06 MONTHS TRAINING COURSE IN

MEDICAL LAB TECHNICIAN & ECG OF CTs/GDS & PARAMEDIC STAFF SPONSORED BY

CENTRAL RESERVE POLICE FORCE

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TAILOR MADE NOTES ON HUMAN ANATOMY & PHYSIOLOGY

FOR

06 MONTHS TRAINING COURSE IN MEDICAL LAB TECHNICIAN & ECG OF CTs/GDS & PARAMEDIC STAFF SPONSORED BY

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Preface

Notes on Human Anatomy & Physiology will be highly informative for the basic knowledge of the students undergoing Six Months Training in Medical Laboratory Technician and ECG Course under the sponsorship of Central Reserve Police Force.

These notes consist of precise information on all the basic and essential topics to be covered in Human Anatomy & Physiology for beginners. It will give a fair idea about the basic information of the subject and help them understand the human body and its functioning and also to diagnose the diseases associated with various body systems like heart, lungs, digestive system, endocrine system etc.

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PAPER – I
ANATOMY & PHYSIOLOGY

Anatomy: - It is the branch of science which deals with study of the internal structure of the various body parts.

Physiology: - It is the branch of science which deals with the study of functioning of various parts and system of the body.

Anatomical Positions of Body

[1] Supine position: - Person lying on back arms by side palms facing upwards and feet put together.

[2] Prone position: - Person lying on face, chest and abdomen.

[3] Lithotomy position: - Person lying on back with legs up and feet supported in straps.

Plains of Body

1. Transverse plane
2. Coronal plane
3. Medial plane

Anterior	-	In front
Posterior	-	Behind
Superior	-	Upper
Inferior	-	Lower

Cell Structure

Cell: - The smallest unit of body is called as cell.

It contains:

1. Cell membrane
2. Cytoplasm
3. Nucleus

(1) Cell wall:- Boundary wall of cell
(2) Cytoplasm:- contains, Mitochondria, Golgi apparatus, Centro some Fat bodies
(3) Nucleus:- contains Chromosomes and Nucleolus

Tissue

A group of cells is called tissue.

(A) Epithelial
- Simple
- Compound

(B) Muscular tissue
- Skeletal - attached with bones
- Smooth - present in internal organs
- Cardiac - in heart

(C) Nervous tissue

Present in central nervous system only. Contains Neurons

Ex: - Brain and Spinal cord.

(D) Connective tissue

I) Areolar
II) Fibrous
III) Mucoid
IV) Adipose
V) Reticular

SYSTEMS OF THE BODY

DIGESTIVE SYSTEM

Definition: - Digestive system is that system which is related with the intake of food, its digestion absorption, assimilation and excretion of undigested waste material out of body.

Digestive system contains the

[A] Alimentary canal
[B] Digestive glands

[A] Alimentary canal: - It contains the following parts:

1.	Mouth	:	opening of alimentary canal
2.	Buccal cavity	:	contains Tongue, Teeth, Tonsils
3.	Pharynx	:	next part of Buccal cavity
4.	Oesophagus	:	also called food pipe
5.	Stomach	:	stores food capacity 2/3 liters
6.	Duodenum	:	next part of stomach
7.	Small Intestine	:	tube like structure length 6.5 meters, help in complete food digestion
8.	Large Intestine	:	next part to Small Intestine, length 1.5 meters
9.	Rectum	:	organ for storage of waste material
10.	Anus	:	Posterior opening of alimentary canal for excretion

[B] Digestive glands:-

1] Liver:- Largest gland of body secretes Bile juice for fat digestion and stores water , weight 1.5 to 2 kg.

2] Gall bladder:- Pear shaped gland present in liver, helps in storage of Bile juice and concentrates it.

Contains

1) Fundus
2) Body
3) Neck
4) Cystic duct

2] Pancreas:- Double gland. Exocrine and endocrine. It secretes

1) Digestive enzymes

2) Hormones like – Insulin

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CIRCULATORY SYSTEM

Definition: - It is that system related with blood circulation in body

It contains the following parts –

1] Heart

2] Blood

3] Blood vessels

1] Blood: - This is a fluid connective tissue, contains RBC, WBC, Platelets and Plasma.

Composition: Plasma – 55 % part of blood is plasma contains 90 % water.

Formed elements: - contains – RBC, WBC, and Platelets.

Blood functions:-

1) Transport
2) Respiration
3) Body temp.
4) Excretion
5) Carrier of Hormones
6) ph – value
7) Blood clotting

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8) Anti clotting

Heart: - It is a hollow muscular organ

Internal structure:- Contains Right and Left parts, Right part contains CO_2 blood , Left part contains O_2 blood. Heart has 4 chambers, upper 2 are called as Auricles, and Lower2 are called as Ventricles.

From Right auricle large blood vessel Pulmonary artery arises (CO_2) blood from left ventricle aortic arch arises carries (O_2) blood.

Bicuspid valve – Left side of heart

Tricuspid valve – Right side of heart

[C] Blood vessels:- are the routes by which the blood flows. Two types

1] Arteries
2] Veins
1] Arteries:- Red in colour, present deep inside body, carry O_2 blood, thick walls.
2] Veins:- Bluish green in color, present in upper surface of body, carry CO_2 blood, thin walls.

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URINARY SYSTEM

Definition:- Urinary system is that system which is related with the formation of urine and its excretion out of body

It contains the following parts

1] Kidney
2] Ureter
3] Urinary bladder
4] Urethra

1] Kidney:- These are bean shaped structure. Two in number one on Right, one on Left side of abdomen.

L = 10 cm

B = 5 cm

Thickness = 2.5 to 4 cm

Weight = 140 gm approx.

Colour – Dark redish brown

Internal structure:- It contains

Outer layer of tissue – cortex

Middle layer of tissue – medulla

Inner layer of tissue – pelvis

Each Kidney contain 1.2 millions Nephrons which manufacture urine

2] Ureter:- Tube like structures carry urine from kidney to urinary bladder. L = 25 cm

3] Urinary bladder:- Bag like structure for the storage of urine.

4] Urethra:- opening of urinary bladder is called as urethra. Urine is passed out from urethra.

RESPIRATORY SYSTEM

Definition:- It is that system which is related with the exchange of O2 and CO2 between the living body and the atmosphere.

Respiratory system contains the following parts

1) Nose
2) Nasal cavity
3) Naso pharynx
4) Larynx
5) Trachea
6) Bronchus
7) Bronchioles
8) Lungs

Nose: Opening of Respiratory system contain two openings.

Nasal cavity: Next part to nasal opening, filters the air and makes it slightly hot or cool.

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Naso pharynx: Upper part of pharynx

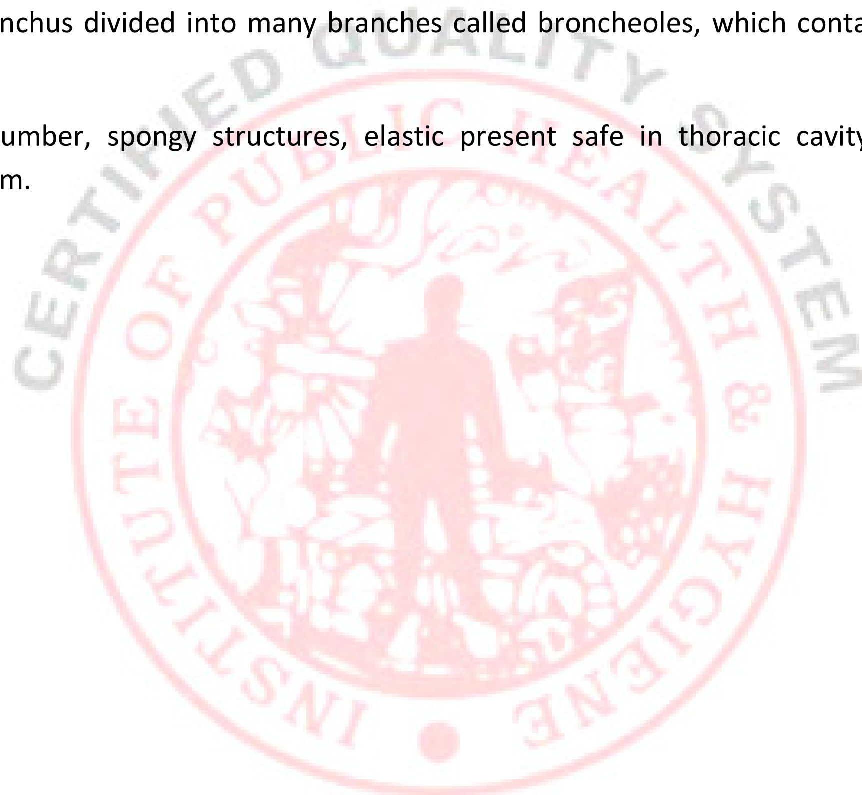
Larynx: Contains vocal cords present in Neck

Trachea: Also called as wind pipe, 11 cm in length

Bronchus: Trachea divides into two bronchi Right and Left enters in each lung.

Broncheoles: Bronchus divided into many branches called broncheoles, which contain air cells at the endings.

Lungs: Two in number, spongy structures, elastic present safe in thoracic cavity. Main organ of Respiratory System.



NERVOUS SYSTEM

Nervous system is that system which is related with the different sensations like vision, hearing, smell, feeling pain in the body and controlling all the muscles of the body.

It can be divided in three parts –

1] CNS – Central Nervous System

2] PNS - Peripheral Nervous System

3] Autonomic – Nervous system

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1] CNS

- Brain

- Spinal Cord

BRAIN: It is the largest organ present in nervous system. Present in upper part of skull cranium, weight in adults about 3 pounds, upper portion contains Grey matter inner contains White matter.

BRAIN has three parts –

1] Fore brain: it contains Cortex + Thalamus + Hypothalamus + Pineal body

2] Mid brain: Shortest part of brain connected fore brain and hind brain, 2 cm in size.

3] Hind brain: contains Pons veroli + Medulla oblongata + Cerebellum

SPINAL CORD: Cord like structure, arise from the lower part of the Medulla oblongata, Length in adults about 45 cm, present in back bone 31 pairs of spinal nerves arise from spinal cord as following

8 pairs – Cranial
12 pairs – Thoracic
5 pairs – Lumbar
5 pairs – Sacral
1 pair – Coccyxgeal
31 pairs = TOTAL

Spinal Cord is protected by C.S.F.

C.S.F.: Cerebrospinal fluid clear alkaline fluid resembling to Plasma protect Brain and Spinal cord.

2] PNS

12 pairs of cranial nerves arising from brain

31 pairs of spinal nerves arising from spinal cord

Cranial nerves are as following –

1. Olfactory nerve – Nose
2. Optic – Eye
3. Ocoulometer - External muscles of eye

4. Trochlear- Sup. Oblique muscle of eye
5. Trigeminal – Largest nerve
6. Abducens – One muscle of eye
7. Facial – Face
8. Auditory – Ear

9. Glossopharyngeal – Pharynx, Laryn

10. Vagus – Heart, Lungs, Kidney, Small Int.

11. Spinal accessory nerve – Pharynx and Larynx and Neck muscle

12. Hypoglossal nerve – Tongue

31 Pairs of Spinal nerves are as following in groups –

8 pairs Cervical – Neck

12 pairs Thoracic - Thorax

5 pairs Lumbar – Lumbar part

5 pairs sacral – Sacral region

1 pair coccyxgeal – Lower part of body

----

31 pairs = TOTAL

3] Autonomic Nervous System

Function separately but directly or indirectly related with brain or spinal cord, Divided in two parts

- Sympathetic
- Parasympathetic

If one is Stimulatory other is inhibitory, supplied almost in all organs.

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SKELETAL SYSTEM

Skeletal system is that system which is related with the study of different bones which make skeleton of body, provide shape and size of body, protect all internal organs of body and help in movement.

It can be divided in two parts –

1] Axial: - Skull, Sternum, Ribs vertebral column, Hyoid bone.

2] Appendicular:-

Bones of upper limb

Bones of lower limbs

1] Classification of bone

(I) long bones

(II) Short bones

(III) Irregular

(IV) Flat

(V) Sesamoid

2] Functions of bones

(I) Body protection

(II) Movement

(III) Muscle attachment

(IV) Bones manufacture RBC

2] Structure of bone

Bone hardest

Connective tissue

Contains Calcium and Phosphorus

Upper and Lower heads and shaft

Covering of bone is called as Periostium

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Joint: - It is a point where two or more than two bones unite.

<u>Classification:-</u>

1) Fibrous joint e.g. Skull
2) Cartilagenous joint e.g. Backbone
3) Synovial joint e.g. Hip joint, Shoulder joint

[1] Skull: - skull contains 22 bones firmly fixed with each other. Lower jaw can move.

[2] Vertebral column: - Also called as back bone. It contains 33 bones. All are irregular bones. These are as following-

7 – Cervical

12 – Thoracic

5 – Lumbar

5 – Sacral (fused)

4 – Coccyxgeal (fused)

33 TOTAL

[3] Ribs:-

24 in number, present in 12 pairs, protect the Lungs, example of flat bones.

[4] Clavicle:-

Two in number, one on right side and one on left side, also called as Collar bone.

[5] Scapula:-

Two in number, one on right side and one on left side, help in forming the shoulder joint.

[6] Sternum:-

Single bone present in Thorax, it helps to make Thoracic cavity, Ribs attach with it.

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Bones of upper limb

1) Humurus – 1

2) Radius - 1
3) Ulna - 1
4) Carpals - 8
5) Meta carpals-5
6) Phalanges - 14

TOTAL - 30 bones

Bones of lower limb

1) Femur - 1
2) Tibia - 1
3) Fibula - 1
4) Tarsals - 7
5) Meta tarsals - 5
6) Phalanges - 14
7) Patella - 1

TOTAL = 30

Pelvis bone (Hip bone)

Two in number, Right and left, help to form Hip joint.

