# FREQUENTLY ASKED QUESTIONS ( FAQs)

# AUTOPSY AND HISTO TECHNIQUES



-BY DR. CHERRY SHAH

# AUTOPSY

## TEMPERATURE:

- Main cold storage temperature is just about $4^{o}c$
- Deep freeze unit temperature is $-20^{o}c$

## INSTRUMENTS:

- Weighing scale for weighing of body & organs.
- Measuring scale for measuring of length

## AUTOPSY TECHNIQUES:

**(1)VIRCHOW:** In this technique organ is removed one by one

**(2) ROKITANSKY**: In this technique enbloc removed with insitu dissection

**(3) A. GHON:** In this technique thoracic, cervical and abdominal organ removed separetely.Urogenital organ enbloc removed

**(4) M.LETTULE:** In this technique thoracic, cervical, abdominal and pelvic organ removed eumasse and dissected separately.



**DR. CHERRY SHAH**

## PEDIATRICS:

- Potter & Langley technique is used for pediatric autopsy
- Normal time to submit Autopsy report is 3 weeks
- Search of malformation (a) cleft palate
  (b) Choanal atresia
  (c) Stenosis
  (d) renal agenesis
- Chest cavity opened under wall for pneumothorax
- Enman-removal for anamalous pulmonary venous connection
- HPE of lung, liver, kidney, thymus, rib, brain, fetal membrance,placenta and umbilical cord.

## STEPS OF ADULT AUTOPSY:

- Body weight measurement
- Body length measurement
- Y shaped primary incision
- Removal of material from abdomen for microbiological studies
- Blood for various investigation from descending aorta

## POST OPERATIVE AUTOPSY:

- Incision should not be through wound
- Communication with surgeon should be made
- Chest x-rays to be seen
- Fistula should be stained with contrast media
- Drains should be not removed
- Smears, material for microbiological examination should be taken
- Pneumo thorax should be seen
- Photographs should be taken

## NEEDLE AUTOPSY:

- To obtain tissue samples when more invasive procedures are not possible
- Wide core needles should be used
- Special autopsy needles with projecting trocar-10-15 cm in length should be used
- With 2-3mm bore should be used

## ENDOSCOPIC AUTOPSIES:

- Used for Neoplasm
- Traumatic lesion with or without hemorrhages

## OBJECTIVES OF AUTOPSY:

## EXTERNAL EXAMINATION:

- (1)Body built
- (2)Height- from crown to heel by measure tap to be taken
  - (a) Gigantism- (1)pituitary adenoma
    (2) delayed epiphyseal closure
  - (b) Acromegaly- in pituitary lesion
  - (c) Arachnodactyly- Marfan's, look for Aorta & Heart (Dissecting aneurysm)
  - (d)Dwarfism- (1) hypothyroidism,
    (2)rickets
    (3) CHD.
- (3) Weight- should be done by weighing machine
- (4) Rigor mortis is due to breakdown of ATP to ADP. It causes muscle fibers to contract when ATP synthesises- rigor passes off
- (5) Livor mortis also known as hypostasis. It is seen in dependent parts due to gravitation. Begins after 35-40min. Cherry pink colour due to CO poisoning
- (6) Cyanosis: Two types:
- (a) central cyanosis :
  Site: tongue, conjunctiva, tip of nose, skin, nail, finger toe in cardiac and respiratory disease
  (b) Peripheral cyanosis:
  Site: skin, nail ,toes in cold and shock
- (7) Bone deformity can be congenital
  (a) Kyphosis- (1) postural problems
  (2) rickets
  (3) osteomalacia

(4) collapse of vertebral bodies

(b) Scoliosis- (1) poliomyelitis
(2) injury to vertebral bodies
(c) Lordosis- (1) congenital
(2)postural weakness

- (8) General condition of body
  (a) Nourishment – (1) malnourished in tuberculosis,
  (2)malignancy
  (3)diabetes
  (b) Obesity can show changes of atherosclerosis, only Cushing's in Endocrine diseases
  (c) Cachexia
  (d) Oedema – Two types
  (a) Pitting oedema -(1) nutritional
  (2) cardiac
  (3) kidney disease
  (b) Non pitting oedema -filariasis
  (c) general- (1) nephrotic
  (2) nutritional disease
  (d) Localized – (1) face- nephritic syndrome
  (2)feet – cardiac, thrombosis.
  (e) Ascities –liver and portal hypertension.

- (9) Pigmentation, trauma, birth marks and tattooing

(a)Pallor- (1) anemia
(2) bleeding disorder
(3) malignancy

(b) Hyper pigmentation –

Generalized: (1)addison's disease,
(2)haemochromatism
(3) malaria,
(4) thyrotoxicosis

localized : (1) pellagra
(2) neurofibromatosis
(3) chronic liver disease
(4)urticaria

(c) Hypopigmentation: (1) leprosy
(2) fungal
(3) albinism
(4) vitiligo
(5) leucoderma

(d) Icterus - skin, sclera & nail in jaundice

(e) Clubbing of fingers: (1) chronic disease
(2)malignancy
(3) cyanotic heart disease

(f)Skin- Dehydrated skin - GIT disturbances
-Tightening of skin - odema

- Strae –(1) pregnancy
(2) obesity
(3) abdominal tumor

- Petchie –(1) purpura,
  (2) thrombosis
  (3)septicemia
- Spider angiomata is 2to5 mm flat in size red blue complexes seen on face ,arms, neck and thorax in cirrhosis and exterior aspects of body and hands in hereditary telengictasia

➢ (10) Hair- loss of hair –(1) cirrhosis
(2)hormonal disturbances
(3) Chemotherapy

-Thick coarse hair - microcephaly

-Thinning & drying of scalp - myxedema

➢ (11) Lymph nodes of body are to be examined

## EXAMINATION FROM HEAD TO TOES:

1) Skull to be examined for a) trauma
   b) hematoma
   c) hydrocephalus

2) Face to be examined for a) cyanosis
   b) edema

3) Eyes to be seen for a) exophthalmos
   b) sclera for pallor

4) Cornea to be seen for a) cataract
   b) Kayser Fleischer ring – a) seniling
   b) Wilson disease

