MEDICAL MICROBIOLOGY PRACTICAL

S.No	Name of the Practical
1.	Identification of Pathogenic Bacteria (any two) based on cultural, morphological and biochemical characteristics
2.	Growth curve of a bacterium
3.	To perform antibacterial testing by Kirby bauer Method
4.	To prepare temporary mounts of *Aspergillus* and *Candida* by appropriate staining
5.	Staining methods: Gram's staining permanent slides showing acid fast staining, Capsule staining and spore staining

Exp. No: 1

Isolation and Identification of Pathogens- Morphological, Biochemical Method

Objectives:

- To learn the medical significance of the staphylococci.
- To learn selected laboratory tests designed to differentiate among the major staphylococcal species.

Background:

The genus staphylococcus is composed of both pathogenic and nonpathogenic organisms. They are gram-positive cocci and occur most commonly as irregular clusters or spherical cells. They are mesophilic non- spore –formers; however, they are generally highly resistant to drying, especially when sequestered in organic matter such as blood, pus and tissue fluids. They are capable of surviving outside of the body for extended periods of time, even up to several months. Many staphylococci are indigenous to skin surfaces and mucous membranes of the upper respiratory tract. Breaks in the skin and mucous linings may serve as portals of entry into underlying tissues, with the possibility of infection by virulent strains.

The three major species include *S. aureus, S. saprophyticus, and S. Epidermidis*. Strains of the last two species are generally avirulent; however, under special circumstances in which a suitable portal of entry is provided, S. *epidermidis* may be the etiological agent for skin lesions and endocarditis and S. *saprophyticus* has been implicated in some urinary tract infections.

Materials and Methods

Catalase test: during aerobic respiration, microorganisms produce hydrogen peroxide, accumulation of this substance will result in death of the organism unless they can be enzymatically degraded. Organisms capable of producing catalase rapidly degrade hydrogen peroxide ad illustrated

Laboratory tests for Identification of Staphylococcal sp.

Gram +ve cocci

↓

Staphylococcus sp. +ve ← Catalase test -ve → Streptococcus sp.

Test	*S. aureus*	*S. epidermidis*	*S. saprophyticus*
Mannitol salt agar Growth Fermentation	 + +	 + -	 + -
Colonial pigmentation	Golden yellow	White	White
Coagulase	+	-	-
DNase	+	-	-
Hemolysis	Generally Beta	-	-
Novobiocin sensitivity	sensitive	Sensitive	Resistant

Observations and results:

Gram stain:

Microscopically examine the gram-stained smears of the test organisms. Record your observation s of their cellular morphology and Gram reactions.

Identification of Human Streptococcal Pathogens

Objectives:

- To learn the medical significance of streptococci.
- To learn selected laboratory tests to differentiate streptococci on the basis of their hemolytic activity and biochemical patterns associated with lancefield group classification.

Background:

Members of the genus streptococcus are perhaps responsible for a greater number of infectious diseases than any other group of microorganisms. Morphologically, they are cocci that divide in a single plane forming chains. They form circular, translucent to opaque, pinpoint colonies on solid media. All members of this group are gram-positive, and many are nutritionally fastidious, requiring enriched media such as blood for growth.

The streptococci are classified by means of two major methods: (1) Their hemolytic activity, and (2) the serologic classification of lancefield. The observed hemolytic reactions on blood agar are of the following three types:

Classification

I- Hemolytic activity on Blood Agar

β-hemolytic	**α- hemolytic**	**γ- hemolytic**
Complete lyses to RBCs	Partial lyses	Non-
hemolytic due to production of	*Pneumococcus*	e.g. *Enterococcus*
- Streptolysin O	Viridans group	Esculin +ve
- Streptolysin S		

Optochin test

Pneumococcus → Sensitive

Viridans group → Resistant

Viridans group
(oral strepotococci)
S. mutans
S. salivarius
S. sangius
S. melleri
S. mitis

II- ***Lancefield grouping***

A - U
OOOO- C- carbohydrate
on cell well

III- ***Bacitracin sensitivity test***

Group A → Sensitive Group
B-U → Resistant

IV- Group A OOOO← M-protein
80 serotypes

Optochin test: this is a growth inhibition test in which 6 mm filter paper discs impregnated with 5 mg of **ethylhydrocupreine hydrochloride** (optochin) and called P-discs are applied to the surface of a blood agar plated streaked with the test organisms. The *S. pneumoniae*, being sensitive to this surface- active agent, are lysed with the resultant formation of a zone of inhibition greater than 15 mm surrounding the P-disc. Nonpneumococcal alpha-hemolytic streptococci are resistant to optochin and fail to show a zone of inhibition or produce a zone less than 15 mm.

Bacitracin test: a filter paper disc impregnated with 0.04 unit of bacitracin is applied to the surface of a blood agar plate previously streaked with the organism to be identified. Following incubation, the appearance of a zone of growth inhibition surrounding the disc is indicative of group **A** streptococci. Absence of this zone suggests a non-group **A** organism.

CAMP test: group **B** streptococci produce a peptide, the CAMP substance that acts

in concert with the beta-hemolysins produced by some strains of *staphylococcus aureus*, causing an increased hemolytic effect. Following inoculation and incubation, the resultant effect appears as an arrow-shaped zone of hemolysis adjacent to the central streak of *S. aureus* growth. The non group **B** streptococci do not produce this reaction.

Bile esculin test: in the presence of bile, group **D** streptococci hydrolyze the glycoside esculin to **6,7-dihydroxy-coumarin** that reacts with the iron salts in the medium to produce a brown to black coloration of the medium following incubation . Lack of this dark coloration is indicative of a non-group **D** organism.