Difference between male and female pelvis

Male	Female
Funnel shaped	Wider
Angel less	Angel wide

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REPRODUCTIVE SYSTEM

MALE REPRODUCTIVE SYSTEM

Contains the primary and secondary sex organs

1. Testis
2. Epididymis
3. Vas deferens
4. Seminal vesicle
5. Ejaculatory ducts
6. Prostate gland
7. Bulbourethral glands
8. Penis

(1) Testis: - Two in numbers. Primary sex organ. Produces sperms and male sex hormone Testosterone.
(2) Epididymis:- Next structure to testis. Help in the storage of sperms temporary.
(3) Vas deferens:- two duct like structure, 45 cm in length bring sperms to seminal vesicle.
(4) Seminal vesicle:- Two in numbers, present over urinary bladder, store sperms till ejaculation.
(5) Prostate gland:- Single gland, present below urinary bladder, ejaculatory ducts open in prostate gland, size about WALNUT, secrets important secretion for sperms.
(6) Bulbourethral glands:- Two small pea sized glands present below the prostate gland.
(7) Penis:- Muscular organ used for mating and passing out the sperms after ejaculation.

Sperm: - Male sex cell.

FEMALE REPRODUCTIVE SYSTEM

It contains the primary and secondary sex organs as following-

1. Ovary
2. Fallopian tubes
3. Uterus
4. Cervix
5. Vagina
6. Mammary glands

(1) **Ovary:-** Primary sex organ, secretes ovum and female sex hormone progesterone and estrogen, oval or almond in shape

L = 3 cm

B = 2 cm

Th = 1 cm

Wt. = 3 gm

(Right and Left side)

(2) **Fallopian tubes:-** Two in number, one on right side and one on left side, Length about 10 cm, open part near ovary, closed part attached with uterus.

(3) **Uterus:-** Muscular structure having two parts

I) Body

II) Cervix

L = 8 cm

B = 5 cm

Th. = 2 cm

Wt. = 50 – 80 gm

(4) **Cervix:-** Lower part of uterus,

L = 2.5 cm

(5) **Vagina:-** Cervix opens in muscular tube like structure Vagina

Length = 8cm

(6) **Mammary gland:** - Also called as female breast. Two in number contains lactiferous ducts, which produce milk after delivery.

Menstruation Cycle:- Monthly bleeding during reproductive phase containing unfertilized ovum is called menstruation cycle.

SENSE ORGANS

EYE

Important organ of sight , Rounded ball like structure, contains three layers-

(I) Sclera
(II) Choroid
(III) Retina

Contains Sclera, Iris, Pupil, and Lens. Image is formed on Retina.

TONGUE

Tongue is a muscular sense organ of taste present in buccal cavity.

Contains three parts-

Tip – sweet taste saltish

Body – sour

Root – Bitter

Anterior side is free

Posterior side is fixed

EAR

Sense organ of hearing, Contains three parts

(1) External ear
(2) Middle ear
(3) Internal ear

(1) External ear:- contains Pinna + Auditory canal + Ear drum
(2) Middle ear:- contains three short hones : Malleus + Incus + Stapes
(3) Internal ear:- contains Cochlea + Vestibule + 3 semicircular canals + 8th Auditory nerve

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NOSE

Divided in two parts

(1) External nose:- External nasal openings + Bone + Cartilage
(2) Internal nose:- contains Nasal cavity. It contains the 4 Air pockets present within skull-

I. Maxillary sinus
II. Frontal
III. Ethmoidal
IV. Sphenoidal

SKIN

Largest organ of the body, contains two parts-

(I) Epidermis (outer)
(II) Dermis (inner)

(I) Epidermis:- contains three layers-
   1) Stratum corneum
   2) Stratum granulosum
   3) Stratum germinativum

(II) Dermis:- called true skin and contains the following structure
   1) Sweat glands
   2) Sebaceous glands
   3) Hair follicle
   4) Hair bulb
   5) Nerve endings
   6) Blood capillaries
   7) Fat cells

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ENDOCRINE SYSTEM

It is that system which is related with the ductless glands which secrets the chemical Hormones directly in blood.

There are following glands-

(1) Pituitatory gland:- Smallest gland in our body, weight ½ gm, size ½ inch, secrets maximum hormones in body, present in brain.
(2) Thyroid & Parathyroid glands:- Present in the neck around larynx. Parathyroid are four or five small gland present in the Thyroid tissue, secrete important hormones like TH,PTH
(3) Adrenal glands:- Present at the top of kidneys. Two glands one on each kidney, secretes important hormone like Corticosteroids and Epinephrine.

LYMPHATIC SYSTEM

Part of the circulatory system, contains the following parts-

(1) Lymph – resembles to plasma
(2) Lymph vessels – like blood vessels
(3) Thymus, Tonsils, Spleen Lymph nodes.

Function: Important Functions

(a) Defense mechanism in body
(b) Fluid balance in cell & Tissues of the body

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CLINICAL BIOCHEMISTRY

FOR

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Preface

The Medical Laboratory Technology has been an invincible tool in diagnosis and study of the various diseases for proper treatment for decades and the demand for trained Medical Laboratory Technologists is growing continuously .It is a booming field with rewarding career. With latest and innovative technologies being introduced everyday in the field, the role of Medical lab Technologist is becoming even much crucial and will continue to change and expand in years to come.

Notes of clinical biochemistry have been developed with an objective to orient the students enrolled for 6 Months Medical Laboratory Technology & ECG Training Course under the banner of CRPF, with basic techniques of Clinical Biochemistry. Owing to paucity of time, these short notes will help the students to get an elaborated idea of diagnostic tests performed for biomolecules for diagnosis of various diseases and conditions like diabetes mellitus, heart attack, jaundice malnutrition, infections, cancer etc. The notes will also highlight about various instruments used in clinical biochemistry. It has also focused on various types of chemicals, reagents and solutions used in clinical biochemistry laboratory.

Dr. Anita Mukherjee

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CLINICAL BIOCHEMISTRY

Unit 1 Introduction

Biochemistry

Biochemistry is a subject in which chemical reactions inside body is studied

Clinical biochemistry

Clinical biochemistry is a subject in which tests of biomolecules for diagnosis of disease is studied

Scope of clinical biochemistry

Scope of biochemistry includes

1. Study of tests of various biochemical parameters performed for diagnosis of disease
2. Study of various instruments used for diagnosis
3. Research of various methods of diagnosis
4. Study of normal values of various biochemical parameters
5. Study of clinical significances of various biochemical parameters

Unit 2 Equipments and instrument

Colorimeter and Spectrophotometer

Principle

Principle of colorimeter and spectrophotometer is based on Lambert-Beer's Law

Lambert's Law

Lambert's Law states that absorption of light is directly proportional to the thickness of the container

Beer's Law

Beer's Law states that absorption of light is directly proportional to the concentration of solution

Concentration of test = OD_t/OD_s × concentration of standard

Optical density, OD= % log of absorbance

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Application of Colorimeter and spectrophotometer in Medical Laboratory Technology

Estimation of following

1. Carbohydrate (Glucose, Fructose)
2. Protein (Albumin, Globulin, Haemoglobin)
3. Lipids (Cholesterol, Lipoprotein,Triglyceride)
4. Waste products (Biirubin, Urea, Uric acid, Creatinine)
5. Enzyme activity
6. Minerals and electrolytes

Incubator

Principle

In incubator electrical energy is converted into heat energy. It has an inbuilt thermostat to maintain the temperature.

Applications of incubator

1. Growth of micro organism
2. Increase of enzyme activity
3. Keeping preterm immature babies

Hot air oven

Principle

In hot air oven electrical energy is converted to heat energy.

Application s of hot air oven

1. Killing of microorganisms (sterilization)
2. Drying

Water bath

Principle

In water bath electrical energy is converted to heat energy. Water is heated by convection; hence heat is retained for longer time

Applications of water bath

1. Heating those substances which cannot be heated directly like
   A Substances which catches fire on exposure to direct flame e.g. alcohol
   B Substances which gets damaged in flame e.g. blood sample
   C Substances which melts in direct flame
2. Heating reaction mixtures which needs constant temperature for longer time

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Centrifuge

Principle

Centrifugal force is generated by spinning or revolutions. It helps gravity to separate particles with little difference in weights. Heavier particles settle down as sediments and lighter particles forms supernatant

Applications of centrifuge

1. Separation of
   A Serum
   B Plasma
   C Protein free filtrate
   D Lipoprotein
2. Sub cellular localization
3. Purification of protein

Unit 3 Knowledge of reagent

Types of reagent

Acid, base, salt, buffer, indicator

Acid

Acid is H^+ or proton donor or electron pair acceptor

Base

Base is $H^{=}$ or proton donor or electron pair acceptor

Buffer

Buffer is a solution which maintains pH. pH is negative logarithm of hydrogen ion concentration

Indicator

Indicator is substance which changes color in acidic and basic medium and oxidized and reduced state

Solution, solute, solvent

Solution

Solution is homogenous mixture of two or more substances.

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Aqueous solution

Aqueous solution is a solution which is made in water

Non- aqueous solution

Non aqueous solution is a solution which is not made in water

Solute

Solute is a component of solution which is present in lesser amount

Solvent

Solvent is a component of solution which is present in larger amount

Saturated solution

Saturated solution is that solution in which no more solute can be dissolved

Unsaturated solution

Unsaturated solution is that solution in which more solutes can be dissolved

Concentration

Concentration is mass per unit volume.

Percentage

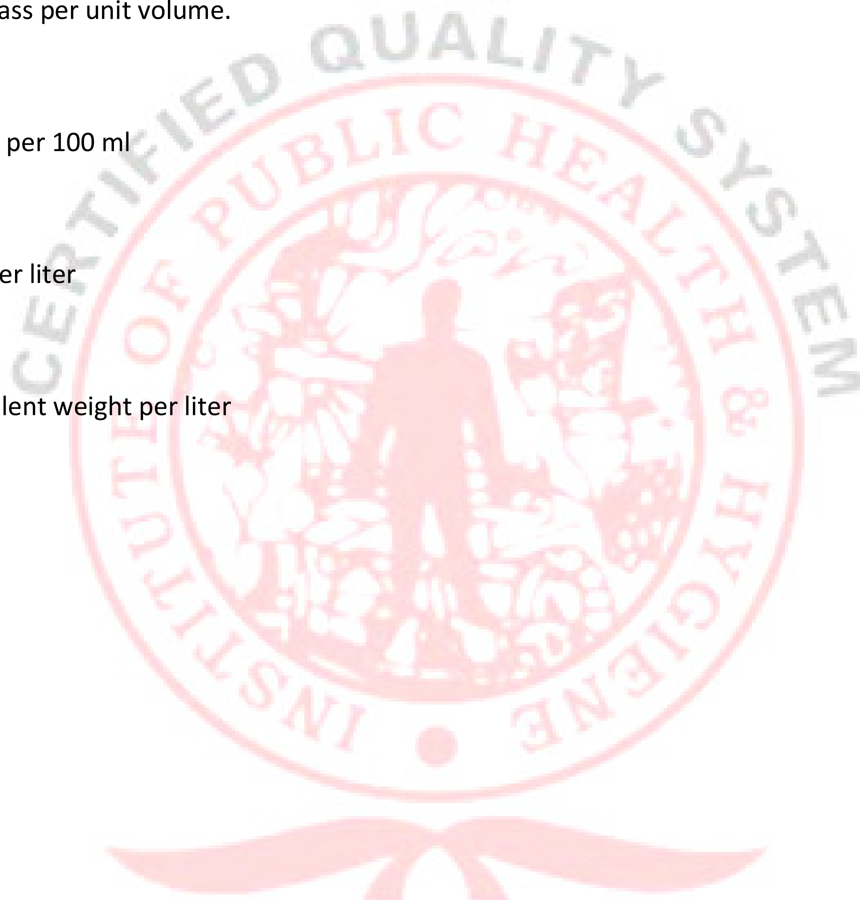
Percentage is gram per 100 ml

Molarity

Molarity is moles per liter

Normality

Normality is equivalent weight per liter



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Unit 4 Carbohydrates

Carbohydrates are polyhydroxy aldehydes or ketones. e.g. glucose, fructose

Classification of carbohydrates

Carbohydrates are of four types

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1. Monosaccharides
2. Disaccharides
3. Oligosaccharides
4. Polysaccharides

1. Monosaccharides
   Monosaccharides are single units of carbohydrate. e.g. glucose, fructose
2. Disaccharides
   Disaccharides are two units of monosaccharides joined by glycosidic bond. e.g. Scrosae, Maltose, Lactose
3. Oligosaccharides
   Oligosaccharides are two to five units of monosaccharides joined by glycosidic bond. e.g. Maltriose
4. Polysaccharides
   Polysaccharides are more than five units of monosaccharides joined by glycosidic bond. e.g. Starch, Glycogen

Glucose estimation in blood and CSF (Cerebro Spinal Fluid)

Introduction

Glucose is main energy giving molecule in body.