5) Nose, mouth ,tongue are to be observed

6) Ears to be seen for pus/fluid indicates CSF leakage

7) Neck to be seen for a) thyroid
   b) lymph node
   c) veins

8) Thorax to be seen if it is a) barrel shaped
   b) Pigeon shaped

9) Back for Livor mortis

10) Breast specially for gynecomastia in male

11) Abdomen, genitals and lymph nodes to be seen

**SYSTEM CORRELATION RELATED TO CAUSES:**

1) Urinary system is related to edema a) facial
b) Generalized

2) CVS system is related to a) edema –feet
b) Central cyanosis

3) RS system is related to a)tuberculosis- lymph node, cachexia
b) COPD- barrel chest, clubbing

4) Hepato billiary is seen for a)jaundice
b) edema
c) ascities

5) Lymph node for leukemia

6) Reticulo-endotheial system to seen

7) GIT, genital and CNS to seen

## CRANIAL CAVITY:

### SKULL VAULT OPEN:

- Transverse incision over scalp right to left mastoid process & reflection of scalp form posterior to anterior up to supra orbital process.

(1) Hemorrhage

- Extra dural hemorrhage is seen in head injury. Atleast 50 ml blood to be present clinically. Maximum 400-500ml.
- Sub dural hemorrhage is due to direct head injury
- Sub arachnoid hemorrhage- a) hypertension
  b) Berry aneurysm
  c) Leukemia
  d) Bleeding disorder
  e) Trauma

(2) Cerebral oedma is indicated by widening of gyri and narrowing of sulci

(3) Cerebral malaria shows slate gray appearance

(4) Yellowish green exudate: purulent meningitis

- Plastic membrane like exudate :tuberculosis
- Slimy appearance of brain: cryptococcal meningitis

(5) Cerebral infarct if present is soft and depressed.

(6) Localized swelling is seen in SOL

## THORACIC CAVITY :

(1) Pericardium: Normal Fluid: 5-50 ml
Maximum: 500-800ml to 1 liter
a) Pericarditis
b) Effusion
c) Brown atrophy
d) Cardiomegaly

- Dry pericardium is seen in dehydration like cholera

(2) Petechial hemorrhage: a) Leukemia
b) Bleeding disorders

## PULOMONARY EMBOLISM:

- Irregular, twisted ,dry brittle feeling on palpitation of pulmonary artery
- Y shaped incision on pulmonary trunk
- Insertion of finger in pulmonary trunk for embolism from deep veins of calf, thigh, pelvis.

## (2) PLEURAL CAVITY

- Pleural effusion
- Raised swollen ,localized pleural area- infraction of lung
- Metastatic nodules-(a)carcinoma
  (b) Sarcoma
- Mesothelioma of pleura –plaque -2-3cm thick
- Transudate: (a)Circulatory corset
  (b) Lymphatic obstruction
  (c) Hypoproteinemia
  (d) Obstruction to SVC

- Exudate: (a) Microbial infection
  (b) Rheumatic fever
  (c) Collagen disorder

**(3) LUNGS**

(a)Adhesion- Fibrocaseous tuberculosis

(b) Multiple foci over one or both lungs- Tb broncho pneumonia

(c) Millet seed like foci- Miliary Tuberculosis

(d) Small wrinkling lung- collapse

(e) Consolidation –pneumonia

(f) Metastasis-generally peripheral part of lung

**ABDOMINAL CAVITY:**

(a) Ascitis- when more than 100 to150 ml of transudate.
Normal -50ml ,clear

(b) Dry peritoneum-Dehydration

(c) Hemorrhage- anterior peritoneum :A) surgical
B)trauma
C) fracture
D)rupture of urinary bladder

- Retro peritoneum- Aorta rupture due to AS, aneurysm
- Sub peritoneal- due to break in internal illiac artery close to uterus

## FLUIDS IN ABDOMINAL CAVITY:

Ascites- yellow green –jaundice
-Blood tagged- tuberculosis, malignancy

## TUMORS OF PERITONEUM:

(a) Primary tumors of peritoneum- rare

(b) Secondary- mucin-gelatinous
-Malignant melanoma- pigment
-White nodule- tuberculosis,
Carcinoma
fat necrosis
lumphoid agenensis

## LIVER:

1) Hepatomegaly: search for causes

2) Atrophy: mainly seen in a) Necrosis
b) Old age
c) Cirrhosis (biliary)

3) Consistency: mainly varies in a) necrosis
b) Fatty changes
c) Amylodosis,
d)Cirrhosis

4) Colour: Deep blue: CPC
Green: bile from gall bladder(biliary cirrhosis)

5) Nutmeg- CPC

6) Obstruction of hepatic artery distal to its last branch- anemic infarct yellow in colour

7) Amyloidosis- Translucent, waxy with blurring of lobular pattern

8) Old age- dark brown atrophy

9) Dark brown colour – Iron deposition

10) Malaria – Slate grey colour

11) Abscess- Amoebic

12) Metastasis- multiple grayish white umblication, scalloped edges

**G.I.T**

1) Perforation
2) Tuberculosis
3) Gangrene
4) Malignancy
5) Ulcer

**SPLEEN:**

- Size of spleen whether enlarged or diminished is judged by splenic capsule
- WRINKLING : weight: 100-150
- Hemochromatosis-red brown
- Malaria-Slate grey
- Infract- pale
  Red( due to embolus from acute bacterial endocarditis)
- Amyloidosis : Sago
  Lardaceous

**KIDNEYS:**

1) Scarring
2) Abscess
3) Infarction
4)Tumor
5)Cyst

**PELVIC ORGAN:**

Frozen pelvis- cases of stage IV carcinoma cervix with massive infiltration & encasing of female pelvic organs is known as FROZEN PELVIS.

