Medical Laboratory Techniques and Quality Control

تقنيات مختبرية طبية وسيطرة نوعية

النظري

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(MICROBIOLOGY)

Pre-test:

Circle the correct answer:-

1. **The role of using alab is to assist in the: -**

a- diagnosis. b- Treatment
c- Control of disease c- all

2. **Medical lab technician analyze: -**

a- blood b- cells and body fluid
c- Body fluids only d- blood cells &body fluids

3. **The technician plays a key role in:**

a- diagnosis b- treatment
c- a &b d- none

4. **Modern medicine would be :**

a- impossible without lab test b- possible without alab test
c-occasionally depends on lab test d- none

5. **There are many types of techniques used which may be :**

a-either manual or mechanical b- manual &mechanical
c-manual, mechanical &others d- none

What is Medical Laboratory Technology?

I've tracked down the answers to some common questions about medical laboratory technology.

Medical laboratory techniques:

A medical laboratory is a branch of medical technology science which is acomplex subject branching several **different disciplines** study different types of techniques in association with **analytical pathology** and only the diagnostic supports help in differentiating functional from organic and idiopathic from non-idiopathic.

? What Do Medical Laboratory Technicians and Medical Technologists Do?

They analyze blood, and cells, and body fluids, in search of clues that aid in the detection, diagnosis, and treatment of disease.
They are the "detectives" of the health care team, working behind the scenes to ensure quality patient care.

So the role of using laboratory is to assist in the:

a) **Diagnosis.** التشخيص **b) Treatment.** العلاج **c) control of the disease.** السيطرة

Medical Laboratory Technicians and Medical Technologists who work in Microbiology: ماهو دور المايكروبايولوجيين والفنيين

1- Help to diagnose bacterial infections.
2- They identify viruses, parasites, and fungi that cause infections.
3- They work with **specimens** such as **blood**, **urine**, **sputum**, **feces**, **body fluids**, and **tissues** and are trained in appropriate infection control procedures to protect themselves and others from infectious agents.
4- They play a key role in both the diagnosis and treatment of infections and stand ready to recognize and the medical diagnostic laboratory is more than the backbone of treating a person, it is the very soul of diagnosis making a **better diagnosis does mean better patient care**.

In the laboratory, a highly skilled medical team of pathologists, specialists, technologists, and technicians work together to determine the presence, extent, or absence of disease and provide valuable data needed to evaluate the effectiveness of treatment. The fact is, the practice of **modern medicine would be impossible without the tests performed in the laboratory**.

Quiz / 1 : What is the role of using thelab?

Medical Lab Technician Job Description

Your typical duties as a medical Laboratory Technicians will likely include the following:

- Monitoring tests and procedures
- Preparing blood, urine, and tissue specimens for analysis
- Using sophisticated laboratory equipment to look for bacteria, parasites, and other microorganisms
- Analyzing the chemical content of fluids

- Matching blood for transfusions
- Testing for drug levels in the blood to show how a patient is responding to treatment

Where do Medical Laboratory Technicians and Medical Technologists work?

When it comes to the challenges and rewards of medicine, the medical technologist has the best of both worlds. They work in all areas of the clinical laboratory and test for everything from the AIDS virus to zinc toxicity. The information that they give to the doctor influences the medical treatment that the patient will receive

Medical Technologists and Laboratory Technicians work in

- Hospital laboratories
- Reference laboratories
- Public health laboratories
- Blood banks
- Physician offices Laboratory
- Research centers
- Forensic laboratories
- Environmental and food industry laboratories
- Fertility Clinic
- Pharmaceutical Company
- Research Laboratory
- Veterinary Laboratory
- And many other places

Quiz /Where do Medical Laboratory Technicians work?

Types of techniques:

The subject provides information regarding the methods used in laboratory analysis. There are many types of techniques used, which may be:

1-Manual.

2- Mechanical.

The increased demands on the laboratory inevitably resulted in the introduction of more specialized and sophisticated procedures including:

- **Mechanization**
- **Automation**
- **Data processing**
- **How to interpret the reports of investigations conducted**.

e.g., for reading the results of any test, there are many ways to know the results like:

a) Visual techniques: methods which were used in the past, depending on the eye to give the results such as: - Read the pH of thesolution by filter paper methods (litmus paper: red = acid and blue= alkaline).

b) Color techniques: modern than the first that gives the results of any test by reading the density of its color by using different types of instruments like acolorimeter, photometer, and spectrophotometer.

c) Automation techniques. Methods using a machine to complete analysis like autoanalyzer, hemoglobinometer, Ms-9

For all above this technique are useful in all kinds of medical laboratory technique that includes many diagnostics brunches such as:

- Hematology
- Blood banking
- Chemistry
- Clinical Biochemistry
- Cytology
- Histopathology
- Cytogenetic
- Genetic
- Parasitology
- Immunology } Microbiology
- Bacteriology
- Virology

Post-test :

Circle the correct answer: -

1. **Lab test is the job of: -**

a- technicians. b- Doctors
c- Nurses c- health care workers

2. **The lab tests play an important role in: -**

a- controlling disease b- all
c- Treatment d- diagnosis

3. **Without lab tests modern medicine is:**

a- impossible b- possible
c- No relationship d- none

4. The technician plays a key role in:

a- treatment b- diagnosis
c- Diagnosis & treatment d- none

5. There are ----------main types of techniques:

a- two b-three
c- four d- on

Pretest: -

Circle the correct answer: -

1. The scientist that discovered the first compound microscope is: -

a- Joseph Lister. b- Louis Pasteur
c- Zacharias Janssen c- Watson

2. First electron microscope was discovered at: -

a- 1938 b- 1953
c- 1910 d- 1929

3. Eukaryote is :

a- Animal b- Plant
c-Protista d- Prokaryote

4. Mastigophora moves by:

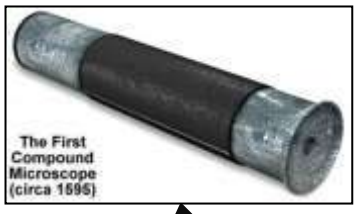
a- pseudopodia b- flagella
c-cilia d- do not move

5. The capsule is the structure of:

a- all bacteria b-some bacteria
c- all viruses d- none

Introduction to microbiology

HISTORICAL SURVEY



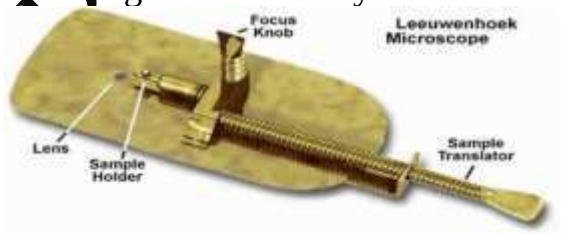
- 1590 – Zacharias Janssen ———First compound light microscope
  - 1938 – First Electron Microscope discovered
- 1953 Watson & Crick- Structure of DNA discovered

- 1676 – Anton Von Leeuwenhoek——first observation of bacteria "animalcules"
- 1796 – Edward Jenner——— First vaccine (smallpox)
- 1867 – Joseph Lister ———Antiseptic Surgery
- 1857 – Louis Pasteur ——— Germ Theory of Disease
- And in 1885 discovered Vaccine against Rabies
- 1884 – Robert Koch: Koch's Postulates of Disease Transmission
- 1929 - Alexander Fleming —— Discovered Penicillin (first antibiotic)

Penicillium

Antony van Leeuwenhoek (1632-1723)

A draper living in Holland, in 1675 **described "little animals**" he found when examining stagnant rainwater under his homemade **microscope making of lenses. Many of the first "animalcules"**, as he called them, were **protozoa**, but later experiments yielded the first recorded account of **microorganisms**. After his death, very little progress was made in determining the relation between bacteria and disease, until towards the end of the eighteenth century.



Edward Jenner (1749-1825) substantiated the belief **that cowpox gave protection to people against smallpox.** He introduced the term **vaccine** (from the **Latin *Vacca*-cow**) and established the idea of **immunity.** The quality of microscopes was rapidly improving and many more microorganisms were being discovered, but it was still not generally accepted that they were the cause of disease.

Barri in 1836 helped to establish that micro-organisms could cause disease when using a **heat-sterilized**. Even after evidence such as this, the real science of bacteriology did not begin until the middle of the nineteenth century.

Louis Pasteur (1822-1895) wasa French chemist much credit must be given to him. It was through his work on

- Sterilization of liquids.
- Fermentation provedto chat the breakdown of sugar to alcohol was the result of the activity of micro-organisms.
- He learned how to isolate and cultivate bacteria and how to study their effect on animals.
- In 1878 he read a paper on the germ theory of disease which helped to establish that specific organisms can give rise to specific diseases.

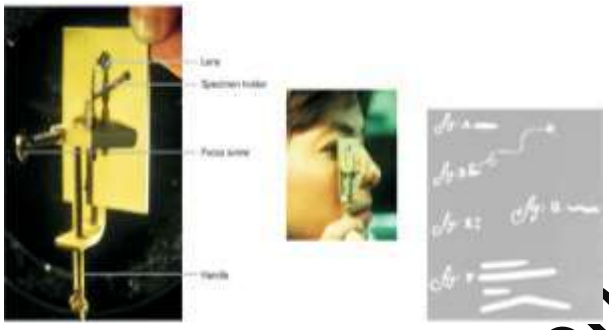
Robert Koch (1843-1910) was making enormous contributions to bacteriology practical. He developed

- Methods of fixing and staining bacteria.
- He discovered the tubercle bacillus (TB).
- He isolates the anthrax bacillus in pure culture.
- He discovered the cause of cholera.
- In 1881 he published a method of producing **pure cultures** of bacteria by growing them on the surface of a solid medium. The medium he devised was a **meat infusion broth** solidi-fied with gelatin, and poured onto a glass place. This was the be-ginning of our present-day culture media. Agar soon superseded gelatin and lacer Petri introduced his masterpiece dish.

Many others, such as **Lister,** with his introduction of **antiseptic** and aseptic techniques, contributed to the vast amount of knowledge after

discovering the electronic microscope which has developed into the science of **Microbiology**. And after Today, with our ever-increasing knowledge of bacteria, fungi and yeasts, rickettsia, viruses, and protozoa.

Quiz / 1: What do you know about Robert Koch?



