
Bacteriology
(culture & sensitivity)

What is meant by culture and sensitivity?

- a laboratory test by which samples from body specimens are cultivated in a special growth medium in order to isolate the microorganisms that may be present. Culture is a highly effective laboratory method for identifying the microorganisms that cause infectious disease and for obtaining a definitive diagnosis.

Why It Is Done

- Generally culture and sensitivity test is done to:
- Detect and identify bacteria that may be causing an infection
- Identify the best antibiotic to treat the infection.

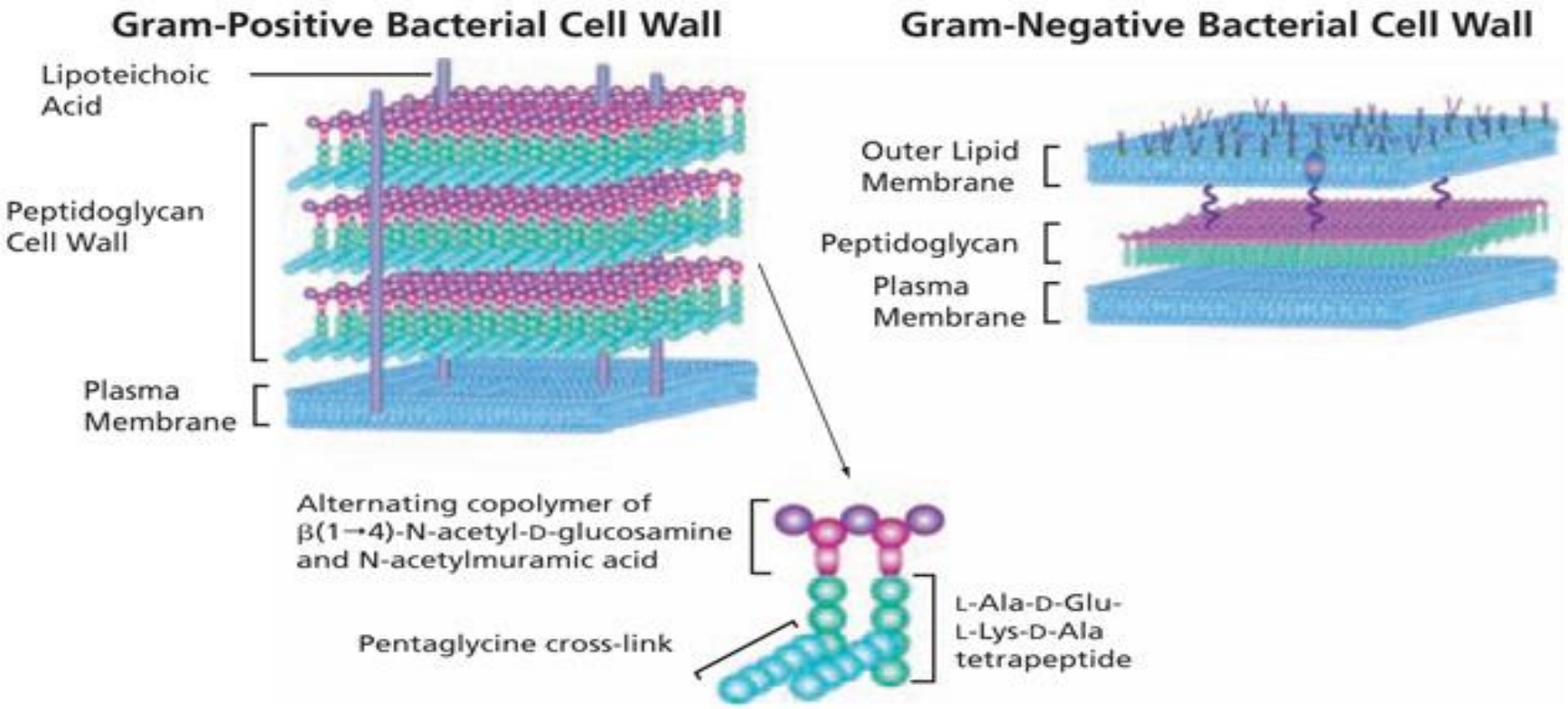
LAB SAFETY

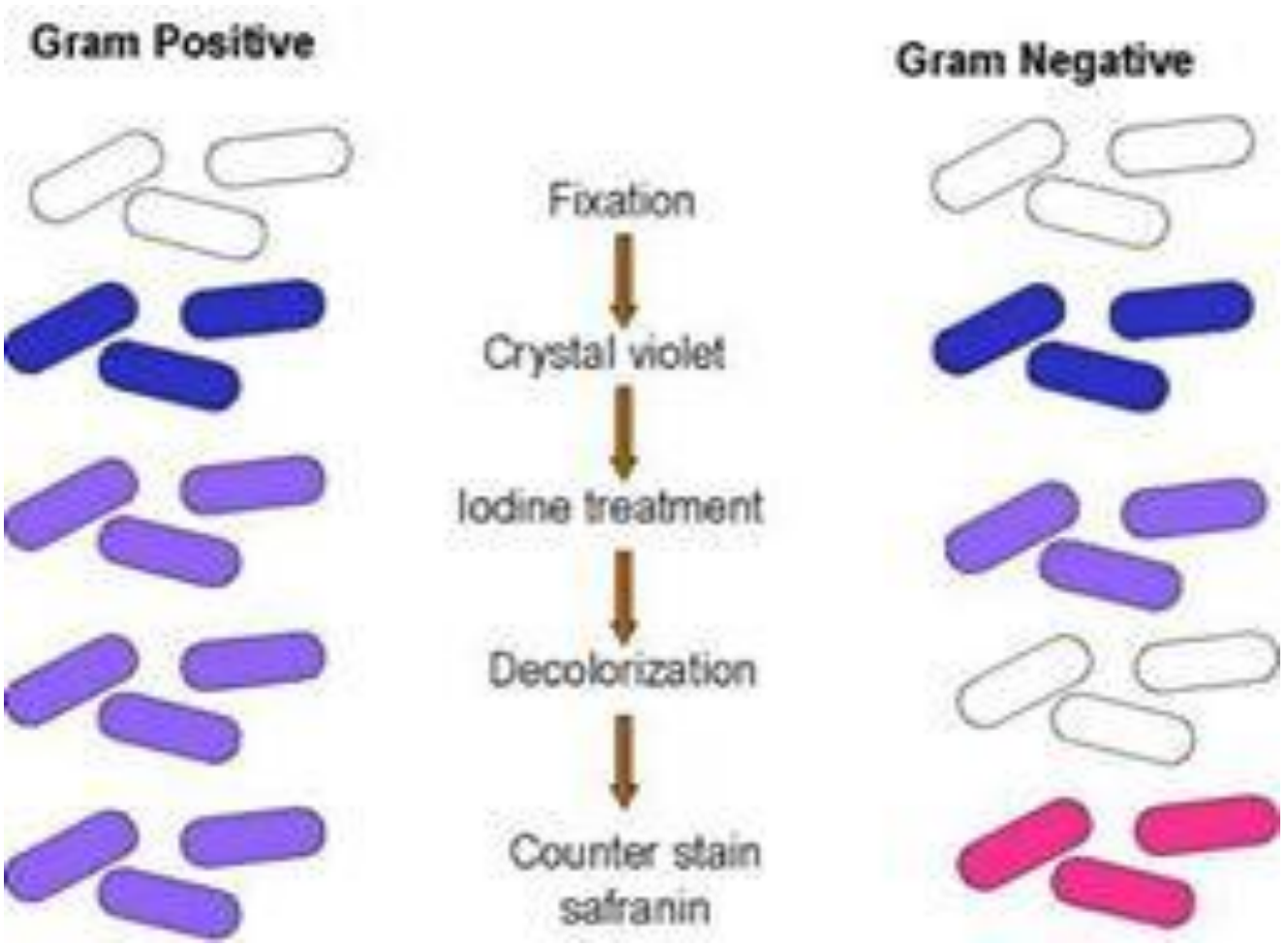
Gram +ve and Gram –ve bacteria

