14 January 2022

#Microbiology

Laboratory 1: Microscopes and streaking teqnique

* Light microscope: Light microscope uses visible light to illuminate specimens.

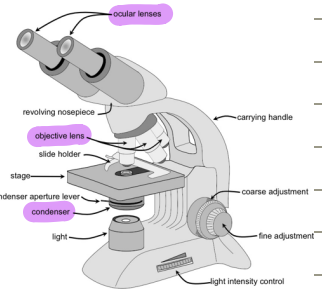
↳ it uses 2 factors that contribute the ability to see and distinguish structures

Magnification
the ability of a microscope to produce an image of an object at a larger scale
Larger than it's actual size. ✓

Resolution
the ability to distinguish two different objects. For the light microscope Resolution is 0.2 micrm → so if distance between 2 objects is smaller than 0.2 micrm → we cannot see them as two separate objects.

→ Light microscope has 3 optical components (three systems of lenses)

- Condenser: Collect and focuses light producing a cone of light that illuminate the object to be observed. ✓
- Objective lenses: enlarge and projects the image in the direction of the eye peice. ✓
- Ocular lense (Eye piece): Further magnifies the image of the object and projects it onto the observers retina. (x10) ✓.



* What is the total magnification?

→ the product of the magnification of the objective and ocular lense. (maximum magnification is 1600 times)
↓
Product of a 10X of the ocular lense 150x of the objective.

→ Resolution → is a function of wavelength + ability of objective lense to gather light

* Numerical Apeture: the number which indicate the ability of gathering light.

- Resolution is expressed by: $R = 0.5\lambda / NA$

Blue light → has the highest Resolution + high NA.

- One of the objective lenses is called Oil immersion lens. → Increases the ability of the objective to gather light + increasing Resolution.

* a higher magnification → higher NA

Sometimes we use stains to → improve contrast between the cell and the surrounding → which makes it more visible.

x100 magnification

The oil immersion lens: We have to add a drop of immersion oil on the slide

- if oil is not added light waves reflect off the slide specimen through the glass cause slip

this type of lense is used for increasing the resolving power of a microscope → How this can be achieved?
it can be achived by immersing both the objective lens and the specimen in transparent oil of high refractive index → increasing NA of the objective lens. → So it increases the light gathering property.

Focal point? A specified place which light is focused on.

Focal length? Distance between center of lens and the focal point.

↓ Focal length → ↑ magnification → Shorter wavelenght → ↑ Resolution

* Electron microscope

Such as transition electron microscope → Focus a beam of electrons which have shorter wave lenght resulting in much higher Resolution.

→ Incubator: An insulated and enclosed device that provides Optimal condition of temperature, Humidity that are required for the grow of organisms. + water bath may be used for this proccess also

→ Autoclave: Is used to decontaminate certain biological waste and sterilize media, instruments and lab wear

*Streaking:

We use this teqnique to produce a pure cultures of microorganisms why? characterize and identify microorganisms.

What is a pure culture? is a culture in which all microorganisms are descendants of the same organisms

to grow microorganisms we must have a sterile nutrient → medium to grow up microorganisms

↳ A sterile medium → free from all life forms and it's stabilized by heating into a temperature that kills all forms of microorganisms.

there are several ways to grow microorganisms:

When cultures need to kept for small period (days/weeks)
↳ we use to a solid medium to grow them which contains AGAR → a complex of polysaccaride

AGAR has many important properties:

A) Some microorganisms can degregade agar → so solid medium is an inert substance that keeps it solid.

B) it liquifies at 100°C or when temperature drops about 40°C. → so it can be incubated.

the agar can be poured in a petri dish → shallow dishes with lid → it need to be set on the bottom to prevent contamination

we can also pour agar in test tubes in 2 ways:

A) Slant tubes: contains nutrient medium + agar → and it solidfies at an angle → Flat surface

B) Stab tubes: tubes of hardened agar → inoculated by stabing the inoculum into the agar.

Broth tubes can be also used for culturing microorganism

↳ Are used to culture organisms for a long period → why? → Because microorganisms grow faster in a liquid media and can reach a considerable density.

