جامعة الملك سعود
King Saud University
1957

Practical of General Microbiology

MIC 140

قسم النبات والأحياء الدقيقة
Botany & Microbiology Department

جامعة الملك سعود
King Saud University

(علم الاحياء الدقيقة) 140 حدق

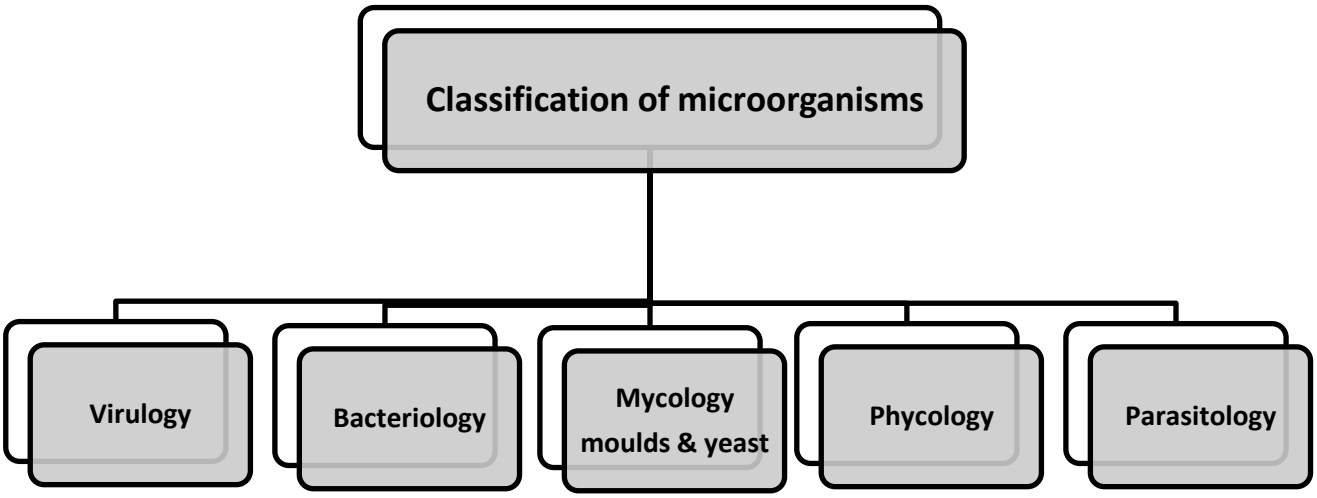
نرحب بالطلاب المسجلين في مقرر ونتمنى لهم التوفيق والسداد وهناك بعض التعليمات التي يجب على الطالب الالتزام بها:

1- الالتزام بالحضور في وقت العملي والطالب المتأخر لن يسمح له بدخول المعمل.
2- يجب إحضار البالطو الخاص بالعملي.
3- سيتم تقسيم الطلاب إلى مجموعات داخل المعمل وكل مجموعة تكون مسئوله عن مكان العمل المخصص لها.
4- على كل طالب كتابة تقرير عن كل عملي بحيث يتم تسليمه في الأسبوع الذي يليه.
5- درجات الجزء العملي 30 درجة.

توزيع عملي مقرر 140 حدق على الاسابيع

No. week	Practice title
First week	Safety roles in the laboratory, Introduction of microbiology.
Second week	Laboratory equipment's and materials.
Third week	Light microscope parts and examination.
Forth week	Sterilization Techniques Preparation culture media of microbiology.
Fifth week	
sixth week	Isolation organisms from different sources.
Seventh week	Purification of organisms.
Eighth week	Morphology colonies of bacteria & fungi.
Ninth week	Smear preparation and simple stain.
Tenth week	Staining of bacteria by gram stain.
Eleventh week	Staining & identification of fungi.
Twelfth week	Cultivation and examination of Algae
Thirteen week	serial dilutions of bacteria
fourteen week	**Final exam**

Introduction of Microbiology



PRACTICAL No: 1

Safety Roles in Microbiology Laboratory

A- Safety rules in the laboratory in general:

1-Don’t eat, smoke or drink inside the laboratory.

2-Don’t put anything in your mouth such as pens, fingers, ect.

3-Don’t take your personal belongings that that don’t you need.

4-Wash your hand frequently after work by soap and water.

5-Wear lab coat, gloves, musk and goggles.

6- Change your coat twice per week.

7-Avoid un necessary talking and walking in the laboratory.

8-Clean the bench before and after the work by disinfectant.

B- Equipment safety rules:

1-If the equipment not work properly stop using it and inform the senior.

2-Don’t carry hot flasks of media by the neck, use a basket and wear heat resistant gloves.

3-Don’t open the centrifuge until the rotator has stopped.

4-Don’t open the autoclave until the contents have cool to about 45c.

C- Safety rules in the reagent and chemicals:

1-Read in the instruction on the bottle before transporting or using any chemicals or reagents.

2-Wear gloves and goggles when handling dangerous liquid or chemicals.

3-When diluting acid always add acid slowly to water.

D-Safety rules at the end practical:

1-Put way all culture and equipment.

2-Wipe down the bench with disinfectant.

3-Turn off and unplug equipment that is not required.

4-Turn off and all Bunsen

5-Turn off any gas cylinder not in use.

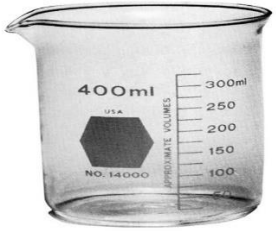
6-Remove the gloves and wash your hands by soap and water.

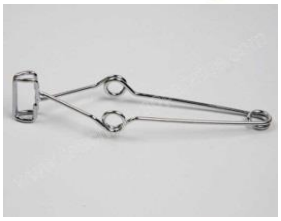
PRACTICAL No: 2

Microbiology lab equipment's and apparatuses

Introduction

Microbiology equipment is a large category covering all kinds of items used in microbiology laboratories. Many industries use microbiology for quality control purposes, to prove there are no living contaminants or to figure out what type of contaminants there are so they know how to fix the problem. Microbiology equipment include microscopes; slides; test tubes; petri dishes; growth mediums, both solid and liquid; inoculation loops; pipettes and tips; incubators; autoclaves, and laminar flow hoods ….etc. Some equipment, like the microscopes and hoods, are permanent items, whereas others, such as pipette tips, are disposable.

Name	Equipment	Use
Forceps (metal/plastic)		Transfer of sterile paper/antibiotic discs; also plant material, e.g. short lengths of root with nodules
Spreader (glass/plastic)		Making spread/lawn plates
Bunsen burner		Sterilization of wire loops and (with alcohol) metal forceps and glass spreaders
- Flask **-Test tube** **- Beaker**	  	-Used for making solutions or for holding, containing, collecting, or sometimes volumetrically measuring chemicals, samples, solutions. -Is a cylindrical container used to store, mix and heat liquids in laboratories. -Small volumes of liquid media/agar slopes/sterile solutions for inoculation (held in test tube rack; dry non-absorbent cotton wool plug or plastic cap prevents contamination)

-Graduated Cylinder **- Pipette (glass/plastic)**	 	- used to measure the volume of a liquid. -Transfer of measured volumes/drops of culture/sterile solutions (dry,non-absorbent cotton wool plug in neck prevents contamination)
Petri dish (plastic/glass)		Plastic: pre-sterilized for streak/spread/lawn/pour plates Glass: only for materials for sterilization by hot air oven, e.g. paper discs
-Loop (wire/plastic) **-Needel (wire/plastic)**		Routine inoculation of agar slopes/deeps and small volumes of liquid media (up to ca 10 cm3); making streak plates
-Test Tube Rack **-Test Tube Clamp**	 	- Used to hold upright multiple test tubes at the same time. - Is used to hold test tubes

Funnel		Used for pouring liquids or powder through a small opening and for holding the filter paper in filtration. Used in transferring liquids in small containers
Autoclave		Sterilization of media, solutions and equipment before use and contaminated items afterwards; melting solidified agar media for use 121 °C for around 15–20 minutes.
Incubator		Incubation of cultures (but many cultures will grow at room temperature in the interval between lessons)
Personal protective equipment (hood)		protection of clothing, containment of dust on clothing Safety spectacles: not considered essential when dealing with suitable cultures and observing GMLP, but may be required by local regulations and for dealing with chemicals
Digital balance		Is a class of balance designed to measure small mass in the sub-milligram range
-Slides -Cover Slips		- Used to hold objects for examination under a microscope.

