MEDICAL LAB TECHNICIAN

Paper - II

MICROBIOLOGY & PATHOLOGY

INDEX

UNIT 1

History of Microbiological Scientists

Structure

1.0 History of Medicine

1.1 Antony Von Leeuwenhoek

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1.0 History of Medicine

Comparision of Indias Vedic sages (Ancient Indian Hindu Scientists) with Western and Europian scientist.

Birds eye view about medical lab technician course.

This contains the following steps.

Subjects and their definition

Role of Medical Lab Technician

On the Job Training (OJT)

Apprenticeship Training

Jobmela(Employement)

Birds Eye view about medical Lab Technician Course

1. Subjects and their defition

1. Biochemistry in both 1st and 2nd year.
2. Microbiology and pathology in 1st year and Microbiology in 2nd year.
3. Anatomy and Physiology in 1st year and Pathology in 2nd year.

Biochemistry : Biochemistry is the science of study of biochemical reactions taking place in living matter.

Microbiology : The branch of science dealing wit hthe study of microorganisms in called Microbiology.

Anatomy : The branch of science dealing with the study of structures of human body is called as Anatomy.

Physiology : The branch of science dealing with the study of functions of human body is called physiology

Pathology : The branch of science dealing with the study of diseases is called pathology.

2. Role of Medical lab Technician

Medical lab technician collect samples and performs tests to analyze body fluids, tissues and other substances.

A medical lab technician searches for basic clues to the absence, presence, extent and causes of diseases. This skilled individual is responsible for performing lab tests efficiently and accurately for high quality patient care.

Medical lab technicians are playing a vital role in the diagnosis and prevention of disease. Medical lab technician perform less complex tests and less lab procedures.

Medical Lab Technician may

- Collect specimens at specimen collecting centers
- Prepare specimens and operate automated analysers
- Perform manual tests in accordance with detailed instructions.
- Work under the supervision of concerned dept heads or lab managers.

Medical lab Technicians should have the following other skills

- Good analytical judgement and the ability to work under pressure
- Utmost attention and care is essential for med lab technicians because small differences or changes in test substances or numerical readouts can be crucial to a diagnosis.
- Mannual dexterity (skill in performing tests) and normal color vision are highly desirable.
- Because of wide spread use of automated lab equipment, computer skills are important.

3.On the Job Training

Both MLT 1st and 2nd year students will undergo OJT in the month of December every year, under the supervision of concerned class incharge lecturer for 30 days in the government hospitals or recognized well organized medical diagnostic center (preferably in government hospitals).

4. Apprenticeship Training

The 2nd year MLT students will be given apprenticeship training for one year in the various medical laboratories in the district head quarters and in the surrounding places of that zone after the announcement of their 2nd year results, under the chairmanship of Board of apprenticeship Training (BOAT) Chennai with the assistance of SIVE staff and guidance. This apprenticeship training is a must for every MLT 2^{nd} year student to get a job a government hospitals and primary health centers.

5. JOB Mela (Employment)

The board of apprenticehsip training (BOAT) Chennai officers will conduct JOB mela every year in the month of June at zonal level in the state with the assitance of SIVE staff at a single stretch. Every MLT pass out 2nd year student should and must attend the JOB mela after completing their apprenticeship training. Only apprenticeship training completed fresh students will be given importance in the JOB Mela.

Medical lab technician's course has a wide range of job potentiality and talented, hard working, worthy students flourish like any thing and even pass out students also settle easily in their life.

1.1 Antonyvan Leeuwenhoek

Bacteria, and other microorganisms, were first observed by 'Antonie van Leeuwenhoek' in 1676 using a single-lens microscope of his own design. He doing so Leeuwenhoek made one of the most important discoveries in biology and initiated the scientific fields of bacteriology and microbiology. He is the first microbiologist.

Fig. 1.1 Antony Van Leeuwenhoek

He is best known for his work on the improvement of the microscope and for his contributions towards the establishment of microbiology.

1.2 Robert Koch

Robert Koch was born on December 11th 1843 in Germany. Robert Koch was the Father of medical microbiology and discovered the Bacteriology. Robert postulated the germ theory, has seperated the Anthrax Bacillus, tuberculosis, and Vibrio cholera. Robert Koch introduced the staining techniques. Robert Koch got the Noble Prize in Medicine in 1905. Robert Koch was died on 27th May 1910.

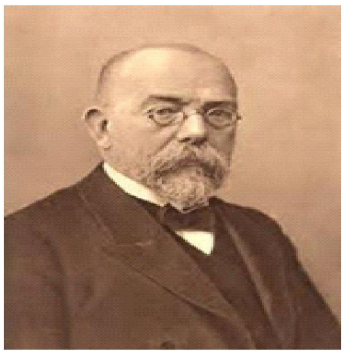

Fig. 1.2 Robert Koch

1.3 Edward Jenner

Edward Jenner was born on 17th May 1749 in London. Jenner used to study his natural surroundings. Edward Jenner was the Poineer of SmallPox Vaccine. Jenner is also sometimes called as the "Father of Immunology". Edward Jenners Works gave the life to many than any others Works. Edward Jenner has prepared the Cow Pox Vaccine to protect the smallpox. Edward Jenner was died on 26th January 1823.

Fig. 1.3 Edward Jenner

1.4 Joseph Lister

Lister was born on 5th April,1827 in United Kingdom.Lister was Famous for surgical sterile techniques.Lister used the Carbolic Acid in antiseptic surgery

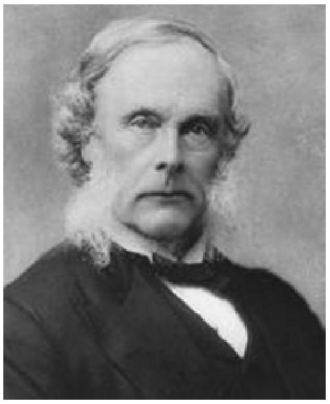

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Fig. 1.4 Joseph Lister

Lister confirmed Pasteurs conclusions(to eliminate the microorganisms responsible for gangrene by filtration, exposure to heat or exposure to chemical

solutions) by conducting experiments on his own and developed the antiseptic techniques for Wounds. Lister died on February 10th 1912.

1.5 Louis Pasteur (Father of Modern Microbiology)

Louis Pasteur is often referred to as the "Father of Microbiology" (along with scientist Robert Koch) for his contributions to discerning the cause and prevention of disease. He is best known for inventing the process of pasteurization, creating the first vaccine for rabies, and the germ theory of disease. Louis Pasteur was born on December 27th 1822 in France (Dole). He was a French Chemist and microbiologist.Pasteur was the first microbiologist to prepare the vaccine for the rabies and anthrax. Experiments of Pasteur supported the germ theory of disease. Pasteur invented the pasteurisation and sterilisation techniques. Pasteur is the one of the main founders of microbiology together with Ferdinand Cohn and Robert Koch.Pasteur explained about Fermentation theory.Pasteur worked in the fields of chemistry,mostly notably assymetry of certain crystals on the molecular basis.Pasteur died on September 28th 1822 in France.

Fig. 1.5 Louis Pasteur

Key Terms

Microbiologist: the person who is engaged with scientific study of topics of microbiology

Microscope : Instrument/tool used to observe the objects and the microorganisms which can not be seen with our naked eye.

Immunology : The branch of science dealing with the study of immunity to infection.

Spermatozoa : The male sex cell of animal/human being that fertilizes with the egg/ovum.

Bacteria : A group of microorganisms most of which can cause diseases

Acid Fast : Not readily decolourised by acids after staining

Bacilli : The rod shaped bacteria is called as bacilli

AFB : Acid Fast Bacillus

Vaccine : A substance prepared from the microorganisms it self and when injected onto the body provides a immunity against a disease is called as vaccine.

Smallpox : A serious disease which forms vrious layers or blisters on the skin and leaves the permanent scars.

Germ : A microorganism which causes disease/a part of an oorganism developing into a new one.

Pasteurisation : The process of heating and cooling to destroy the germs present in the milk

Fermentation : The process of undergoing a chemical change by the action of yeast or bacteria.

Infection : The process of causing and producing a disease to a person or animal by a germ or bacteria

Surgery : The process of medical treatment which involves cutting open the body and repairing or removing the parts.

Short Answer Type Questons

1) Name the scientists who contributed their services to the Microbiology field.
2) Mention any two contributions of Louis Pasteur
3) Write the contribution of Robert Koch and and Edward Jenner in MicroBiology
4) Write the contribution of Joseph Lister in the MicroBiology.
5) Mention the contributions of Edward Jenner and Joseph Lister in MicroBiology.
6) Mention the contributions of Robert Koch and Antony Van Leeuwen hoek.
7) Mention the achievements of Joseph Lister and Louis Pasteur in Microbiology.

Long Answer Type Question

a) Write thecontributions of following scientists in Microbiology
   - Antony Van Leeuwen Hoek
   - Louis Pasteur
   - Joseph Lister

b) Mention the contribution of following scientists in Microbiology
   - Robert Koch
   - Edward jenner
   - Louis Pasteur

UNIT 2

Microscopy

Structure

2.1 Principle, Working and maintenance of Compound microscope.

2.2 Principle of Fluorescent microscope and Dark field microscope

Introduction

A Compound microscope is used to produce an enlarged and well defined object images which can not be seen with our naked eyes.The degree of enlargement is called as magnification.With the invention of Microscopes,it has become easy for the Microbiologists and Pathologists in identifying the microorganisms and blood components and slide preperations for finding out the abnormal condition of a patient.With the use of compound Microscope,the morphology of bacteria,both Gram positive and Gram negitive bacteria, can be known.Various pathological slides were screened and observed with the help of Compound Microscope and results were given.With the help of Flouroscent Microscope, Mycobacteria can be identified.Very slender organisms like Spirochaetes can be identified with the help of Dark field Microscope.

2.1 Compound Microscope

Principle and Working

The Microscope is used to Visualise Stained and Unstained Microrganisms like Bacteria and Fungi and to identify them. The Microscope magnifies the image of the Object to be visualised through it. The laboratory Microscopeprovides a magnification of

40(scanner) x100(low power) x 400(High power) x 1000 (Oil immersion)

The total magnification is obtained by multiplying the magnification of the objective with that of eyepiece.

Total Magnification= Magnification of eye piece x Magnification of the objective.

Parts of the Microscope

The Microscope consists of

a) Stand
b) Mechanical adjustments
c) Optics or the Lens.

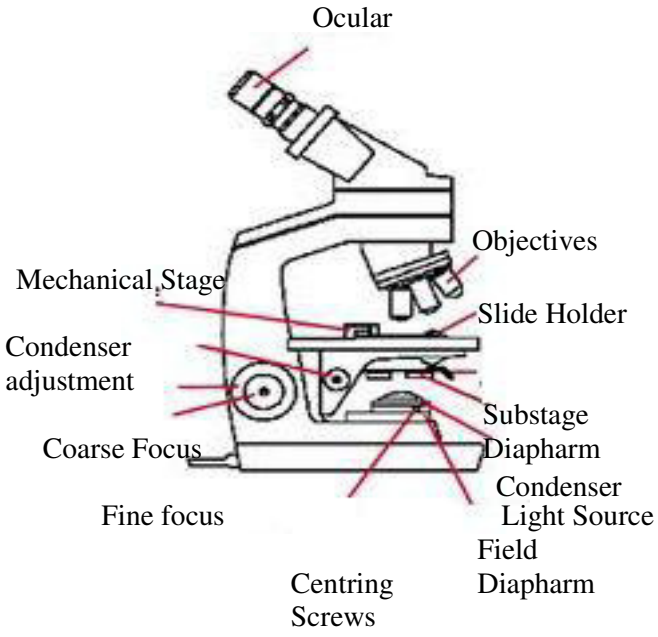

Fig.2.1 Mono-Ocular Microscope

BINOCULAR

1. Stand

The Stand consists of

- Tube-Supports objective and eye piece
- Body-Gives support to the tube
- Arm-The Microscope is held with the arm.It gives correct height and angle to the body and the tube.
- The stage has a pair of spring clips to hold the microslide.
- The sub-stage holds the condenser lense with the iris diaphragm and a holder for fitting.
- The horse shoe shaped foot which supports the body of the microscope

2. Mechanical Adjustment

The different adjustments are

- Coarse adjustment
- Fine adjustment
- Inclination
- Condenser adjustment
- Aperture adjustments
- Stage

Coarse Adjustment: Controlled by a pair of large knobs, one on each side of the body. It helps to move the tube with its lenses.

Fine Adjustment: Necessary for high power lences and oil immersion lenses. It is controlled by two smaller knobs on each side of the body.

Draw Tube: Used for adjusting distance between objective and eye piece lence

Inclination: Arm is tilted upon the foot by a finger.

Condenser Adjustments: The condenser is focused by rotating a knob below the stage.

Aperture Adjustments: This is done by iris diaphragm.

Stage: It has the knobs for the to and fro, side to side movements of slidesover the stage.

3. Microscope Optics

The following are the different parts of the microscopic optics

- Monocular microscope
- Binocular microscope
- Eye piece
- Objective
- Numerical aperture
- Oil immersion objectives
- Condenser and Iris
- Mirror
- Light

Monocular Microscope

Simple compound microscope is the example for this.This has only one eye piece

Working of the Compound Microscope

This constitutes the following two steps

I. Setting of the Microscope
II. Focussing the object

I. Setting of the Microscope

This again consists of the following steps

- Positioning of the microscope
- Fitting the accessories of the microscope.
- Positioning the lamp (if electric illumation is to be used)
- Preliminary adjustment of the mirror.
- Centring the condenser.
- Adjustment of the diaphragm.
- Adjustment of eye pieces.

Positioning of the Microscope

- Microscope should be placed on a firm level bench or on a piece of heavy cloth.The ideal height of the platform is 2 ft 8 in.
- The bench should be placed near the window,if day light is to be is used.
- Place the microscope away from the window,if electricillumination is to be used.

Fitting the Accessories of the Microscope

- In a clock wise Direction, fit the objectives into the revolving nosepiece.
- Put the eye pieces in proper place.
- Fit the condensor under the stage.
- Fix the mirror on the foot

Positioning the lamp(if electric illumunation is to be used)

- Facing the mirror,keep the lamp about 20 cms infront of the mirror
- At about an angle of 45 degrees fix the mirror

Preliminary adjustment of the mirror

- Use the plain side of the mirror
- To the maximum,open the iris diaphragm
- Next raise the condenser
- Then on the top of the condenser,place a piece of thin white paper
- To get the image of the bulb in the exact center of light circle,adjust themirror

Centering the Condenser

- On the stage (without condenser) place slide preparation.
- Open the iris diaphragm after lowering the condenser.
- With lowest power objective, examine through eye piece. Bring the object into focus
- Raise the condenser slowly until the edges of light ring circle are in sharp focus and then close the diaphragm.
- Adjust the position of the mirror if necessary in such a manner that light circle is in exact center of the bright area surrounded by the dark zone.
- Then adjust the condensers centring screws accordingly.
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Adjustment of the Diaphragm

- First open the diaphragm completely
- Look down the tube after removing the eyepie
- Until the light circle on the objective takes up only 2/3 of the surface,slowly close the diaphragm.
- For each objective repeat this procedure.

Adjustment of Eyepieces

- Select the eyepiece(X5orX6)
- If binocular is used,the distance between the pupils should be adjusted

II. Focussing the Objective

Focussing the objective consists of the following steps

- Use of lowpower objective
- Use of high power objective
- Use of oil immersion objective

Use of low power objective(X5orX10)

- To the lowest position,adjust the condenser
- Until the objective is just above the slide preparation,lower the objective
- Until a clear image is seen in the eye piece,using the coarse adjustment

Use of high power objective(X40)

- About half way down,adjust the condenser.
- If already the object is set to a low power objective,until a blurred image appears on the field,raise the objective very slowly by using the coarse adjustment.
- Using the fine adjustment,bring the image into focus.for sufficient illumination,raise the condenser.

Use of oil immersion objective(X100)

- To the uppermost position, adjust the condenser.Open the iris diaphragm fully
- On the dry stained slide preparation,place a small drop of immersion oil
- Until the objective is in contact with the oil,lower the objective.

Care and Maintenance of the Microscope

This involves the following steps.

- Requirements: Tissue paper, Xylene, Soft cloth, Petrolium jelly, Plastic cover,a fine paint brush.
- Cleaning the objectives
- Cleaning the eyepieces
- Cleaning the condenser and mirror
- Cleaning the support and the stage

Precautions

- When microscopic examination is carried out under oil immersion lens,Cedar wood oil is widely used
- Since paraffin oil or mineral oil often enters the objective,it should not be preferred.
- When microscope is not in use, dont leave the slides on it. Dont touch the lenses at any time.
- Cover the microscope with pastic cover when it is not in use and put the lower objective in the focussing position

2.2 Fluorescent Mircoscope and Darkfield Microscope

2.2.1 Fluorescent Microscope

Principle

This microscopes works on the principle of fluorescence.This involves the use of ultrvoilet lightwhich strikes the fluoroscent substances and visible light is emitted.Then the material stained with dye is visible.

This method causes illumination of microorganisms which are stained with fluoroscent dyes,which converts ultravoilet light into visible light by lengthening their wavelength.

Fig. 2.3 Fluoroescent microscope

This procedure is used to visualise the Mycobacteria against dark back ground. Special filter is used to remove all unwanted fluoroscent light.The second filter is placed above the eye piece to prevent damage to the eye by UV light.

Equipment : This microscope consists of the following parts

- A fluoroscent lamp
- A blue filter(primary filter)
- A yellow filter(secondary filter)
- An immersion dark ground condenser
- A non fluorescent immersion oil eg. liquid paraffin oil

Uses

- Identification of microorganiosms-Mycobacteria
- Fluoroscent antibody techniques-bacteriology and parasitology
- Histopathology
- Cytogenetics

Short Answer Type Questions

- Write the principle of compound microscope
- Write the principle of fluoroscent microscope
- Wha it is the use of numerical aperture in compound microscope?
- Write the uses of fluoroscent microscope
- Write the parts of fluoroscent microscope
- Define the objective and mention the various objectives of compound microscope

Long Answer Type Questions

- Write the construction, care and maintenance of compound microscope.
- Write about the Fluoroscent microscope.
- Draw the neat diagram of compound microscope and locate the variousParts of it.

UNIT 3

Sterilisation

Structure

3.1 Introduction
3.2 Classification of Sterilisation

3.1 Introduction

Microorganisms are ubiquitous.Since they cause contamination, infection, and decay, it becomes necessary to remove or destroy them from materials and from areas. This is the object of sterilisation.The process of sterilisation finds application in microbiology for prevention of contamination by extraneous organisms,in surgery for maintenance asepsis, in food and drug manufacture for ensuring safety from contaminating organisms and in many other situations. In todays dialy life any where across the globe, sterlisation is playing an imporatant role and gaining prominance day by day, because of health awareness among the people. Sterilisationhas wide range of applications right from diary, food industies to biological products.Sterilisation also playing an important role especially in the pharmaceutical industries,which is having direct link with the human beings.

Sterilization: Sterilisation is defined as the process by which an article, surface or medium is freed of all microorganisms either in vegetative or spore state .

Disinfection: Disinfection means the destruction of all pathogenic organisms or organisms capable of giving rise to infection.

Chemical disinfectants which can be safely applied to skin or mucous membrane surfaces and are used to prevent infection by inhibiting the growth of bacteria is called as antisepsis

Antisepsis: The term antisepsis is used to indicate the prevention of infection, usually by inhibiting the growth of bacteria in wounds or tissues.

Bactericidal agents: The agents which are used to destroy and kill the bacteria completely are called as the bactericida agents.

Bacteriostatic agents: The agents which are used to prevent the multiplication of bacteria by stopping the growth of bacteria are called as the bacteriostatic agents.

3.2 Classification of Sterilization

The various agent used in sterisation can be classified as follows;

Physical Agents

- Sun light
- Drying
- Dry Heat-Flaming,Incineration,Hot air oven

- Moist Heat-Pasteurisation,Boiling under normal pressure,Steam under pressure
- Filtration-Candle filters,asbestos disc filters,membrane filters
- Radiation.
- Ultrasonic and sonic vibrations

Chemical Agents

- Phenol and Phenolic compounds
- Alcohols
- Aldehydes
- Halogens
- Dyes
- Acids and Alkalis

Gaseous Agents

- Ethylene oxide
- Beta-propiolactone

3.2.1 Physical Agents

1. Sunlight: Sun light possesses appreciable bactericidal activity and plays an important role in spontaneous sterilisation that occurs natural cinditions.The action is primarily due to its content of ultraviolet rays. Bateria suspended in water are readily destroyed by exposure to sunlight. This is one of natural methods of sterilisation in cases of water in tanks, rivers and lakes.

2. Drying: Moisture is essential for the growth of the bacteria. Drying in air has therefore, deleterious effect on many bacteria. Susceptibility to drying varies with different bacteria and also with the conditions under which they are exposed to drying

Dry Heat Sterilisation-Heat is the most reliable method of sterilization

a) Flaming:

Fig. 3.1 Electric Gas Burner

Inoculating loops or wires,points of forceps and spatulas are held in Bunsen flame till they become red hot for sterilising them.If the loops contain infective proteinaceous material,they should be first dipped in chemical disinfectants before flaming to prevent spattering. Scalpels,needles,mouths of culture tubes,glass slides,cover slips etc,could be passed a few times through the bunsen flame without allowing them to become red hot.

(b) Red Heat: By exposing the materials to be sterilised till they get become as red hot to the flames of bunsen burner.

(c) Incineration: Through the process of burning,the conversion of material to be sterilised into ashes is called as Incineration.This is an excellent method for rapidly destroying materials such as soiled dressings,animal carcasses,bedding and pathological material.

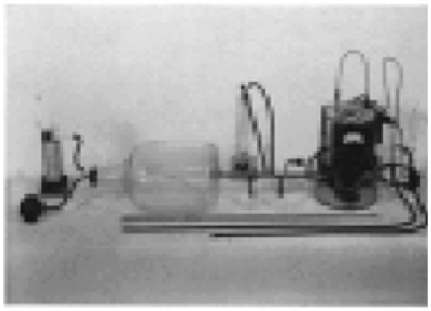


Fig. 3.2 An incinerator and aerosol chamber for testing

(d) Hot Air Oven

Fig. 3.3 Hot Air Oven

Construction and Working: This is the most widely used method of sterisation by dry heat.A holding period of 160^{o} C for one hour is used. Hot air oven is used to sterilise glassware,foreceps,scissors, scalpels, all glass syringes, and swabs. Some pharmaceutical products such as liquid paraffin, sulphonamides, dusting powder, fats, greases etc.
The oven is usually heated by electricity, with heating elements in the wall of chamber and it must be fitted with a fan to ensure even distribution of air and elimination of air pockets.It

should not be overloaded.The material should be arranged in a manner that allows free circulation of air in between.

Glassware should be perfectly dry before being placed in the oven.Test tubes,flasks,etc should be plugged with cotton wool.Other glassware such as petridishes and pipettes should be wrapped in kraft papper.For cutting instruments such as those used in ophthalmic surgery, a sterilising time of 2 hours at 150o is recommended.The oven must be allowed to cool slowly for about 2 hours before the door is opened,since the glassware may get cracked by sudden or uneven cooling.

4. Moist Heat Sterilisation

a) Temperature below 100^0C

For Pasteurisation of Milk

Boiling of milk to kill the pathogens present in the milk is called as the pasteurisation of milk. The temperature employed is either 60°C for 30 minuteswhich is alled as the holder method or 72 °C for 15 to 20 seconds followed by cooling quickly to 13 °C or lower is called as flash process.

Fig. 3.4 Pasteurisation Equipment

By these processes all nonsporing pathogens such as mycobacteria, Brucellae, and salmonae are destroyed.

(b) Inspissation

Fig. 3.5 Laboratory Inspissator

Heating at 80-85^{o}C for half an hour on three successive days in an inspissator to kill the bacteria is called as Inspissation.With this media such as Lowenstein Jensens and Loefflers serum are rendered sterile.

(c) Tyndallisation: The process of exposure of media containing sugars or gelatin to 100^{o}C for 20 minutes on three successive days is called as tyndallisation or intermittent sterilisation.
The principle is that the first exposure kills all vegetative bacteria,and if any spores present, being in a favourable medium, will germinate and be killed on sunsequent conditions

Autoclave

Principle

The principle of autoclave or steam steriliser is that water boils at which its water vapour equals that of the surrounding atmosphere

Construction and Working

The autoclave consists of a vertical or horizontal cylinder of gunmetal or stainless steel, in a supporting sheet-iron case. The lid or door is fastened by screw clamps and made air tight by an asbestos washer. The autoclave has on its lid or upper side a discharge tap for air and steam, a pressure gauge and safety valve that can be set to blow off at any desired pressure. In this autoclave,material for sterilisation is exposed to 121^{o}C for 15 to 20 minutes at 15 lbs pressure per square inch. Saturated steam heats the article to be sterilisedby releasing the latent heat. On condensation 1600 ml of steam at 100^{o}C and at atmospheric pressure condenses into 1 ml of water and liberates 518 calories of heat. The condensed water ensures moist conditions for killing bacteria. The air is poor conductor of heat and must be removed from chamber. The contents must be so packed that free circulation of steam occurs.Autoclave is used to sterilise the culture media,rubber goods,syringes and dressings.

Fig. 3.6 Auto Clave

3.2.2 Chemical Agents

Disinfection: Disinfection means the destruction of all pathogenic organisms or organisms capable of giving rise to infection

Mode of action

The chemical substances act as bactericidal agents as under:

- Coagulation of bacterial protoplasm
- Disruption of cell membrane by chemical substances. They may alter physical and chemical property of cell membrane.Thus results in killing or inhibiting the bacterial cell.
- Oxidation or burning out the bacterial protoplasm
- By affecting bacterial enzymes or coenzymes systems,thus causing interference of bacterial metabolism.

Chemical Disinfectants

The chemicals which are used for killing the organisms responsible for causing the infection are called as the chemical disinfectants.

1. Phenol and Phenolic compounds

Phenol(carbolic acid) is a powerful microbicidal substance.

Mode of action: The lethal effect of phenols is due to their capacity to cause cell membrane damage,thus releasing cell contents and causing lysis.

Uses: Phenolic disinfectants derived from coaltar are widely used as disinfectants for various purposes in hospitals.Lysol and cresols are active against a wide range of organisms.

2. Alcohols

Ethyl alcohol and Isopropyl alcohols are most frequently used.

Mode of Action: Alcohols act by denaturating the bacterial proteins.These have no action on spores and virusses. To be effective,these must be used at a concentration of 60 to 70 percent in water.

Uses: Isopropyl alcohol is used to disinfect thermometer.Methyl alcoholis effective againsat fungal spores and is used for treating the cabinets and incubators affected by them.Alcohols are mainly used as skin antiseptics.

3. Aldehydes

Formaldehyde and glutaraldehyde are the two aldehydes mainly used.

Mode of action: Formaldehyde is active against the amino group in the protein molecule.In aqueous solutions, it is bactericidal and sporicidal and also has lethal effect on viruses.

Glutaraldehyde has an action similar to that of formaldehyde.It is specially effective against tubercle bacilli,fungi and viruses.It is less toxic and irritant to the eyes and skin than formaldehyde

Uses: Formaldehyde is used to preserve anotomical species and for destroying anthrax spores in hair and wool.Glutaraldehyde is used to disinfect endoscopes.

4. Halogens

Mode of action: Iodine compounds act as bactericidal agents with a moderate activity against spores.These are active against the tubercle bacilli and a number of viruses.Clorine and hypochlorites are also bactericidal in activity.

Uses: Iodine compounds are used as skin disinfctants.Clorine and its compounds are also used as disinfectants for many years in water supplies, swimming baths, and food and diary industries.

5. Dyes

The substsnces which are used to impart colour to some thing and at the same time having the bacteriostatic and bactericidal activity are called as the dyes.Aniline and Acridine dyes belong to this category.

Mode of action: Both the aniline and acridine dyes in high dilution act as bacteriostatic and are having low bactericidal activity.These are more active against gram positive than the gram negitive bacteria. The aniline dyes in use arebrilliant green, malachite green, and crystal voilet.

Uses: The aniline and acridine dyes are extensively used as skin and wound antiseptics

6. Acids and Alkalies

Inorganic acids like boric acid and chromic acid,organic acids like benzoic acid,salicylic acid are used as effective disinfectants.

3.2.3 Gaseous Agents (Gaseous Methods of Sterilisation)

Ethylene oxide and beta propio lactone are used as gaseous agents

Fig. 3.9 An Ethylene Oxide Sterilisation Gas Chamber

Key Terms

Microorganisms: The organisms which we can not see with our nakedeye.
Pathogens: The microorganisms capable of causing diseases
Sterilisation: The process by which an article,surface,or medium is freed of all microorganisms either in vegetative or spore state
Disinfection : The process of destruction of all pathogenic organisms
Antisepsis : The process of prevention of infection usually by inhibiting the growth of bacteria.
Bactericidal Agents : The agents used to stop the multiplication of bacteria completely by killing all the bacteria.
Bacteriostatic Agents : The agents used to prevent the multiplication of bacteria by keeping the bacterial growth to a standstil.
Dry Heat Sterilisation : The sterilisation which utilises the only dry heat
Incineration : The process of converting ,materials to be sterilised ,directly into ashes, through burning
Hot Air Oven : The instrument used to sterilse the glasswares, foreceps, scalpels, all glasssyringes, swabs, etc by dry heat at 160^{o}C for 1 hour
Moist Heat Sterilisation : The sterilisation which involves the utilisation of steam
Psteurisation of Milk : Boiling of the milk by holder method(60 ^{o}C for 30 min) and flash process(72 ^{o}C for 20 sec)
Insipissation : The process of sterilising the media in an in an insipissator on three successive days at 80 to 85 ^{o}C for 30 minutes
Tyndallisation : The process of sterilising the media containg sugars or gelatin at 100 ^{o}C for 20 minutes on three seccessive days
Autoclave : The instrument used to sterilise the culture mediqa,rubber goods,syringes and dressings at 121 ^{o}C for 15 to 20 minutes
Filtration : The process of sterilisation utilised to sterilise the heat sensitive antibiotic solutions, serum and carbohydrate solutions used in the preparation of culture media
Radiation : Emission of subatomic particles (electrons)in the form of energy which is used to kill the bacteria.
Ionising Radiation : Emission of electrons in the form of energy by Ionisation.
Chemical Disinfectants : The chemicals which are used to kill all pathogenic organisms
Ethylalcohol : The chemical which is used as skin antiseptic
Isopropylalcohol: The chemical which is used to disinfect the thermometer and also used as skin antiseptic
Aniline&Acridine dyes: The dyes which are used as skin and wound anti septics.

Short Answer Type Questions

- Define sterilisation
- Define Disinfection
- Define chemical disinfectants
- What are the articles sterilised in hot air oven
- How many methods are there in moist heat sterilisation?
- What is Incineration?
- Define pasteurisation of milk.
- What is Tyndallisation?
- Define Bactericidal agent and disinfectant
- Define Bacteriostatic agent and Inspissation.
- Mention the two agents of gaseous sterilisation.
- What are the articles sterilised by the Autoclave?

Long Answer Type Questions

- Define sterilisation and mention the classification of sterilisation.
- Describe the dry heat sterilisation method briefly.
- Explain in detail about Hot air oven and Incineration
- Write the classification of sterilisation and briefly describe the moist heat heat sterilisation.
- Explain in detail about the Autoclave and pasteurisation of milk.

