AVIT
AARUPADAI VEEDU INSTITUTE OF TECHNOLOGY
VINAYAKA MISSION'S RESEARCH FOUNDATION
(Deemed to be University under section 3 of the UGC Act 1956)
Accredited by NAAC
Approved by AICTE

DEPARTMENT OF BIOTECHNOLOGY

B.TECH
MICROBIOLOGY LABORATORY
(17BTCC83)

HOD/BIOTECH

LIST OF EXPERIMENTS

S.No	List of Experiments
1	**Principles Of Microscopy**
2	**Sterilization**
3	**Preparation Of Culture Media For Micro Organisms**
4	**Preparation Of Agar Slants And Agar Plates**
5	**Pure Culture Techniques**
6	**Simple Staining**
7	**Gram's Staining**
8	**Enumeration Of Bacteria From Soil**
9	**Environmental Sample Analysis**
10	**Analysis of Milk Sample**
11	***In vitro* Determination Of Anti Microbial Sensitivity**
12	**Bacterial Growth Curve**
13	**Quantitation Of Microbes**
14	**Effect Of Different pH on Bacterial Growth**
15	**Biochemical Test-Indole production test**
16	**Urease test**

1. PRINCIPLES OF MICROSCOPY

Exp:
Date:

Microscopy is the method used to view, identify and study the microorganisms.

Terms Involved:

Resolution: Ability of an instrument to optically separate two very close objects so that they can be distinguished individually and not as a single object. It is numerically equal to the ratio of wavelength of light used to the numerical aperture of the objective and condenser.

***Numerical Aperture*:** Measure of the amount of light that can be gathered by the objective and is a function of the diffraction pattern formed in the eyepiece.

Types:

- Bright field microscopy
- Dark field microscopy
- Phase contrast microscopy
- Fluorescence microscopy
- Interference microscopy

Bright field microscopy is the most widely used optical instrument and requires least amount of training to use.

Useful magnification: 100X magnification produces a fuzzy image.

Parts of the microscope:

***Objective lens* :** Commonly used objective lens: 10x, 40X,100X working distance is the space between the objective and the specimen and it decrease with the focal length.

***Quis Diaphragm*:** It is used to control light cone entering the objective. Size of light cone passing into the microscope differs with the objective. The working distance with the iris diaphragm is the shortest with oil immersion and iris diaphragm is opened move.

High resolution requires that parallel ways of light enter the condenser and the condenser be focused on the object. Here natural light is used and the condenser is focused on the edges of the lamp housing. A variable transformer controls light intensity.

Using the compound microscope:

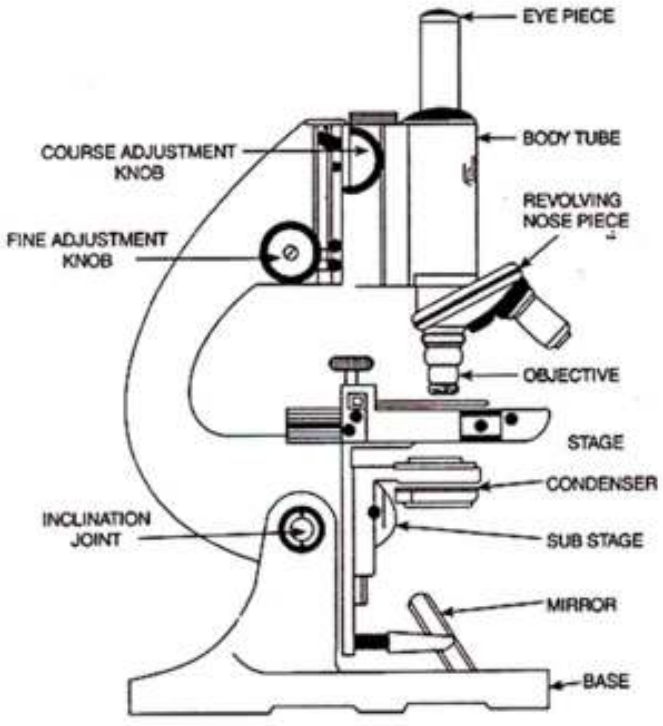
- Place a drop of sample on the slide and keep on the stage for viewing at the centre.
- The light source is adjusted until sufficient intensity is obtained.
- With low power objective in position lower the lens using the adjustment until about ¾ th of an inch.
- Look through the eyepiece and slowly raise the objective with coarse adjustment until the specimen is in appropriate focus. Bring specimen into sharp focus with fine adjustment.
- Raise the sub stage condenser all the way up and open the diaphragm until the edges just disappear from view.
- After the exact field of view is set with low power objective, the nosepiece is rotated to bring in the high power objective, which then clicks into place as the object is brought into sharp focus.

Focusing the oil immersion lens:

- First the low power objective is used to locate the portion of the specimen to be examined.
- Raise the body of the tube and rotate the nosepiece until oil immersion objective clicks into place.
- Place a drop of immersion oil on the portion of the slide to be viewed. Carefully lower the objective into oil.
- Without letting the objective touch the slide, look through the ocular and upward with the fine adjustment to get a sharp focus. Adjust the light intensity and iris diaphragm to obtain optimum illumination.

***Precaution while handling the microscope*:**

- Lens should not be touched. If lens are dirt, wipe off with tissue paper gently. Never leave a slide on the microscope when not in use.
- Remove all immersion oil from objective after use. Keep the stage of the microscope clean and dry.
- Don't tilt the microscope while working on oil immersion system.



2. STERILIZATION

Exp:
Date:

Aim:

To sterilize the materials used for culturing microorganisms

Background:

It is the process to destroy all micro organisms without damaging or altering the substance being sterilized.

***Types*:**

***Dry heat*:**

Dry heat done using hot air ovens used to sterilize glassware, metal instruments and the materials that cannot be penetrated by steam and it was done at 180^{o} C for 2-3 hrs.

Moist heat:

Moist heat is done using autoclaves Steam is bactericidal and kills microbes by denaturing proteins present in the cell wall. Moist heat is used in sterilization of culture media and other liquids used.

Gaseous chemicals:

Highly effective for sterilization

Filter sterilization:

This type of sterilization used to sterilize solutions that are thermo labile. Function by entrapping micro organisms within the porous structure of the filter matrix. Ex: antibiotics, sugars, amino acids, vitamins.

Radiation:

Working area routinely sterilized using UV radiation and these lamps are called UV lamps, which emit radiation chiefly at wavelength at 2537 A.

Chemical disinfectants:

They are used to kill vegetative bacteria, fungi, viruses and occasionally spores. Example: phenolic compounds and hypo chlorides.

Procedure:

- Keep the glassware in the inoculation chamber and switch on UV light and let it glow for 30 minutes.
- Switch off the UV lamp.
- Check the sterility of working area by swabbing with sterile cotton plugs. The plates were filled with suitable agar and inoculated with swabs.
- Plates were incubated at 37^{o}C.

Observation:

3. PREPARATION OF CULTURE MEDIA FOR MICRO ORGANISMS (liquid media)

Exp:
Date:

Aim:

To learn the principles, and techniques of preparing nutrient broth for the microbial culture.