↳ Broth contains typical nutrients, microbial growth substance, Sodium chloride and water

growth in the broth may be observed as one or combination of three forms:

1) Pellicle: Organisms floting on top
2) Turbidity: Cloudiness throughout the broth
3) Sediment: Organisms apear as deposit at the bottom

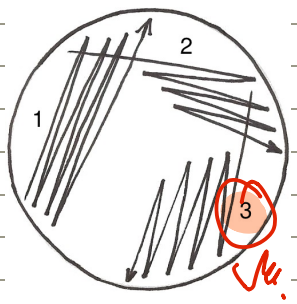
Agar plates and tubes are better to grow microorganisms because:

1) Solid culture is less prone to contamination in comparision to liquid cultures
2) they can be easily stacked and take up much less space than liquid medium.

After growth and after reaching density → growth needs to stop but without killing microorganisms.
↳ So we transfer cultures into a Refrigerator that slows down growth to almost zero.

What is inoculum? method to transfer growing microorganisms from pure culture to sterile medium without introducing unwanted contamination.
↳ the method is Aseptic teqnique → using an inoculating loop

Area number 3 must have pure cultures of the microorganisms



15 January 2022

Laboratory 2 : Enviromental plate for growth of bacteria

* Microorganisms are ubiquitous , Highly adaptive
+
Can live in extream enviroments
↙ ↓ ↘
Extreamly High temposture | High / Low PH | Salinity

→ Microorganisms are Found everywhere Surfaces, secretions of men and animals.
↳ Are heavily colonized with bacteria

* Also we can find microorganisms and bacteria in the Laboratory
+ We can find microorganisms in the air, settling with dust on most of surfaces

• Theory: all enviroments contain microorganisms, unless they are sterile enviroments. It is possible to identify at least some of them by simple techniques application.

• What is the aim of this experiment?
to investigate microbial growth from different environments , to analyse the presence of microbes in the air on the benches in the laboratory and other sources.

An example of collected Bacteria from surrounding Air


Can Be collected from Human's skin.

Human microflora

16th January 2022

Laboratory 3 : Simple staining, Gram staining and Bacteial shape

A) Smears preperation and Simple staining:

→ most common way to observe microorganisms is by Killing and staining them ✓
* Before staining microorganisms they must be fixed. Staining microorganisms with a dye allows us to visualize certain microbial structures and feature
↓
attached to a microscope Slide and Killing the microorganism too.

Staining Specimens helps in:
1) Increasing visibility of specimen.
2) Accentuates specifict morphological features.
3) Preserves specimens

. Fixation: Preserves internal and external structures and fix them in place.
So it usually kill them and firmly attach them to the slide.
Fixation is done in 2 ways: a) chemical fixation b) Heat fixation
Heat Fixation: used for Bacteria and Archea + preserves overall morphology but not internal structures.
Chemical Fixation: used for larger and more delicate organisms. Protects fine cellular substructure and morphology.

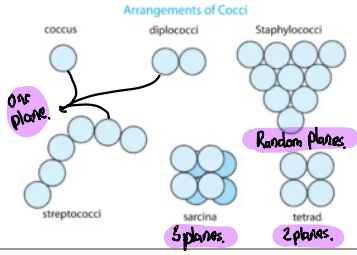
* Smears preperation:
Smears is a thin film of material containing microorganisms on the surface of the slide.
First if smear is taken from a solid medium you need to add a drop of water then adding the culture then we have to mix it with the water 2nd Smear is allowed to by dry by the surrounding air then it need to be fixed by passing it almost 3 times through the flame in the bunsen burner.
it's important that smear is completely dry why? Because the presence of water might lead to the formation of artefacts that may cause the cells to apear in strange shapes and sizes. + if the smear is not fixed properly it may wash off during staining

What are stains? Salts composed of negative and postive ions. → Chromophores.
a) Basic dyes: Are positively charged b) Acidic dyes: (chromophor) // Negatively charged. → Orange G, eosin
↳ crystal violet, methlyn blue, malachite gree, Safranin.
We use Basic dyes because Bacteria are negatively charged at ph 7 So it attract the colored postive ion in this dye. While acidic dyes are not attracted by most of bacteria Because dye has the negative ion which reples the negative charge of the Bacterium surface, So they stain background instead of the cell.

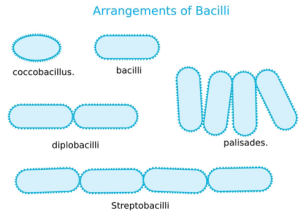
there are 3 types of staining: a) Simple staining b) differential staining c) special staining.