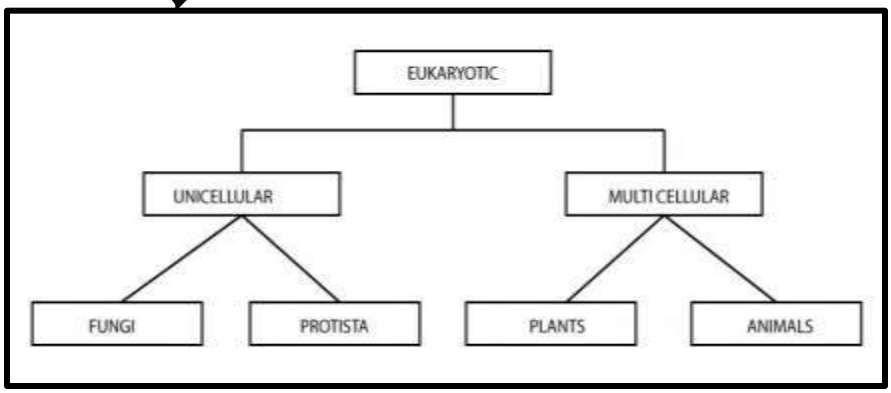
CLASSIFICATION OF MICROORGANISMS

All living things

Plant **Animal** **Protista** **[Heakle (1866)]**

Higher Protista (Eukaryotic)

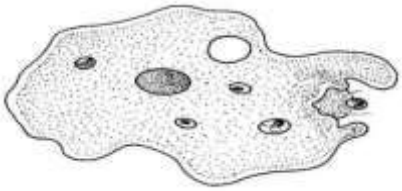
Lower Protista (Prokaryotic)



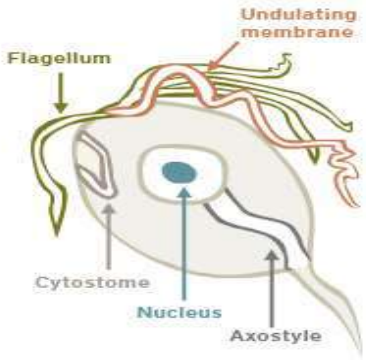
1- Bacteria
2- Rickettsia
3- Mycoplasma
4- Viruses
5- Bacteriophage
6- Chlamydia

Protozoa: These are small, single-cell animals belonging to the lowest division of the animal kingdom. They consist of protoplasm, which is differentiated into nucleus and cytoplasm, and they are non-photosynthetic. There are four classes of protozoa:

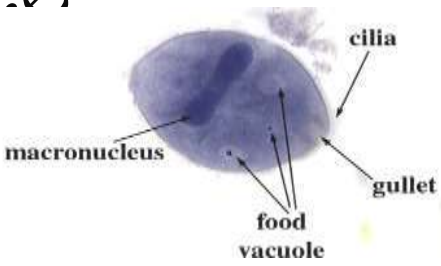
1-**Class I Rhizopoda move using protoplasm production called pseudopodia. An example *is Entamoeba histolytica* which causes amoebic dysentery.**



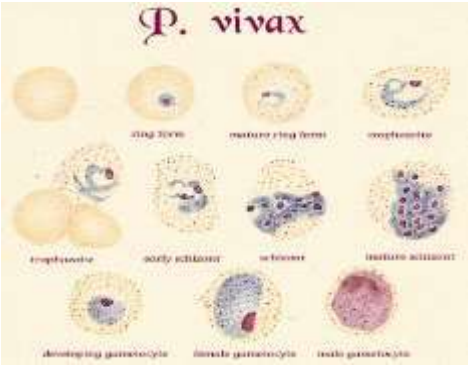
2-**Class II Mastigophora moves using undulating membranes or flagella**. An example *is* ***Trichomonas vaginalis***, which causes a **vaginal discharge**



3-**Class III ciliate: move by the beating of numbers of cilia**, an example *Balantidium* ***coli***, which causes **balantidial dysentery**

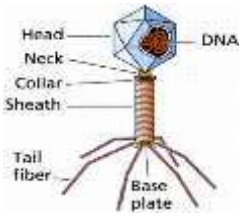


4-**Class IV Sporozoa** are **non-motile organisms** that **live parasitically within the cells of host animals**. An example is***Plasmodium vivax***, which causes **malaria**. Many protozoa when placed under unfavorable conditions pass into a resting phase, often with the formation of a distinctive **cyst** that can be used in identification.



Fungi: **like protozoa**, **fungi are non-photosynthetic organisms**. They **grow either as single cells**, e.g., **yeasts** or as **colonies of multicellular filaments (hyphae), e.g., molds**. They **reproduce using spores** and the recognition of these spores is often an aid to identification. Some species **cause disease in man and animals**. For example, ***Candida albicans***, a type of **yeast**, causes **thrush**.

Viruses: These minute organisms **can only multiply within living cells**. Consist in their simplest form of an **theouter coat of protein and an inner thecore of nucleic acid which may be either Ribonucleic acid (RNA) or desoxyribonucleic acid (DNA)** (no virus has been shown to contain both).



Rickettsia: are **small microorganisms** that are in some ways **intermediate between viruses and bacteria**. They are **similar to bacteria in that they contain both RNA and DNA**, **possess metabolic enzymes, and reproduce by binary fission**; they **resemble viruses by being able to multiply only within living cells**. Although we are mainly concerned with bacteria, a brief description of other microorganisms will be helpful.

Quiz Fill in the blanks with thesuitable answer:

The all living things are classified into _____,_____ and_____.

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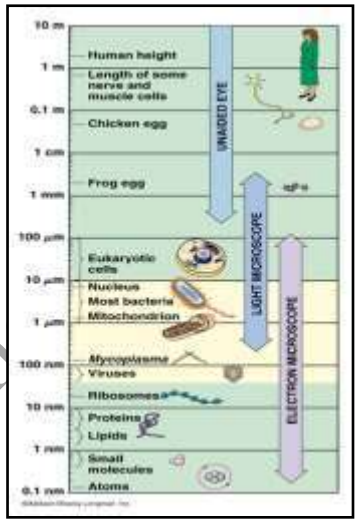
Lec. 2 and 3

Bacteria

Bacteria are **microscopic simple unicellular organisms** whose structure shows little difference and whose main function is simple, **dividing** and growing by simple binary fission.



Size of bacteria: Most bacteria are so small that their size is measured in units called microns. The unit of measurement is the *micro*-meter, written µm (Greek mu). The micrometer is 1/1000 of a millimeter (0.001 **mm)** = 10^{-3} or 1/25000 of an inch. [one micron (µ) or micrometer (µm) = 1/1000 of millimeter]. Generally**,** bacteria are sized about 1-10 µ in length and 0.2-0.5 µ **and** can be visualized only under magnification. The smallest having a diameter of about 0.5m, and Viruses, being smaller than bacteria, are generally measured in milli micrometers (1/1000 of a micrometer, or 0.001 µm or 1.0 µm), now correctly called nanometers (nm).

Units of Measurement

- **1 µm = 10^{-6} m = 10^{-3} mm**
- **1 nm = 10^{-9} m = 10^{-6} mm**
- **1000 nm = 1 µm**
- **0.001 µm = 1 nm**

Bacterial Nomenclature (Name of bacteria)

Bacteria are classified under the national taxonomy like another organism by:

Kingdom→Phylum→ Class→Order→Families→ Genus→Species

Types Strains Varieties

The name of bacteria writes according to:

Genus name	species name	
Salmonella	*typhi*	Salmonella typhi
Salmonella	*paratyphi A*	Salmonella paratyphi A
Salmonella	*paratyphi B*	Salmonella paratyphi B

Bacteria are present in:

- all types of environments
    a. Water
    b. animals
    c. frozen mud
    d. soil
    e. plants
    f. inside us
    g. air
    h. hot springs
    i. on our skin
- Our Food (Cheese, Bread)
- Industry (Molecular Biotechnology, Beer)
- Deep-Sea Vents, Volcanoes
- Hospitals.

Structure of bacteria

1. **The cytoplasmic membrane** consists of a layer of **lipoprotein** and is 5-10 nm thick. It **encloses the Cytoplasm**

2. The cell wall is a complicated lattice structure of **lipoprotein, lipopolysaccharide, and peptidoglycan, which gives the bacterial cell its shape and also protects the cytoplasmic membrane.**

3. **Capsule:** The cell wall of certain bacteria is covered with a capsule, which is usually a loosely attached slime layer consisting of **polymerized sugars and amino sugars** that are secreted by the organism. In many cases, the possession of a **capsule correlates with virulence.**

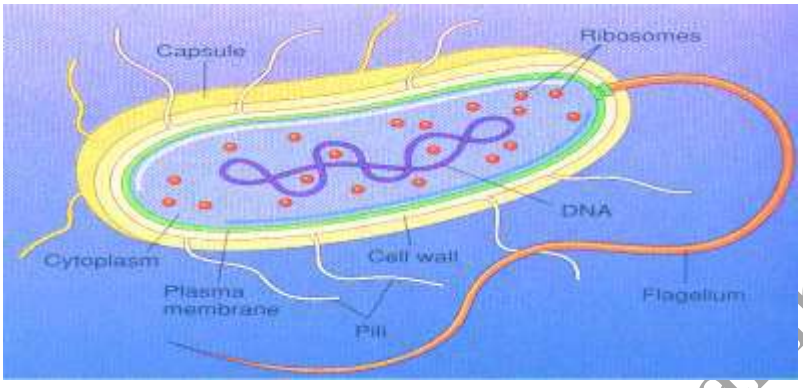
4. **Cytoplasm** which **contains soluble metabolites** and precursors of **macromolecules** together with **organelles such as:**

a) Ribosomes: within the cytoplasm is the bacterial chromosome-usually a single

b) Closed ring of double-stranded DNA. At binary fission, a duplicate copy of the chromosome passes to the new cell

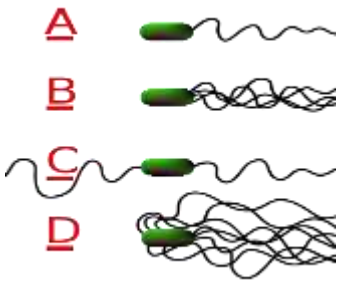
c) Circles of DNA (plasmids): carry genes that confer antibiotic resistance on the cells carrying them. Of greater current interest is the fact

that these plasmids may be **transferred between cells of different types (e.g. non-pathogen to pathogen).**



5- Pili (Pilus): Latin for 'hair'; plural. They are **found in bacterial cells**. These may be important in the **attachment of pathogens to host tissue cells**. Fimbria (Latin for 'thread' or 'fiber'; plural: *fimbriae*)

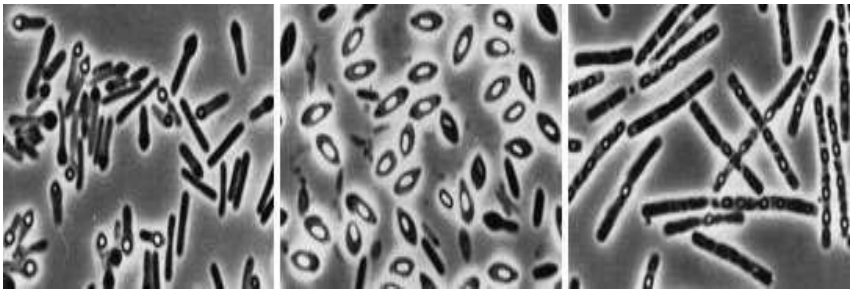
6- Motile organisms possess **flagella**, which are **thread-like appendages** composed of a **protein called flagellin.** Some organisms possess one flagellum, others are more than one. The arrangement of the flagella is:



A-Monotrichous; B-Lophotrichous; C-Amphitrichous; D-Peritrichous.