UNIT 4

Glassware & Biomedical Waste

Structure

4.1 Introduction

New glassware needs special attention because it may contain

- Resistant spores present in the straw and other packing material
- It may give off alkali, which may interfere with the bacterial growth.

Hence it should be placed in 10% HCL overnight, washed in tap water or distilled water and autoclaved Cleaning lab glassware is not as simple as washing the dishes at home.Some cleaning basics have to be learnt while cleaning the glassware.Some times detegents like LIQUINOX OR ALCONOX are used, which are specially designed for cleaning the glassware.But these detergents are not always preferable for cleaning the glassware.Care should be taken while cleaning the special glassware.

Remove the stoppers and stopcocks when they are not in use.Otherwise they may freeze in place.The glassware was sterilised using one of the methods of strilisation.The contaminated material either clinically infective material or inoculated culture media must be disposed, otherwise it will become as health hazard to the people. Biomedical waste consists of solids, liquids, sharps,and lab waste that are potentially infectious or dangerous and considered as biowaste. Biomedical waste differs from other types of hazardous waste such as industrial waste. Biomedical waste comes from biological sources or is used in thediagnosis, prevention or treatment of diseases. Biomedical waste producers include,hospitals,health clinics,nursing homes, medical research labs, physicians' offices,dentists, and home health care. Biomedical waste is handled by placing in specially-labelled bags and containers for removal by biomedical waste transporters. It is most important to know clearly about the cleaning,drying and sterilisation of glassware. And at the same time we have to dispose the contaminated materials like clinical infective material and inoculated culture media other wise,both if not disposed poses greater health hazards to the public.So the goverrnment should strictly frame the amendments regarding this i.e. in disposing the contaminated material from all the medical labs,hospitals,health clinics,nursing homes,medical research labs,physicians offices ,dentists and home health care.

4.2 Cleaning of Glass ware

Basics of the cleaning of the glassware

It is generally easier to clean glassware if we do it in the right way.Sometimes detergent like Liquinox or alconox is used in some special cases to clean the glassware,but always detergent and tapwater is not preferable to clean the glassware.We have to rinse the glassware with proper solvent,then finish up with a couple of rinses with distilled water,followed by final rinses with the deoionised water.

4.2.1 Cleaning of glassware for general laboratory use

Glass ware with discarded culture are placed in 3% lysol after use are transferred to boiling soap solution containing tubercle bacilli or spore bearing organism or must be autoclaved.The discarded slide is boiled for 1hour in 5% soap solution in tap water or distillled water.The glassware is cleaned with test tube brush in hot and cold water.Finally the glass ware is allowed to drain and dried in hot air oven or climate

Cleaning of glassware for biochemical work:

Remove any grease with petroleum and then wash with warm tap water.Place in dichromate sulphuric acid cleaning solution for 12 to 24 hours. Remove,wash with hot water 4-5 times and distil water twice

4.2.2 Cleaning Special Glassware

Cleaning of Pipettes

- If pipettes are contaminated with infected material,discard the used pipettes into 3% lysol solution and leave until it is washed in tap water.
- If necessary keep overnight in dichromate-sulphuric acid cleaning fluid
- Wash with tap water or an antimagnetic pipette washer
- Connect the pipette to a water pump by rubber tubing and draw through distil water followed by acetone.Finally pump air until the internal surface is dry.

Cleaning of Volumetric Flasks

- In some cases we may need to soak the glass ware overnight in soapywater.
- Clean volumetric flasks using warm soapy water.
- The glass ware may require scrubbing with a brush
- Rinse with tap water followed by 3-4 rinses with deionised water

Cleaning of glass ware used for organic chemistry

- Rinse the glassware with appropriate solvent
- Use deionised water for water-soluble contents
- Use ethanol for ethanol-soluble contents,followed by rinses in deionized water
- Rinse with other solvents as needed,followed by ethanol and finally with deionised water.
- If the glassware requires scrubbing,scrub with a brush using hot soapy water, rinse thoroughly with tap water, followed by rinses with deionised water

Cleaning of the glassware containing the common lab chemicals:

Water Soluble Solutions:Rinse 3-4 times with deionised water then put the glassware away(e.g, Sodium chloride or sucrose solutions)

Water Insoluble Solutions:Rinse 2-3 times with ethanol or acetone, rinse 3-4 times with deionised water,then put the glassware away.In some situations some other solvents need to be used for the initial rinse(e.g. Solutions in hexane or chloroform)

Strong Acids:(concentrated HCL or H_2SO_4)Under the fume hood,carefully rinse the glassware with copious volumes of tap water.Rinse 3-4 times with deionised water,then put the glassware away

Strong Bases: (e.g. 6M NaOH or concentrated NH_4OH)Under the fume hood,carefully rinse the glassware with copious volumes of tap water.Rinse 3-4 times with deionised water,then put the glassware away.

Weak Acids: (e.g.acetic acids solutions or dilutions of strong acids such as 0.1M or 1M HCL or H_2SO_4) Rinse 3-4 times with deionised water before putting the glassware away

Weak Bases: (e.g.0.1M and 1M NaoH and NH_4OH) Rinse thoroughly with tap water to remove the base,then rinse 3-4 timres with deionised water before putting the glassware away

4.3 Drying of Glasware

Removal of water and even the moisture completely from the glass ware is called as the drying

- If glassware is to be used immediately after cleaning and must be dry,rinse it 2-3 times with acetone.
- This will remove any water and will evapourate quickly
- Some times we can apply vaccum to evapourate the solvent

or.

- Glassware should be cleaned thoroughly
- Excess of water should be drained
- Articles should be arranged in the hotairoven and door closed
- Power supply to the hotairoven should be turned on
- Vent should be opened and temperature should be set to 65oC
- After reaching 65^{o}C article should be kept for 15 minutes and dried
- After completion of drying,power should be turned off.
- After the internal temperature reaches the room temperarure,door should be opened
- Article should be unloaded from the oven carefully

4.4 Sterilization of Glassware

- The process by which the glassware is freed of all microorganisms either in vegetative or spore state is called as the sterilisation of glassware.
- Mouths of culture tubes,glass slides,coverslips are sterilised by passing a few times through the bunsen flame without allowing them to become red hot.
- Glassware, all glass syringes are sterilised by the hotairoven.Glassware should be perfectly dry before being placed in the oven.Test tubes, flasks etc should be

plugged with the cotton wool.Other glassware such as petridishes and pipettes should be wrapped in kraft paper

- Glass syringes are also sterillised by the autoclave
- Glass syringes are also sterilised by the ionising radiation.

4.5 Disposal of Contaminated material

(i.e.clinical infective material and inoculated culture media):

It is necessary to dispose the contaminated material immediately from the site to prevent the health hazards.It is most important to dispose the contaminated material from the hospitals,health clinics,nursing homes,medical research laborarories,offices of physicians,home health care,to protect the public from narious unwanted diseases.

4.5.1 Disposal of Clinical Infective Material

- Various color bins/buckets should be used for collecting the clinical infective material
- Care should be taken while collecting the clinical infective material,which otherwise pose threat to health of an individual collecting the clinical infective material
- The clinical infective material may include the sample specimens of HIV, malignant tumours, STD diseases, etc,so must be handled carefully
- The material should be disinfected with suitable chemical disinfectants to prevent the infection in some cases and some times the infective material should be burnt.
- The clinical infective material should be taken to a far away place and disposed
- The disinfected material can also be burried by digging a pit.
- The color bins/buckets should be cleaned and disinfected properly before reusage.

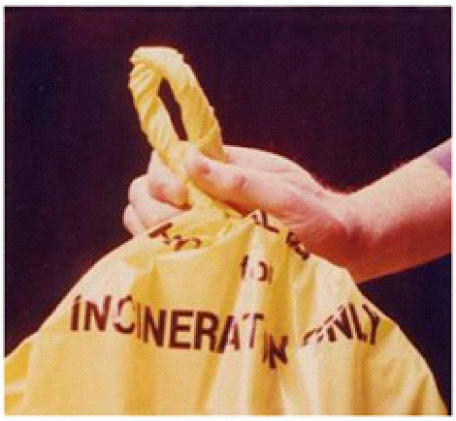


Fig. 4.1 Disposal of clinical infective material

4.5.2 Disposal of Inoculated Culture Media

Culture : The artificial growth of microorganisms is called as culture.

Culture Media : The media consisting of carbon ,nitrogen,oxygen,sources material and inorganic electrolytes to support and enhance the growth of the microorganisms is called as the culture media.

Inoculation: The process of transfer of bacterial specimen using inoculating loop wire onto the culture media asceptically is known as Inoculation.
While handling the preserved cultures and stock cultures,care should be taken to prevent the spread and transmission of diseases
Inorder to prevent the spread and transmission of diseases,the inoculated culture media should be disposed properly
Inoculated culture media should be treated properly before disposal
The inoculated culture media should be autoclaved at temperature of 121^{o}C for 15 minutes or 115^{o}C for 30 minutes
Then this material should be taken away to a far off place and disposed
The clinical infective material and inoculated culture media are placed in buckets and autoclaved seperately at 121 ^{o}C for 15 minutes.
After autoclaving the plates, tubes, other glassware is washed and dried in the hotairoven. The slides also should be boiled, washed and dried in hotairoven.

4.6 Handling and Disposal of Biomedical Waste

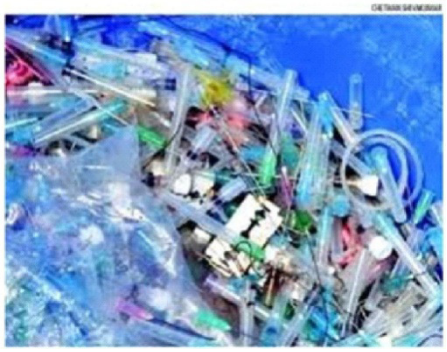


Fig. 4.2 Biomedical waste comprising plastics and biological waste

Biomedical waste(BMW): BMW consists of solids, liquids, sharps,and laboratory waste that are potentially infectious or dangerous and considered as biowaste. BMW must be properly managed to protect the general public, specifically health care and sanitatin workers who are regularly exposed to BMW as an occupational hazard . BMW comes from biological sources during the diagnosis, prevention,or treatment of diseases.common producers of BMW include hospitals, health clinics, nursing homes, medical research laboratories, offices of physicians, dentists, and home health care.

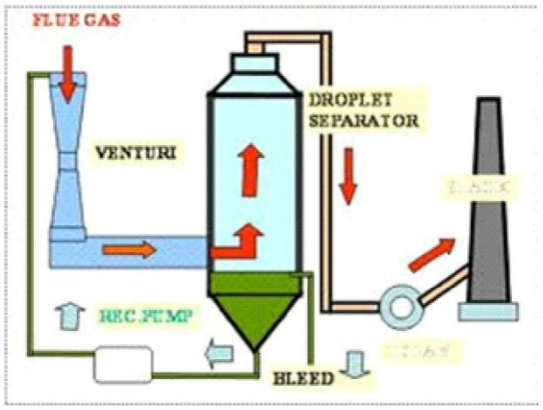


Fig. 4.3 Biomedical Waste Treatment and Disposal

Handling BMW

Sorting of medical waste in hospital is the first step in handling the BMW
At the site where it is generated,BMW is placed in specially-labled bags and containers for removal by BMW transporters
The person handling the BMW should wear disposable latex hand gloves.
Discard the gloves immediately after use.

Fig. 4.4 Biomedical Waste Management and Handling

- The person should wear mask covering the nose and mouth
- The person should wear a cap over the head
- The person should wear foot protectives
- The person should wash his hands with soap and warm water after handling BMW
- The person also should wash all areas of his body with soap and water that he thinks may have come into contact with BMW,even if he is not sure that his body actually touched the BMW
- Keep all sores and cuts covered
- The person should wear an apron or another type of cover to protect his clothes from contact with the waste
- Promptly clean and disinfect soiled,hard-surfaced floors by using a germicidal or bleach solution and mopping up with paper towels

Disposal of Biomedical Waste

Biomedical waste consists of solids,liquids, sharps and lab waste that are potentially infectious or dangerous and are considered as biowaste.It must be properly managed to protect the general public, specifically health care sanitation workers exposed to the biomedical waste Biomedical waste comes from the biological sources during the diagnosis, prevention and treatment of various diseases.Common producers of biomedical medical waste include hospitals, health clinics, nursing homes, medical research labs, offices of physicians and dentists. Householdwaste biomedical waste usually consists of needles and syringes from drugs administered at home(such as insulin) soiled wound dressings, disposable gloves other cloths that have come in to contact with bodily fluids.

- Colour bins/bags should be used for collecting the biomedical waste.
- The biomedical waste should be disinfected with suitable chemical disinfectants to prevent infection.
- The biomedical waste should be taken away to a far away place and disposed
- The biomedical waste which is disinfected can also be burried by digging a pit and filled with soil.
- The used disposable needles should be made into pieces by cutting
- The used syringes should be destroyed
- Biomedical waste after autoclaving should be disposed
- The biomedical waste like swabs,dressings,cotton plugs and dressings should be disinfected,disposed or dried and burnt.
- Dispose the needles lancets,syringes in a plastic soda pop bottlewith a cap

Short Answer Type Questions

- Mention the lab glassware
- How do you clean the glassware containing water soluble solutions?
- How do you clean the pipettes?
- How do you clean the glassware containing strong acids?
- How do you clean the volumetric flasks?
- How do you clean the glassware containing strong bases?
- Mention the chemical used in drying the glassware.
- Define biomedical waste

Long Answer Type Questions

- Write about the cleaning of glassware.
- Define Biomedicalwaste and write about the handling of Biomedicalwaste
- Write about the disposal of Biomedicalwaste

UNIT 5

Bacteria

Structure

5.1 Introduction

With the discovery of microscope only,the presence of bacteria was found. Before that the scientists only assumed that uncurable and illness of the patients was due to some diseases and also assumed that,those diseases are also due to some organisms. All these assumptions were cleared after the invention of microscope, which is playing a crucial role in identifying bacteria.Staining techniques is also paying an important role in identifying the bacteria.

5.2 Morphology and Classification of Bacteria

Bacteria are classified into 7 groups depending on their shape:

1. Cocci-(Kokhos means berry)-These are spherical in shape
   - Bacilli-(Baculus means rod)-These are rod shaped cells
   - Vibrios-These are comma shaped,curved rods possessing vibratory motility
   - Spirilla-These are rigid spiral forms
   - Spirochaetes(Coiled hairs)-These are thin flexuous spiral forms
   - Actinomycetes-These are branching filamentous bacteria.When seen in tissues they appear like radiating rays of the sun(sunray appearance).This is due to the rigid cell wall.
   - Mycoplasma - These are bacteria with defective cell wall.This may be due to spontaneous mutation or due to penicillin administration.Such cells are called as protoplasts,spheroplasts or L forms.

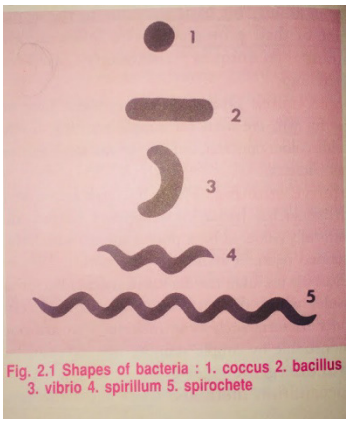


Fig. 2.1 Shapes of bacteria : 1. coccus 2. bacillus 3. vibrio 4. spirillum 5. spirochete

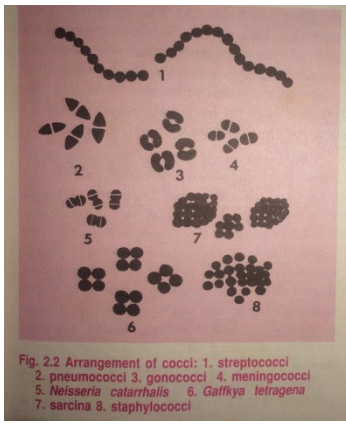


Fig. 2.2 Arrangement of cocci: 1. streptococci 2. pneumococci 3. gonococci 4. meningococci 5. *Neisseria catarrhalis* 6. *Gaffkya tetragena* 7. sarcina 8. staphylococci

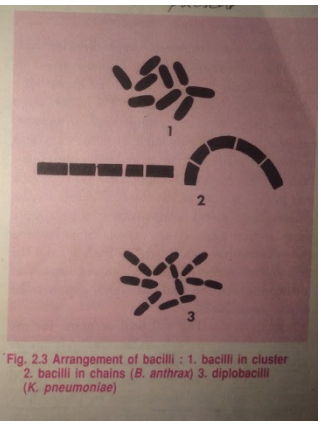


Fig. 2.3 Arrangement of bacilli : 1. bacilli in cluster 2. bacilli in chains (*B. anthrax*) 3. diplobacilli (*K. pneumoniae*)

Arrangement of Bacterial cells

1) Cocci in pairs-Diplococci
2) Cocci in chains-Streptococci
3) Cocci in clusters-Staphylococci
4) Cocci in groups of four- Tetrads
5) Cocci in pockets of eight –Sarcina
6) Bacilli in chains- Streptobacilli
7) Bacilli in chinese letter pattern- Coryne bacteria

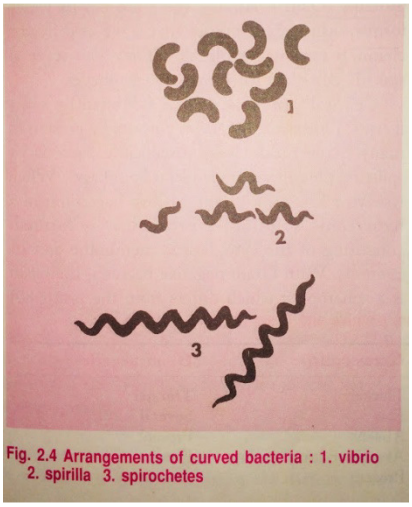


Fig. 2.4 Arrangements of curved bacteria : 1. vibrio 2. spirilla 3. spirochetes

Structure of Bacterial cell : (Bacterial Anatomy)

Like other living cells,bacteria possess protoplasm,cytoplasmic membrane, cellwall. Some intracellular and extracellular structures may be present. Under certain conditions of growth the cell is enclosed in a viscid layer known as capsule.Chemically it is a polysaccharide except Anthracis where it is a polypeptide.

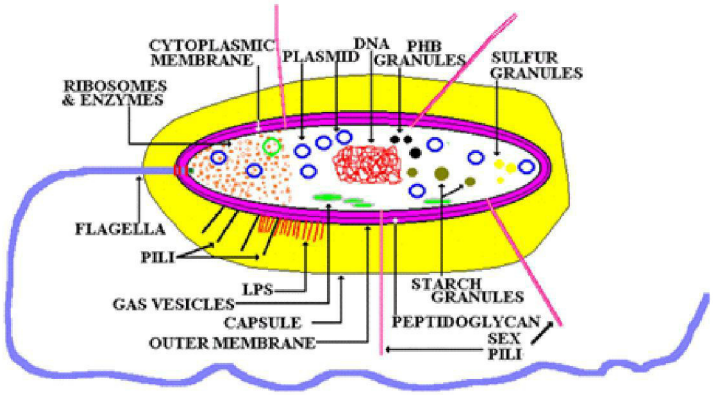


Fig. 5.1 Bacterial Cell Structure

Some bacteria carry filamentous structures protruding from the cell surface,which form the organs of locomotion.These are called as flagella.

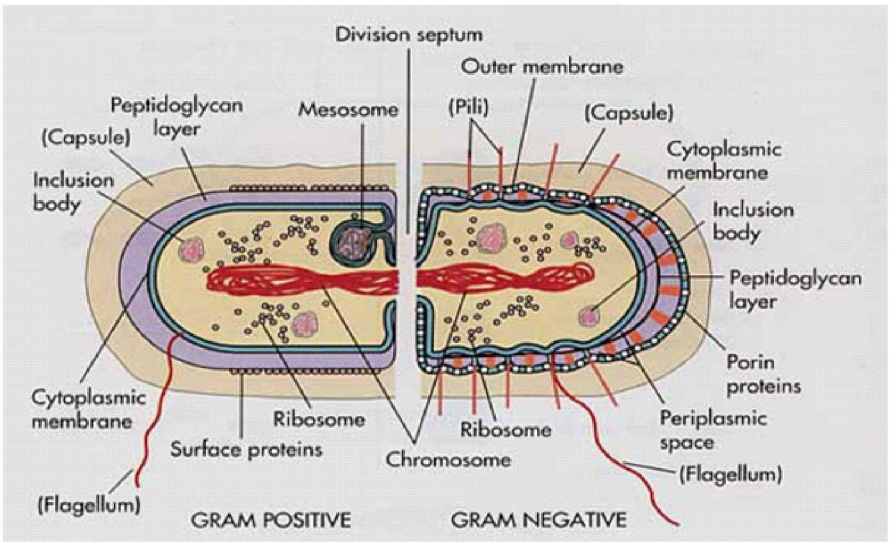


Fig. 5.2 Gram Positive and Gram Negative Bacteria Structure

Fimbriae : These are the organs of adhesion cellwall:

Cellwall is about 10 to 25 micro mtrs in thickness.It maintains the shape and rigidity of bacterial cell. It can not be seen by direct microscope.it can be demonstrated by reaction by Electron microscopy and reaction with specific antibody.Chemically it is made up with mucopeptides(peptidoglycon), N-acetyl glucosamine and N-acetyl muramic acid molecules cross linked by peptide chains.

The cellwall of Gram positive bacteria has simpler chemical nature than Gram negitive bacteria.

Cellwall carries bacterial antigens,which are important in virulence and immunity. The cellwall of Gram negative bacteria has lipopolysaccharide which are associated with endotxic activity. LipidA is responsible for this.

The outermost layer of gram negative has a proteinaceous outer membrane called as porins They form diffusion channels for small molecules.Cellwall synthesis is inhibited by lysozyme.

Cytoplasmic membrane: This is 5 to 10 micro mtrs in thickness.It allows the to and fro movement of metabolites from the cell.

Cytoplasm

This contains ribosomes,mesosomes, inclusions and vacuoles.It is a viscous watery sloution.It stains uniformly with basic dyes.

Ribosomes

These are the centeres of protein synthesis.

Mesosomes

These are the primary sites of respiratory enzymes.They coordinate nuclear and cytoplasmic division

Nucleus

This is seen by the electron microscope. It contains a double stranded DNA arranging in the form of a circle measuring 1000 micro mtre length. Bacteria possess extranuclear genetic elements consisting of DNA called as Plasmids and Episomes. Bacterial nuclei can be demonstrated by acid or ribonuclease hydrolysis and subsequent staining for nuclear material. Bacterial nuclei have no nuclear membrane or nucleolus.

Capsule and Slime layer

Many bacteria secrete a viscid material around the cell surface.When this viscid material is organised into a sharply defined structure, it is known as capsule.When the material not forms a defined structure but releases loose secretion,then it is called as a slime layer.capsules which are too thin and seen under light microscope are called as microcapsules.

Capsular material is antigenic in nature and may be demonstrated by serological methods.

Flagella

The organs of locomotion of bacterial cells are called as flagella.Each flagella has a hook,filament and a basal body. They are 3 to 20 micro mtrs long and possess a protein called flagellin. Flagella have antigens and antibodies which are useful in serodiagnosis. Flagella of different genera of bacteria will have the same chemical composition but are antigenically different. Flagellar antibodies are not protective but are useful in serodiagnosis.

Arrangement of Flagella

1. Peritrichous : Flagella may be arranged all around the cell
2. Polar : Flagella are arranged at both ends of the cell
3. Monotrichous : The polar flagella may be single as in cholera vibrio
4. Lophotrichous : Polar flagella may be in tufts as in spirilla
5. Amphitrichous : Flagella are present at both the poles

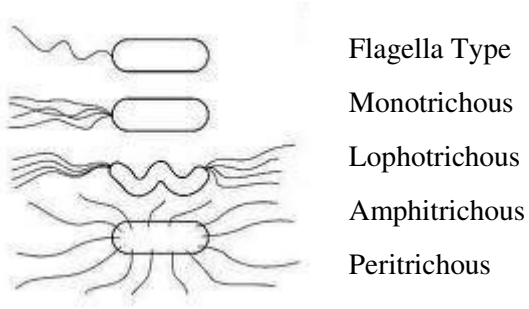


Fig 5.3 Arrangement of Flagella

Bacterial Spore

The highly resistant resting stage of bacteria is called as bacterial spore.Each bacterium forms one spore which on germination forms single vegetative cell. Bacterial spore formed inside the bacteria are called as the endospores.Spores are not a method of reproduction. They are formed due to depletion of nutrients in adverse conditions.

The bacterial spore has a core and a nuclear body surrounded by a spore wall. Again the spore wall is surrounded by the spore cortex,which in turn is enclosed by a multilayered tough spore coat. The shape and the position of the spore are characteristic for each species.

Types of Spores

1. Central or equatorial
2. Subterminal
3. Terminal
4. Oval or Spherical
5. May or may not distend the bacterial cell

Spores are resistant to boiling for prolonged periods.Spores are destroyed by autoclaving at 121degreeC for 15 minutes.

Sporulation helps bacteria to survive for long periods under unfavourable conditions.Spores germinate to form the vegetative bacterium.

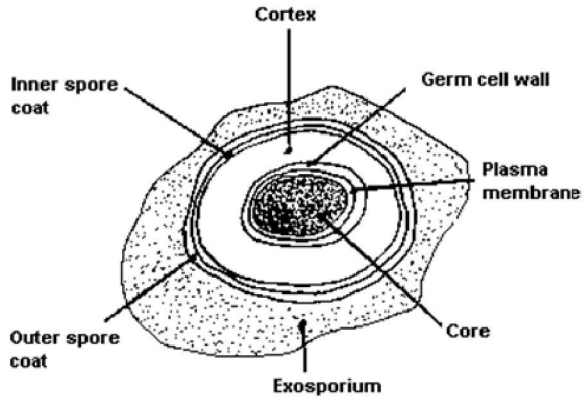


Fig. 5.4 Bacterial Spore

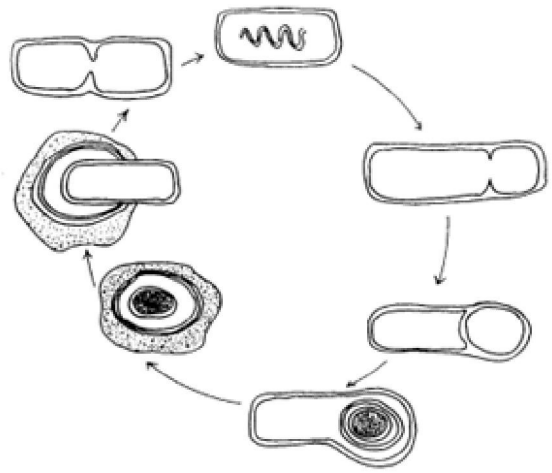


Fig. 5.5 Cycle of Spore Formation and Germination

Key Terms

Aerobes : Bacteria which requires oxygen for their growth
Anaerobes: Bacteria which do not require oxygen for their growth
Obligate anaerobes: Bacteria which die on exposure to oxygen
Microaerophilc bacteria: Bacteria which grow in the presence of low oxygen tension
Cocci: Spherical shaped bacteria
Bacilli : Rod shaped bacteria
Spore : Resistant resting stage of bacteria
Endospore: The spore formed with in the bacteria

Short Answer Type Questions

- Write the two functions of cellwall
- Mention two important functions of nucleus
- Write about ribosomes
- Write about Flagella
- Name the types of flagella
- What is spore? Name the type of spores.
- What is Cocci & Bacili?
- Expand DNA & RNA

Long Answer Type Questions

- Describe the morphological classification of bacteria
- Draw the neat diagram of bacteria and mark the different parts of it
- Explain about the bacterial spore and its types
- Write about the following
  a)Nucleus b) Mesosomes c) Cellwall

UNIT 6

Micro Biological investigation

Structure

6.1 Introduction

Diagnosis of bacterial diseases should preferably be made by direct method e.g., by finding of bacteria in smears of specimens or by isolation of the organisms by culture. Indirect methods are also helpful in many cases.

Succesful diagnosis depends on the selection of specimen, its time of collection in proper way followed by quick transport of the specimen to the laboratory. If delay in transport is inevitable,it should be refrigerated and sent in cold condition or be collected and sent in transport media.

6.2 Collection of Specimen

The following general rules may be followed

Time of Collection : Specimens particularly for culture should be collected before any anti-microbial agents have been used.If antibiotics have already been started,information should be given to the microbiologists particularly when blood for culture is wanted.Stage of the disease when the sample is collected is also very important for succesful diagnosis.

Site of Collection : The specimen should be from those sites where the suspected organismsare most likely to be present with as little external contamination as possible.

Amount of Specimens to be collected : A sufficient quantity of the specimen should be collected in proper container for complete examination.

A strict precaution should be taken during collection so that the inner sides of the container are not soiled by any sort of external contamination.

Labeling: The specimen should be properly labled with particulars of the patient e.g.name,age,address,along with patient's signs and symptoms and other relevant information including type of investigations asked for.

Transport of specimen: All specimens should be sent to the lab quickly where it should be processed without delay. If delay is inevitable,the samples may be kept in refrigerator before they can't be transported to the lab.

Methods Of Collection Of Clinical Specimen For Micro-Biological investigation

Sputum: Coughed sputum instead of saliva is collected in a wide mouthed sterile container.In case of children who cannot cough out sputm but swallow it, a gastric aspirate may be taken instead of sputum.

Urine: For diagnosis of acute urinary tract infection mid stream morning sample of urine is advised.The extermal genitalia is washed with soap and water and when the patient voids

urine the first and the last part of urine are avoided and only the mid stream urine is collected in a wide mouthed sterile container.

Swab: A swab is cotton tipped applicator stick contained in a cotton-plugged test tube.Specimens are collected by swabs,preferably from deeper tissues avoiding the surface.
Stool: The stool sample is collected in a sterile container and send to the lab without delay.If delay is inevitable help of transport media is taken.Eg.V.R fluid for cholerae,Cary Blair transport media for pathogenic members of the enterobacteriacae family.
Blood : It is collected from vein and kept undisturbed till serum is seperated.Serological test can be done with that serum. Understrict sterile conditions about 5-10ml of blood is drawn by vein puncture and transferred to blood culture bottle containibg 50ml of 1% glucose broth.The dilution of blood should be 1 in 50 to 1 in 10.Various anticoagulants may be added.
Cerebrospinal Fluid : Under strict aseptic procedure the Cerebrospinal fluid is usually collected by lumbar puncture in 3 test tubes.One is sent for biochemical test,second for culture and third used for staining micriscopical examination for total and differential cell count.
Aspiration : Abscess and closed wound pus can be collected by aspiration from lesions by a sterilized syringe and needle.

Sputums Petroffs method of Concentration
This is the most widely employed method.The sputum is incubated with an equal volume of 4% sodium hydroxide at $37^{o}C$ with frequent shaking till it becomes clear,on an average for 20 minutes.It is then centrifuged at 3000rpm for 30 minutes and the sediment neutralised with 0.1 NHCL and used for smear,culture and animal inoculation.

Key Terms

- Clinical Specimen : The sample collected for the detecting the presence of pathogen.
- Sputum : The sample consisting of mucus coughed out from the lungs but not from the salivary glands.
- Swab : A pad used for cleaning a wound or taking liquid from the body for testing.