Summary of the differences between Gram positive & Gram negative bacteria

Property of bacteria	Gram Positive	Gram Negative
Thickness of wall	20-80 nm	10 nm
Number of layers in wall	1	2
Peptidoglycan content	>50%	10-20%
Teichoic acid in wall	+	-
Lipid & lipoprotein content	0-3%	58%
Protein content	0%	9%
Lipopolysaccharide	0	13%
Sensitive to penicillin	Yes	Less sensitive
Digested by lysozyme	Yes	Weakly

Cell wall structure of gram +ve and gram-ve bacteria

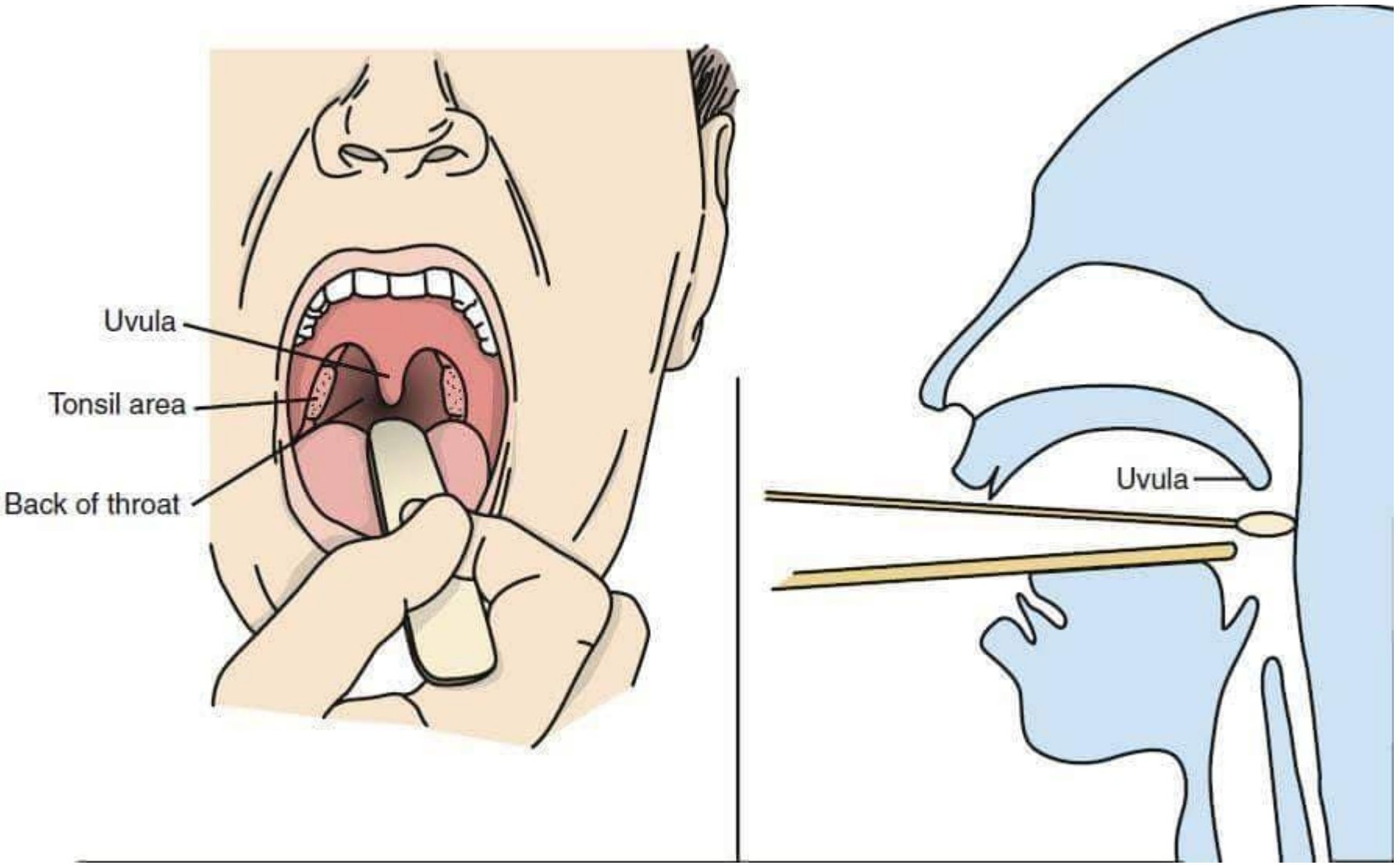



Gram Positive
Gram Negative
Fixation
Crystal violet
Iodine treatment
Decolorization
Counter stain
safranin

Precautions

- The sample should be in sterile cup
- No Antibiotic before testing by 48 hours
- Morning mid-stream specimen is preferable