% sodium chloride broth: the group **D** enterococci can be separated from the nonenterococci by the ability of the former to grow in this medium.

Laboratory test for the identification of Streptococcal species:

Gram +ve cocci

Observation and results:

You are provided with *S. pnuemoniae, S, pyogenes, S. agalactiae, enterococcus faecalis* and *S. bovis.*

Gram stain:

Microscopically examine the gram-stained smears of the test organisms. Record your observation s of their cellular morphology and Gram reactions.

Exp.No: 2

Bacterial Growth Curve

GROWTH CURVE OF BACTERIA

Introduction

If a microorganism is inoculated into a flask of medium and the growth rate of this organism reproducing by binary fission is plotted as the logarithm of cell number versus incubation time, a growth curve consisting of four distinct phases results:

1. The initial phase, lag phase, refers to the fact that cell division does not occur immediately; the microorganisms must adjust to their new medium. The length of this stage will depend on the condition of the cells at the time of inoculation and the type of medium into which they were inoculated. If a young culture is transferred into the same medium under the same conditions, no lag time is seen.

2. During the next phase, the exponential phase, microorganisms are growing and dividing at the maximal rate possible given their genetic potential, the nature of the medium and environmental conditions. The population is most uniform in terms of chemical and physiological properties. During this stage the generation time (doubling time) can be determined directly from a graph of log cell number versus time. See the MB 302 textbook for more details.

3. In the stationary phase, growth ceases. Normally this occurs once the population level has reached 109 cells/ml. This stage is reached because available nutrients are depleted, toxic waste products have accumulated, physical space is limited, and/or quorum sensing has occurred.

4. If incubation continues, the culture will enter a fourth phase, the death or decline phase, whereby the number of viable cells decreases exponentially.

Growth curves are typically determined using the viable plate method. This method employs spreading a diluted sample of bacteria over a solid medium and determining the number of colonies that arise. The number of viable microorganisms in the sample is calculated from the number of colonies formed multiplied by the dilution factor (inverse of the dilution). This method works well as long as microbes are well dispersed so that you increase the chance that each colony arises from an individual cell. On the Petri plates used, this method is only

statistically valid if the sample yields between 25-250 colonies. Plates with higher or lower counts are not used normally, although the data should still be recorded.

Bacterial cell numbers can also be approximated by measuring cell mass by means of optical density (O.D.) or turbidity, once the population has reached about 107 cells/ml. For exponentially growing cells this method is fairly reliable and provides an almost instantaneous estimation of the bacterial concentration for a culture. If cell number versus O.D. are graphed a straight line should result, indicating correlation between the two values. This follows the Beer-Lambert Law of Solutes, which typically applies to molecules in a solution but which has been shown to also apply for bacteria in suspension.

A microbiologist can produce a standard curve by measuring both O.D. and cell numbers by plate count for a bacterial population. The resulting graph can be used to extrapolate cell concentration by O.D. in future experiments, thus eliminating the delay caused by incubation. Cell size influences the results obtained by spectrophotometry so the same bacterium must be used and the values are only valid for cells in the exponential phase of growth.

In this experiment, we will determine the growth curve of E. coli under various nutritional and environmental conditions.

Objectives

Lab objectives are not required in the lab notebook for this experiment. The results for this experiment will be written up separately from the lab notebook.

Materials and Methods

E. coli culture grown overnight

50 ml of minimal salts broth in side arm flask

Tube of BHI or minimal salts broth as blank

Spectronic 21or 20D with black velvet cloth

TSA plates (2 for each sampling time)

1 can of sterile 1 ml pipettes

% peptone dilution blanks: 9.0 ml (clear liquid, blue cap)

% peptone dilution blanks: 9.9 ml (clear liquid, silver cap)

package of Kimwipes

hockey stick, ethanol

Procedure

Overview: each group will inoculate a flask with a specified amount of an overnight culture of E.coli. Immediately after inoculation, each group will take both an initial O.D. and a viable cell count sample. Additional O.D. measurements will be taken every 15 minutes over a 3 hour period, while additional viable plate count samples will be taken every 30 minutes over a 3 hour period. The dilution schemes are shown in Figure 12.1. For an O.D. measurement at any time point: if the O.D. is below 0.5, use dilution scheme A. If the O.D. is over 0.5, use dilution scheme B.

1. Blank your spectrophotometer with an appropriate blank (uninoculated BHI or minimal salts broth) at 600 nm. Use the black velvet cloth to prevent light from entering while you are measuring and use Kimwipes to wipe your tubes before measuring. Re-blank before every reading.

2. Vortex the overnight E. coli culture for a few seconds, to get all the cells in suspension. Immediately inoculate the side-arm flask with the specified amount of culture, for an O.D. value of ~ 0.07. (Note: this is less than 0.5, so every group should start with Dilution Scheme A shown on Figure 12.1)

3. Immediately after inoculating flask with E. coli, measure and record the time zero O.D. reading of the culture in the flask at 600 nm. Fill out the data chart provided as you take readings. It may take two people to measure the O.D. with the slightly cumbersome side arm flasks. Fill side arm with culture, wipe glass with Kimwipe. Place the arm into the chamber, in the same direction each time you take a measurement. Make sure the side arm is straight upright and snugly fit against the right side of the measurement chamber, otherwise the O.D. will be drastically off. Cover the flask with black velvet cloth during the reading. If you are having trouble, ask TA for help.

4. After measuring the O.D. at time zero, pour side arm contents back into main flask and then pull sample for the time zero dilution scheme. Remove 1 ml of culture from the flask (using aseptic technique), dispense into a labeled 9.0 ml dilution blank and then set it aside.