Background:

A common medium (Nutrient broth) used for growing bacteria (non fastidious organism). It contains beef extract, peptone and NaCl as ingredients. This liquid medium is nutrient broth and can be supplemented with other substances like sugars, and other inorganic salts to meet the requirements of any particular organisms. These media are always kept sterile until they are used.

Requirements:

Beef extract, peptone, NaCl, agar, conical flask, cotton wool, measuring cylinder, pH meter etc.

Composition of Nutrient broth:

Beef extract	-	1g
Peptone	-	1g
NaCl	-	0.5 g
Distilled water	-	100 ml

Procedure:

- Hundred milliliter of distilled water is taken in a conical flask.
- Weigh the above ingredients and dissolve in distilled water.
- The pH of the medium is adjusted to 7.2 with the help of 0.1N sodium hydroxide.
- Distribute equal quantity of the nutrient broth in test tubes and plug the tubes with cotton.
- Sterilize the medium at 121^{o}C for 15 minutes in an autoclave.
- After 15 minutes remove the test tube and keep in a clean place.

Observation:

4. PREPARION OF AGAR SLANTS AND AGAR PLATES

Exp:
Date:

Aim:

To prepare the solid agar medium for culturing the micro organisms.

Materials required:

Test tubes, Petri plates, measuring cylinder, pH meter, auto clave etc

Nutrient agar composition:

Beef extract	- 1 g
Peptone	- 1 g
NaCl	- 0.5 g
Agar	- 1.5 g
Distilled water	- 100 ml

Procedure:

Agar slant preparation:

Allow the test tube containing the agar medium to cool down to about 45 – 50^{o} C and keep them in a slanting position by resting the plugged end over the glass rod on the table and leave the tubes in this position till the medium has cooled to room temperature and solidified.

***Agar plate preparation*:**

- Label the Petri dishes and organize them in inoculation chamber. Grasp the Erlenmeyer flask containing sterile agar medium.
- Turn your left hand palm side up and clamp the cotton plug between two fingers. Remove the plug and flasks the mouth of the Erlenmeyer flask.
- Use the hand holding cotton plug to lift the lid of the Petri dish. Now pour about 20 - 25 ml of sterilized nutrient agar medium.
- Hold the lid so that it partially covers the bottom of the dish as you pour. This help to prevent microbes and air born dust dropping into your sterile plate and contaminating it.
- Immediately replace the lid, flame the mouth of the flask and pour the next plate in the same way.
- If air bubbles occur on the surface of the medium, break aseptically by quickly passing the Bunsen flame over the media surface.
- Allow the plates to cool at room-temperature and store the plates in sterile place.

Observation:

PROCEDURE:

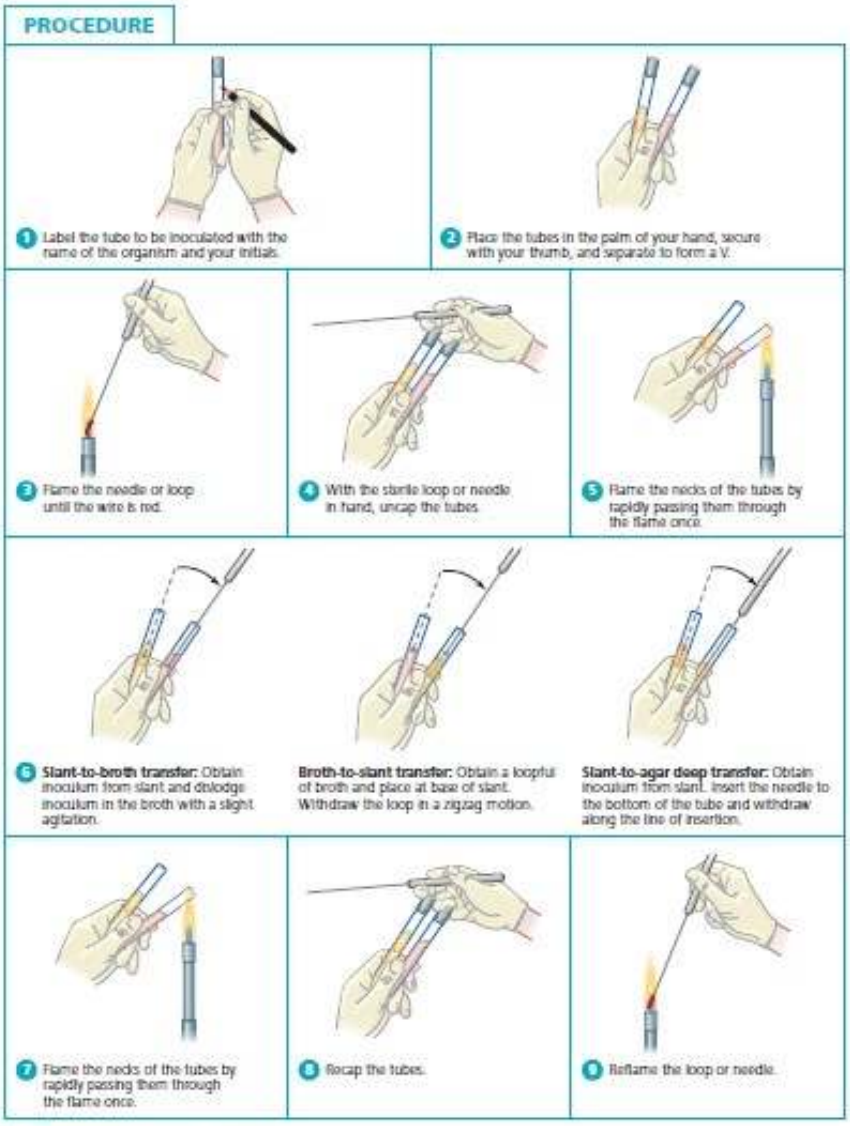


Figure 2.1 **Subculturing procedure**

PROCEDURE:

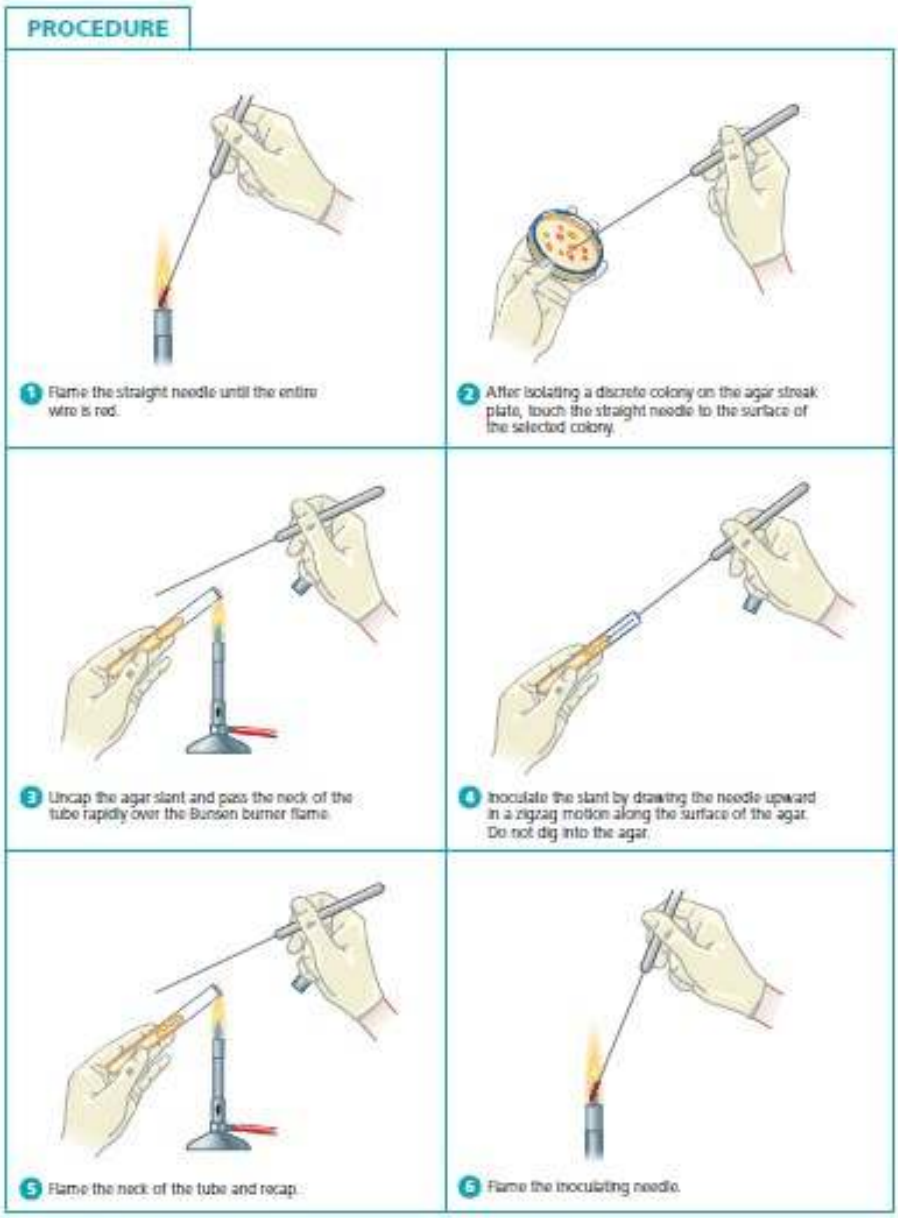


Figure 3.4 **Procedure for the preparation of a pure culture**

5. PURE CULTURE TECHNIQUES

Exp:

Date:

Aim :

To learn the pure culture techniques, and isolate pure culture of bacteria.

Back ground:

Micro organisms are ubiquitous in nature so it is necessary to isolate and culturing a pure culture of microorganisms in order to study the properties of a particular microorganisms.

Pure culture represent a population of organisms of a single species in the absence of living cells of any other species in this there are various techniques, where by the different species in a natural population can be isolated and grown as pure culture. They are

a) Streak plate culture
b) Pour plate culture
c) Spread plate culture

***Materials required*:**

Petri plates, test tubes, ' L' shaped glass rod, Inoculation loop, 70% ethanol, nutrient broth, pipettes etc.

a. ***Streak plate culture*:**

- Pour the autoclaved nutrient agar to the Petri plates under sterile condtions.
- Sterilize the inoculation needle, by flaming in red-hot and allow it to cool for 10 seconds.
- By holding the culture tube in your left hand near flame, remove cotton plug with your right hand and flame sterilize the mouth of the tube for few seconds.
- Touch the culture with the inoculation loop and take the culture.
- Plate it on agar plate and streak it on the plate once resterllize the loop and cool it as above and take the culture from on end of the plate to other end complete the streak as it.

***B) Pour plate technique*:**

Liquefy the nutrient agar in the tubes by heating them in water bath cool the tubes to 45^{o}C and hold at this temperature until ready to pour into the plate.

Label the tubes and corresponding Petri dishes serially dilute the given mixture and from each dilution take 0.1ml of the sample.

Mix the sample with agar medium by gently rotating the tube between your plates. Pour the contents of the tubes into the corresponding labeled Petri dishes and allow solidifying and incubating the plates in inverted position at room temperature.

C). Spread plate technique:

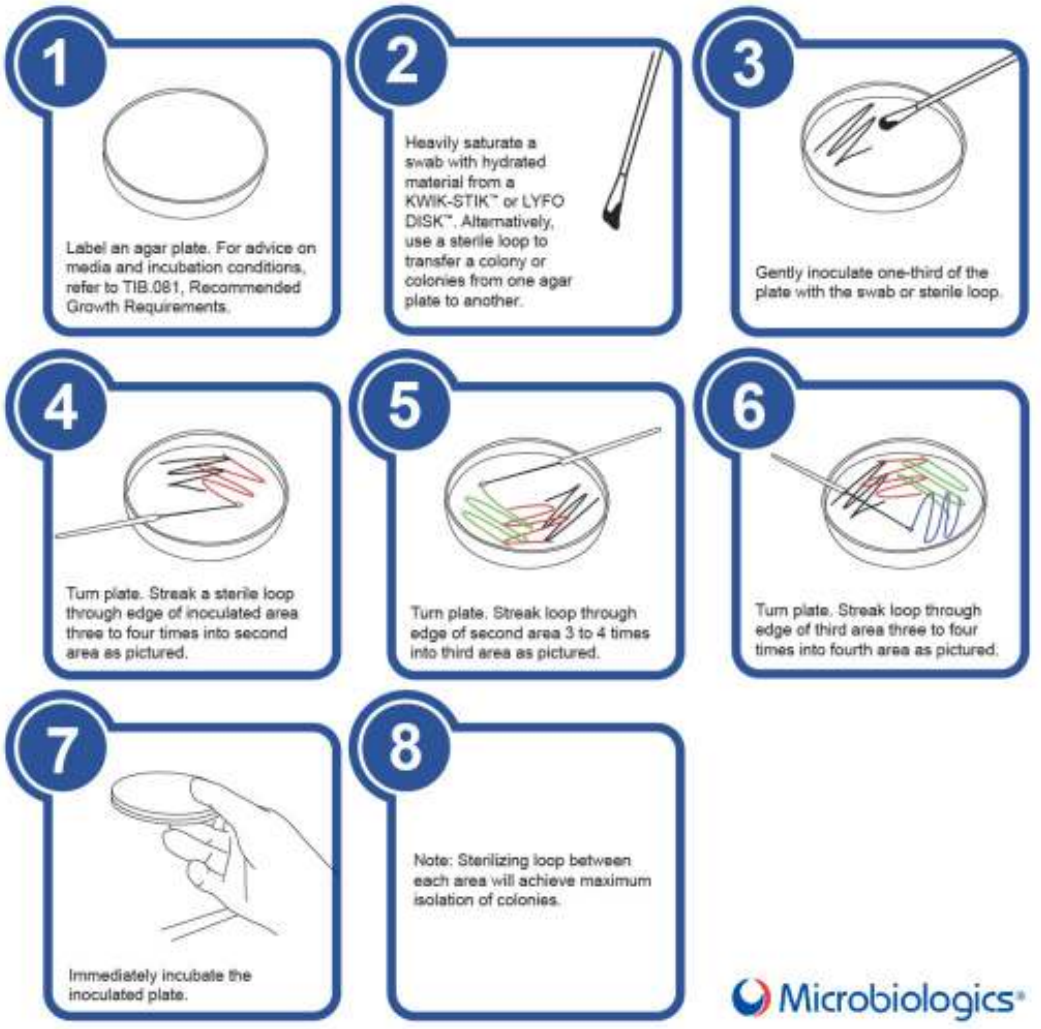
Use the nutrient agar plates as prepared earlier serially dilute the given culture and label the nutrient agar plates corresponding to the dilution.