Throat Swab sample



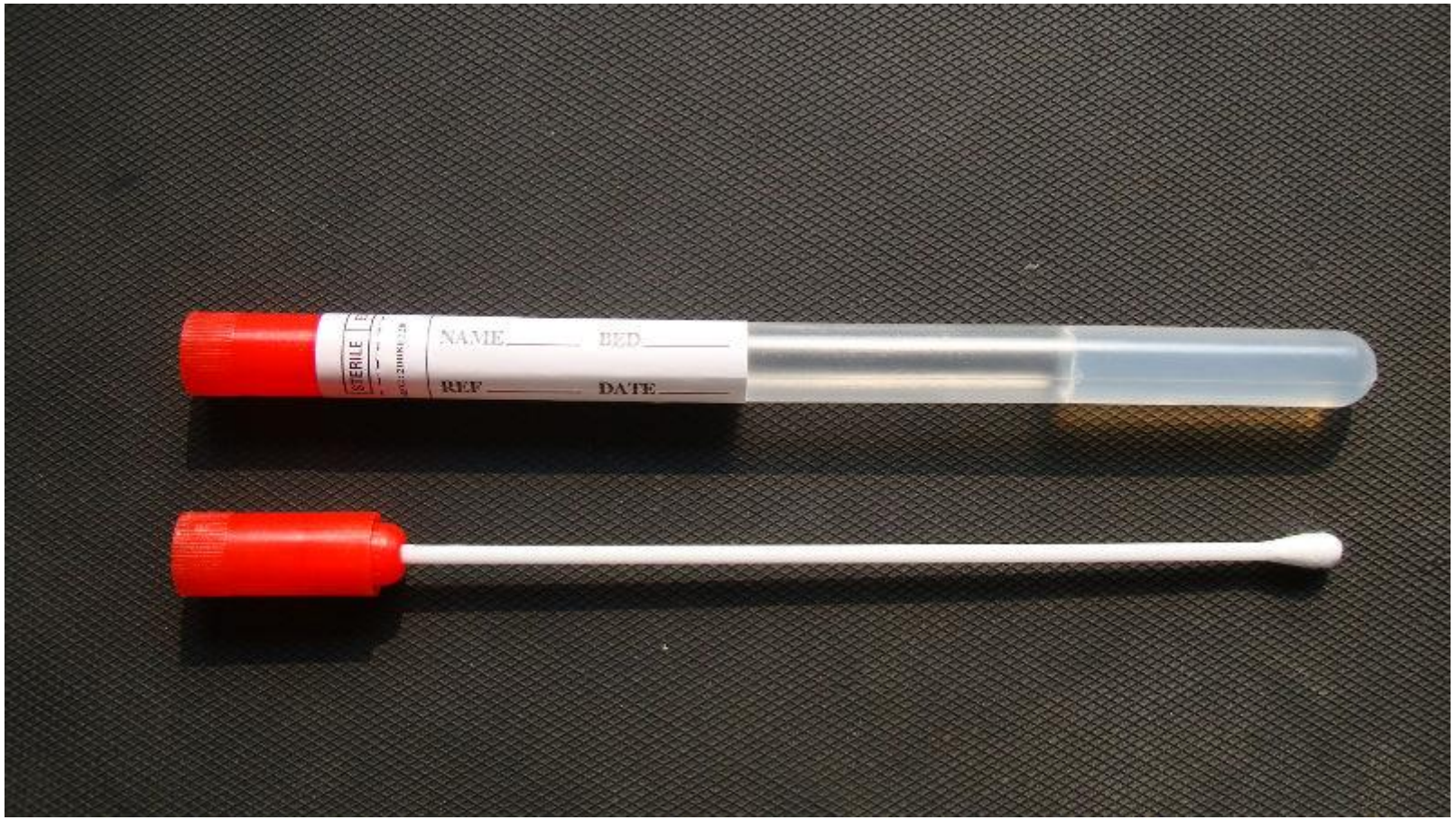
Throat Swab con.

- A throat culture may be done to determine the cause of a sore throat.
- A throat culture helps to distinguish a bacterial infection from a viral infection.
- Identifying the organism that is causing the sore throat.

Throat Swab con.

- Sampling
- Place the patient's mouth in good visual light.
- Use a sterile throat culture swab.
- Tilt head back. Depress the patient's tongue with
- a tongue blade and visualize the throat as well as possible.
- Rotate the swab firmly and gently over the back of the throat, around both tonsils, and on areas of inflammation, exudation, or ulceration.

swab



Pus sample

- A skin or wound culture is a test to detect and identify bacteria that may be infecting the skin or a wounds such as injuries, burn, surgical wound or animal bites.
- Note: symptoms of an infection often include pain at the site, redness, tenderness, swelling, warmth, red streaks toward the body, swollen lymph nodes, and the presence of pus.

Blood culture

- A blood culture can detect and identify bacterial infection in the blood. The blood does not normally contain any bacteria but bacteria can enter the bloodstream as a severe complication of infections during surgery (especially when involving mucous membranes such as the gastrointestinal tract), and other foreign bodies entering the arteries or veins (including intravenous drug abuse). A bacterial infection is usually serious because the blood can spread the bacteria to any part of the body.

Sampling blood culture

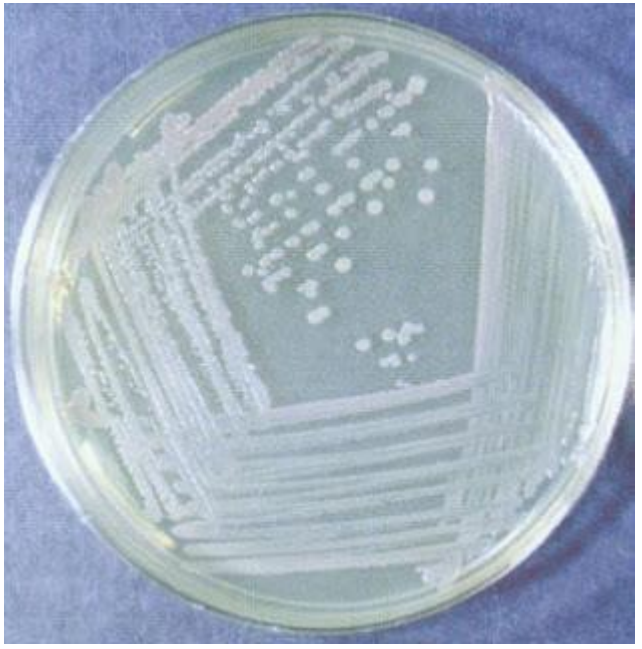
- Incubate media bottles before taking the sample at 37 degree for at least10 minutes
- put the label on the bottle indicating the patient identification
- Disinfect the skin carefully to avoid contamination with skin microorganisms.
- Blood is obtained by inserting a needle into the vein then draw about 10 ml of blood and put it into two culture bottles containing broth to grow aerobic and anaerobic microorganisms.
- Inoculate the culture bottles carefully and incubate the bottles in incubator
- Note: do not store inoculated bottles in the refrigerator
- Note: Multiple samples (two or three blood samples from different veins) are usually taken to increase the chances of identifying bacteria in the blood and to have a better chance of ruling out a false positive blood culture (results from skin contaminant)

Classification of media

- Classification based on the basis of purpose/ functional use/ application
- Many special purpose media are needed to facilitate recognition, enumeration, and isolation of certain types of bacteria. To meet these needs, numerous media are available.