5. Place your flask into the appropriate shaker incubator (if your group is treatment 2, leave the flask on the lab bench at room temperature, no shaking). Be sure to use caution when handling and removing flask from shaker. These flasks break easily and are very expensive. The time the flask is out of the shaker should be kept to a minimum.

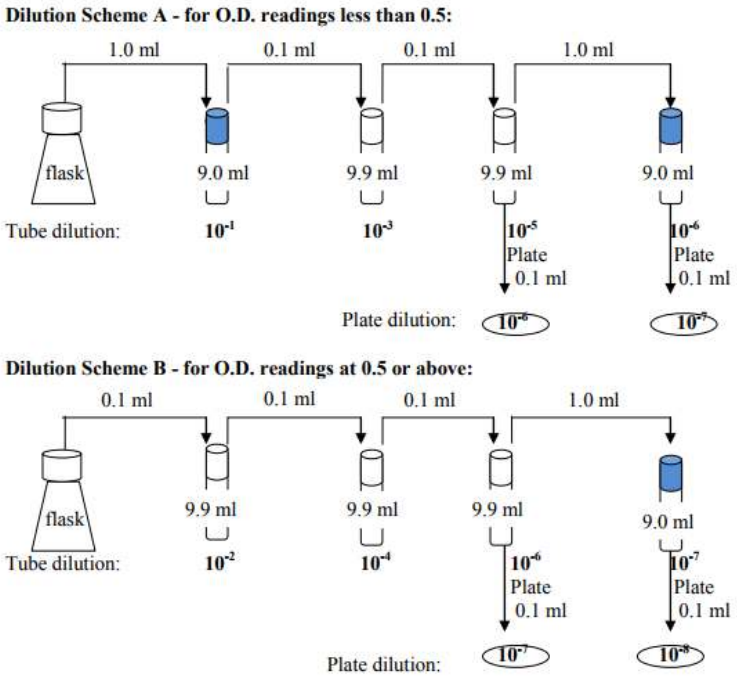
6. Vortex the dilution tube that you set aside in Step 4 for several seconds. This is to separate and homogenize the bacteria. Finish Dilution Scheme A on Fig. 12.1. Vortex subsequent dilution tubes thoroughly before transferring to next dilution. Make sure to use a new sterile pipette for each dilution. The same pipette can be used to make a dilution and a plate if both samples come from the same dilution tube. Remember to sterilize hockey sticks by dipping in alcohol and flaming. Plates should be spread immediately after sample is deposited onto them. Do NOT allow the plates to sit before spreading them. Be sure to mark each plate with group ID, treatment #, time of plating, and final dilution. Every group should be preparing 2 plates for each sampling time (i.e. every 30 minutes). Once the 2 plates are prepared, it is recommended that used dilution blanks be discarded before the next sampling time, to prevent confusion.

7. Groups should continue to take O.D. readings of the culture every 15 minutes for 3 hours, filling out the data sheet provided as readings are obtained. Dilutions/platings should be performed every 30 minutes for 3 hours. The OD reading will determine which dilution scheme (A or B) is appropriate. Perform dilution scheme A if the O.D. is less than 0.5. Perform dilution scheme B if the O.D. is at or greater than 0.5.

8. After the last reading, place a rubber band around all the plates for your group and place in the appropriate incubation tub. The plates will be incubated at 37°C for 48 h. Remove the tape from your side arm flask and carefully place it into a metal discard coffin.

9. Hand in the group data sheet to your TA with the names & seat #s of all the group members. 2.5 inclass points.

Figure 12.1 Flow chart of dilutions for viable cell count



Next Lab Period (after plates have been incubated)

Count the colonies on the plates that your group prepared. If the count goes above 250 colonieson a single plate, record the colony count for that plate as TNTC. Record the information on the data sheet provided and hand in to your TA at the end of lab. 2.5 in-class points.

A complete set of data for all treatments will be posted on Canvas. Use this information tocomplete the assignment described on the following pages.

Note: it is not required to record anything in your notebook for this experiment. However, each student should make note of the treatment that their group was assigned.

Exp. No: 3

Kirby Baeur Method

The Kirby-Bauer test, known as the disk-diffusion method, is the most widely used antibiotic susceptibility test in determining what choice of antibiotics should be used when treating an infection. This method relies on the inhibition of bacterial growth measured under standard conditions. For this test, a culture medium, specifically the Mueller-Hinton agar, is uniformly and aseptically inoculated with the test organism and then filter paper discs, which are impregnated with a specific concentration of a particular antibiotic, are placed on the medium. The organism will grow on the agar plate while the antibiotic "works" to inhibit the growth. If the organism is susceptible to a specific antibiotic, there will be no growth around the disc containing the antibiotic. Thus, a "zone of inhibition" can be observed and measured to determine the susceptibility to an antibiotic for that particular organism. The measurement is compared to the criteria set by the Clinical and Laboratory Standards Institute (CLSI). Based on the criteria, the organism can be classified as being Resistant (R), Intermediate (I) or Susceptible (S).

Principle of the method:

The media used in this test has to be the Mueller-Hinton (15x150mm) agar because it is an agar that is thoroughly tested for its composition and its pH level. Also, using this agar ensures that zones of inhibitions can be reproduced from the same organism, and this agar does not inhibit sulfonamides. The agar itself must also only be 4mm deep. This further ensures standardization and reproducibility.

The size of the inoculated organism must also be standardized (using the MCFarland 0.5 Latex standard with the Wickersham Card. The reasons are because if the size of the inoculum is too small, the zone of inhibition will be larger than what it is supposed to be ("the antibiotics will have a distinct advantage") and if the inoculum is too large, the zone of inhibition will be smaller.