Short Answer Type Questions

- How do you collect sputum for microiological investigation?
- Write about procedure for collection of urine ?
- What is swab and mention its prominence?
- How do you collect the blood specimen for microbiologcal investigation?
- Write about the petroff's method of sputum concentration
- Name different clinical specimens for microbiological investigation?

Long Answer Type Questions

- What are the general rules followed while collecting the specimens?
- How do you collect various clinical specimens for microbiological investigation?

UNIT 7

Processing of Clinical specimen

Structure

7.1 Preparation of Direct Smear and Staining
7.2 Different techniques of Inoculation
7.3 Hanging drop preparation and its use
7.4 Preparation and Inoculation of various media

7.1 Preparation of Direct Smear and Staining

Clinical specimens collected when converted into thin films on the glass slide either with a slide or with a match stick(in case of sputum) is called as a smear.And the prepared smear is subjected to related staining. For the staining refer staining chapter.

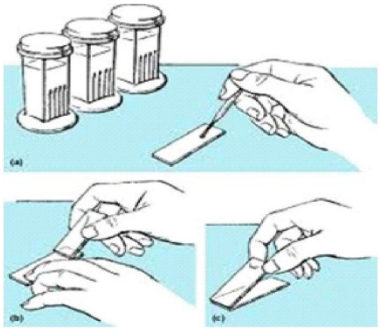


Fig. 7.1 Preparation of Direct Smear

Collection Of Specimen : Specimens are taken with two sterilized swabs,which are rubbed on the affected site under direct vision by using the tongue depressor. One swab is used for making smears and the other for culture. For collection of clinical specimen refer the collection of clinical specimen for microbiological inestigation

Smear Examination

Prepared smears are stained by the following methods and examined under oil immersion lens.

I. Gram's Stain: Useful for detection of streptococcus, staphylococcus, haemophilus, corynebacterium, etc.

II. Alberts Stain: For demonstration of metachromatic granules of C.diptheriae,though confirmation of toxigenic C.diphtheriae cannot be done on this basis only.

7.2 Different Techniques of Inoculation For Isolation Of Bacteria

- The streak culture(surface plating)
- The lawn or carpet culture
- The stroke culture
- The stab culture
- Pour plate culture
- Liquid cultures

The Streak Culture: This method is routinely employed for the isolation of bacteria in pure culture from clinical specimens.With platinum loop,one loopful of the specimen is transferred onto the surface of a well dried plate,on which it is spread over a small area at the periphery. The inoculum is then distributed thinly over the plate by streaking it with the loop in a series of parallel lines, in different segments of the plate. The loop should be flamed and cooled in between the different sets of the streaks. On incubation growth may be observed.

The Lawn or Carpet Culture Culture: Lawn cultures are prepared by flooding the surface of the plate with a liquid culture or suspension of the bacterium.Alternatively,the surface of the plate may be be inoculated by applying a swab soaked in the bacterial culture or suspension. The lawn culture provides a uniform surface growth of the bacterium and is useful for bacteriophage typing and antibiotic sensitivity testing.It may also be employed when a large amount of growth is required in solid media as in the preparation of bacterial antigens and vaccines.

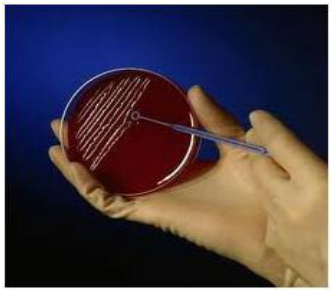


Fig. 7.2 Streak Plate Culture

The Stroke Culture: These cultures are made in tubes containing agar slopes(slants) and is employed for providing a pure growth of the bacterium for slide agglutination and other diagnostic tests.

The Stab Cultures: These cultures are prepared by puncturing with a charged long,straight wire into a suitable medium such as nutrient gelatin or glucose agar.

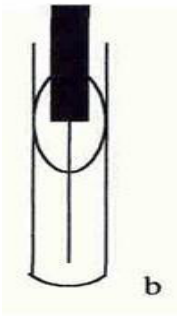


Fig. 7.3 Stab Culture

Stab cultures are employed mainly for demonstration of gelatin liquifaction and oxygen requirement of bacterium under study.They are also used in the maintenance of ssstock cultures.

Pour Plate Culture: Tubes containing 15ml of the agar medium are melted and left to cool in a water bath at 45-50^{o}C.Appropriate dilutions of the inoculum are added in 1ml volume to the molten agar, mixed well,and the contents of the tube poured into a sterile petridish and allowed to set. After incubation,colonies will be seen well distributed throught the depth of the medium.The pour plate method gives an estmate of the viable bacterial count in a suspension and is the recommended method for quantitative urine cultures.

Liquid Cultures: Liquid cultures in tubes,bottles or flasks,may be inoculated by touching with a charged loop or by adding the inoculum with pipettes or syringes.Large inocula can be employed in liquid cultures and hence this is the method adopted for blood culture and for sterility tests.

The major disadvantage of liquid culture is that it does not provide a pure culture from mixed inocula.

This is essential for the identification of the organism by performing different tetsts, e.g.staining,biochemical tests,motility test,serological tests.

Isolation of Micro-Organism

Isolation can be done by inoculating the specimen on solid media or liquid media.

Methods of Inoculating in Solid Media: If any ordinary media or an enriched media is used, the surface of media is dried well by keeping it in an incubator.For this the lid of the petri-dish is placed looking upwards and the plate containing media are kept in slanting way inside the lid keeping the media surface looking downwards obliquely.

A platinum loop is well sterilized by keeping it vertically on a flame and making it red hot and allowed to cool.The loop is touched with well mixed specimens and rubbed on one side of dry media in a little area.The loop is again sterilized and the plate is rotated for 90. The loop is rubbed 3-4 times on media touching a little part of media of previously inoculated side and non-inoculated area.Again the plate is rotated and the above process repeated.

7.3 Hanging Drop Preparation

Aim : To study the morphology and motility of bacteriain the given suspension.

Requirements : Cavity slide,Coverslip, Vaseline, Microscope and the Bacterial suspension to be examined.

Procedure

- Vaseline is applied to the four corners of a clean coverslip.
- Using a sterile loop,a loopful of the given suspension is placed on the center of the coverslip.

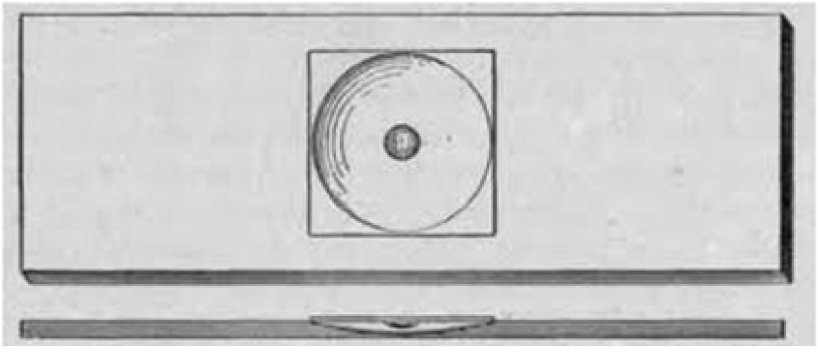


Fig. 7.4 Top View

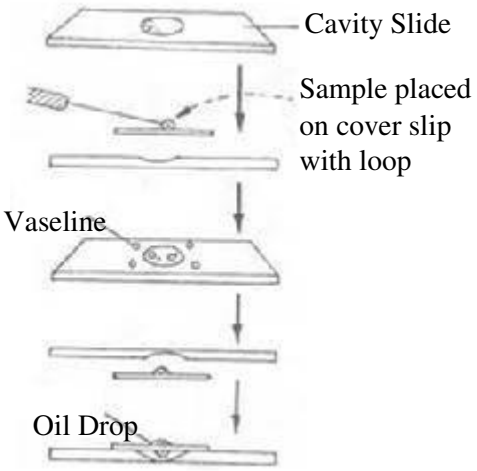


Fig. 7.5 Cavity Slide Preparation

- A cavity slide is inverted over the coverslip so thet the drop of suspension is in the center of the cavity.
- The slide is quickly and carefully turned over so that the cover slip is on the top with the drop hanging in the cavity.
- The microscope is adjusted for reduced light by lowering the condenser and using the concave mirror.
- The edge of the drop is focussed under low power.The microscope is then turned to high power to observr the morphology of the bacteria.
- The shape and motility of the bacteria in thr given suspension are observed.

Short Answer Type Questions

- How do you prepare the direct smear?
- What is inoculation?
- What is the use of hanging drop preparation?
- Explain about the following:
  a) Streak culture b) Lawn or carpet culture c) Stroke culture d) Stab culture
  e) Pour plate culture f) Liquid cultures

Long Answer Type Questions

- Write about the preparation of direct smear and staining.
- Write about the different techniques of inoculation for isolation of bacteria
- Write about the Hanging drop preparation and its use

UNIT 8

Staining Techniques

Techniques

8.1 Introduction

Live bacteria do not show much structural detail under the light microscope due to lack of contrast. Hence it is customary to use staining techniques to produce colour contrast. Bacteria may be stained in the living state. However, many dyes are toxic and kill the cell on staining. This type of staining during which the cell is killed is known as supravital staining. Non-toxic staining during which the cells retain their viability is known as vital staining. Routine methods of staining bacteria employ drying and fixation of smears-procedures that kill them. Bacteria have an affinity for basic dyes due to acidic nature of their protoplasm. Staining techniques in common use in bacteriology are the following

- Simple staining
- Grams staining
- Zeihl Neelson staining
- Negative staining

Fig. 8.1 Simple Staining

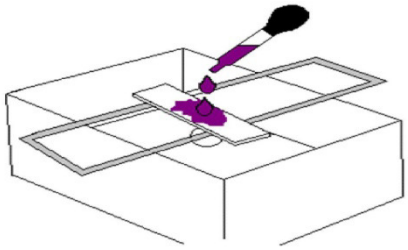


Fig. 8.2 Method of Simple Staining

8.2 Simple Staining

Definition : The staining which uses the simple staining reagents is called as the simple staining.

Composition : (Composition and Preparation)

Loefflers Methylene blue reagent orDilute Carbol Fuschin reagent

Procedure

1. Take the clean grease free microscopic slide
2. Mark the specimen
3. Mark 2 lines in the centre of the slide for preparing smears.
4. Smears are made on the opposite side of the line.
5. Loop is sterilized by flaming till it becomes red hot. Allow it cool near the flame
6. Place a loop full of emulsion in the centre of lines and make a uniform smear
7. Strerlize the loop and keep it a side
8. Smear is allowed to dry in air
9. Fix the smear by passing through flame 3-4 times
10. The slide is placed on the rack and is flooded with Loefflers methylene blue or Dil.Carbol Fuschin
11. The stain is allowed to act for 3 minutes for methylene blue and 30 seconds for Dilute.Carbol fuschin respectively
12. The slide is then washed with distilled water and gently blotted to dry
13. A drop of cedar Wood oil/Liquid parrafin is placed on the smear
14. The microscope is adjusted for increased light by raising the condenser,and slide is examined with oil immersion objective using the plane mirror.

Result

- Only the presence of bacteria can be detected by this staining.
- The bacteria can not be differentiated by this staining.

8.3 Grams Staining

The staining technique which differentiates bacteria into gram positive and gram negative bacteria is called as the gram staining

Reagents (Composition and Preparation)

1.Crystal voilet stain

Solution A

i) Crystal voilet : 2g
ii) Ethyl alcohol : 20ml

Solution B

i) Ammonium oxalate : 0.8g
ii) Distilled water : 80ml

Mix solutions A and B. Keep for 24 hours and filter. Store in an amber coloured dropping bottle.

2. Grams Iodine solution

(i) Iodine : 1.og
(ii) Potassiun iodide : 2g
(iii) Distilled Water : 100ml

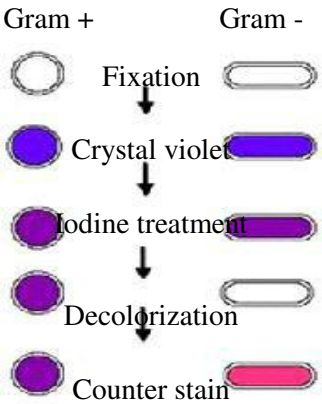


Fig. 8.3 Steps of Gram Staining

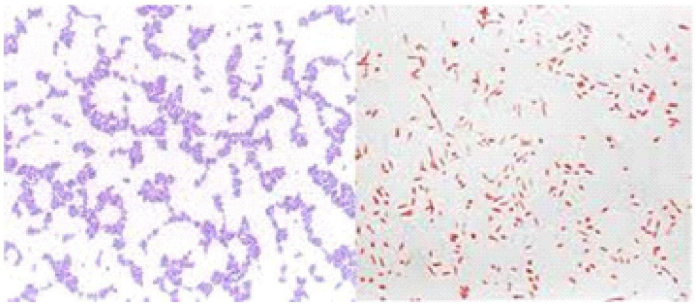


Fig. 8.4 Gram Positive & Gram Negative Bacteria

Store in amber coloured bottle

3. Decolouriser

Mix 95% alcohol and acetone in equal proportion.Store in a white dropping bottle.

4. Safranine solution

a) Safranine O : 0.34
b)Absolute alcohol : 10ml
c)Distilled water : 90ml

Dissolve the saffranine in alcohol and then dilute it with distilled water.Filter and store in an amber coloured dropping bottle.

Procedure

1. Primary Staining

The fixed smear is covered with gentian voilet for 1 minute and washed with water.

Gentian voilet is basic dye which combines chemically with the cytoplasm of the bacterial cell.It renders all organisms voilet

2. Mordanting
It is then covered with Grams Iodine,kept for 1 minute and washed with water.

Grams iodine fixes basic dyes to the smear,and thus acts as a mordant

3. Decolourisation
The smear is covered with alcohol for a few seconds and is washed withwater immediately Alcohol acts as a decolouriser,Gram positive organisms retain the primary dye while Gram negative organisms get declourised

4. Counter Staining
The smear is then covered with Dil carbol fuschin,kept for 1 minute and washed with water. Using filtr paper the slide is gently blotted to dry

A drop of Cedar wood oil/Liquid paraffin is placed on the smear.
The microscope is adjusted for increased light by raising the condenser and the slide is examined under the oil immersion objective using the plane mirror.

Result
Gram positive organisms remain as voilet,while Gram since negative organisms are in decolourised state take up the counter stain and turn pink.

Gram Positive – Violet color

Gram Negative- Pink color

8.4 Zeihl Neelsons Staining
This staining technique is used to detect the presence of Acid Fast bacilli. In this sputum smear is used. The organisms such as Mycobacterium tuberculosis and Mycobacterium leprae are extremely difficult to stain by ordinary methods because of the lipid containing cellwalls. Thats why this staining technique was used to stain the acid fast bacilli.

This staining consists of two methods

- Hot staining method
- Cold staining method.

I. Ziehl Neelson Hot staining method

This staining technique was used to detect the presence of M.Tuberculosis
Specimen-Sputum
Reagents(composition and Preparation)
1.Stock carbol fuschin staining solution:

Solution A

(i) Basic fuschin powder :3g
(ii) 95%v/v ethyl alcohol :to 100ml

Dissolve the powder in alcoholby using mortar and pestle.If necessary heat carefully in aboiling watrbath

Solution B

50 % w/v phenol solution
Mix both the solutuin A and B before using

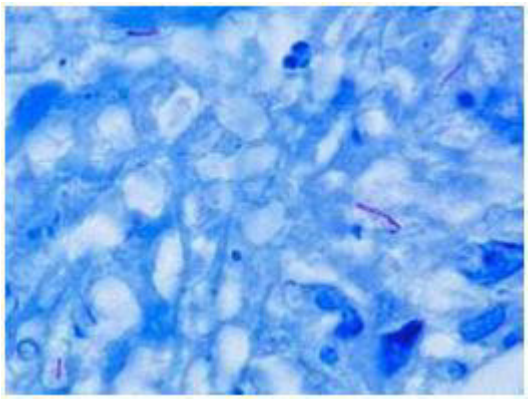


Fig. 8.5 Mycobacterium Tuberculosis with Hot Staining Method

2. Working carbol fuschin staining solution:

Mix 10ml of solutionA with 90 ml of solutionB.Keep at room temperature overnight,filter and store in amber coloured dropping bottle.

3. 20%v/v Sulphuric acid
4. Methylene blue counter stain

(i) Methylene blue: 0.3g
(ii) Distilled water: 100 ml

Dissolve the powder in distilled water,filter and store in amber coloured dropping bottle

Procedure

- Prepare the smear with sputum and fix it on a glass slide by passing it over a bunsen flame for few seconds
- Place the heat fixed slide on the staining rack or rods and flood the smear with working carbolfischin stain.
- Heat gently by bunsen burner flame,until steam raises.Avoid boiling and continue heating for about 5 minutes. Do not allow the stain to dry on the slide.Add more stain if necessary
- Wash the stain off the slide with water and continue rinsing until until the water that runs off is colourless
- Declourisation : Cover the slide with 20% sulphuric acid for about oneminute
- Counter staining:Cover the slide with methylene blue stain for one minute
- Wash with tap water,allow ware to drain, and allow it to dry in air or blot carefully
- Microscopic examination:
- Observe the slide under lower power objective and then observe under oil immersion objective.

Results

Acid Fast organisms : Bright red bacilli on blue back ground
Other organisms : Dark blue

II. Zeihl Neelsons Cold Staining Method

This staining technique was used to detect the presence of Mycobacterium leprae.

Fig. 8.6 Myco Bacterium Leprae with Cold Staining Method Reagents

Composition and preparation :Same as for the previous a hot staining technique

Specimen : Skin smears from visible lesions

Procedure

- Flood the fixed smear with working carbol fuchsin stain.Wait for 12-15 minutes without heating.
- Wash the smear with running tap water.
- Decolourize with 5% sulphuric acid for one minute.
- Counterstain with methylene blue for one minute.
- Wash with water, drain and blot dry.
- Observe under low power objective and examine under oil immersion objective.

Result

- M.Leprae:Bright red bacilli on blue background.
- Macrophage cells:Blue

8.5 Negative Staining

The staining technique which is used to demonstrate the bacterial capsule in which the bacteria will never get stained is called as Negative Staining

Fig. 8.7 Bacterium Prepared by Negative Staining

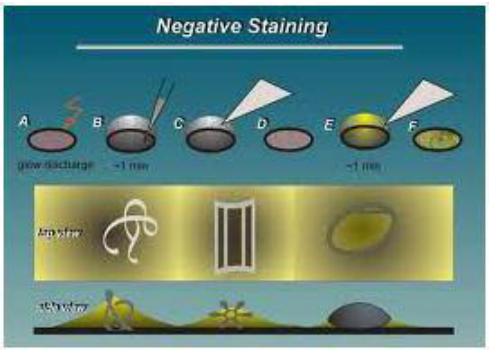


Fig. 8.8 Negative Staining Scheme

Reagents: 10gm/dl nigrosine solutiom.
Sample :A given culture.

Procedure

- Transfer asepitcally a loopful of culture on a clean and dry slide.
- Mix it with loopful stain.
- Make smear by using a glas slide.
- Allow it to dry slowly at room temperature.
- Observe under oil immersion lens.

Results

- Background:Bluish Black
- Organisms:Colourless (hollow bodies)

Key Terms

Staining : The process of using a dye or chemical to impart colour to the bacteria in order to see the important components of the bacteria.

Simple Staining : With this presence of bacteria can be known by using simple dye

Gram Staining : With this Bacteria are classified into gram positive and Gram negative bacteria.

Negative Staining : In this bacteria remains unstained.Bacterial capsules and spirochaetes can be observed with staining.

Ziehl Neelson Staining : With this Acid Fast bacilli can be observed

Short Answer Type Questions

- What is the importance of simple staining?
- Mention the colours of Gram positive and Gram negative bacteria?
- What are the steps involved in gram staining?
- Give two examples for Acid Fast Bacilli?
- How do you detect Mycobacterium leprae?
- Name the reagents used in Gram staining?
- Define staining?
- What is negative staining?
- What is the color of AFB?

Long Answer Type Questions

- Explain about the Gram staining technique.
- Explain about the Ziehl neelson hot staining method
- Write about the Simple staining and Negative staining
- Explain about the Ziehl Neelson Cold staining method

UNIT 9

Culture media

Structure

9.1 Introduction

It is essential to grow the organisms from infected material to identify the cause of infection.Only after growing them and isolating them in pure culture,it is usually possible to identify them.For studying their characteristics as well,it is necessary to culture them.

The majority of bacteria to be studied are pathogenic. Hence to obtainsuitable growth of bacteria,the culture media should approximate to the composition and reaction of the tissues and body fluids in which these bacteria grow. No single medium can satisfy all the requirements.The food requirements of bacteria vary with their natural environments and the particular role they play in nature.They derive their energy by oxidation and by decomposition of food material such as proteins and carbohydrates

It was Louis Pasteur who first introduced the use of complex media Blood,chicken and meat broth were in use.All were liquid media and though growth could be obtained, growth characteristics and purity of cultures could not be made.Robert Koch introduced solid media. On the suggestion of Frau Hesse, Robert Koch Introduced agar agar as a base for preparing solid media. Peptone, Blood, Serum, Yeast extract are the other ingredients

A Culture medium can be defined as an artificially prepared substance containing various microbial nutritional elements at optimum pH,which will allow growth of most of the microorganisms.

Classification of Media

A. Media have been classified into various ways according to the consistency.
   a) Liquid Media
   b) Solid Media
   c) Semi-Solid-Media

Liquid Media

Liquid Media are used for particular sub-culturing and bio-chemical tests like motility test,sensitivity test.However,in liquid media,the growth usually does not exhibit special characteristic appearence.

When there is more than one type of organism,they cannot be seperated by growing in liquid media.

Solid Media

Growth usually shows special characteristic appearance that helps in identification of the organism.

Micro-organism can be separated with certainty from mixtures by growth in solid media.

Semi-Solid Media: It enables motile organisms to spread. So a motile organism can be separated from a non-motile organism.

9.2 Simple media or Basal media

Simple media or Basal media contains only the basic requirements for growth of bacteria

a) Peptone water
b) Nutrient agar
c) glucose broth

Peptone water Compostion

Peptone: 5 g
Sodium chloride:5g
Water: 500 ml

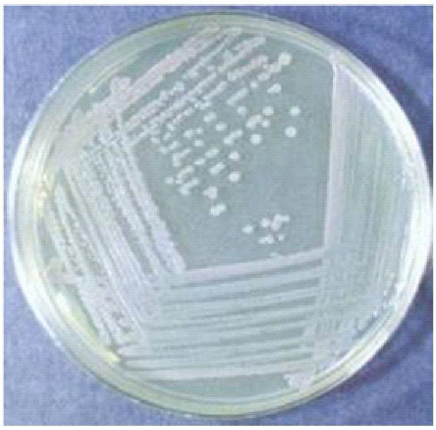


Fig. 9.1 Growth on Basal Media

Preparation: Dissolve the ingredients in warm water.Adjust the PH to 7.4-7.5 and filter.Distribute 5ml in test tube and autoclave at 121^{o}C for 15 minutes

Uses

For subculturing the gram negative bacillifor sugar fermentation tests
For Indole test

Nutrient Agar	
Composition	
Peptone	5.0g
Beefextract	3.0g
Sodium chloride	8.0g
Agar	15.0g
Distilled water	1000ml

Preparation:

- Place the solid ingradients(except agar) in one litre conical flask
- Add about 8ooml of distilled water
- Dissolve the ingradients by using a glass rod.If necessary heat the solution to dissolve the chemicals
- Adjust to Ph7.3 by using 1N sodium hydroxide or 1N hydrochloric acid
- Add agar and boil the solution for 1 or 2 minutes
- Make volume 1000ml by adding distilled water.
- Distribute in conical flasks(100ml),bottles and tubes.Sterilize by autoclaving at 121degreeC for 15 minutes.

Uses

- Used as basic culture medium in the preparation of blood agar and other media
- Also used in the form of slope medium to subculture pathogens isolated on carbohydrate containing media
- Used to maintain Cultures of control organisms in semisolid form and in solid form

Glucose broth:
Composition:

peptone --10g

Meat Extract --10g

Sodium chloride--5g
Glucose-------
- 10g

Water------- 1000ml

Preparation

- Mix the ingradients and dissolve them by heating .Adjust the PH to 7.5-7.6. Distribute 5ml in test tubeand autoclave at 121degreeC for 15minutes

Uses:

- Supports the growth of microorganisms that do not have special nutritional requirements
- To maintain the stock cultures of control strains of bacteria

a) Enriched Media

- Blood agar medium
- Loefflers serum slope
- Chocolate agar medium
- Blood agar medium:

Composition:

Nutrient agar	-	500ml
Sterile defibrinated blood	-	25ml

Preparation

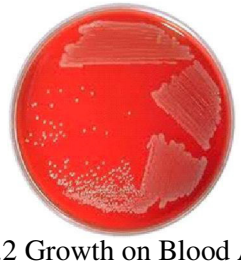


Fig. 9.2 Growth on Blood Agar Media

Preparation

- Prepare and sterilise glucose broth(refer preparation of glucose broth)
- Add sterile serum and mix well
- PH of the medium should be adjusted to 7.0-7.4
- Dispense 2.5ml amounts in test tubes
- Inspissate at 75degreeC for 1 hour in a sloped position
- Inspissate the next day for further 1 hour at 75degreeC

Uses

This medium is mainly used to culture the corynebacteriun diphtheriae to show the volutin granules.

Chocolate Agar medium

Composition:

Nutrient agar	100 ml
Sterile sheep blood	10 ml

Preparation:

Melt 100ml nutrient agar,cool it in a water bath at 75degreeC.Add 10ml sterile sheep blood. Mix blood and agar by gentle agitation from time to time,until it becomes brown in 10 minutes.

Uses

Used to grow Gonococci, H.influenza, Pneumococci.

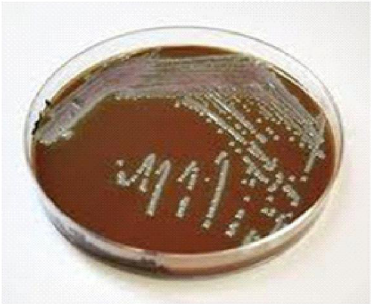


Fig. 9.3 Growth on Chocolate Agar (Enriched Media)

9.4 Differential Media

Mac Conkeys Agar Medium

Composition:

Peptone	20g
Sodium Taurocholate	5g
Agar	20g
Water	1000ml
Lactose(10%)	100ml
Neutral red solution(2% in 50% Ethanol)	3.5ml

Preparation:

- Dissolve peptone and taurocholate in water by heating and cool, adjust PH to 7.5
- Add agar and dissolve it
- Adjust PH to 7.5
- Add Lactose and neutral redwhich should be well shaken before use Autoclace at 115oC for 15minutes
- Pour the medium into plates
- It should be reddish brown(15ml per plate)

Fig. 9.5 Lactose +ve & -ve Bacteria on Maconkeys Agar Medium

Uses

- Used for differentiating lactose ferminting bacteria from non-lactose fermenting bacteria
- Media for biochemical reactions.

9.9 Anaerobic Media

Anaerobic organisms which can not grow in the presence of oxygen can be grown in suitable media by using oxygen free environment, in which reducing substances are added to remove oxygen and also to maintain anaerobic atmosphere.

- Robertsons cooked meat medium
- Thioglycolate broth

Robertsons cooked meat medium

Compostion

Fresh bullock heart	500g
Water	500ml
Sodium hydroxide	1.5ml

Preparation:

Mince the meat,Place alkaline boiling water and simmer for 20 minutes to neutralise the Lactic acid.Drain off the liquid.While still hot,press the minced meat in a cloth and dry completely.Place the dried minced meat in big test tube to a depth of 2.5cm.And add Nutrient broth in such a way the broth column will be atleast 1cm above the meat particles.

Fig . 9.21 Robert Son Cooked Meat Media

Now autoclave these tubes at 121oc for 20 minutes.A tall column meat is essential because conditions are anaerobic only when there are meat particles.

Uses:

Used to culture anaerobes in blood

(ii) Thioglycolate broth:

Compostion:

Yeast extract powder	5.0g
Tryptone	15.0g
Glucose	5.5g
Sodium thioglycolate	0.5g
Sodium chloride	2.5g
L-Cystine	0.5g
Methylene blue or Resazurine	0.001g
Agar	0.5g
Distilled water	100ml

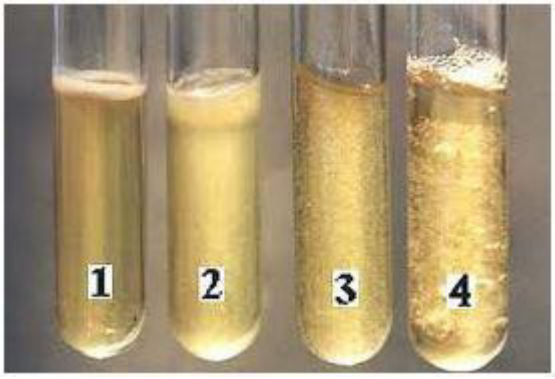


Fig. 9.22 Growth in Thioglycolate Medium

Preparation:

Heat the solid ingradients in distilled water until clear solution is obtained. PH should be adjusted to PH7.2. Dispense in 10ml amounts in test tubes and sterilise by autoclaving at 121oC for 15 minutes. Store at room temperature in a cool and dark place.

Uses:

Used to culture anaerobes in blood

9.8MediaforBiochemicalReactions

Basedonbiochemicalreactionsthebiochemicaltestswereperformed using variousmedia, thesebiochemicaltestwhichinturnidentifiesthevarioubacteria.

1) IndoleTest

MotolityIndoleureaMedium:

Composition:

Tryptone	30.0g
Potassiumdihydrogenphosphate	1.0g
Sodiumchloride	5.0g
Agar	4.0g
0.25%v/vphenolred	2ml
Distilledwater	900ml
20g/dlurea(indistilledwater)	

Fig.9.11IndoleProductioninIndoleTest

Preparation

- Placethedryingradientsinaconicalflaskandaddabout900mlofdistilled water.
- Boiltillthecontentsaredissolved.
- Coolto50-55^{o}Candmakevolume 1000mlbyadding20g/dlureasolution.
- PHofthesolutionshouldbeadjustedto 6.0-7.3.
- Dispensein95mlamountsinscrewcapbottles.
- Autoclaveat121^{o}C for15minutes.
- Dispenseasceptic allyinpetridishesaftercoolingto about50-55^{o}C.

Uses:

UsedtodifferentiateEnterobacteriaspecies

2)**<u>Voges-ProskaurTest</u>**

Glucosephosphatepeptonewatermedium:

Composition:

Peptone	5g
Dipotassiumhydrogenphosphate	5g
Glucose	5g
Distilledwater	1000ml

Preparation :

Dissolvetheingradientsindistilledwaterandadjustthe PH7.6.Dispense themediumin5mlamountsintoatesttubeandauto-claveat121°Cfor 15 minutes.

Uses

UsedtoassistinthedifferentiationofEnterobacteria

2) MethylRedTest

Glucosephosphatepeptonewatermedium:

Composition

Peptone	5g
Dipotassiumhydrogenphosphate	5g
Glucose	5g
Water	1000ml

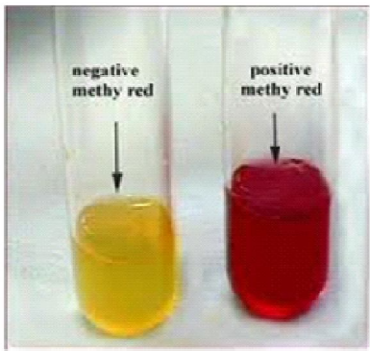


Fig.9.13MethyleRedTestwith+ve&-veresult

Preparation

Dissolvetheingradientsindistilledwaterandadjustp Hto7.6.Dispense themediumin5mlamountsinatesttube.Auto-clavethemediumintesttubesat 121°Cfor15minutes.