Method

GOD- POD (Glucose oxidase- Peroxidase method)

Principle

Glucose reacts with water and oxygen in presence of GOD to form Gluconic acid and hydrogen peroxide. Hydrogen peroxide converts to water and nascent oxygen. Nascent oxygen reacts with amino antipyrine and phenol to be quininimine complex of pink-red color, OD of which is measured at 540 nm. OD is directly proportional to the glucose concentration

Reagents

1. GOD
2. POD
3. 4 Amino anti pyrine
4. Phenol
5. Buffer
6. Glucose Standard (100mg/dl)
7. Distilled water
8. Serum

Procedure

1, Pipette in tubes labeled as follows

S.No.	Reagents	blank	Standard	Test
1	GOD-POD reagent	1ml	1ml	1ml
2	Distilled water	10µl	-	-
3	Standard	-	10µl	-
4	Serum	-	-	10µl

2 .Incubate for 15 minutes at 37^{0}C or 30 minutes at room temperature

3. Take OD of test and standard against blank, at 540 nm.

Calculation

Serum glucose $=OD_T/OD_s \times 100$

Normal value

Fasting blood glucose 60-100 mg/dl

Post prandiel blood glucose 80- 140 mg/dl

Random blood glucose 60-140 mg/dl

Clinical significance

1. Hyperglycemia
   It is increase of blood glucose. It occurs in case of
   (i) Diabetes Mellitus
   (ii) Hyperthyroidism
   (iii) Cushing's syndrome
   (iv) Alpha cell tumor

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2. Hypoglycemia
   It is decrease of blood glucose. It occurs in case of
   (i) Malnutrition
   (ii) Malabsorption
   (iii) Addison's disease
   (iv) Hypothyroidism

Glucometer

It is a portable instrument for measuring blood glucose

GTT (Glucose Tolerance Test)

It is confirmatory test of Diabetes Mellitus

HbA_{1c} (Glycated Hemoglobin)

It is a test which shows trends of increase or decrease of blood glucose

Unit 5 Protein

Proteins are biomolecules made up of amino acids joined by peptide bond.

Classification of protein

Proteins are of three types

1. Simple protein
2. Complex protein
3. Derived Protein

Simple protein

Simple proteins are proteins made up of amino acids joined by peptide bond. e.g. Albumin, Globulin

Complex Protein

Complex proteins are those proteins which are made up of amino acids as well as some non protein part. They include

Glycoprotein (Carbohydrate + Protein) mucin

Lipoprotein (Lipid + protein) Chylomicron, High density lipoprotein, Low density lipoprotein, very low density lipoprotein

Chromo protein (Chromogen + protein) e.g. Haemoglobin

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Derived protein

Derived proteins are derivatives of proteins. They include

Amino acids e.g. Alanine

Polypeptides e.g. Oxytocin

Estimation of total protein in blood and CSF (Cerebro Spinal Fluid)

Introduction

Total protein include Albumin (60%), and Globulin (40 %)

Method

Biuret method

Principle

Protein reacts with copper sulphate in presence of Sodium Hydrroxide to give pink-purple colored Biuret copper complex, OD of which is measured at 540nm. OD is directly proportional to the protein concentration.

Reagents

1. Biuret reagent
2. Albumin standard (6%)
3. Serum
4. Distilled water

Procedure

1, Pipette in tubes labeled as follows

S.No.	Reagents	blank	Standard	Test
1	Biuret reagent	1ml	1ml	1ml
2	Distilled water	10µl	-	-
3	Standard	-	10µl	-
4	Serum	-	-	10µl

2 .Incubate for 1o minutes at room temperature

3.Take OD of test and standard against blank, at 540 nm.

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Calculation

Serum total protein =$OD_T/OD_S \times 6$

Normal value

Serum total protein 6 -8 %

Clinical significance

3. Hyperproteinimia

   It is increase of total protein in blood . It occurs in caseof

   (i) High protein diet
   (ii) Infection
   (iii) Multiple Myeloma
   (iv) Dehydration

4. Hypoproteinimia

   It is decrease of total protein in blood . It occurs in case of

   (i) Malnutrition
   (ii) Malabsorption
   (iii) Liver disease
   (iv) Kidney Disease

Estimation of Albumin , Globulin, A:G ratio

Introduction

Albumin is major protein of blood. It is used for maintaining osmotic pressure, buffering and transportation. Globulin is used for defense

Method

BCG (Bromo-cresol Green Method)

Principle

Albumin forms blue green complex with Bromo-cresol Green, in presence of succinate buffer, OD of which is measured at 630 nm. OD is directly proportional to the protein concentration

Reagents

1. BCG
2. Succinate buffer
3. Albumin standard
4. Serum
5. Distilled water

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Procedure

1. Pipette in tubes labeled as follows

S.No.	Reagents	Blank	Standard	Test
1	BCG reagent	1ml	1ml	1ml
2	Distilled water	10µl	-	-
3	Standard	-	10µl	-
4	Serum	-	-	10µl

2 .Incubate for 5 minutes at room temperature

3.Take OD of test and standard against blank, at 630 nm.

Calculation

Serum albumin $=OD_T/OD_s \times 4$

Normal value

Serum albumin 4-6 %

Clinical significance

1. Hyperalbuminia
   It is increase of albumin in blood. It occurs in case of
   (i) High protein diet
   (ii) Dehydration
2. Hypoalbuminia
   It is decrease of albumin in blood. It occurs in case of
   (i) Malnutrition
   (ii) Malabsorption
   (iii) Liver disease
   (iv) Kidney disease

Calculation of Globulin

Serum Globulin = Serum total protein – Serum albumin

Normal value of Globulin

Serum Globulin 2-4 %

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Clinical significance of Globulin

1. Hyperglobulinimia
   It is increase of Globulin in blood. It occurs in case of
   (i) Infection
   (ii) Multiple Myeloma
   (iii) Dehydration
2. Hypoglobulinimia
   It is decrease of Globulin in Blood. It occurs in case of
   (i) Immuno deficiency diseases

Normal value of A:G ratio

Normal value of Albumin : Globulin or A:G ratio is 1.2 to 2.5

Unit 6 Lipids

Lipids

Lipids are esters of fatty acids and alcohol. e.g. soyabean oil, butter etc

Classification of lipids

Lipids are of four types

1. Simple lipids
2. Complex Lipids
3. Derived Lipids

Simple lipids

Simple lipids are those lipids which are made up of fatty acids and alcohols only. They are of two types

(i) Triglycerides
(ii) Wax

Triglycerides

Triglycerides are esters of fatty acid and glycerol. They are of two types

A Oil

B Fats

Oils

Oils are triglycerides which are liquids at 20^0C. e.g. mustard oil, soyabean oil

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Fats

Fats are triglycerides which are solid at 20^0C. e.g. coconut oil, butter etc

Waxes

Waxes are esters of fatty acids and monohydric alcohol. e.g. cholesterol ester

Complex Lipids

Complex lipids are lipids which are made up of fatty acids, alcohol as well as some non lipid part.

They include

(i) Glycolipid
(ii) Lipoprotein
(iii) Phospholipid

Glycolipids (Carbohydrate + lipid) e.g. ceramide

Lipoprotein (Lipid + protein) e.g. HDL, LDL, VLDL, chylomicron

Phospholipid (Phosphoric acid + Lipid) e.g. cephalin

Derived lipid

Derived lipids are derivatives of lipids. They include

Fatty acids and

Alcohol

Fatty acids

Fatty acids are long chain carboxylic acid . They are of two types

(i) Saturated Fatty acid
(ii) Unsaturated Fatty acids

Saturated Fatty acids

Saturated fatty acids are fatty acids which do not have any carbon-carbon double bond. e.g. Palmitic acid

Unsaturated Fatty Acid

Unsaturated fatty acids are fatty acids which have at least one carbon –carbon double bond. e.g. Oleic acid, Linoleic acid

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Unsaturated fatty acids are of two types

a. MUFA
b. PUFA

MUFA

Monounsaturated fatty acids are fatty acids which contains only one carbon –carbon double bond. e.g. Oleic acid

PUFA

Polyunsaturated fatty acids are fatty acids which contains more than one carbon-carbon double bond. e.g. linoleic acid

Test of lipid profile

Test of lipid profile include estimation of

!. Total cholesterol

2 Triglycerides

3 .High density lipoprotein

4 Low density lipoprotein

5. Very low density lipoprotein

Estimation of serum total cholesterol(TC)

Introduction

Cholesterol is 27 carbon monohydric alcohol. It is precursor of vitamin D, steroid hormones and bile salt. Increase of cholesterol causes heart disease

Method

Cholesterol oxidase (CHOD) method

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Principle

Cholesterol ester in presence of cholesterol esterase converts into free cholesterol and fatty acid. Free cholesterol converts into cholest 4 ene 3 one and hydrogen peroxide, in presence of cholesterol oxidase. Hydrogen peroxide converts into water and nascent oxygen. Nascent oxygen reacts with 4 Amino anti pyrine and phenol to give pink red quininimine complex, OD of which is measured at 540nm. OD is directly proportional to the cholesterol concentration.

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Reagent

1. Cholesterol oxidase
2. Cholesterol esterase
3. Peroxidase
4. 4 Amino anti pyrine
5. Phenol
6. Buffer
7. Cholesterol standard (200 mg /dl)
8. Serum
9. Solvent

Procedure

1. Pipette in tubes labeled as follows

S.NO.	Reagent	Blank	Standard	Test
1.	CHOD reagent	1ml	1ml	1ml
2.	Distilled water	20µl	-	-
3.	Standard	-	20µl	-
4.	Serum	-	-	20µl

2. Keep at 37^0C for 10 minutes
3. Take OD of standard and test against blank

Calculation

Serum Cholesterol =OD_T/OD_s ×200

Normal value

Serum Cholesterol – 140 -250 mg/dl

Estimation of serum high density lipoprotein (HDL)

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Introduction

HDL is called good cholesterol as it removes cholesterol from extra hepatic cells and brings it back to liver.

Method

Wybenga-Pileggi method

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Principle

LDL and VLDL is precipitated by Magnesium Chloride and Phospotungustic acid. HDL forms the supernatant after centrifugation., HDL reacts with ferric per chlotate in presence of concentrated sulphuric acid to form Lavender color complex, OD of which is measured at 540 nm. OD is directly proportional to the HDL concentration

Reagents

1. $MgCl_2$ (Magnesium Chloride)
2. PTA (Phospho tungstic acid)
3. Ferric per chlorate
4. Concentrated Sulphuric acid
5. Cholesterol standard (50 mg/dl)
6. Solvent
7. Serum

Procedure

1. 0.1 ml serum + o.1 ml precipitating reagent ($MgCl_2$ + PTA)
2. Keep at room temperature for 10 minutes
3. Centrifuge for 15 minutes at 2000 rpm
4. Take clear supernatant
5. Pipette in tubes labeled as follows

S.No.	Reagents	Blank	Standard	Test
1	Cholesterol reagent	1ml	1ml	1ml
2	Solvent	10µl	-	-
3	Standard	-	10µl	-
4	Serum	-	-	10µl

6. Keep at boiling water bath or 90 minutes and cool at running tap water

7. Take OD of standard and test against blank at 540nm

Calculation

Serum glucose $=OD_T/OD_S \times 50$

Normal value

Serum HDL Male 25-65 mg/dl
Female 35-75 mg/dl

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Clinical significance

Increase of HDL decreases chances of heart disease

Decreased level of HDL occurs in case of

1. Administration of androgen, neomycin and propanol
2. Maturity onset Diabetes Mellitus
3. Chronic renal dialysis
4. Nephrosis
5. Cystic Fibrosis
6. Hepato cellular disease
7. Cigarette smoking
8. Alcoholism
9. Atherosclerosis
10. Myocardial Infarction

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Estimation of serum triglyceride

Introduction

Triglycerides are both endogenous and exogenous

Method

GPO (Glycerol phosphate Oxidase) method

Principle

Triglycerides converts into free fatty acid and glycerol in presence of lipoprotein lipase..Glycerol converts into Glycerol 3 phosphate in presence of glycerol kinase. Glycerol 3 phosphate converts to hydrogen peroxide and dihydroxy acetone in presence of Glycerol phosphate oxidase. Hydrogen peroxide converts to water and nascent oxygen. Nascent oxygen reacts with 4 amino anti pyrine and phenol and forms pink red quininimine complex , OD of which is measured at 540 nm. OD is directly proportional to the protein concentration

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Reagents

1. Lipoprotein lipase
2. Glycerol Kinase
3. Glycerol 3 phosphate oxidase
4. ATP (Adenosine Tri Phosphate)
5 POD
6 Buffer
7 4 amino anti pyrine
8 Phenol
9 Serum
10 Solvent
11 Glycerol standard (200 mg/dl)

Procedure

1. Pipette in tubes labeled as follows

S.No.	Reagents	Blank	Standard	Test
1	GPO reagent	1ml	1ml	1ml
2	Distilled water	10µl	-	-
3	Standard	-	10µl	-
4	Serum	-	-	10µl

2 .Incubate for 10 minutes at 37^{0}C

3.Take OD of test and standard against blank, at 540 nm.