Reagents and Supplies:

Mueller Hinton 50x150 mm agar plates

Hardy Diagnostics Susceptibility Disks (various antimicrobials as in QC above)

37 C Incubator

Sterile polyester or cotton swabs

Hardy Diagnostic Saline tubes

McFarland Latex 0.5 Standard and Wickerham Card

Calipers, ruler, or template for measuring the diameters of inhibitory zones.

Procedure:

Preparation of Bacterial Suspension

Remove a Hardy Diagnostic Saline 0.85%, 1.8mL tube from the box, label with the patient name and place in a test tube rack.

Using a 1ul loop, pick several isolated colonies from the agar surface.

Immerse the loop in a labeled saline tube. Vortex.

Using a Wickerham Card and a vortexed McFarland Latex 0.5 standard, compare the turbidity of the inoculated saline tube with the Standard. If the turbidity is comparable, proceed with the inoculation of the Mueller Hinton Plate. If not, adjust the turbidity by adding more isolated colonies in the same manner if the turbidity is less than the standard or more saline if the turbidity is greater. Once the turbidity is comparable to the standard, proceed with the inoculation of the labeled Mueller Hinton plate.

The bacterial suspension should be used within 6 h of preparation. If not used immediately after preparation, shake vigorously to resuspend the bacteria just prior to use.

B. Inoculation of Mueller Hinton Agar

Allow plates to come to room temperature before use.

Dip a sterile cotton swab into the bacterial suspension. To remove excess liquid, rotate the swab several times with a firm pressure on the inside wall of the tube above the fluid level.

Using the swab, streak the Mueller-Hinton agar plate to form a bacterial lawn.

To obtain uniform growth, streak the plate with the swab in one direction, rotate the plate 90° and streak the plate again in that direction.

Repeat this rotation 3 times.

Allow the plate to dry for approximately 5 minutes.

Use an Antibiotic Disc Dispenser to dispense disks containing specific antibiotics onto the plate.

Using sterile sticks or loops, gently press each disc to the agar to ensure that the disc is attached to the agar.

Plates should be incubated overnight at an incubation temperature of 37°C.

C. Reading and Interpreting Zone Sizes

After overnight incubation measure the zone sizes (area of no growth around the disk) in millimeters using a ruler or template.

Enter the zone sizes into the Kirby Bauer sensitivities log along with the patients information.

Interpret the results as Resistant, Intermediate or Sensitive for each antimicrobial according to the ranges listed on the log for Enteric gram negative rods.

Enter the results (R, I or S) into the LIS.

For Staph species that are Cefoxitin (Oxacillin) Resistant, Penicillin and Cefazolin will be reported as "not effective for MRSA".

Limitations

This system is only set up for Enteric gram negative rods or a Staph aureus gram positive cocci. Therefore make sure the gram negative organism is lactose fermenting,and oxidase negative before setting up.

Non-enterics, streptococci/enterococci, gram positive rods and gram negative cocci must be sent out to Quest if sensitivities are needed.

Specimen Requirement:

Well isolated colonies from BAP agar plate only.

Quality Control:

Perform weekly Quality Control for the antimicrobial disks by setting up the stock culture of E. coli (ATCC 25922), S. aureus (ATCC 25923) and E. coli (ATCC 35216) Kirby Bauer sensitivities using the procedure specified for "Preparation of Bacterial Suspension

Exp.No: 4

Fungus are eukaryotic organism and they are classified into two main groups that is yeast and molds. Its cell wall is made up of chitin. The fungal structures include mycelium, sporangiospore, spores etc. The Lactophenol Cotton Blue wet mount is simple and widely used staining method for fungi.

Lactophenol Cotton Blue Stain (LCB)

Cotton Blue 0.05g

Phenol Crystals 20g

Glycerol 40ml

Lactic Acid 20ml

Distilled water 20ml

Method

Preparation of staining requires two days.

1. Dissolve the Cotton Blue in distilled water and leave overnight to eliminate insoluble dye.
2. Next day, add phenol crystals to the lactic acid in a glass beaker and stir it on magnetic stirrer until the phenol is dissolved.
3. Add the glycerol and filter the cotton blue solution into the Phenol + Glycerol + lactic acid solution.
4. Mix and store at room temperature.

The main components of LCB staining are :

1. Phenol: Fungicidal in nature
2. Lactic Acid :Preserves fungal structures
3. Cotton Blue: Stains the chitin in the fungal cell walls & the cytoplasm (in light blue).

Staining of Clinical Specimens (Non-keratinized) Procedure (LCB Staining)

1. Place a drop of 70% alcohol on the slide.
2. Add the specimen to the drop of alcohol.
3. Add one or two drops of Lactophenol Cotton Blue Stain before alcohol gets off.

4. Place the coverslip on the drop avoiding air bubbles to be trapped.
5. Examine under Microscope using 10X and 40X objective.

Staining of fungus from culture

1. Take a grease free slide.
2. Add a drop of lactophenol cotton blue solution on a slide.
3. Sterilize the inoculation loop or needle and cool it then transfer mycellial growth onto the LCB stain and press it gently so that it easily mix with the stain.
4. Take a clean cover slip and with the help of a forcep place the cover slip on mycellial gowth + LCB.
5. With the help of blotting paper, wipe the excess stain .
6. Observe the preparation under low & high power objectives of the microscope.

Slide Culture Technique

For accurate identification of fungi, it is required that the precise arrangement of the conidiophores and the way in which the spores are produced is essential. The simple method of slide culturing used widely is described here, which permits fungi to be studied virtually in-situ with as little disturbance as possible.