Centrifuge		Is a laboratory device that is used for the separation of fluids, gas or liquid, based on density.
Water bath		Suitable temperature for keeping melted agar media molten for use (50 °C); accurate temperature control
Hot air oven		Sterilization of glass Petri dishes and pipettes and paper discs (but not essential as autoclaves/pressure cookers serve virtually all needs)

PRACTICAL No: 3

Type of microscopes and Microscopic examination

Introduction

Microscope: A device for magnifying objects that are too small to be seen with the naked eye. Used to observe very small organisms.

Kinds of microscopes:

1. Optical Microscope:

The optical microscope has one or two lenses that work to enlarge and enhance images placed between the lower-most lens and the light source. It was the first device ever created.

a. Simple Optical Microscope:

b. Compound Optical Microscope:

2. Inverted Microscope:

This kind of microscope views objects from an inverted position than that of regular microscopes. It used to the study cell cultures in liquid media.

3. Fluorescence microscopy:

Fluorescence microscope is widely used device in the life sciences and biology. It is a light microscope used to study properties of organic or inorganic

substances using the phenomena of fluorescence and phosphorescence. Both of an excitation and emission filters used in the fluorescent microscope.

4. Electron Microscopes:

Electron microscopy employs electron waves running parallel to a magnetic field providing higher resolution. Electron microscopy allows one to visualize objects that are as small as 1 nm. Electron microscopy is a high-cost technology use very expensive materials such as osomium gold-palladium or carbon or platinum.

a. Scanning electron microscope (SEM):

Scanning electron microscope used to visualize the surface of tissues, macromolecular aggregates.

b. Transmission electron microscope (TEM):

Transmission electron microscope used to study the inner structure of objects (tissues, cells, viruses).

Examination by optical Microscope

Learning Objectives

Each student should be able to

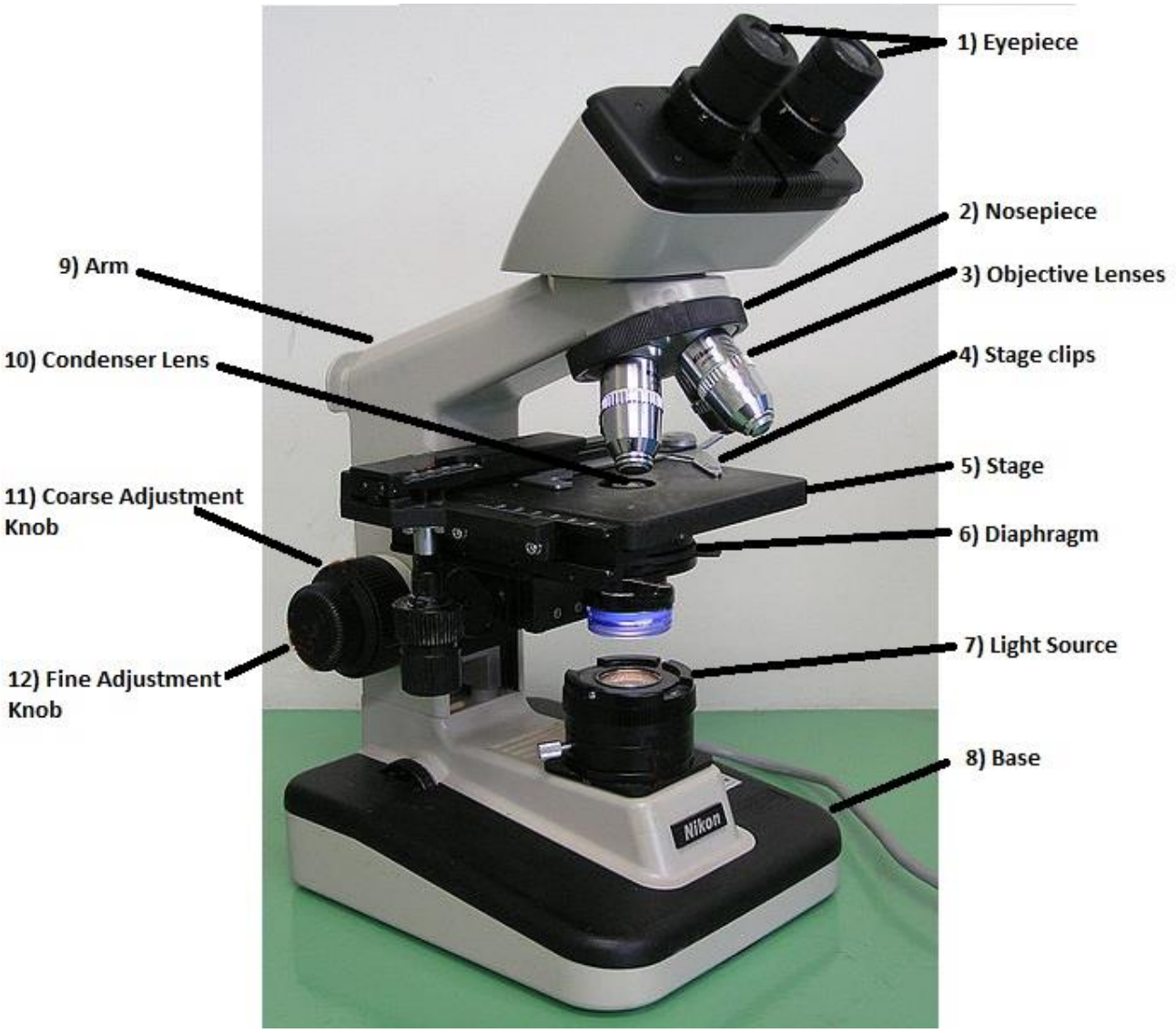
1. Identify all the parts of a compound microscope
2. Know how to correctly use the microscope especially the oil immersion lens
3. Understand how microorganisms can be measured under the light microscope
4. Perform some measurements on different microorganisms

Materials

Optical Microscope, ready slides for bacteria, sedar oil

Procedure

1. Always carry the microscope with two hands and place it on the desk with the open part away from you.
2. Clean all of the microscope's lenses only with lens paper and lens cleaner if necessary. Do not use paper towels.
3. Place the glass slide on the stage of the microscope and secure it firmly using stage clips.
4. Move the slide until open in the stage.
5. Look into the microscope and slowly raise the tube by turning the coarse adjustment knob counterclockwise until the object comes into view. Once the object is in view, use the fine adjustment knob to focus the desired image**.**
6. Open and close the diaphragm, and lower and raise the condenser, noting what effect these actions have on the appearance of the object being viewed.
7. Use the oil immersion lens to examine the stained bacteria
8. After you are finished with the microscope, place the low-power objective in line with the ocular, lower the tube to its lowest position, clean the oil from the oil immersion lens with lens paper and lens cleaner, cover, and return the microscope to its proper storage place.



1. **Eyepiece**: contains the ocular lens, which provides a magnification power of 10x to 15x, usually. This is where you look through.
2. **Nosepiece**: holds the objective lenses and can be rotated easily to change magnification.
3. **Objective lenses**: usually, there are three or four objective lenses on a microscope, consisting of 4x, 10x, 40x and 100x magnification powers. In order to obtain the total magnification of an image, you need to multiply the eyepiece lens power by the objective lens power. So, if you

couple a 10x eyepiece lens with a 40x objective lens, the total magnification is of 10 x 40 = 400 times.

4. **Stage clips**: hold the slide in place.
5. **Stage**: it is a flat platform that supports the slide being analyzed.
6. **Diaphragm**: it controls the intensity and size of the cone light projected on the specimen. As a rule of thumb, the more transparent the specimen, less light is required.
7. **Light source**: it projects light upwards through the diaphragm, slide and lenses.
8. **Base**: supports the microscope.
9. **Condenser lens**: it helps to focus the light onto the sample analyzed. They are particularly helpful when coupled with the highest objective lens.
10. **Arm**: supports the microscope when carried.
11. **Coarse adjustment knob**: when the knob is turned, the stage moves up or down, in order to coarse adjust the focus.
12. **Fine adjustment knob**: used fine adjust the focus.

PRACTICAL No: 4

Sterilization and disinfectant techniques

Objectives*:*

Sterilization:

- Freeing of an environment from all living microorganisms includes bacteria and their spores, fungi, parasites and viruses.

Disinfection:

- Is removing of pathogenic microorganism or reducing their number on the exposed area.