Calculation

Serum triglyceride $= OD_T / OD_S \times 200$

Normal value

Serum triglyceride (TG) 25-160 mg/dl

Calculation of VLDL, LDL

Serum VLDL = TG/5

Serum LDL= TC – (HDL+VLDL)

Normal value

Serum VLDL 5-36 mg/dl

Serum LDL 100-150 mg/dl

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Clinical significance Total cholesterol, LDL, VLDL and triglyceride

Total cholesterol, LDL, VLDL and triglyceride increases in case of

1. Atherosclerosis
2. Myocardial Infarction
3. Nephrotic syndrome
4. Myxaedema
5. Obstructive Jaundice
6. Diabetes Mellitus

Total cholesterol, LDL, VLDL and triglyceride decreases I n case of

1. Malnutrition
2. Malabsorption
3. Acute infection
4. Anemia
5. Hemolytic Jaundice

Unit 7 Liver function test (LFT)

Liver function test includes estimation

1. Direct and indirect bilirubin

2. SGPT/ALT (Serum glutamate pyruvate transainase/Alanine Transaminase

3. SGOT/AST (Serum glutamate oxaloacetate trasaminase/Aspartate Tranaminase

4. ALP (Alkaline Transaminase)

Estimation of Direct and indirect Bilirubin

Introduction

Bilirubin is breakdown product of hemoglobin. Increase of bilirubin in blood is called jaundice

Method

Diazo method

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Principle

Direct bilirubin reacts with Diazotized sulphanilic acid in presence of HCl to form azobiliuibin which is pink in color and OD is measured at 540nm. Total bilirubin reacts with diazotized suphanilic acid in presence of sulphanilic acid surfactant. OD is directly proportional to bilirubin concentration

Reagents

1. R1 (Sulphanilic acid, HCl and surfactant)
2. R2 (Sulphanilic acid and HCl)
3. R3 (Sodium nitrite)
4. Artificial standard, methyl red (10 mg/dl)
5. Distilled water
6. Serum
7. Working reagent

   Total Bilirubin R1 (10 ml) + R3 (0.2 ml

   Direct bilirubin R2 (10ml) + R3 (0.1 ml)

Procedure

1. Pipette in tubes labeled as follows

S.NO.	Reagent	Blank	Standard	Test
1	Working reagent	500µl	500µl	500µl
2	Distilled water	20µl	-	-
3	Standard	-	20µl	-
4	Serum	-	-	20µl

2. Incubate for 5 minutes at 37^0C

3. Take OD of test and standard against blank at 540 nm

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Calculation

Direct Bilirubin = OD_{TD}/ OD_s×10

Total Bilirubin = OD_{Tt}/ OD_s×10

Indirect Bilirubin -= Total bilirubin – Direct bilirubin

Normal Value

Direct Bilirubin 0.0 – 0.2 mg/dl

Indirect Bilirubin 0.2 – 0.8 mg/dl

Total Bilirubin 0.2 - 0.8 mg/dl

Clinical Significance

Increase of bilirubin in blood is called jaundice. It is of four types

(i) Pre hepatic or hemolytic jaundice

In this indirect bilirubin increases due to breakdown of hemoglobin. It occurs in case of

a Untreated malaria

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b Mismatch of blood

c Erythroblastosis foetalis

(ii) Neonatal or physiological jaundice

This type of jaundice occurs in newborn, in which indirect bilirubin increases as the liver is not ready to conjugate them

(iii) Hepatic jaundice

In this type of jaundice, direct bilirubin increases due to liver diseases like

a. Hepatitis

b. Cirrhosis of liver

(iv) Post hepatic or obstructive jaundice

In this type of jaundice, direct bilirubin increases due to stones or tumor in between liver and urinary tract

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Unit 8 Kidney Function Test (KFT) or Renal function Test (RFT)

Kidney function test includes

1. Estimation of urea in blood
2. Estimation of uric acid in blood
3. Estimation of creatinine
4. Clearance test
5. Estimation of electrolyte

Estimation of urea in blood

Introduction

Urea is breakdown product of protein

Method

Uriase UV kinetic method

Principle

Urea reacts with water in presence of urease to form ammonia and carbon dioxide. Ammonia reacts with alpha keto gluterate in presence of NADH (Reduced Niacinamide Adenosine Dinucleotide) to form glutamic acid. OD of NADH is measured at 340 nm. OD is inversely proportional to the urea concentration

Reagent

1. Urease
2. NADH
3. Buffer
4. Urea standard (50 mg/dl)
5. Serum

Procedure

1. Pipette in tubes labeled as follows (one by one)

S.NO.	Reagent	Standard	Test
1	Urease reagent	1 ml	1ml
2	Standard	20µl	-
3	Serum	-	20µl

2. Take OD after 1, 2 and 3 minutes

Calculation

Serum urea = Delta A_T/Delta A_s × 50

Normal Value

Serum urea 15- 45 mg/dl

Clinical significance

1. Hyperuremia

   Increase of blood urea is called is called hyperuremia. It is of three types

(i) Perennial hyperuremia

In this urea is increased due to excess protein loss. It occurs in case of

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a. Diabetes Mellitus
b. Bleeding in stomach and intestine
c. Severe burn
d. Fever
e. High altitude
f. Hot water bath

(ii) Renal hyperuremia

In this urea is increased due to renal diseases

(iii) Post renal hyperuremia

In this urea is increased in blood due to obstruction due to tumor or stone in between kidney and urinary tract

2. Hypouremia

Decrease of blood urea is called hypouremia. It occurs in case of

(i) Liver disease

(ii) Pregnancy

(iii) Lactation

Estimation of uric acid in blood

Introduction

Uric acid is breakdown product of purine

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Method

Trinder and Point method

Principle

Uric acid reacts with water and oxygen in presence of uricase to form carbon dioxide, allantoin and hydrogen peroxide. Hydrogen peroxide in presence POD converts to nascent oxygen and water. Nascent oxygen reacts with 4 amino anti pyrine and phenol to give pink- red quininimine complex, OD of which is measured at 540 nm. OD is directly proportional to the uric acid concentration.

Reagent

1. Uricase
2. POD

3. Buffer
4. Uric acid standard (6 mg/dl)
5. Distilled water
6. Serum

Procedure

1. Pipette in tubes labeled as follows

S.NO.	Reagents	Blank	Standard	Test
1	Uricase reagent	1ml	1ml	1ml
2	Distilled water	20µl	-	-
3	Standard	-	20µl	-
4	Serum	-	-	20µl

2. Incubate for 5 minutes at 37°C

3. Take OD of standard and test at 540 nm

Calculation

Serum uric acid = $OD_T/OD_S \times 6$

Normal Value

Serum uric acid male 3.5 – 7.2 mg/dl

Female 2.5 – 6.2 mg/dl

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Clinical significance

1. Hyperuricemia

   It is increase of uric acid in blood. It occurs in case of

   (i) Acute gout
   (ii) Sepsis
   (iii) Pneumonia
   (iv) Anemia
   (v) Polycythemia

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(vi) Diabetes Mellitus
(vii) Leukemia
(viii) Renal disease

2. Hypouricemia
It is decrease of uric acid in blood. It occurs in case of

(i) Acromegaly
(ii) Wilson's disease

Estimation of creatinine in blood and urine

Introduction

Creatinine is a waste product formed from creatine phosphate

Method

Modified Jaffe's method

Principle

Creatinine reacts with alkaline picrate to give orange red color, OD of which is measured at 540nm. Od is directly proportional to creatinine concentration.

Reagent

1. Picric acid
2. NaOH
3. Creatinine standard (2 mg/dl)
4. Serum
5. Working reagent Picric acid + NaOH (1:1)

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Procedure

1. Pipette in tubes labeled as follows (one by one)

S.NO.	Reagent	Standard	Blank
1	Working reagent	1ml	1ml

2	Standard	100µl	
3	Serum		100µl

2. Take OD after 20 and 80 seconds

Calculations

Serum creatinine = $(OD_{T\ 80\ sec} - OD_{T\ 20\ sec})/(OD_{s\ 80\ sec^{-}} - OD_{s\ 20sec}) \times 2$

Normal Value

Serum creatinine male 0.7 -1.4 mg/dl

Female 0.6 – 1.2 mg/dl

Clinical significance

1. Hypocreatininemia

   It is increase of creatinine in blood. It occurs in case of

   (i) Diabetes mellitus
   (ii) Muscular trauma
   (iii) Renal disease
   (iv) Myocardial Infarction

2. Hypocreatininemia

   It is decrease of creatinine in blood. It occurs in case of

   (i) Muscular dystrophy

   (ii) Mysthenia gravis

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TAILOR MADE NOTES ON

CLINICAL MICROBIOLOGY

FOR

06 MONTHS TRAINING COURSE IN

MEDICAL LAB TECHNICIAN & ECG OF CTs/GDS & PARAMEDIC STAFF SPONSORED BY

CENTRAL RESERVE POLICE FORCE

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PREFACE

The Short Notes on Microbiology have been developed exclusively to meet the need of 6 months Training in Medical Lab Technician & ECG Course for CRPF Sponsored candidates. These notes consist of a general and brief introduction about micro-organisms and diagnostic procedures used in diagnosis of various microbial diseases.

This booklet will also help the learners to understand the subject before entering into the application field and whenever they need the subject notes at a glance

Paper-III

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MICROBIOLOGY

Unit-I

Introduction:

Microbiology – Microbiology is a branch of biology which deals with study of micro organisms.

Medical Microbiology – This is a special branch of microbiology which deals with medically important must organisms.

* Disease causing microorganisms are called Pathogen.

Micro-organism – These are small living organism which cannot be seen by naked eyes, but can be studied by microscope.

e.g.- Bacteria, virus etc.

Branch of Microbiology –

Virology

Bacteriology

Mycology

Phycology

Parasitology

Virology: This branch of microbiology deals with study of virus.

	Example	Disease
1.	HIV Human Immuno Deficiency virus	AIDS Acquired immune deficiency syndrome
2.	Hepatitis virus HAV, HBV, HCV, HDV, HEV, HGV	Hepatitis Hepatitis A,B,C,D,E,G
3.	Polio Virus	Polio
4.	Rabies Virus	Rabies
5.	Influenza Virus	Influenza

Bacteriology: This branch of microbiology deals with study of bacteria.

	Example	Disease
1.	Ecoli	UTI
2.	Mycobacterium Tuberculosis	Tuberculosis
3.	Salmonella Typhi	Typhoid
4.	Streptococcus pneumonia	Pneumonia
5.	Clostridium tetani	Tetanus

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Mycology: This branch of microbiology deals with study of fungi

	Example	Disease
1.	Candida sp	Oral thrush
2.	Aspergillus sp	RTI
3.	Dermatophytes	Skin infection

Phycology: Phycology is study of Algae.

	Example	Disease
1.	Anabaena	Non pathogenic
2.	Volvox	Non pathogenic
3.	Chlorella	Non pathogenic

Parasitology: In this branch of microbiology we study about parasites.

	Example	Disease
1.	Entamoeba histolytica	Amocbiasis
2.	Giardia Lamblia	Giardiasis
3.	Plasmodium	Malaria
4.	Ascaris	Ascariasis
5.	Ancylo stoma	Ancylostomiasis

General Character of Microorganism

1. Virus:

* Acellular – Ultra microscopic
* Connective link between living and non-living.
* Parasite at genetic level have either DNA or RNA but never the both.
* Obligate ultra cellular parasite.

2. Bacteria:

* Small prokaryotes
* Size 0.2 um – 8 um
* may be saprophytic and pathogenic
* Can be useful, harmful and pathogenic
* Reproduce by Binary fission

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3. Fungi:

* Non-photosynthetic, Eukaryotes
* Saprophytic hetero trophs
* May be microscopic or microscopic
* Can be unicellular or multi cellular

4. Algae :

* Photosynthetic Eukaryotes
* Usually non-pathogenic
* Responsible for Eutrophication
* Rich source of Protein

5. Parasites :

* Non photosynthetic eukaryotes which depend on other living organism for their food and shelter.
* May be unicellular like protozoal parasites
* May be multi cellular like Helminthes parasites

Unit-2

IMPORTANT LAB INSTRUMENTS

Incubator

Incubator is an electrically heated cabinet, where electric energy is converted into heat energy and this heat energy is used to incubate the sample.

Incubator provides optimum temperature to micro organism as well as to enzymatic reactions. In microbiology lab Incubator is operated at 37^0C for 24-48 hrs.

Autoclave

Autoclave work as a pressure cooker for sterilization

Principle: - Moist heat sterilization by steam under pressure

Working parameter:-

Temp - 121^0C

Pressure - 15 PSI

Time - 15 minutes

Procedure –

(1) Check water level
(2) Put the material to be sterilized in basket of autoclave
(3) Close the lid
(4) Switch on power supply
(5) Open steam releasing valve till steam escapes out
(6) Close steam releasing valve
(7) Let pressure elevate up to 15 PSI
(8) At 15 PSI Ist whistle will be there
(9) Wait for next 15 minutes
(10) Switch off power supply
(11) Open steam releasing valve slowly
(12) Take out the sterilized material

Laminar Air Flow: They are also called as bio safety cabinets. They contain heap filters which remove 99.9% particles which are sized more than 0.3 microns. The cabinet also has UV light which is switched on about 15-20 mins earlier before starting the work. Laminar Air Flow is employed in research labs and industries for conducting assays preparing media and culturing micro-organisms

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Refrigerators: Are basic requirement of microbiological lab. Here temperature is maintained below 8 degree celcius for

a. Maintaining media
b. Maintaining microbial cultures
c. Preventing dehydration of prepared media
d. Storing thermolabile solutions, reagents, antibiotics, serum and biochemical agents

Fumigator- Is an apparatus to decontaminate microbiology lab from various micro-organisms and their spores by the process of fumigation. Fumigation is a process where the fumes of an effective biocidal chemical are dispersed in a leak proof lab to kill all contaminants

Formaldehyde papers are extremely effective biocidal agents which are prepared by heating the formalin

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Unit-3: Microscope

Microscope is an important instrument used to visualize microscopic object by magnifying them

Types of Microscope:-

a. Simple Microscope
b. Compound Microscope
c. Phase Contrast Microscope
d. Dark Field Microscope
e. UV Microscope
f. Electronic Microscope

Parts of Microscope: The components of Microscope are

a. Light Source
b. Abbe Condensor
c. Stage
d. Body Tube which consists of Eye piece, revolving nose piece and objective lens

Principles of Microscopy: Magnification means enlargement. In compound microscope two lend systems-objective and ocular lens work together for this

Objective lens produces magnified real image which is again magnified by the ocular lenses to produce final magnified image

Microscopes should not only produce enlarged image but clear image also

The efficiency of Microscope lies in its resolving power

Resolving power is the minimum distance (d) between two objects that reveals them as separate entities:

$D = 0.5 \lambda / \eta \text{ Sine } \theta$

Where λ= wave length of light and

η Sine θ = Numerical aperture

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Numerical apertures is characteristics for each lens and its printed on the lens itself. It can be refined as function of diameter of the objective lens in relation to its focal length. It depends on η and θ where η= refractive index of the medium in which lens works and θ= half of the angle of cone of light entering the objective

Sine θ can not be more than one

Applications:

1. Diagnostic cytology
2. Diagnostic microbiology
3. Diagnostic urology
4. Diagnostic Histopathology

Use and Care of Microscope

Proper care and maintenance of microscope is needed following point s should be kept in mind while handling the microscope

a. Instrument should be kept in special cabinets while not in use
b. Microscope should be held firmly by holding the arm with right hand and base with left
c. All the lens system should be cleaned with lens tissues to remove dust and oil which may decrease
d. Blotting paper cloth or towel should not be used for cleaning of lenses

e. If lenses are oily or sticky they can be wiped off with Xylol followed by 95% alcohol

UNIT-4: Sterilization Disinfection

Sterilization and disinfection are the two procedure to control the microbial growth.