7- Some bacteria produced a highly resistant dormant state called spores like *Bacillus* and *Clostridium*, they make bacterial survival possible under unfavorable conditions, spores are resistant to heat, drying, freezing, and toxic chemical. They are killed by autoclaving (15 b pressure at 121°C for

20 min.). Spores may oval or spherical found as free or central or sub-terminal or terminal in the cells.



Terminal **central** **sub-terminal**

Quiz: **Name the bacteria scientifically?**

Bacterial Physiology

Bacterial metabolism: the properties and processes of life essentially the same in all living things, whatever size or they are plants or animals. If an individual organism is to survive it must be able to react to changes in its environment it can **feed**, **respire** and it must be able to **reproduce**. The essential condition for growth (mean= increase in size and increase in number {multiplication}).

1. **Nutrition.**
2. **Energy.**
3. **Oxygen (O_2)**
4. **Temperature (Temp.)**
5. **pH (acidity)**
6. **Pressure.**
7. **Osmotic pressure.**
8. **Moisture.**
9. **Light.**
10. **Radiation.**
11. **Others.**

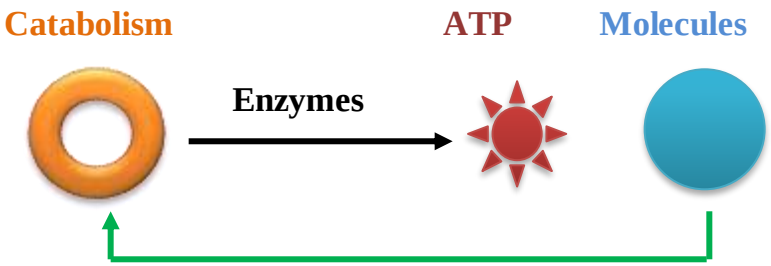
Physical factors that effect on growth

To obtain optimal bacterial growth, it is necessary to understand the metabolic role of nutrients. Metabolism: an interacting set of chemical reactions of which very few occur spontaneously and most to be catalyzed by specific proteins called **Enzyme**.

There are two main types of metabolism reactions:

- **Catabolism reaction**: It is a breakdown of molecules.
- **Anabolism reaction**: it is a synthesis of molecules

The energy needed to drive the synthetic reaction comes from a breakdown reaction and the enzymes which may number about 1000 in single cells are involved in its transfer. The action of enzymes on their specific substrate is often used in the identification of bacteria.



Quiz/ **Fill in the blanks with suitable answer: Catabolism reaction is_____ of molecules while anabolism is _____of molecules.**

Nutrients:

A. **Water:** bacteria require water for growth, dryness may kill most bacteria, and 80-90% of bacteria dry weight is water.

B. **Minerals**: all bacteria must be supplied :

a- Inorganic elementary (salts) like Na, P, Cl, Co, Cu, Zn, Fe, Ca, Mg, Mn, K, S

b- Organic source (C, O_2, H_2, and N_2).

i. Carbon source for carbohydrate metabolism.

ii. Nitrogen source for protein and nucleic metabolism.

Bacteria can be divided into groups based on the nutritional requirements into:

A. On how they obtain their energy.

B. On how they obtain carbon needed for the synthesis of all organic molecules.

Energy sources

Chemotrophic
(Obtain energy for growth from oxidation or fermentation of chemical components)

Phototrophs
(Obtain energy from sunlight)

- Photolithotrophs
- Photorganotrophs

Chemoorganotrophs
Source of energy from oxidation or fermentation of organic compounds
Ex.: all pathogenic bacteria

Chemolithotrophs
Source of energy from oxidation of inorganic compounds

Paratrophs
Obtain energy from metabolism of host cells

Carbon sources

Autotrohpes
It is phototrophs and chemotrohps are able to grow with CO_2 + sunlight

Heterotrophs
It is need or require to supplied with organ carbon molecules for carbon molecules for growth (all bacteria)

Bacterial association

1. **Symbiosis**: benefit relationship between both host and parasite.
2. **Saprophyte**: Organism living on dead matter.
3. **Parasitism**: Organism living and multiplying with the living body.
4. **Commensalism**: Harmless to the host.

Q- What are the sources of energy?

Groups of bacteria according to **Oxygen** requirement:

1. obligatory aerobic bacteria ex.→ ***Pseudomonas***
2. obligatory anaerobic bacteria ex. → ***Clostridium***
3. facultative anaerobic bacteria ex. → ***E. coli***
4. microaerophilic bacteria ex. → ***Neisseria***

Groups of bacteria according **to pH** requirement:

1-Neutrophilic bacteria (optimal pH 6.8-7-7.2) ex. → ***E. coli***
2-Acidophilic bacteria (optimal pH 0.2-2) ex. →***Lactobacillus***
3-Basophilic bacteria (optimal pH 8.9-9) ex. → ***Vibrio***

Groups of bacteria according to **Temperature** requirement:

1. Thermophilic bacteria (optimal temp. 55-75°C).
2. Mesophilic bacteria (optimal temp. 30-45°C).
   [The optimal temperature for all medical bacteria is 37 °C]
3. Psychrophilic bacteria (optimal temp. 0-10°C).

There are other physical factors:

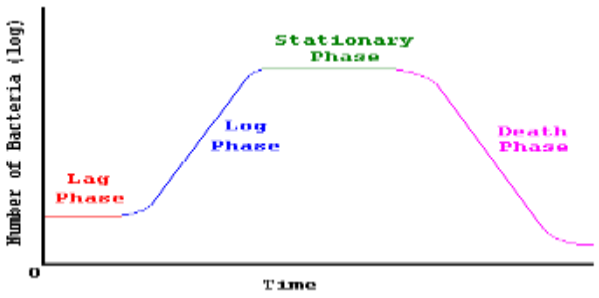
1- Pressure. 2- Radiation. 3- Osmotic pressure. 4- Light. 5-Moisture 6-Mechanical 7-sonication

Substances produced by bacteria:

1. Stains (pigments). (**extracellular or intracellular**)
2. Enzymes **ex.→** (**Haemolysin …coagulase ...oxidase….)**
3. Toxins (**Exotoxin or Endotoxin**)
4. Antibiotic
5. Acids

Growth Curve

When organisms are cultured in appropriate broth media (liquid medium): **Counting** of bacteria at the different periods after inoculation is represented one's graph which is called **growth curve**.



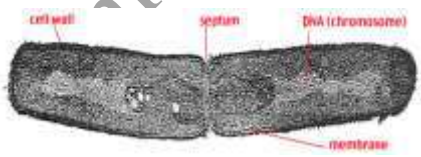
1- Lag Phase: During this phase:

- Organism takes a little time to adjust itself (adaptation) to its new environment

- Increase in size of the cell without any multiplication.
- Increase in metabolic rate

The length of the lag phase depends upon:

1. Type of bacteria → *E. coli*, T.B.
2. Good and better medium, shorter this phase.
3. The phase of culture from which inoculation takes.
4. Size of inoculums.
5. Environmental factors like temp., pH, …



2- Log phase (Logarithmic phase): The organisms become to multiply by simple binary fission there will be an increase in the number of bacteria to the extent that the medium looks turbid to the naked eye. As multiplication is by geometric progression. Logarithms of viable count plotting against time gives a straight line, during this period occurs. Bacteria have a high rate of metabolism.

1. Bacteria increase in number.
2. Bacteria will be more sensitive to antibiotics.
3. control of log phase is brought about by:
   a. Nature of bacteria. طبيعة البكتريا
   b. Environment of medium (temp.). حرارة الوسط
   c. Concentration of material in media. تركيز المواد في الوسط

3- Stationary phase: After some time, due to the exhaustion of nutritional factors of the medium and the accumulation of waste products, some bacteria die, and there is a balance of dead and living bacteria. This is the number of bacteria multiplying is equivalent to (=) the number of bacteria dying. Production may occur during his stage. This is called the stationary phase.

An equal rate of multiplication and death may be due to:

1- Depletion of nutrients.
2- Accumulation of toxic products.

3- **Decline phase**: In some bacteria after this short period of equilibrium, **the number of dying is greater than the number of multiplying** and the stage is referred to as the decline phase. During this phase, the population decreases due to the death of cells.

Factors responsible for this phase are:

1. Nutritional exhaustion.
2. Toxic accumulation.
3. Autolytic enzymes.

Quiz/ **What do you know about the stationary phase?**

Post-test:

Circle the correct answer:

1. **Autolytic enzymes is: -**

a- decline phase. b- Stationary phase
c- Log phase c- lag phase

2. **Increase of size of bacteria without any multiplication is called: -**

a- stationary phase b- log phase
c- lag phase d- decline

3. **Hemolysis is a substance produced by :**

a- virus b- hemolysin
c- Bacteria d- all

4. **Heterotrophs requires :**

a- carbon source for growth b- no carbon source
c- oxygen for oxidation d- nitrogen for growth

5. **Synthesis of molecules is called :**

a-catabolism b-metabolism
c- a & d d- anabolism

Medical Laboratory Techniques and Quality Control

تقنيات مختبرية طبية وسيطرة نوعية

النظري

أ.م. د. تغريد خضر محمد
المرحلة الأولى/ قسم التحليلات المرضية
المعهد الطبي التقني/ المنصور

2020 - 2019

Lec. 4 and 5

Sterilization

Pre test :-

Circle the correct answer:-

1. Sterilization means:-
a- without M.O. **b- Contamination**
c- Kill M.O. and their spores' **c- without viruses**

2. Physical methods of sterilization is :-
a- heat only **b- heat, gases, radiation**
c- Filtration **d- dry heat**

3. Moist heat sterilization means using :
a- steamed heat **b- some chemicals**
c- boiling **d- flaming**

4. The advantages of autoclaving :
a- can be used for antibiotics **b- less toxic**
C-effective and rapid **d- more toxic**

5. Milk is sterilized by :
A-formalin **b-flaming**
c- Autoclaving **d- pasteurization**

6. Filtration is a :
a- mechanical method **b- chemical method**
c- Physical method **d- other method**

7. Gamma radiation is used to sterile :
a- inoculating loop **b- milk**
c- Glass wares **c- food factory**

8. Disinfectant is a chemical substance used for :
a- living objects **b- non living objects**
c- Living and non living objects **d-none**

9. Bacteriostatic agent is a substance that :
a- stops the growth of bacteria **b- stops the multiplication of bacteria**
c- Kill the bacteria **d- limit the spread of the bacteria**

10- Contamination:
a- is the opposite of sterility **b- means sterility**
C-always air borne **d- can not be minimized**