Sterilization methods:

- **Physical methods.**
- **Chemical methods.**

Physical methods:

A-Dry Heat (Flaming):

- Exposure of the objects to heat will kills microbes by coagulation of protein, denaturation of enzymes and oxidation.
- Exposure of wires and forceps to the Bunsen flame until it becomes red hot, then cool down and use.
- Used for loop, forceps, and metal rods.
- Used for sterilization of the mouth of bottle, flasks, containers and test tubes, smear slides etc,,,.

B-Hot air oven:

* Instruments consist of heater, oven.

* Used for sand, powder, metal, glass.

* Thermal death point and Thermal death time:

- 160C for 60 min.
- 180C for 30 min.

C- Moist heat:

<u>Less than 100C</u>

- Pasteurization of milk:
  - Holding method (65C for 30 min)
  - Flash method (72C for 20 sec)
- Preparation of vaccine:
  - By heating at 56C for 30-60 min.
- Steaming:
  - Single exposure of the microbe to steam at 100C for 90 min.
- Boiling:
  - Boiling water is the most common form of application of moist heat but is not capable of killing endospores or killing all viruses
  - At 100º C for 30 min.

<u>Above 100C (Autoclaving)</u>

- Depends on steam and pressure.
  - Steam is a hot air able to penetrate through things.
  - Pressure will rise the temperature from 100C to 121C.
- Moist heat is more effective than dry heat at a given temperature or length of exposure. also more penetrating than dry heat

- Make complete killing of bacteria, their spores, fungi and their spores, parasites and viruses including Envelop and non Envelop virus.
- Thermal death point and thermal death time:
  - 121C for 15 - 20 min.
  - Flash autoclaving at 134C for 4-5min.

D-Filtration:

- Sterilization through removing of microbes from fluids by exposing to small size filter. Used for heat sensitive fluids like serum, antibiotic, suger, and urea.

E-Radiation:

- Exposure to irradiation causes denaturation of proteins and enzymes.

Chemical methods:

Disinfection

Phenolic group of disinfectant:

- E.g.: Phenol crystal, Dittol, Lysol, Cresol.
- Remain active, stable and persist for long period of time.
- Active against G+ve, G-ve, Mycobacterium & viruses.

Alcohols:

- E.g.: Anti-bacterial, sanitizer.
- They able to act and Evaporate, short period of time.

Sterilization and disinfictant
disinfitant
Chemical
alcohols
phinolic group
Sterilization
Physical
filtaration
radiation
ionizing
uv radiation
Heat
moist heat
above 100º C
at 100º C
less than 100º C
dry heat
Hot air oven
Flaming

PRACTICAL No: 5

Culture Media Preparation

Objectives*:*

- To be familiar with different types of culture media used in a microbiology laboratory.
- To be familiar with the pure technique for pouring sterilized media into Petri dishes or tubes.
- To prepare antigen, toxins, vaccines.
- To isolate pathogens from pathological specimens in pure culture to identify them and test their sensitivity to antibiotics for helping treatment the patients.

Introduction:

- **Common ingredients of culture media:**

1- **Peptone:** provides nitrogen for growing microorganisms may be Carbohydrates, minerals and vitamins.

2- **Meat extract:** provide microorganisms with amino acids vitamins and mineral salts.

3- **Yeast extract:** a bacterial growth stimulant.

4- **Mieral salts:** sulphates, phosphates, sodium chloride, potassium, iron and calcium.

5- **Carbohydrates:** simple and complex sugars are add to media for following purposes:

A- Provide pacteria with carbon and energy.

B- Assist in the differentiation of bacteria, example: lactose is added to MacConkey agar to differentiate enterobacteria. Fermentation of sugar with acid production is detected by change in color of indicator.

6- **Agar:** An inert polysaccharide extracted from some seaweed, assist solidify of culture media, and its solidify at 45˚C and dissolved at 90-100˚C.

7- **Water:** it is essential for growth all microorganisms.
Distilled water is better used in preparation of culture media and must be free from chemicals which inhibit bacterial growth.

- ## Classification of Culture media:

1-Culture media can be classified by consistency (physical state) as:

1) Liqid:	•also know as Broth •e.g. Peptone Water, Nutrient Broth (tubes or bottle)
2) Solid:	•consist (1-2%) agar. •e.g. Nutrient Agar, Blood agar (Petri dishes, bottle or tubes in tow forms slant and deep)
3) Semisolid:	• consist (0.5%) agar •e.g. Amies transport medium (tubes or bottle)

2-Culture media can also be classified based on the function and the chemical components:

Type of media	Remark
1- Simple (Basic) Media	These are simple media such as(**nutrient agar**, **nutrient broth** and **potato dextrose agar**)that will support the growth of microorganisms that don't have special nutritional requirement.
2- Selective and Differential Media	An example of this is **MacConkey Agar** which is **selective** for enteric and other Gram-negative bacteria while inhibits gram-positive bacteria, and has an **indicator (neutral red)** to differentiate between **lactose** and **non-lactose** fermenting organisms. Another example, **MSA** (**Mannitol Salt Agar**) is selective and differential to grow ***Staphylococcus aureus***.
3- Enrichment media	Fluid media containing substance which stimulate the growth of certain bacteria such as ***salmonella*** and ***Shigella*** species. Example: **Tetrathionate broth** and **Selenite broth**.
4- Transport media	These are mostly semi-solid media> their use is important when specimens cannot be culture soon after collection, when transporting microbiological specimens (swabs) from health centers to microbiological laboratory. Example: **Amies transport medium** for swabs.

Materials:

1-Powder media.
2- Balance.
3- Distilled water.
4- Spoon.
5- Bottle or flask.
6- Cylinder.
7-Filter paper.
8- Time Med labeling.
9-Marker pen.
10- Autoclave.
11-Petri-dishes or tubes.
12- Cotton or tissue.
13-Water path.
14- Safety cabinet.
15-Incubator.
16- Parafilm.
17-Refrigerator.

Procedure (Methods):

1- Read the label on the powdered media for instructions, on how to use, before preparation of the media.
2- Rinse all glassware in sterile distilled water before using.
3- Weighting powder media in a clean dry and dust free atmosphere. Use glassware (flask or bottle). The capacity of bottle or flask should be twice the volume media being prepare.
4- Label the flask or bottle (name of media).
5- Dissolving powder media with distilled water. Mix wills the bottle or flask until dissolve the powder. If media not dissolved, use heat and avoid boiling.
6- Autoclaving the media. Use autoclave by correct method. Use Time Med Labeling. Autoclaving at 121-126˚C under 15 Pa for 15-20 minutes.
7- Dispenses media. Cooled media until 48-50˚C. pour media in petri dishes about 15-20ml.
8- Labeling all petri dishes or tubes.
9- Perform sterile testing before using media. Incubation all petri dishes or tubes at 37˚C for 24h.
10-Store media at 2-8˚C.

Results:

Student group	Name of media	Solidify media	Type of media	Remark

Discussion:

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PRACTICAL No: 6

Isolation microorganisms from different sources

Objectives*:*

- To Learn how to isolate bacteria from body site and natural places.
- Appearance of the colonies in the culture medium

Materials:

1. Aseptic tools.
2. Petri plates with media (Nutrient agar/broth medium, MacConkey agar media and Potato dextrose agar medium)
3. Different Sources (Flora like mouth swabs, soil, rotten fruits, yoghurt,..)
4. Incubators 25ºC and 37º C.
5. Inoculating loops and needles.

Procedure (Methods):

Isolation of bacteria

For isolation of bacteria, prepare (Nutrient agar medium and MacConkey agar media) according lab 5.

From Soil:

1. Sprinkle a pinch of soil on the solid media plate (Nutrient agar medium).
2. Close the lid and incubate at 37 °C.
3. After 18 -24 hours observe for growth.

From Mouth:

1. Take some saliva with the help of a cotton swab.
2. Inoculate it on the media and incubate at 37 °C for 18 -24 hours.

From Hand:

1. Touch the surface of the solid media plate.
2. Incubate at 37 °C for 18 -24 hours.

From Yoghurt:

1. Put a drop of diluted yoghurt on the solid media plate of bacteria.
2. Incubate at 37 °C for 18 -24 hours.

- These plates of bacteria will be incubated at 37°C for 24 hours and then stored at refrigerator until next week when you will observe for results.

Isolation of fungi

For isolation of fungi, prepare (Potato dextrose agar medium) according lab 5.

From Air:

1. Expose the prepared plate of solid media in air for 5 min
2. Close the lid and incubate at 25- 28 °C.
3. After 2-5 days fungus observe for grow.