Sterilization : Sterilization is a process which all articles medium or surface are made free from all types of micro-organism by physical and chemical method.

Disinfection : Method to control the growth of pathogenic and non sporing micro-organism from non living surface by chemical method.

In this method spores are not eliminated purposely.
The chemicals used for disinfection are called disinfectatits
Eg. – Phenol, Aldehyde

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Physical Methods	Chemical Methods
Sun Light	Alcohols
Heat (a) Dry heat	Phenols
(b) Moist heat	Aldehyde
Radiation	Halogens
Filtration	

Sun Light : Sunlight is a natural method to control microbial growth by UV rays from natural water reservoirs bedding, clothing etc.

Heat : Heat is very reliable method to control microbial growth. Two types of heat are used for sterilization.
* Dry heat
* Moist heat

Dry heat : Dry heat kills the micro-organism by oxidizing cellular component.
Dry heat can be used in

1. Red heat method
2. Flamming method
3. Hot Air Oven
4. Incineration

Red heat : method is used for sterilization of metallic articles.

Flaming : method is used for glass wares.

Hot air oven : is most widely used method of sterilization for metallic and glass wares.

Hot air oven is a electrically heated cabinet were articles are sterilized by hot air.

Components :

a) Double walls
b) Heat coil
c) Electric fan
d) Porous shelves
e) Thermo regulator
f) Thermo state control

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Working Parameter :

Hot air oven works on combination of time and temperature factors.

Temperature	Time
80°C	3 hrs
160°C	2 hrs
170°C	1 hr
180°C	½ hr

Incineration : Is a method to control reduce and reuse biomedical waste here biomedical waste is burnt into ashes in a large furnace.

Moist Heat Sterilization : is used in

a) Pasteurisation
b) Boiling
c) Autoclaving

Pasteurisation : is used for sterilization of milk and milk products here two methods are used
1) Flash method
2) Holder method

Boiling : is used for domestic purpose. In boiling vegetative form are eliminated but it is uneffective for spores.

Autoclave : is used for sterilization of metallic and glass wares. It works on the principle of moist heat sterilization by steam under pressure. Autoclave works like a pressure cooker at 121°C and 15 PSI pressure for 15 min.

Chemical Methods : The chemicals which are used for control of microbial growth are known as disinfectants and process is called as disinfection.

Different disinfectants used are

1. Alcohol
2. Phenols
3. Aldehydes
4. Halogens
5. Surface Active Agent
6. Vapour phase disinfectant

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Unit-5: Bacterial Cell Structure

Bacteria are small unicellular prokaryotic microorganisms which multiply by biniary fission they have various shape

Shapes Shape's name

Round	Cocci
Rod Shaped	Bacilli
Oval	Coccobacilli
Spring Shaped	Spirochaete
Ray Shaped	Actinomycetes
Coma Shaped	Vibrio
Pleomorphic	Mycoplasma

Bacterial Cell Structure

Important cell organelles and their functions are as follows:

a. Capsule: Outermost protective covering encapsulated bacteria. It inhibits Phagocytosis
b. Flagella: Is a fine thread like structures which help in movement
c. Fimbri: Short hair like structures help in attachment
d. Cell Wall: Protective covering made up of Peptidoglycan and lipids. On the basis of cello wall bacteria may be of two types

1. Gram Positive
2. Gram negative

e. Cell membrane: It is a selective semi permeable membrane present under cell wall governs inflow and outflow of metabolites
f. Cytoplasm: Color less viscous fluid which provides nutrition to cell organelles
g. Nucleoids: It is incomplete nucleus made up of single circular chromosome which transfers genetic characters from one generation tom other
h. Mesosomes: These are bag like structures which act as power house
i. Ribosomes: These are Dumb-bell shaped bodies and work as centre of protein synthesis
j. Inclusion Bodies: they work as preserved food material

Bacterial growth: It depends on two types of factors

1. Nutrition factors
2. Environmental factors

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1. Nutritional factors:

A. Source of Energy
B. Source of Electrons
C. Source of carbon
D. Source of Nitrogen
E. Source of Phosphorous
F. Source of Sulpher
G. Source of Metals and
H. Water

2. Environmental factors:
3. A. Temperature
4. Oxygen
5. pH
6. Salt concentration and
7. Light

Unit-6

Culture Media

Culture media is an artificial food used for growth of micro-organism is vitro.

Component of Culture Media :

* Source of protein
* Source of carbohydrate
* Source of electrolytes
* Source of growth factors
* Water

Types of media

As per Consistency :

* Solid media
* Semi solid media
* liquid media

As per composition :

* Synthetic media
* Non-synthetic

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As per purpose :

* Basal media
* enriched media
* Enrichment media
* Selective media
* Differential media
* Transport media
* Sugar media

Composition of nutrient agar :

Peptone	– 5.0 gm
Beef extract	– 3.0 gm
NaCl	– 2.0 gm
Agra	– 2.0 gm
Dist. water	– 1000 ml.

Composition of Mac Conkey Agar :

Peptone	– 17.0 gm
Protease Peptone	-3.0 gm
Lactose	- 10.0 gm
Bile salt	- 1.5 gm
Nacl	- 5.0 gm
Neutral Red	- 0.03 gm
Crystal violet	-0.001 gm
Dist. Water	- 1000ml

Mac Conkey broths :

Peptone	- 20.0 gm
Lactose	- 10.0 gm
Bile salts	- 1.5 gm
Nacl	- 5.0 gm
Neutral Red	- 0.03 gm
Crystal Violet	-0.001 gm

Dist. Water -1000 ml

Blood Agar :
Same as nutrient Agar + 5-10% blood agar.

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Unit-7: Staining Techniques

Staining stands for art of colouring, micro-organism are colourless, so they can be seen clearly if they are stained.

Staining is done by some chemical called as stains.

Stains may be of two types:

a) Acidic satin:- stain the basic components
b) Basic stain:- stain the acidic components

Staining Techniques:

There are many staining techniques which are used for various purposes

1. Differential staining
2. Capsule staining
3. Albert staining

1) Differential staining

Differential staining is used to differentiate between 2 groups of bacteria, on the basis of their colour 2 imp. Differential staining are

a) Gram staining
b) ZN staining

a) Gram staining

Gram staining was introduced by Christian Gram in 1884, to differentiate between Gram+ve and Gram-ve bacteria

Gram+ve bacteria are violet in colour where Gram-ve bacteria appear in pink colour.

Principle:- When a heat fixed bacterial smear is stained by crystal violet in presence of Gram Iodine; and then decolorized with alcohol or acetone.

a) Some bacteria retain their colour after decolourization and appear as violet, even after secondary staining with safranine those bacteria are called as Gram+ve.

b) Some bacteria loose their colour after decolourization and take the colour of counter stain safranine and appear as pink; such bacteria are called as Gram-ve.

Procedure:-

1) Take a neat and clean glass slide
2) Prepare a smear of bacterial culture on glass slide with the help of normal saline
3) Air dry and heat fix the smear
4) Cover the smear with crystal violet for 1 minute and then wash with running tap water
5) Cover the smear with gram`s iodine wait for 1 minute and wash it
6) Decolourize the smear with Acetone or alcohol for few seconds
7) Wash with running tap water
8) Now cover the smear again with safranine and wait for 1 minute and then wash with running tap water
9) Air dry the smear and then observe under 100X lens in presence of Immersion ort.

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Observation:-

I. Gram+ve will appear in violet colour
II. Gram-ve will appear in pink colour

Example:- Gram+ve

Staphylococcus, streptococcus, Bacillus, corynebacterium, clostridium.

Example:- Gram-ve

E-coli
Salmonella
Shigella
Klebsiella
Neisseria

ZN Staining

ZN Staining is also known as AFB staining. It is used to differentiate between AFB and NAFB. AFB are present in Red colour and NAFB are present in Blue colour. This staining is used to trace out disease Tuberculosis.

Principle:-

Mycobacteria are very difficult to stain due to presence of waxy covering but if these are coloured once with hot carbol fuchsin , they are again very difficult to destain, even in the presence of mineral acid, due to the presence of mycolic acids and appear as Red.

This property of not leasing the colour by Mycobactrium even by acid is called as acid fasteners; hence called as acid fast bacteria.

On the other hand, all other bacteria loose their primary colour in presence of acid and take the colour of methylene Blue and appear as Blue.

Procedure:-

1. Take a neat and clean glass slide
2. Prepare a smear from clinical sample
3. Air dry and heat fix the smear
4. Pour carbol fuchsin on the slide and heat up the slide upto steaming and wait for 5 minutes
5. Wash with running tap water
6. Decolourize with acid alcohol, and or 20% H_2SO_4 till smear is completely decolourized in to faint pink colour
7. Wash with running tap water
8. Now counter stain with Methylene Blue for 2 minutes
9. Wash with running tap water
10. Air dry and observe under 100 X lens of microscope in presence of Immersion oil.

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Observation:-

1. AFB will be seen in Red colour
2. NAFB will be seen in Blue colour, all cellular component like epithetical cells, pus cells will also be seen in Blue colour.

Example:-

AFB

1. Mycobacterium tuberculosis
2. Mycobacterium leprae
3. Mycobacterium avium
4. Mycobacterium bovis

Example:-

1. E-coli

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2. Salmonella
3. Shigella
4. Streptococcus

Capsule Staining

Capsule staining is used to demonstrate capsule in capsulated bacteria.

Capsule is mucoid, gelatinous covering on cell wall of a few bacteria, which does not take the colour of any dye, so it can be demonstrated by negative staining where capsule is present as colour less covering in a coloured background.

Procedure:-

1. Take a neat and clean glass slide
2. Put a big drop of India ink/ nigrosin dye, and mix loop full of bacteria in this drop.
3. Spread this drop in to a tongue shape smear, with help of spreader.
4. Air dry it and observe under 100X lens of microscope.

Observation:-

Capsule will appear as colourless, hollow, structure against a coloured back ground.

Examples of capsulated bacteria-

1. Strepto coccus pneumonia
2. Klebsiella pneumonia
3. Bacillus anthracis
4. Streptococcus pyogenes

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Albert Staining

Albert staining is used to demonstrate metachromatic granules especially in corynebacterium diptheria to rule out disease diptheria.

Procedure:-

1. Take a neat and clean glass slide
2. Prepare a smear from pharyngeal swab
3. Air dry the smear

4. Cover the smear by Albert A staining for 5 minutes
5. Drain out the excess stain
6. Cover the smear by Albert B
7. Drain out the excess stain after 3 minutes
8. Blot the slide and observe under 100X lens of microscope.

Observation:-

Bacillary body will appear in Blue green colour and metachromatic granules will appear in Blue-Black colours.

Unit-8

Salmonella Typhi, Typhoid and Widal Test

Typhoid is a enteric fever, caused by salmonella typhi, which is Gram-ve, non spore forming, non capsulated, bacilli.

Salmonella typhi establish typhoid in 3 stages

(I) Intestinal phase
(II) Septicemia phase
(III) Phase of localization

Symptom of Typhoid –

(I) High grade fever
(II) Malaise
(III) Lethargy
(IV) Epigastric discomfort
(V) Spots on belly region
(VI) Jaundice
(VII) Imbalance in liver enzymes

Diagnosis-

(i) In Internal phase Typhoid can be diagnosed by culture
(ii) Septicemia phase can be diagnosed by Blood culture
(iii) Phase of localization can be detected by Widal test

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Widal Test

- Serological test
- Used to detect Salmonella antibodies against four salmonella antigens
  Salmonella typhi `O` Ag
  Salmonella typhi `H` Ag
  Salmonella paratyphi `AH` ag
  Salmonella paratyphi `BH` Ag

  Type of test – Agglutination test
  Variants of widal test
  a) Qualitative slide test - which tells +ve/-ve test only
  b) Quantitative Tube Test – which declares titer. A titer more than 1:80 is considered as positive.

     If antibodies against any of the antigen `O`, `H`, and 'AH' & `BH` are traced in sample, sample is considered as positive.

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Unit-9: ABST

Antibiotic Sensitivity Test: This test is used to select more suitable antibiotic against a clinical isolate. Antibiotics are the chemicals or secondary metabolites which are produced by some microorganisms to inhibit or kill other microorganisms. ABST test may be of two types

1. Diffusion test
2. Dilution Test

Diffusion Test: This can be done by

1. Karbybaur Method
2. Stokes Method
3. Epsilometric Method

Dilution test: Is used to calculate

a. MIC (Minimum Inhibitory concentration
b. MLC- Minimum Lethal Concentration

Unit-10

Mycology

Mycology is the study of fungi

Important fungi are

1. Dermatophytes
2. Candida
3. Aspergillus

1. Dermatophytes:- are skin infectious agents

   The members of this group are

   (a) Trichophyton
   (b) Microsporum
   (c) Epidermatophyton

   Disease caused by dermatophytes are termed as:
   Ringworm, Tinea and Dermatophytosis.