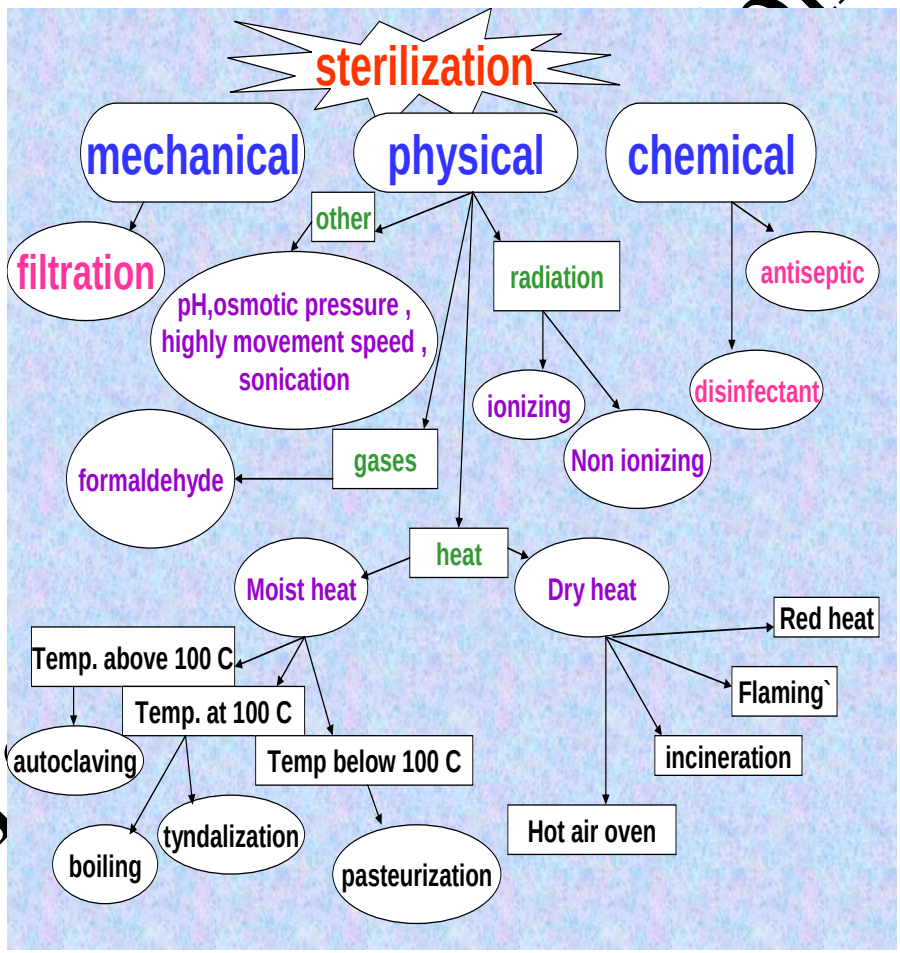
Sterilization

Sterilization: is a freeing of an article from all living microorganisms including bacteria and their spores, viruses, yeasts, molds (pathogenic and nonpathogenic).

هو خلو الادوات من الاحياء المجهرية المتضمنة البكتريا وسبوراتها والفايروسات والخمائر والاعفان (الممرضة والغير ممرضة)

Methods of Sterilization :- طرق التعقيم

- Physical methods . الفيزياوية



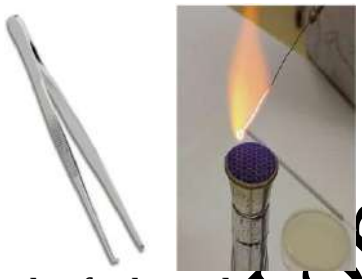
- Chemical methods . الكيمياوية
- Mechanical methods الميكانيكية

Physical methods of sterilization :-

A -Heat sterilization التعقيم بالحرارة

1) Dry heat sterilization الحرارة الجافة

a. **Red heat** used to sterilize wire loops ,point end of forceps الحرارة الحمراء تستخدم لتعقيم الناقل ونهايات الملاقط



b. **Flaming:** used to sterilize mouth of tubes, glass spreaders (which are flamed in ethanol). الألسنة تستخدم لتعقيم فوهات الانابيب

c. **Incineration** الحرق:used in pathological fuming materials

d. **Hot air oven:** (160-180) ˚C for 2-4 hr., used to sterilize glass wares (pipette ماصات زجاجية , syringes سرنجات زجاجية, flask فرن الحرارة الساخن اطباق بترى زجاجية glass Petri dishes, دوارق زجاجية

Conical flask, volumetric flask, Beaker, Graduated cylinder, glass Petri dish

2) Moist heat sterilization الرطبة

A. Temperature **below 100˚C**, **pasteurization** (63˚C for 30 min), to sterilize milk . حرارة أقل من 100 م لتعقيم الحليب

B. Temperature **at 100 ˚C**

Boiling (5-10 min) to sterilize rubber tubes, glass syringes (kills all non-spore forming bacteria).

- **Steaming** (**tyndillization**) steam 30 min for 3 days ,used to sterilize gelatin media, sugar media. بخار بدرجة 100 م لتعقيم الاوساط الجيلاتينة والاوساط السكرية

- C .Temperature **above 100 °C** (**autoclave**) the condition used in this instrument (121°C ,15 min, 15 p/inch^2),used for sterilization of :

- surgical tools and clothes الادوات الجراحية وملابس العلميات
- culture media and to sterilize inoculated media الاوساط الزرعية
- Swab المسحة
- D.W. ماء مقطر, Solutions sealed in containers ampuls, vials ,
- Bulk Solutions محاليل بحجوم كبيرة
- Glassware
- Instruments Intraoperative sterilization of metallic devices اجهزة معدنية
- metallic surgical instruments

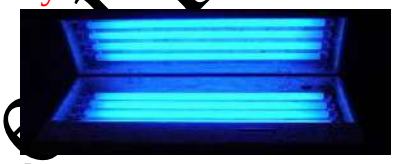
B -Radiation sterilization

- Non ionizing type, like ultra-violate rays , infra-red rays
- Ionizing type, like Gamma rays , X ray , Beta rays

Ultraviolet Lampe (UV rays)

Wavelengths 2500-2600 A° (300 – 400 nanometers)

Limited uses that even thin glass or moisture protect from UV rays



Used in

- **sterile inoculations cabinets**
- **sterile operation rooms**

C- GASEOUS STERILIZATION التعقيم بالغازات

Gases: formaldehyde, ethylene oxide

Ethylene Oxide : Kills germs by damaging their DNA-RNA

Application: plastic syringes (disposable syringes), disposable Petri dish.

Mechanical methods:

Filtration: The material is effect by heat (Heat sensitive solutions)
Ex. (serum, protein, sugar, vaccine, antibiotics, toxins,) are sterilized by filtration.

D-Others:

- pH
- Pressure
- Osmotic pressure

Chemical methods of sterilization

A- Antiseptic:

It is chemical substance that kills micro-organisms on living tissues, ex. 70% alcohol, heptane, 10% Dettol to sterilize hands المادة الكيمياوية المستخدمة لقتل الاحياء المجهرية وتستخدم مع الانسجة مثل الانسان والايدي

B- Disinfectant:

IT is a chemical substance used to sterilize non -living objects لتعقيم الاشياء غير الحية , ex. Phenol , 5% formalin to sterilize refrigerator الثلاجة , bench البنجات , floor الارضية

The disinfectant may be described either as:

- **Bacteriostatic:** any chemical substance which inhibits the growth and multiplication of bacteria but do not necessarily kill them.
- **Bacteriocidal:** any chemical substance which kills the bacteria and their spores.
- **Sepsis**: Presence of infection (M.O) in living tissue.
- **Asepsis**: Absence of infection (M.O) in living tissue.

CULTURE MEDIA

Culture Medium is an artificial food prepared in laboratories.

Use of culture media الاهداف من أستخدام الأوساط الزرعية

1. **Isolate the microorganism (bacteria).**
2. **Identify the microorganism, and study the characteristic of microorganism.**
3. **Study the antibiotics sensitivity.**
4. **Maintain stock culture.**

Basic requirements for bacteria culture media: المتطلبات الواجب توفرها في الوسط الزرعي

1- **Energy sources and Nutrition**
   - **A- Carbon source: Ex: sugars, carbohydrate, CO_2**
   - **B- Nitrogen source**
   - **C- Others (like; Sulfurs, phosphorous, metal Salts, trace elements, vitamins, essential metabolites).**

3- **pH (acid, alkaline, natural).**

4- **O_2 (aerobic or anaerobic).**

5- **Time for incubation.**

6- **Temperature**: **The optimum temperature of pathogenic bacteria is 37 $^{\circ}$C (Body temperature) well used the incubator.**

7- **Moisture**

Culture Media prepared in suitable containers according to solidity of culture media

- **flasks** or **tubes** with suitable sizes
  plugged cotton bottles, or in **screw capped bottles**
  Media dispense in plate Petri-dish for solid media, **if colonial characteristics of an organism are to be examined**, the Petri dish is an excellent container for the medium. The dish should be flat-bottomed, and either of heat-resistance **glass or plastic**.

Quiz / **Mention the basic requirements for bacterial culture media.**

Kinds of media

Culture media are divided into 3 types according their solidity:

1. **Solid.** صلبة
2. **liquid** سائلة
3. **Semisolid.**شبه صلبة

In liquid media the bacteria are free to move, but when grown **in solid media, they multiply at the site of inoculation and form colonies.** The appearance of these colonies is often typical of these species; this makes **possible the isolation of single species of bacteria from a mixture.**

Liquid media are solidified by the addition for example of gelatin or agar.

Agar is a long-chain carbohydrate, obtained from sea algae it melt at 80 – 100 C° and solidified at 35- 42 C°, which does not affect the nutrient properties of the original media (doesn't provided any nutrition to the bacteria). **It acts as solidifying agent.**

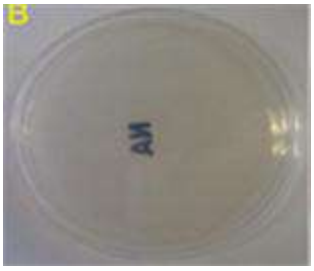
Gelatin is a protein derived from the collagen of bone, skin, and melting point 24 C°.

Classification of media according to components (Function): حسب الوظيفة

Media according to component	Liquid	Solid	Semi-Solid
1. Simple	Nutrient broth	Nutrient agar	Motility test medium
2. Enrichment	Meat extract broth	Blood agar, Chocolate agar	
3. Differential	Sugar media	MacConkey agar	
4. Selective	Thioglycollate media	T.C.B.S S-S agar medium	
5. Special	Stuart transport media		

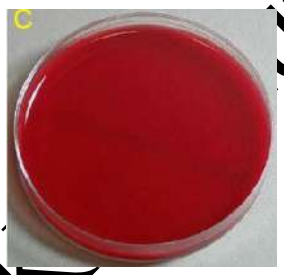
Culture Media Storage: For longer periods (1 week – 3 week) store at 4 – 6 ^{o}C in (refrigerator).