Uses

ThistestisperformedtodifferentiatetheEntrobacteria

4)CitrateUtilisationTest

SimmonsCitrateMedium

Composition:

Sodiumchloride	5.0g
Magnesiumsulphate	0.2g
Sodiumcitrate	2.0g
Dipotassiumphosphate	1.0g
Ammoniumdihydrogen phosphate	1.0g
Agar	20.0g
0.4%w/vbromothymolblue	4.0ml
Distilledwater	1000ml

Fig.9.14CitrtateTestwithPositiveandNegativeresult

Preparation

Dissolvetheingradientsindistilledwaterbygentleheat.PHofthemedium shouldbeadjustedto6.9.Dispensein5mlamountsincottonplugged tubes. Sterilizebyauto-clavingat121^{o}Cfor15minutes.Allowtosolidifyinslanting posotion.

Uses:

UsedtoidentifythepathogenicbacteriasuchasEnterobacteria,Serraria, and Klebsiella.

5)**<u>UreaTest</u>**

MotilityIndoleUreaMedium

Forcomposiotion,preparationandusesreferIndoletest

6)**OxidaseTest**

Requirements

i) Oxidasereagent:

1.0g/dltetramethyl-p-phenylenediaminedihydrochlorideindistilledwater(it shouldbepreparedfreshifitappearsblueincolour)

ii) **FilterPaperStrips**

Fig.9.15Leftsidewith+vetest&Rightwith-vetest(Ureasetest)

Fig.9.16Oxidase+veTest

Uses

Usedtohelpintheidentificationoftheorganismswhichproduceoxidase enzyme

7)**CatalaseTest**

NutrientAgarSlope:

Composition

Peptone: 5.0g

Beefextract: 3.0g

Sodiumchloride :8.0g

Agar: 15.0g

Distilledwater :1000ml

Fig.9.17CatalasePositiveTest

Preparation

1. Dissolveallthe ingradientsindistilledwaterbyheatingandallowthemto coolbykeepingthedispensedtesttubesinslantingposition.
   10vol Hydrogen peroxide 1ml

Uses:

Usedtodifferentiatecatalaseproducingbacteriafromnon-catalase producingbacteria.

8) NitrateReductionTest

i) Nitratebroth:

Composition:

Potassiumnitrate 0.2g

Peptone 5.0g

Distilledwater 1000ml

Preparation

Dissolveallthe ingredientsindistilledwater.AdjustthePHto7.4.Dispense themediumin5mlamountsintesttubeandsterilizeby autoclaving at121oCfor 15minutes.

TestreagentispreparedwithsolutionA(SulphanilicAcidin5nAcetic Acid)solutionB(AlphanaphthylAminein5nAceticAcid)beforeuse.Add1 mlofthisreagenttothetestculture.

Formationofredcolourindicates+vetestotherwise –vetest

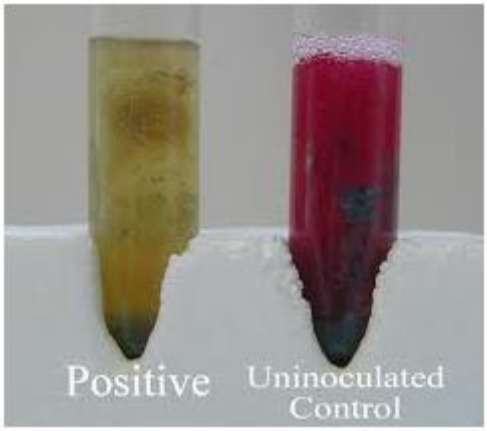


Fig.9.18NitrateReductionTestResults

Uses

ThistesthelpstodifferentiatemembersoftheEnterobacteriaceaethat producetheenzymenitratereductase,fromGramnegativebacteriathatdonot producetheenzyme.

9) PhenylalanineDeaminationTest

PhenylalanineAgarMedium

Composition:

YeastExtract	:3g
DL-Phenylalanineor	:2g
L-Phenylalnine	:1g
Disodiumhydrogenphosphate	:1g
Sodiumchloride	:5g
Agar	:12g
Distilledwater	:1000ml

Preparation:

Dissolveallthe ingredients in distilled water, if required by heating. Distribute and sterilize by auto clavingat121oCfor15minutes.Allowtosolidifyintubesas longslopes

Fig.9.19GreenColourwith+vetest,Otherwise-vetest

Uses

Usedmainlytoassistintheidentificationof Proteus species

10) GelatinLiquifactionTest

NutrientGelatinMedium

Composition:

Peptone	10.0g
Beefextract	3.0g
Gelatin	120.0g
Distilledwater	1000ml

Preparation

Dissolvethesolid ingredientsin100mlofdistilledwaterbyheating.Dispense in7mlamountsinscrewcaptesttubes.Sterilizebyautoclavingat121^{o}C for 15 minutes

Uses

Thistestisperformedintheidentificationoforganismssuchas PseudomonasandVibriocholerae

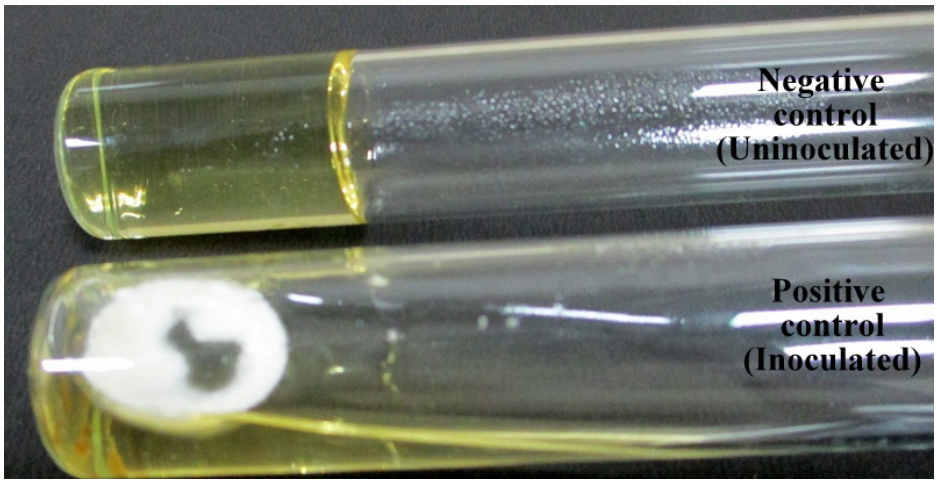


Fig.9.20Tubewith –vetestinthebottomandwith+vetestattopside

Short Answer Type Questions

- Define culture media
- Give the examples for basal media
- Write the composition of peptone water
- Define Enriched media
- What is an enrichment medium?
- Write the examples for enriched media
- Define differential media
- Give the examples for blood culture media
- What is an anaerobic media?
- Write the media for biochemical reactions
- Give the example for fungal media
- Write the examples of ANAEROBIC MEDIA
- Expand IMViC tests

Long Answer Type Questions

- Define Basal media and write composition,preparaton and uses of any two basal media

- Write the composition,preparation and uses of Macconkeys agar medium
- Write the compostion,preparation and uses of Robertsons cooked meat medium
- Write the classification of culture media
- Explain in detail about enriched media with examples

PATHOLOGY

PATHOLOGY

Introduction

The branch of Biological science which deals with the study of diseases nature,its cause and symptoms is known as Pathology.

There will be structural and functional alterations,after patient being infected with microorganism or if there is any abnormality in the normal human being.The symptoms will be produced due to the disturbances in the normal functions of the effected cells of the body which leads to the illness of a person.Pathology deals with the study of disturbed functions and how the effect,how they arise,how they progress and how they effect other cell systems.

Fig. 1 Rudolf Virchow, Father of Pathology

UNIT 10

URINE ANALYSIS

Structure

10.1 Introduction

Analysis of urine physically,chemically and microscopically and even the collection of urine sample is called as the urine analysis.Collection of urine sample also plays an important role.Sterilised and clean containers are used for the collection of urine samples.After collection of the urine sample physical examination of urine sample is the next step in urine analysis.Presence of albumin in urine can be detected carefully with boiling test for albumin.Chemical examination of urine plays crucial role in urine analysis which has prominant role in diagnosing the disease.Ketonebodies,bilesalts,bilepigments and blood presence in urine can be detected by various tests.Microscopic examination of urine is the last step of the urine analysis.Cells,Casts,Crystals and atypical cells can be detected after preparaing the slide preparation from the centrifuged urine sample i.e taking the sediment of urine sample after centrifugation.

10.2 Collection of Urine Samples

Types of Urine Samples

Types of urine samples: a)Single specimen b) 24 Hrs urine specimen

Single Specimen: Urine specimen collected any time randomly is called as Single urine Specimen. It is useful in qualitatives analysis.

24 hrs Urine Specimen: Specimen of urine collected in a period of 24 hrs duration is called as 24 hrs Urine Specimen.It is required in quantitatives determination. A big container is required for collection.Preservative is needed to prevent any charges. Sometime, say 8.am has to be selected in the morning.At that time bladder has to be emptied and urine passed has to be discarded.Subsequently pased urine specimens up to same time in the next morning have to be collected.Container has to be covered and kept in a cool place during this period.

Labelling & Preservatives

Labelling: Labelling of sample plays an important role in medical laboratories.Samples have to be labelled after collection to enable their identification during testing and reporting.Eventhough the volume of sample is more and if it is not labelled due to forgetfullness it is useless.Proper numbering should be marked on the label seeing the previous samples.After labelling on the sample,the same numbers should be noted in the records with patients name.this will avoid the confusion in the identification of patients sample.

Preservatives: The chemicals are used to preserve the urine specimens are called as Preservatives.

Freshly passed urine has to be examined.On standing,chemical changes take place.Bacterial growth also further causes chemical changes.If urine is to be kept prior to examination,it has to be stored at 2-8`C in refregirator.

Different Urinary Preservatives are

1) Conc.HCL	2) 2N HCL	3) Acetic Acid	4) Metaphosphoric Acid
5) Formalin	6) Boric Acid	7) Thymol	8) Toluol
9) Chloroform			

10.3 Physical Examination

- General Appearance
- Quantity (Volume)
- Odour
- Colour
- Specific Gravity

- PH (Reaction)
- Albumin

1. **General Appearence:** Normally freshly voided urine is usually clear, slightly cloudy occasionally.But when it is allowed to stand a faint cloudiness develops due to the settlement of pus cells. RBC's, epithelial cells and mucus. It is allowed for a long time settlement. Then the sediments will increase due to the precipitation of various salts and whole urine will become cloudy with bacterial growth.

Abnormal Findings (Clinical Significance)

Abnormal cloudness develops due to the settelements of phosphates, urates, urinary deposits and occasionally chemicals.

Report: Urine is clear / Cloudy / Turbid with or without sediment.

2. **Quantity (Volume):** Children - infants : 100 - 500ml. / 24-hrs

1- 8 years : 500 - 1000ml. / 24-hrs

Usually 1000 ml. to 1600 ml. volume of urine will be excreted by a normal person per 24-hrs in adults.

Abnormal Findings (Clinical Significance)

1. If volume of urine is more than 500ml, it indicates poly urea,diabetes mellitus,diabetes insipidus.
   If volume of urine is less than 200ml,then it indicates oliguria,aneuria,and renal conditions.
3. **Odour:** Normally urine has Aromatic Odour.

Abnormal Findings(Clinical Significance)

It is ammonical/pungent in cystitis and in urinary retention due to presence of ammonia.Fruity odour is due to presence of ketone bodies in urine

4. **Colour:** Normal urine appears as amber to yellow colour.Colour of the urine is due to urochrome.

Abnormal Findings(Clinical Significanse)

- Cloudy urine is due to phosphates,proteinuria,pus and chyluri.
- Uniform opalescence is due to bacterial growth.
- Smoky colour is due to haematuria.
- Turbideness of urine is due to fat droplets,chyluria and pus.
- Red urine is due to haemaglobinuria and myoglobinuria.
- Deep yellow to brown or greenish brown urine is due to bile pigments namely bilirubin.
- Orange red or orange brown urine containing haemoglobin.Other causes are homogentisic acid and melanin.
- **Specific Gravity**: The presence of various solutes in the urine changes the specific gravity of urine.Specific Gravity depends up on the cincentration of various solutes in the urine.

Specific Gravity of the urine can be measured with:

1) Urinometer 2) Refractometer 3) Can be tested with dip sticks
4) Osmometry:

This method gives the most accurate assessment

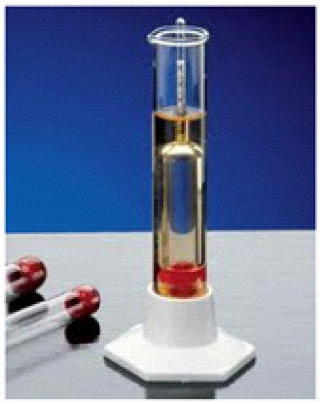
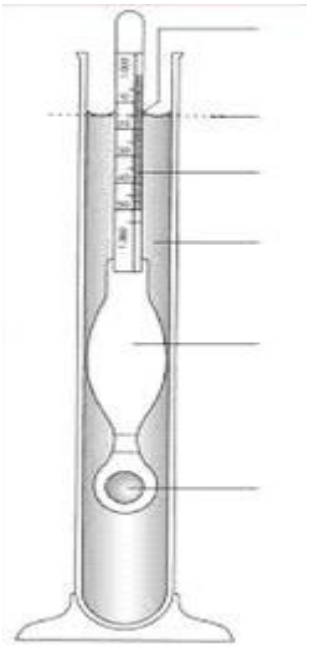


Fig. 10.1 Urinometer



Meniscus

Reading Level

Specific gravity scale

Urine in cylinder

Hydrometer

Mercury bulb

Measurement of specific gravity of urine with urinometer:

Take the urine sample into a beaker up to nearly to full.By means of filterpaper remove the froth of bubbles.Float the urinometer,so that it does not touch the bottom or sides of the beaker.Take the reading from the bottom of the meniscus,If the urine amount is less, dilute the urine to raise the volume upto 70-80ml,take the reading and multiply the last 2 digits by the dilution factor

Normal values: 1.003-1.060

Fig. 10.2 Urinometer in detail

Abnormal findings (clinical significance)

High specific gravity values are found in

Diabetes Mellitus
Fever
Acute Nephritis
Excessive sweating

Low specific gravity values are found in

Chronic Nephritis
Diabetes Nephritis

6. Reaction:(PH)

Reaction indicates the PH of the urine.PH of the urine can be measured with the PH meter and indicated by litmus papers.

Normal Findings:

PH range 5-7(slightly acidic)
To confirm the test,blue and red litmus papers should be dipped in urine.
If blue litmus paper turns to red colour then the urine is acidic in nature.
If red litmus paper turns to blue colour then the urine is alkaline in nature.
Usually specific collected urine shows PH value of 6.0

Fig 10.3 Urine PH meter

Abnormal Findings (Clinical Significance)

PH of urine <4.7 is more acidic

PH of urine>7.5 is alkaline

7. Albumin(Protein): There are 5 main proteins found in the urine.

1) Serum or plasma globulin 2) Serum or plasma albumin
3) Mucus or mucin 4) Haemoglobin
5) Bence jones protein.

There are three main albumin proteins tests found in the urine.Those were
i) **Boiling Test for Albumin (Coagulation test or heat and acetic acid test)**

Procedure: If the collected urine (patients) sample looks turbid, filter it and if it is alkaline make it slightly acidic to litmus by adding few drop of 3% acetic acid. Fill the urine into a clean dry test tube upto 1/3 rd from top portion of the test tube. Boil the urine present in the top one third portion test tube over a spirit lamp. If any cloudiness appears add a few drops of 3% of acetic acid. If the cloudiness appears while the tube is being heated but disappears when it reached the boiling point Bence Jones protein is present. If the cloudiness disappears on addition of 3% acetic acid,it indicates the presence of phosphates in urine. If the cloudiness still remains after adding acetic acid, then it is due to the presence of the album in urine sample. If the urine remains clear no albumin is present.

Fig. 10.4 Boiling Test for Albumin

Normal Findings: Albumin is absent in normal urine.

Abnormals Findings: Presence of albumin in urine indicates the nephritis or kidney damage.State whether there is only a faint haziness (+),a cloud (++),a dense cloud(+++) or a definite flocculent precipitate(++++) to give a rough idea of the quantity of albumin present.

Clinical Significance: Urine generally contains 2-8 mg. of proteins,when their quantity increases to 150 mg.,one can conclude that filtration of urine in the kidney is improper and the condition is referred to as proteinurea or albuminurea. By these diseases like myelo and pyelo nephritis,diseases of

urinary tract can be diagnosed. Proteinurea reveals the irregular functioning of the kidneys. Diseases like glomerullo nephritis,toxemia during conceptions and diabetic nephropathy can be identified through this test.

(ii) Sulphosalicylic Acid Test for Albumin(Precipitation of Protein)

Procedure: Take a clean test tube and to it add 5ml. of clear urine and then to it add 10 drops of 20% sulphosalicylic acid.

Report: If protein is present there will be a white precipitation increasing with the amount of protein.

(iii) Nitric Acid Test for Albumin (Hellers Test)

Procedure: Take a clean test tube and it to add 2 to 3 ml. of nitric acid and then gently float equal volume of clear urine.

Report: Presence of white ring at the junction indicates the presence of albumin.

10.4 Chemical Examination

1. Sugar (Glucose) i) Benedicts Qualitative Test ii) Fehlings Test

- Ketone Bodies (Acetone Bodies)
- Bile Salts.
- Bile Pigments
- Blood in Urine
- Strip Method
- Albumin

Sugar (Glucose): Presence of sugar in urine is detected by Benedicts Qualitative Test & Fehlings Test.

Benedicts Qualitative Test

Principle: In this method the cupric ion is reduced to cuprous oxide.

Procedure: Take 5ml. of Benedicts qualitative reagent and subject it to boiling,and then add 7-8 drops oe urine with the help of pipette.Sugar condition can be decided basing on the colour developed.Sometime colour cannot be change then it might have to be reboilrd or deep cooled.Conclusions are to drawn on thorough examination.

Report: A Bluish white precipitate,a light green colour or no change is recorded as negative.

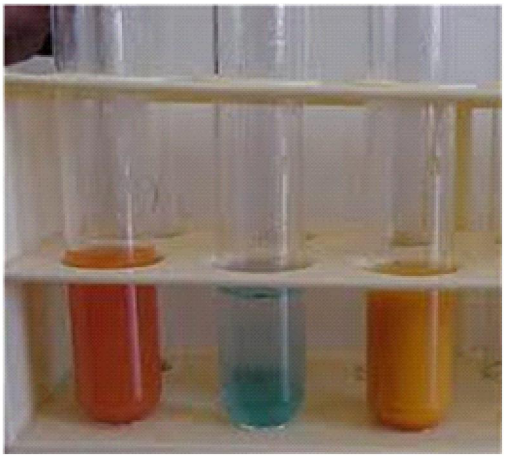


Fig. 10.5 Benedicts Qualitative Test

A greenish colour with a little yellow deposit is reported as a trace(+).Green yelloowish precipitate indicates sugar presence(++).Presence of orange colour in urine indicates glucose(+++).Presence of brick red in urine indicates presence of sugar in urine(++++).

(ii) Fehlings Test

This test can be also used for detecting the presence of sugar in urine.

Procedure: Take a dry clean test tube and to it add 1ml. each of solution - A and solution-B and then add 6ml distilled water mix and boil this solution present in the test tube. Add urine, a few at a time to the solution,but should not boiled now.The volume of urine should not be more than volume of the reagent.

Report: If the sugar is present a heavy red or yellow precipitate appears.

Clinical Significance: Glycosuria with Hyperglycaemia is indicated in diabetes mellitus,other endocrinedisorders,pancreatic disease,CNS dysfunction,massive metabolic derangement.hypoglycaemia indicates the presence of los levels of glucose in urine.

2. Ketone Bodies(Acetone Bodies)

Ketone Bodies are the products of incomplete fat metabolism and thgeir presence is indicative of acidosis(ketosis).Ketonuria is commonly seen in uncontrolled diabetes mellitus.

The ketone bodies are 1) Acetone. 2) Acetoacetic acid. 3)Beta-hydroxy bityric acid.

The presence of Ketone bodies in human body can detected by tests.
i) Rothera's Test ii) Gerhard's Test

Rothera's Test: Acetone and Aceto-acetic acid react with sodium nitroprusside in the presence of alkali to produce a purple coloured complex.

Sodium Nitroprusside is decomposed to Sodium Ferro Cyanide,Sodium Nitrite and Ferric Hydride in an alkaline solution. These chemicals are strong oxidising agents.thye ammonium sulphate acts as a buffer maintaining the alkalinity within the range at which complex has a purple colour.

Rothera's Mixture

Ammonium Sulphate - 99 parts

Sodium Nitroprusside - 1 part Grind and Mix well

Procedure: Take 5ml. of urine in a test tube, Saturate it with crystals of ammonium sulphate. To it add 2 drops of freshly prepared sodium nitroprusside reagent.

Mix well, overlay with about 14ml. of concentrated ammonium hyderoxide.A positive shows the appearence of a reddish purple ring at the interface within 1mt. and 30sec. A brown ring is not a positive reaction.

Interpretation

- No ring or brown ring-negative.
- A faint pinkish-purple ring appearing slowly.trace.
- Narrow dark purple ring.-++
- Wide dark purple ring appearing rapidly-++++

Clinical Significance: Presence of ketone bodies is observed in diabetes mellitus and starvation.

Gerhard's test:

Take a clean dry test tube and add about 5ml. of urine & 10% of ferric chloride solution precipitate will be formed.Filter it to the filtrate add o0ne or two drops of 10% ferric chloride solution.

Result: Formation of a red colour in test tube indicates the presence of ketone bodies in the patient. This is 20 times less sensitive than Rothera's test.

Fig. 10.5 Detection of Ketone Bodies by strips

3. Bile Salts (Hays Test)

Sodium glycocholate and sodium taurocochlate are called as Bile Sallts.These are helpful in the emulsification of the fat globules.(Fat Digestion).

Principle: Bile Salts reduce the surface tension.

Procedure: Fill the urine sample up to the 3/4th height of a clean beaker sprinkle little dry sulphur powder over the surface of about 1/2 inch height.

Result: If the Bile salts are present sulphur particles will sink.In normal urine sulphur practices will float.

4) Bile Pigments

Liver secretes bile juice.It contains yellow green substance bile pigment(bilirubin).In some hepatic diseases,bile enters to the blood and is eliminated through urine. The presence of bile pigment bilirubin can be detected by four tests.

- Fouchets Test (Harrison's Spot Test)
- Smith's Test
- Gmelin Test
- Lugols Test.

i) **Fouchet's Test(Harrison's Spot Test)**

This test is used for detecting the bile pigments in urine.

Principle: After precipitation of the bile pigments by Barium Chloride it is oxidized by acids to derivatives like biliverdine(green) and bilicyanine(blue).

Procedure: To about 2ml. of acid urine add 1ml.of 10% of barium chloride. Mix and wait for few minutes,for the precipitate ti appear(if there is no precipitate add 1 or 2 drops of saturated ammonia sulphate solution).Filter through a filter paper and add a drop or two of Fouchet's reagent(Trichloracetic acid and ferric chloride).

Result: A positive reaction is indicated by a blue or green colour.This test is more sensitive than the iodine or nitric acid ring test.the sensitive of the test is 0.05 to 0.01 mg/100ml. of urine.

Clinical Significance: Bile pigments are increased in obstructicve jaundice and hepatitis.

(ii) Smiths Test

This is another test for detecting the presence of bile pigments in urine.

Procedure: Take 3ml. of urine in a test tube,over the urine layer add equal amount of diluted tincture iodine.

Result: A bright green ring develops at the junction of the liquids if bile pigment is present.

(iii) Gmelin Test

This is also another test for detecting the presence of bile pigments in urine.

Procedure: Take about 2ml. of Nitric acid in a test tube.Add vertically urine at the centre portion of acid layer in a test tube.

Result: A green or blue ring indicates the presence of bile pigments.

(iv) Lugol's Iodine Test

Procedure: Take 4ml. of urine in to a test tube and add 4 drops of lugol iodine solution and stir it.Afterwards observe the colour in th etest tube.

Result: Yellow or brownish yellow colour formation indicates the absence of bile pigments in urine.

Formation of green colour indicates the presence of bile pigments in the urine.

Pale Green:(+)

Intence Green:(++)

5. Blood In Urine:(Haematuria)

The presence of blood in urine is known as Haematuria.

The presence of blood in urine or haematuria can be detected by the following tests.

Benzidine Test (Occult Blood Test)
Paper Strips

Benzidine Test(Occult Blood Test)

Principle: The peroxidase activity of haemoglobin decomposes Hydrogen peroxide and the liberated oxygen oxidises the benzidine.

Procedure: To 2ml. of urine (previously boiled and cooled) and 1ml. of clear benzidine solution(benzidine powder and glacial acetic acid),mix well,add 1ml.of freshly prepared 3% hydrogen peroxide and mix.

Result: A deep blue colour indicates presence of haemoglobin in urine.
Clinical Significance: The appearence of green or blue colour within 5 minutes.indicatesthe presence of blood or haemoglobin or myoglobin in the urine.

(iii) Paper Strips

Blood present in urine reacts with the peroxide-orthotoluidine reagent of paper strip to produce a blue colour.

This indicates the presence of blood in urine.

Microscopical examination:

Introduction

Identification or detection of normal or abnormal components like cells, casts, crystals, mucus threads, parasites and bacteria using microscope is known as Microscopic Examination.

First collect the urine sample(clear,fresh morning specimen).Obtain urinary sediment by centrifusing urine at 3000 rpm for 5 minutes.Withdraw the clear supernatant fluid,place a drop of the sediment on a glas slide and cover it with a cover slip.Examine first under low power objective,then observe under high power objectives of a microscope.Vary the high intensity for screening casts. If protein is present, look for casts R.B.C;S pus cella and epithelial cells.

- Cells
- Casts
- Crystals
- Atypical Cells
- Mucuc Threads

- Yeast Cells
- Bacteria
- Spermetozoa
- Parasites & Parasiticova

Urates

a - Calcium, Magnesium and potassium (mostly amorphous)

b - Ammonium (Spherical)

c - Sodium (thorn - apple forms)

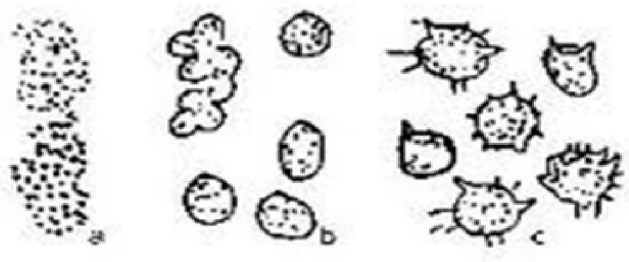


Fig. 10.7 Microscopial examination of Urine

(I) Cells

(i) RBC'S

Under high power objective of a microscope RBC appears as pale disks. More than one RBC for high power field is abnormal.The red blood cells swell up and broken down in diluted urine.The red cells may show crenated margings.

Clinical Significanse: Increased red blood cells are found in (a) Polynephritis (b) Renal Stones (c) Cystitis (d) Polycythemiavera

Decreased red blood cells are found in Anemias.

(ii) WBC

Normal values 0-5 per high power field.

Clinical Significance: Large number of WBC'S indicates bacterial infection of urinary tract.

Increased white blood cells are found in Leucytosis,Leukacmia.

Decreased white blood cells are found in openia.

(iii) Epithelial Cells

These cells have a single rounded nucleus Squamous epithelial cells present in urine in moderate numbers have no pathological importance.

Clinical Significance: Presence of all other epithelial cells indicates pathological condition.

(iv) Renal Tubular Epithelial Cells

Unstained cells have almost the same size as that of a neutrophill but contain

a large round nucleus.Oval fat bodies are these cells containing fat globules,the nucleus,then,is not visible.

(v) Blader Epithelial Cells

Unstained cella are larger than renal tubular cells,have a round nuclear and vary in size depending on depth of origin in transitional.

(vi) Squamous Epithelial Cells

These are unstained large flattened cells with abudant cyptoplasm and a small round nucleus.The cell may be folded or rolled.

Casts

Urinary casts are formed in the lumen of the tubules of the nephrons.
The following casts are observed after microscopical examination of urine sediment covered with coverslip on a glass slide.

- Hyaline Casts
- Granular Casts
- Waxy Casts
- Epithelial Casts
- Cylindroids
- Fatty Casts
- Fibrinous Casts
- Pus Casts
- Pseudo Casts
- Blood Casts

Crystals

Generally many of the crystals which are found in the urine have little clinical significance although they may be found in calculus formation,metabolic disorders and in regulation of medication.

(a) Crystals Present In Acidic Urine

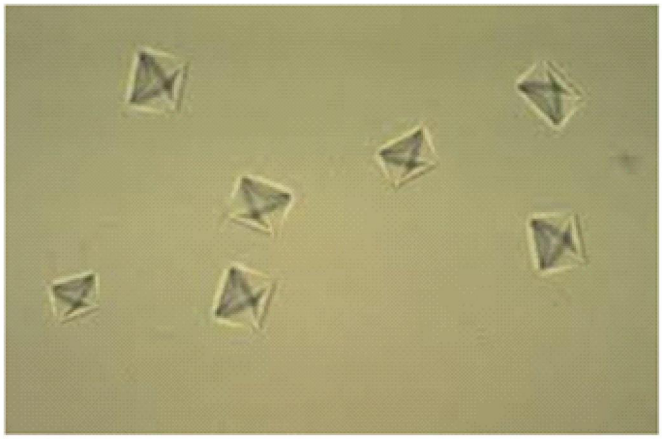


Fig. 10.8 Crystine Crystals present in the Neonates Urine

- Uric Acid Crystals
- Calcium Oxalate Crystals
- Amorphous urate crystals
- Sodium urate crystals
- Calcium sulphate crystals
- Hippuric acid crystals
- Cysteine crystals
- Tyrosine Crystals
- Leucine Crystals
- Cholestrol Crystals
- Sulphur Crystals

b) Crystals Found In Alkaline Urine

- Triole Phosphate (Ammonium Magnesium Phosphate) Crystals
- Amorphous Phosphate Crystals
- Calcium Carbonate Crystals
- Ammonium Biurate Crystals
- Calcium Phosphate Crystals

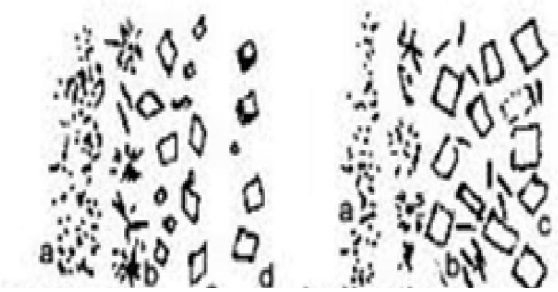


Fig. 10.9 Microscopic Examination of Urine

Atypical Cells

- Mucus Threads
- Yeast Cells
- Bacteria
- Spermatozoa

Key Terms

Sediment: The solid portion whch gets settled at the bottom of the testtube after centrifugation of urine

Microscopic Examination: Identification oe identification of normal or abnormal components using microscope is known as microscopic examination of urine.