They can be diagnosed by

a) Microscopy
b) Culture
c) KOH mount

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(2) Candida:- Candida is an opportunistic pathogen which is responsible for candidiasis.

Disease due to Candida

a) Superficial candidiasis
b) Muco-cutaneous candidiasis
c) Systemic candidiasis

Candida can be diagnosed by

a) KOH staining
b) Gram`s staining
c) Culture
d) Germ tube test

(3) Aspergillus:- Aspergillus is also an opportunist mould which is responsible for RTI

Disease due to Aspergillus

a) Cutanoeous Aspergillus
b) Respiratory Aspergillus
c) Systemic Aspergillus

Lab diagnosis:-

(1) Microscopy
(2) Culture
(3) KOH mount

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Unit-11: Virology

Study of viruses is called virology

Virus can be of two types on the basis of Nucleic acid

(I) DNA Virus
(II) RNA Virus

They cannot be seen by naked eyes or compound Microscope. They need to be seen by electron Microscope.

Important Virus which act as occupational hazard for technicians is known as HIV

HIV is a Human Immuno deficiency Virus which is responsible for an incurable condition called as AIDS.

Modes of transmission are:-

a) Sexual transmission
b) Parenteral transmission
c) Perinatal transmission

Phases of HIV infection:

a) Acute HIV infection

b) Asymptomatic HIV infection
c) PGL
d) ARC and AIDS

Lab diagnosis:

I. Ag detection
II. HIV viral RNA detection
III. HIV cultivation
IV. HIV Ab detection

HIV Ab can be detected by-

a) Screening test – TDT, ELISA
b) Confirmatory test Western Blot Test

TDT – Tri Dot Test

ELISA – Enzyme Linked Immuno Sorbant Assay

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Unit-12: Parasitology

This is a branch of Microbiology which deals with study of parasites.

Parasites may be of 2 types

a) Unicellular – (e.g.) Protozoa
b) Multicellular – (e.g.) Helminthes

Protozoal Parasite

(1) Entamoeba histolytica
Disease:- Amoebiasis

Morphology:- 3 forms exists

a) Trophozoit
b) Cyst
c) Precyst

Pathogenesis

(I) Intestinal Amoebiasis
(II) Extra intestinal Amoebiasis

Lab diagnosis:-

a) Normal saline mount
b) Lugol's iodine mount
c) Iron haematoxylin Mount

Giardia Lamblea

- Intestinal flagellate
- Disease caused- Giardiasis

Morphology:- Two forms exist-

(1) Trophozite
(2) Cyst

Pathogeneity
Epigastric pain
Nausea
Vomiting
Fat malabsorption

Lab diagnosis:-

a) Wet mount
b) Lugol's Iodine mount
c) Iron haematoxylin mount

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Ascaris

Common Name:- Round Worm
Habitat – small Intestine
Disease caused – Ascariasis

- White, thread like nematodes.
- 4 types of eggs can be laid by female ascaris
    a) Embryonated egg (double walled)
    b) Embryonated egg (semi decorticated)
    c) Unfertilized egg (double walled)
    d) Unfertilized egg (semi decorticarticated)

Pathogenesis may be due to

(I) Adult worm in intestine

(II) Larva in lungs

Lab diagnosed is :-

a) Wet mount by Normal saliva
b) Wet mount by Lugol`s Iodine

Ancyclostoma duodenales

Common name – Hook worm

Habitat – Small Intestine

Disease - Ancyclostomiasis

Morphology – 2 types of adult worms can be seen

a) Male adult worm
b) Female adult worm

Female adult worm can lay 1 type of egg only

- Embryonated egg with 4 blastomers

Pathogenecity:- Anaemia, Hypovitaminosis, Malnutrition, Diarrhoea.

Lab diagnosis:-

1) Wet mount by normal saliva
2) Wet mount by Lugol`s iodine

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Tape Worm (Tenia)

These are long parasites

Size may be 3 cm – 2.5 meters

Responsible for – Teniasis (Intestinal Infection)

2 important speceas are

Tenia solium
Tenia sagimate

Pathogenicity:- Anorexia, and cyst formation.

Lab diagnosis – Wet mount preparation

Plasmodium

Malarial Parasite
Vector – female Anopheneles mosquito
Inter mediate host – Man.

Important species

- P. vivax
- P. falciparium
- P. ovale
- P. malaria

P. vivax cause Intermittent fever in malaria but falciparum is responsible for complications such as cerebral malaria and black water fever which may be life threatening.

Lab diagnosis:-

(I) RDK
(II) Thin smear preparation
(III) Thick smear preparation

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TAILOR MADE NOTES ON

HISTOPATHOLOGY

FOR

06 MONTHS TRAINING COURSE IN

MEDICAL LAB TECHNICIAN & ECG OF CTs/GDS & PARAMEDIC STAFF SPONSORED BY

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PREFACE

Notes of Histotechnology will help the students to acquire the basic knowledge of Histotechnology and various techniques which are involved during the tissue processing and instruments used. Students will be able to prepare the slides of tissues

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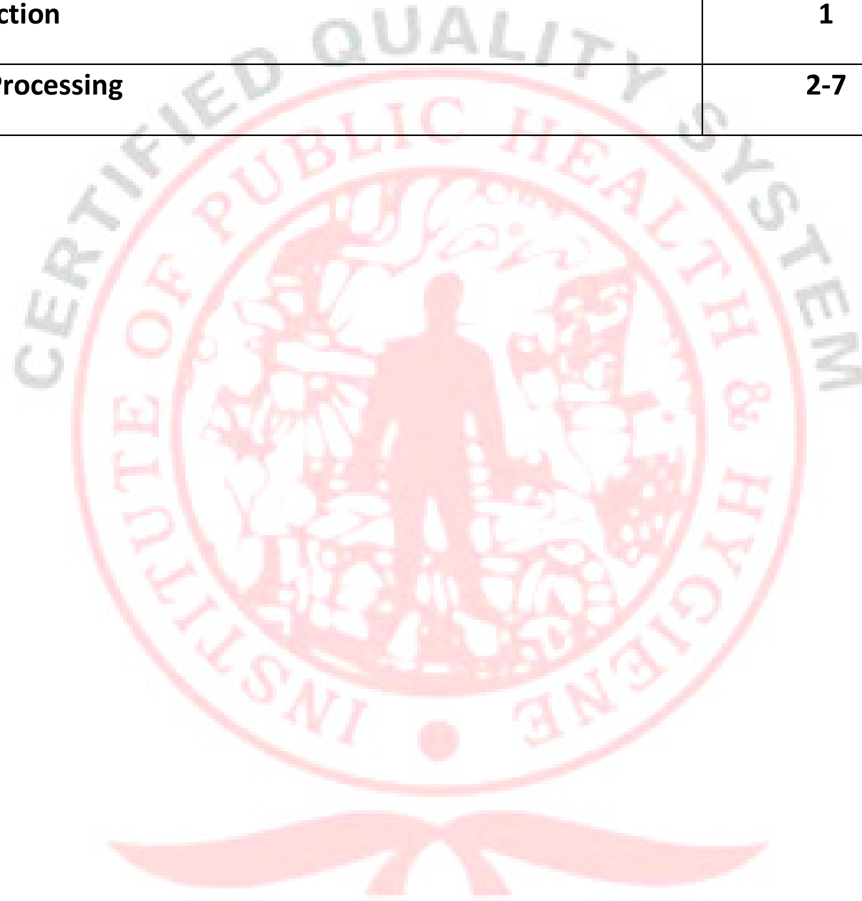

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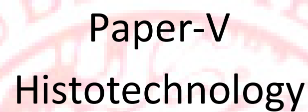

Paper-V
Histotechnology

Unit-I

Introduction: Histology is the branch of medical science which deals with study of tissue which may be diseased or not.

Histopathology: It is the branch of medical science which deals with diseased tissue.

Histotechnology: It is the branch of medical science which deals with the techniques involved in studying the tissue.

Histotechnician: The person who knows the technique involved in studying the tissue.

The Histopathology helps in diagnosis of disease mostly the cancer patients.

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Unit-II: Tissue Processing

Tissue Processing: It is the process by which tissue is made firm (hard) so that thin sections can be cut and studied under microscope. It can be done either manually or with the help of a machine known as automatic tissue processor. It involves the following steps.

1. Reception and logging
2. Grossing
3. Fixation
4. Decalcification (if required)
5. Dehydration
6. Cleaning
7. Impregnation
8. Embedding
9. Section cutting
10. Staining
11. Mounting
12. Microscopy

1. Reception and logging : The specimen sent for histopathological examination is collected in plastic or glass contain. Once specimen is received in the laboratory ensure that
   - (a) Specimen is accompanied by a requisition form with relevant details of the patient i.e. name, age, sex, hospital number, diagnosis history site of biopsy.
   - (b) The specimen and the requisition form are of the same patient.
   - (c) Ascertain the presence of the tissue in the bottle.

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(d) In case more than one specimen check whether the specimen number consider with that on the requisition form.

Providing proper number to the tissue for its proper identification is known as logging or labelling. It helps in removing errors in pre analytical and post analytical stage. Number is given as S/108/19 in this number –S- represents the Sr. No. of the tissue while 19 indicates the year in which sample has come this number remains with the tissue throughout the tissue processing.

2. Grossing : Grossing means gross examination of the tissue. It includes the macroscopic examination of tissue that is to observe its shape, size, colour texture etc. and the tissue in thin slices of 3 to 5 mm.
After grossing the tissue is placed in tissue cassette along with the label on which number is written with the help of the pencil. The tissue cassette helps in prevent change in morphology of tissue while transferring it from one container to another container, its another advantage is that we can process more than one tissue in a single container.

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3. Fixation : To preserve the tissue from any physical or chemical change after it is removed from patient body is known as fixation. The physical change involves change in morphology i.e shape and size of tissue the tissue should not shrink or there should not be any swelling in the tissue. While chemical change involves Autolysis and purification.
Autolysis : Self destruction or damaging of tissue by the enzymes which is secreted by cell itself, name of the enzyme is hydrolytic enzyme and it is secreted by lysosome.

Putrefaction: Destruction or damaging of tissue by the bacteria which are present in surrounding atmosphere.

When fixation is done these changes do not occur.
Fixation is done with the help of chemicals known as fixatives the most commonly used fixative is 10% Formalin. Other fixatives are formal calcium, Zenker's fluid, Bouin's fluid, Heidenhains susa etc.

4. Decalcification: Removal of calcium from the tissue is known as decalcification. It is done in order to make the tissue soft so that its proper section cutting can be done and microtome knife do not damage. It is done only in those tissues which contains calcium i.e bones and cartilage. If the given sample does not contain calcium thin step is not required for example skin, intestine liver etc. Decalcification is done with the help of chemicals known as decalcifying fluids some examples are 5 to 10% nitric acid, 10% Formic acid, Gooding Stewart reagents, formal nitric acid etc.

5. Dehydration: Removal of water from the tissue is known as dehydration. It is done because in further tissue processing there is a step of impregnation in which we have to introduce paraffin wax inside the tissue for proper section cutting. As paraffin wax and water are not soluble with each other. In order to enter paraffin wax inside the tissue we will have to remove water from the tissue. Dehydration is done with the help of dehydrating agents e.g Graded ethyl alcohol, Acetone, isopropyl alcohol, dioxane etc. Most commonly used dehydrating agent is graded ethyl alcohol. We take seven containers of ethyl alcohol i.e 70%, 80%, 95%, 95% and then three containers of absolute alcohol and keep the tissue for one hour in each container in this way water is removed from the tissue and alcohol enter inside the tissue.

   In order to check the complete dehydration we place the anhydrous copper sulphate in last container of absolute alcohol the anhydrous copper sulphate is hygroscopic in nature and white in colour, if water will be there it will be absorbed by copper sulphate and it will change into hydrated copper sulphate which is blue in colour. So if colour of anhydrous copper sulphate changes from white to blue means dehydration is incomplete and if it remains white means dehydration is complete.

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6. Clearing : Removal of dehydrating agent from the tissue is known as clearing, it is done because the dehydrating agent alcohol is also not soluble with wax so we have to remove the alcohol. It is done with the help of clearing agents which are soluble both in alcohol and wax. Most commonly used clearing agents is xylene. We take two containers of xylene and place the tissue for one hour in each container. Other examples of dehydrating agents are Benzene, tolerance cidar wood oil, clove oil, chloroform phenol etc.

7. Impregnation: Entering of paraffin wax inside the tissue and removal of clearing agent is known as impregnation. It is done in order to provide proper hardness to the tissue for proper section cutting. The impregnation media should be solid and room temperature and its melting point should not be too high. Example of impregnation media or supporting media are paraffin wax, paraplast, paraplast plus, celliodine, tissue mat ester wax etc. It is done in paraffin oven.

8. Embedding or Blocking : Embedding is the process of placing the impregnated tissue is precisely arranged position into a mould containing the embedding media and causing this medium to solidify. Embedding is done to give the tissue proper shape and size so that it can be attached to microtome for proper section cutting. Moulds used in embedding are Leuckhart's piece or L-piece which are made up of grass (an alloy of copper and zinc) or tissue tek.

Technique of embedding:

(a) The mould is placed on a tray, tile or a plain surface.
(b) Fresh molten wax is poured from stock jug into the mould.
(c) The tissue is lifted from final wax bath and placed at the bottom of the mould.
(d) The surface to be cut is pressed gently against the solid layer with no trapped air bubbles.
(e) The labelled bearing number of the specimen is fixed to the corner of the solidifying wax.
(f) When block has cooled to form a skin on the surface, it should be immersed in cold water.
(g) When the block hardens, remove the mould.
(h) A fresh labelled with typed number is fixed to the side of the block by pressing hot forceps. The block is now ready for cutting or storing.
(i) A block has to be cut, the surface of the block away from the surface to be cut is pressed against chuck. Chuck helps in attachment of a embedded block to the microtome.