## POST MORTEM INVESTIGATION:

## BLOOD:

- Femoral vein or neck veins ,Right side of heart seared by hot spatula

## GLUCOSE:

- Hamiltan Patarsan Sohusan in 1940 for 1st time
  Low level- peripheral blood while due to glycolysis
  High level- right atrium due to glycogenolysis

- Hill (1941)- Non diabetic condition with terminal elevation of glucose up to 350gm/dl
  a) CO poisoning
  b) Increased intracranial pressure
  c) CA occlusion
  d) Cerebral hemorrhage

- In true diabetes glucose is more than 350mg/dl

## PROTEINS:

- Decreased in albumin
- Increased in globulins (beta, gamma)

**ENZYMES ELEVATED:**

- Acid phosphatase
- Alkaline phosphatase
- Amylase
- SGOT
- LDH

**CSF:**

- Spinal tap or cisternal tap collection by exposing midbrain or pons, also from corpus callosum
- Glucose increased in diabetics
- Increased level of a) Lactic acid
  b) Urea
  c) NPN
  d)$K^+$
- Decreased level of a) $Na^+$
  b)$Cl^-$

**FAECES:**

- From large intestine
- For protozoa-5% formalin preservation for cyst & eggs
- Polyvinyl alcohol for trophozoites

**FALSE POSITIVE DUE TO :**

- Clotting, hemolysis, bacterial contamination.
- Multiplication of bacteria after death
- If blood and CSF are not possible to be taken, VITREOUS HUMOR should be taken.
- IT IS IMPORTANT FOR ALCOHOL VALUE DETERMINATION if blood not obtained
- It is also important in embalmed body
- Early postmortem period – time between death and intra vascular hemolysis (2-3 hours)

**FROZEN SECTION :**

- For fat embolism in lung
- Iron- Prussian blue
- MI- TT2
- Amyloid- Lugol's iodine
  Sulfuric acid

## CVS AUTOPSY:

- External examination for a) cyanosis
  b) Oedema
  c) CHD
- Situs- Situs solitus- normal position of organ
  -Situs invertus- complete reversal of normal position
  Situs ambiguous- situs not clear, some normal other inverses
- Asplenia- absence of spleen
- Polyspenia- 7/8 splenicules

## HEART:

- 2/3 anterior surface by RV left to midline, 1/3 LV
- Atrium covered by lungs
- Levo cardia- Apex to left
- Meso cardia- Apex to midline
- Dextro cardia- Apex to right

## FIXATION OF HEART:

- Should be kept in formalin for 24 hours. Perfusion at height of 25 cms through cannula into cava should be done.
- Cut as flow of blood
- Thickness of myocardium is measured 1 cm before PV OR MV
- Valve circumference is measured by graded glass after opening – by ruler
- Weight should be 4to 5% of body weight

- Other techniques- Schlesinger and Rodriguez Reinen, Lum Haidy

- RA-IVC,SVC
- LA- PV
- SA node- Junction of SVC & RA
- AV node- triangle of Koch by opening of cor sinus ,tricuspid & fibrous body

- All valves involved –Rheumatic heart disease seen as Bread butter pan carditis

**ENLARGEMENTS:**

- RA/RV- Pulmonary hypertension
- LA- MS, MI
- LV- AS,AI

**VEGETATIONS(NON BACTERIAL ENDOCARDITIS)**

- Hypercoagulable states associated with collagen disorders (Lupus) No bacteria seen
- Antiphospholipid syndrome

**INFECTIVE ENDOCARDITIS :**

- More common fungus
- Left sided valves more involved
- Extra cardiac embolism may occur

**IHD:**

- Coronary artery examination should be done by
  1) Angiography
  2) Perfusion with 10% formalin
  3) Dissection
  4) Cross section areas of thrombi
     Grade-1- 25%
     Grade -2-50%
     Grade-3-75%
     Grade-4-100%

**MYOCARDIUM:**

- Look for location of infarct a) Lateral wall
  b) Posterior wall
- Extent of infarct a) Basal
  b) Middle
  c) Apical
- Distribution of infarct a) Subendocaridal less than ½ thickness
  b) Transmural more than ½ thickness
- Sections taken transversely at 1:0 to 1:5cm intervals from apex to 2 cms below AV sulcus

## AUTOPSY RESPIRATORY SYSTEM:

## COMMON CAUSES OF

(1) Pulmonary embolism- a) Cyanosis
b)H/O CVC dissection
c) Bed ridden patient
d) Post partum

(2) Pneumothorax- a) Tuberculosis
b) COPD

(3) Aspiration- a) Foreign body
b) Alcoholic

(4) Infections- a) Pneumonia
b) Septicemia

(5) Chronic Respiratory failure –a) COPD
b) Tuberculosis
c) Pneumoconiosis

## DISSECTION:

- First pulmonary artery, then lungs from hilum
- Slices from apex to base from periphery of hilum or from lateral to medial aspect

**FIXING:**

(1) Wet fixing

- Inflate lungs using syringe to infuse 10% formalin till lungs are inflated and then bronchus
- Before infusion confirm there are no tears or vents in pleura and lung
- Infused lungs should be kept in tub of formalin covered by cotton for 2-3 days

(2) Pressure fixation (vacuum fixation)

- Isolated lung is kept in air tight container of 10% formalin

(3) Slicing of lungs after fixation long bladed knife, Stored in plastic bags containing formalin

**TEST FOR PNEUMOTHORAX:**

- Pulmonary angiography

**HPE:**

- Section from all lobes should be taken
- Additional sections from pathological sites should be taken

## G.I.T AUTOPSY:

- Abdominal cavity- midline incision
- Pus,fluid or blood to be seen
- Microbiological exam should be done
- Stomach along greater curvature should be demarked
- Duodenum should be demarked

## GALL BLADDER:

- Gall bladder is squeezed gently so that bile is seen coming out in ampulla of vater in second part of duodenum

## LYMPHOMA IN G.I.T

- Plaque in mucosa
- Sub mucosal nodule

## CARCINOID IN G.I.T

- Localized thickened with yellowish discoloration
- Tip of appendix- common

## ULCER OF INTESTINE

- Tuberculosis
- Typhoid
- Amoebic
- Bacillary
- UC
- Crohn's disease

**STRICTURE IN INTESTINE :**

- Tuberculosis
- Crohn's disease
- Neoplasia
- Ischemia

**FIXING**

- Relevant strips of intestine flattened on filter paper with mucosa up and fixed in buffered formalin along filter paper
- Polyp fixed first initial cut through core

**HEPATOBILIARY AUTOPSY:**

- Manifestations of liver disease
  (1) Jaundice
  (2)Portal hypertension
  (3) Ascitis
  (4) Hepatic encephalopathy

- Hemolytic Jaundice- Pale liver due to increased RES activity, Enzymes to be tested.