Procedure:

1. With the help of a sterile blade cut out an agar block (6 x 6 mm) enough to fit under the coverslip.
2. Flip the block up onto the surface of the agar.
3. Inoculate the sides of the agar block with spores or mycelia of the fungus to be grown.
4. Flame the coverslip and place it on agar block.
5. Incubate at 26OC until growth and sporulation take place.
6. After attaining the growth, remove the cover slip from the agar block.
7. Apply a drop of 95% alcohol as a wetting agent and gently lower the coverslip onto a small drop of Lactophenol cotton blue on a grease free glass slide.
8. The slide can be left overnight to dry and later sealed with nail polish.

9. When sealing with nail polish use a coat of clear polish followed by one coat of red coloured polish.

Exp.No: 5

Gram Positive and Negative Staining

Background:

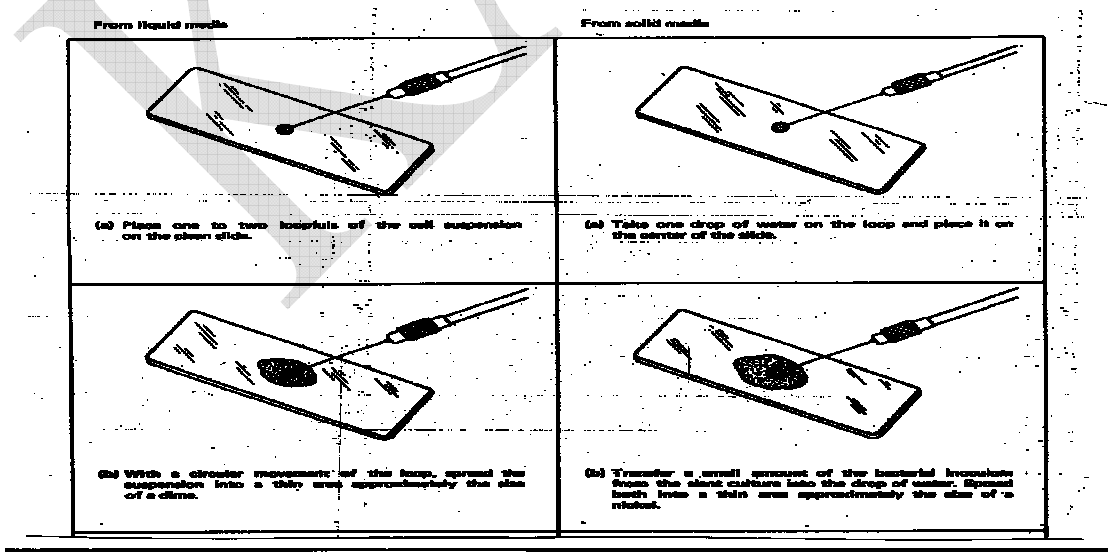
Since bacteria are so small, they are difficult to see with light microscope. Bacteria are usually colorless and therefore cannot be seen because of the lack of contrast with the surrounding medium. Simple stain is the use of basic dye to increase the contrast of cells for microscopy; the scope of simple staining technique to determine cell morphology (shape) relative size and arrangement.

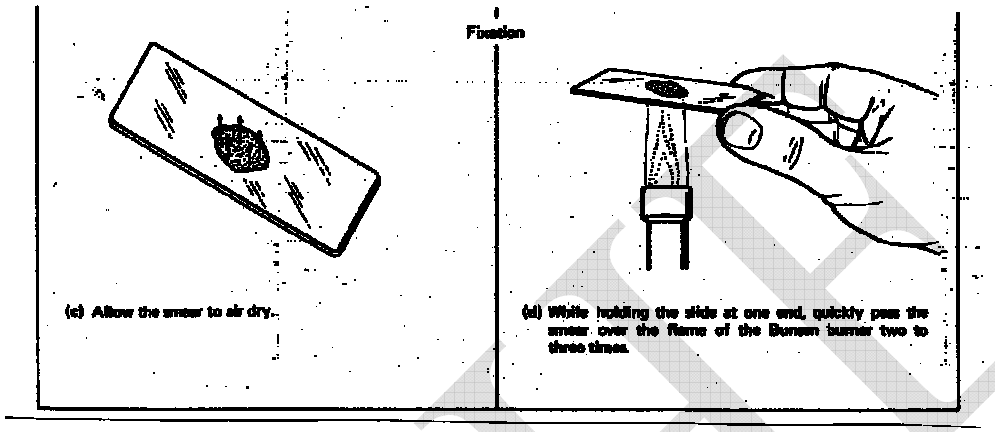
Materials:

1. Culture plates of the previous lab.
2. Basic dye (crystal violet, safranine or methylene blue

Procedure:

1. Smear preparation as in figure.
2. Place a slide on the staining tray and flood the smear with one of the indicated stains, using the appropriate exposure time.
3. Wash smear with tape water to remove excess stain.
4. Using filter paper, blot dry but do not wipe the slide.
5. Examine all stained slides under oil immersion





Observations and Results:

- Draw a representative field for each organism.
- Describe the morphology of the organism with reference to their shapes and arrangements

Background:

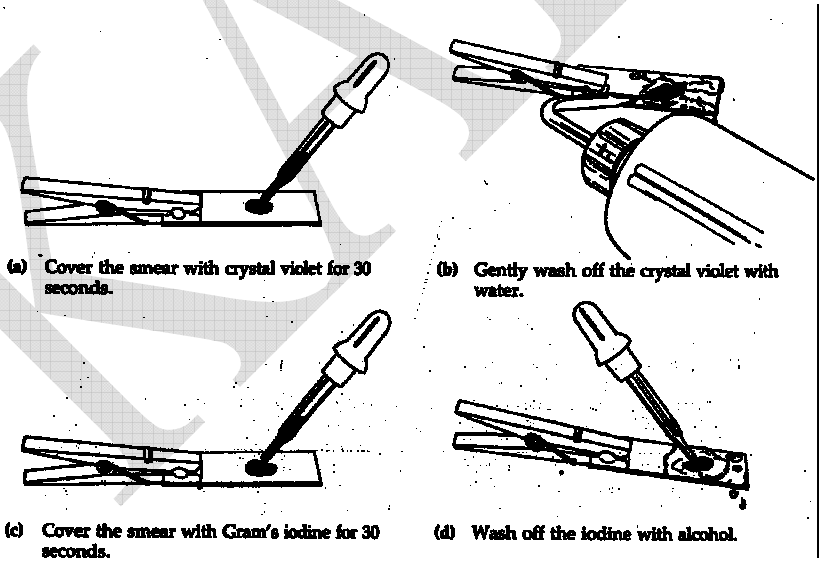
Developed in 1884 by the Danish physician Christian gram, is one of the most important and widely used procedures in microbiology for characterizing most bacteria into two groups based on the structure and chemical difference of the cell wall.

Those organisms which retain the crystal violet (appear dark blue or violet) are designate gram positive; those which lose the crystal violet and stained by the safranine (appear red) are designated gram negative.

Materials:

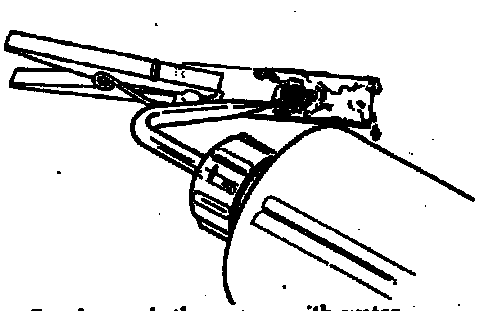
1. Gram stain kit
2. *Staphylococcus epidermidis* and *E. coli* strains.

Procedure:

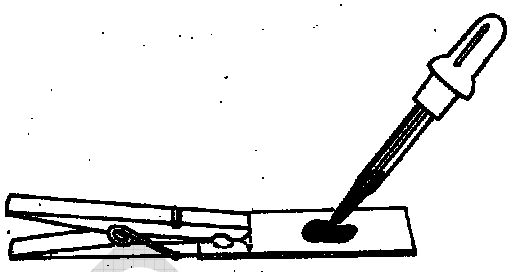


Smear preparation as in simple stain.

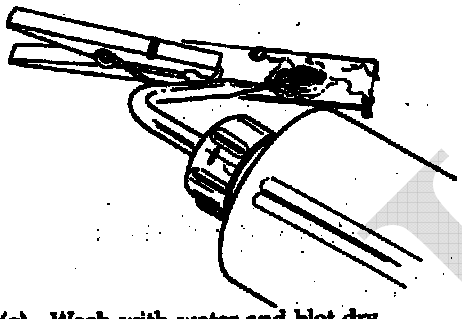
Follow the diagram.



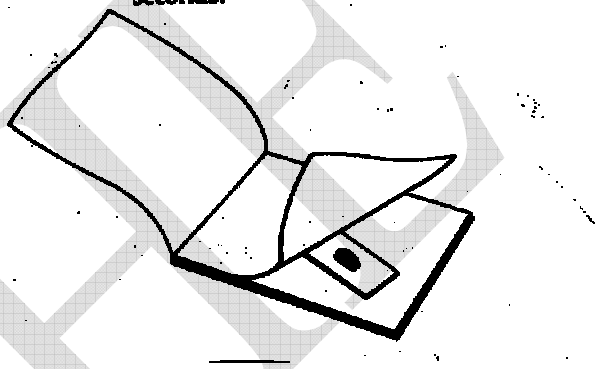
(e) Gently wash the smear with water.



(f) Cover the smear with safranin for 30 seconds.



(g) Wash with water and blot dry.



Observations and Results:

- Draw the representative microscopic field.

Describe the cells according to their color, morphology and

Exp.No: 5

Acid fast Staining

Objectives:

- To know the chemical basis of the acid-fast stain.
- Performance of the procedure for differentiation of bacteria into acid-fast and non-acid-fast.