Pus cells: A yellowish liuid formed in certain infections consisting of tissue fluid,bacteria and WBC

Alveolar: Pertaining to lungs

Chronic: Persisting for a long time

Cystinuria: Excretion of cystine in urine

Leucocytosis: Increased levels of WBC count

Leucopenia: The condition in which leucocytes count is decreased

Leukaemia: Abnormal in WBC count with immature cells among them

Physical Examination: The tests which does not require any kind of chemical substances is called as chemical examination.

Diabetes Mellitus: Lack of insulin secretion

Diabetes Insipidus: Lack of secretion of Anti diuretic hormone

Calculi: Related to stones and Kidneys

Haematuria: Excretion of blood in urine

Aneuria: Stoppage of urine formation or excretion

Centrifuge: Instrument used to separate solid substances from liquid mixture by centrifugal force

Glycosuria: Presence of sugar in urine

Hypoglycaemia: Low levels of sugar in urine

Hyperglycaemia: High levels of sugar in urine

Ketonuria: Excretion of ketone bodies in urine

Mucus: The viscid fluid secreted by mucous glands

Nephritis: Inflammation of nephron.

Chemical Examination: The test performed with chemicals to detect/ identify the presence of normal or abnormal components of human body is called as the chemical examination

Albumin: It is a variety of protein found in animal,vegetable matter,which is soluble in water and coagulates on heating

Albuminuria: Excretion of albumin in urine

Benedicts Qualitative Test: Test used to detect the presence of sugar in urine

Ketonuria: Excretion of ketone bodies in urine

Short Answer Type Questions

What is granular cast and mention about its clinical significance?

Write notes on parasites and parasitic ova present in urine.

Write about clinical significance of tyrosine crystals

Explain about mucus threads

Mention the names of different casts

Define microscopic examination of urine

What is leukaemia?

What is the normal urine out put or volume of urine in 24hrs in adults?

What are the causes of oliguria?

Define oliguria

What are the causes of polyuria?

Define polyuria and aneuria

In which conditions urine will appear as red urine and deep yellow?

What is the odour of normal urine?

What is the normal PH of urine?

What is the use of urinometer?

What is the specific gravity of normal urine.

What are the bile salts and mention their use?

Name the bilepigments

What is the significance of benzidine test?

Define ketonuria

Mention the names of ketonebodies

What is the importance of Hays test?

Write the names of bilesalts

What is the significance of Fouchets test?

Define Haematuria

What is Diabetes mellitus?

Mention the names of bilepigments

Define chemical examination

Long Answer Type Questions

Mention the different types of casts in urine

Write notes on various types crystals present in urine

Explain in detail about the cells present in urine and about its clinical significance

Write notes on physical examination of urine

Write about the Benedicts qualitative test and Fehlings test

Explain about the boiling test for albumin

Write about the following tests

a) Rotheras test b) Hays test c) Fouchets test

Define Haematuria and write about benzidine test

Write briefly about the chemical examination of urine by strip method

UNIT 11

REAGENTS USED IN PATHOLOGY

Structure

11.1 Introduction

The term Reagent can be applied in particular to any chemical compound or mixture of compounds,usually in solution,employed in chemical analysis or for the detection of biological constituents.A solution is a combination of two substances a solute and a solvent.The dissolved portion is called as solute and the substance present in relatively greater proportions in the solution is called the solvent.

Various types of reagents and solutions used in a pathological laboratory Are

a) Normal solutions b) Molar solutions c) Percent solutions d) Buffered solutions e) Indicators f) Primary standards g) Other complex reagents

Note: All the reagent bottles should be labelled appropriately

11.2 Preparation of Reagents, Principles and Interpretation

The following are the various reagents used in pathological laboratory

- Haemoglobin diluting fluids
  - 0.1N Hydrochloric acid
  - Drabkins reagent
- Benedicts solution
- RBC diluting fluid
- WBC diluting fluid
- 4.0g/dl EDTA solution (anticoagulant for completeblood count)
- Absolute eosinophil diluting fluid(Hinglemans solution)
- Blood banking reagents.
- Semen diluting fluid
- Cytology and Histopathology Fixtures

Haemoglobin Diluting Fluids

a) 0.1N HCL(200ml):

Method of Preparation

Take accurately quantity of HCL equivalent to 0.73 g of HCL

Add gradually to approximately 150ml of distilled water with constant stirring and cool

Dilute to 200ml with distilled water

Standardise and adjust normality if necessary

Principle

Brown coloured acid haematin is produced from haemoglobin,when blood is added to 0.1N HCL. After dilution the resulting or final colour formed is compared with standard brown glass reference blocks of a sahlis haemoglobinometer.

I**nterpretatio**n

0.1 NHCL is used in the estimation of Hb by Sahlis acid haematin method Decreased Hb values indicates anaemia.High Hb values are observed in congenital heart disease,in polycythemia vera and in emphysema

b) Drabkins reagent:

Sodium bicarbonate : 1.og

Potassium cyanide : 0.2g

Potassium ferricyanide : 0.2g

Distilled water : 1000ml

This reagent is stable in polythene container at 2-8^{0}C

To prepare the Drabkin's Solution, reconstitute one vial of the Drabkin's Reagent with 1000 ml of water.

Principle

When blood is mixed with Drabskins reagent containing potassium cyanide and potassium ferricyanide,haemoglobin reacts with ferricyanide to form methaemoglobin which is converted to stable yanmethaemoglobin(HiCN) by the cyanide.The intensity of colour is proportional to haemoglobin concentration and it is compared with a known cyanmethaemoglobin standard at 540nm

Interpretation

Haemoglobin: It is normally cinfined to the corpuscles but may be present free in the plasma(haemoglobinaemia)under conditions when there is appreciable intravascular haemolysis e.g,

In severe haemolytic anaemia or malaria
In septicaemia(blood poisoning)due to haemolytic streptococcus
As a result of transfusion with incompatible blood

Benedicts Reagent Method of Preparation

One litre of Benedict's reagent can be prepared from 100 g of anhydrous sodium carbonate, 173 g of sodium citrate and 17.3 g of copper(II) sulfate pentahydrate. It is often used in place of Fehling's solution.

Dissolve 173gm of sodium citrate with 100gm of sodium carbonate anhydrous in about 600ml of distilled water and gently heat it in 1000ml beaker or flask.In another beaker dissove 17.3g of copper sulphate in 100ml of distilled water and pour it into the carbonate citrate solution with constant stirring.Transfer to a 1000ml volumetric flask quantitatively wasing the beaker

with distilled water.Mixwell and bring the volume to 1000ml.When it cools to room temperature with disrilled water.If it is not clear, filter.

Principle

When sugars containing free aldehyde or keto groups are treated with Benedicts reagent,sugars are converted to enediols.Enediols are powerful reducing agents.

They reduce cupric ions to cuprous ions,which is responsible for change in colour of the Benedicts reagent.

Benedicts solution is a chemical solution used to detect the presence of glucose and other reducing sugars. Medically, it is used to test the urine of diabetics

Interpretation

This test is used in clinical practice to detect the reducing substance present in the urine.It is a semiquantitative test.When sugars are present at different concentrations,different colours are developed,which is interpretated and reported as follows.

Colour	Approximate amount of Sugar	Reported as
Green	0.5%	+
yellow	1.0%	++
orange	1.5%	+++
Red	2.0%	++++

RBC Diluting Fluid

Formal citrate sloution

Trisodium Citrate : 3 gm

Formalin : 1 ml

Distilled water : 99ml

Preparation

Trisodiumcitrate is dissolved in few ml of distilled water and to that formalin is added and finally volume is made to 100ml with distilled water.

b) Hayems Fluid

Sodium chloride 0.5 gm

Sodium sulphate 2.5 gm

Merecuric chloride 0.25gm

Distilled water 100ml

Peparation

All the contents are dissolved in few ml of distilled water and finally the volume is make up with 100 ml distilled water

Principle

The blood specimen is diluted to 1:200 with the RBC diluting fuid and cells are counted under high power(40 x objective) by using a counting chamber.The number of cells in undiluted blood are caluclated and reported as the number of red cells per cmm of whole blood

Interpretation

The RBC is a count of the number of red blood cells per cubic millimeter of blood. In response to hypoxia, the hormone erthyropoietin, secreted by the kidneys, stimulates the bone marrow to produce red blood cells. The formation of red blood cells is known as erthyropoiesis.

Normal red blood cell values at various ages are:
Adults: (males): 4.6 - 5.9 million
(Females): 4.2-5.4 million
Pregnancy: Slightly lower than normal adult values
Newborns : 5.5 - 6 million
Children : 4.6 - 4.8 million

4. WBC Diluting Fluid

Composition:

Glacial acetic acid	: 2.0ml
1%(w/v)gentian voilet	: 1.0ml
Distilled water	: 97ml

Preparation

Both the components are dissolved in few ml of distilled water and volume is made to 100ml. This solution is stable at room temperature(25+ r – 5^{o}C.A pinch of thymol may be added as preservative.

Principle

The glacial acetic acid lyses the red cells while the gentian voilet slightly stains the nuclei of the leucolytes.The blood specimen is diluted to 1:20 in a WBC pipette with diluting fluid and the cells are counted under the low power of the microscope by using a counting chamber.The number of cells in undiluted blood are reported per cubic mm of whole blood.

Interpretation

Total WBC: 4,500 - 10,000 millions per cmm

The numbers of leukocytes changes with age and during pregnancy.

On the day of birth, a newborn has a high white blood cell count, ranging from 9,000 to 30,000 leukocytes. This number falls to adult levels within two weeks.

The percentage of neutrophils is high for the first few weeks after birth, but then lymphocyte predominance is seen.

Until about 8 years of age, lymphocytes are more predominant than neutrophils.

In the elderly, the total WBC decreases slightly.

Pregnancy results in a leukocytosis, primarily due to an increase in neutrophils with a slight increase in lymphocytes.

Leukocytosis, a WBC above 10,000, is usually due to an increase in one of the five types of white blood cells and is given the name of the cell that shows the primary increase.

Neutrophilic leukocytosis	= Neutrophilia
Lymphocytic leukocytosis	= Lymphocytosis
Eosinophilic leukocytosis	= Eosinophilia
Monocytic leukocytosis	= Monocytosis
Basophilic leukocytosis	= Basophilia

6. Platelet Diluting Fluid

Compositiion

Sodium Citrate	: 3.8gm
Formalin	: 0.2ml
Brilliant cresyl blue	: 0.1gm
Distilled water	: 100ml

Preparation

Sodium citrate is dissolved in little amount of distilled water.To this formalin is added.Brilliant cresyl blue is also dissolved in little amount of distilled water, above two portions are added to one another and the final volume is made with distilled water to 100ml. Filter this fluid each time before use.

Principle

Blood is diluted with the diluting fluid to give a dilution of 1:200 and the cells are counted in the improved Neubauer chamber.

Interpretation

- Normal Results
- 150,000 - 400,000 platelets per microliter (mcL).

Increased Values of ESR

Are found with all diseases associated with a modification of the plasma proteins like globulin, albumin and fibrinogen. ESR shows especially high values in

- Tuberculosis
- Leishmaniases
- Malignant condition
- Hepatic Amoebiasis
- Acute and Chronic Inflammation
- 4.0g/dl EDTA (Ethylene Diamine Tetra Acetic Acid)
- EDTA - 4gm
- Distilled water - 100ml

Preparation

EDTA (Ethylene Diamine Tetra Acetic Acid)

4gm of EDTA is dissolved in distilled water and solution is prepared.

Principle

It acts as powerful calcium chelating agewnt.The calcium in blood is bound in an unionised and forms soluble complex with EDTA.

Interpretation

As the results are indirectly correlated with the following tests,refer the following tests in the previous topics.

Tests performed with EDTA are

- Haemoglobin
- WBC count
- RBC count
- PCV determination
- ESR byWintrobes method
- Platelet count *Differential WBC count

Absolute Eosinophil Diluting Fluid (Hinglrmans solution)Composition

The most commonly used diluting fluid is Hingleman's fluid. Hingleman's solution

Yellow Eosin – 0.5 g – stains eosinophil granules

95% phenol – 0.5 ml
Formalin – 0.5 ml – fixes the cells
Distilled water – 99 ml – lyse the RBCs

Preparation

Dissolve all the components in few ml of distilled water and made up to

100ml volume with distilled water.

Principle

Blood is diluted with a special diluting fluid which lyses the RBCs and stains the eosinophils red. These cells are counted under low power objective using Levy chamber with improved Neubauer ruling.

Interpretation

Normal values

40-440/cu mm (micro liter) i.e. 0.04 - 0.44 x 109/L

4.6.6 Eosinophilia - Increase in the absolute eosinophil count beyond the upper limit of normal (> 440 cells / cu mm)

Causes for Eosinophilia

Allergic Diseases: Bronchial asthma, Hay fever. In asthma - eosinophil count correlates with pulmonary performance and also indicates the adequacy of steroid therapy.

Skin Disorders: Eczema, atopic dermatitis.

Parasitic infections: Hook worm, Filariasis, Trichinosis, Cysticercosis.

Infectious diseases: Scarlet fever.

Semen Diluting Fluid

Semen diluting fuid is used in sperm counting

Composition

Semen Diluting Fluid

Sodium bicarbonate – 5 gm.

Formalin – 1 gm

Distilled water – 99 ml.

Preparation

Both the solid and liquid ingradients are dissolved in few ml of distilled water and made to 100ml with the distilled water.

Principle

Sodium bicarbonate provides basic medium to semen by keeping it as lively onFormalin acts preservative by restoring the natural condition of the semen

Water is used for diluting purpose

Interpretation

Sperm Vitality

Normal Observation

1. Spermatozoa Head caps :Light blue
2. Nuclear posterior : Dark blue
3. Bodies and tails : Red or pink
4. Spermatozoa size : 50-70 microns
5. Head size:3-6microns x 2-3 microns.

Low sperm counts are observed when there is supression of endogenous gonadotrophin production by exogenous estrogens or androgens or by anabolic agents.If sperm count is less than 60millions/ml,then there are less chances for fertility.

Normal sperm count is 100-150 millions/ml

Abnormal forms of Spermatozoa

Giant head spermatozoa
Pin head spermatozoa
Immature spermatozoan(spermatid)
Constricted head spermatozoa
Double tail spermatozoa
Amorphous form spermatozoa
Acute tapering form spermatozoa

Short Answer Type Questions

- Write the principle of Drabkins reagent.
- Write the interpretation of Drabkins reagent.
- Write the principle of Benedicts solution.
- Write the interpretion of Benedicts solution.
- Write the composition and preparation of RBC diluting fluid.
- Write the principle of RBC diluting fluid.
- Wite the interpretation of RC diluting fluid.
- Write the principle of WBC diluting fluid.
- Write the interpretition of WBC diluting fluid.
- Write the principle of 4g/dl EDTA solution.
- Write preparation of Absolute eosinophol diluting fluid.
- Write the principle of Absolute eosinophil dilting fluid.
- Write the interpretition of Absolute eosinophil diluting fluid.
- Write preparation of semen diluting fluid.
- Write principle of semen diluting fluid.
- Write interpretition of semen diluting fluid.
- Write interpretition of double oxalate mixture.

Long Anwer Type Questions

Write preparation,principle and interpretition of the following regents

(a) 0.1N HCL (b) Drabkins reagent (c) Benedicts solution

RBC AND WBC diluting fluids

Write the preaparation,principle and interpretition of following reagents

(a) 3.8g/dl Trisodium citrate (b) 4.0g/dl EDTA solution

(c) Absolute eosinophil diluting fluid (d) Reticulocyte dliuting fluid

UNIT 12

Sputum Analysis

Structure

12.0 Introduction

Matter which is expectorated from the lungs is known as the sputum.The analysis of sputum for finding out normal and abnormal constituents of a patient by physical,chemical and microscopical examination using laboratory techniques is known as sputum analysis.Normal sputum is colourless,watery and odourless tracheobronchial secretion.It is a constant mixture of plasma,mucin,electrolytes and water.When this secretion mixture passes through the lower and upper respiratory tract,the secretory mixture contaminated with cells removed from tissues in layers,nasal,and salivary gland secretions and normal bacterial flora of the oral cavity.Only sputum specimen is used to detect the presence of Mycobacterium tuberculosis bacteria and Mycobacterium leprae bacteria by Ziehl neelsen hot and cold staining methods.Physical examination also helps inidentification and in the diagnosis of certain diseases concerned to Lungs.

The lower respiratory tract has been kept sterile by

Alveolar Macrophage System

Mucociliary System: Early morning specimen or the entire 24-hr specimen should be collected into a sterile wide moth glass bottle and closed with a screw cap.

12.1 Physical Examination

Quantity: The amount varies from disease to disease.

Volume: 24 hrs volume sputum specimen should be collected and measured from the patients with chronic bronchitis,lung abscesses and bronichal asthma.

Abnormal Findings:Decreasing sputum volume indicates the normal state of the person.Increasing sputum volume indicates abnormal state of the patient.

Consistency And Appearence: Normal sputum is colourless water

and opalascent.Based on consistency and appearence,sputum can be classified in to the following types,which indicates abnormal of patient.

(i) Serous Sputum (ii) Mucoid Sputum(tough Sticky) iii) Purulent Sputum iv) Bloody Sputum. Ex : Seropurulent, Mucopurulent.

iv) C o l o u r: Normal sputum is clear and colouless.

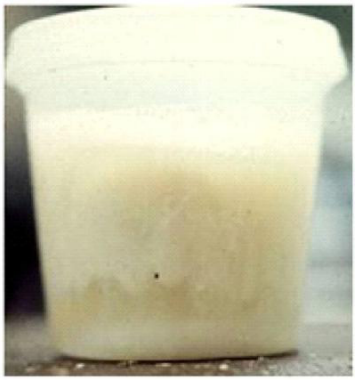


Fig 12.1 Sputum Collection

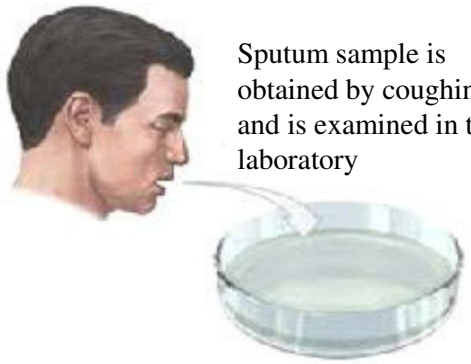


Fig. 12.2 Physical Examination of Sputum

Abnormal Findings: Yellow coloured sputum of the patient indicates the pus&epithelial cells as seen in pnemonic process.Greenish coloured sputum of the sputum patient indicates pseudomonas infection.Rust coloured sputum of the patient indicates the decomposition of haemoglobin as seen in pneumococcal pheumonia or pulmonary gangrene.Bright red coloured sputum of the patient indicates recent haemorrhage,pulomonary infraction,breakdown of a blood vesselpulomonary tuberculosisBlack coloured sputum of the patient is due to inhalation of dirt.Coal,dust or due to the decomposition of anthracotic tissue.

Odour: Normal odour has no odour.

Abnormal Findings: Sputum with putrid odour of the patient indicates lung abcess bronchiectasis and gangrene of the lung.

Sputum with sweetish odour of the patient indicates pulomonary tuberculosis with cavities, bronchomoniliasis and bronchiectasis.

Sputum with cheesy odour of the patient indicates the necrosis of malignant tumours and perforating empyemas.

12.2 Preparation or Making a Sputum Smear

Sputum should be collected from the patient in to sterile container.From the sterile container a portion of sputum has been transferred on to the glass slide.With the help of a match stick or thick sterile needle and sputum has been made in the form of a film and covered with a cover slip and observed under the microscope.

12.3 Staining of Sputum Smear

Sputum smear on the glass slide has been dried in the air.After that sputum smear has been fixed by passing over a fame of a spirit lamp.After fixation of the sputum smear,sputum smear should be subjected to the following stainig techniques.

(i) Gram staining (ii)Acid Fast Staining (iii)Wrights staining

(iv) Buffered Crystal Voilet Staining (v) Paps staining

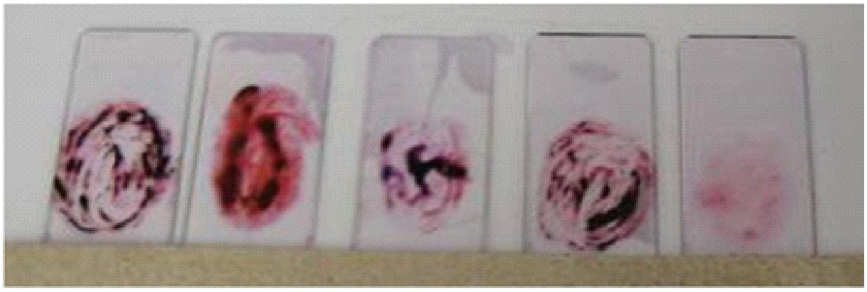


Fig. 12.3 Gram Stained Sputum Smears

(i) Grams Staining Of Sputum Smear

Christian gram has been discovered the Grams Staining Technique.By this technique the presence of gram +ve or gram -ve bacteria in a sputum smear can be identified.

Required Reagent

- Crystal Voilate Solution
- Grams Iodine Solution
- Ethyl Alcohol
- Safranine Solution

Staining Procedure

First take a clean dry slide and prepare sputum smear then dry it in air.After that mix the sputum smear on glass slide by passing over a flame of a spirit lamp.After fixation crystal voilate solution is added to the smear and after one minute wash with tap water.After that add gram iodine solution to the sputum smear and after one minute remove the grams iodine solution.After that decolourise the sputum smear on glass slide with 95% alcohol for 20 or 30 seconds.After that alcohol is removed from the slide by washing with tap water.After that again counter stain the sputum smear on glass slide with safranin solution.After that safranin solution is removed from the sputum smear of glass slide,dry the sputum smear in air and first observe under low power objective and secondly under high power objective of simple microscope.

Result: Gram +ve bacteria appear as in voilet colour.

Gram -ve bacteria appear as in pink colour.

Acid Fast Staining For Sputum Smear(Ziehl Neelsen Stain Method): Sir Ehrich had been discovered the Acid Fast Stainig. Mycobacteriumtuerculosis has been identified with Ziehl neelsen hot stain technique by examining sputum after acid fast staining under sample microscope.

Ziehl Neelson Hot Stain Method

Required Reagents

Carbol Fuchsin Solution.
Working Carbol Fuchsin Solution.
Stock Carbol Fuchsin Solution.
20% Sulphuric Acid.
3% Mythelene Blue Solution.

Procedure

Prepare the sputum smear on glass slide,dry in air and fix by passing through a flame of bumsen burner.Keep the sputum smear present on the gkass slide on a rack or on glass rods of a tray.Then stain it with working carbol fuchsin.Afterwards heat the slide withy sputum smear over a bunsen burner flames till the fumes comes out of the sputum smear.Afterwards wash it with water.Next counter stain the glass slide with sputum smear with methylene blue.Afterwards wash it with tep water and dry it in air.After then observe or examine under microscope.

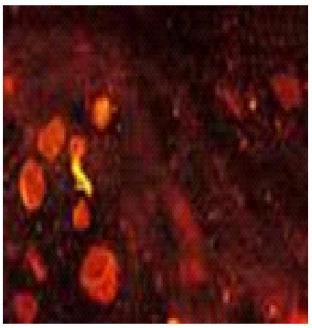


Fig. 12.4 Mycobacterium Tuberculosis in Sputum Sample

Result: Acid Fast organisms appears as red bacilli on blue black ground.

Ziehl Neelson Modified Method or Cold Stainig Technique:

By these technique mycobacterium leprae can be identified.

Required Reagents

- Stock Carbol Fuchsin Solution.
- Working Carbol Fuchsin Solution.
- 3% Mythelene Blue Solution.
- 5% Sulphuric Acid.

Procedure: Prepare the Sputum Smear as usual using glass slide and then stain it with working carbon fuchsin for15 minutes.And then wash it with to water.Then decolourise the sputum smear with5% sulphuric acid. Afterwards counter stain with methylene blue for 1 minute.Wash the sputum smear slide with water,dry it and observe under microscope.

Result: Acid fast bacili appear as bright red bacili on blue background.

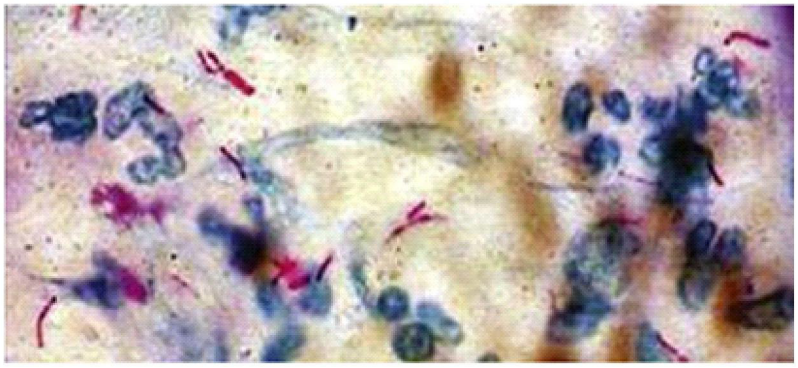


Fig. 12.5 Sputum specimen showing AFB

Wright Staining Of Sputum Smear: The presence of blood cells in sputum can be detected with the help of wrights stain.

Buffered Crystal Voilet Stainig: The presence of epithelial cells in sputum can be detected with this stainig technique.

Paps Stain: The stainig technique is useful for studying cytology of sputum.

12.4 Mounting

The covering 0f sputum specimen taken onto the glass slide with a coverslip for for diagnostic purposes by examining microscopically is called as mounting.

In certain cases for mounting the specimen mouting media are used in special situations.

12.5 Microscopical Examination of Sputum Smear

The detction or identification of normal or abnormal components of a sputum stained smear using microscope is known as Microscopical Examination of Sputum Smear.

Procedure: Transfer sputum on to clean glass slide and prepare smear and cover it with cover slip.Then observe under simple microscope.When ever you observe sputum smear under simple microscope,normally the following abnormal components can be detected. Those are Elastic Fibres

Abnormal Findings

The presence of elastic fibres in sputum smear indicate destruction of lung tissue,whether from grangrene or abcess.These elastic fibres can be detected with the number of a wet cover slip preperations.

Bronchial casts

Heart Failure Cell

Myelin globules

Elastic Fibers

Curshmann's Spiral

Fig. 12.6 Sputum Examination Microscopic Findings

(ii) Curschmans Spiral

Abnormal Findings: These structures are sometimes found in asthmatic patient.But their exact nature is not known.

(iii) Charcot Leyden Crystals

Abnormal Findings: The presence of these crystals in sputum smear indicate the asthma.

(iv) Pigment Cells

Many mono nuclear cells containig pigments may be found in the sputum.These cells may show dark brown blood pigments or black carbon pigments.

(v) Sulpher Granules

The presence of sulphur granules indicates Actinomycosis of the lungs.

vi) Bronchial Cast

In such conditions fibrinous cast may form inside the bronchial tree and may be found in the sputum as branching Structures.

Cellular Structure

From granular degeneration pus cells may be observed in sputum smear.

Red Blood Cells

Abnormal Findings : The presence of Eosinophil cells is sputum smear indicates asthma of a patient.

(ix) Parasites:

Abnormal Findings: The presence of hooklets ajnd still often of fragments of the laminated Octocyst of echnicoccus hydatid indicate hydatid disease of the lung.

(x) Asbestosis Bodies

Abnormal Findings: The presence of asbestosis in sputum smear indicate the asbestosis.

Short Answer Type Questions

- Define sputum.
- Define sputum analysis.
- What is the volume of sputum?
- Write the types of sputum.
- What is the clinical significance of volume?
- What is the colour of sputum?
- Write the odour of sputum.
- What is the clinical significance of colour?
- What is the clinical significance of odour?
- .How do you prepare sputum smear?
- Abbreviate AFB.
- Give examples for AFB.
- Mention the methods of staining of sputum smear.

Long Answer Type Questions

- Write down the physical examination of sputum.
- How will examine sputum smear microscopically?
- Write down the procedure of Ziehl neelsen hot staining method
- Explain about the Ziehl neelsen cold staining method

UNIT 13

Semen Analysis

Structure

13.1 Introduction

The secretion from the testicles and accessory male sex Organs.ex.Prostate containg spermatozoa is known as semen.Spermatozoa is suspened in seminal plasma.The seminal plasma activates the spermatozoa to a greater motility.The analysis of semen plays an important role in finding out the functioning of gonads..Semen because of containing the sperms which are playing prominant role in forming zygote after getting fertilised with ovum.Many of the childless male individual wont come forward in giving semen for analysis.

Nothing wrong is there in knowing the reason behind not bearing (getting) child.It is not only the responsibility of male individual to consult the doctor to know reason behind not bearing(getting) the child and at the same time the female individual also should consult the doctor to know the reason. Both the married couple should consult the doctor without hesitation for finding out reason.If there is no possibilty of conceiving,then the couple should either adapt a child or should approach the test tube baby center.

The couple should not feel shy regarding this.So many organs are getting replaced,transplanted in the todays human beings existing life.So in the same way the childless couple can approach the test tube care center if all the doors are closed.Research is going on in preparing the artificial ovum and sperm from the human cells in a congenial environment. Once this is achieved then childless couple can have ready made ova and sperm. If these are prepared from childless individuals then it will be a great achievement in the medical technology. I hope India too will try in this direction in the quest of new achievements in the field of medical technology in the coming future. Lets hope with positive attitude.

Composition of Semen

Semen is viscid,neutral or slightly alkaline and pale yellow coloured due to its flavin ocntent. Approxmately 60% of semen volume is derivede from the seminal vescicles ,which are also the major source of the the high fructose

content of semen.The other constituents of semen are potassium, critic acid, ascorbic acid, ergothionine and phosphoryl choline.

13.2 Collection of Specimen

Before collecting the semen sample the person should not participate in intercourse up to three days.After three days the person can collect the semen specimen.In clinical pathological laboratory the specimen is collected by masturabation.

Another Method: In home patient can collect semen in to wide mouth clean bottle powder free in dry. In patients home by coitus interruptus he can collect the semen in to the wide mouth container.The specimen should be delivered with in 30 minutes to the laboratory.

Fig. 13.1 Semen collection from animal

Storage: The semen specimen should be examined immediately after collection.If it is necesary to store,it should be kept at room temperature but do not store in the refregirator.

Couselling Before Collection

The Individual from which the semen was collected should be given counselling before collection due to lack of knowledge regarding the purpose of semen collection.Guide lines and way of collecting the semen should be explained by the technician(coitus interuptus or by masturbation).

The individuals should be made psychologically alert strong before the collection of semen.The individual should feel comfortable in giving semen.They should have awarenesss regarding the semen collection.

13.3 Physical Examination

(a) Volume

Measure the semen immediately after collection.From few drops to 5ml. of semen should be present.Normally volume of semen is in between 3-4ml.If semen volume is below 1.5ml. then the semen volume should be considered as below normal.

(b) Viscosity

Viscosity of semen should be observed by pouring from a pastuer pipette drop by drop.The semen specimen has normal viscosity,if semen is poured drop by drop.

(c) Liquefacation

Semen should be converted into liquid state with in 30 minutes.