- Portal hypertension- Portal venous congestion, collateral vessel formation
- Caput medusa radiate from umbilicus

- Splenomegaly

- Ascites- a)Cirrhosis
  b) Chronic Renal disease

**DISSECTION:**

- Liver and gall bladder removed together
- Technique 1- Enbloc removed
  2. Separate removal of liver and gall bladder
- Liver sliced 2 cm in thickened in frontal plane

- Gallbladder: Removed intact and then opened to prevent spilling
- Remove bile with syringe without opening if needed intact

**RES AUTOPSY:**

- Organs are mainly A) Thymus
  B) Lymph node
  C) Spleen- a) hyperplasia
  b) Septicemia
  c) Cardiac failure
  d) Hematological disease

**COLLECTION OF BLOOD:**

- Blood collected cannot be used for smear examination/ Hematocrit values
- Blood viscosity, Hematocrit value begin to rise rapidly
- Blood coagulation factors alter

## COLLECTION OF BM:

- Sections of sternum, vertebra,illac crest- Zenker's fixative

## SPLEEN:

- Capsule should be seen
- Thin parallel sections should be fixed without washing
- Imprint smears should be made
- In sickle cell diseases-immediate fixation is must

## CONGENITAL ANOMALIES :

- Asplenia
- Splenic rupture generally occurs in a) Trauma
  b) Malaria
  c) Typhoid
- Atrophy- is seen in sickle cell disease
- Infection should be observed
- Infarct- a) Myocardial infection
  b) Myeloproliferative disease
  c) Cone shape is seen in polycythemia
- Congested- CPVC
- Amyloidosis-a)Sago
  b) Lardaceous

- Pink diffuse spleen in Niemann Pick disease
- Cysts are seen in Echinococcal
- White nodules are seen in NHL/Leukemia
- Red purple marbling is seen in Hodgkins lymphoma
- Metastasis is mostly from Breast/ lung carcinoma, malignant melanoma

- Massive splenomegaly- a) CML
  b) Malaria
  c) ITP

**SPECIAL STAINS :**

- Hemochromatosis- Iron
- Amyloid- Congo red

**URINARY SYSTEM AUTOPSY:**

- Kidney- a) Agenesis
  b) Supernumerary
  c) Small
  d) Horse shoe
  e) Cystic

**COLLECTION OF URINE:**

- Culture- sterile needle

**DISSECTION :**

- Cut along convex border capsule stripped

1) Hypoplasia -number of calyces less than or equal to five
2) Atrophy- Granular surface, calyces >5
3) Hypertrophy- a) Congenital
   b) Nephritic syndrome
   c) Leukemia
   d) Diabetes

4) Coarse granularity- Chronic pyelonephritis
5) Fine granularity- a) CGN
b) Benign nephrosclerosis
6) Scars of old infarct- Cardiac
7) Flea bitten- a) Acute GN
b) Malignant HT
8) Blotchy hemorrhage- DIC, TTP
9) Cysts – true- Polycystic
Cystic dilatation- Hydronephrosis
10) Contracted kidney – a) Chronic PN
b) Hypertension

**CUT SURFACES:**

1) Cortico-medullary ratio- 1:3
- Increased ratio in Acute tubular necrosis
- Decreased ratio in CRN

2) Pallor- Acute tubular necrosis

**FEMALE GENITAL TRACT**

- Maternal mortality – death of a woman who is pregnant or within 42 days of termination of pregnancy
- Ovaries- Longitudinal sections taken
- Tubes- Cross sections at intema
- Uterus- Two short incision in fundus each towards cornium

## DIFFERENTIAL DIAGNOSIS OF WHITE OR RED PATCHES IN VULVAR REGION

- Dermatoses like lichen planus, psoriasis
- Squamous intra-epithelial lesion
- Chronic vulvar dystrophy
- Lichen sclerosis- Atypia is seen
- Keratosis- No atypia

# CNS

## SCALP:

## CSF:

- Cisternal puncture
- LP needle above second vertebra in midline
- Spinal canal anteriorly through inter peduncular fossa
- Laterally into corpus callosum

## FIXATION OF BRAIN :

- Suspended by thread
- Cut across corpus callosum
- 10% formalin- 10to14 days
- Bucket tub with 3/4 of formalin

**NEONATAL :**

(1) 10ml formalin through anterior fontanalle, wait for 45 to 60 min for fixation
(2) Beneke- cut along fontanalle and suture lines
(3) Vaides dapena and huff – two elliptical /D shaped incision parallel to sagital suture
(4) Hangley- Removal of brain by gravity by raising lower limbs of baby

**CUTTING :**

- Examine Circle of Willis
- Cerebrum separated from cerebellum.
- Brain sliced in coronal section with 1 cm thickness
- Brainstem perpendicular to neural axis
- Cerebellum parallel or perpendicular to long axis

**INFARCTION**:

- In fixed brain 12 hours for old infarct ,necrotic area is not fixed so velvety compared to normal fixed brain tissue

**GROSS EXAMINATION:**

- Weight- 1200 to 1400
- Symmetry
- Atrophy- a) aging
  b) Alzheimer disease
- Softening

- Focal abnormality
- Meninges
- In malaria brain is gray in colour

## AUTOPSY IN AIDS :

### UNUSUAL LESIONS FOUND:

- Tuberculosis of thyroid, prostate
- Cryptococcosis of skin/ various organs
- Lack of granuloma in tuberculosis
- Simultaneous infection, tuberculosis with CMV with Cryptococcus with Aspergillus
- Disseminated infections