From Fruit:

1. Clean the rotten part of fruit with alcohol
2. Cut a piece and inoculate it on the media plate
3. Then incubate, after 2-5 days fungus will grow

From Soil:

1- Sprinkle a pinch of soil on the solid media plate

2- Close the lid and incubate at 25 °C

3- After 2-3 days fungus observe for growth.

- These plates of fungus will be incubated straight at 28°C for 2-5 days and then stored at refrigerator until next week when you will observe for results.

Results:

Student group	Source of Sample	Bacterial growth		Fungal growth
		Nutrient agar(NA)	MacConkey agar (Mac)	Potato dextrose agar medium (PDA)

Discussion:

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PRACTICAL No: 7

Purification of microorganisms from mixed cultures (Inoculation and incubation)

Objectives*:*

- To obtain a pure culture by isolating single colonies.
- To be familiar streak plate technique (third streaking).
- To describe bacterial/fungal colony shapes.
- learn aseptic techniques for working with bacterial/fungul cultures.
- learn methods for transferring and working with bacterial/fungal cultures aseptically.

Introduction:

Glossary:

- **Culture:** The growing organism onto the media plate.
- **Pure Culture:** Only one type of microorganism growing on the media plate.
- **Contaminated (mixed) culture :** More than one type of microorganism growing on the media plate.
- **Colony:** The number of cells of any organism living together.
- **Broth culture:** Microorganisms growing in a liquid medium.
- **Inoculum:** A few number of cells transferred to other media for isolation.

Materials:

1-Bacterial/ fungal cultures.	2- media plates.	3-Wire or plastic loop/Needle.
4- Bunsen burner.	5-Marker pen.	6-Petri dishes.
7-Disinfectant.	8-Incubator.	9-Cotton or tissue.
10-Safety cabinet.	11-Colony count.	

Procedure (Methods):

Purification of bacteria and fungal (yeast)

Using a wire loop

Wire loops are sterilised using red heat in a Bunsen flame before and after use. They must be heated to red hot to make sure that any contaminating bacterial spores are destroyed.

Streak plate (Single colony)

1- After sterilizing the loop on the flame, cool it, and then take a loopful from the culture.

2. Inoculate plate with a inoculating loop taken from the culture to make a primary inoculum.

3. Sterilize loop after step "2" and allow it to cool. Turn the plate anti-clock wise and by going back across the initial inoculum, streak across the surface of the agar.

4. Sterilize loop and allow it to cool. Repeat step"3" again, this time goes back across the first streak and spread across the surface of the agar.

5. Again sterilize, then repeat the streak one last time across the second streak and spread across the surface.

6. Finally, sterilize inoculating loop then streak the remainder of the plate in a zig-zag fashion.

7. Incubate the plate at 37 ˚C for 24 hours.

(See figure:1)

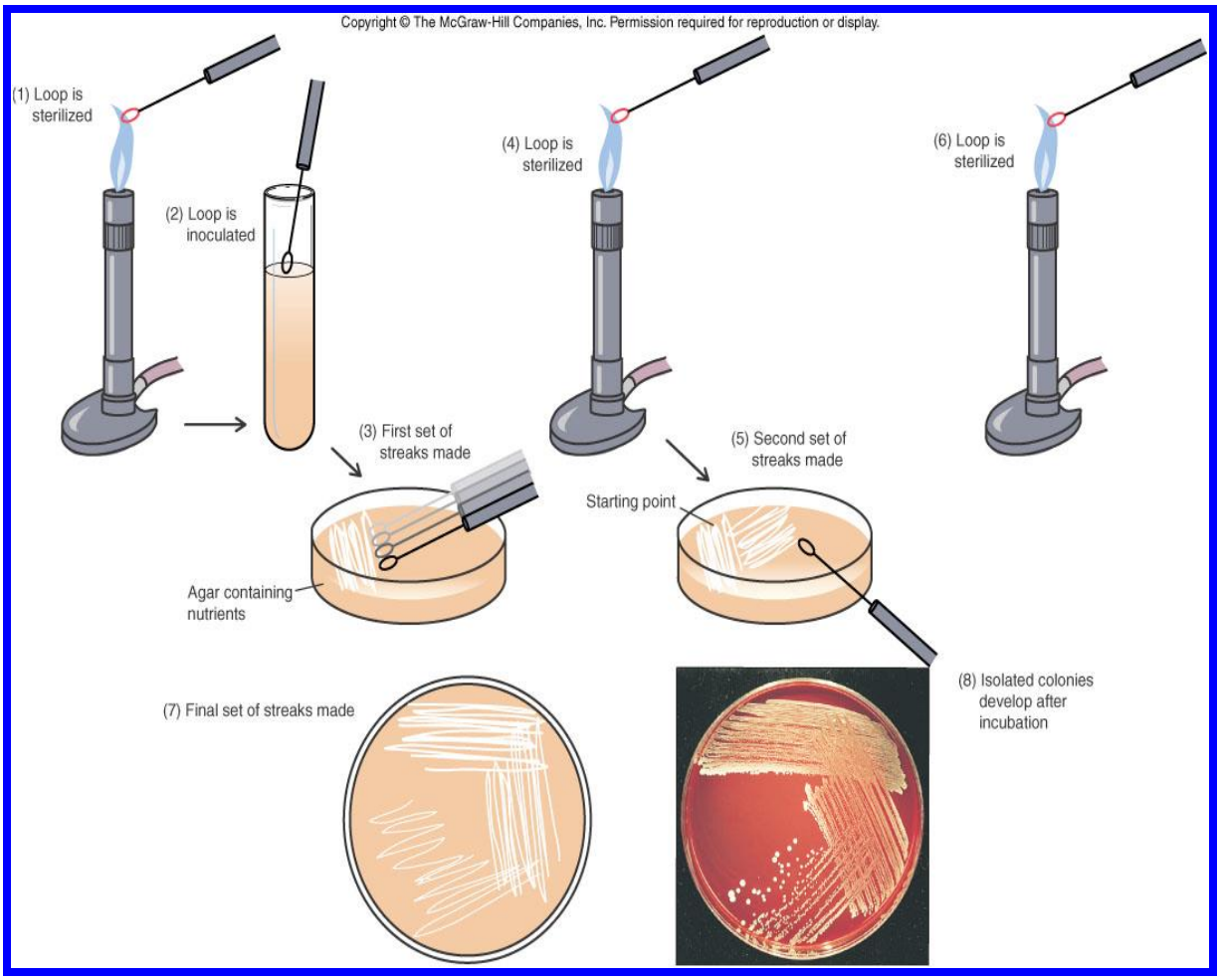

Figure1: streak plate technique (third streaking)

Purification of fungal (moulds)

1. Use a cork borer or needle.
2. Flame cork borer using alcohol and allow to cool.
3. Cut few discs from the edge of an actively growing fungal colony.
4. Inoculate it (surface facing down) on the center another media plate with the help of flamed forceps.
5. Incubate it at 25-28˚C for 2-5 days.
6. Pure culture of the organism will grow.

Result:

Record your observations

Discussion:

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PRACTICAL No: 8

Morphological Characteristics of microbial colonies

Objectives*:*

Describe the character of the different colonies.

Introduction:

Bacteria

Bacteria grow as colonies on solid media. A colony is a visible mas of microorganism that originated from a single mother cell. Hence, a colony of bacteria is a clone of genetically alike bacteria.