1) Nutrient agar

- Is a microbiological growth medium commonly used for the routine cultivation of non-fastidious bacteria.



Nutrient media

- Agar is a polysaccharide formed of agarose and agaropectin, it is used to solidify culture media because:
- It has a highly gelling capacity
- Free of substances toxic to bacteria
- Note: This medium is the base of different types of media.

2. Enriched medium (Added growth factors):

- Addition of extra nutrients in the form of blood, serum, etc, to basal medium makes them enriched media. Enriched media are used to grow nutritionally exacting (fastidious) bacteria. Blood agar, chocolate agar, Loeffler's serum slope etc are few of the enriched media. Blood agar is prepared by adding 5-10% (by volume) to a blood agar base. Chocolate agar is also known as heated blood agar or lysed blood agar.

3. Selective and enrichment media

- are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria. While selective media are agar based, enrichment media are liquid in consistency. Both these media serve the same purpose

3.Selective and Enrichment media

- . Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen of interest. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these.
- ex: Macconkey

4. Differential/ indicator medium:

- Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony colour. Various approaches include incorporation of dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently coloured colonies. Such media are called differential media or indicator media. Differential media allow the growth of more than one microorganism of interest but with morphologically distinguishable colonies.
- Ex:blood agar ,Mackonkey

5. Anaerobic media:

- anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation –reduction potential and extra nutrients.

- Media for anaerobes may have to be supplemented with nutrients vitamin K.. Boiling the medium serves to expel any dissolved oxygen. Addition of 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cysteine or red hot iron filings can render a medium reduced. Before use the medium must be boiled in water bath to expel any dissolved oxygen and then sealed with sterile liquid paraffin.

Samples and media

- Urine : CLED,Macconkey
- Stool:blood ,Macconkey,salmonella and shigella media
- Semen :blood ,Macconkey,chocolate
- Pus :blood ,Macconkey,chocolate and thioglycolate(An aerobic media)

Bacterial Cultivation
Culturing Methods

in Broth medium

- by direct **inoculation** of clinical specimen using cotton swab or plastic pipette.

on Agar plate

1- Streak-plate method: (commonest & routinely-used).

2- Pour-plate method.

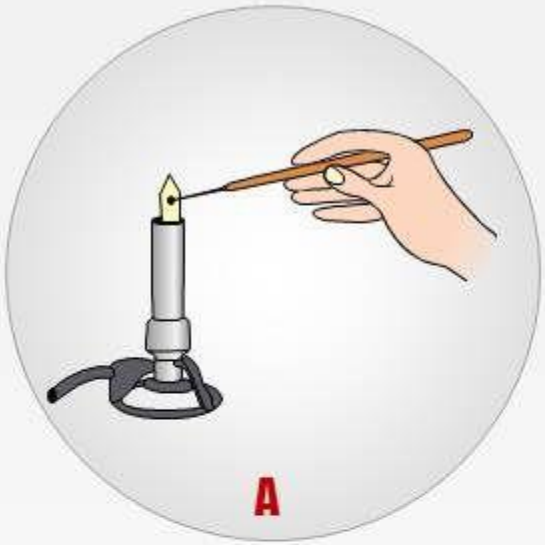
3- Spread-plate method.

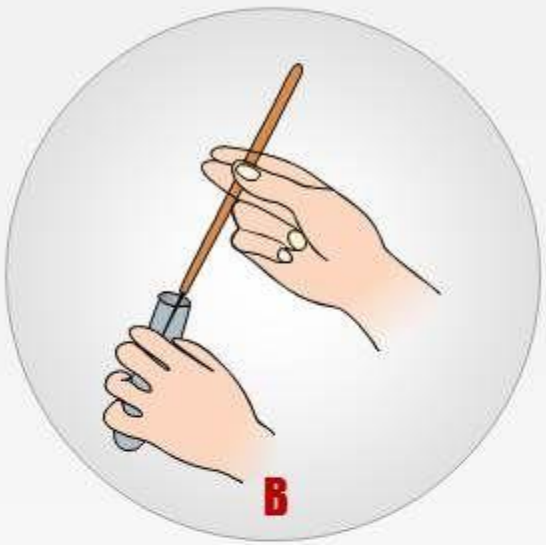
(2 & 3 uncommonly used).