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9. <u>Section cutting or microtome</u> : In order to view the structure of the cell, a tissue is properly cut in very thin sections of the order of the microns. It is done with the help of

a machine known as microtome. The most commonly used microtome is rotary microtome. When sections are thin, it can be stained in better way and viewed properly in the microscope generally thickness of the tissue is 3 to 5 microns or advised by the pathologist. Technique of cutting paraffin embedded sections.

Technique of cutting paraffin embedded sections :

(a) Trim the block to remove the surplus paraffin on the surface.
(b) The cutting surface should be even and parallel.
(c) Attach the block to the chuck and seal the sides of the block with warm forceps.
(d) Attach the block to the microtome.
(e) Turn back the feed mechanism as far as it goes.
(f) Insert the knife in the knife holder with slight tilt to produce the clearance angle.
(g) Adjust the knife holder or move the block holder, so that wax block just touches the knife.
(h) Block should move parallel to the knife edge.
(i) If not minimized earlier set the gauge to 15 microns and cut till complete sections are obtained.
(j) If it is already trimmed set the gauge to required thickness.
(k) Operate the microtome till complete sections are cut and ribbon is formed.
(l) With forceps hold the ribbon at its free margin and separate the sections attached to the knife by brush.
(m) Float the sections on tissue floatation water bath maintain at temperature 5 to 6° below melting point of wax.
(n) Immerse the albumenized slide in the water and bring the section gently into the center of the slide.
(o) Keep the slide with section vertically on the rack till the water is drained.
(p) Write the login number on the glass slide with the help of diamond pencil.
(q) Place the slide on slide warming table maintained at temperature 1 to 2°C above melting point of the wax. Then remove moisture, left out wrinkles and make attachment of tissue better.

10. Staining : Colouring a dying the tissue to known as staining. Staining of tissue sections enables us to study the characteristics of the tissues and their constituent cells. The most commonly used stain is hematoxylin and Eosin stain it is also known as routine stain.

Composition of Hematoxylin stain

Harris Hematoxylin

Hematoxylin - 2.5 gm
Absolute alcohol - 25 ml
Potash alum - 50 gms
Dist. Water - 500 ml
Mercuric oxide - 1.25 gm
Or
Sodium Iodate - 0.5 gm

Eosin - Eosin alcoholic

Stock solution : - Eosin - 1 gm
-Dist water – 20 ml

Dissolve eosin in water by heating it gently. Cool the mixture and add 80 ml of 95% ethyl alcohol.

Working Solution :

Stock Solution : 25 ml
80 % ethyl alcohol = 75 ml

Adding 15 ml glacial acetic acid to 100 ml of stain gives deeper shades of red colour.

Procedure :

(a) Deparaffination : Removal of paraffin wax is known as deparaffiniation. It is done with three container of xylene for 5.5 and two minutes.

(b) Hydration : Bringing of water inside the tissue is known as hydration. It is done as the stains are soluble in water. It is done with the help of decreasing con of alcohol i.e 100% alcohol, 95% alc, 80% alc, 70% alc, 50% alcohol than in water for one minute each.

(c) Primary Stain : Now slide is placed in Harris hematoxylin for 4 to 5 minutes.

(d) Give one washing with water.

(e) Differentiation : In this step selective removal of stain is done. It is done with the help of 0.5% Hu or 1% acid alcohol in this step the stain is removed from cytoplasm and retained by the nucleus but due to acidic pH blue colour of hematoxylin changes to red.

(f) Bluing : Regaining of original blue colour of hematoxylin which was changed to red during acidic pH is known as bluing. It is done with the help of Lithium carbonate solution or dilute ammonia for one minute. It can also be done by tap water for five minute.

(g) Give one washing with water.

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(h) Counter stain Eosin : Now slide is placed in eosin stain for 2 minutes in order to stain cytoplasm.

(I) Give one washing with water.

(j) Dehydration : It is done with increasing concentration of alcohol or three container of acetone.

(k) Clearing : Removal of dehydrating agent, it is done with the help of xylene for 5, 5 and 2 minutes.

11. Mounting : Covering the stained slide with the help of mounting media (DPX) and cover slip is known as mounting. It is done in order to

(a) Protect the tissue section from physical injury or deterioration of stain due to oxidation.

(b) Fix the slide to cover slip.

(c) Fill the tissue space and cavities.

(d) Remove and trapped air bubbles.

(e) Avoid distortion of tissue and loss of stain or long periods.

(f) Visibility becomes better.

12. Microscopy: Viewing the stained slide under microscope is known as microscopy after staining following results are seen.

Nucleus = Blue

Cytoplasm = Pink

RBC = Red

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Unit-III: Instruments Used in Histotechnology

1. Microtome : It is a type of machine which is used to cut thin sections of the tissues of the order of microns. There are 5 classes of microtome.

(a) Rotary microtome.
(b) Rocking microtome
(c) Base sledge or sliding microtome
(d) Freezing microtome
(e) Ultra thin section microtome for electron-microscopy work.

The most commonly used microtome is Rotary Microtome.

Basic Principle : The pawl or panel is brought in contact with ratchet wheel which is connected to mill head micrometer screw. This action turns the wheel and rotate the screw. As a result the block is moved towards the knife at a predetermined thickness.

Parts of Rotary Microtome :

(a) Block holder , (b) Knife holder (c) knife clamp, (d) knife clamp screw, (e) Block adjustment screw, (f) Thickness gauges, (g) Thickness adjustment screw, (h) Operating handle, (i) Operating wheel, (j) Reverse wheel, (k) Angle of tilt adjustment

Mode of Action : It is the most popular microtome, a rotary action of hand wheel starts the cutting movements block is mounted on the steel carriage the fly wheel is turn towards the top of upwards stroke. The pawl comes in connected with ratchet wheel which in turn rotates the micrometer screw. The

block moves forward and block hold moves up and down vertically in front of knife.

Advantage :

(a) The microtome is heavy and stable
(b) Knife angle is adjustable
(c) Knife holder is moveable
(d) Sections are cut flat and plain
(e) Good serial sections are obtain
(f) Its is useful for routine and research purpose

Disadvantage :

(a) It is very heavy, so difficult to move
(b) Microtome knife is costly, so needs extra care

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Automatic Tissue Processor (Autotechnichon)

The automatic tissue processor is a excellent device which can perform the different steps of tissue processing automatically. It is electrically operated. However, it create serious problem, if there is interception in the supply of electricity, in addition if there is no proper maintenance of the instrument it is a matter of trouble for most of the technician. It consist of main body having beaker platform with beaker, a transfer arm to shift the basket containing tissue capsule through different reagents . It has a metal disc know as timer disc which is used to set the time schedule for tissue processing. It has a plastic cover plate which covers all the baskets to prevent evaporation of reagents. It also contains thermostatically controlled wax bath for impregnation some time delay timer facility also available which helps inn delaying the time if required. It is also known as Autotechnician.

Mode of Action :

(a) The transfer arm moves enroller which is responsible for shifting tissue basket from one container to another container.

(b) The transfer arm moves the tissue through processing reagents by lifting it in and out of beaker.

(c) The timer unit consist of an electric clock which is attested to melt disc in which slots are cut at require intervals with the help of metal cutter.

(d) The disc rotates against the spring loaded liver, which when slips into slots caused the transfer arm to lift and tuning mechanism is set in motion, shifting the tissue to the next position in the cycle.

(e) Delay mechanism is available to process the tissue latter on.

Bone Decalcifier : It is a machine with the help of which tissue is decalcified. It is electrically operated. It reduces the time of decalcification by 1/3 of that which is taken when decalcification is done manually.

Mode of Operation :

(a) assemble the base plate with vertical pillar

(b) Mount the rotor motor and clamp it on horizontal pillar

(c) Keep the tissue with calcium in the stainless steel perforated basket

(d) Suspend the basket in pins of the clamps

(e) Place the beaker with decalcifying fluid on heating unit

(f) By unscrewing the knob on the pillar of the stand lower the rotor so that basket is immersed in the beaker fluid know clamp the rotor at this position by tightening the knob of the pillar.

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(g) Connect the rotor motor lead wire plug to the socket of the control box.

(h) Connect the control box wire to the socket.

(i) Switch on the main supply to control box.

(j) Set the digital temp. controller temperature generally maintained is 37°C.

(k) The basket along with tissue will be provided rotary and translator motion by the motor rotor. It rotates at 30 rpm.

(l) Run the process for required time.

(m) After desired time, switch off the rotor and heating mantle by switching off the main supply.

(n) The process is now complete.

(o) Raise the motor and basket, basket will come out of the beaker.

(p) Remove the tissue from the basket for further processing.

Microtome Knife Sharpener

It is a machine with the help of which we sharpen the microtome knife.

Mode of Operation :

(a) Clean the plate thoroughly to provide the surface completely free from dust and dirt clean it with solution of calcium carbonate and water if necessary.

(b) After washing the glass plate thoroughly with the solution, rub the glass surface vigorously with cotton. Presence of water on glass surface indicates that glass place is not clean. The glass plate must always be free from dirt, oil or grease.

(c) Insert knife carefully and tight it with the help of screw provided with the knife holder.

(d) The knife should not be removed from the holder, until the sharpening operation is completed. To remove the knife from the holder, carefully release the screw and remove the knife.

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Autopsy

An Autopsy is also known as postmortem examination, is a specialized surgical procedure used to determine the cause and manner of death. The cause of death is the medical reason explaining why patient passed. The manner of death is the circumstances surrounding the death. It recognizes the following manner of death:

Natural, accident, homicide, suicide and unknown

Autopsy is continually advance our understand of disease. What we learn from Autopsies allows clinicians to better understand disease process, accurately diagnose the disease improve therapy and potentially aid other patients who are currently suffering from similar disease.

Sometime a forensic expert is also present if death takes place in ambiguous circumstances.

TAILOR MADE NOTES ON CLINICAL BIOCHEMISTRY

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FOR

06 MONTHS TRAINING COURSE IN

MEDICAL LAB TECHNICIAN & ECG OF CTs/GDS & PARAMEDIC STAFF SPONSORED BY

CENTRAL RESERVE POLICE FORCE

PREFACE

Notes of Lab Management and Ethics will help the student to acquire the knowledge about the Laboratory, different departments of Laboratory, precautions taken against the lab incidents. It will help the students to know how to maintain the quality in Lab i.e. about quality control, different types of glassware's in laboratories. Ultimately it will help in management of Lab

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DUTIES AND RESPONSIBILITIES OF LABORATORY TECHNICIAN

UNIT-I

A medical Laboratory technician is like any other professional worker. However in the field of medicine where one is providing services to others especially in handling patients, care should be taken. The following points related to the conduct expected of a medical laboratory technician should be followed.

1. The well being and service of the ill should always be placed above one's interest.
2. One should work scientifically and with complete honesty.
3. Professional skills or knowledge should not be used for undue personnel gain.
4. The results of the investigation should not be revealed to any unauthorized person.
5. Any personal information about the patient should be kept confidential.
6. One should respect the other members of the laboratory or health centre.
7. One should work in harmony with his/her colleagues.
8. Other than personal belongings, nothing should ever be taken out of the laboratory.
9. All the time, one should be courteous patient and considerate with the sick and their attendants.
10. Preventive health care and control of disease should be promoted at all the times.
11. Safety precautions should always be followed.
12. Application of first aid should be learned diligently.
13. Drinking of alcohol or use of other intoxicants when on duty should not be done.
14. Equipment should be well maintained and cared for.
15. Reagents or other laboratory supplies should not be wasted.
16. Conditions of employment should always be fulfilled.
17. He/she should consult the pathologist and senior hospital staff, if any problem arises. He/she should not try to play a role of the doctor.

18. He/she should arrive early to the laboratory and unexpected delay or absence should be reported to the laboratory supervisor as soon as possible. If necessary alternative arrangement should be made in advance.

19. He/she should have pleasant personality. The technician should wear neat and clean laboratory coat while in the laboratory.

Organizational Structure of the Clinical Laboratory

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UNIT-II

The clinical Laboratory should have well defined organizational structure so that it can perform its job effectively. The clinical laboratory can be attached to the hospital or it can be independent laboratory. The medical laboratory should be under pathologist, who should be a medical graduate with specialization in pathology. The laboratory in-charge is responsible for all activities of the lab. like work distribution, problem solving, and decision making. The pathologist is also responsible for supervising all laboratory work such as dealing with external agencies, documentation purchasing, maintaining all laboratory equipments and taking care of all safety regulations. A Lab technician performs various tests in the laboratory records results and sends the result to laboratory in-charge who sends these results to the pathologist for his signature. The final report after it is signed by the pathologist is then sent to physician.

<u>Functional components of the Laboratory</u> : The functional component of the laboratory include various sections of the laboratory. In tertiary level laboratory each section is headed by a specialist of that field. The major sections of laboratory can be classified as under.

ADMINISTRATOR
↓
PATHOLOGIST
↓
LABORATORY SUPERVISOR
↓

↓	↓	↓	↓	↓	↓	↓
1. Specimen Collection	2. Hematology Lab.	3. Blood Bank	4. Biochemistry Lab.	5. Microbiology Bacteriology & Parasitology	6. Clinical Pathology	7. Histopath & Cytology Lab

1. <u>Specimen Collection</u> : In this section blood, urine and other specimens are collected as per the requirement . The specimen is then dispatched to the concern department. The accuracy of the test depends upon the quality of the specimen received by various labs.