## PROCEDURES

- Total body barrier protection with water repellant clothing-cap, face shield, goggles with mask to cover nose
- Water protective boots
- Double surgical gloves
- After autopsy, body washed with detergent, sodium hypo chlorite
- Tables, instruments, floor washed and decontaminated with Na hypochlorite
- Dispose disinfected material by incineration
- Training

## STAINS USE FOR HIV BIOPSY

- H&E
- GMS for fungus
- ZN for Tuberculosis
- Gram stain for bacteria
- Mucicarmine for Cryptococcus
- Frozen section fixed with formalin

❖ Cryostat decontaminated with 95% ethanol or formalin
❖ Culture with 1 to 6 days using laminar air flow
❖ Centrifugation in capped tube
❖ Spillage- 5% Hypochlorite to be used

## OPPERTUNISTIC INFECTIONS

- Virus- CMV,,HPV
- Fungal- Candida, Cryptococcus, Histoplasma,Coccidiomycosis
- Neoplasm- NHL, Kaposi's sarcoma, Encephalopathy, Nephropathy, cardiopathy



**DR. CHERRY SHAH**

## EMBALMING

- Method of preserving dead bodies by injection of chemical substances

## PRINCIPLE:

- Changes protein colloidal nature and form latices of long lasting from substances which cannot broken by enzymes or bacteria and destroy all bacteria

## PRESERVATIONS

- Formalin, Methyl alcohol, Phenol
- Germicides- a)Zephiran chloride
  b) Glutaraldehyde
- Buffers- Sodium Borate
- Wetly Agent- a) Glycerine
  b) Glycol
  c) Sorbitol
- Anticoagulant- Sodium citrate
- Dyes- a) Eosin
  b) Poncue acid fuschin
  c) Rhodamine
- Vehicle- a)Water with glycerin
  b) Alcohol

- Total 6L embalming fluid needed for one body
- Formalin-3parts
- Alcohol-2parts
- Glycerine - 1 part
- Done by Cadaverous injector
- Method- Arterial embalming
  Cavity embalming
  Hypodyne embalming
  Surfaces embalming
- Body transported in Aluminum lined coffin

# HISTO TECHNIQUE

## HAZARDS

- Formaldehyde- sensitizers
- Carcinogen- Formaldehyde, chloroform
  Dyes- auramine, basic fuchsin
  Congo red
- Toxic- Methanol- death on ingestion
  Xylene ,toluene- Neurotoxins
- Acetone- Narcotic
- Dyes- Containing benzidine cause cancer
- Ethanol- Flammable
- Formaldehyde- Respiratory system carcinogen
- Methanol- Blindness, death, irritable, flammable
- Xylene- Skin, eye irritant , neurotoxic, flammable

## PROTECTIVE EQUIPMENT

- Acrylic fibers dissolve with xylene
- Goggles
- Apron
- Gloves- Latex thick 8 mi (normal -1-1.5mi) break through after 12 minutes by formaldehyde
- Nitrite gloves- Best option in histology

## QUALITY CONTROL IN HISTOLOGY

- Internal : Experienced members
  IHC endothelium
- External : Equa system: Q track – Superficial
  Q probe- deep
  Fixation Score given: Adequate
  Pigmentation
- Decalcification: (a) Under
  (b) Over
- Processing – Adequacy
- Embedding- Grouping
- Microtomy
- Heamotoxylin - (a)low power
  (b)Color differentiation
- Eosin- (a)Selectivity
  (b) Uneven staining
- Artefacts- (a)Bubbling
  (b) Melt down
  (c) Residual wax
- Finishing – (a) Dehydration
  (b) Air bubble removal

## LIGHT MICROSCOPE

- Resolution- Smallest distance between two dots or lines that can be seen as separate entities
- Magnification – Product of magnification, values of objective & eyepiece.

## FIXATION

- **Aims**- (1) Prevent autolysis
  (2) Bacterial attack
  (3) Do not change shape, volume
  (4) Allows cleaning for Staining
  (5) Remain close to living state except Lipids
  (6) Proteins well preserved
- **Classification : (**a) Aldehydes- a) Formaldehyde
  b) Glutaraldehyde
  (b) Oxidising agent- a) KMNO4
  b) Potassium dichromate
  (c) Protein denaturing agent- a) Acetic acid
  b) Methyl/Ethyl alcohol
  (d) Physical- a) Heat
  b) Microwave
  (e) Miscellaneous- a) Mercuric chloride
  b) Picric acid
  c) Dye stuff

- **<u>PROTEINS</u>**:

- Form cross links between each others
- With formaldehyde – reversible with excess water
- With glutaraldehyde – irreversible

- Microwave- temp 45-55$^{o}$
  Under heating- poor sectioning
  Over heating- Vacuolation, over stained cytoplasm

- Ethanol/Methanol- act on nucleic acid
  Example: Carnoy's fixative
  Bouin's fluid
- **<u>LIPIDS:</u>**

  - Cryostat/ frozen sections needed
  - On fixation- they are removed
  - Formal calcium better for lipids
  - Formaldehyde reacts with unsaturated fatty acid, so not demonstrated

- **<u>CARBOHYDRATES:</u>**

  - Alcoholic fixative for glycogen

## ➢ MAIN FACTORS INVOLVED IN FIXATION

- PH
- Temperature
- Penetration
- Osmolality
- Concentration
- Duration- (6 hours)- Prolonged causes shrinkage

## ➢ FIXATION ARTEFACTS:

- Nuclei appear bigger in frozen section
- Tissue shrink by 33%
- Formalin pigment is deposited
- Crust effect associated with intense eosinophilia at centre of tissue in H&E stain.
  Example- in liver biopsy
- Protein coagulation ,incomplete wax impregnation

- **FORMALDEHYDE(4%)/30-40%FORMALIN**

For routine tissue- 10% formalin

For brain- 15% formalin

Buffer- Phosphate

**Brain**:

5-6 days, Perfusion

**Renal**:

3 parts

**G.I.T**:

Small biopsies in buffered formaldehyde

Large biopsies- submucosa down in buffer formaldehyde

Larger specimen- opened along greater curvature or anti mesenteric border and floated in buffered formaldehyde.