Colony Morphology

These are the characteristics used to accurately and consistently describe the morphology of a bacterial colony:

1-Size 2-Shape 3-Color (also known as pigmentation) 4-Texture

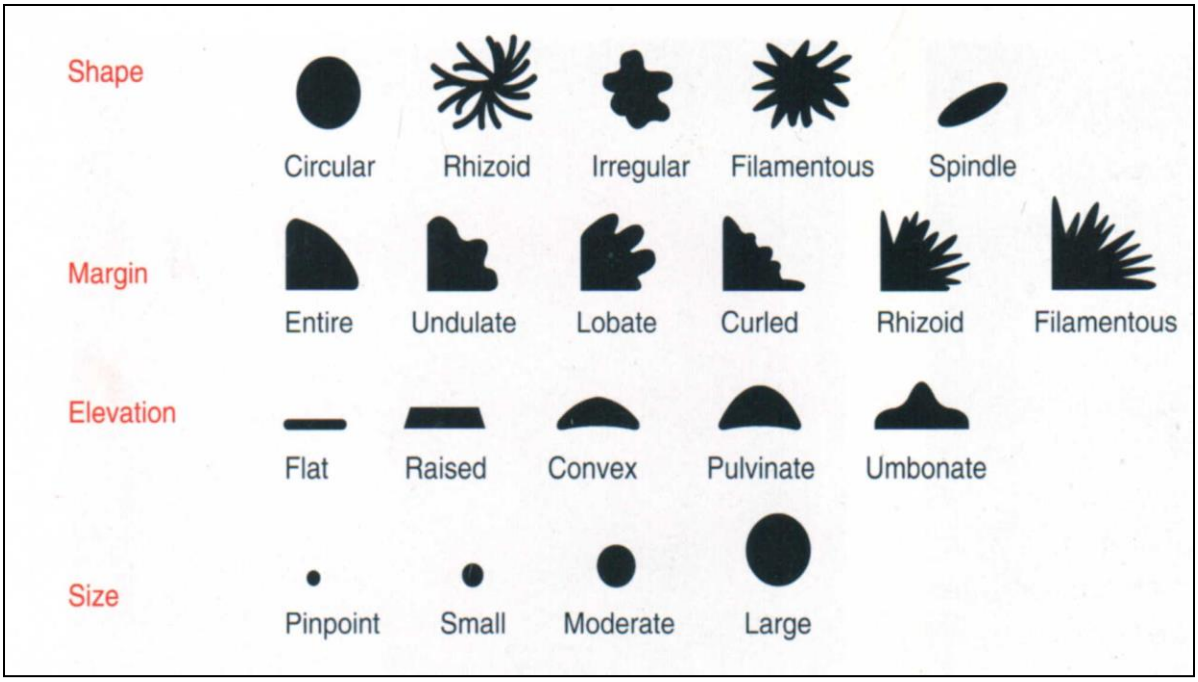
5- Height (elevation) 6-Edge (margin)

Fungi

Colony morphology:

Surface texture: cottony, granular, powdery, smooth, creamy or waxy.

Pigmentation: Fungi may be colorless or brightly colored. Color may be on fungus itself, on the agar, or on the bottom of the colony.



The image shows the colony morphology of bacteria.

Materials & procedure:

-Bacterial culture's (fresh plates).

-Fungal culture (mould and yeast).

Procedure:

1-Use a plate which has well-isolated colonies according lab 7.

2- Observe one colony from each of the different pure cultures.

3- Determine general shape and color.

4-Use microscope for more detail bacterial colonies (4X).

Result:

After incubation period, observe the plate for single isolated colonies on media agar.

Bacteria colonies:

Student group	Type of Samples	Single Colonies			
		Colony shape	Colony size	Colony color	Colony surface

Fungal colonies:

Record your observations (color, texture, pigment)

Discussion:

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PRACTICAL No: 9

Staining techniques of bacteria

A) Simple stain techniques

Objectives:

- Use Aseptic Technique to prepare smears for staining.
- Prepare bacterial smears for the microscopic visualization of bacteria.
- Examine smears to determine bacterial morphology, arrangement.
- Perform a simple staining procedure.
- Compare the shapes and arrangements of bacterial cells.

Introduction:

Smear:

Much of your success in staining microbes will come from preparing a good smear. In doing so, you must keep in mind three key goals. First, you want to adhere the cells to the slide so that they are not washed off in subsequent procedures. Second, in doing so, you do not want to overheat the smear so that the cells shrink and you are left with distortion and artifacts. Third, you need to prepare a thin smear, because the thickness will determine whether or not you can visualize individual cells, their arrangement, or details regarding gram reaction or internal structure.

Simple stain:

The use of a single stain or dye to color a bacterium is called a simple stain. These types of dyes, called basic dyes. Some common basic dyes used in staining are methylene blue, crystal violet, and basic fuchsin.

Materials:

1- Bacterial growth (fresh).	2- Wire or plastic loop.	3-Pasture pipettes.
4- Filter paper or tissue.	5- Bunsen burner.	6- Gloves.
7- Clean microscope slides.	8- Safety cabinet.	9-Marker pen.
10- simple stain	11- Light microscope.	12-Disinfectant.
13- oil emersion.	14- Microscope.	15-Distilled water.

Procedure (Methods):

A-Smear preparation:

1. Clean and dry microscope slides thoroughly.
2. With the pen (permanent ink)/pencil, mark the name of the bacterial culture in the far left corner on each slides.
3. Flame the inoculating loop.
4. For the broth culture, shake the culture tube and, with an inoculating loop, aseptically transfer 1 to 2 loopfuls of bacteria to the center of the slide. Spread this out to about a 1/2-inch area.
5. preparation a smear from a slant or plate, place a loopful of water in the center of the slide.
6. With the inoculating loop, aseptically pick up a very small amount of culture and mix into the drop of water. Spread this out as above.
7. Allow the slide to air dry, or place it on a slide warmer.
8. Pass the slide through a Bunsen burner flame three times to heat-fix and kill the bacteria.

Please note: It is very important to prevent preparing thick, dense smears which contain an excess of the bacterial sample. A very thick smear diminishes the amount of light that can pass through, thus making it

difficult to visualize the morphology of single cells. Smears typically require only a small amount of bacterial culture. An effective smear appears as a thin whitish layer or film after heat-fixing.

B- simple stain procedure:

1. Prepare a heat fixed smear of the culture to examination.
2. Cover the smear with methylene blue.
3. Allow the dye to remain on the smear for approximately 1 minute. (Note staining time is not critical. Somewhere between 30 seconds and 2 minutes should give you an acceptable stain. The longer you leave the dye on, in general, the darker the stain).
4. Wash the excess stain off the slide Pick up the slide by one end and hold it at an angle over the staining tray.
5. Wash off any stain that got on the bottom of the slide as well.
6. Blot off excess stain using bibulous paper. DO NOT rub the slide, rather place the slide between two sheets of bibulous paper and press down gently. Paper will absorb excess dye.
7. Examine the slide under the bright field microscope.
8. Record the shape, arrangement, and approximate size of the organisms. (see figure1).