2. <u>Hematology</u> : Hematology deals with investigations related to blood bank and its components. It measures the quantity of various elements of the blood such as RBC, WBC platelets, raticulocytes. Examination of Bone marrow is also performed in hematology lab. apart from these it also deals with the properties and variances of the constituents of the blood such as hemoglobin concentration, pack cell volume, ESR etc. It also comprises the study of

coagulation mechanism such as prothrombin time, activated partial thromboplastin time, tests for loipus anti coagulant etc.

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3. Blood Bank : The main function of the blood bank is blood collection and preservation of the blood of the donor. The blood bank also caters to cross matching the donor blood with that of recipient blood. The blood bank also indulge in separating plasma, platelets, packed red cells etc. from the blood and issue them as per specific needs. Blood banks in tertiary level health centers may even provide coagulation assessment facilities.

4. Microbiology : Microbiology can be classified into sub sections of bacteriology, mycology, virology and parasitology in various laboratories. Immunology is also considered subsection where as in other labs it is considered as independent sections.

a) Bacteriology : A bacteriology laboratories carries out the lab diagnosis of various disease caused by bacteria, sample collection bacterial culture and identification antibiotic sensitivity test etc. are major tasks performed in this section. Microscopic examination of samples by using gram stain Ziehl-Nelsen stain, Albert stain, capsule stain etc. are performed to identify the bacteria present in this specimen.

b) Mycology : Mycology deals with the study of various fungi, apart from fungal cultures, potassium Hydro-oxide preparation of various clinical samples, lacto phenol cotton, blue stain for identification of fungal cultures, anit-fungal sensitivity tests are also done.

c) Virology : Virology deals with the study of viruses. Only a few medical colleges and research labs have this facility. Isolation of the viruses their culture, egg inoculation, compliment fixation test, ELISA and PCR are done in this laboratories.

d) Parasitology : Parasitology deals with the study of parasites of medical importance and their hosts. Various tests performed in parasitology section includes examination of the stools for parasites, parasitic eggs and cysts examination of blood for malarial parasite of the plasma and various stool concentration techniques culture of various protozoan's such as entamoeba, tryponema etc are also done in the section, Now a days PCR is also used in parasitology to diagnose various infections.

e) Immunology : Immunology is usually considered as independent section. It deals with the detection of antigens of infectious organisms and anti bodies formed in response to them. Auto immune disorders are also studying in this section. It employs many different techniques such as precipitation, agglutination, haemagglutination, latex agglutination , latex agglutination,

ELISA, RIA (Radio immuno Assay), CLIA (Chemilumine sense) etc. to detect these antigens and antibodies.

f) Clinical Pathology : Clinical pathology mainly deals with the examination of the urine and body fluids. The tests commonly performed in this section includes physical, chemical and microscope examination of urine, stool, CSF, peritoneal fluid (abdominal) Synvoil fluid (joints),plural fluids (lungs), pari cardial fluids (heart) etc. in addition to examination of semen, sputum, pregnancy test etc. also carried out in this section.

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g) Clinical Biochemistry : various chemicals and enzymes are found in our blood, urine and other body fluids. Their detection and quantification is the domain of this section, blood sugar, blood urea, serum glutamic oxaloacatic transaminase (SGOT) serum glutamic pyruvic transaminase (SGPT), amylase, serum bilirubin, serum cholesterol etc. are estimated in this section. It is one of the most automated section in a clinical laboratory.

h) Histopathology & Cytology : Histopathology laboratory deals with the collection, preparation, staining and examination of biopsy specimen for detection of cancer. In a cytology laboratory cells are collected from various body sites (eg. cerviex) and are smeared and examined for information of cancer. These laborites are usually limited to tertiary care hospitals.

i) Medico Legal Problems : The setting up of a laboratory requires appropriate licensing before any operation can commence. Designing, staffing, waste disposal and appropriate ventilation should be in accordance with established guidelines and regulations of the local government. The licensed certificate should be displayed at the reception are the data pertaining to the qualification of the doctors and technician of the laborites should be recorded and verified. The relevant documents such as agreements with the bio medical waste disposal agencies should also be recorded.

All records of the patients including reports and reference should be maintained for atleast three years. In case of histopathology the records should be maintain for seven years, so that tissue or samples is preserved in its natural state for further examination. In case of other conditions arise related to aliment patient for routine test specimen should be preserved atleast for 24 hours. The standard procedure and practices should be followed for preservation of the samples. Rechecking of all the reports is mandatory before they are dispatched for avoiding clerical mistakes. All reports must be signed either by consultant heading the section or by head of the laboratory. In case of emergency a technician can issue a presumptive report which has to be authenticated by pathologist as soon as possible. In case of any dispute regarding reports issued by laborites, it should follow the legal advise.

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LABORATORY ACCIDENTS AND LABORATORY SAFETY

UNIT-III

Types of laboratory Accidents :

There can be many type of accidents example are

1. Infections : The samples handled in laboratory, especially a clinical laboratory are likely to contain many potentially infectious agents, which can infect the laboratory personnel in following way.

   (a) Inhalation, (b) Ingestion (if Hands are contaminated), (c) injection (pricks, cuts, wounds, scratches etc.)

2. Burn : Burn can occur either due to fire or inflammable chemicals. The typical causes of fire are the Bunsen flame or sprit lamp, electric malfunctioning inflammable substance etc. Some chemicals like acids are also corrosive in nature. Mouth pipetting is also one of the causes.

3. Cuts & Pricks : Cuts and pricks are caused either by needles or by broken glass, improper handling of sharp instruments like microtome knife can causes serious cut injuries. Most common causes is needle prick which happens when needle recapped handling broken glass wares, walking barefoot on the floor etc.

4. Toxic effect of chemicals : Toxic chemicals when not handled properly eg. opening of containers containing inflammable liquids near a flame. Many chemicals give out fumes when opened and they are toxic in nature when inhaled. Mouth pipetting can lead to ingestion of these chemicals certain chemicals used in laboratory are radioactive in nature their improper storage, leakage from containers spillage during transportation and use and improper disposal can lead to exposure of staff to radiation.

5. Electric shock : Electric shock are generated when human body comes in contact with an unguarded source of electricity. Electric shock commonly occur due to faulting wiring in proper installations of equipments, loose connection improper wiring etc.

   We have to take precaution against these accidents which are as under :

   i) Always wear protective clothing like laboratory coat/gowns over normal clothing.

   ii) Wear closed shoes in laboratory.

   iii) Never wake bear foot in the laboratory.

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iv) Wear gloves while handling and processing specimen.

v) Treat all samples as being potentially infectious.

vi) Do not eat, drink or chewgum in the laboratory area.

vii) Prohibit smoking in and around laboratory area.

viii) Do not lick adhesive labels.

ix) Do not put pen or pencil in mouth while in laboratory.

x) Ban mouth pipetting in the laboratory.

xi) Never recap needle ever. Discard them in sharp shape containers and dispose them using incinerators.

xii) Culture samples and other specimens containing suspected infection should be processed in a safety cabinet.

xiii) Keep your nails cut short and hair tacked properly.

xiv) Keep a jar filled with disinfectant on each bench/table. Change disinfectant routinely.

xv) In case of any spillage, pour 1% hypochlorite on it and keep from 15 minutes before cleaning up.

xvi) Soak infected glass ware over night in 1% hypochlorite solution then clear it thoroughly under running tap water before reuse.

xvii) Disinfect or infected or contaminated material before disposal.

xviii) Always wash your hand with soap and water before leaving the lab. Even if your visit is really short.

Safety measures against chemical accidents :

Laboratory use many chemicals, which can be classified in two categories of flammable, corrosive, toxic, carcinogenic , radioactive etc. Therefore, it is mandatory for manufactures of such chemicals to label them with symbols that signify their hazards of reagents are prepared from these chemicals. In the

laboratory then they too should be labelled with hazard symbols. Some commonly used hazard symbols are as under.

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Apart from these the labelled should also include, the chemical name, strength of the solution, the date of preparation, expiry date, conditions of storage and any other relevant detail or warning.

Toxic Sign flammable chemicals

Health hazard Oxidizing chemicals

Radioactive hazard Corrosive hazard

Biomedical hazard Hazardous to environment

Explosive hazard

Following instructions should be adhered for various types of hazardous chemicals.

1. Flammable chemicals: These chemicals has the tendency to catch fir easily. These chemicals should store in fire proof metal containers in a cool and guarded place. As far as possible it is advisable to store them on ground floor and always outside the laboratory. Only small quantity should be stored inside the laboratory examples of flammable chemicals are - carbon tetra Chloride, ether, chloroform, acetone alcohol etc. The containers of flammable chemicals should ever be opened near a flame. Smoking should be strictly not allowed in the laboratory.

2. Corrosive Chemicals : Corrosive Chemicals either classified into strong acids (hydrochloric acid, nitric acid, sulphuric acid) or strong alkalis (NaOH, KOH etc.). These chemicals should be store near floor level on wooden planks. These chemicals should never be pipeted by the mouth. Mechanical or automatic pipetes should be used. While mixing acid and water always put acid into cold water slowly. Never add water to the acid as large quantity of heat will produced in case of spillage large quantity of water or sodium bicarbonate solution should

be used before cleaning the spillage. If corrosive chemical come in contact with the skin or mucous membrane wash it with running water immediately.

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3. Toxic Chemicals : Toxic chemicals like formaldehyde, ammonia, potassium cyanide, ninhydrin etc. can cause serious illness or even death when swallowed or inhaled . They can cause irritation when they come in contact with skin or mucous membrane. These toxic chemicals must be kept under lock and key. Always put on gloves and mask while using them.

4. Carcinogenic Chemicals: These chemicals cause cancer if they are inhaled or ingested. While using them take all precautions and their exposure should be minimum. After using these chemicals the hands should be properly washed. The examples are benzidine, o-toludine, B.napthloamine, nitrosoamine etc.

5. Explosive chemicals : Some chemicals are explosive in nature and when they are subjected to high pressure and temperature they may explode. Chemicals like picric acid which are kept under water may explode when they are not stored proper or water is dried up.

6. Radio Active Chemicals : These chemicals are stored in prohibited area and they must be properly labelled and warning signals should be displayed on the containers or in the area so that their explosure should be minimized and all safety measures should be taken.

7. Precautions against fire Accidents : All the fire fighting equipments must be installed in the laboratory as well as in the stores. Fire extinguishers should be installed and staff should be given training to use them and fire sighting drills should be conducted periodically.

8. Safety against electric shocks : All electrical wires and connections should be properly insulated, working bare foot should not be allowed. The earthing of all

Lab equipments and fits must be done properly. If any instrument is not working properly, its maintenance should be conducted immediately.

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GLASS EQUIPMENT, PLASTIC WARES, INSTRUMENTS

UNIT-IV

Laboratory glass wares refers to variety of equipments made of glass used for performing various types of procedures in a clinical laboratory. Glass ware used in laboratory are usually made of borosilicate glass. Laboratory glass wares can be classified as general purpose glassware and volumetric glass ware. Volumetric glass wares are used for measuring volume of a liquid accurately. It includes pipettes, burettes, volumetric flask and graduated cylinders. All other glass wares used in laboratory comes under general purpose glass wares.

Some of the glass wares are as under :

1. Flask : There are so many types of flask like conical flask, round and flat bottom flask, volumetric flask.
2. Beakers : Beakers are containers used for measuring, storing, sterring, heating or boiling liquids. These are manufactured in different sizes.
3. Bottles : Different types of bottles are used for various purposes. Specimen bottles are used for collecting specimen. Reagent bottles are used for storing and dropped bottles are used for dispensing of liquids in drops. They have special tops.
4. Funnels : Funnels are used for holding filter paper during the filtering of a liquid. They are also used for poring liquids into containers of narrow neck.

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5. Tubes : These are also of various size and shape. Test tubes are used for conducting different types of tests. The centrifuge tubes are used for centrifugation.

6. Pipettes : There are many types of pipettes generally used for measuring volume. They include Pasteur pipettes, volumetric pipettes, micro pipettes, RBC pipettes, WBC pipettes, graduated pipettes, serological pipettes.

   Graduated pipettes are further of two types :

   a) Mohr pipettes : They are calibrated between two marks on the stamp and calibrations starts only at the tip.

   b) Serological pipettes : These pipettes are graduated upto tip.

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Cleaning of glass wares: cleaning of glass wares is very important for the laboratory. This is necessary to obtain correct result of the test carried out in the lab.

Cleaning of new glass wares: Glass ware that has never been used is slightly alkaline. So the new glass ware should be immersed in 2% hydrochloric acid for 24 hours before using them. The glass ware should be washed with tap water and then with Distilled water and after that it should be kept on hot air oven at 60°C for two to three hours to make it dry.

Cleaning of dirty glass wares : The dirty glass wares should be kept in chromic acid solution over night and then next day it should be washed with running water and then with distill water.

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CLEANING AND MAINTENANCE OF COMMON EQUIPMENTS

UNIT-V

A medical laboratory technician should have sufficient knowledge about the functioning use and maintenance of various types of equipments.

a) Service manuals must be provided for each and every equipment for its proper maintenance and cleaning on regular basis.

b) Daily care of equipments should be emphasized.

c) Equipments should be maintained as per the manual provider, provided by the manufacturer.

d) The laboratory staff should know the Standard Operating Procedures (SOP's) of all the equipments and instruments.

e) They must strictly operator the instruments as the directions provided by the manufacturer.

f) The lab staff should operate the equipments like Auto clave, hot air oven, centrifuge machines, microscopes, laminar air flow as per the SOP's as directed by the manufacturer.

g) The maintenance of the equipments periodically as per AMC, in case of any problem the equipment should be get repaired by the supplier or by qualified technicians.

All safety measures should be taken while using these instruments and equipments.