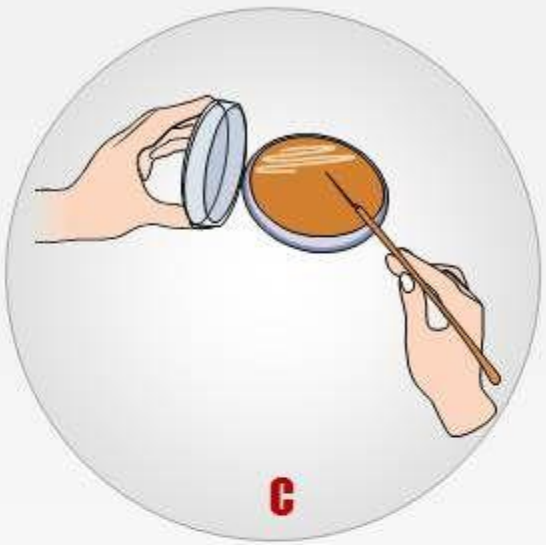
TARGET : **Pure Separate Colonies**

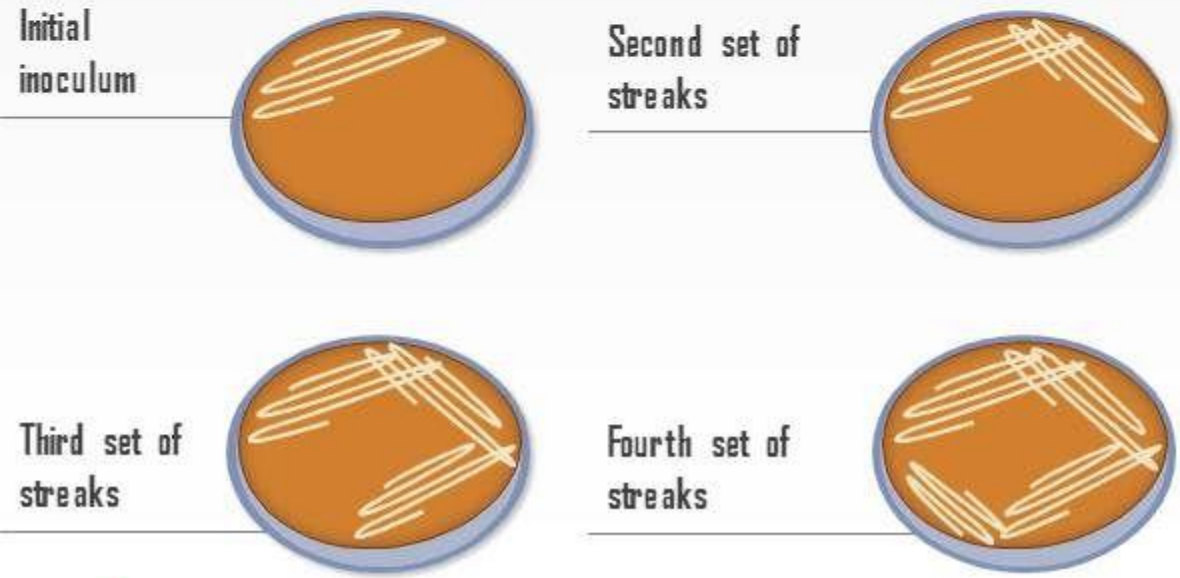
Streak Plate Method

The Streak Plate Isolation Method



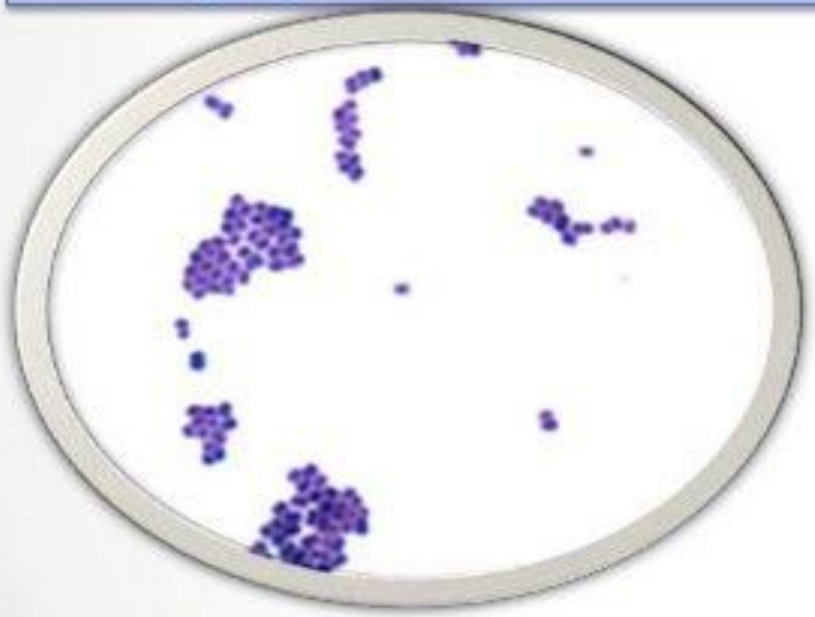






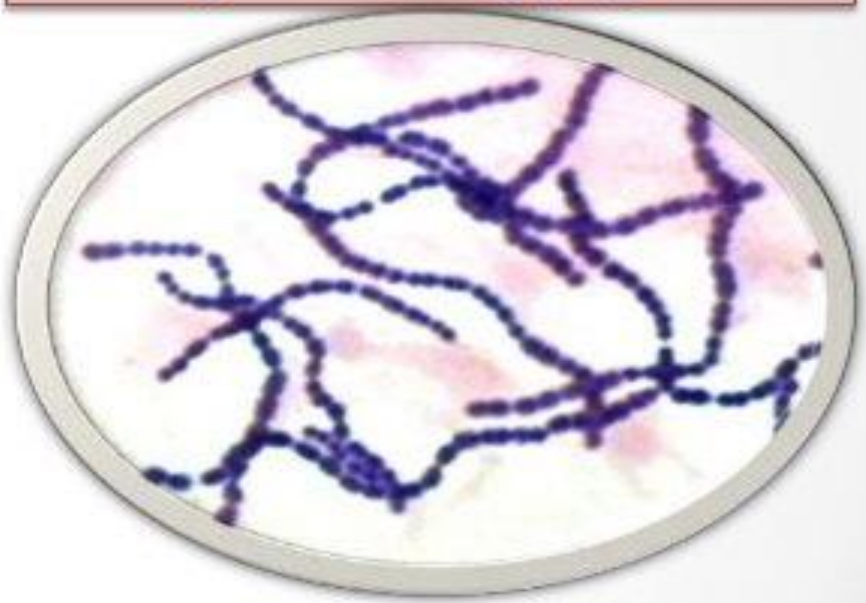
Distinguishing Features
Morphology

Staphylococcus aureus



Gram positive
Cocci
Grape like clusters

Streptococcus pyogenes



Gram positive
Cocci
Chains

Biochemical Reactions

- Bacteria **vary** in their metabolic & enzymatic activities.
- Used in **identification** of different genera & species of bacteria.
- Biochemical reactions are done on bacteria grown in **pure culture**.

Biochemical Reactions

1. Sugar fermentation
2. Oxidase test
3. Catalase test
4. Coagulase test
5. API Test

Catalase test

- Used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as streptococci.

Catalase test

Principle:

Demonstrate the presence of an enzyme catalase.

Catalase is an enzyme that decomposes hydrogen peroxide into water and oxygen. Chemically,it is a hemoprotein structurally similar to hemoglobin

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2 \text{ (gas bubbles)}$$

Uses of Antibiotic Sensitivity Testing

- **Antibiotic sensitivity test:** A laboratory test which determines how effective antibiotic therapy is against a bacterial infections.
- Antibiotic sensitivity testing will control the use of **Antibiotics** in clinical practice
- Testing will assist the clinicians in the choice of drugs for the treatment of infections.