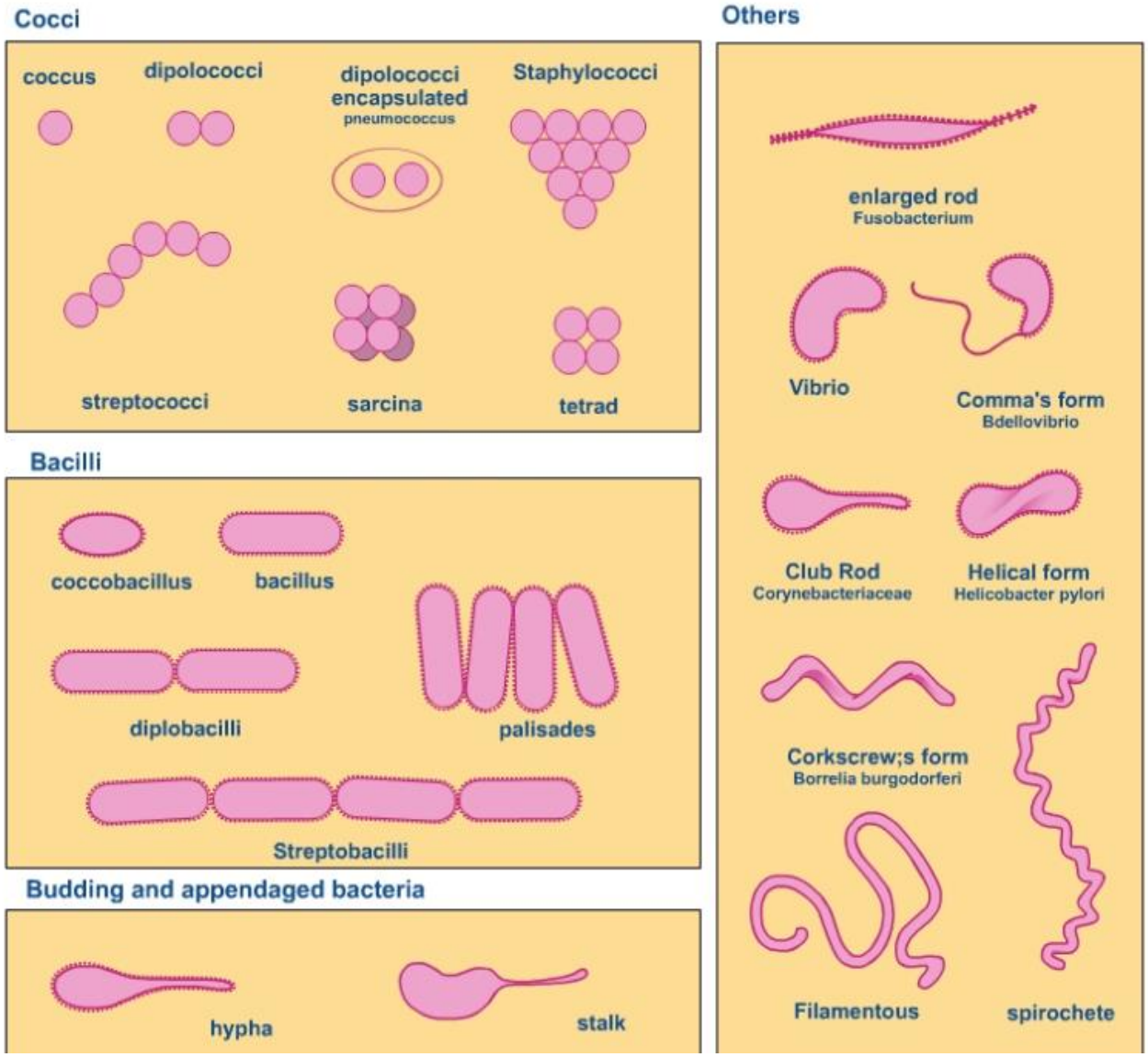

Fig1: Different bacterial morphology

Results and observations:

Student group	No. of slide	Shape of bacteria	Arrangement

Discussion:

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PRACTICAL No: 10

Staining techniques of bacteria

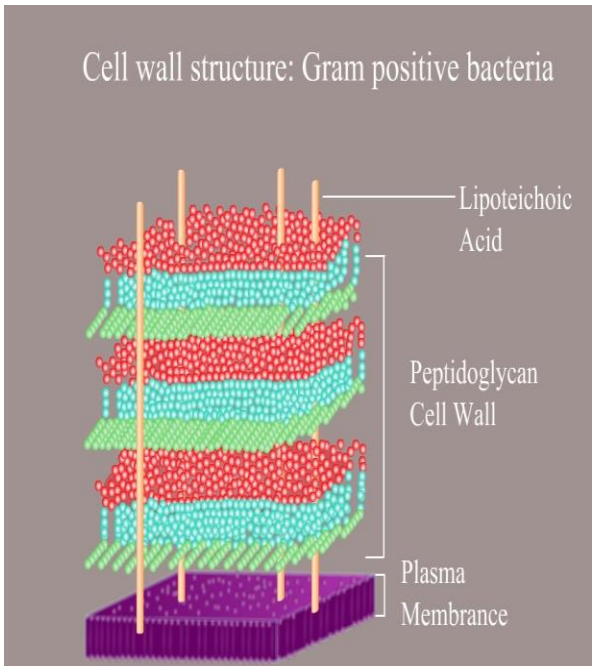
B) Gram Stain Technique

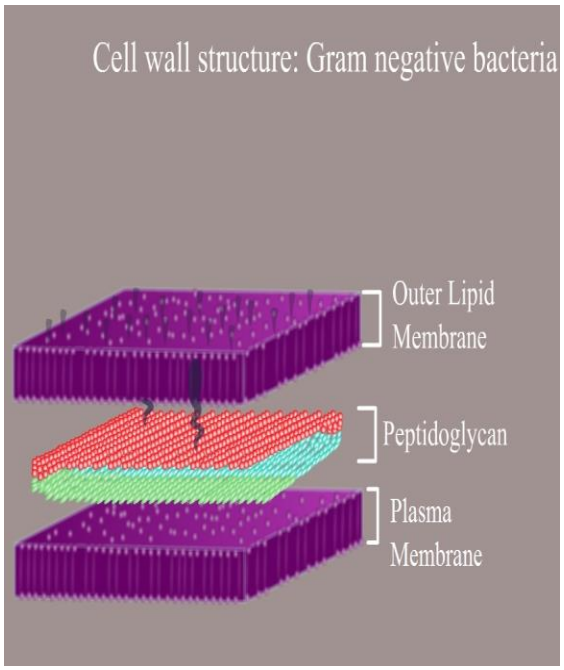
Objectives:

- To differentiate between the two major categories of bacteria: Gram positive and Gram negative.
- To understand how the Gram stain reaction affects Gram positive and Gram negative bacteria based on the biochemical and structural differences of their cell walls.

Principle:

Bacteria morphology could be determined using the simple staining, but further staining method is required to distinguish between bacteria of similar morphology. Gram stain (differential staining) could be used in this particular. It stains gram positive bacteria by violet color wile gram negative by red color.





Materials Required:

1-Clean glass slides. 2- Inoculating loop. 3- Bunsen burner.

4- Bibulous paper. 5- Microscope. 6- Immersion.

7- Lens paper and lens cleaner. 8- Distilled water.

9-18 to 24 hour cultures of organisms.

Reagents:

1. Primary Stain - Crystal Violet.
2. Mordant - Grams Iodine.
3. Decolourizer - Ethyl Alcohol.
4. Secondary Stain – Safranin.

Procedure:

Part 1: Preparation of the glass microscopic slide according lab 9**.**

Part 2: Labeling of the slides according lab 9**.**

Part 3: Preparation of the smear according lab 9.

Part 4: Heat Fixing according lab 9.

Part 5: Gram Stain Procedure:

1. Place slide with heat fixed smear on staining tray.
2. Gently flood smear with crystal violet and let stand for 1 minute.
3. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
4. Gently flood the smear with Gram's iodine and let stand for 1 minute.
5. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle. The smear will appear as a purple circle on the slide.
6. Decolorize using 95% ethyl alcohol or acetone. Tilt the slide slightly and apply the alcohol drop by drop for 5 to 10 seconds until the alcohol runs almost clear. Be careful not to over-decolorize.
7. Immediately rinse with water.
8. Gently flood with safranin to counter-stain and let stand for 45 seconds.
9. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
10. Blot dry the slide with bibulous paper.
11. View the smear using a light-microscope under oil-immersion. (see figure1)

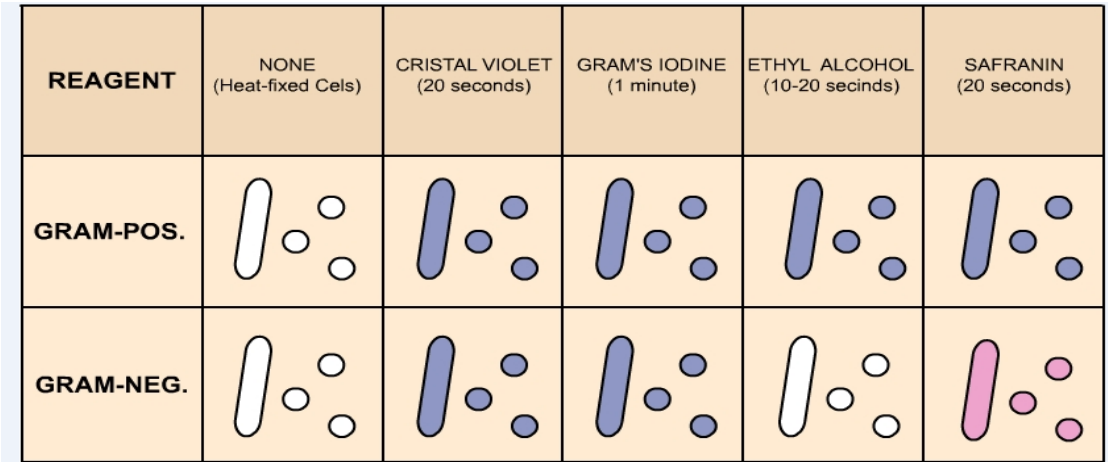


Fig1: Color changes that occur at each step in the staining process

Typical Gram-negative bacteria:

Gram-negative bacilli:

1. *Salmonella typhi,*
2. *Vibrio cholera*
3. *Escherichia coli*
4. *Proteus* sp

Gram-negative cocci: ***Neisseria* sp**

Typical Gram-positive bacteria:

Gram-positive cocci:

1- ***Streptococcus sp***.
2- ***Staphylococcus sp***
3- ***Micrococcus*** sp

Gram-positive bacilli:

1. *Bacillus* sp
2. *Corynebacterium listeria*

Results and observations:

Gram positive bacteria→→→→ Violet or blue color.

Gram Negative bacteria→→→→ Red or pink color.