The motility of spemetozoa is stopped if the semen did not convert in to l;iquid state.i.e. motility of spermetozoa is restricted,if semen remains highly viscous.

(d) Reaction

The pH of semen always remains in the alkaline side.The normal value is 7.2 to 8.9.

(e) Motility

Take one drop of semen om to the glass slide and cover with a coverslip and observe under low power and high power objective.

If spermetozoa is absent in the semen then that condition is called as Azoospermia.

If semen contains few spermetozoa which have motility then that conditions called as Oligozoospermia .

If semen contains spermetozoa which has no motility then that condition is called as Necrozoospermia .

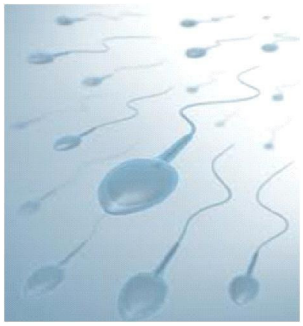


Fig. 13.2 Sperm Insemination

(f) Colour

It is opaque,white or grey white coagulum.

(g) Odour

Musty or Acrid

13.4 Microscopic Examination of Semen

Study of Motility of Sperms

Transfer a small drop of liquified semen in a glass slide and cover it with a cover slip.

Observe the cover slip preperation under the high power objective with reduced illumination.

Count the number of sperms which are actively motile out of the total count of 200.

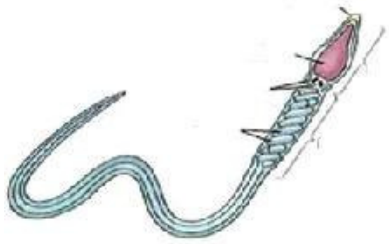


Fig. 13.3 Sperm Structure

Calculate the percentage of sperm showing actual progressive motion.

Observe the slide after 2 hours,3 hours and 6 hours. Care should be taken to present drying of he sperm.

Observe for pus cells,epithelial cells and or the other findings.

Determination of Sperm Count

After liquefaction gently mix the specimen.

Draw semen up to the 0.5 mark of a WBC pipette.

Draw the semen diluting fluid up to 11 mark and mix well.

Load the Neubauer chamber and allow the sperms to settle for about 5 minutes.

Count the sperms in the four corner squares(as in WBC count)

Calculation: Sperms /ml. of semen = (Sperms counted in four Squares x 10 x 20 x 1000) / 4

Normal sperm count = 100 to 150 millions/ml.

Determination of Morphology of Sperms

Semen after (liquefacation) getting liquified transfer a drop of semen on to a clean glass slide and prepare a smear.

Dry the semen smear in air and heat very gently to fix.If necessary remove the mucus by dipping semen smear in semen diluting fluid and then in buffer distilled water.

Using Leishman stain,stain the smear or stain in 0.25% aqueous basic fuchsin for 5 minutes.

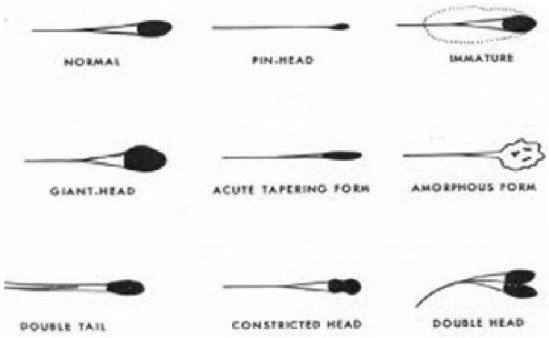


Fig. 13.4 Morphology of Sperms

NORMAL OBSERVATIONS	ABNORMAL FORMS OF SPERMS
1. Spermetozoa Head Caps: Light Blue.	Giant head Spermatozoa Pin head Spermatozoa Immature Spermatozoa
2. Nuclear Posterior: Dark Blue.	Constricted Head spermatozoa Double tail Spermatozoa
3. Bodies And Tails: Red Or Pink.	Double head Spermatozoa Amorphous Form spermatozoa
4. Spermetozoa Size: 50-70 Microns.	Acute tapering form spermatozoa
5. Head Size: 3-6 Microns X 2-3 Microns.	

Clinical Significance

Low sperm counts are observed when there is supression of endogenous gonadotrophin production by exogenous estrogens or by anabolic agents. Hypothyrodism and hyper thyrodism oligozoospermia. Trauma infections,irradiation and antimitotic chemotherapy can damage the testes. These patients often have oligospermic or azoospermia. Loss of libido,testicular atrophy and azoospermia can result from a pituiatary and hyper thalamic turnor. If sperm count is less than 60millions/ml. these are less chances for fertility.Normal sperm count 100-150 millions/ml.

Conclusion

Many of the individuals feel discomfortable in consulting the doctor regarding childlessness. Many factors play role in effecting either in the release of ovum in right time or presence of fully motile spermatozoa. Especially in India manyindivduals feel shy and won't consult the doctor.

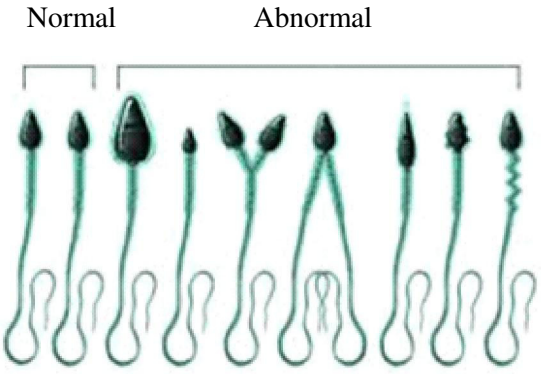


Fig. 13.5 Abnormal sperm Morphology

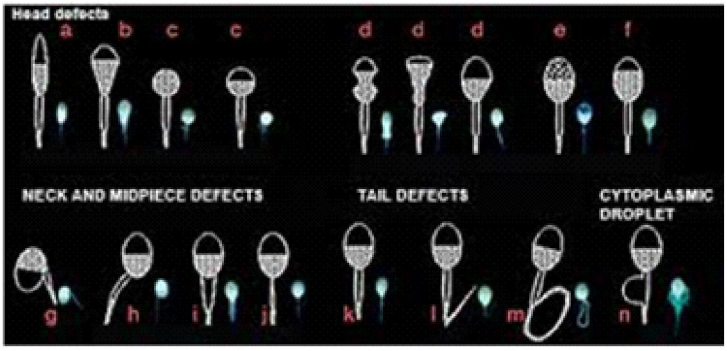


Fig. 13.6 Abnormal forms of Human Sperm Etozoa

Some times with minor problems also the individuals may not bear the child. And the collection of semen by both the methods (masturbation and coitus interuptus) are not easy methods of collecting the semen.With minor surgeries also the problems of women can be solved. So the individuals in the quest of children should approach or consult the doctor.Some times the persons (society) surrounding the individuals and his family members also prick them with harsh words.

The society should change their attitude towards the individuals not bearing the child.Any how Sputum analysis plays ultimately an important role in knowing the status of sperms present in the semen.

Key Terms

Semen : The secretion from the testicles and accessory male sex organs ex. prostate containing spermatozoa is the semen

Seminal plasma : The fluid portion in which sperms are suspended

Semen analysis : Analysis of semen to know about the spermatozoa condition

Masturbation : The process of stimulating the individuals genital organ with hands for the collection of semen

Coitus Interuptus : The process of collecting the semen by interupting sexual intercourse

Azoospermia : The condition in which spermatozoa is absent in semen

Oligozoospermia : The condition in which semen contains few spermatozoa which have motility

Necrozoospermia : The condition in which the semen contains few spermatozoa which has no motility

Liquefaction : The process of conversion of semen from solid state to lquid state

Short Answer Type Questions

Define semen

How do you collect semen specimen

What is Masturbation?

What is the normal volume of semen?

What is the PH of the semen?

What is the odour of semen?

Write the composition of semen

What are the various parameters present in physical examination of semen

Mention the normal sperm count

What is the PH of semen

Long Answer Type Questions

How will you examine different parameters of a semen by physical examination?

Write notes on microscopic examination of ssemen

Explain about the determination of morphology and sperm count.

UNIT 14

Body Fluids

Structure

14.1 Introduction

The commonly examined body fluids in the pathological laboratory are

1.Serous fluids such as

(a) Pleural (around the lungs)

(b) Pericardial (around the heart)

(c) Peritoneal fluids (around the abdominal and pelvic cavities).

2.Synovial fluids(around the joints).

14.2 Peritonial Fluid

Definition: The double serous membrane which covers the organs present abdominal cavity and pelvic cavity is known as peritonial cavity.The fluid present in the peritonial cavity is known as peritonial fluid.Generally peritonial fluid contains less than 100ml.

Normal composition of Peritonial Fluid

Peritonial fluid appears as clear or pale yellow in colour. Peritonial fluid do not consists of albumin. The pH of peritonial fluid is 7.4.Peritonial fluie consistitutes alkaline phosphatase,ammonia,cholestrol, glucose, lactic acid, lactate dehydrogenous and white blood cells.

Collection of Peritionial Fluid (Specimen)

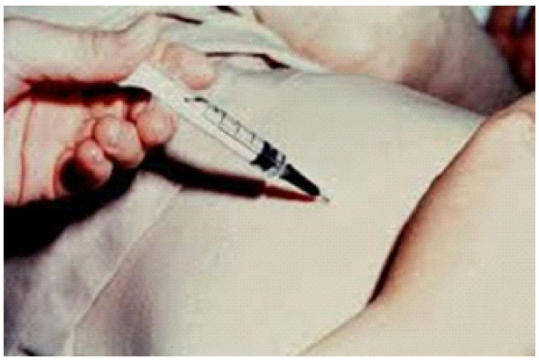


Fig. 14.1 Peritoneal Fluid Collection

The specimen is collected in fluoride oxolate tube, EDTA tube and plain tube. The peritonial fluid is collected into 3 asceptic tubes as follows:

Add 5ml of peritonial fluid into a testtube containing 15mg of fluoride oxolate.This is used for determination of protein and sugar.

Add 5ml of peritonial fluid into a testtube containing 15mg of EDTA.This is used for microscopic examination.

Add 5ml of peritonial fluid into a plain tube which is used for bacteriological tests.

Diluting Fluid and Labelling

Diluting Fluid

If the specimen is clear do not dilute it.Charge neubauer chamber directly by the specimen.If the specimen is turbid then dilute it by using saline.Acetic acid present in WBC diluting fluid may cause turbidity by reacting with the high protein content of the fluid.

Labelling

Labelling of sample plays an important role in medical laboratories. Samples have to be labelled after collection to enable their identification during testing and reporting. Eventhough the volume of sample is more and if it is not labelled due to forgetfullness it is useless. Proper numbering should be marked on the label seeing the previous samples. After labelling on the sample,the same numbers should be noted in the records with patients name.this will avoid the confusion in the identification of patients sample

Physical Examination of Peritoneal Fluid (Clinical Significance)

Peritoneal fluid is turbid in the following conditions.

(a)Appendicitis
(b) Pancreatitis

(c) Infected intestine

(d) Ruptured bowel due to the trauma

Peritoneal fluid is pale yellow in colour or ambered colour in the following.

(a)Hepatic vein obstruction
(b) Cirrhosis
(c) Nephrotic syndrome
(d)Congestive heart failure

Peritoneal fluid is greenish in colour in the following conditions.

(a)Perforated intestine
(b) Perforated gall bladder
(c) Appendicitis
(d)Perforated duodenal ulcer.

Peritoneal fluid is milky white in colour in the following conditions
(a)Parasitic infections
(b) Nephriotic Syndrom
(c) Carcinoma
(d)Lymphoma

Peritonial fluid is bloody in the following conditions.

a)Haemorrhagic Pancreatitis

b) Ruptured Liver

Microscopical Examination of Peritonial Fluid

In peritonial fluid total leucocyte count more than 500/cmm or RBC count more than 10,000/cmm are considered as abnormal.Increased total leucocyte count,mainly neutrophils in peritoneal fluid indicate acute peritonitis from any cause.

14.3 Pericardial Fluid

The fluid which is present in double membranous sac which covers the heart is known as Pericardial Fluid.

Normal Composition of the Pericardial Fluid

Pericardial fluid appears as pale yellow col;oured one and is clear.Volume of pericardial fluod is 20 to 50 ml. normally.If volume pf pericardial fluid exceeds 200ml., that condition should be considered an abnormal one.Pericardial fluid constitutes glucose and lactate dehydrogenase.

Collection of Pericardial Fluid (or Pericardial Fluid Aspiration)

Aspirate pericardial fluid into 3 sterile tubes.Use EDTA tube for gross examination and for microscopic examination.Plain or heparinised tube should be used for microbiological examination.

Heparinised tube is used for chemical examination.

Collection of Pericardial Fluid

Note: Pericardial fluid aspiration should be done under CT scan guidance.

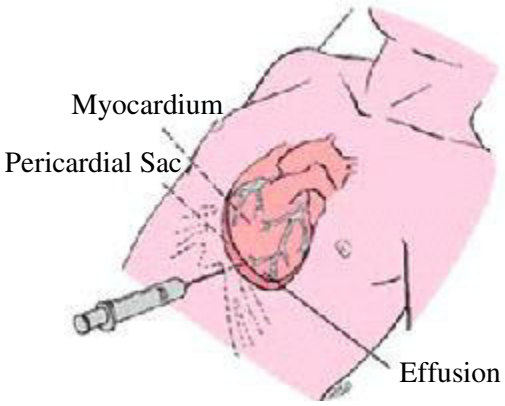


Fig. 14.2 Collection of Pericardial Fluid

Diluting Fluid and Labelling

Diluting

If the specimen is clear do not dilute it.Charge neubauer chamber directly by the specimen.If the specimen is turbid then dilute it by using saline.Acetic acid present in WBC diluting fluid may cause turbidity by reacting with the high protein content of the fluid.

Labelling

Labelling of sample plays an important role in medical laboratories.Samples have to be labelled after collection to enable their identification during testing and reporting.Eventhough the volume of sample is more and if it is not labelled due to forgetfullness it is useless.Proper

numbering should be marked on the label seeing the previous samples.After labelling on the sample,the same numbers should be noted in the records with patients name.this will avoid the confusion in the identification of patients sample

Clinical Significance

Pericardial fluid may be clear,cluody grossly bloody,milky or similar to gold point.

Increased amounts of normal appearing pericardial fluid may be found in
(a) Congestive Heart Failure
(b) Earky Stages of Inflammation
Cloudy appearence may be associated with
a)Septic/nonseptic inflammation
b) Chronic effusions of any etiology

c) Myxedema
d) Idiopathic
e) Post myocardial infraction syndrom.

Blood tinged pericardial fluid is seen in traumatic tap but it clears on aspirating more fluid.

Grossly Bloody Fluid may be caused by

(a) Post myocardial infraction syndrome
(b) Post pericardiectomy syndrome
(c) Tuberculosis
(d) Rheumatoid arthritis
(e) Systemic lupus erythematosus
(f) Metastatic Carcinoma
(g) Bacterial pericarditis

Milky Pericardial May Be Due to the

(a) True chylopericardium
(b) Bacterial
(c) Fungal
(d) Tuberculosis
(e) Rheumatoid Pericarditis
(f) Myxedema

Microscopial Examination of Pericardial Fluid

Total and differential counts are done as CSF. Increased leucocytes with more neutrophils indicates bacterial pericarditis but this condition may also seen in viral pericarditis.A high percentage of lymphocytes suggests tuberculosis pericarditis.

Microbiological Examination of Pericardial Fluid

Cultures for bacteria,fungi and tuberculosis should be performed in all effusions of unknown ethiology.

14.4 Pleural Fluid

The fluid which is present in the cavity between the layers sorrounding the lungs is called as PLEURAL FLUID.

Normal Composition of Pleural Fluids

Pleural fluid is clear and slightly amber in colour.The pH of pleural fluid is 7.4 and specific gravity of pleural fluid is 1.016.The volume of pleural fluid

isless than 25ml.Pleural fluid consisits, cholestraol, glucose, lactate dehydrogenase, total protein and White Blood Cells.

Collection of Pleural Fluid

Collect pleural fluid in to 3 sterile EDTA tubes and label the test rubes.Use the first test tube for culturing and Grams Staining. Use the remaining two test tubes for cell counts, differential counts, total protein, glucose and cytology.

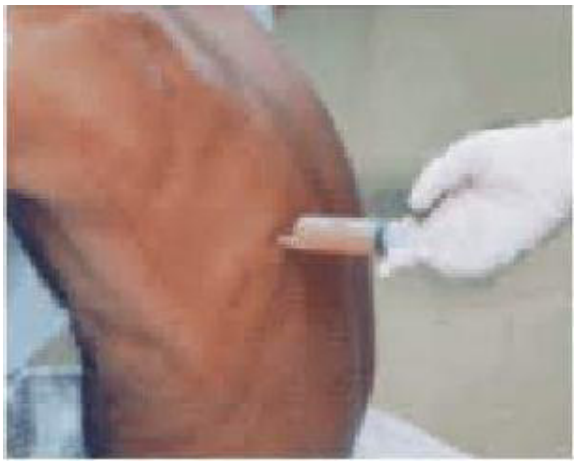


Fig. 14.3 Pleural Fluid Collection

Diluting Fluid and Labelling

Same as in Peritoneal fluid

Clinical Significance

Haemorrhogic pleural fluid can be observed in the following conditions

(a) Intra Pleural Malignancy
(b) Pancreatitis
(c) Pulmonary Infraction
(d) Pleural Infection
(e) Closed Chest Trauma
(f) Tuberculosis
(g) Hepatic Cirrosis

Microscopial Examination of Pleural Fluid

Follow the procedure of total count and differential count of CSF for pleural fluid also. A WBC count of more than 1000/cmm.or over 50% of neutrophills suggests inflammation(septic or nonseptic).

Sometimes lymphocytic effusion may be seen in

(a) Cardiopulmonary Disease
(b) Cirrhosis
(c) Infectious Mononucleosis
(d) Subacute Bacterial Pulmonary Infection

14.5 Cerebrospinal Fluid

Definition : The fluid whih is formed by selective dialysis of the plasma by the choroid plexus of the ventricles of the brain is known as CSF.CSF is present in the cavity present between the layers that sorrounds the brain in the skull and the spinal chord in the spinal column.The volume of CSF in adults is 150ml.

Collections of CSF

The CSF specimen should be cillected by a specially trained technician,a physician or a nurse. The sterile lumbar puncture needle is inserted between the fourth and fifth lumbar vertabrae to a depth of 4-5 cm. After collection of CSF specimen the fluid is collected through the needle in to two test tubes.

In to first test tube about 0.5ml. or few drops of CSF is collected.

In to second test tube about 3-5ml. of CSF is collected.

Note :1. The specimen in the test tube no.1 is used for bacterial culture.
.2.Specimen second test tube is centrifuged.
3.Supernatent liquid is used for biochemical tests such as glucose, protein, globulin and chlorides.
4.Use the sediment for grams staining,acid fast staining and differential leucocyte count after preparing a smear

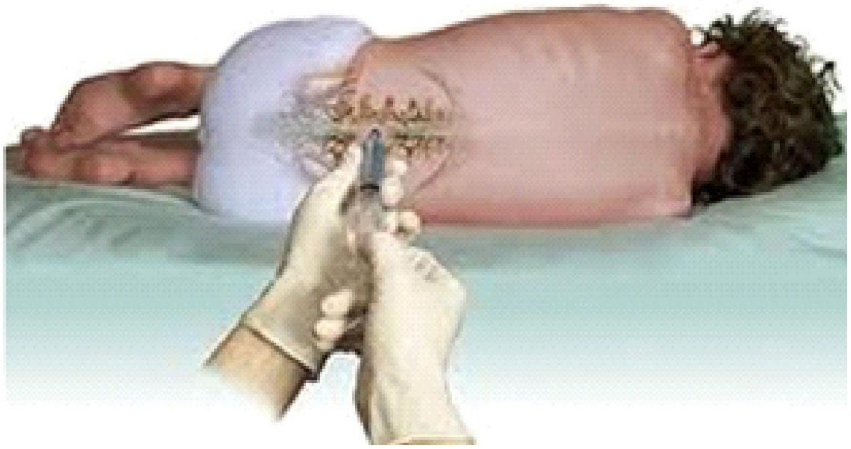


Fig. 14.4 Collection of CSF

Normal Composition of CSF

Colour : CSF has no colour i.e. colourless.

The pH of CSF is 7.3 -7.4

Appearance : CSF appears to be clear

CSF will not form clot on standing.

Specific Gravity : 1.003 - 1.008

Total Solids : Urea,Globulns,Sugar,Uric acid and Proteins etc.- 1.70g/dl.

Volume : Volume of CSF is 100-150 ml.

Routine Examination of CSF : It should be performed by

- Physical examination
- Microscopic examination
- Chemical examination

Physical Examination

Collect the specimen and note down the observations for the following aspects.

Colour : CSF has no colour in normal condition.

Appearence :CSF appears to be clear in normal condition.

Presence Of Blood : This will be observed in cerebral haemorrhages, **meningitis.**

Presence of clot or fibrin web in CSF take place due to blood brain barrier disturbance.

pH: The pH of CSF is 2-10.5

Microscopic Examination

Requirements: 1. Fuchs rosenthal counting chamber or improved neubaur counting chamber with cover slip. 2. Glass slides 3. Pasteur pipettes 4. Leishman stain and buffer solution pH 7.0 5.CSF diluting fluid 6. Grams stainig reagents 7. Acid fast staining reagents 8.Centrifuge 9. Microscope

Test Principle

Glucose in CSF specimen reacts with ortho-toluidine in hot acidic medium to form a green coloured complex.The intensity of ultimate colour produced is measured by using a photometer at 620nm to 660nm.The measured colour intensity is directly proportional to the concentration of glucose in the specimen.

Normal Values : (fasting) 70-110 mg/dl

Serum/plasma (post prandial) : Up to 130mg/dl.

(2 hrs. after lunch)

Specimen Collection

Fasting sample,post-glucose sample and post prandial samples should be collected for quantitative determination of glucose.

Clinical Significance: Increased levels of glucose can be observed in diabetes mellitus, hyper thyroidism and hyper pituitarism.

Globulin: Presence of globulin in CSF can be detected by a) Pandys test b) Nonne-apelt test

andys Test: Take test tube and to that add 1ml. of pands reagent and to that add 1 drop of CSF.

Result: If bluish white cloud is formed arround CSF drop, increased globulin level is observed in CSF.

Nonne-Apelt Test: Take clean test tube and to it add 1ml saturated ammonium sulphate.To this add 1mof CSF.If white ring is formed between the junction of two liquids,it indicates the presence of globulin in CSF.

Clinical Significance of Pandys Test: This test gives rough ideas about the increased levels of globulin in bacvterial infection.

Clinical Significance of CSF Examination: CSF examination is carried out in the pathological laboratory mainly for the diagnosis of meningitis.CSF examination is required in encephalitis,subarrachnoid haemorrhage,spinal chord tumor and CNS syphylis.

Key Terms

Bodyfluids: The fluids which are present in the various regions of the bodyi.e between different layers and in cavities are known as body fluids

CSF: The fluid which is formed by selective dialysis of plasma by the choroid plexus of the ventricles of the brain is known as CSF

DLC: Differential leucocyte count

Pleural Fluid: The fluid which is present in cavity between the layers surrounding the lungs is called as pleural fluid

Pericardial Fluid: The fluid which is present in double membranous sac which covers the heart is known as pericardial fluid

Peritoneal Fluid: The double serous membrane which covers the organs present in abdominal cavity and pelvic cavity is known as peritoneal fluid

Trauma: Bodily injury:emotional shock
Pancreatitis: Inflamation of the pancreas
Cardial: Related to heart
Choroid plexus: Related to brain
Inflammation: Swollen part of the skin
Intrapleural Malignancy: Cancer of the pleural cavity

Short Answer Type Question

Define CSF
Define pleural fluid
HOW do you collect CSF?

Write down the physical examination of CSF
What is pandys test?
Define pericardial fluid
Write down the normal composition of pleural fluid
How do you collect pericardial fluid?
Define peritoneal fluid
How do you collect peritoneal fluid?

Long Answer Type Questions

Write notes on microscopical examination of CSF
Explain in detail about Pleural fluid
Write notes on peritoneal fluid
Write notes on chemical examination of CSF
Explain about the pericardial fluid.

UNIT 15

Haematology

Structure

15.1 Introduction

The branch dealing with the study of blood and blood components is called as haematology. Haematology encompases the study of blood components and coagulation. Blood may be described as a specialised connective tissue which circulates in a closed system of blood vessels. The circulating blood consists of suspensiion of formed elements such as erythrocytes, leucocytes and platelets in a pale yellow coloured fluid called plasma.

Hematology, also spelled haematology, is the branch of medicine concerned with the study of the cause, prognosis, treatment, and prevention of diseases related to blood. It involves treating diseases that affect the production of blood and its components, such as bloodcells, hemoglobin, blood proteins, bone marrow, platelets, blood vessels, spleen, and the mechanism of coagulation. Such diseases might include hemophilia, blood clots, other bleeding disorders and blood cancers such as leukemia, multiple myeloma, and lymphoma. The laboratory work that goes into the study of blood is frequently performed by a medical technologist or medical laboratory scientist. Many hematologists work as hematologist-oncologists, also providing medical treatment for all types of cancer.

The functions of the blood are as follows:

- Respiration
- Excretion
- Acid-base balance maintenance
- Nutrition
- Regulation of water balance
- Regulation of body temperature
- Transport of harmones, vitamins and salts
- Transport of metabolites
- Defensive action
- Coagulation against haemorrhages

15.2 Collection of Blood

Definition: The way of obtaining blood from veins and capillaries of a human body using disposable syringes is known as collection of blood.

Universal precautions were designed for doctors, nurses, patients, and health care support workers who were required to come into contact with patients or bodily fluids. This included staff and others who might not come into direct contact with patients.

Pathogens fall into two broad categories, bloodborne (carried in the body fluids) and airborne.

Uses

Universal precautions were typically practiced in any environment where workers were exposed to bodily fluids, such as:

- Blood
- Semen
- Vaginal secretions
- Synovial fluid
- Amniotic fluid
- Cerebrospinal fluid
- Pleural fluid
- Peritoneal fluid
- Pericardial fluid
- Bodily fluids that did not require such precautions included:
- Feces
- Nasal secretions Urine
- Vomitus
- Perspiration
- Sputum , Saliva

Methods of Collection

Definition

The various methods which are used to collect the blood from various routes is called as the methods of collection.

The following methods are used to collect the blood

- Venous Method
- Capitallary Method
- Vacutainer Methods

Collection of Venous Blood

Aim: Collection of Venous Blood.

Requirements: Disposable syringe, tourniquet, cotton, spirit.

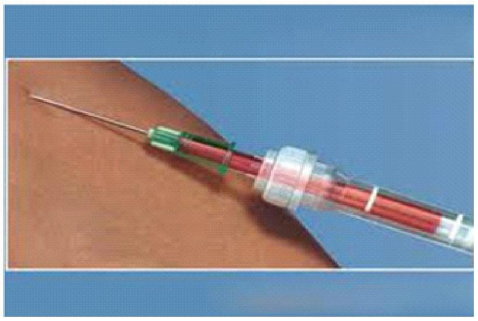


Fig. 15.1 Collection of Venous blood

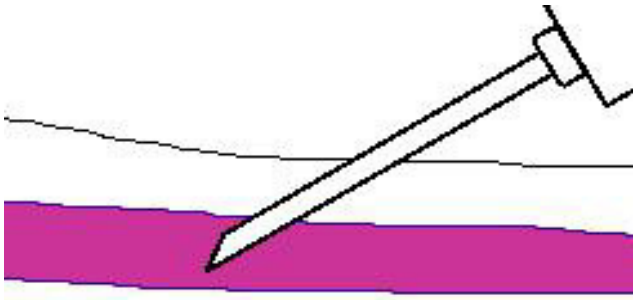
Blood Specimen Collection and Processing

The first step in acquiring a quality lab test result for any patient is the specimen Collection.

Venipuncture Procedure:

1. A phlebotomist must have a professional, courteous, and understanding manner in all contact with all patients.
2. The first step to the collection is to positively identify the patient by two forms of identification; ask the patient to state and spell his/her name and give you his/her birth date. Check these against the requisition (paper or electronic).
3. Check the requisition form for requested tests, other patient information and any special draw requirements. Gather the tubes and supplies that you will need for the draw.
4. Position the patient in a chair, or sitting or lying on a bed.
5. Wash your hands.
6. Select a suitable site for venipuncture, by placing the tourniquet 3 to 4 inches above the selected puncture site on the patient.
7. Do not put the tourniquet on too tightly or leave it on the patient longer than 1 minute.
8. Next, put on non-latex gloves, and palpate for a vein.
9. When a vein is selected, cleanse the area in a circular motion, the area needs to be re-cleansed before the venipuncture is performed.

10. Insert the needle through the skin into the lumen of the vein. The needle should form a 15-30 degree angle with the arm surface.



11. When the last tube is filling, remove the tourniquet.
12. Remove the needle from the patient's arm using a swift backward motion.
13. Apply and hold adequate pressure to avoid formation of a hematoma. After holding pressure for 1-2 minutes, tape a fresh piece of gauze or Band-Aid to the puncture site.
14. Dispose of contaminated materials.

Precautions

1. Void healed burn areas
2. Take steps to avoid haemolys is
3. Always Torniquetor aclothlikeh and key is used to tighten the top portion of the hand, which pools up blood in the collecting site.
4. Always asceptic conditions are followed and disposable syringes and needles are to be used

Advantages

- For separation serum from venous blood(clotted blood as specimen)
- Separated serum from venous blood is used in chemistry testing, serological testing and blood banking (serum as a specimen)
- Whole blood is used in haemotology testing.
- Plasma is used in coagulation studies and in plasma chemistries (plasma as specimen)

Finger Puncture:(Collection of capillary blood)

Definition:The blood which is collected from the capillaries by puncturing the skin is called as the collection of capillary blood)

Collection of Capillary Blood

Aim: Collection of Capillary Blood.

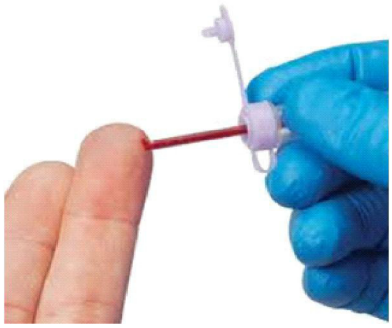
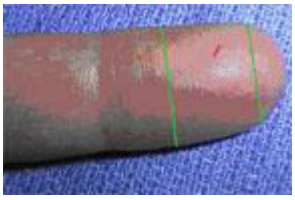


Fig. 15.2 Capillary Blood Collection

Requirements: Disposable needle, spirit, cotton.

Fingerstick Procedure:

1. Follow steps #1 through #5 of the procedure for venipuncture as outlined above.
2. The best locations for fingersticks are the 3rd (middle) and 4th (ring) fingers. Do not use the tip of the finger or the center of the finger. Use the finger where vessels and nerves located.
3. When a site is selected, put on gloves, and cleanse the selected puncture area.
4. Massage the finger toward the selected site prior to the puncture.
5. Using a sterile safety lancet, make a skin puncture just off the center of the finger pad. The puncture should be made perpendicular to the ridges of the fingerprint so that the drop of blood does not run down the ridges.
6. Wipe away the first drop of blood, which tends to contain excess tissue fluid.