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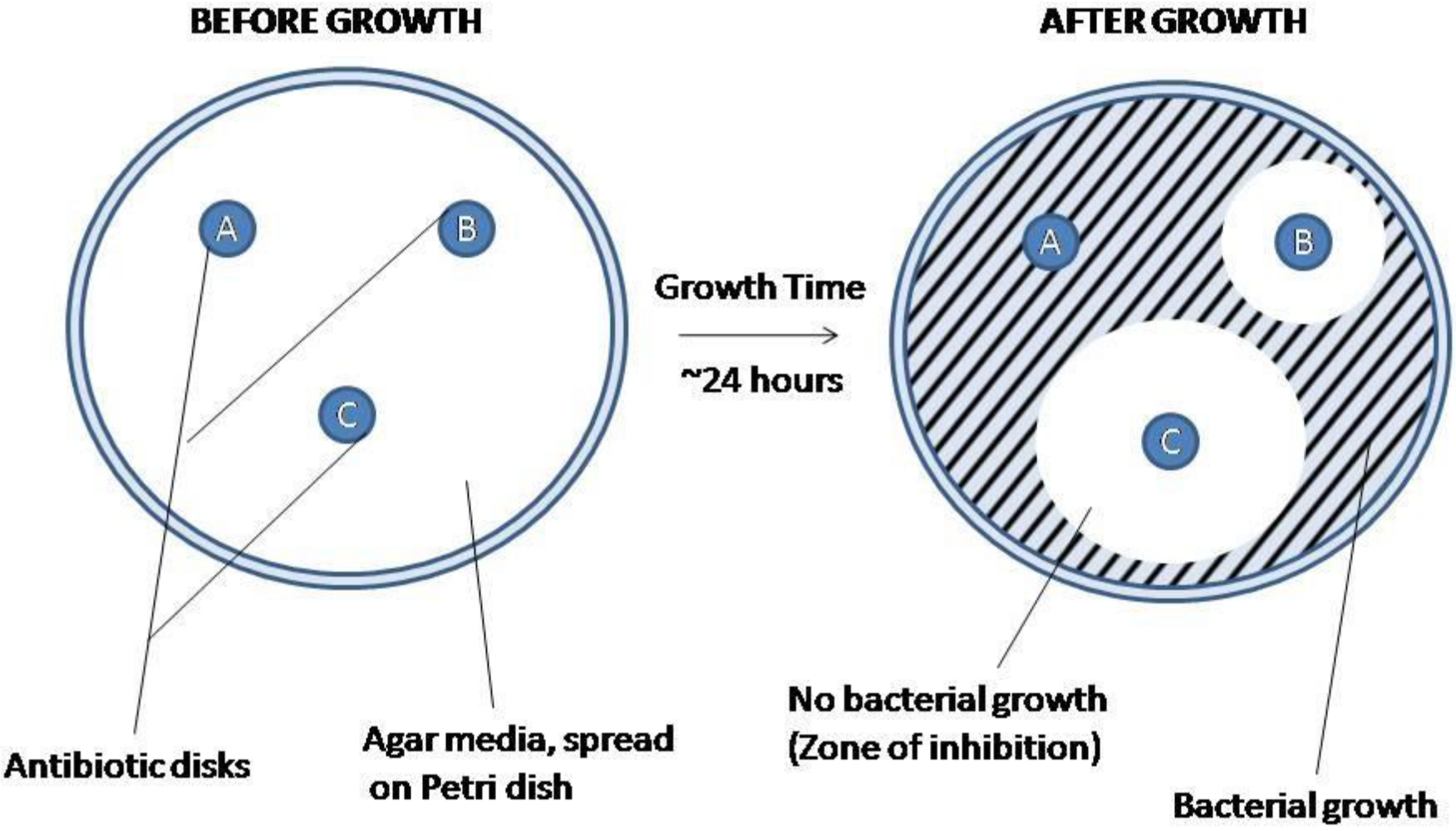
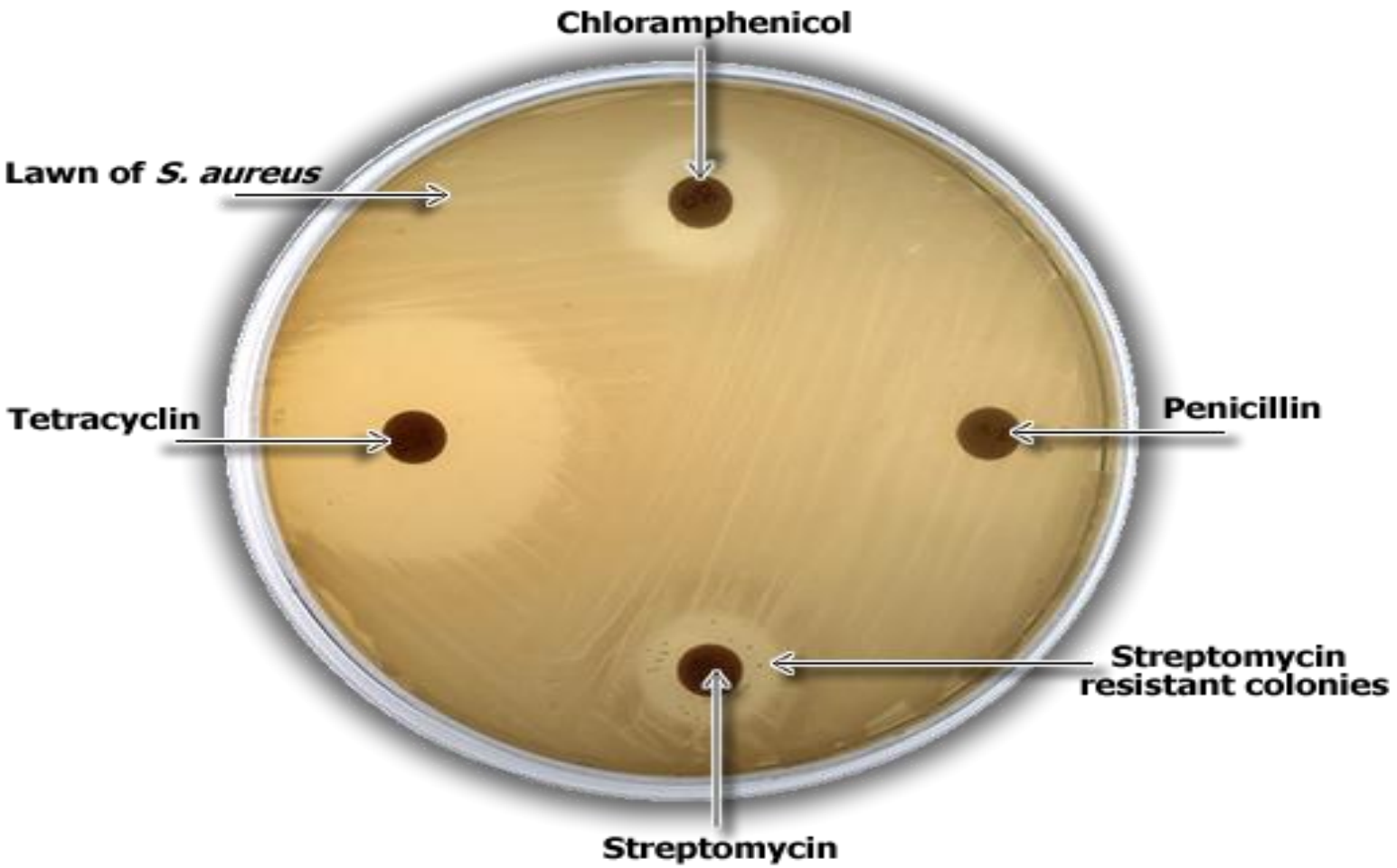
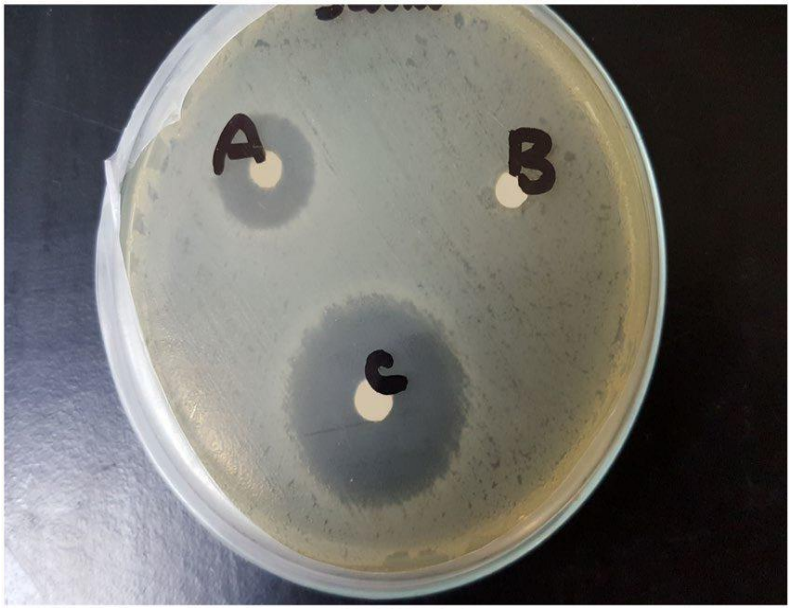