Student group	Sample number	Color cells	Shape cells	Result read	Example

Discussion:

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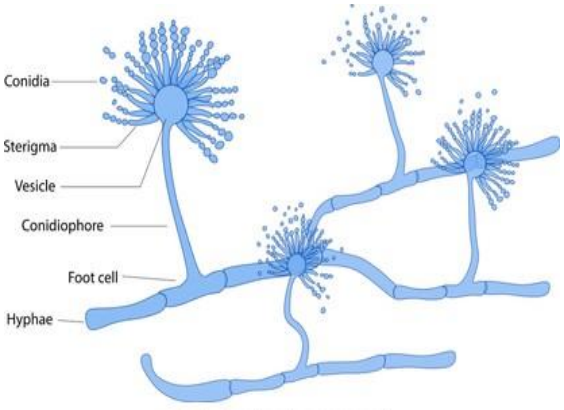
PRACTICAL No: 11

Staining and identification of Fungi

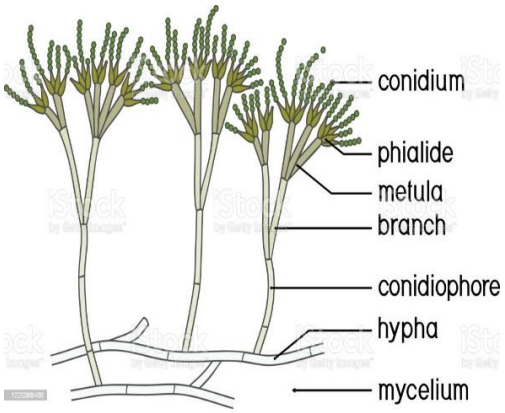
Objectives:

Fungi are eukaryotic organisms that includes yeasts and molds. Their cell walls contain chitin.

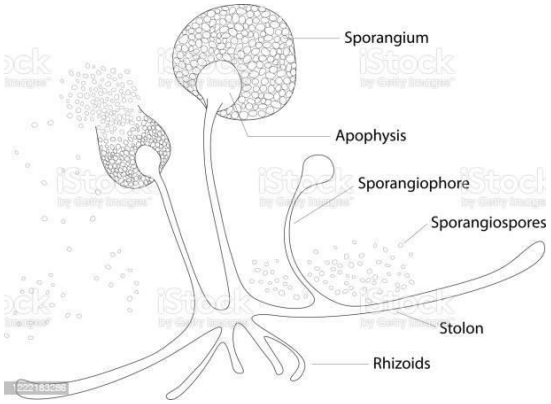
Usually, **Lactophenol Cotton Blue (LPCB)** Staining method is used to detect fungi under the microscope, as **Lactic acid** is used for preserve the fungal structures, **Phenol** is used as a disinfectant by killing any living organisms and **Cotton blue** is give color to the chitin.



A*spergillus* sp.



Penicillium sp.



Rhizopus sp.

Materials Required:

1-Clean glass slides. 2- Inoculating needle. 3- Bunsen burner.
4-Microscope. 5- Lens paper and lens cleaner.
6- Cultures of organisms A*spergillus, Penicillium, Rhizopus, Fusarium* and *saccharomyces* (yeast).

Procedure:

1-label the needed clean slides.

2-Add a drop of the **Lactophenol Cotton Blue (LPCB) on slide.**

3-Transfer a part of mycelium by sterile needle to slide and mix with the stain drop.

4- Cover the stain with a clean sterile coverslip without making air bubbles.
5- Examine the stain microscopically at 40X.

Results and observations:

Record your observations

Discussion:

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PRACTICAL No: 12

Cultivation and examination of Algae

Definition of microalgae:

Prokaryotic or eukaryotic photosynthetic microorganisms that grow rapidly and live in different conditions due to their unicellular or simple multicellular structure.

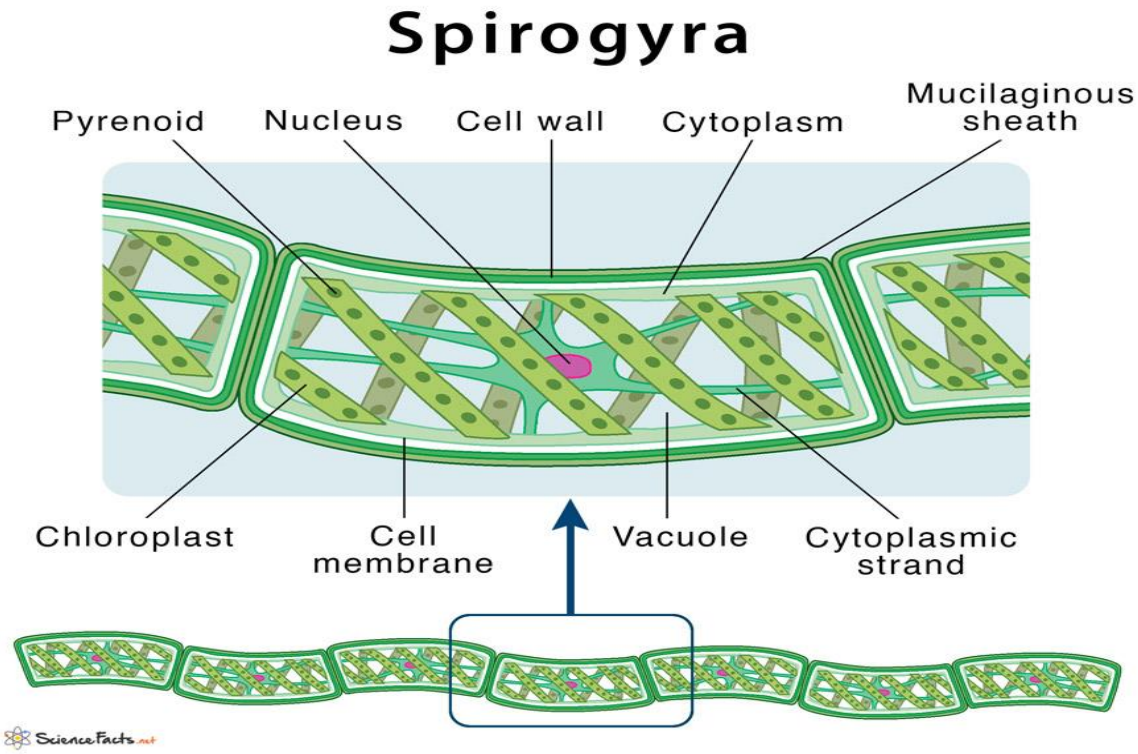
Prokaryotic microorganisms for example, cyanophyta

Eukaryotic microorganisms for example, chlorophyta and diatoms.

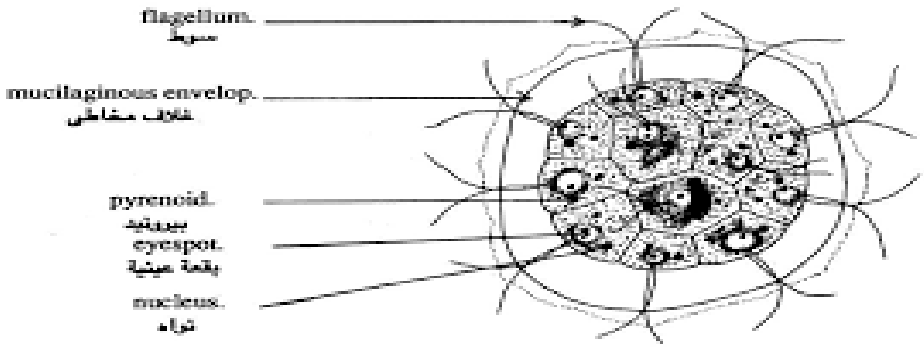
Culture media: cho 10 and BG11

Some of Classification algae:

1. **Green algae:** Example:



Pandorina sp



Pandorina sp.
باندورينا

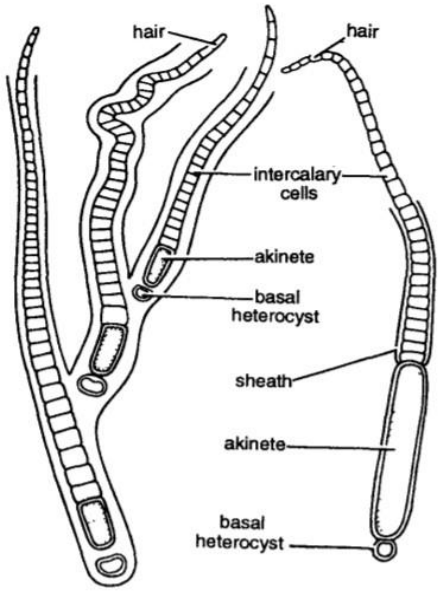
2. Red algae:

Example:

***Polysiphonia* sp**

3. **Brown algae:** Example: **Diatoms**

4. **Cyanobacteria:** Example: ***Gloeotrichia* sp**



Gloeotrichia. A filament to show heterocyst and akinete.