7. Collect drops of blood into the collection tube/device by gentle pressure on the finger.
8. Cap, rotate and invert the collection device to mix the blood collected.
9. Have the patient hold a small gauze pad over the puncture site for a few minutes to stop the bleeding.
10. Dispose of contaminated materials/supplies in designated containers.
11. Label all appropriate tubes at the patient bedside.

Precautions

- Blood pipettes used should be clean and dry.
- Clean and dry pipettes ensure filling with ease.
- The site from which capillary blood is collected should be cleansed with cotton soaked in spirit
- After collection of blood from the site, it has to be covered with cotton by pressing it

Advantages

- Collection of capillary blood is very easy when compared to the venous blood collection
- No special technique is needed in collecting the capillary blood
- Majority of haematological tests like, Hb estm, RBC count, WBC count
- DLC, Platelet count are determined
- Less expensive equipment like ,a single disposable needle is needed
- Easy way of collecting the blood

iii) **Vacutainers methods**

Definition: The method of collection of blood by using Vacutainer blood collection tube is called as vacutainer method.

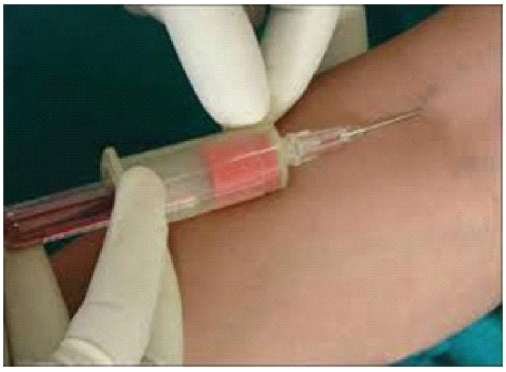


Fig. 15.3 Blood Collection by Vacutainer Method

A **Vacutainer** blood collection tube is a sterile glass or plastic test tube with a colored rubber stopper creating a vacuum seal inside of the tube, facilitating the drawing of a predetermined volume of liquid. Vacutainer tubes are available with a safety-engineered stopper, with a variety of labeling options and draw volumes. The color of the top indicates the additives in the vial.

Vacutainer tubes

Blood Collection byusing Vacutainers

During the bloodcollection process,the rear cannula pushes through the rubber sleeve and puncture the rubber stopper,allowing the vaccum in the tube todrawblood from the vein.

Note

1. All vaccutaner tubes and needles are sterile and intended for single use.
   The needle holders can be reused after sterilisation by autoclaving.
2. The tube stoppers are colour coded so that the person using them find out at glance the type of additive used.

Advantages of Vacutainer System

- It eliminates the preparation of anticoagulated bulbs and tubes
- There is no processing of containers
- It minimises haemolyses in specimens
- It assures accurate blood to additive ratio

POCT

- Point-of-care testing (POCT) is defined as medical testing at or near the site of patient care. POCT includes: blood glucose testing, blood gas and electrolytes analysis, rapid coagulation testing(PT/ INR, Alere), rapid cardiac markers diagnostics (TRIAGE,Alere), drugs of abuse screening, urine strips testing, pregnancy testing, fecal occult blood analysis, food pathogens screening, hemoglobin diagnostics, infectious disease testing and cholesterol screening.
- These tests require only a single drop of whole blood, urine or saliva, and they can be performed and interpreted by any general physician within minutes.

Fig. 15.4 POCT Collection of Blood

POCT has become established worldwide and finds vital roles in public health.

Fig. 15.5 Blood collection at Bed side in case of Children

Fig. 15.6 Blood Collection at Bed side in case of Adults

15.3 Preparation of Anticoagulants

Definition: The chemicals which are used to prevent the coagulation or clooting of blood are called as anticoagulants.

The following are the various types of anticoagulants:

- Double Oxalate
- Sodium Citrate
- Edta
- Heparine

Double Oxalate

Preparation: The solution is prepared with a concentration of two parts of potassium oxalate and three parts of ammonium oxalate by disolving in 0.08 grams of potassium oxalate and 1.2 grams of Ammonium oxalate in 100ml. of distilled water.

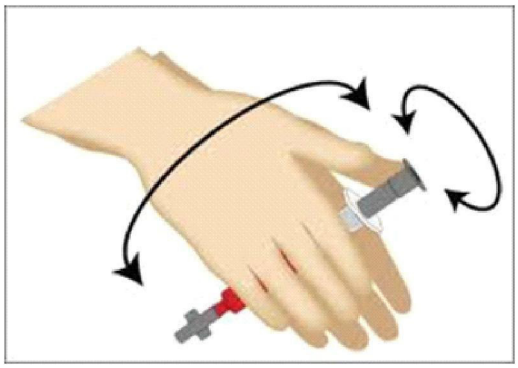


Fig. 15.7 Mixing Anticoagulant with Blood to Prevent Stacking of red blood

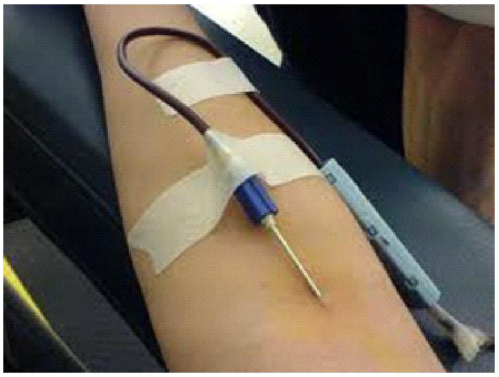


Fig. 15.8 Plastic Bags containing Anticoagulant for Blood Collection

2. **Sodium Citrates 3.8%**

   Preparation: 3.8gm of sodium citrate is dissolved in distilled water and labelled as 3.8% sodium citrate

3. **EDTA(Ethylene diamine tetra acetic Acid):** of EDTA is dissolved in distilled water and solution is prepared.

4. **Heparine**

Preparation

This used in the concentration of 0.1to 0.2mg/ml of blood

15.4 RBC and WBC Count

Introduction

Diagnostics plays prominent role in the field of Medicine. Without proper diagnosis, proper conclusions regarding Medical treatment/ surgery can not be obtained.

The RBC count and WBC count decides about major clinical conditions and status of the patient. Some times in certain cases both the two counts may rise and in certain cases both the two counts may fall down. Fall or rise of both the two counts are having diagnostic importance. So RBC and WBC counts plays an important role in giving information about clinical conditiion of the patient.

RBC Count

A red blood cell (RBC) count is a blood test that tells you how many red blood cells you have.

Red blood cells contain a substance called haemoglobin which transports oxygen around the body. The amount of oxygen that's delivered to your body's tissues will depend on the number of red blood cells you have and how well they work.

A RBC count is usually carried out as part of a full blood cell (FBC) count. A normal RBC count would be:

male–4.7 to 6.1 million cells per micro litre(cells/mcL)

female–4.2 to 5.4 million cells/mcL

The results of an RBC count can be used to help diagnose blood-related conditions, such as irondeficiency anaemia (where there are less red blood cells than normal).

A low RBC count could also indicate a vitamin B6, B12 or folate deficiency. It may also signify internal bleeding, kidney disease or malnutrition (where a person's diet doesn't contain enough nutrients to meet their body's needs).

A high RBC count could be due to a number of health conditions or health-related factors including:

- smoking
- congenital heart disease
- dehydration – for example, from severe diarrhoea low blood oxygen levels (hypoxia)
- pulmonary fibrosis – a lung condition that causes scarring of the lungs

Counting of the number of RBC’s in human blood using haemocytometer is known as RBC Count.

Haemocytometer: The haemocytometer Neubauer counting chamber has a total ruled area of 9sq.mm. It consists of centrally heavy ruled area of 1sq.mm.in size and four other of the same size in each corner. The central area is divided into 25 squares is further subdivided into 16 subsquares. For total RBC counts use 5 squares of the central area. That is 80 ml squares.

Requirements: Haemocytometer, RBC pipette, sterilized needles, microscope, cover glass, cotton, spirit, RBC diluting fluid etc.

RBC pipette: The RBC pipette has red bead. It has two graduations, namely 0.5 and 101.

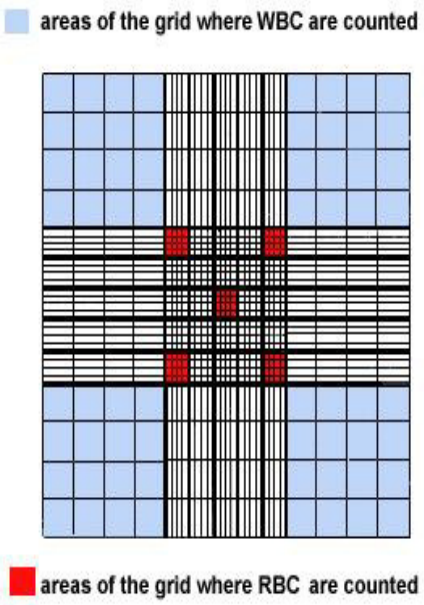


Fig. 15.9 Neubaur Counting Chamber with circles for both RBC count and WBC count

RBC Diluting Fluid: The area two fluids and any one can be prepared and used

(a) FORMAL CITRATE SOLUTION

Trisodium - 3g.
Distilled water - 99ml
Formalin - 1ml.

(b) HAYEM'S FLUID

Sodium Chloride - 0.5g
Sodium Sulphate - 2.5g
Mercuric Chloride - 0.25g.
Distilled Water - 100ml.

Procedure for RBC Count

1. The tip of the index finger is sterilised by rubbing with a cotton soaked in spirit.
2. Make gentle prick with the help of sterilised pin or needlr.
3. The tip of the finger is pressed and blood oozes out.

4. The first drop is wiped out with the help of cotton.
5. Then the blood is aspirated into the RBC pipette exactly upto 0.5 mark.
6. Immediately RBC diluting the fluid is loaded up to the 101 mark.
7. The pipette is rotated between the thumb and forefinger. This will give a dilution of 1:200.
8. Clean the counting chamber and cover glass thoroughly.
9. Place the coverglass in position over the ruled area,using gentle pressure.
10. Mix the suspension thoroughly by rotating the pipitte for about a minute holding it in horizontal position.
11. Fill the chamber by holding the pipette at an angle of 45 degrees
12. Allow two to three minutes for the red corpuscels to settle.
13. Count the number of RBC's in 80 small squares.(4 squares at the four corners and right hand lines, but count the cells touching the upper and left hand lines).

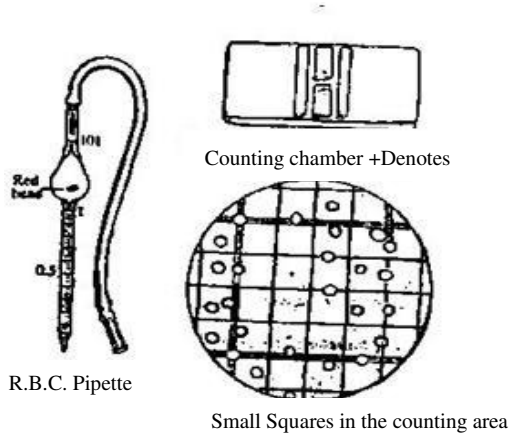


Fig. 15.10 RBC Counting

Trisodium citrate is dissolved in little amount of distilled water and to this 1ml of Formalin is added. Then this is diluted with distilled water and volume is made upto 100ml with distilled water

Calculation

The area of a small square is = 1/400sq.mm.

The depth of the counting chamber is = 1/10mm.

Therfore, the volume of a small square is=1/4000c.mm.

The dilution of the blood is = 1/200

$$\text{Total RBC's} = \frac{423 \times 4000 \times 200}{80 \times 1 \times 1} = 42,30,000 \text{ per c.mm.}$$

Normal: Men 4.5 to 6.5 millions per c.mm. Women 3.9 to 5.6 millions per c.mm.

Clinical Significance

RBC count increases in polycythemia.RBC count decreases in anaemia.

Precautions

1. Care should be taken while puncturing the finger & taking the sample
2. Use sterile needles or lancets.
3. Diluting fluid must be taken accurately up to the mark

This method helps in minimising the pipetting and the dilution errors which are encountered while using the micropipettes. In this method large volumes of blood and diluting fluid are used.

WBC Count:

Counting of the number of WBCs in human blood using haemocytometer is known as WBC Count.

Requirements

Haemocytometer, WBC pipette, sterilised needles, microscope, cover glass,cotton, spirit, WBC diluting fluid etc.

WBC PIPETTE: The WBC pipette has a white bead. It has also two graduations, namely 0.5 and 11 markings.

WBC Diluting Fluid (Truck's Fluid)

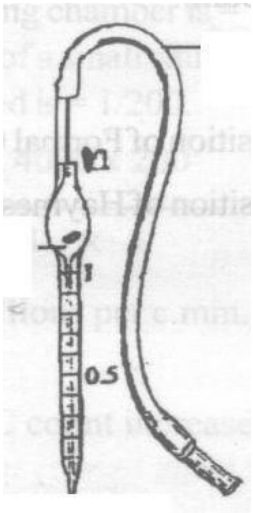
Acetic acid, glass 3ml.

Distilled Water 97ml.

Add Gention voilet to give a pale voilet colour.

Preparation of Diluting Fluid

Add the two lquids. Add gentian voilet to give a pale voilet colour



W.B.C. Pipette

Fig. 15.11

Procedure for White Blood Cell (Leucocyte) Counts

1. Draw the blood upto the 0.5 mark in WBC pipette and dilute upto mark 11 with WBC fluid and fill the counting chamber.
2. Allow 3 minutes for cells to settle.
3. Count the cells in the four corner blocks by using the low power objective
4. In counting the cells, include those cells touching on the inner lines on the right and top, but mot count the cells touching the lines on the left and bottom.

Clinical Significance

WBC count increases in leucocytosis and leukaemia.

WBC count decreases in leucopenia.

Calculation:

No.of WBC's countedin fourcorners=104

Thevolumeof a square =1/10c.mm.

The blood was diluted to =1/20

Therefore numberof cellspercmm $= \frac{104 \times 10 \times 120}{4}$

= 5200 cells per c.mm of undiluted blood

Normal : WBC's per c.mm. of blood 4,000 to 10,000 per c.mm.

Precautions

1. Care should be taken while puncturing the finger & taking the sample
2. Use sterile needles or lancets.
3. Diluting fluid must be taken accurately up to the mark

15.5 Platelet Count

Introduction

The methods used for automated platelet counting are impedance, optical scatter, optical fluorescence, and immunologic flow cytometry. The introduction of the international reference method (IRM) for platelet counting by flow cytometry has improved the precision and accuracy of platelet counting at thrombocytopenic levels and offers a suitable comparator for routine platelet counting methods.

Morphology Platelets . Functions of Platelets. Platelet Diluting Fluid

Procedure of Platelet Counting

Morphology Platelets

Platelets look as round oroval plates with biconvex surfaces when observed microscopically. These are named as thrombocytes platelets are having the 2-4 diameter Platelets contain the granules in the cytoplasm without nucleus. Normal platelets count ie 2,50,000 to 5,00,000 per common of blood. They are the fragments of giant cells called Megakaryocytes produced in the bone marrow. Platelets are non-necleated and irregular in shape. When disintegreted, they release the enzyme thromboplastic and initiate the clotting process, which is very complex one.The blood platelets also help in sealing small vascular openings by forming a platelet plug. Decreased platelet count is seen in thrombocytopenia.

Function Of Platelets

- Initiation of blood clotting by disitegretion and liberation of thromboplatin.
- Speedy repair of capillary endothelial lining.
- Haemostatic mechnism by means of aggulutination and coagulation.
- Hastening the retraction of clot.It is dependent on thrombosthenin.
- Liberating of 5-HT and Histamine to exert vaso constriction which helps in haemostasis.

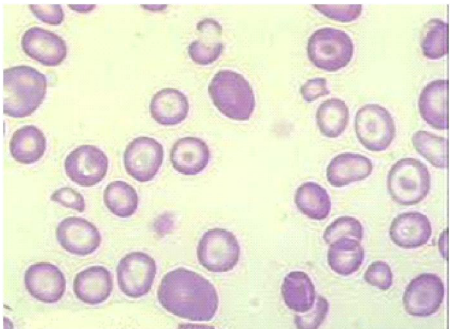


Fig. 15.12 Morphology of Platelets

Platelet Diluting Fluid

Composition

Procaine hydrochloride	3.0gm
Sodium chloride	10.0gm
Distilled water	100ml

Preparation

Above two components are dissolved in few amount of water and volume is made to 100 ml with distilled water

Procedure of Platelet Counting

Requirements

(1) Microscope (2) Improved Neubaur Counting Chamber

(3) RBC Pipette (4) Platelet Diluting Fluid

Procedure

1. Mix the blood specimen carefully.
2. By using RBC pipette draw blood upto 0.5 mark.
3. Wipe excess blood on the outside of the pipette.
4. The diluting drawn upto the mark 101.
5. Mix the contents in the bulb thoroughly.
6. After 5 minutes, discard the first drop, then transfer a small drop on one side of the counting chamber.
7. Place the filled mounted counting chamber under a petridish with a moust filter paper.

8. Let it stay undisturbed for 15 minutes.
9. Place the counting chamber carefully on the stage of the microscope. Under low power magnification focus red cell counting area.
10. Move to view the corner square the red cell area and change to high power objective.
11. Keep the condenser down and reduces the light by adjusting the diaphragm.
12. The platelets will appear like highly refractile particles.Count platelets in all 25 small squares.

Normal Ranges

2,50,000 - 5,00,000 / cumm (Micro Liters)

Clinical Significance

Detrmination of platelts is requested in the investigation of bleeding disorders.Decreased platelets count is often associated with prolonged bleeding and poor clot retraction.Decreased platelet count also occurs in aplastic anaemia, megaloblastic anaemia & acute leukaemia.Increased platelet count is found in polycythemia vera, following spleen ectomy.

15.7 Haemoglobin Estimation

Introduction

HB% in the given sample is estimated by following methods

- Tallquist method
- Sahlis acid haematin method
- Alkali method
- Cyanmethhaemoglobin method

Sahlis Acid Haematin Method

To Determination of HB by Sahlis acid haematin method

Requirements:

- Sahli haemoglobinometer
- 0.1N HCL
- Distilled water
- Pasteur pipettes

The Sahli haemometer method utilizes the conversion of haemoglobin into acid haematin which has a brown colour in solution. The intensity of the colour is related to the amount of

haemoglobin in the blood sample. Water is added to dilute the brown solution until it matches that of a standard. The more haemoglobin, the more water required to obtain a colour match. Haemoglobin values are read at the meniscus of the brown solution.

5.14 Sahlis Acid Haematin Method

Parts-

a. **Sahli'sgraduated hemoglobin tube** (marked in grams percent g% (2-24) and percentage % (10 -140)

b. **Comparator** with a brown glass standard. opaque white glass is present at the back to provide uniform illumination.

c. **Sahli'spipette or hemoglobin pipette** (marked at 20µl or 0.02 ml). No bulb

d. **Stirrer:** Thin glass rod .

INDICATIONS OF HAEMOGLOBIN ESTIMATION

- To determine presence and severity of anemia
- Screening for polycythemia
- To assess response to specific therapy in anemia.
- Estimation of red cell indices
- Selection of blood donors.

PRINCIPLE:

Blood is mixed with an acid solution so that hemoglobin is converted to brown-colored acid hematin. This is then diluted with water till the brown colormatches that of the brown glass standard. The hemoglobin value is read directly from the scale.

Procedure

- The diluent is N/10 Hydrochloric acid (HCL). Add it from the dropping bottle provided to the graduated tube, up to mark 2.
- Measure 0.2 ml (20 µl) of well-mixed blood, with the provided micropipette (Sahli's pipette) and transfer it to the HCL in the tube.
- With the pipette beneath the surface of the acid, gently blow the blood.
- Rinse the pipette by sucking up and blowing out diluent 2-3 times.
- Thoroughly mix blood and acid using a fine glass rod (HCL will react with the haemoglobin and convert it into acid-haematin, which has a brown color).
- Wait up to 3 minutes after adding the blood to allow the color to develop sufficiently to achieve an accurate comparison.
- Add distilled water gradually to the mixture and mix the solution with glass rode.
- Place the tube in the haemoglobinometer and compare it with the standard.
- Continue to add distilled water until the sample firstly appears to be detectably pallor than the standard.

Note the level of the liquid in the tube.

Normal values:	Hb,g/dl
Men	14-18
Women	11.5-16.5
Children(up to 1 yr)	11.0-13.0
Children(10-12yrs)	11.5-14.5
Infants(full term cord)	13.5-19.5

Advantages

- This method is useful for places where a photometer is not available
- Sahlis method is easy to perform and convenient.
- This method is inexpensive.
- It is not very time consuming (maximum takes 15 minutes.

Clinical Significance

Decreased in anemia, hemorrhage, and hemolytic reactions;increased in dehydration, heart and lung disease

A decrease in haemoglobin below normal range is an indication of anaemia. An increase in haemoglobin concentration occurs in haemoconcentration due to loss of body fluid in severe diarrhoea and vomiting.High values are also observed in congenital heart disease and in polycythemia.

CYANMETHHAEMOGLOBIN METHOD

This is the preferred and the most accurate method for determining the hemoglobin concentration. It is the standard method used in most of the centers.

This method is optional for estimation of hemoglobin and this method is recommended by International Committee for Standardization in hemotology. This is because in this method all type of hemoglobin are transformed to cyanmethemoglobin (except sulfhemoglobin), and a firm and trustworthy standard is available.

Principle

When Blood is mixed with a solution of potassium cynide, potassium ferricyanide and Drabkin's solution, the erythrocytes are lysed by producing evenly disturbed hemoglobin solution. Potassium ferricyanide transforms hemoglobin to methemoglobin, and methemoglobin combines with potassium cyanide to produce cyanmethemoglobin. This way all types of hemoglobin present in blood are entirely transformed to a single compound cyanmethemoglobin. When the reaction is entire, absorbance of the solution is deliberate in a spectrophotometer at 540 nanometer. Cyanmethemoglobin has a wide absorbance peak at this wavelength. The absorbance is compared with that of the standard cyanmethemoglobin solution by using a formula to obtain the amount of hemoglobin. The Formed cyanmethemoglobin has a wide absorbance peak at 540nm wave length.

Equipment

- Spectrophotometer or photoelectric colorimeter
- Pipette 5 ml
- Sahli's pipette

Reagent

- Drabkin's Solution
- Cyanmethemoglobin standard solution with known hemoglobin value

Specimen

Blood obtained from skin puncture or EDTA-anticoagulated venous blood.

Procedure

- Take 5 ml of Drabkin's solution in a test tube and add 20 µl of blood. This way, we will get the dilution of 1:25. Now mix the mixture and allow to stand for atleast 5 minutes. This time is adequate for transformation of hemoglobin to cyanmethemoglobin.
- Pour the test sample to a cuvette and read the absorbance of the test sample in a spectrophotometer at 540 nanometer or in a photoelectric colorimeter using a yellow-green filter. Also read the absorbance of the standard solution. Absorbance must be read against Drabkin's solution.
- From the formula given below, the hemoglobin value is derived.

Hemoglobin in gm/dl = [Absorbance of test sample ÷ Absorbance of standard] x concentration of standard x [Dilution factor ÷ 100]

Result

The result is calculated by using the formulae

Hbgm/dl=Absorbanceoftest/AbsorbanceofStandard×conc.of

Standard..

Advantages

- All forms of Hb except Sulphahemoglobin are readily converted to cyanmethemoglobin
- Direct comparison with cyanmethemoglobin standardpossible.
- Stabilityof the diluted sample, readings made at operator's convenience.
- Easy toperform the test.
- Reagents are readily available.
- The standardis stable.

–

Disadvantages

- Increasedabsorbancenotduetohaemoglobinmaybecausedby turbidity due to abnormal plasma proteins, hyperlipaemia, high WBC countor fat droplets.
- Potassium cyanide in the solutions is poisonous, though it is present only in a very low concentration hence the reagents should be handled carefully.

Clinical Significance

Hemoglobin (Hb) has the major function of supplying oxygen tothe tissue cells. Hb estimation is one ofthe commonest screening tests for the diagnosis of Anemia. Decreasedlevelsof hemoglobin concentrations areobserved in all varieties of anemia, resulting from hemorrhage or fromdeficiencyof iron, Vitamin B12 or Folic acid. Increased levels of hemoglobin concentration is observed in polycythemia vera, congenital cyanotic heart disease and in hemoconcentration due to various clinical causes like heat stroke and dehydration

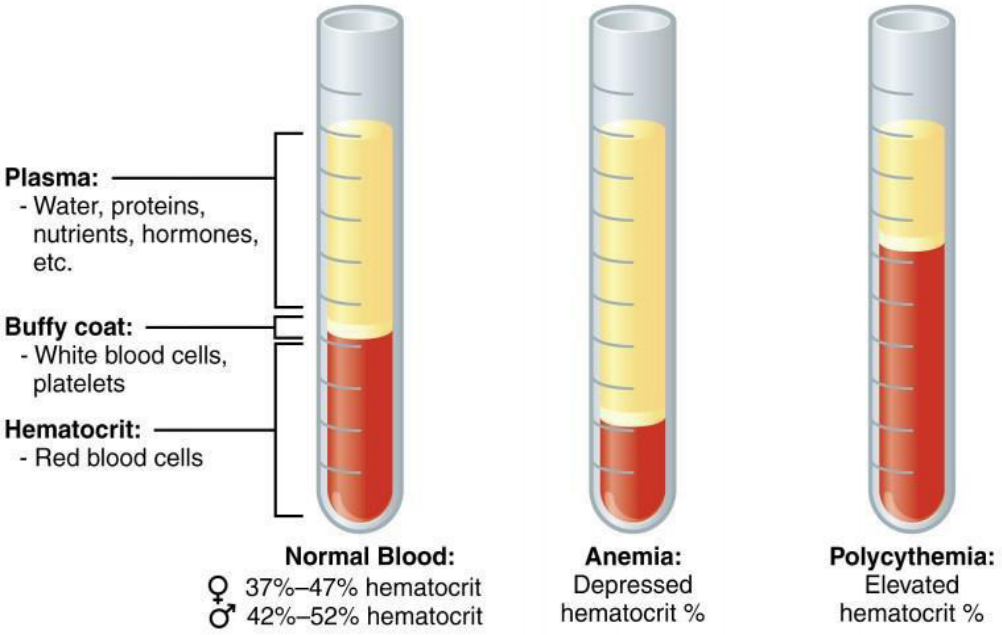
15.8 Estimation of PCV

Introduction

The packed cell volume (PCV) is the measure of the ratio of the volume occupied by the red cells to the volume of whole blood in a sample of capillary, venous, or arterial blood. The ratio is measured after appropriate centrifugation and is expressed as a decimal fraction.

The PCV is an easily obtained measure for detecting anemia or polycythemia and can be useful in estimating changes in hemodilution or hemoconcentration. The PCV is used, together with the red cellcount, in calculating the mean cell volume (MCV) and, together with the hemoglobin content, incalculating the mean corpuscular hemoglobin concentration (MCHC).
Estimation of PCV can be done by Macro method & Micro method:

MACRO METHOD (WINTROBE METHOD) FOR ESTIMATION OF PACKED CELL VOLUME (PCV) OR HEMATOCRIT



Principle

Anticoagulated whole blood is centrifuged in a Wintrobe tube to completely pack the red cells. The volume of packed red cells is read directly from the tube. An advantage with this method is that before performing PCV, test for erythrocyte sedimentation rate can be set up.

Equipment

Wintrobe tube: This tube is about 110 mm in length and has 100 markings, each at theinterval of 1 mm. Internal diameter is 3 mm. It can hold about 3 ml of blood.

Pasteur pipette with a rubber bulb and a sufficient length of capillary to reach the bottom of the Wintrobe tube.

Specimen

Venous blood collected in EDTA (1.5 mg EDTA for 1 ml of blood) or in double oxalate. Test should be performed within 6 hours of collection.

Method

- Mix the anticoagulated blood sample thoroughly.
- Draw the blood sample in a Pasteur pipette and introduce the pipette up to the bottom of the Wintrobe tube. Fill the tube from the bottom exactly up to the 100 mark. During filling, tip of the pipette is raised, but should remain under the rising meniscus to avoid foaming.
- Centrifuge the sample at 2300 g for 30 min (To counterbalance a second Wintrobe tube filled with blood from another patient or water should be placed in the centrifuge).
- Take the reading of the length of the column of red cells.

After centrifugation of anticoagulated whole blood, three zones can be distinguished in the Wintrobe tube from above downwards-plasma, buffy coat layer (a small greyish layer of white cells and platelets, about 1 mm thick), and packed red cells. Normal plasma is straw-colored. It is colorless in iron deficiency anemia, pink in the presence of hemolysis or hemoglobinemia, and yellow if serum bilirubin is raised (jaundice). In hypertriglyceridemia, plasma appears milky. Increased thickness of buffy coat layer occur if white cells or platelets are increased in number (e.g. in leukocytosis, thrombocytosis, or leukemia). Smears can be made from the buffy coat layer for demonstration of lupus erythematosus (LE) cells, malaria parasites, or immature cells

- Plasma colour
- Yellow colour of plasma indicates jaundice.
- Opaque plasma is due tolipaemia.
- Pink colour denotes haemoglobinemia.

Normal Values:

- Men 40-54%
- Women 36-47%
- Atbirth 44-62%

- Oneyear 35%(approx)
- Tenyears 37.5%(approx)

Clinical Significance

Avalue below an individual's normal or below the reference range for the age and sex indicates anaemia and a higher value indicates polycythemia.

Fall of haematocritvaluesare observedin

1. Anaemias 2. Hydraemia(occurrence ofexcessivefluidinbloodin pregnancy)

Increased haematocrit values are observed in

1. Polycythemia vera 2. Dehydration 3.Emphsema 4.Congenital heart disease.

Estimation of PCV(Haematocrit) by Microhaematocrit method

This method is used whenever there is a difficulty in drawing sufficient amount of blood.It is useful particularly in paediatric patients.This is most ideal for skin puncture.

Specimen:1. EDTAor oxalated specimen(use plain capillary tubes)

2. Capillary blood(ysed heparinsed capillaries)

Requirements

- Haematocrit centrifuge
- Haematocrit reader
- Capillary haematocrit tubes
- Soft wax or modelling clay which is used to seal the end of the capillary tube

Procedure

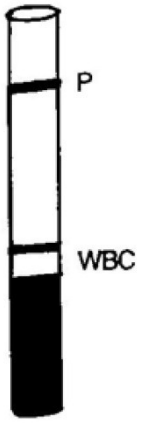
1. In to the capillary tube draw the specimen(anticoagulated blood) appropriately.
2. Fill the capillary tube with anticoagulated blood upto about3/4 length.
3. Seal both ends of the capillary tube wiyh soft wax or modelling clay to a depth of about 1cm.
4. Place the sealed capillary tube in centrifuge cup and place another similar sealed capillary tube at opposite side.
5. Close the centrifuge with cover plate and centrifuge the tubes at high speeds(at 1500RPM) for 5 minutes.
6. Remove the capillary tube.
7. Three layers will be formed after centrifugation.

a) Clear plasma layer will be formed at the top

b) Whitish buffy coat later will be formed at the middle portion

c) Column of red blood cells is formed at the bottom

The bottom of the column of the red blood cells is brought to the same line with horizontal zero line by holding the tube against the haematocrit scale 1.0 mark of haematocrit scale should pass through upper line of plasma(top of the plasma column). Reading on the haematocrit scale corresponing to the upper level of the RBC column has to be noted which is to be taken as the PCV value.