Nutrient agar



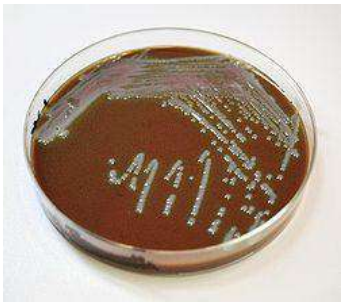
Blood agar

(Nutrient agar or blood agar) + Distal water, dissolve by heating &then sterilized with autoclave and then cool to 55 C$^{\circ}$, after that add 5-10 % blood.



Chocolate agar

(Nutrient agar or blood agar) + Distal water, dissolve by heating &then sterilized with autoclave &then cool to 80 C$^{\circ}$ and then add 5-10 % blood (blood is hemolysis release X , V factor).



MacConkey Agar

Is selective for Gram negative organisms, and helps to differentiate **lactose fermenting** gram negative rods from **Non lactose fermenting** gram

negative rods. It is primarily used for detection and isolation of members of family enterobacteriaceae and *Pseudomonas* spp. Composition of MacConeky Agar:

1. Enzymatic Digest of Gelatin, Casein: provides nitrogen, vitamins, minerals and amino acids essential for growth.
2. Lactose: fermentable carbohydrate providing carbon and energy.
3. Bile Salts: selective agents and inhibit Gram positive organisms.
4. Crystal Violet: Gram positive bacteria are generally inhibited.
5. Sodium Chloride: supplies essential electrolytes for transport and osmotic balance.
6. Neutral Red: pH indicator. which is red in color at pH's below 6.8. When lactose is fermented, the pH of the medium decreases, changing the color of neutral red to pink.

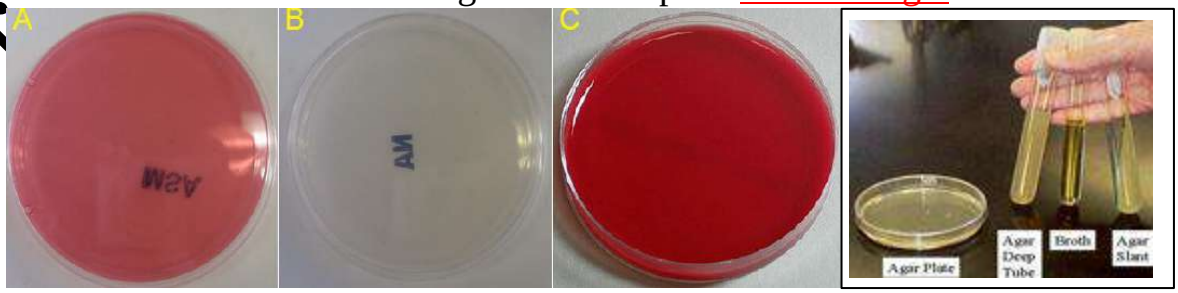
7- Agar : (Solidifying agent) polysaccharide extract from sea weedy (red algae) , used for solidification of culture media , its solidify at 42 C^0 & melted at 95 C^0 .

Classification of media according to solubility (Form) :

1 – Liquid media (broth media): contain all ingredients except agar (0%).

2 – Semi solid media: contain all ingredient except and 0.2 – 0.4% agar. (Motility test medium).

3 – Solid media: contain all ingredient except & 1.5 – 2% agar.



MacConkey agar **Nutrient agar** **Blood agar**

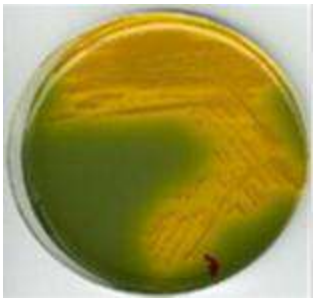
Quiz / **What is the purpose of streaking?**

2-What is the T.C.B.S. agar? Thiosulfate citrate bile sucrose agar to isolate Vibrio cholera

1 Bacterium —Culture on Solid media→ **one colony**

37C° for (18-24) hr

In incubator



Medical Laboratory Techniques and Quality Control

تقنيات مختبرية طبية وسيطرة نوعية

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2020 - 2019

Lec.6

Dr.Taghreed Khudhur Mohammad

CULTURE MEDIA

Preparation of Culture media

Equipment:

1- Balance
1- Conical flask.
2- Graduated cylinder.
3- Spatula.
4- Source of heat (Bunsen burner).
5- Filter paper
6- Autoclave
7- powder base of media






Procedure:

1. Weight media powder by using a balance.
2. Dissolve the powder in D. W.
3. Using a heat to complete dissolving of powder.
4. Put cotton plug on 1 mouth of conical flask.
5. Sterilize by using Autoclave.
6. Cool the media to (45-50) C °.
7. Pour the culture medium in sterile Petri dishes (about 20 mL for each).
8. Let the plates for some time to solidify a medium.
9. Put plates in refrigerator upside down until using.





Inoculation on Solid culture media

Streaking on Solid culturemedia

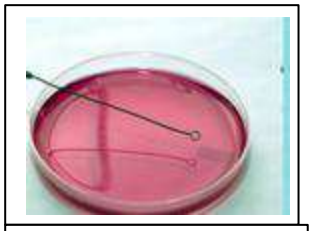
The purpose of streaking

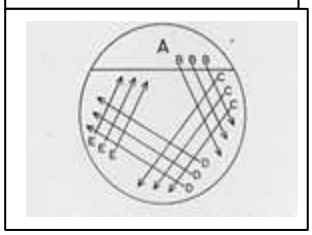
- To get an isolated colony.
- To detect and identification of bacteria .

Equipment:

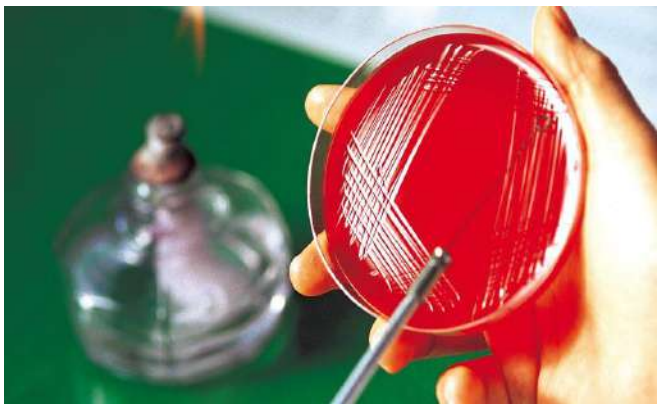
1. **Spirit lamp or Bunsen burner.**
2. **Bacteriological loop.**
3. **Solid culture media.**

Procedure:





1. **Prepare Solid media in a Petri dish.**
2. **Sterilize the loop by flaming.**
3. **Cool it by touching the loop on side of medium.**
4. **Hold a piece of colony by loop and transfer it to a new media .as in A, this area termed inoculum area.**
5. **Desterilize the loop and repeat point 3.**
6. **Make 4 parallel lines as in B.**
7. **Incubation the culture medium in incubator at 37 C for 24 hr.**



Which is help to diagnosis of organism which cause disease?

1 Bacterium → **Culture on Solid media** → **one colony**

37C° for (18-24) hr

In incubator

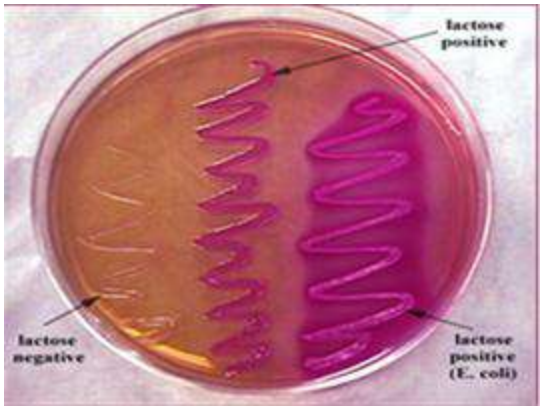
***Nutrient agar**

25 g → 1 L Distilled water (D.W)

2.5 g 100 ml D.W

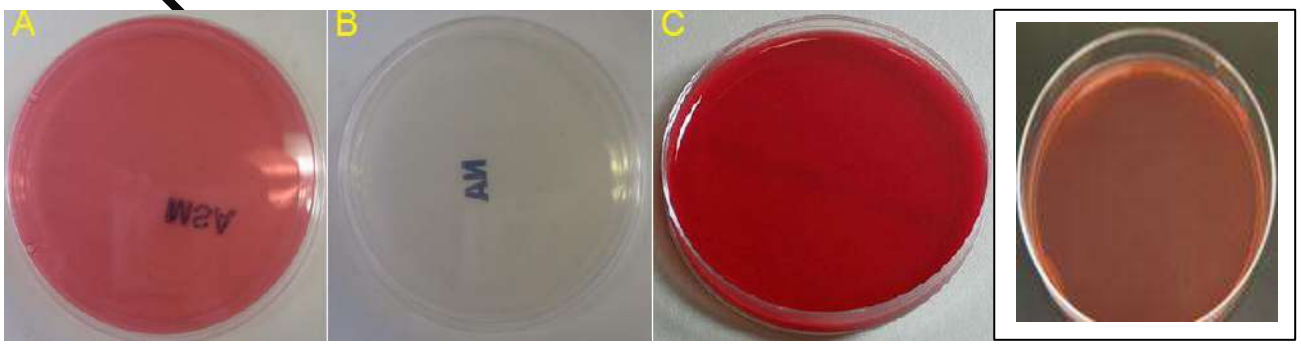
***MacConkey agar:**

16g → 1 L D.W



***Blood agar:**

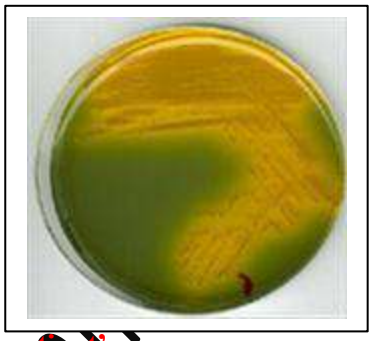
Nutrient agar + 5-10 % Blood



MacConkey agar **Nutrient agar** **Blood agar** **Chocolate agar**

Quiz / 3

What is the purpose of streaking?