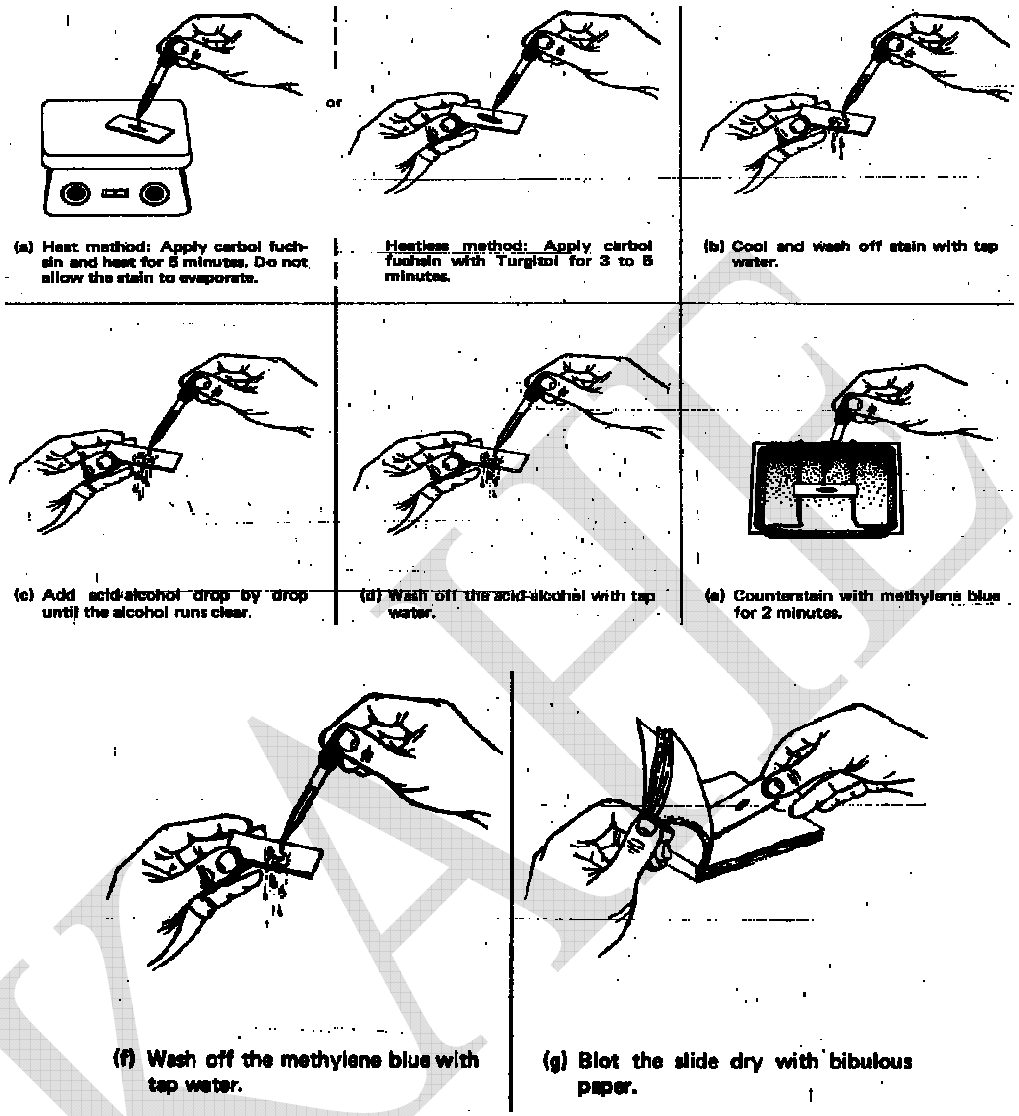
Background:

While the majority of bacterial organisms are stainable by either simple or gram's staining procedures. A few genera, particularly the members of the genus mycobacterium, are resistant and can only be visualized by the acid-fast method. Since M. tuberculosis and M. leprae represent bacteria that are pathogenic to humans, the stain is of diagnostic value in identifying these organisms.

The characteristic difference between mycobacteria and other microorganisms is the presence of a thick waxy (lipoidal) wall that makes penetration by stains extremely difficult. Once the stain has penetrated, however, it can not be readily removed even with the vigorous use of acid alcohol as a decolorizing agent. Because of this property, these organisms are called acid-fact, while all other microorganisms, which are easily decolorized by acid-alcohol, are non-acid- fast.

Materials:

1. Atypical Mycobacterial culture.
2. Carbol fuchsin
3. Acid alcohol
4. Methylene blue



Observations and Results:

- You are provided with stained smear of sputum.
- Record your observations; describing the cells shapes arrangements and the color of the stained smear.

Structural stain or Special stain

Negative stain

Objectives:

- To learn the application and mechanism of the negative stain.
- Prepare a negative stain.

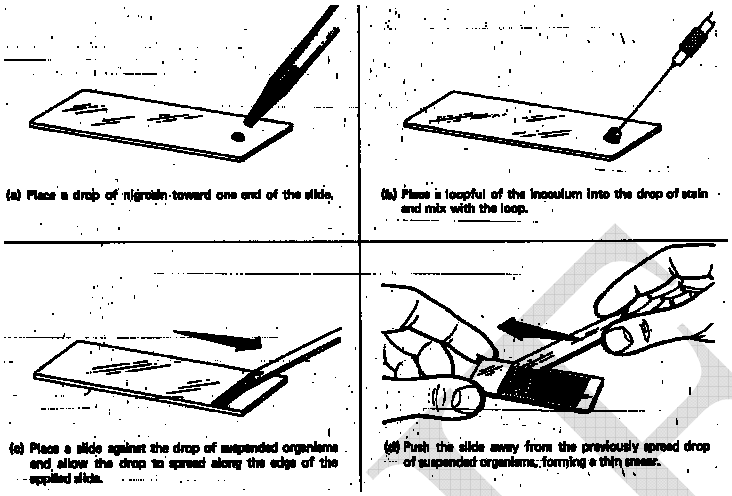
Background:

The negative stain technique does not stain the bacteria but stains the background. The bacteria will appear clear against a stained background. The negative stain does not stain the bacteria due to the ionic repulsion of the negative charge on the bacterial surface and the acidic stain (negative charge). No heat fixing or strong chemical are used, negative stain can be used to determine cell morphology, size and capsule.

Materials:

1. Nigrosine or India ink
2. Clean slide
3. Dis. Water.
4. Culture of E. coli or *staphylococcus epidermidis*.

Procedure:



Follow the diagram.

Observations and Results:

Draw a representative field of your microscopic observation

Endospore stain

Objectives:

- Prepare and interpret Endospore stain.
- Visualize different position of the Endospores.

Background:

Endospores are formed by members o two genera bacillus and clostridium which are of great medical importance.

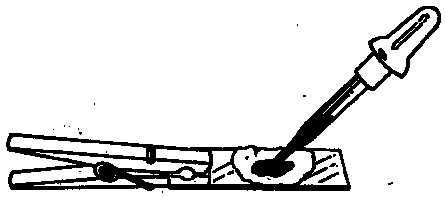
Endospores are metabolically inactive and are resistant to heating, various chemicals and many harsh environmental conditions. **Sporogensis** is not for reproduction, but it is resistant to unfavorable environment, Endospore can remain dormant for long time. However, Endospore may return to its vegetative or growing state.