BEFORE GROWTH
AFTER GROWTH
A
B
C
Growth Time
~24 hours
Antibiotic disks
Agar media, spread on Petri dish
No bacterial growth (Zone of inhibition)
Bacterial growth

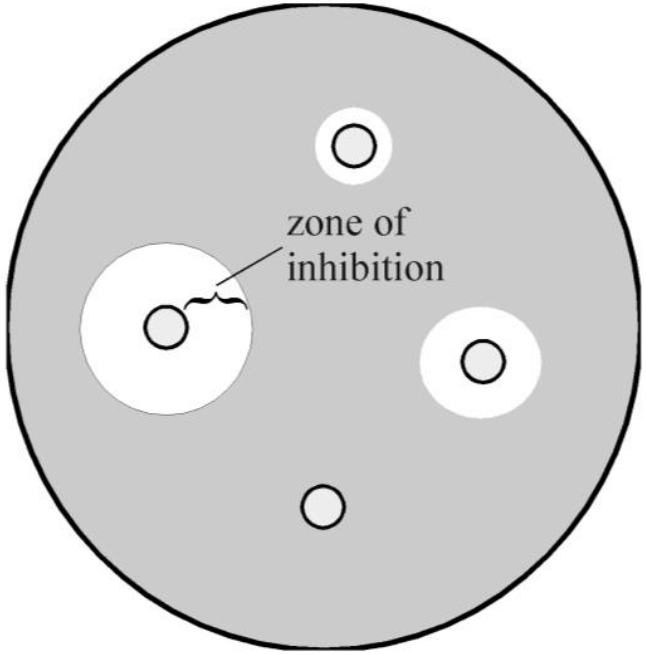
Antibiotics - Testing Sensitivity

Kirby-Bauer Method

Staphylococcus aureus




A
B
C


zone of
inhibition


Antibiotics

Types of Antibiotics (Based on their mode of action)

Bacteriostatic Antibiotics

- Tetracyclines
- Spectinomycin
- Sulphonamides
- Macrolides
- Chloramphenicol
- Trimethoprim

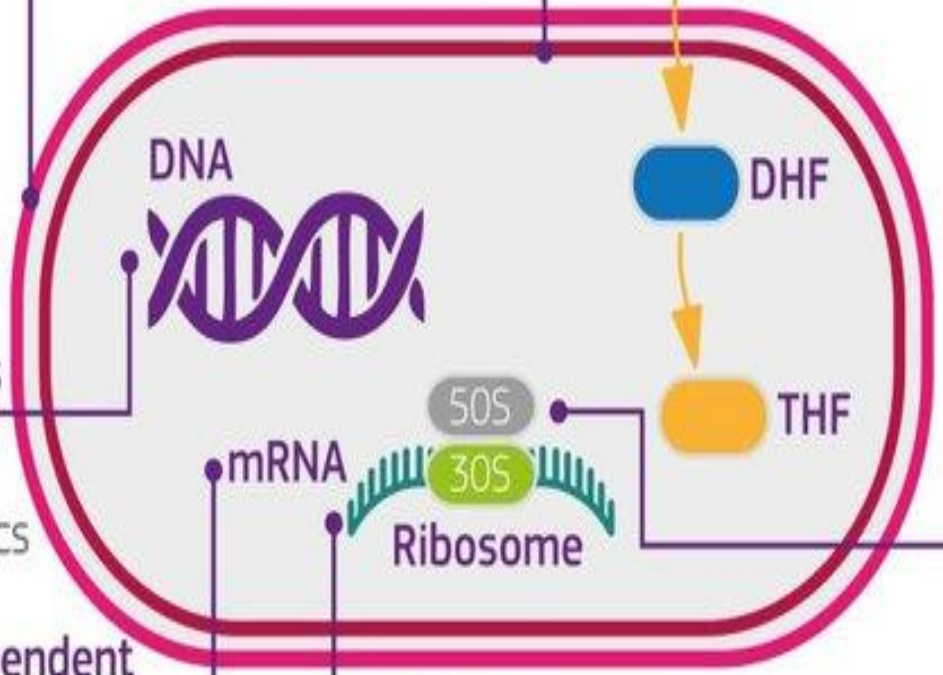
Bactericidal Antibiotics

- Penicillins
- Cephalosporins
- Fluoroquinolones (Ciprofloxacin)
- Glycopeptides (Vancomycin)
- Monobactams
- Carbapenems

❑ According to chemical structure:

- *Sulphonamides- Sulfone, PAS.*
- *Quinolones- Nalidixic acid, ciprofloxacin.*
- *Beta lactum- Penicillin, cephalosporin.*
- *Amino glycoside- Streptomycin,Gentamycin.*
- *Tetracyclin- Oxytetracyclin, doxytetracyclin.*
- *Nitrobengene derivatives-Chloramphenicol.*
- *Macrolids- Azithromycin, Erthromycin.*
- *Polypeptide-Polymyxin B.*

Mode of action of antibiotics



Cell wall synthesis inhibitors

β-lactams
Glycopeptides
Fosfomycin
Bacitracin
Alafosfalin

Cell membrane synthesis disruptors

Lipopeptides

Folate synthesis inhibitors

Sulfonamides

DNA gyrase inhibitors

Quinolones
Coumermycin antibiotics

Protein synthesis (30S and 50S inhibitors)

Tetracyclines
Aminoglycosides
Macrolides
Lincosamides
Amphenicols
Pleuromutilins
Oxazolidinones

Inhibition of DNA-dependent RNA polymerase

Rifampicin

RNA synthesis inhibitors

Ansamycines

Antibiotic	Sensitivity
Levofloxacin	++
Amikacin	-
Ciprofloxacin	-
Gentamicin	+++
Ceftriaxone	+++
Cefotaxime	-
Chloramphenicol	-
Cloxacillin	-
Amoxicillin & Clavulanic acid	++++
Enrofloxacin	++++
Moxiflox	-
Cefetrixone & Salbactum	-
Cobactan	++++

Our time with **ANTIBIOTICS** is running out.