What is the T.C.B.S. agar ? Thiosulfate citrate bile sucrose agar For Vibrio cholera

GENERAL PROCEDURE

1. **The specimen should be properly labeled with the patient's name, hospital number, word and date of collection. This is essential in order to prevent confusion of specimens from patients similar.**
2. **The request from should state the provisional diagnosis and the nature of examination required. This facilitates selection of techniques; for example, if a specimen of sputum is sent to detection of *Mycobacterium Tuberculosis* (in a suspected case of pulmonary tuberculosis) detailed examination for other organisms is obviously not required.**
3. **Any information with regard to the chemotherapy should be noted. Certain precautions may be necessary; for example, specimens from patients receiving sulphonamides should be inoculated on to the media containing *p*-aminobenzoic acid, which prevents the bacteriostatic action of sulphonamides on the organisms. Similarly, the use of penicillinase may be required for the isolation of organisms from a patient receiving penicillin.**
4. **The correct container should be used. Most specimens for bacteriological examination should be received in a sterile container.**
5. **With all specimens extreme care must be taken with all manipulations and they should be carried out under the protection of an exhaust inoculating cabinet.**

6. THE INOCULATING CABIENT (SAFTY HOOD) . All work involving the handling of such specimens and cultures should be performed under cover of an inoculating cabinet.

FURTHER PRECAUTIONS

Other precautions when dealing with all specimens or cultures are:

- **Never lay a culture tube on the bench ; always place it in a rack or tin.**
- **Label clearly *every* tube or plate with the specimen's number.**
- **When finished always discard the cultures into an appropriate discard receptacle for sterilizing. Never remove the cultures once discarded until they have been sterilized.**
- **Keep the working space on the bench clear so that if an accident occurs the minimum number of articles will be involved.**

- **Handle all apparatus and materials carefully.**
- **Do not smoke when working with specimens or cultures.**
- **Never lay a pipette mouthpiece on the bench**
- **When pipetting always use a teat, never pipette by mouth.**
- **Do not lick gummed labels.**
- **Report any accident, however trivial, to the senior person in the laboratory.**
- **Always wash your hands with soap and water after handling cultures and specimens, and before going of duty. It is recommended that disposable paper or continuos roller towels be provided to minimize any possibility of cross infection.**

Safety Method
General Exempt Laboratory Requirements

Those using the General Safe Method of Use must have also read and comply with:
1. Safe Method of Use for Basic Laboratory Safety
2. Safe Method of Use for Personal Protective Equipment.Note that the word 'must' connotes a mandatory requirement and that the word 'should' connotes a recommendation

A. Requirements for Use and Storage of Chemicals

1. MSDS sheets must be consulted prior to handling any chemical whose properties the user is not familiar with.
2. Flammable solvents *must* be kept in cabinets (if volumes are large then these cabinets must be flameproof). No more than 100 liters of flammable solvent can be stored in each cabinet.

3. Flammable solvents and combustible organics must not be stored with oxidizers (hydrogen peroxide, sodium nitrate, hypochlorite) or any oxidizing acid (conc. nitric or perchloric acids).
4. If highly Flammable solvents (e.g. ethers) are stored in refrigerators, these refrigerators *must* be spark-proofed (and the refrigerator labeled as such). Use of highly flammable solvents is restricted to fume hoods.
5. Ethers such as diethyl ether and tetrahydrofuran *must* have date of purchase clearly written on container. Once opened, ethers such as diethyl ether *must* not be kept longer than 18 months without being tested for peroxides. If testing is not available, the ethers *must* be disposed.
6. Bulk flammable solvents in the laboratory *must* be kept to a minimum – use DCC stores for bulk solvent.
7. Flammable liquids *must* not to be stored or used near sources of ignition.
8. Flammable liquids *must* be decanted and used in fume hood. The only exceptions will be Class 3.1B and C liquids that are diluted in aqueous solvents (e.g. ethanol and methanol).
9. No waste solvents are to be stored outside flameproof cabinets, unless the waste receptacles are attached to analytical machines.
10. Where liquids are stored inside cabinets there should be some form of secondary containment – flameproof cabinets have sumps which provide secondary containment.
11. Individual Winchesters of liquid should be transported in a carrier.
12. Fume hoods *must* not be used for storage of chemicals with the exception of highly toxic gases or chemicals that leak toxic fumes.
13. Reticulating fume hoods *must* be clearly labeled with limitations of use.
14. All gas cylinders *must* be secured.
15. Cylinders of flammable gases *must* be used with a flashback arrestor when attached to a source of ignition.
16. Cylinders of flammable, toxic and oxidising gases must have cylinder key attached to cylinder when in use.
17. Particular care *must* be taken when storing and handling toxic and flammable compressed gases.
18. Particular care *must* be taken when storing and handling
19. Chemical compounds *must* be segregated from combustible organics and flammable liquids.
20. Fume hoods will be used for handling toxic compounds and concentrated acids.
21. Highly toxic chemicals (Oral LD50 < 5 mg/kg) ***must*** be stored in a secure area or locked cupboard/refrigerator if the laboratory is not secure. A register should be kept of these toxic compounds

B. Highly Hazardous Chemicals

Where chemicals have highly hazardous properties, they may require additional measures over and above general safety rules provided by the General Safe Methods of Use.

Particular Storage Requirements

1. Water sensitive compounds must be stored away from sources of water
2. Acids must be stored away from alkalis.

3. Oxidisers ***must*** be stored separately from flammable or combustible organic compounds and ***must*** never be stored with flammable solvents
4. Purchasing date of time sensitive compounds such as ethers ***must*** be recorded on bottle. Compound will be tested or discarded at the prescribed date (see SMOU for Peroxide –forming chemicals).
5. Desensitized explosive compounds such as picric acid ***must*** be checked every 6 months to ensure adequate levels water desensitizing agent are present.

C. Compounds with Chronic Toxicity

Care ***must*** be taken to reduce exposure to any chemical (through the use of fume hoods and gloves). The adverse effects of some compounds are only evident after repeated low-level exposures (e.g. sensitizing agents). Particular care should be taken when MSDS sheets indicate a compound is teratogenic, mutagenic or sensitizing agent. Fume hoods ***must*** be used when handling sensitizing agents such as formaldehyde as well as many common solvents(xylene) to reduce inhalation hazard not only to the user but also to other laboratory personnel. Gloves of appropriate resistance) must also be worn when handling these compounds.

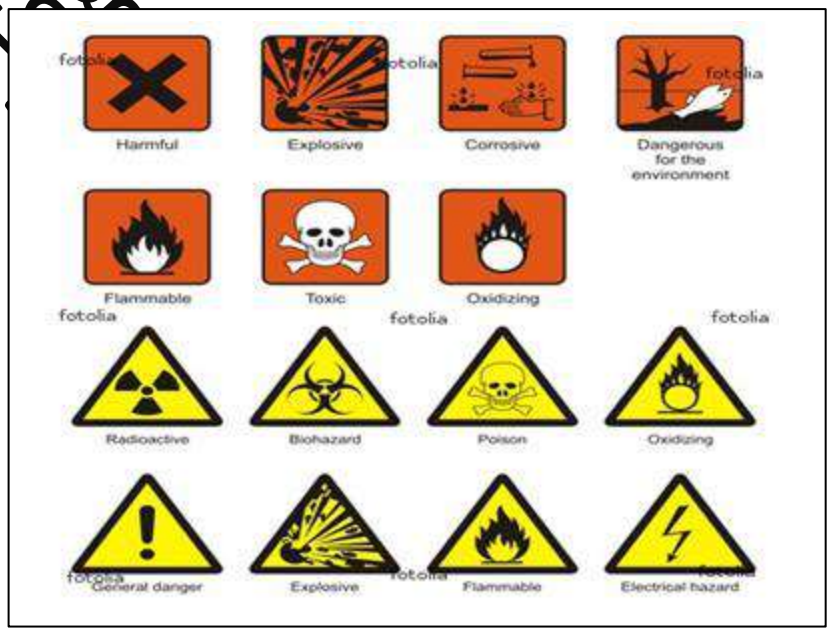
D. Disposal of Chemicals With few exceptions all chemicals must be disposed by a licensed chemical waste contractor (see Chemical Safety Website for more details).

E. Work Alone or After-hours

1. Laboratory personnel working alone or after-hours must ensure they obtain prior permission from their supervisor.
2. Laboratory personnel working alone or after hours must familiarize themselves with emergency procedures and have easy access to emergency telephone numbers.
3. It is strongly recommended that any person working after-hours notifies Unsafe when they enter the building and expected time of leaving.
4. In the case of some specific chemicals (e.g. HF) work alone or afterhours is specifically prohibited (these prohibitions are specific to chemical concerned and are included in the SMOU for that chemical).

The Most Signs that present on the chemical and biological laboratories

اذكر مع الرسم خمس علامات ارشادية وتحذيرية في مختبر البكتريا؟



Post test:-

Circle the correct answer:-

1. **Hanging drop is used for :-**

a- visualizing live bacteria. b- Dry smear
c-a & b c- none

2. **Differential stain:-**

a- is used to differentiate between 2 groups of bacteria b- is used more than one dye
c- The result will be either G+ve or G-ve d- all

3. **Negative stain :**

a- stains around the cell b- used one dye or more
c- none d- a & b

4. **Heat fixation used to :**

a- kill & fix b- kill the bacteria only
c-fix only d- none

5. **The fixative in Gram's stain is :**

a-iodin b-safranin
c- ethanol d- decolorizer

Circle the correct answer :-

1. **Agar is :-**

a- melt at 80 C. b- melt at 40 C
c- solidify at 80 C c- solidify at 100 C

2. **T.C.B.S. is a :-**

a- selective media b-liquid media
c- simple media d- enriched media

3. **Nutrient broth is considered :**

a- solid b- semi solid
c- liquid d- none

4. **Streaking is done to :**

a- obtain single colony for diagnosis of bacteria
b- observe shape & aggregation of bacteria
c-do draw growth curve of bacteria
d- none

5. **Incubation condition for most bacteria is :**

a-37 C for 2weeks b-37 C for 24 hours

c-4 C for a week d- 18 C for 24 hours

Circle the correct answer :-

1. chocolate agar is :-

a- liquid b- enriched
c- special c- simple

2. Culture media is used to :-

a-identify bacteria only b- isolate bacteria only
c- isolate & identify d- none

3. The optimum temperature for pathogenic bacterial growth is :

a- 48 C b- 37 C
c- 4 C d- 20 C

4. Amino acids is :

a- carbohydrate source b- carbon source
c-vitamins d- nitrogen source

5. Culture media is sterilized by :

a-formalin b-flaming
c- autoclaving d- pasteurization

STAINING OF BACTERIA

Pre test:-

Circle the correct answer:-

1. **Hanging drop is used in :-**

a- wet smears. b- Dry smear
c- Visualizing dead bacteria c- none

2. **Unstained preparation used to study :-**

a- both shape & motility b- shape of bacteria
c- Aggregation of bacteria d- only motility

3. **Gram's negative bacteria :**

a- remains purple b-get pink or red color
c- Colorless d- none

Microscopic examination of bacteria

The morphology of bacteria can be studied by the microscopic examination

A- **Unstained preparations** used to study both **shape and motility of bacteria** suspended **in a fluid** using:

- **Hanging drop method.**
- **Wet smear.**

B- **Staining technique must be used** to render the structure of cells visible. These will only differentiate relatively gross individual structures

C- **Electron microscopy** complex techniques are needed to reveal those not shown by staining.