Take 0.1ml from each dilution and pour over the surface of nutrient agar plates. Sterilize the 'L'- rod with alcohol and then by flaming it. Cool the rod and place the rod gently on the surface of the agar. Spread the sample over the surface of the agar medium by rotating the Petri plate manually or by placing on the rotating plate disc.

Incubate the plate in inverted position at the room temperature.

Observation:

PROCEDURE:



6. SIMPLE STAINING

Exp:
Date:

Aim:

To learn the staining technique, for studying the morphology of microorganisms.

Principle:

Staining is the method of artificially producing colors in microbes to allow for the visualization under the microscope. Stains are employed not only to make the organisms visual but also understand their structure and chemical nature.

Dyes or stains are synthetic chemicals products of aniline type. They can be divided into acid dyes and basic dyes with respect to their color properly in the anionic acid cationic form. Acid dyes have shorter tendency to combine with the cytoplasm but basic dyes have greater affinity to the nuclear region of the cells. Basic dyes are generally used in bacteriological studies.

Simple stain contain only one dye may allow visualization of morphological characteristics of bacterial cells.

***Materials required*:**

24 hrs old bacterial culture, stain, clean glass slide, cover slips, microscope, Methylene blue, Carbol fuchsin, Crystal violet, Saffranin, Malachite green etc.

Procedure:

Clean the slides in any detergent solution. Rinse with tap water and wipe the slides. Dry with a clean cloth or tissue paper. There after handle the slides only by grasping them at the edges.

Flame the inoculation loop and the mouth of the culture tube containing microbial culture. Allow the loop to cool.

Pick up a loopful of bacterial colonies from the agar surface.

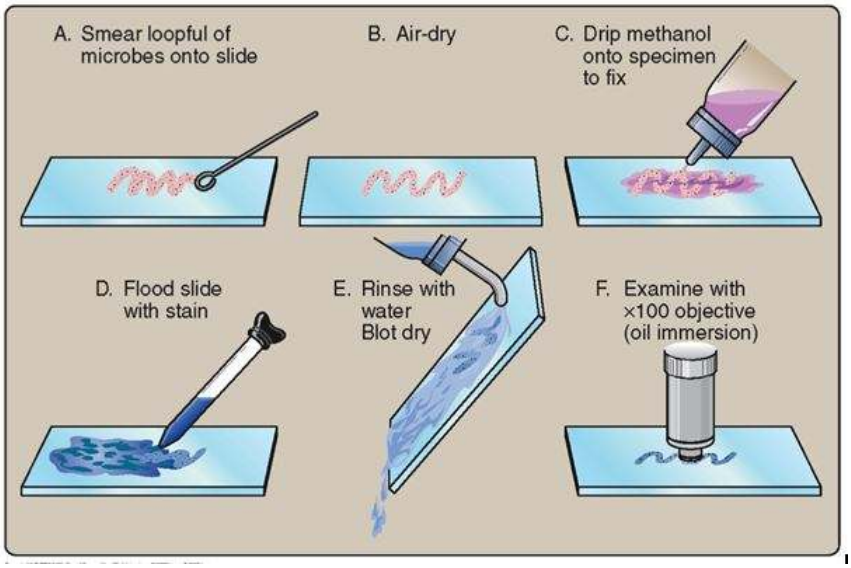
Transfer the loopful of suspension on to a clean slide and spread it onto a small area and air dry, thus making a smear.

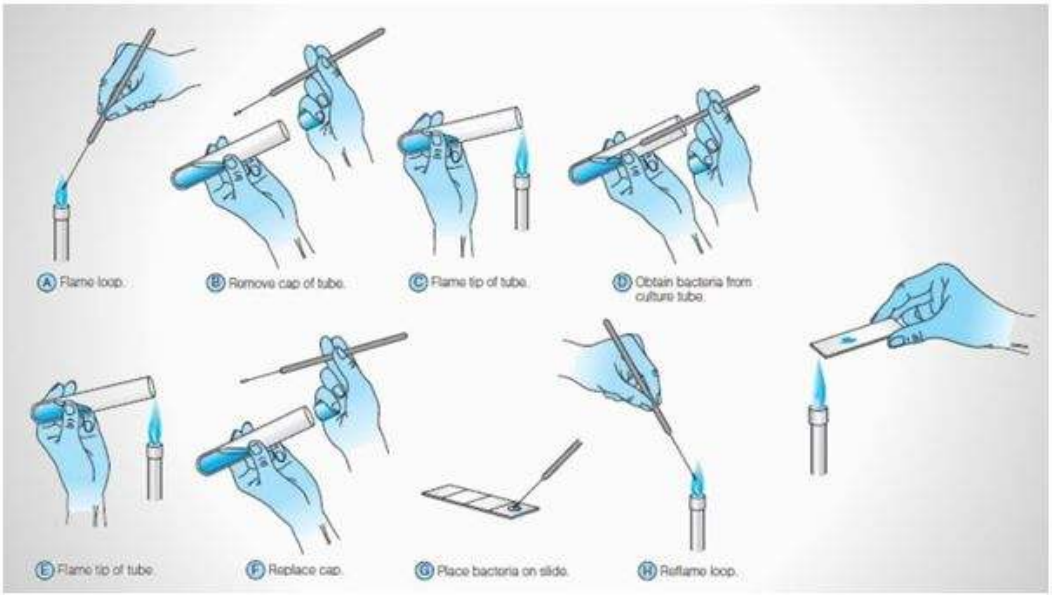
Heat fixes the smear by passing the slide through the Bunsen flame two to three times so that the heat is just tolerable to the skin of the arm.

Flood the smear with any one of the dyes mentioned above for about 3 minutes. Wash the slide in a gentle steam of tap water to remove excess stain air dry. Examine the preparation with the oil immersion objective.

***Observation* :**

PROCEDURE:





7. GRAM'S STAINING

Exp:
Date:

Aim:

To study and identify whether the given bacterial culture is gram positive of gram negative.

Principle:

Gram's stain was introduced in 1880 by the Danish bacteriologist Christian Gram. Gram's staining is employed to visualize and differentiate between organisms. It is useful of presumptive identification of organisms before carry out the other serological and biochemical test.

Bacteria can be classified into two classes: gram positive and gram negative. The bacteria, which retain the crystal violet, are termed as gram positive and the bacteria which lose the crystal violet are termed as gram negative.

The cell walls of negative bacteria are thinner and has high liquid content during the alcohol treatment it extracts the lipid this increases the porosity of cell. So the gram negative bacteria are decolorized and take up the color of the counter stain.

Iodine is used as a mordant, saffranin is used as a counter stain.

Materials required:

Crystal violet, gram's iodine, ethanol and saffranin, glass rod, hot plate, glass slides, 24 hrs old culture, microscope, distilled water etc.