Antibiotics are in danger of losing their effectiveness due to misuse and overuse, and in many cases they aren't even needed.

Always seek the advice of a healthcare professional before taking antibiotics.

HANDLE ANTIBIOTICS WITH CARE

World Health Organization

DRUG ADDICTION & ABUSE

توجد انواع كثيرة من المواد المخدرة ومن أشهر هذه الانواع خمس مجموعات

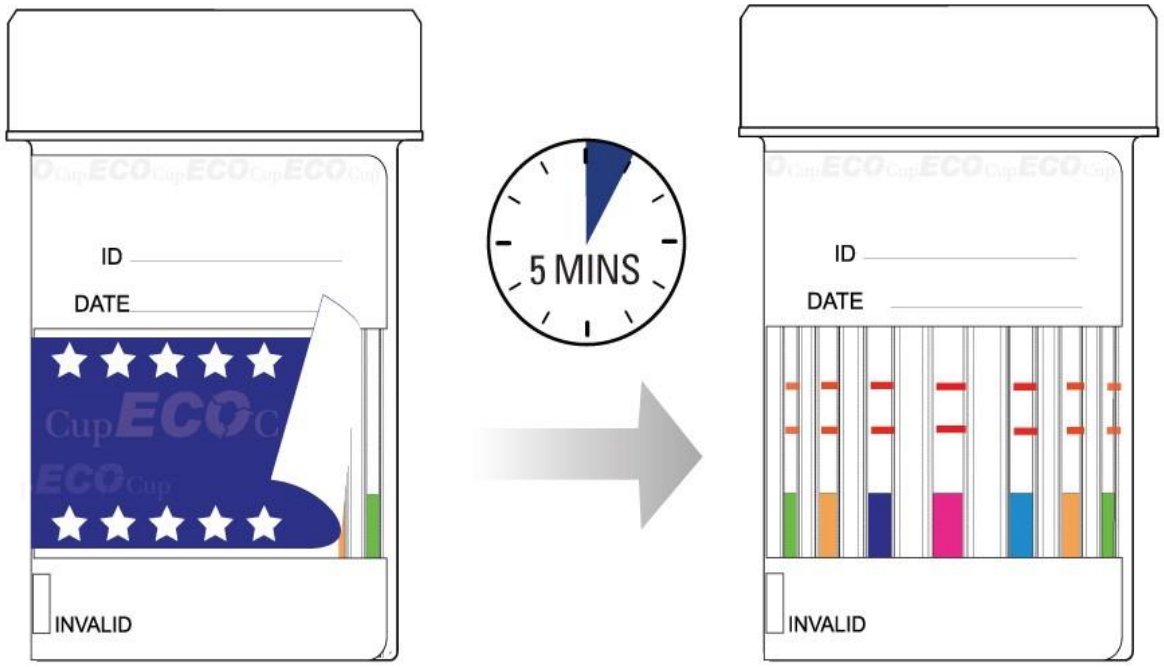
المجموعة الاولى وتشمل الهيروين والمورفين والكودايين opiates

المجموعة الثانية وتشمل المواد المنشطة amphetamins

المجموعة الثالثة وتشمل المواد المنومة barbiturates

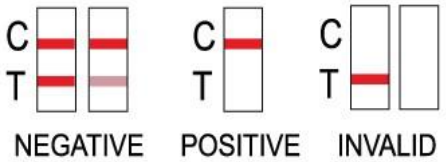
المجموعة الرابعة وتشمل المواد المهدئة benzodiazepine

المجموعة الخامسة عبارة عن القنبيات الحشيش والبانجو والماريجوانا cannabinoid



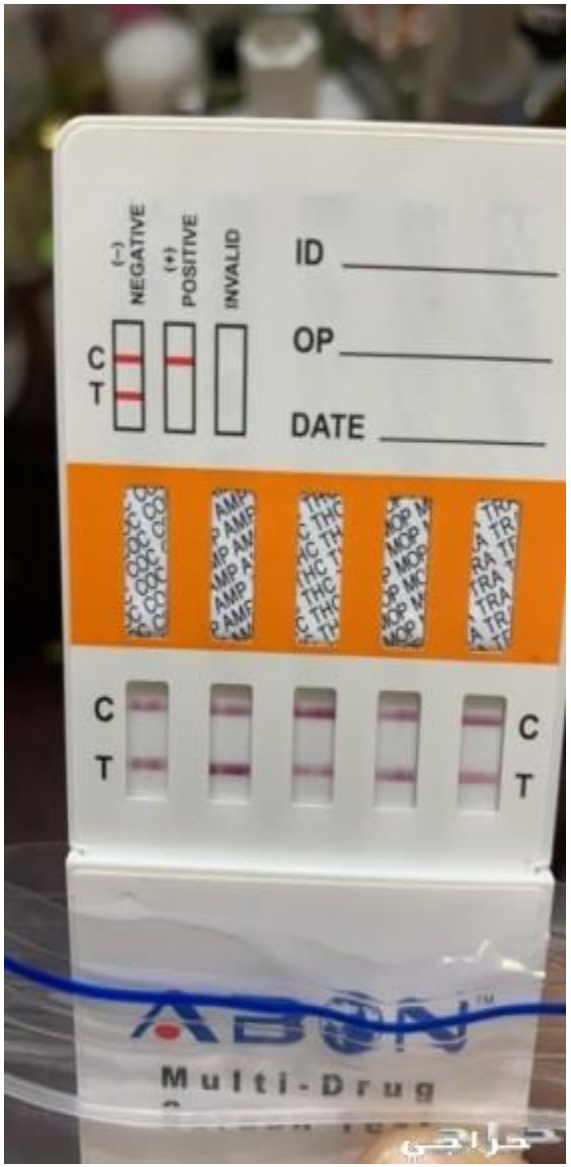
Open the test cup lid. Urinate directly into the test cup, filling between minimum and maximum as marked on the cup with the urine specimen

Read test results at 5 mins. DO NOT INTERPRET RESULTS AFTER 10 MINS.



RESULTS

NEGATIVE



POSITIVE -THC


CLASSIC
Coca-Cola
ENJOY
Chicken
SIMPLY
SOUTHERN