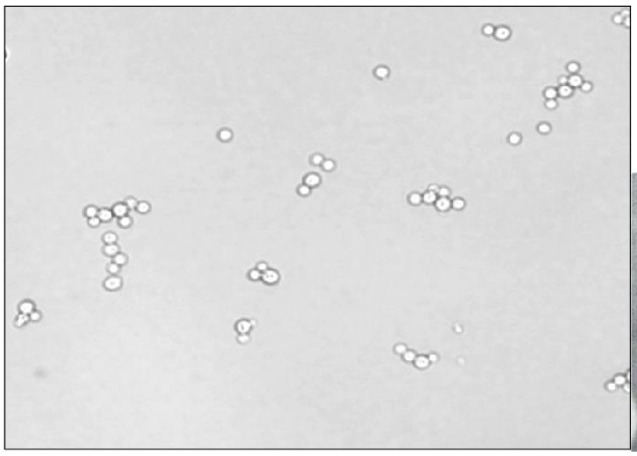
2-incubate at 37 c for 3 hours and read result

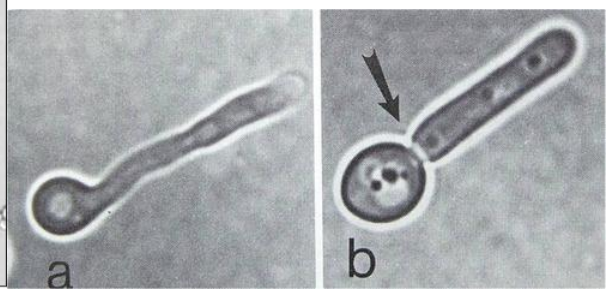
germ tube





Germ tube positive Candida albicans





Germ tube negative (+)positive False positive