Procedure:

Prepare a bacterial smear on the slide with a given bacterial culture as performed earlier. This smear is first treated with a basic dye crystal violet and allows it to stand for one or two minutes and then wash the slide with sterile water.

After washing, in second step, the smear is treated with iodine is added after waiting for 1 minute the slide is again washed with distilled water.

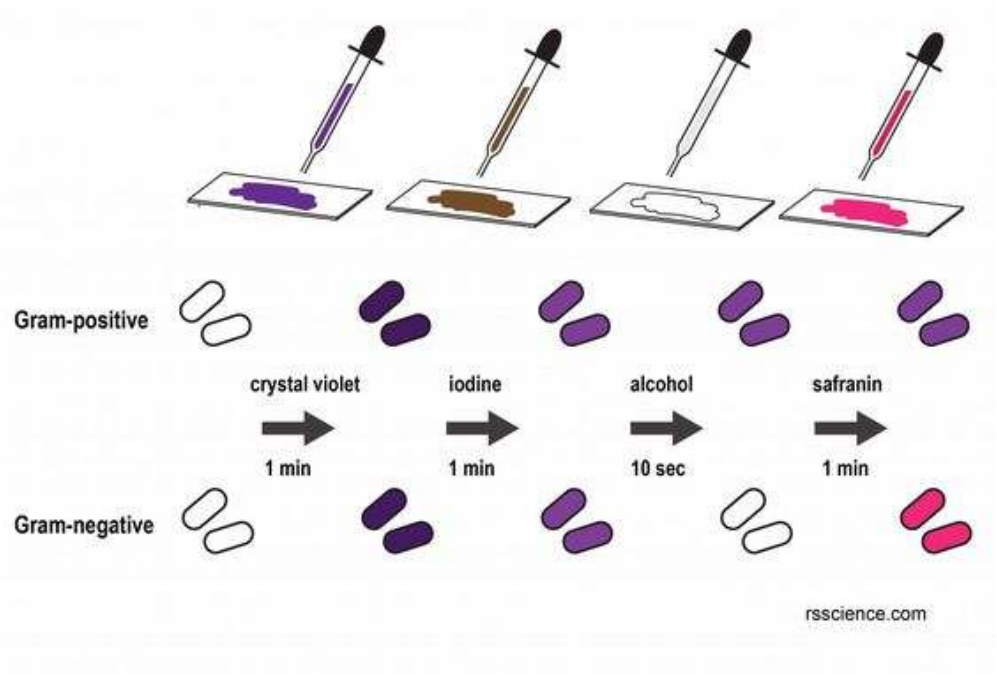
After treating with iodine solution decolorize with alcohol. Alcohol is added drop by drop on slide kept in staining position till all blue color was removed; wash it with distilled water and air dry.

Finally saffranin (Counter stain) was added and after waiting for 1 minute the slide is then wash with distilled water. Then slide is allowed to dry.

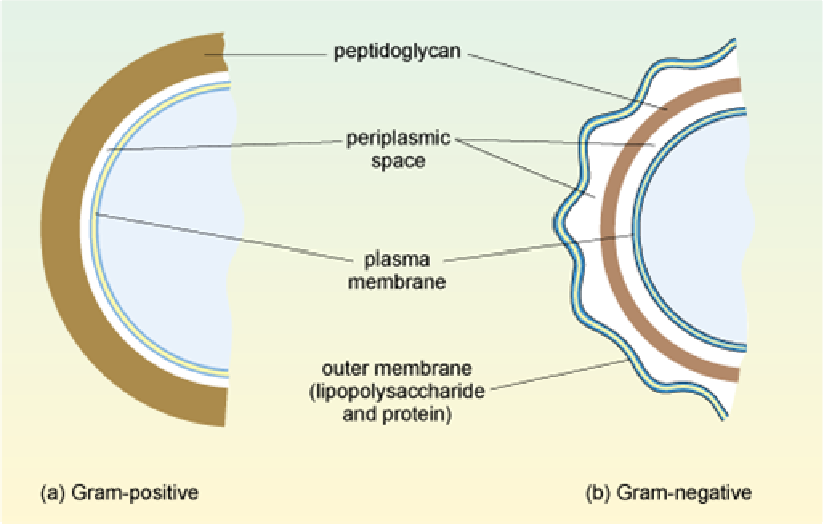
After completion of all four steps, the slide is examined that the stained smear in oil immersion objective were observed and determines whether the organism is gram positive of gram negative.

Observation:

PROCEDURE:



GRAME +VE AND GRAME –VE CELL



8. ENUMERATION OF BACTERIA FROM SOIL

Exp:

Date:

Aim:

To enumerate the number of microorganisms present in the soil sample.

Principle:

Soil contains all source of microorganism (Bacteria, fungi, algae, protozoa). The amount the microorganism present in the soil can be quantified by serial dilution technique.

***Materials required*:**

Petri plates, test tubes, sterile tip box, 'L' rod, pipette, distilled water, conical flask, autoclave, nutrient agar, NaCl etc.

Procedure:

The analysis of soil sample involves the following steps:

Sample collection
Sample preparation (serial dilution)
Media preparation
Plating of sample in nutrient agar plate

Sample collection:

The soil sample was collected from the environment from which 1g was taken and suspended in 10 ml of sterile dis.water. It was allowed to stand on the worktable for 5 minutes. This is considered as 10^{-1} dilution.

Sample preparation:

Sample preparation was done using serial dilution technique. Sterile test tubes were arranged in a test tube stand which was cotton plugged and the test tube were labeled as 10^{-2},10^{-4},10^{-6} and 10^{-7}.

Nine ml of saline solution was distributed asepectically to each tube.

One ml of sample was taken from 10^{-1} dilution and transferred to second test tube and mixed well.

Take 1ml of sample from the 10^{-2} dilution test tube and transfer to next tube vice versa.

This kind of serial dilution from one test tube to next was repeated till the last tube. Finally, take 1ml of sample from the last test tube and discard the sample. Thus the sample dilution was prepared by serial dilution technique.

Media preparation

For bacterial enumeration nutrient agar was prepared in conical flask. Autoclave the nutrient agar containing flasks and Petri plates simultaneously.

After sterilization the media can be poured on the Petri plate and allow solidifying the media.

Plating of sample:

Pour plate technique:

0.1 ml of the diluted sample was transferred to the boiling tubes contain 20 ml of nutrient agar.(The sample should be add immediately after autoclaving around 37^{o}C and then the sample is transferred to the tubes).

The inoculated media transfer to the sterile Petri plates and allow solidifying.

After the media is solidified the plate are kept inverted position in incubator at 37^{o}C.