**Liver**:

Wedge biopsy- buffered formaldehyde

Core biopsy (2x10cm) - buffered in formaldehyde

**Lungs**:

inflation with buffered 4% formaldehyde through cannula

**Lymph node:**

lymphoma –smear air dried given before b

**Testis:**

Bouin's fluid - clear nuclear detachment

**Uterus, cervix**:

Uterus- Opened by lateral incision leaving two halves in contact at fundus

Cone biopsy at cervix with suture at 12'O clock - opened with endocervix upper most

- **EYE**: Phenol added to alcohol to soften tissue
  Sclera is less affected
  Chloroform as clearing agent less harsher than xylene.

## PROCESSING

- Frozen : (A) Neuropathology
  (B)Lipid demonstration
  (C) Urgent frozen section
- Paraffin wax not miscible with water(most satisfactory embedding material)
- If inadequately fixed, completed by dehydrating alcohols alter staining characteristics.
- To help identify small pieces of tissue few drops of 1% eosin is added 30 minutes before transferring
- Picric acid form water soluble picrates placed in 70% ethanol

Factors influencing rate of processing
(1)Agitation
(2)Heat-45˚
(3)Viscosity- cedar wood oil altered viscosity
(4)vaccum

## DEHYDRATION:

- Remove fixative and water from tissue and replace them with dehydrating fluid.
  Ethanol: (a)70%
  (b)95%
  (c)100%
  For delicate tissue 30% ethanol miscible with water
  others -Methanol
  Acetone
- For hard substance like nail, keratin glycol alcohol mixture or phenol can be added to dehydrating agent to soften.
- Anhydrous $CUSO_4$ dehydrating and indicator
  layer 1-2 cm of anhydrous $CUSO_4$ in final dehydrating bath, cover with filter paper, If water is present $CUSO_4$ will form blue colour and ethanol is to be changed.

## CLEARING

- Replacing dehydrating fluid with a fluid which is miscible with both dehydrating and embedding medium.
- Most are flammable
- Low boiling point ,are readily replaced except chloroform
  Prolonged exposure- tissue becomes brittle
  Example :(a) Xylene
  (b)Toluene

  (c)Chloroform(noninflammable):(i)Large tissue block
  (ii)CNS
  (d)Paraffin

Citrus fruit oil:

Orange and lemon rinds

Disadvantage: sour odor

Cu and $Ca^{++}$dissolve out of tissue

**EMBEDDING**:

- Replacing clearing agent by embedding agent
- Paraffin wax- cheap, easily handled embedding agent
  wide range of melting point(40˚to 70˚)
  for good ribbons- 54-58˚C temperature is used
- Alternative embedding media:
  Resins, polyester wax, gelatin, cellodin, agar.

**ORIENTATION OF TISSUE**

- Tubular structures: cross section is taken.
- Skin/Epithelial biopsy: plane at right angle to surface
- Muscle: transverse/longitudinal section
  Nicking opposite aspect for correct orientation or marking with Indian ink or dye like Alcian blue

## PROCESSING MACHINES

- There are two types of machines
  (1)Carousel type
  (2)Enclosed type
  If any fault, machine stops and sounds an alarm.
- Processing takes 16-18 hours. Minimum time at each stage is 15-30 min.
- There are 12 seperate stages.
- Recent- microwave technology.

- **Maintainence of machine:**
  (1) Any odor of clearing agent in final paraffin wax, indicates that wax should be changed.
  (2)Discard first 100% ethanol bath.
  (3)Regularly change clearing agent and ethanol once per week.
  (4)Fluid and wax to be filled at appropriate level
  (5)Thermostats set 3˚above melting point of wax.

- Advantage of enclosed pump:
  (1) Vaccum and heat at any stage is rapid.
  (2) Less fluid spillage.
  (3) Less toxic fumes

- Size of slides universally used - 76×25mm.
  1.0 to 1.2 mm thickness, as do not break easily.
  Slides marked with diamond marker at frost end side.

- Floating of section occurs in:
  - Strong alkali solution during staining
  - In cryostat

- In High temperature
- Following tissue generally floats:
  (a)CNS tissue
  (b)Blood clots
  (c)Decalcified tissue

➢ Proteins like albumin, gelatin, starch are not recommended as adhesive ,as they stain heavily with dye.

➢ Two adhesives are there:
- Poly-L-lysm (0.1%)
- Aminopropyltriethoxy silane(APES)

**CUTTING**:

- Type of microtomes used:
  (1) Clearance angle-3˚ to 4˚
  (2) Angle of slope- 40˚
- Floating out bath- distilled water to be changed frequently by adding 2-3 crystals of thymol.
- Air bubbles on slide to be removed by tapping.
- For trimming adjust 15 mm thickness
- Before cutting, block to be placed on melting ice
  (1) It gives same consistency to tissue and wax
  (2)water absorbed in tissue allow it to swell out well.

➢ Routine surgical size in 3-4 µm.

➢ If difficulty in cutting flat section, gently exhale breath on block surface as it is cuts expanding block and giving thicker section.

**FLOATING OUT :**

- Trailing end of ribbon coming in contact first.
- In 30 sec, flatten section on water will expand to distort tissue.

**DRYING SECTION:**

- By applying heat as melting point of wax
- Can be kept in oven for 5 minutes.
- Over heating, distort tissue and melt collagen.

**CUTTING HAND TISSUE**:

- Prolonged melting ice treatment of block.
- Exposing block surface to running tap water for 30 minutes.
- A slight reduction in knife slant.

**SURFACE DECALCIFICATION**:

- While trimming, small foci of $Ca^{++}$ revealed.
- This is removed by placing face down on pad of cotton wool saturated with 10% hydrochloric acid for 1 hour.

**FROZEN SECTION**:

- Methods:
  (1) Liquid nitrogen (-190˚C)
  - Most rapid method
  - Tissue crakes in this method
  - Damage to blade and tissue occurs
  - Freezing artefact occurs due to vapor which can lead to problem in diagnosis.