Staining will help to identify organisms and place them in their own particular group by their individual reactions to certain stains. An example is the **gram stain**.

Preparing of bacterial smears

A-Making of wet preparations:

Aim:

Procedure:

1. Make a smear on a clean and dry microscope slide :
   a-From **culture grow in liquid media** by putting 1-3 drops of liquid cultures on the slide using the loop or Pasteur pipette

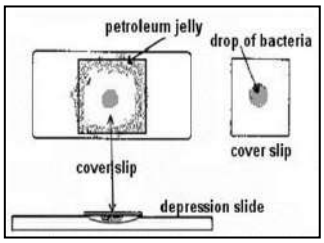
   b-From **culture grow on solid media** by emulsify the colony in a small drop of saline

   c-Emulsify the specimens such as **feces** in a small drop of **saline**, **iodine**, or the required **stain**.

2- Carefully place a cover slip on to the suspension taking care that no fluid extrudes beyond the edges of the cover slip.

3- Examine microscopically as for hanging drop under power **10X or 40X.**

Hanging drop technique

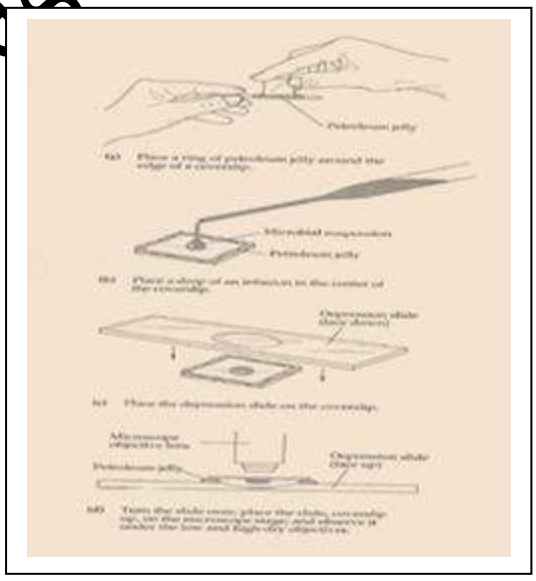


drop of liquid containing microorganisms on a slide covered with a cover slip & suspended over a depression slide

Advantages of the hanging drop:

*Easy to prepare

*Bacteria are live so we can see bacterial motility:



Disadvantages of the hanging drop:

*Requires special depression slide

*Difficult to visualize since microbes are not stained

*Bacteria are live and therefore slides must be disinfected

Viewing under a Microscope

B-Making of dry smears:

Aims:

General notes:

1. Use clean slides free from grease.
2. Mark the slide with a glass writing diamond - grease pencil is easily rubbed away.
3. From liquid cultures make fairly heavy smears.
4. From cultures on solid media, make thin smears.
5. Do not used water taken form rubber tubing attached to taps for making smears, as organisms may be transferred from the rubber.
6. When blotting slides, use a fresh portion of paper for each slide, to prevent transference of material.

Bacterial smear

Equipment:-

- Bacteriological **Loop**
- **Slide** (clean and dry).
- **Drop of water**

(If the culture medium is solid)

- **Bunsen burner**
- **Specimens** [growth of microorganisms in:
  - **Liquid media**
  - **Solid media**
  - **Pathological samples** (liquid or solid)

Swab (pus, ear, wound, vaginal ,urethral) **stool,tissue**

Sputum

C.S.F. (Cerebrospinal fluid)

Urine (after centrifugation)

A//Making of dry smears from liquid media:

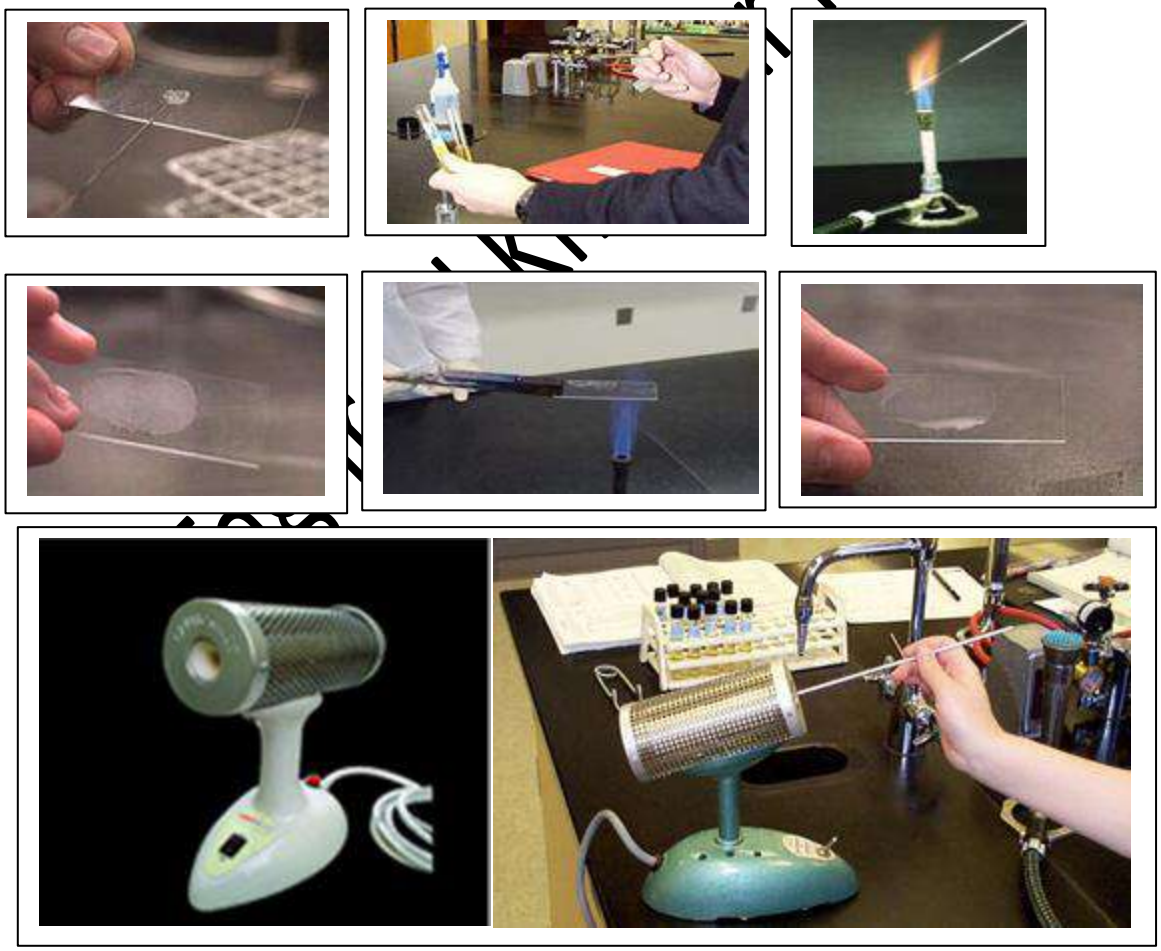
Procedure:

1. Sterilize loop in Bunsen flame.
2. Draw one loop full of liquid culture.
3. Transfer this to a clean slide
4. Spread it with the loop to form a thick film of liquid.
5. Sterilize the loop.

6-Allow the film to dry in air (without heating).

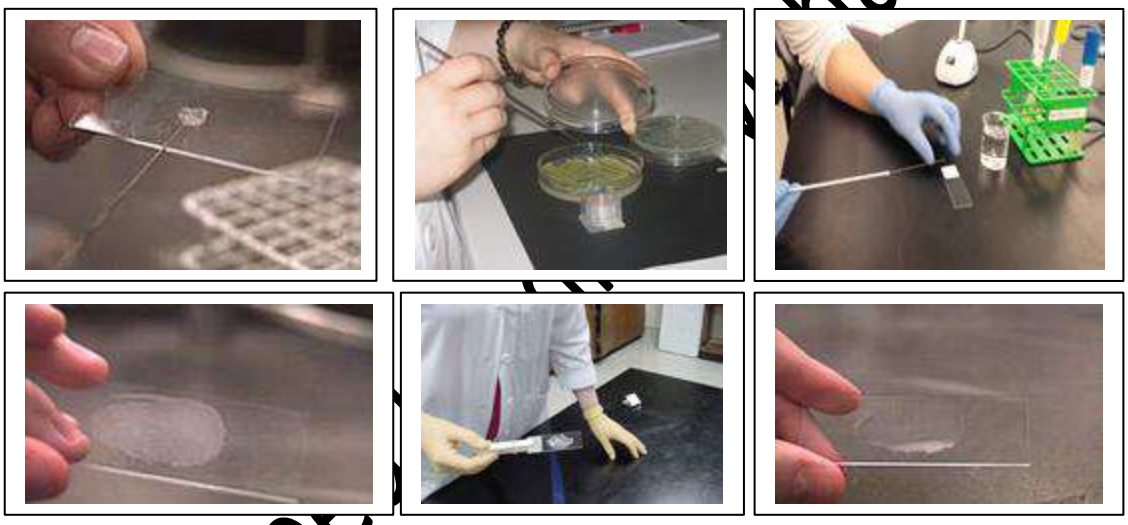
7- Pass the slide 3 times through the Bunsen film flame. This **fixes and killing the** bacteria on the slide.

8-Allow the slide to dry, and then **stain the film** by the requisite method.



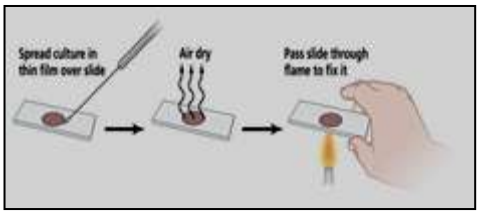
B//Making of dry smears from solid media:

1. Place one **drop of distilled water** on a clean slide by sterilized loop
2. Sterilized loop.
3. With the loop transfer to the slide a small portion of the growth to be examined and emulsify it in the drop of water until a thin homogenous film is produced.
4. Sterilize loop in Bunsen flame.
5. **Allow the smear to dry (air)**
6. **Fix by rapidly pass the slide 3 times through the Bunsen film flame**.