Spread plate technique:

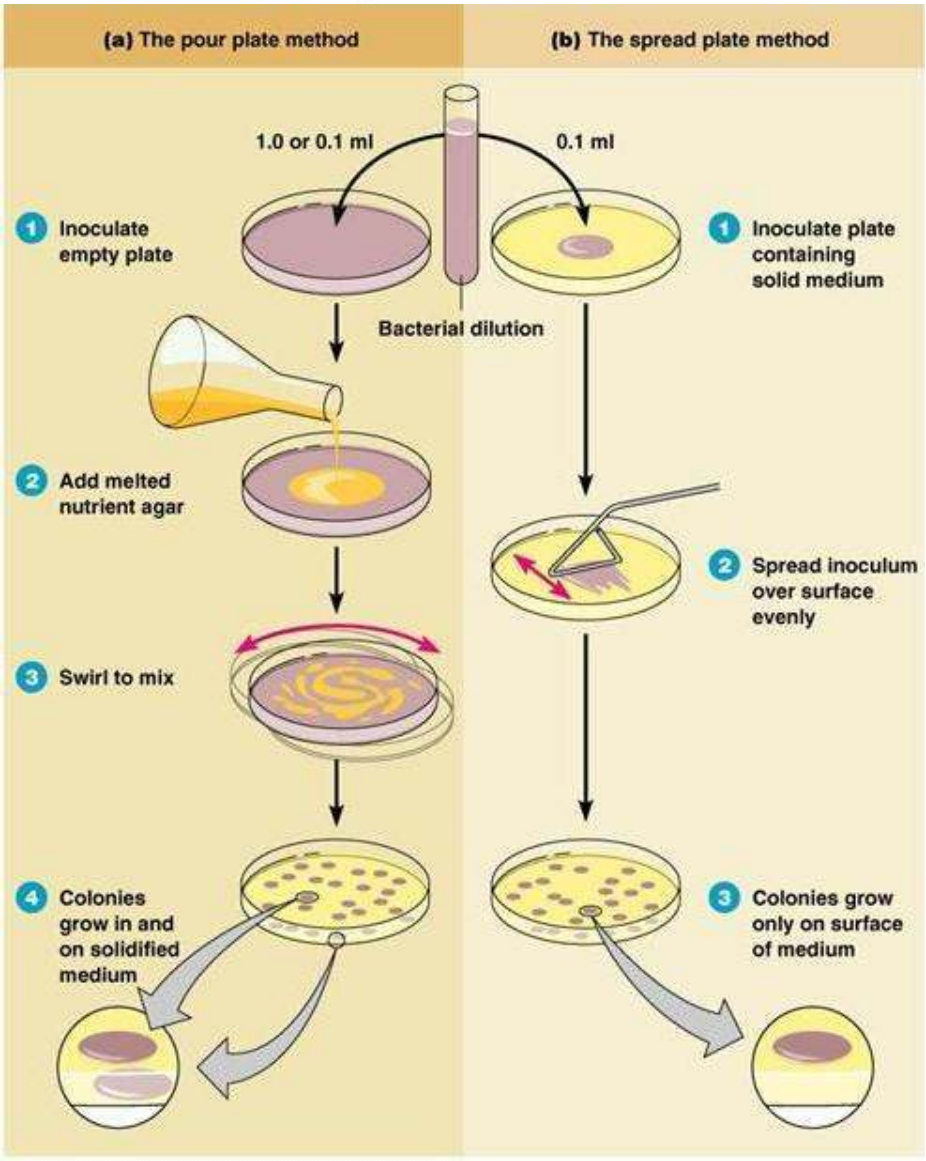
The prepared nutrient agar containing plates was taken and kept in inoculation chamber.

Take 0.1 ml of sample from diluted tube (using sterile tip) and pour over the surface of the nutrient agar.

Dip the 'L' rod in alcohol and flaming in Bunsen burner and allowed to cool for few seconds.

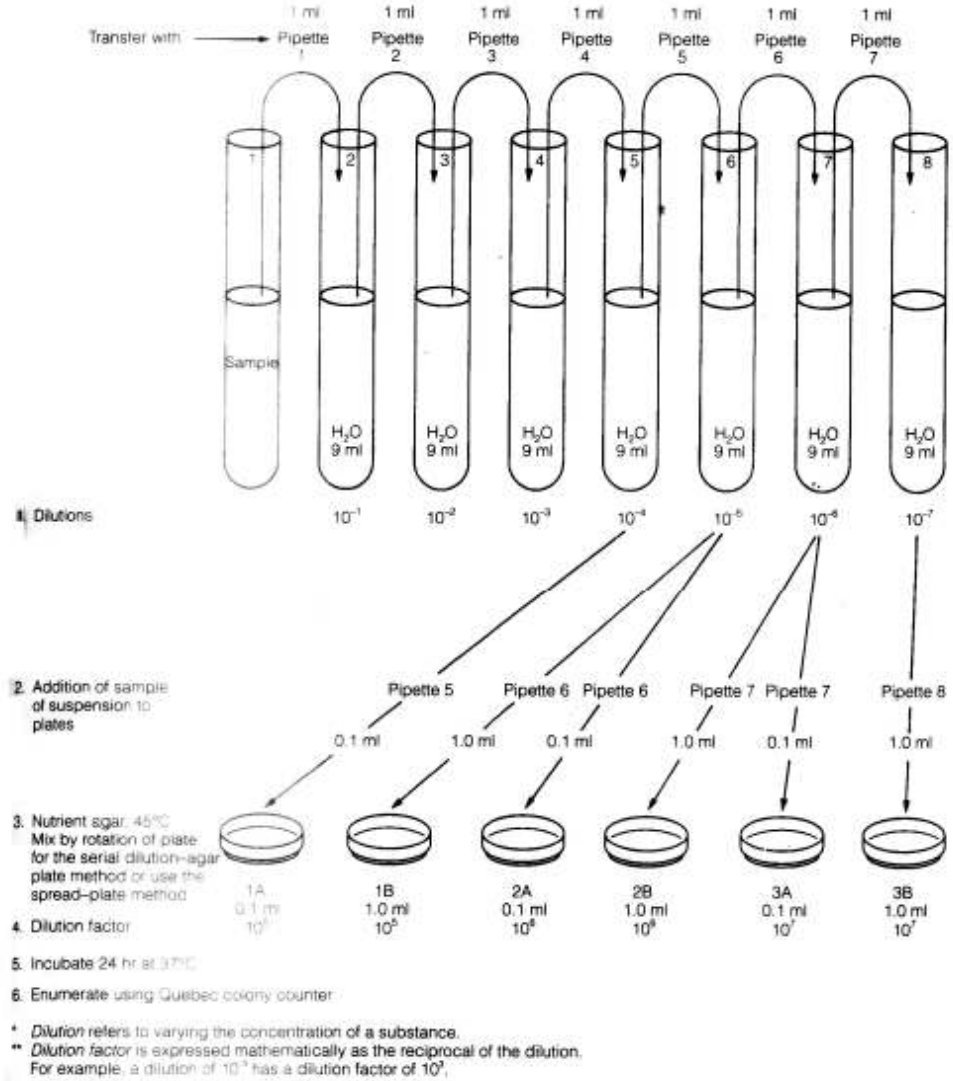
By using this sterile "L" rod evenly spread the sample over the surface of the agar medium. Then the plates allowed settling for few minutes and incubating in the incubator at 37^{o}C.

Observation:



Copyright © 2007 Pearson Education, Inc., publishing as Benjamin Cummings.

SERIAL DILUTION TECHNIQUE



* *Dilution* refers to varying the concentration of a substance.
** *Dilution factor* is expressed mathematically as the reciprocal of the dilution. For example, a dilution of 10^{-3} has a dilution factor of 10^{3}.

9. ENVIRONMENTAL SAMPLE ANALYSIS
ANALYSIS OF WATER SAMPLES

Exp:
Date:

Aim:

To analyze the water samples for the presence of various microorganisms.