A) Simple staining: we use a single stain and it's used for determing size, shape and arrangment of bacteria.
↳ in an aqueous or alcohol.

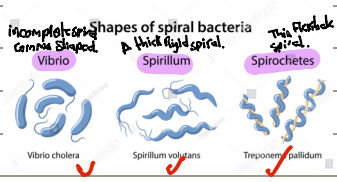
. Bacteial shapes: 1) Coccus // cocci: it's usually spherial / oval, most of them 0.5-1 mcm.



2) Bacillus (Rod shaped):



3) Spirillum: have 3 forms of shapes



B) Differential staining: React differently with different types of Bacteria and used to distinguish among them.

We have 2 types of differential staining:
A) Gram staining B) Acidic fast staining.

Gram staining differentiate between 2 main groups of Bacteria, gram positive bacteria and gram negative bacteria. Because each one reacts differently to the gram stain and thats because of the structural differences in their cell walls which affect the retention or escape one of the dyes that are used

. Gram positive Bacteria:
peptidoglycan
Plasma membrane

. Gram negative Bacteria:
outer membrane
Peptidoglycan
plasma membrane

.if the bacteria stains purple with gram staining → gram positive Bacteria.

.And if it staies Pink with gram staining → gram negative Bacteria.

* Simple Staining procedure:

1) Place the fixed slide on the staining rack and stain with one type of stains: methylene blue, Crystal violate, Safranin for almost 30 sec to 1 min. Dont alwo stain to dry out so you have to keep adding stain.
2) Wash off the stain gentaly using a constant flow of water.
3) Dry the slide with a paper towel
4) And finally you can examine slide under the microscope.

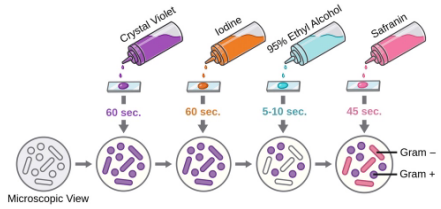
* Gram Staining procedure: we use Iodine, 95% ethanol, crystal violet and Safranin.

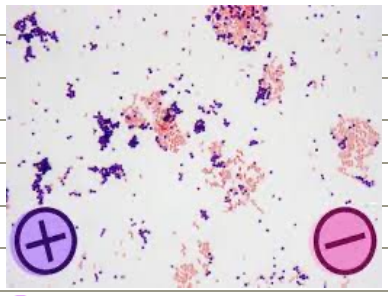
1) Add crystal violate stain to the fixed smear for about 1 min, and if it dry's out keep adding stain.
2) Rinse the stain with water.
3) Apply Iodine. For 1 min (it acts as a mordant and Fix the dye.)
4) Wash with 95% Ethanol (work as Decolorizer) use it until all Free color is washed "For 10 seconds Almost"
5) Rinse again with water
6) Apply Safranin (As a counter stain, Directly stains the bacteria that has been decolorized with Alcohol)
7) Rinse Again and dry it with a paper towel
8) And now your slide is Ready to be examined.

. So As a summary when adding crystal violet cells stain purple then after adding Iodine they Remain purple But After adding Alcohol color disappear if the cell is gram negative and stays purple if its gram positive And Finally Adding Safranin Keep gram positive Bacteria in a purple color while gram negative Strains with Pink

Why do the color of gram negative Bacteria disapear when adding Alcohol?

Because Alcohol disolves lipids found in the outer membrane of the bacteria, Allowing crystal violet- iodine complex to leak out of the thinner peptidoglycan layer. Gram postive bacteria has a thick layer of peptidoglycan. So stain is highly attached to the microorganism.





Pink color → gram negative

Purple color → gram positive.

Bacterial shapes and differentiation

Bacteria	Shape	-\+
2) P.mirabilis →	Rod shaped →	Negative
S.aureus	Cocci/round	Positive
M.lateus	Cocci/round	Positive
S.epidermidis	Cocci/round	Positive
E.faecalis	Cocci/round	Positive
1) E.coli →	Rod shaped →	
3) K.pneumonia →	Rod shaped →	Negative
4) P.aeruginosa →	Rod shaped →	Negative
5) B.subtitles →	Rod shaped →	Positive

17th January 2022

Laboratory 4 :

Endospore Staining and special Staining

* What are special stains? to color and isolate specific parts of the microorganisms (Endospore, Flagella and Revealing the presence of capsules)

We have 3 types of special staining:

A) Negative staining for Capsules: Some bacteria produce slimy and viscous covering → Capsule / Glycocalyx

the ability to produce such a structure is Inherited property of the Organism. (what does it composed of?

it's not an essential cellular component. it's composed of polysaccharide + Polypeptide

* Grow under specific growth conditions. + it helps Bacteria to survive in nature.