Fixation

- **preservation of morphology but NOT internal structures**
  - -Cellular enzymes are inactivated
  - -Cell structures are hardened
- **kills organisms** (Organism dies) [usually results in the death of the attached microorganisms]
- **adheres specimen strongly to the glass slide**
- **promotes stain ability of specimen**



Types of fixation:

A//Heat fixing: Flame heating bacterial film : **Pass slide through flame quickly 3-4 times**

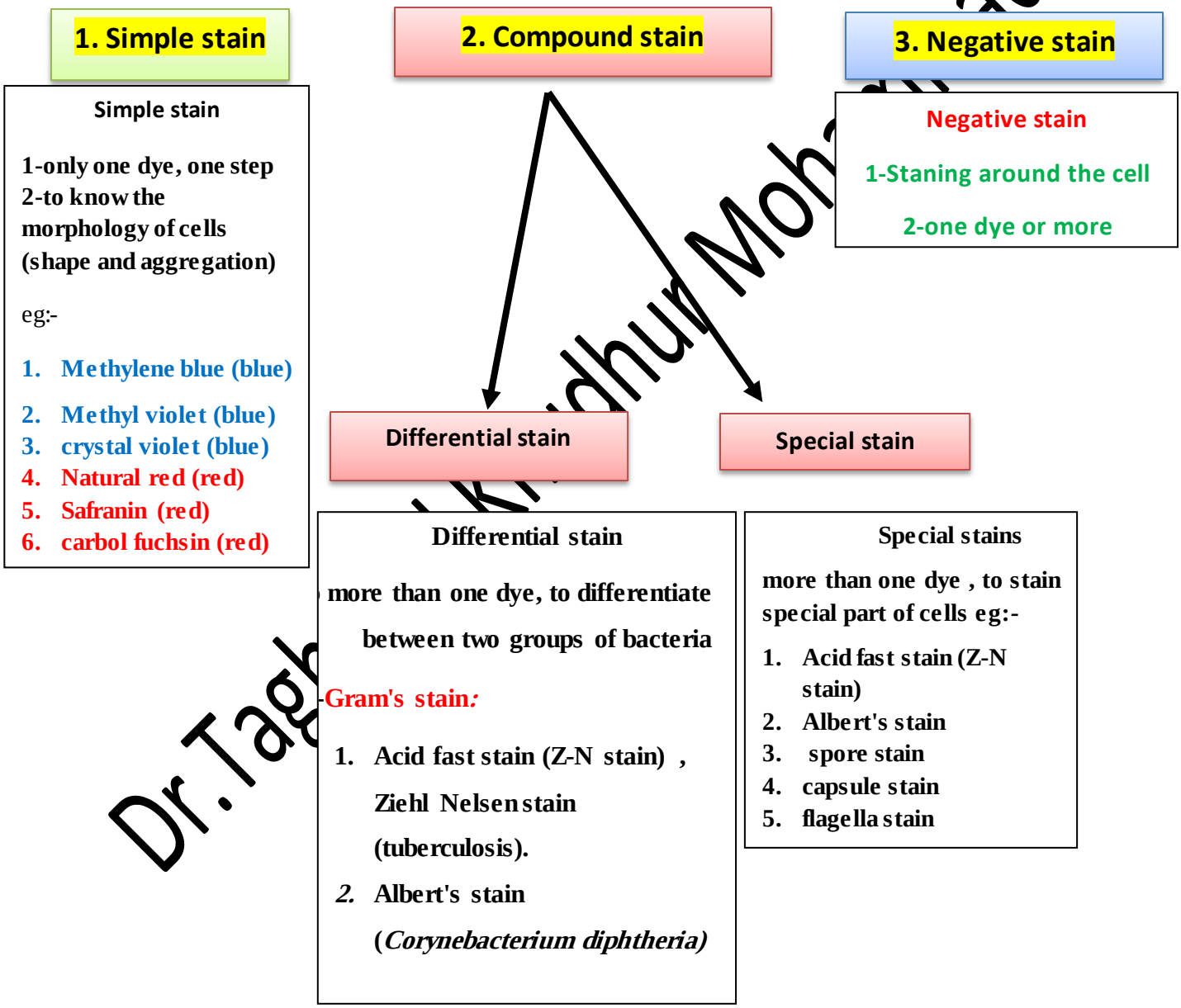
- **Heat fix too little and organisms may wash off slide**
- **Heat fix too much and organisms may be distorted**

B//Chemical fixation:

*chemical fixatives penetrate cells
*Preserves fine substructures and morphology

Staining of smears

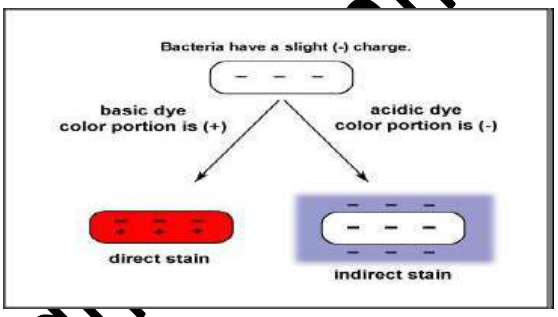
Kinds of bacterial stains



Simple stain

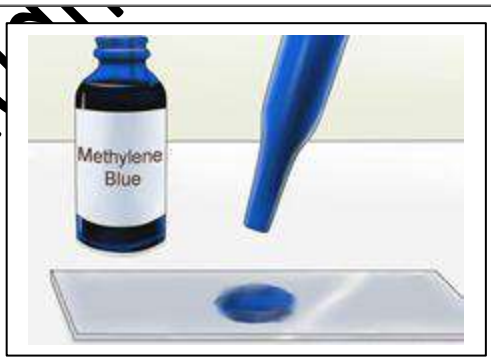
- One reagent
- Usually involves basic dyes
  - Crystal violet
  - Methylene blue
  - Carbol fuchsin

- Most microbes bind basic stains (+ charged dye) because surfaces have lots of negative charges
- Typical examples are **crystal violet and methylene blue**)
- Used to stain outer surface, so used to look at morphology, size and cell arrangement



Equipment:-

- Staining rack.
- loop
- Slide.
- Source of heat.
- bacterial growth
- simple stain (**Crystal violet** or diluted **Carbol fuchsin**)
- filter paper
- distil water
- 70%alcohol



procedure :-

1- Clean and dry slide with 70%alcohol
2-prepare bacterial smear.
3- Fix the smear.
4- Put the slide on staining rack.
5- Stain the smear using any simple stain folded the smear by few drops of stain, let it for 1-2 min.
6-pour the stain on the slide.
7-wash the slide by tab water upside.
8-Let it to dry in air or with filter paper.
9-examin the stained smear under light microscope by oil (100X).

Differential stain

Gram's stain:

In 1884, Gram described this method which is the most important stain in routine bacteriology. It divides bacteria into two categories depending on whether they can be decolorized with acetone, alcohol, or aniline oil after staining with one of the rosaniline dyes such as crystal violet, methyl violet, or gentian violet, and treating with iodine. Those that resist decolorization remain blue or violet in color and are designated gram positive, those that are decolorized and take up the red counterstain such as natural red, safranin or dilute carbol-fuchsin are termed gram negative. Although many investigators have tried to uncover the mechanism of the gram reaction, no universal answer has yet been found and it is possible that more than one mechanism exists.

Reagents of gram staim

Solution 1: (primary stain) ….. Methyl violet

Methyl violet ……..0.5 g

Distilled water ……100 ml

Dissolve the methyl violet in distilled water and filter. Record date and label.

Solution 2 (mordant): Logols iodine

iodine……..……10 g

potassium iodide…….……20 g

distilled water……..……1000 ml

Dissolve the potassium iodide in about 50 ml of water, add the iodine, dissolve by shaking and make up to the final volume. Record date, label and store in a tightly stopper bottle.

Solution 3: (Decolorizor) : Absolute ethyl alcohol 95%or Acetone

Solution 4 (Counter stain) : Natural red OR Safranin OR dilute carbol fuchsin

Procedure:

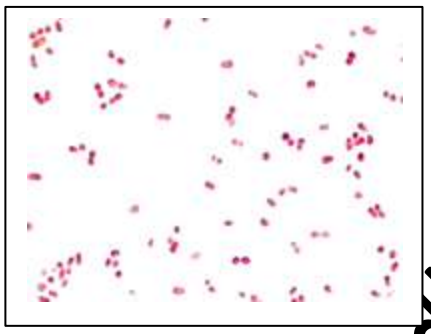
1. **Prepare bacterial smear, allow to dry and fix with gentle heat.**
2. **Stain with crystal violet (primary stain) for 1-2 min.**
3. **Add Iodine (solution 2) (mordant) for 1-2 min. to increase interaction between cell and dye.**
4. **Add the Decolorizer 95% ethanol (solution 3) and continue application until no more color appears to flow from the preparation.30sec.**

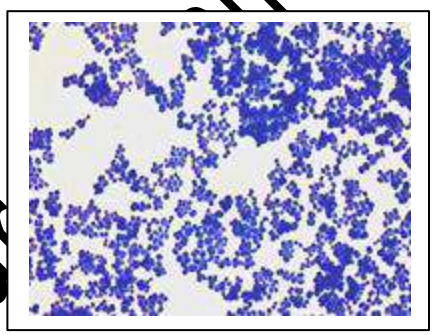
5. **Wash with water.**

6. **Apply Counter stain (solution 4) safranin, for 1 min. (If dilute fuchsin is sued, stain for 30 sec.).**

7. **Rinse with water. Blot carefully and dry with filter paper.**

8. **examine the stained smear under Microscope by oil (100X)**





Gram-negative bacteria pink to red **Gram-positive bacteria dark purple**

Table: reaction of some organisms to gram's stain

Gram positive	Gram negative
Staphylococcus	Coliforms
Streptococcus	Neisseria
Pneumococci	Vibrios
Corynebacterium	Spirochetes
Mycobacteria	Salmonella
Bacillus group	Shigella
	Hemophylus group

Quiz 3

1. **What are the main types of staining with examples?**
2. **What is the procedure of Gram's stain?**

Steps of Gram stain		G-ve	G+ve
Primary stain	Crystal violet	Blue	Blue
fixation	Lugals Iodine	Blue	Blue
decolonization	Ethanol alcohol	Blue	Colorless
counter Stain	Safranin	Red	Blue

5/ Post test:-

Circle the correct answer:-

1. **Hanging drop is used for :-**

a- visualizing live bacteria. **b- Dry smear**
c-a & b **c- none**

2. **Differential stain:-**

a- is used to differentiate between 2 groups of bacteria **b- is used more tan one dye**
c- The result will be either G+ve or G-ve **d- all**

3. **Negative stain :**

a- stains around the cell **b- used one dye or more**
c- none **d- a & b**

4. **Heat fixation used to :**

a- kill & fix. **b- kill the bacteria only**
c- fix only **d- none**

5. **The fixative in Gram's stain is :**

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CULTURE MEDIA

3/ Pre test :-

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a- melt at 80 C. **b- Melt at 40 C**
c- Solidify at 80 C **c- solidify at 100 C**

2. **T.C.B.S. is a :-**

a- selective media **b-liquid media**
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3. **Nutrient broth is considered :**

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c- Liquid **d- none**

4. **Streaking is done to :**

a- obtain single colony for diagnosis of bacteria
b- Observe shape & aggregation of bacteria
c- do draw growth curve of bacteria
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5. **Incubation condition for most bacteria is :**

a-37 C for 2weeks **b-37 C for 24 hours**
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