Material:

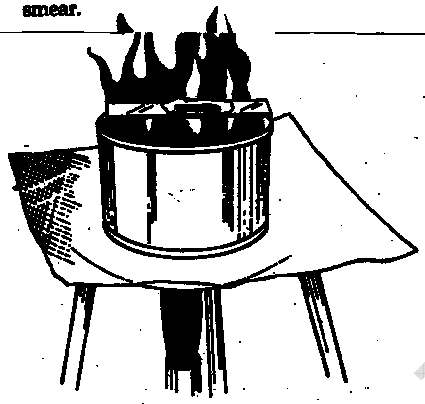
1. Culture, 2-3 days old of Bacillus sp. And Clostridium sp.
2. Malachite green.
3. Safranin.
4. Beaker of boiling water.
5. Piece of filter paper.
6.

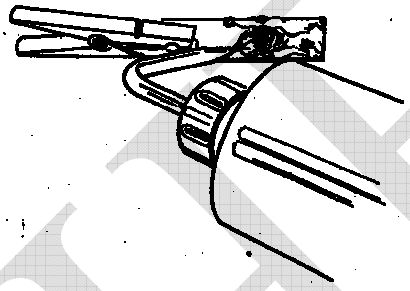
Procedure:

(a) Place a piece of absorbent paper over the smear.

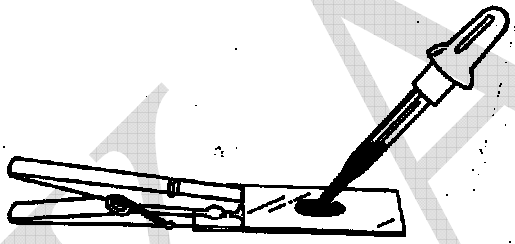


(b) Cover the paper with malachite green.

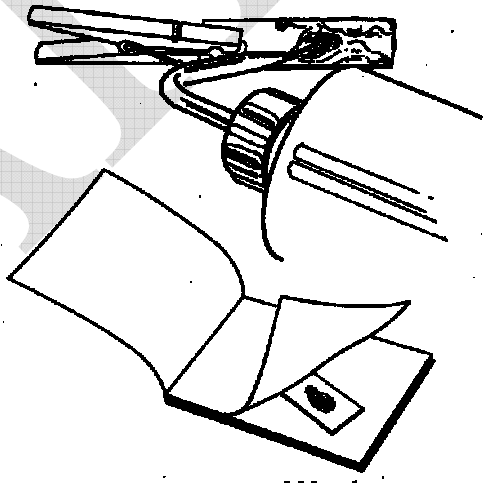




(c) After steaming for 5 minutes, wash the smear with water.



(d) Cover the smear with safranin for 30 seconds.



(e) Wash with water and blot dry.

Results:

You are provided with stained smear of spore forming bacteria

- Draw a representative microscopic field, describing location of the endospore, color of the spore and the vegetative cell.

Motility of bacteria

Objectives:

- To learn different methods of determining motility.
- To distinguish between true and false motility of bacteria.

Background:

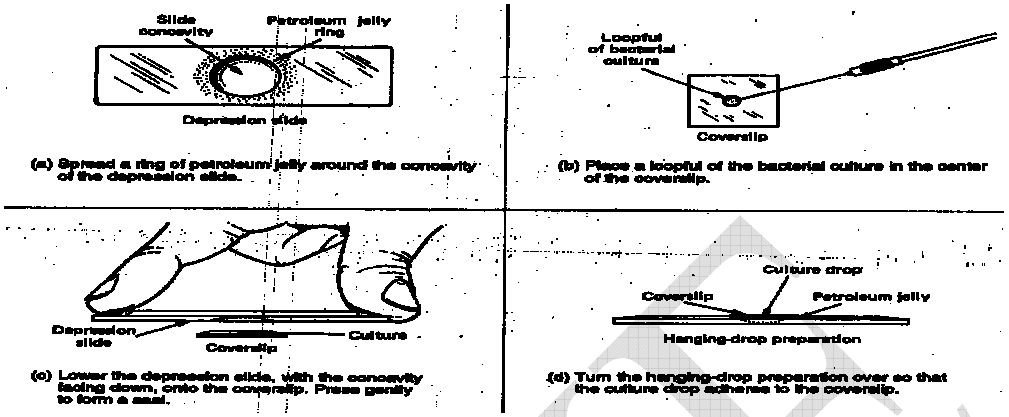
Organisms that possess the ability to propel themselves are said to be motile. This is an important characteristic that is used for identification of bacterial organisms. There are two types of movement that can be observed, one is Brownian movement caused by molecules colliding with the organism and moving around in an irregular pattern, but is not true motility. True motility appears as movement in consistent direction. There are two different methods of determining motility

1. Hanging drop method.
2. Inoculation of semisolid medium with the organism.
3. Inoculation of solid medium with the microorganism.

Materials:

1. Broth culture of motile bacteria.
2. Concave slides.
3. Cover slips.
4. Vaseline
5. Tubes of semisolid motility media.
6. Chocolate agar.

Procedure:



(a) Spread a ring of petroleum jelly around the concavity of the depression slide.

(b) Place a loopful of the bacterial culture in the center of the coverslip.

(c) Lower the depression slide, with the concavity facing down, onto the coverslip. Press gently to form a seal.

(d) Turn the hanging-drop preparation over so that the culture drop adheres to the coverslip.