P (plasma) WBC(white blood cells) RBC(red blood cells)

PCV by micro haematocrit method.

After centrifugation the capillary tube will show three layers

- Plasma(P)
- WBC(White blood cells)
- RBC(Red blood cells)

Observations

Haemolysed specimen will yield false low values.Proper mixing of blood and EDTA should be done. Otherwise it gives false values

Advantages

- Only small amount of blood is needed for the test.
- Even capillary blood can be sampled.
- Less time is needed for the test.
- Easier procedure.
- Amount of trapped plasma is much less.Fig. 15.17 Micro method

Clinical Significance

Observe any abnormal findings such as

1. Colour of plasma Yellow colour may indicate Jaundice Reddish colour indicate haemolysis
2. Increased white blood cells will indicate the increased buffy coat layer.

Estimation of Erythrocyte Indices

When a patient has a lower than normal hemoglobin, it is important to determine whether red blood cells are of normal size and if they have a normal concentration of hemoglobin. These measurements, known as erythrocyte or red blood cell indices, provide important information about various types of anemias.

Using haemoglobin concentration,PCV and total RBC count,RBC indices are caluclated.The commonly employed RBC indices are

Mean cell volume(MCV)

Mean cell haemoglobin(MCH)

Mean corpuscular haemoglobin concentration(MCHC) RDW

Colour index

Mean cell volume:

Mean corpuscular volume (MCV) measures the mean or average size of individual red blood cells.

Calculation

MCV=PCV X 10

RBC in millions/cmm

Normal values:82 to 92 cubic microns

Clinical Significance

Increased MCV is observed in Macrocytic anaemia

Decreased MCV is observed in Microcytic anaemia

MCV

MCV values are higher in newborns and infants

Men: 80-98 fl (femoliters)

Women: 96-108 fl

Mean Corpuscular Haemoglobin(MCH)

Mean corpuscular hemoglobin (MCH) measures the amount, or the mass, of hemoglobin present in one RBC. The weight of hemoglobin in an average cell is obtained by dividing the

hemoglobin by the total RBC count. The result is reported by a very small weight called a picogram (pg).

Normal values:

MCH - 17-31 pg (picograms)

27-32 micro micrograms

Clinical Significance

Increased values are observed in Macrocytic anaemia.Decreased values are observed in Hypochromic anaemia.

Mean Corpscular Haemoglobin Concentration (MCHC):

The concentration of the average red blood cell containing haemoglobin is called as MCHC.

$$MCHC = \frac{Hb \times 100}{PCV}$$

Normal values: 32-36%

Clinical Significance:

Decreased values are observed in Hypochromic anaemias.Increased values are observed in Spherocytosis.

RDW

The red blood cell distribution width (RDW or RCDW) is a measure of the variation of red blood cell (RBC) width that is reported as part of a standard complete blood count. Usually red blood cells are a standard size of about 6–8 μm. Certain disorders, however, cause a significant variation in cell size. Higher RDW values indicate greater variation in size. Normal reference range in human red blood cells is 11–15%. If anemia is observed.

Calculations

The "width" in RDW is sometimes thought of as "misleading," since it in fact is a measure of deviation of the volume of RBCs, and not directly the diameter. However, "width" refers to the width of the volume curve (distribution width), not the width of the cells.

Thus, it is a reasonably accurate term.

Mathematically the RDW is calculated with the following formula:

$$RDW = (\text{Standard deviation of MCV} \div \text{mean MCV}) \times 100.$$

Clinical Significance

- High RDW
- Iron Deficiency Anemia: high RDW with low MCV
- Folate and vitamin B12 deficiency anemia: High RDW with High MCV
- Recent Hemmorrhage: High RDW with Normal MCV

15.9ESR (ErythrocyticSedimentationRate)Introduction

The ESR is a simple non-specific screening test that indirectlymeasures the presence of inflammation in the body. Changes in redcell shape or numbers m ay alsoaffectthe ESR.Twolayers are formed,the upper plasmalayer andlower o ne of the red bloodcells. The rate atwhich the redcells fallsis known as erythrocytic sedimentation rate.

ESR is greater in women than in men and it is related tothe differencein PCV. During pregnancy ESR graduallyincreases after 3rd month and returns tonormal in about3-4weeks after delivery

METHOS OF ESR DETERMINATION

There are two main methods to determine ESR :

- Wintrobe's method
- Westergren's method

Each method produces slightly different results. Mosely and Bull (1991) concluded that Wintrobe's method is more sensitive when the ESR is low, whereas, when the ESR is high, the Westergren's method is preferably an indication of patient's clinical state.

WINTROBE'S METHOD

This method uses Wintrobe's tube, a narrow glass tube closed at the lower end only. The Wintrobe's tube has a length of 11 cm and internal diameter of 2.5 mm. It contains 0.7-1 ml of blood. The lower 10 cm are in cm and mm. The marking is 0 at the top and 10 at the bottom for ESR. This tube can also be used for PCV. The marking is 10 at the top and 0 at the bottom for PCV.

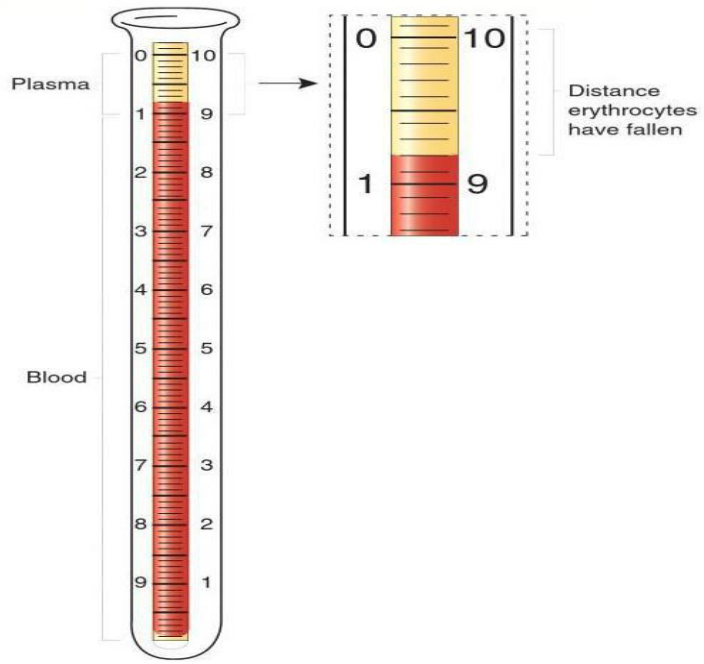
REQUIREMENTS :

- Anticoagulated blood (EDTA, double oxalate)
- Pasteur pipette
- Timer
- Wintrobe's tube Wintrobe's stand

PROCEDURE :

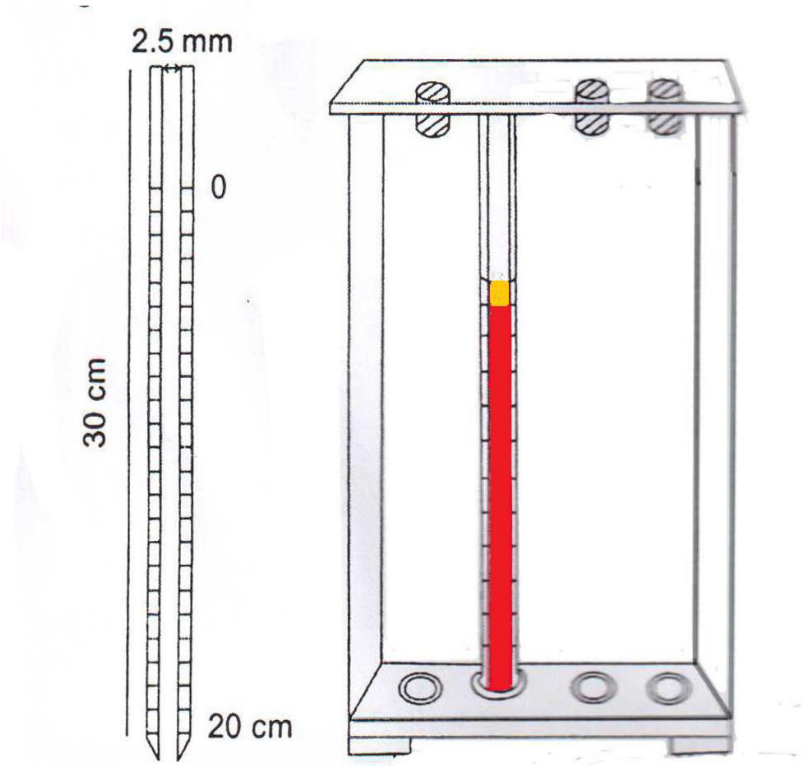
- Mix the anticoagulated blood thoroughly.
- By using Pasteur pipette, fill the Wintrobe's tube upto '0' mark. There should be no bubbles in the blood.
- Place the tube vertically in ESR stand and leave undisturbed for 1 hour.
- At the end of 1 hour, read the result.

NORMAL VALUE: males:0-9mm/hr,females:0-20mm/hr



WESTERGREN'S METHOD

qIt is better method than Wintrobe's method. The reading obtain is magnified as the column is lengtheir. The Westregren tube is open at both ends. It is 30 cm in length and 2.5 mm in diameter. The lower 20 cm are marked with 0 at the top and 200 at the buttom. It contains about 2 ml of blood.



a) Westergren tube b) Westergren tube on the rack

REQUIREMENTS :

Anticoagulated blood (0.4 ml of 3.13% trisodium citrate solution + 1.6 ml blood) Westergren tube

- Westergren stand
- Rubber bulb (sucker)

PROCEDURE :

- Mix the anticoagulated blood thoroughly.
- Draw the blood into the tube upto 0 mark with the help of rubber bulb.
- Wipe out blood from bottom of the tube with cotton.
- Set the tube upright in stand. Make sure the pipette fits snugly to eliminate possible leakage and that the pipette is in vertical position.
- Leave the tube undisturbed for 1 hour.

- At the end of 1 hour, read the result.

NORMAL VALUE :

For males: 0-10 mm/hr

For females: 0-15 mm/hr

Clinical Significance of ESR

The erythrocyte sedimentation rate (ESR) is a non-specific test. It is raised in a wide range of infectious, inflammatory, degenerative, and malignant conditions associated with changes in plasma proteins, particularly increases in fibrinogen, immunoglobulins, and C-reactive protein. The ESR is also affected by many other factors including anaemia, pregnancy, haemoglobinopathies, haemoconcentration and treatment with anti-inflammatory drugs.

Causes of a significantly raised ESR :

All types of anemias except sickle cell anemia

Acute and chronic inflammatory conditions and infections including:

- HIVdisease
- Tuberculosis
- Acuteviralhepatitis
- Arthritis
- Bacterialendocarditis
- Pelvicinflammatorydisease
- Rupturedectopicpregnancy
- Systemic lupus erythematosus

African trypanosomiasis (rises rapidly) Visceral leishmaniasis

Myelomatosis, lymphoma, Hodgkins disease, some tumours Drugs, including oral contraceptives

Causes of Reduced ESR :

- Polycythaemia Poikilocytosis
- Newborn infants Dehydration

- Dengue haemorrhagic feverand other conditions associated with haemoconcentration

Precaution

- Wash the tubes as early as possible, under running tap water.Rinse in deionized water and dry in the incubator between 40-50oC
- Wash the tubes under running tap water by introducing a thick wire in the tube repeatedly to remove the packed cells completely. Afterwards dry the tubes in the incubator.
- In the case of infants and if the blood quantity is insufficient use "Landau" method.

KeyTerms

- **Haematology:** The branch dealing with the study of blood components and coagulation is known as haematology.
- **Blood :** Afluid connective t issue in which blood cells are suspended in fuid plasma
- **Plasma:** The fuid portioned blood
- **Plasma Proteins :** Serum albumim, serum globulin and serum fibrinogen are the plasma proteins
- **Anticoagulants :** Chemicals used to prevent the process of coagulation
- **Coagulation :** The formation of fibrin threads mesh in which blood cells are entangled
- **EDTA :** Ethylene diamine tetra acetic acid
- **ACD :** Acid citrate dextrose
- **E.S.R :** Erythrocyte sedimentation rate
- **Heparine :** Chemical used to prevent coagulation
- **Haemocytometer :** It is an instrument for measuring the number of blood cells
- **Polycythemia Vera:** Condition in which RBC count is high is called as polycythemia vera
- **Anemia:** It is the condition where the levels of RBCS are low.
- **Leucocytosis:** The condition of increased levels of leucocytes is called as leucocytosis
- **Leukaemia :** Abnormal increase in wbc count with immature cells among them
- **Leucopenia:** Decreased levels of wbc count

- **Haemolytic Anaemia :** It is due to excessive or abnormal destruction of red blood cells
- **Haemorrhage :** Escape of blood from a vessel
- **Thrombocytopenia:** Low count of platelets
- **Oedema :** Accumulation of water (fluids) in between body tissues
- **Polyuria:** Secretion of large quantities of urine
- **BT :** Bleeding Time
- **BleedingTime :** The time required for the blood to stop flowing from a deep prickin the finger.
- **ClottingTime :** The interval of time between the appearance of blood and appearance of fibrin threads during the breakage of capillary tubes is known as clotting time.
- **Polynephritis :** Inflammation of the more number of nephrons
- **Pulmonary :** Relating to lungs
- **Erythropoiesis :** Formation of red blood cells
- **Bonemarrow :** Lively material from which blood cells are formed and send to blood circulation
- **Haemolysis :** Breakage of RBC with the release of Haemoglobin
- **Haemolytic Anaemia :** It is due to excessive or abnormal destruction of red blood cells
- **Haematuria :** Excretion of blood in urine
- **Haemoglobin :** The respiratory pigment of the RBC is called as haemoglobin anaemia: the condition where there is reduced levels of RBCS
- **Drabkins Reagent :** Reagent used in cyanmethaemoglobin method of Hb estimation
- **Specific Gravity Method :** One of the methods of Hb estimation
- **Jaundice:** A condition characterized by raised bilirubin level in the blood. Urine appears as yellowish in this condition
- **Lipaemia:** Increased levels of lipoids (especially cholesterol) in the blood.
- **ESR :** The rate at which red cells falls is known as ESR

- **Sedimentation :** Settling of solid components to the bottom of the tube
- **Plasma :** The separated fluid portion of the blood after anticoagulant is added
- **Serum :** The fluid portion formed after formation of clotting

Short Answer Type Questions

- Define coagulation
- What is an anticoagulant?
- Abbreviate EDTA
- What is the sodium citrate?
- Expand ACD
- Where sodium citrate is used?
- How does heparin will act as an anticoagulant?
- For what purposes ammonium and potassium oxalate mixture is used. Write down the composition of wbc diluting fluid.
- Define leucocytosis.
- Write down the normal values of wbc count.
- Define anaemia.
- What is leukaemia?
- Define polycythemia vera.
- Define bleeding time
- What is normal value of platelet count
- Define thrombocytopenia
- Write the compostion of platelet diluting fluid
- What is the importance of platelet count?
- Define Erythropoiesis
- What is anaemia?
- Define haematology
- Write the morphology of reticulocytes
- Expand the term EDTA.
- How do you estimate Hb by specific gravity method?
- What are the normal values of Hb in men and women?

- What are the advantages of specific gravity method?
- What are the disadvantages of specific gravity method?
- What are the advantages of alkaline haematin method?
- What are the disadvantages of alkaline haematin method?
- What are the advantages of Talquist method?
- Write the disadvantages and clinical significance of Talquist method.
- Define the term Haematology
- Define PCV.
- What is the clinicalsignificance of PCV
- Define the Mean cel volume
- Define Mean corpuscular haemaglobin
- Define Mean corpscular haemaglobin consentration
- Define ESR
- Write the methods of ESR determination
- Write the normal values of ESR
- Define roulex formation
- Write the factors affecting the ESR

Long Answer Type Question

- Write notes on RBC count.
- Explain about WBC COUNT.
- Write the morphology and functions of platelets
- Write down the procedure of platelet counting .
- Write notes on reticulocyte counting
- Write notes on advantages of reticulocyte count and morpholgy of reticulocytes.
- How do you estimate haemoglobin by Sahlis acid haematin method?
- Write down the haemoglobin estimation of cyanmethaemoglobin method.
- Explain about the HB estimation by alkaline haematine method
- How do you estimate Hb by specific gravity method?
- Write notes on Hb estimation by Haldanes method.
- Write the advantages and disadvantages of Hb estimation by specific gravity method.
- Write about the importance of haemeglobin in the human body system.
- How do you determine PCV by microhaematocrit method
- How do you determine PCV by macrohaematocrit method
- Write notes on RBC indices
- How do you determine ESR by westergrens method?
- Write the determination of ESR by Wntrobes method

UNIT-16

Disposal of Hospital Waste

Structure

16.1 Introduction

Hospital is a place of Almighty, a place to serve the patient. Hospital waste is a potential health hazard to the health care workers, public and flora and fauna of the area. Hospital acquired infection, transfusion transmitted diseases, rising incidence of Hepatitis B, and HIV, increasing land and water pollution lead to increasing possibility of catching many diseases.

What is hospital waste

Hospital waste refers to all waste generated, discarded and not intended for further use in the hospital.

16.2 Classification of Hospital waste

General waste: Largely composed of domestic or house hold type waste. It is non-hazardous to human beings, e.g. kitchen waste, packaging material, paper, wrappers, plastics.

Pathological waste: Consists of tissue, organ, body part, human foetuses, blood and body fluid. It is hazardous waste.

Fig. 16.1 Hospital waste

Infectious waste: The wastes which contain pathogens in sufficient concentration or quantity that could cause diseases. It is hazardous e.g. culture and stocks of infectious agents from laboratories, waste from surgery, waste originating from infectious patients.

Sharps: Waste materials which could cause the person handling it, a cut or puncture of skin e.g. needles, broken glass, saws, nail, blades, scalpels.

Pharmaceutical waste: This includes pharmaceutical products, drugs, and chemicals that have been returned from wards, have been spilled, are outdated, or contaminated.

Chemical waste: This comprises discarded solid, liquid and gaseous chemicals e.g. cleaning, house keeping, and disinfecting product.

Radioactive waste: It includes solid, liquid, and gaseous waste that is contaminated with radionucleides generated from in-vitro analysis of body tissues and fluid, in-vivo body organ imaging and tumour localization and therapeutic procedures.

Fig. 16.2 Hospital Waste Management Programme

Hospital waste management programme

- Identification of waste types
- Segregation of waste
- Transport & storage of waste
- Proper disposal of waste
- Implementation of contingency plans
- Identify the need for use of personal protective equipment
- Segregation by color coding system
- Three categories
- Infectious waste - Red bags
- Domestic waste - Green Bags
- Sharps - Needle cutters / Puncture proof containers
- Segregation at Source (ward, operation theater, laboratory, labour room, other places)

Transportation

Containers: Puncture proof, leak proof,

Bags: Sturdy, properly tied

Transport trolleys: Designated & Timely

Staff protection: Provided with protective clothing and other items

All infectious waste and sharps containers: Incineration

All Domestic waste: Landfill

All hazardous waste: Chemical treatment before disposal

Implementation of contingency plans

Fig. 16.3 Transportation of Hospital waste

16.3 Disposal of cotton and Swabs

1. Cotton and swabs constitutes the Infectious agents
2. The wastes which contain pathogens in sufficient concentration or quantity that could cause diseases. It is hazardous e.g. culture and stocks of infectious agents from laboratories, waste from surgery, waste originating from infectious patients.

Fig. 16.4 A Portable Incinerator to dispose hospital waste

Fig. 16.5 A Pit to dispose Biodegradable Waste

- Sorting of medical waste in hospital is the first step in handling the Infectious agents
- At th site where it is generated,infectious waste is placed in specially-labled bags and containers for removal by Infectious waste transporters
- The person should wear mask covering the nose and mouth
- The person should wear a cap over the head
- The person should wear foot protectives
- The person should wash his hands with soap and warm water after handling infectious wastes
- The person also should wash all areas of his body with soap and water that he thinks may have come into contact with infectious wastes, even if he is not sure that his body actually touched the infectious wastes
- Keep all sores and cuts covered
- The person should wear an apron or another type of cover to protect his clothes from contact with the waste
- Promptly clean and disinfect soiled, hard-surfaced floors by using a germicidal or bleach solution and mopping up with paper towels
- Red coloured bins/bags should be used for collecting the infectious
- waste.
- The Infectious waste should be disinfected with suitable chemical disinfectants to prevent infection.
- TheInfectious waste should be taken away to a far away place and disposed

- The Infectious waste which is disinfected can also be burried by digging a pit and filled with soil.
- The infectious waste after getting disinfected should be dried and burnt.

16.4 Diposal of Blood and body fluids

- Both solid and pathological waste constitutes the blood and body fluids
- The persons handling these waste should take care while collecting because of transmission of diseases
- These pathological wastes are collected in the concerned coloured bags properly
- The persons handling these wastes should see that,there should be no leakage from the bins or bags.
- The bins or bags should not be overloaded with pathological wastes
- The person handling thein fectious wastes should wear disposable latex hand gloves. Discard the gloves immediately after use.
- The person should wear mask covering the nose and mouth
- The person should wear a cap over the head
- The person should wear foot protectives

- The person should wash his hands with soap and warm water after handling infectious wastes.
- The person should wear an apron or another type of cover to protect his clothes from contact with the waste
- Promptly clean and disinfect soiled, hard-surfaced floors by using a germicidal or bleach solution and mopping up with paper towels.
- These solid and pathological wastes hane to be autoclaved
- Then treated with chemical disinfectants
- And finally burried deep in the soil after digging a pit and closed with filling.

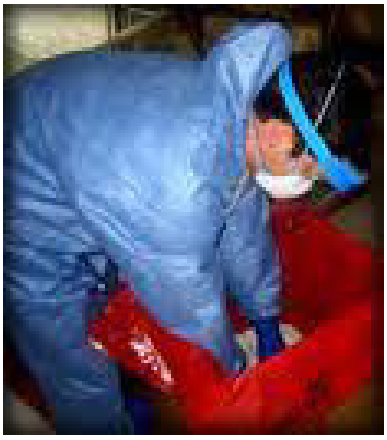


Fig. 16.6 Disposal of blood

16.5Disposal of Syringes and Neddles

1. Waste materials which could cause the person handling it, a cut or puncture of skin e.g. Syringes ,needles, broken glass, saws, nail, blades, scalpels constitute the Sharps.
2. The used disposable needles should be made into pieces by cutting.
3. The used syringes should be destroyed.

Fig. 16.7 Disposal of used syringes in a safety box

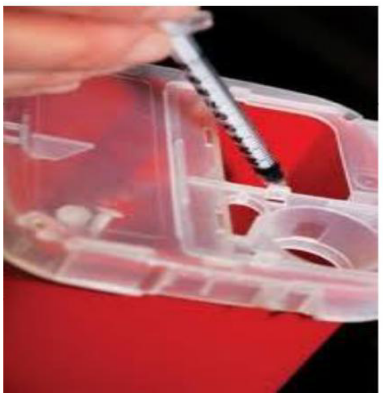


Fig. 16.8 Disposal of home generated syrengis

Fig. 16.9 Syringe Needle Clipper

4. After these are taken to a far away place
5. Where these are disinfected and subjected to chemical treatment
6. And finally these mutilated, disinfected, chemically treated sharps are subjected to Incineration.
7. Sometimes liquid wastes like blood after disinfecting with chemicals are discharged into the drainage

16.6 Disposal of Urine

In the postpartum rather than in a clinic, from the d/c Foley bags, as much of the urine as possible was emptied, deflate the balloon, and put the whole shebang into the regular trash.

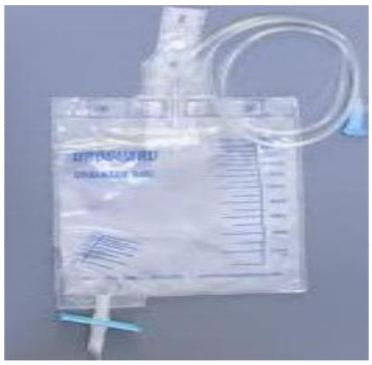


Fig. 16.10 Disposal of Urine in foley bags

The cost of trash disposal is determined by weight, so not emptying the cups/bags would increase the charge for no good reason. The rate for biohazard bags is approximately ten times that of the regular trash, so you don't want anything in there that doesn't have to be.

Emptying the cups into the toilet and then tossing them in the regular trash is the most reasonable method.

Empty the urine out of the cup and place the cups in the red bags. Urine dipsticks go in there, too.

Disposal of Medical Waste

1. Human anotomical are subjected to Incineration and deep burial.
2. Animal waste are also subjected to Incineration and deep burial
3. Microbiology and Biotechnology waste are subjected to Incineration and deep burial

4. Sharps are subjected to the following processes
   i) Incineration ii) Disinfection iii) Chemical treatment iv) Mutilation
5. Medicines and cytotoxic drugs are subjected to
   i) Incineration ii) Destruction and disposal in secured land fill
6. Chemical waste is subjected to chemical treatment and secured landfill
7. Incinerated ash is disposed in Municipal landfil.

Key Terms

Hospital waste : Refers to all waste generated, discarded and not intended for further use in the hospital.

General Waste : Largely composed of domestic or house hold type waste.

Pathological Waste: Consists of tissue, organ, body part, human foetuses, blood and body fluid.

Infectious Waste: The wastes which contain pathogens in sufficient concentration or quantity that could cause diseases.

Sharps : Waste materials which could cause the person handling it, a cut or puncture of skin e.g. needles, broken glass, saws, nail, blades, scalpels.

Pharmaceutical waste: This includes pharmaceutical products, drugs, and chemicals that have been returned from wards, have been spilled, are outdated, or contaminated.

Radioactive waste: It includes solid, liquid, and gaseous waste that is contaminated with radionucleides generated from in-vitro analysis of body tissues and fluid, in-vivo body organ imaging and tumour localization and therapeutic procedures.

Hospital Waste Management Programme : The management programme of hospital waste is called as hospital waste manement preogramme

Chemical Waste: This Comprises discarded solid, liquid and gaseous chemical e.g. cleaning, house keeping, and disinfecting product.

Short Answer Type Questions

- Define hospital waste
- Define general waste
- Define infectious waste
- Define sharps
- Define pharmaceutical waste
- Define chemical waste
- Define Radioactive waste.
- What is hospital waste management programme?.

Long Answer Type Questions

- Write the classification of hospital waste and explain about it in detail
- Explain about the disposal of cotton and swabs
- Write notes on disposal of blood
- Write notes on disposal of syringes, needles and urine.

UNIT-17

Glass Slides, Cover Slips

Structure

17.1 Introduction

History

A microscope slide prepared by Filippo Pacini in 1854, containing reference specimens.

A microscope slide is a thin flat piece of glass, typically 75 by 25 mm (3 by 1 inches) and about 1 mm thick, used to hold objects for examination under a microscope. Microscope slides are often used together with a cover slip or cover glass, a smaller and thinner sheet of glass that is placed over the specimen. Slides are held in place on the microscope's stage by slide clips or slide clamps. Slides are usually made of common glass and their edges are often finely ground or polished. Microscope slides are usually made of glass, such as soda lime glass or borosilicate glass, but specialty plastics are also used. Fused quartz slides are often used when ultraviolet transparency is important, e.g. in fluorescence microscopy.

17.2 Cleaning of glassslides and Cover slips

Load slides and coverslips in the appropriate sized carriers. Fill the Ultrasonic Cleaner with pure water and 1%Micro-90 soap. Submerge the loaded carriers in the cleaner and let sonicate at 60°C for an hour.

Place carriers in a container and rinse under running tap for five minutes. Drain water and repeat the tap waterrinse two more times.

In a similar manner, rinse the loaded carriers under running deionized water for five minutes.

In a similar manner, rinse the loaded carriers by submerging in ultrapure water three times.

Remove carriers from the final rinse and allow excess water to drop for several seconds before placing them in the sealable containers. Fill the carriers with 180 proof ethanol and seal with cover. The glass is now ready for storage.

Before Use

Before using a slide or coverslip, remove it from the ethanol with a pair of flat-ended tweezers and rinse well with a stream of 200 proof ethanol.

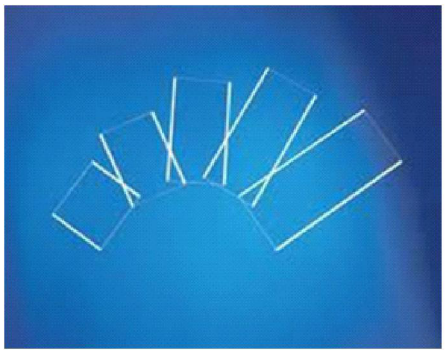
Then thoroughly dry with a clean air gun in a dust free environment. The slide or coverslip will now be ready for use.

Method I.

Squeaky Clean Slides[2]: all done in bath sonicator (Branson 250)

1. Sonicate in Versa Clean and hot water (2%) for 45 minutes
2. Sonicate in just hot water for 30 minutes (to rinse)
3. Sonicate in deionized water for 30 minutes
4. Sonicate in 1 mM EDTA for 30 minutes
5. Sonicate in 70% ethanol for 30 minutes
6. Sonicate in 100% ethanol for 30 minutes
7. Store in ethanol until use

These slides were either flame-dried, air dried, or compressed air dried before use to remove ethanol. The results were not very promising. All the slides had a sort of uniform “fur” covering them with only spots of darkness, indicating a clean area. Plus there were horizontal lines of dirt running across large sections of the slide.



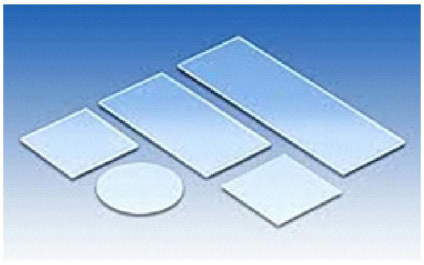


Fig. 8.1 Cover slips for micro array slides Fig. 8.2 Microscope slides and cover slips

Method II

Ultra Clean Slides - Ammonia based glass cleaner and Opti-Clean polymer:

In this method simply used the cleaner with a Kimwipe on the slide. This alone produces poor results under the microscope (darkfield). It does work very well for getting the slide visibly clean (removing the dirt that accumulates on slides over time). After the ammonia-based cleaner is used, it helps to rinse the slides in de-ionized water in a sonicator bath for at least 30 minutes. This removes lint left over from the Kimwipe and other large dust particles.

Key Terms

Glass slides: Used for supporting the specimens to be viewed through microscope

Coverslip : Used to reduce distortion upon viewing and at the same time also protects the specimen from external environment

Wetmount : Used to view microorganisms,stained cross sections or thin slices of tissue

Cleaning : Glass slides after usage washed with cleaning solution is called as cleaning

Ulrasonic Cleaner : Instrument used to clean the used galss slides automatically

Magnification : Enlargement of the image is called as the magnification

Dry mount : Used to observe thin cross sectional segments

Mount : Placement of specimen on the slide and then covering with a cover slip

Maintenance : Protecting the slide right from moumting to finishing of observation of prepared glass slide

Short Anwer Type Questions

Define glass slides and cover slips.

Define wet mount.

Define dry mount.

What is cleaning of glass slides?

Define maitainance of glass slides and cover slips

What are the uses of glass slides and cover slips

What is magnification?

Long Answer Type Questions

Write notes on cleaning of glass slides and cover slips in detail

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