  (2) Isopentane cooled by liquid nitrogen(-150˚C)
  (3) $CO_2$ gas (-70˚c)
  (4) Aerosol Spray(-50 C)

- do not freeze muscle
- Stored easily

(5)Sectioning between -15˚ to -30 C˚

(6) Antiroll plate

- to stop natural tendency of frozen to curl

- IHC:
  - Freeze dry up

**STAINING:**

- Factors contributing to staining are:
  (1) Hydrophobic bonding
  (2) Stain to stain interaction
  (3) Vanderwaal's forces
  (4) Hydrogen bonding
  (5) Covalent bonding

- Natural dyes:
  (1) Hematoxylin
  (2) Carmine

- Synthetic:
  (1) Aniline

- **HEMATOXYLIN**:
  - Extracted from logwood of tree- Haematoxylum Campechianum. ( Mexico origin).
    With hot water, it precipitates out using urea.
  - Hematoxylin, oxidized to form haematein
    (1)Natural oxidation or ripening:
    By exposure to air for 3 to 4 months.
    Example: Ehrlich's hematoxylin
    Delafield hematoxylin
    (2)Chemical oxidation using sodium iodate

Example: Mayer's hematoxylin
Chemical oxidation using mercuric oxide
Example: Harris's hematoxylin

- Examples of hematoxylin according to mordant:
  (1)Alum hematoxylin
  (2)Iron hematoxylin
  (3)Tungstan hematoxylin

➢ STAINS FOR COLLAGEN:
- Van Gieson
- Masson trichrome.

➢ STAINS FOR MUSCLE:
- Van Gieson
- Masson trichrome
- Phosphotungsticacid hematoxylin(PTAH)

➢ STAINS FOR CARTILAGE:
- Van Gieson
- Masson trichrome
- Phosphotungsticacid hematoxylin(PTAH)

➢ STAINS FOR ELASTIN AND RETICULIN FIBERS:
- Van Gieson
- Masson trichrome
- Phosphotungsticacid hematoxylin(PTAH)

❖ Glycogen is present in:

- Carcinoma Bladder
- Carcinoma kidney
- Carcinoma liver
- Carcinoma ovary(Adenocarcinoma)
- Carcinoma pancreas
- Carcinoma lung
- Ewing's sarcoma
- Juvenile Rhabdomyosarcoma
- Stains used are: (1)PAS

  Cytoplasm stains magenta

  Nucleus stains blue

  (2) Best's Carmine

❖ Mucin:

- ➢ Neutral Mucin is present in:
  - Stomach
  - Prostate
  - Brunner's gland
- ➢ Sialo Mucin labile is present in:
  - Salivary gland
- ➢ Sulfated mucin is present in:
  - Goblet cell layer of intestine
  - Prostate

❖ Use of saliva for demonstation of glycogen in histopathology, which is digested by amylase for staining of glycogen by H & E.

- ➢ PAS WITH DIASTASE (PAS-D):

- Use of the PAS in combination with diastase, which is an enzyme that digests the glycogen.
- The purpose of using the PAS-D procedure is to differentiate glycogen from other PAS positive elements in tissue samples.
  Example: to distinguish between mucin and glycogen
  Mucin – diastase resistant
  Glycogen- diastase labile

➢ PAS-DIASTASE STAIN FOR MUCIN
- Positive stain for:
  (1) Neutral mucin
  (2) Sialomucin
- Negative stain for:
  (1) Sulphated mucin
  (2) Connective tissue mucin

➢ PAS-DIASTASE FOR PRESENCE OR ABSENCE OF URONIC ACID
- Positive stain for:
  (1) Hyaluronic chondroitin sulfate
- Negative stain for:
  (1) Keratane sulfate

❖ Mucoproteins in basement membrane:
(1) Of thyroid, colloid
(2) Cerebroside, Gangliosides of nervous tissue

❖ PRUSSIAN BLUE:
- Stains mucin at low PH ,colloidal iron is absorbed and visualized by conversion to ferric ferrocyanide.

**LIPIDS**:

- For lipid frozen section is important
- It is not fixed by formaldehyde
- Only reagents really fixing lipids are:
  (1) Osmium tetroxide
  (2) Chromic Acid
  But they alter chemical reactions.
- Formol calcium made by adding 2% calcium acetate to 10% formalite is used as Ca acetate form lattix of phospholipids with proteins and preserve structure.
- Positive controls are prepared from known cases of lipid storage diseases.
  Examples: Fatty liver
  Brain
  Adrenal
  Atheromatous artery
- Negative controls are prepared by depolarization by:
  Chlorofom: Methanol (2:1)
  Distilled water
- Lipids are stained by:
  (1) Sudan dye: (i) Sudan lll
  (ii) Sudan IV
  (2) Sudan Black B:
  Basic phospholipids stains – Gray
  Neutral fats stains – Blue black
  (3) Oil red O
  Solvent is 70% ethanol
- Bromine helps in staining with Sudan black by converting crystalline cholesterol only at room temperature and is permeable to dye.

**PIGMENTS**:

- Endogenous:
  (1) Hemosiderin
  (2) Hemoglobin
  (3) Bile
  (4) Porphyrin
- Exogenous:
  (1) Minerals
- Artefact pigments:
  (1)Formalin
  (2)Malaria



➢ **<u>HEMOSIDERIN</u>**:

- Stain for hemosiderin in histopathology is Perl's Prussian blue.

  Principle:

  Hemosiderin contains iron in form of ferric hydroxide.