+ Also it may help pathogenic and normal flora Bacteria to initially Resist phagocytosis

+ In Soil and water capsule Prevent Bacteria from being engulfed (preventing fusion of the lysosome with phagosome) by Protozoans + it also may help Bacteria to adhere to surfaces → Resist flushing ✓

↝ Capsules staining is one of the most Difficult type of staining Because it's Soluble in water and capsules are non ionic → Do not accept most Biological dyes. So we use negative staining For them. ✓

We can use (Nigrosin) → Acidic stain.

B) Flagella Staining: Structures used by Bacteria to move in their environment

Flagella become visible when stained with Carbolfuchsin

C) Endospore Staining: Special differentiated state of the Bacteria → Allows them to resist adverse environmental conditions (such as lack of water OR change in temperature)

. Endospore are tough structure which cannot be penetrated easily.

* the most commonly used stain for endospore → Schaeffer-Fulton

Endospore is dormant structure and it's Resistant to harsh Conditions (Heat, Radiation, Chemicals, desiccation)

* Endospore is covered with a Layer which called Exosporium + it is surrounded by A coat (made of thick layers of protein)

+ it has the Cortex + which is beneath the Coat, thick peptidoglycan + Core which has nucleoid and Ribosomes

What makes Endospore so Resistant? A) Calcium (complexed with dipicolinic Acid).

B) Small, Acid Soluble, DNA-binding Protein.

C) Dehydrated core D) Spore Coat + Exosporium → protects it

How does it Form? with a process called sporulation. and it start Forming Because the lack of nutrient.

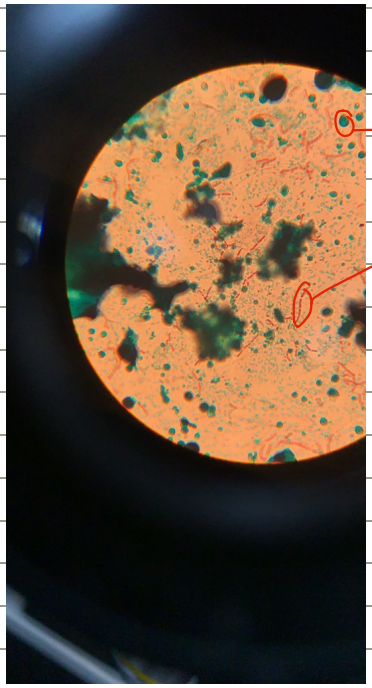
* Formation of Vegitative cell? it starts forming when caused to heat → Activation

↳ Process is called Germination → when nutrients are detected + Absorption of spore coat.

Outgrowth → Emergence of vegitative cell + increased metabolic Activity

* Endospore Staining Procedure:

1) Prepare A smear, Air dry + Heat Fixed.
2) Apply Malachite green
3) Place the Slide over a Beaker with Boiling water And Keep adding stain so it wont Dry (to open tough layers of endospore So stain can get Attached to it)
4) Cooling. + wash with water
5) Apply Safranine → Stains cells Rather than endospores (Vegitative cells).
6) Rinse with water and dry it with paper towel
7) Examine it with Oil immersion lense to view.



18th January 2022

Laboratory 5 : Media preperation and evolution

*What Are Culture media? Nutrient solutions used to grow Microorganisms

*we have 2 classes of culture media: 1) Defined media 2) Undefined Media

- Defined media: Chemically Known and prepared by precise amount of Known chemical substance to distilled water.
  - In simple defined medium → Single source of carbon.
- Undefined / complex media: Media employ digest from animal or plants products
  - Casein → from milk / Yeast cells / Beef / Soyabeans.
  - They are available as powder which can be dissolved in water // UnKnown Exact composition.

If the complex media is Solid → Agar Liquid → Broth

Culture media Are:

- **Selective**: Inhibit the growth of some Microorganisms but not others
- **Enriched**: Medium that allows the growth of Fastidious microorganisms
  - microorganisms that have very specifed Requirment to their growth and cannot be grown on general purpose media.
  - Such as Neisseria gonorrhoeae → grow on medium containing Heat-lysed blood → Heated blood interacts with media Components that are toxic for the organisms and allow for growth that would be otherwise inhibited.
- **Differential**: to differentiation of particular chemical Reaction occuring during growth (Ability to ferment lactose)

*Culture media can be:

- Supportive → Support the growth of many microorganisms

Agar > 13g → Solid Agar < 13 → Semisolid 0 Agar → Liquid.

For this experiment we've used the following tests:

1) Manitol Salt Agar (MSA): Selective + differential

- Selective: Because it contains high concentration of salt → inhibitory for the growth of most of the Bacteria.
- Differential: Because it contains carbohydrate mannitol as a source of carbon which can be fermented by Special type of Bacteria Releasing Acidic products → Phenol Red is the ph indicator.

→ Microorganisms that can grow on high Salt Conc. can survive + ones which can ferment manitol will have Yellow Halo.

test Results:

S. aureus → Postive for growth (Halotolerant)
  - Postive for the fermentation of mannitol.

S. epidermidis → Postive for growth (Halotolerant)
  - Negative for the fermentation of mannitol.

E. coli → Non halotolerant. Thus we can't Know wether it ferments mannitol or not.

2) Blood Agar: Enriched + Differential.

Supports the growth of many fastidious Bacteria → Can be differentiated based on specifed haemolytic propertie

a) Beta Hemolysis: Strains of streptococci which can produce Streptolysin O/S → Can lyse Red blood cells completley So it appears as clear zone → Because of the complete destruction of RBC and the use of Hemoglobin.

b) Alpha Hemolysis: Strains that produce alpha-haemolysin → Cause the reduction of hemoglobin to methemoglobin that change the color around the agar to greenish / Brownish Areas.

c) Gamma Hemolysis: no lysing For RBCs / no leakage / no change in color.

test Results:

S. aureus → β Hemolysis
B. subtilis → α Hemolysis
E. Faecalis → Gamma Hemolysis

3) MacConkey Agar: Selective + differential

- Selective: Inhibits the growth of Gram-positive Bacteria it contains Crystal violet and Bile salts.
- Differential: Because it contains Lactose that only some microorganisms ferments it. (Ph indicator turns Red)

test Results:

K. pneumonia → Positive for growth (Gram negative Bacteria)
  - ✓ positive for fermentation of lactose. which turns the color into Red.

"gram positive Bacteria is inhibited Because Crystal violet dye and bile salt halt the growth of gram positive Bacteria."

S. aureus → Gram positive Bacteria (Inhibited)

P. mirabilis → Gram negative Bacteria / No Fermentation.

4) Eosin Methylene Blue (EMB) Agar: Selective + differential

Inhibits the growth of gram positive and Selective for gram negative Bacteria. Because of the presence of 2 dyes Eosin Y + Methylene blue. And also it can differentiate between bacteria which can ferment lactose.

test Results:

a) K. pneumonia → Gram negative Bacteria + Positive for the fermentation of lactose.
b) S. aureus → Gram positive Bacteria // No growth.
c) P. mirabilis → Gram negative Bacteria + negative for fermentation.
d) E. coli → Gram negative Bacteria + positive for the fermentation.

20th January 2022

Laboratory 6 : Bacterial population Count.

* Microbiologist often has to determine the number of Bacteria given in a sample.

- What is the theory? A) Plate count B) Turbidometry procedure C) Direct cell count with Counting chamber

* Another reason for this experiment is to Compare the amount of Bacterial growth under various Conditions

, Enumeration of Microorganisms is important For Diary, Food and water Microbiology

A) Plate Count (Viable count).

- Usually number of Bacteria given in a sample is too great to be counted directly.

(, But if the sample is serially diluted and the plated on an agar → So single isolated Bacteria Form visible isolated colony

the number of colonies can be used as a measure of the number of the living cell in the known dilution.

* Some organisms Form multiple cell arrangement: Such as Chain

Count

Laboratory 8 → Chemical effects on Bacterial Growth:

1) **Sterilization:** Destroying all living organisms + viruses → A sterile object is free of all life forms.

2) **Disinfection:** Elimination of microorganisms from inanimate objects & surfaces