PRACTICAL No: 13

serial dilutions of bacteria

Objectives:

- There are various ways of counting or monitoring microbial growth in a culture.
- Serial dilution involves taking a sample and diluting it through a series of standard volumes of sterile diluent, e.g. distilled water or 0.9 % saline. Then a small measured volume of each dilution is used to make a series of pour or spread plates.
- By diluting the sample in this controlled way it is possible to obtain an incubated plate with an easily countable number of colonies (30–100) and calculate the number of microbes present in the sample.

Materials Required:

1- sample of natural material
2- 6 sterile test tubes containing 9 ml
3- labelled
4- 12 sterile, Pasteur pipettes
5- Marker pen
6- Petri-dishes
7- Cotton or tissue
8- Incubator
9- media

Procedure (Methods):

1. Take a sterile pipette.

2. Draw up 1 ml of a well mixed sample

4. Add this sample to the first tube. The volume of this tube is now 10 ml . This provides an initial dilution of 10–1.

5. Mix the dilution thoroughly, by emptying and filling the pipette several times.

6. Discard this pipette into the pot of disinfectant, but keep the pipette for making the next dilution.

7. Take a new pipette, fit it to the pipette and draw up a 1 ml sample of the 10–1 dilution and place it in the second tube.

8. Mix well as before. This gives a 10–2 dilution.

9. Discard the pipette in disinfectant.

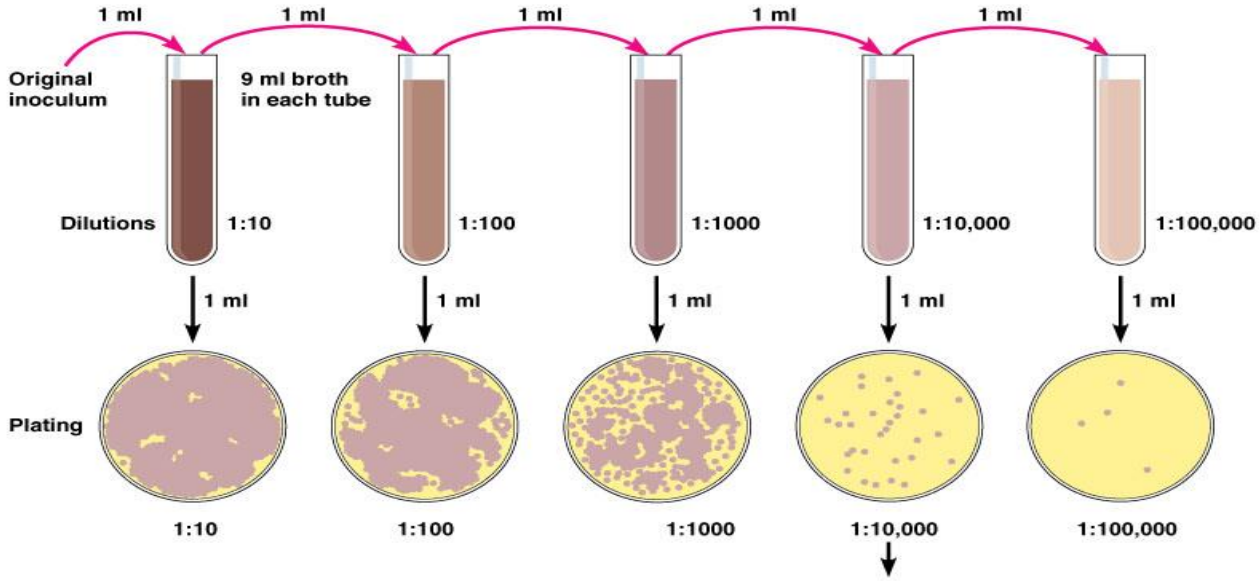
10. Repeat this for the remaining tubes, removing 1ml from the previous dilution and adding it to the next 9 ml of diluent.

If 6 tubes are used, the final dilution for the bacteria will be 10–6 (1 in 1,000,000).

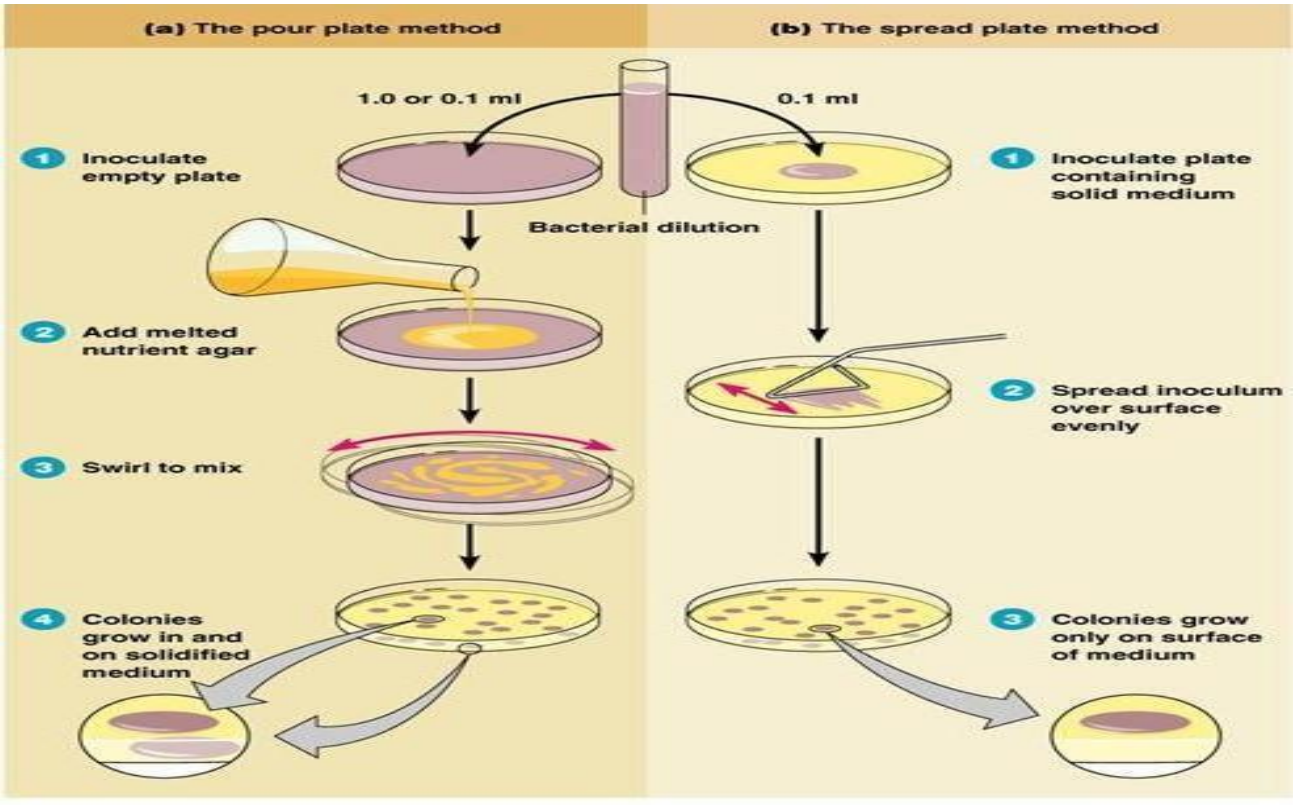
Plating and counting procedure :

Use a known volume of each dilution to make either pour plates or spread plates .By starting with the highest dilution, the same pipette may be used throughout. For statistical purposes, replicate plates should be prepared. After incubation the plates will show a range of numbers of colonies. Choose the plate that has an easily countable number (about 30–100) and carefully count every colony. Using a marker pen helps to avoid counting the same colony twice. Then calculate the number of micro-organisms in the sample:

Number of microbes/ml = number of colonies × dilution of sample



Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000/ml in sample.)



Results and observations:

Student group	Samples number	Dilution	Colonies number	Calculation CFU/ML

Discussion:

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