  The reaction occurs with the treatment of tissue section with acid ferrocyanide solution. Any ferric ion in the tissue combines with ferrocyanide and results in the formation of a bright blue pigment called "Prussian blue" or "ferric ferrocyanide"

➢ **<u>HEMOGLOBIN</u>**:

- Demonstration of enzyme – Hemoglobin peroxidase, by:
  (1) Benzidine nitroprusside (Benzidine is carcinogen)
  (2) Patent blue method
  (3) Amidoblack technique

(4) Red Almond green technique

- **BILE PIGMENT**:
  - Microscopically blue colour – Mixture of biliverdin and conjugated and unconjugated bilirubin.
  - Bile seen in hepatocytes :
    (1) In early stage yellow brown globules
    (2) It must be distinguished from lipofuscin, by Fouchet Technique, in this technique bile stains green in colour.
    (3) Bile also seen in gallbladder mucosa
  - Other method for demonstration is, Gmelin

- **HAEMOTOIDIN**:
  - Pigment related to bilirubin and biliverdin
  - This pigment is bright yellow in color
  - This pigment is seen in (1) Splenic infarcts
    (2) Haemorrhagic area in brain

- **MELANIN** :
  - Common site for melanin:
    (1) SKIN- Basal layer of epithelium
    It is produced by melanocytes
    Phagocytic cells are known as Melanophages
    (2) EYE
    (3) BRAIN
  - Method for demonstration :
    (1) Masson fontana silver
    (2) DOPA enzymes
    (3) Fluorescence

- **<u>LIPOFUSCIN</u>** :
  - Produced by oxidation of lipid and lipoprotein
  - Lipofuscin is found in
    (1) Hepatocytes
    (2) Cardiac muscle – seen in Brown atrophy
    (3) Normal adrenal cortex
    (4) Testis
    (5) Ovary
  - Method for demonstration :
    (1) PAS
    (2) Ferric ferricyanide (Schmorl's method I) reduction test
    (3) Long ZN method
    (4) Sudan black B

- **<u>CALCIUM</u>** :
  - Calcium is seen in form of carbonate and phosphate
  - Calcium is seen in :
    (1) TB
    (2) Infarction (Gamma Gandy bodies)
    (3) Atheroma
    (4) Malakoplakia of bladder (Michaelis Gutman bodies)
  - Stains purple with H&E stain
  - Special stain :
    (1) Alizarin red S
    (2) Purpurin
    (3) Naphtholchrome green

  - Classic method :

(1) Von Kossa

Tissue section are treated with silver nitrate solution, the calcium is reduced by the strong light and replaced with silver deposits, visualized as metallic silver/ black.

**ARTEFACT PIGMENT**:

- Formalin
- Malaria
- Mercury
- Starch
- Schistosome

- **FORMALIN :**
  - It is brown black in color
  - It is used for long fixation
  - Formalin is present in:
    (1) Spleen
    (2) Hemorrhagic lesion
    (3) Large blood vessel
  - It is removed by treating unstained tissue section with saturated alcoholic picric acid or alcoholic solutions of sodium and potassium hydroxide
  - 10% Ammonium hydroxide with 70% alcohol

- **MALARIA** :
  - It is seen in :
    (1) RBCS
    (2) Brain in cerebral malaria
    (3) Kupffer cells of liver
    (4) Sinus lining of lymphnodes and spleen
    (5) Phagocytic cells of bone marrow
  - Malaria pigment is removed by saturated alcoholic picric acid for 12-24 hours.

- **STARCH**:
  - Starch is introduced from talcum powder from gloves of surgeons, nurses and pathologist.

**EXOGENOUS PIGMENT**:

- Tattoo
- Carbon:
  - Differential diagnosis with melanin
  - Treatment with bleaching agent will remove melanin, thus it is differentiated from melanin.
- Silica
- Asbestos
- Lead
- Silver

## BONE

- Bone is made up of :

(1) Bone cells
- Osteoblasts
- Osteocytes
- Osteoclasts

(2) Bone Mineral

(3) Bone collagen

- There are two types of bone:

(1) Cortical or compact bone
- Cortical bone is very dense and strong.
  Example:
  Shaft of long bone.

(2) Cancellous bone
- Cancellous is spongy or trabecular bone
  Example:
  At end of long bone, like epiphysis and metaphysis.

- Fixation of bone by 10% neutral buffered formalin
- Radiography – 3-5 mm thickness of bone pieces ideal
- **Decalcification**:
  - Removing Ca from matrix.
  - Ca removed by two methods:
    (1) Acids
    - Strong acid
    - Weak acid

    (2) Chelating agents.
  - Strong acids like HCL/Nitric acid (5-10%) decalcify bone within 24-48 hours.
  - Weak acid like formic acid, acetic acid, picric acid are for small biopsies done with Jamshidi needle.
  - Buffers used are salt like sodium formate and sodium citrate added to formic acid to counteract injurious effects of acid.

- Chelating agents
  (1) EDTA:
    - Action of EDTA requires specific PH. It acts below PH 3.
    - It takes 6 to 8 weeks.
    - Small biopsy are decalcified within one week.
- Factors affecting decalcification:
    - Concentration of decalcifying agent
    - Temperature ( ideal temperature is 18-30°c)
    - Agitation
    - Suspension

      Suspension should make contact with all surfaces of specimen.
- Completion of decalcification:
    - End point testing to see removal
      With HCL/ Nitric acid daily and near end point every 5 hourly.
      With EDTA weekly
    - Method of end point :
      (1) Radiography
      (2) Weight loss
      (3)Bubble test-

      Acid reacts with $CaCo_3$ in bone to produce $Co_2$,
      Which is seen as bubbles in bone surface.
      Tiny bubble suggest less Ca.
      (4) Ca oxalate for EDTA

      Acidifying used solution to release Ca for precipitate by ammonium oxalate.

- Neutralization after decalcification:
    - By immensing in to saturated lithium carbonate or 10% aqueous sodium bicarbonate.

- Simply rinsing with running tap water for 30 min to 1-4 hours.

- Surface decalcification:
  - When partially decalcified bone is found in paraffin section, paraffin block is placed in 1% HCL or 10 % Formic acid with that side facing down for 15-60 minute then block is rinsed with water and resectioned.

- Cutting of bone done with heavy C- profile knife.

## AMYLOID

- Amyloid is PAS positive
- It stains green or blue with trichrome stain
- It stains khakhi with Van Giesons stain, which is not used for differentiation.
- Purple staining of amyloid with iodine solution changing to blue on addition of weak sulfuric acid stain.
- Paraffin sections by immersing them for 10 seconds in Lugol's iodine containing 0.1 M HCL followed by rinsing in water and mounting in iodine glycol – Amyloid appears delicate pink purple or blue.
- Congo red, Sirius red, Crystal violet, Thioflavin are other stains for amyloid.