Principle:

Qualitative study of the micro organism present in various water samples.

***Materials required*:**

Sterile conical flask, water samples (mineral water, municipal water), Petri plates, inoculation loop, Nutrient agar etc

Procedure:

Water samples from various sources were collected. The composite samples are further taken for analysis.

Take 0.1ml of sample from the conical flask and inoculated in nutrient agar plates and following spread plate technique.

Inoculated plates were incubated at 37^{o}C for 48 hrs.
After 48hrs results were observed.

Observation:

10. ANALYSIS OF MILK SAMPLE

Exp:
Date:

Aim:

To examine the presence of microbes in the milk samples

Background:

Milk is made up of carbohydrates, proteins, fats, vitamins, minerals and water, which support the growth of microorganisms. Milk contains microorganism at the time it is drawn from the cow and it may be further contaminated in subsequent handling and processing.

Materials required:

Raw milk, pasteurized milk, nutrient agar, pipettes, test tubes, sterile screw cap tubes etc

Procedure:

Direct microscopic counting method:

Spread a drop of milk sample onto an area of 1cm^2 on a glass slide and dry gently with heat.

Remove the lipids by dipping the slide in xylene for 2 minutes.

Fix the slide by dipping in isopropyl alcohol for 2 minutes.

Stain for 2 minutes with methylene blue and wash with isopropyl alcohol until it becomes pale blue color.

Dry the slide and count the bacterial cells in each microscopic field.

Plate counting method:

Dilute the milk sample by serial dilution technique.
Pipette 0.1ml of each dilution into labeled nutrient agar plates.
Incubate the inoculated plates for 48 hrs at 37^{o}C. And results were observed.

Observation:

11. *INVITRO* DETEREMINATION OF ANTI MICROBIAL SENSITIVITY(AGAR DIFFUSION METHOD)

Exp:
Date:

Aim:

To determine the antimicrobial agents against various micro organisms.

Principle:

Standard suspension of rapidly growing bacteria is inoculated on the surface of Muller Hinton agar plates. Filter paper discs containing specific concentration of antimicrobial agents are pressed on to the surface and incubated at 35^{o}C over night(18-24 hrs) zone of inhibition of growth around each disc is measured and the susceptibility determined.

Materials required:

Muller-Hinton agar, Bacterial strains, Discs containing anti microbial agents, sterile forceps, sterile buds etc.

Procedure:

Preparation of Muller- Hinton agar(MHA):

Weigh the ingredients, Prepare the medium in sterile conical flasks. Autoclave the medium containing flasks at 115^{o} C for 15 min and cool the medium in water bath at 45-50^{o} C.

Pour the plates up to a uniform thickness of approximately 4mm (25 ml /9 cm plates)

Allow the agar to set and store the plates at 4^{o} C. Use them with in one week.

Inoculation:

Inoculate the surface of the MHA plate with the swab. To ensure that the growth is uniform, pass the swab three times over the entire surface, by repeating the procedure.

***Application of anti microbial discs*:**

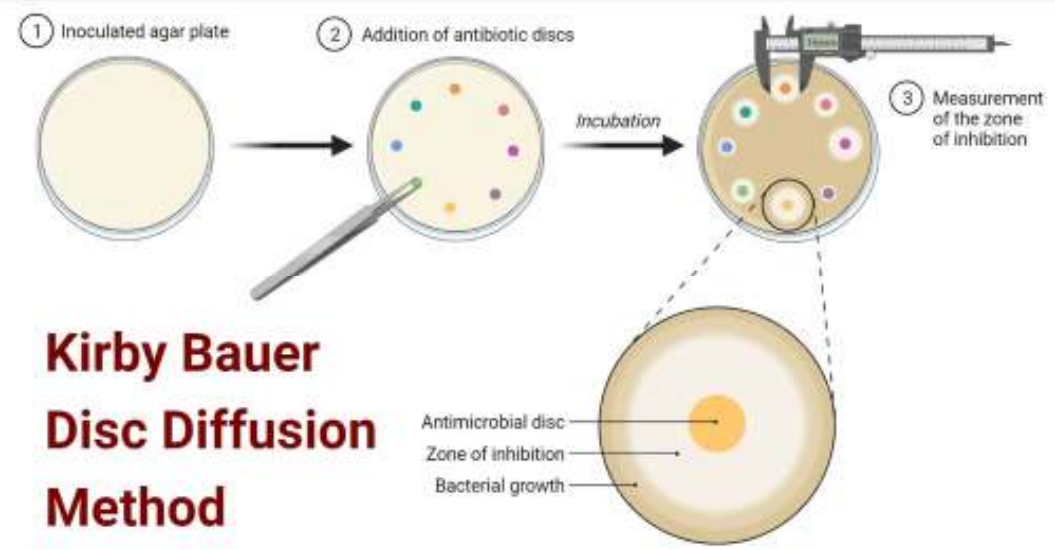
Arrange the discs containing antimicrobial agents on the surface of the inoculated plates by using sterile forceps. The distance between the discs should be at least 20 mm from one another.

Incubation:

Incubate the inoculated plates with the discs with in 15 min from inoculation. And keep the inoculated plate at 37^{o} C for 18 to 24 hrs and read the results.

Following incubation, the plates were examined for the presence of growth inhibition, which is indicated by a clear zone surrounding the disc. The susceptibility of an organism to a drug is determined by the size of this zone.

Observation:


1 Inoculated agar plate
2 Addition of antibiotic discs
Incubation
3 Measurement of the zone of inhibition
Kirby Bauer Disc Diffusion Method
Antimicrobial disc
Zone of inhibition
Bacterial growth

12. BACTERIAL GROWTH CURVE - MEASUREMENET OF GROWTH OF MICROORGANISMS (INDIRECT METHOD)

Exp:
Date:

Aim:

Growth of bacteria like *E.Coli* in liquid medium can be measured by determining the turbidity of culture at different time intervals.

Principle:

Growth is defined as orderly increases of all cellular components with multiplication as consequence. The microbial growth may be measured by variety of techniques. One each technique is turbidity measurements which relate cell number to the turbidity of the broth culture. Turbidity is measured with the colorimeter, an instrument that measures the amount of light transmitted directly through the sample.

With increase in bacterial number the broth becomes more turbid causing the light to reach the photo electric cell. Bacterial growth curve shows four distinct constructions requires that, samples of 25 hrs shake flask culture be measured for population size at regular time intervals during the incubation period.

The bacterial growth curve shows four distinct phases of lag phase, log phase, stationary phase and death phase. The actual length of the each phase varies with the organism and environmental conditions.

***Materials required*:**

10-12hrs broth culture of *E.Coli*, sterile pipette, conical flask, nutrient broth, shaker, colorimeter etc

Procedure:

1. 0.1ml of the given culture was inoculated in to flasks containing nutrient broth.
2. After inoculation cultures were incubated at room temperature and placed on shaker. Optical density of the culture was measured at '0' time and thereafter every one hour. Separate flasks were used for each time interval. The OD was measured at 420nm.
3. Triplicate flasks were maintained for record the growth at various time intervals.
4. A curve with time in 'X' axis and OD value in 'Y' axis was plotted in the graph and the doubling time of the culture was calculated from the growth curve plotted.

Observation:

13. QUANTITATION OF MICROBES
COUNTING OF MICROBIAL CELL USING HAEMOCYTOMETER

Exp:
Date:

Aim:

To enumerate the number of yeast cells present in the culture.

Principle:

The number of cells in the population is measured directly by counting under the microscope. This method is called direct microscopic count.

In the direct microscopic count method a known volume of microbial culture is taken in a special slide and the number of microorganism are counted by examining the slide under a bright field microscope. In this method described below a Neubaur counting chamber is used.

Description of Neubaur counting chamber:

The counting chamber consists of a thick glass slide with a depression in the center and a special cover slip. There is a groove on either side of the depression on the slide. There is a grid of definite area. This area is divided into nine squares each with a area of 1mm square ($1mm^2$). The central square is divided into 25 medium sized squares by triple lines. Each on of these squares has an area of $(0.2m)^2$ or 0.04 mm^2.

Each medium square is further divided into 16 small squares and the area of one small square is 0.0025 mm^2. The thickness of the culture to be introduced between grid and cover slip is 0.1mm that is the volume of liquid will be 0.1m deep. Sample introduced in to this volume contain microbial cells and we can count them easily under a microscope.

Materials required:

Neubaur counting chamber (Haemocytometer), cover slip, yeast culture, alcohol, Pasteur pipette etc.

Procedure:

1.Clean the neubaur counting chamber and cover slip with alcohol.

2. The counting chamber was first examined and the ruling area in haemocytometer was focused with 10x and 40x objective.

3. A small aliquot of the diluted yeast culture was introduced on the grid using a Pasteur pipette and covered with the cover slip.

5. By capillary action, the fluid will be drawn inside the counting chamber.

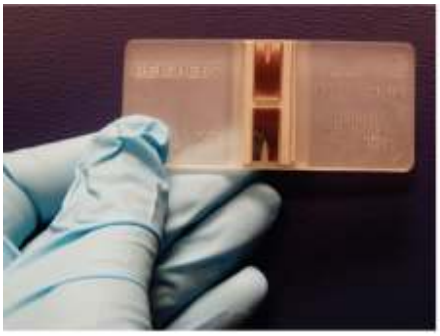
6. Care should be taken to avoid floating of liquid on grid or cover slip.
7. The cells were allowed to settle on the slide.

8. The slide was focused under the microscope and the cell present in the 4 corners and the central medium square was counted. Thus obtained cell number would be multiplied with five to get the total number of cell present in all 25 squares.

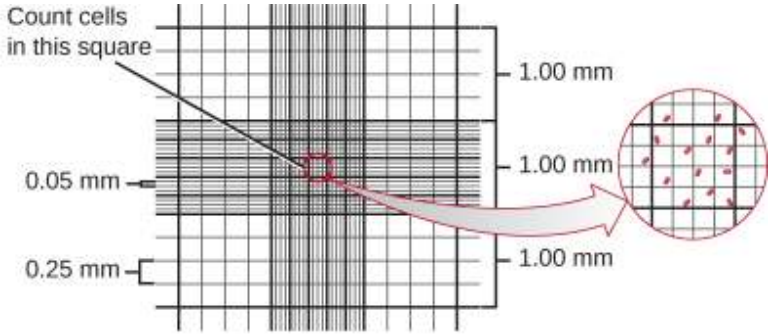
9. The number of cells in each corner and Central Square was recorded and the number of yeast cell present in 1ml of the original sample was calculated.

Observation:

PROCEDURE



(a)



(b)

14. EFFECT OF DIFFERENT PARAMETERS ON BACTERIAL GROWTH
EFFECT OF pH ON MICROBIAL GROWTH

Exp:
Date:

Aim:

To understand how pH affect the growth of the given test organism.

Principle:

Normally pH affects the growth of microorganism and affects the activity of enzyme especially that enzyme involved in biosynthesis and growth. Each species of microorganisms posses a definite pH growth range and a distinct pH growth medium.

Acidophiles have a growth optimum between pH less than 5.5, neutrophiles have between 5.5 and 8 and alkalophiles have 8.5 to 11.5. Majority of bacteria and protozoa are neutrophiles. Many bacteria produce metabolic acids that may lower the pH and inhibit the growth. To prevent this buffers are added to culture media to neutralize acids produced. For example: peptone is complex media act as a buffer. Phosphate salts are often added as buffers in chemically defined media.

Materials required:

Nutrient broths sterilized with pH 3, 5, 7, and 9. Overnight bacterial culture, 1NaoH and 1N HCl to adjust the pH, sterile pipettes (or) tips, colorimeter etc

Procedure:

1. The flasks were labelled indicating the pH of the medium. (pH 3,5,7,9).
2. All flasks were inoculated with 0.1ml culture of the given organism and kept in a shaker at room temperature for 24 hrs.
3. Triplicate flasks were maintained for each pH.
4. After incubation, the optical density of each culture was measured at 420nm nutrient broth was used as blank.
5. The results were recorded in the form of table or graph.

Observation:

15. BIOCHEMICAL TEST - INDOLE PRODUCTION TEST

Exp:
Date:

Aim:

To determine the ability of an organism to produce indole.

Principle:

Tryphotophan present in peptones of the culture media is acted upon by the enzyme tryptophanase an converted into indole, skatol and indole acetic acid. Indole reacts wilth aldehydes to produce a red colored product. So organisms are grown in tryptophan rich medium and tested for the presence of indole.

Materials required:

Tryptone broth, bacterial culture(*E.coli, Klebsiella pneumoniae*), Kovac's reagent, test tube, inoculation loop etc

Kovac's reagent:

Amyl or isoamyl alcohol	– 150ml
Paradimethyl aminobenzaldehyde	– 5.0 gm
Conc. HCL	- 25.0 ml

Procedure:

Indole agar containing test tubes are inoculated with test organism.

After inoculation the tubes are kept in incubator for 48 hrs at 37^{o}C.

Add 0.5 ml of kovac's reagent and shake gently and observe the result.

Observation:

16. UREASE TEST

Exp:
Date:

***Aim*:**

To determine the ability of organism to ferment citrate as a sole carbon source.

Principle:

In the absence of fermentable glucose or lactose, some microorganisms are capable of using citrate as a carbon source for their energy. This ability depends on the presence of a citrate permease that facilitates the transport of citrate in the cell.

Citrate is the first major intermediate in the Krebs cycle and is produced by the condensation of active acetyl with oxaloacetic acid. Citrate is acted on by the enzyme citrase, which produce oxalic acid and acetate. These products are enzymatically converted to pyruvic acid and carbon dioxide. During this reaction the medium becomes alkaline- the carbondioxide that is generated combines with sodium and water to form sodium carbonate, an alkaline product.

The presence of sodium carbonate changes the bromothymol blue indicator incorporated into the medium from green to deep Prussian blue.

Material required:

Simmons citrate agar slants , Bunsen burner, inoculating needle, 24-48 hrs cultures of *E.coli , K. pnemoniae.*

Procedure:

1. Using sterile loop, inoculate test organism into its appropriately labeled tube by means of a stab and streak inoculation. The last tube will be serving as a control.

2. Incubate all cultures for 24 to 48 hrs at 37^0C and observe the result.

Observation: