Tissue Processing

I. Registration

•Lab.no, Date, Name of patient, age, Sex, Occupation, address, reffereing doctor

II. Fixation: preservation of tissue structure

A. Avoid autolysis
B. Common fixatives:
1. formaldehyde,buffered formal-saline 10%
2. glutaraldehyde: for EM
3. 90-100% alcohol: suitable for cytology
4. heat: boiling water, microwave

The purposes of fixation are

A. to inhibit autolytic enzymes and kill microorganisms of decomposition .

B. to preserve tissue as nearly as possible in its original form.

C. to protect tissues against subsequent damage during embding.

D. to give tissue a texture which permits easy sectioning .

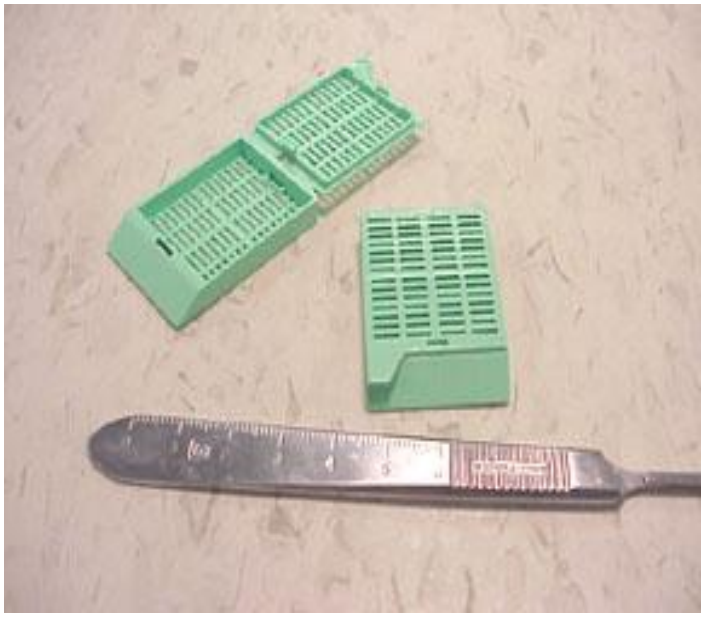
E. to render the various constituents receptive of the proposed stains

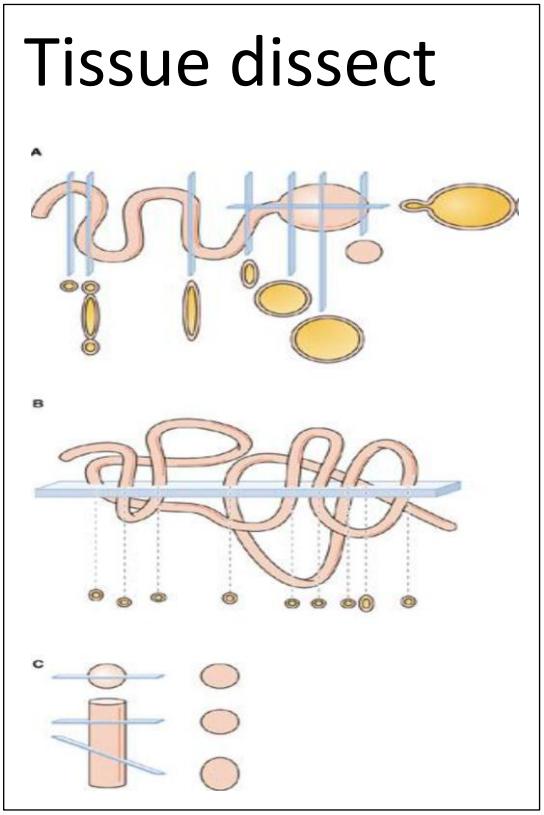
Fixation of tissues with different types of fixatives depend on tissues, large specimen should be sliced . loose tissues max. 10 mm penetration , compact tissue max. penetration 5mm, hollow organs injected or packed with wool socked in formalin .

Gross examination

•Includes description of the specimen : weight , dimensions , color , texture , cutsection , followed by photography

Tissue dissection and taking representative sections followed by labeling . The pieces taken will placed in tissue cassette . Transfer casssette either to automatic tissue processoe or to jars (in case of manual tissue processing)



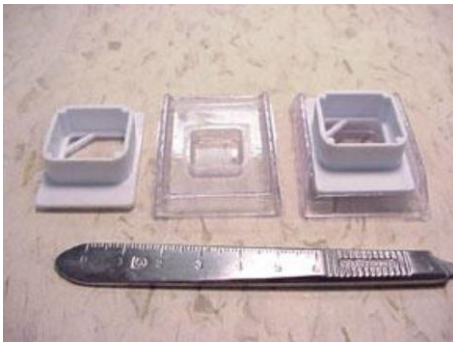


III. Dehydration

A. Definition: removal of water
B. Rationale: for paraffin embedding/sectioning
C. Steps
1. wash out fixative
2. graded series of alcohol
a. 70%, 95%, 100%, 100%
3. replace water by diffusion
4. not too long, not too short
D. Procedure
1. automatic tissue processor
a. overnight
2. Baths: water, 70,95,100,100 % alcohol
3. Clearing agent: 2 baths of xylene

IV. Clearing

A. Paraffin solvent
B. Xylene, “clearing agent”
C. Makes tissue appear “clear”

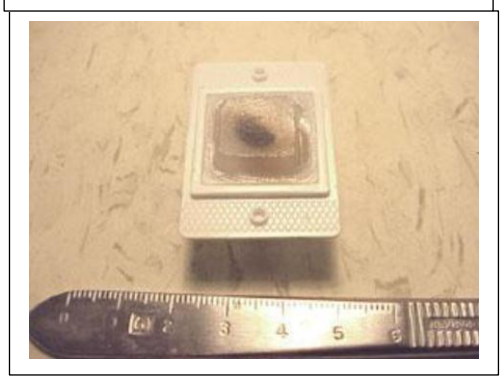


V. Infiltration

A. Replace xylene with paraffin
B. Immerse in melted paraffin
1. ~55oC MP
C. Remove all bubbles, xylene
D. Procedure
1. Two baths of melted paraffin

VI. Embedding

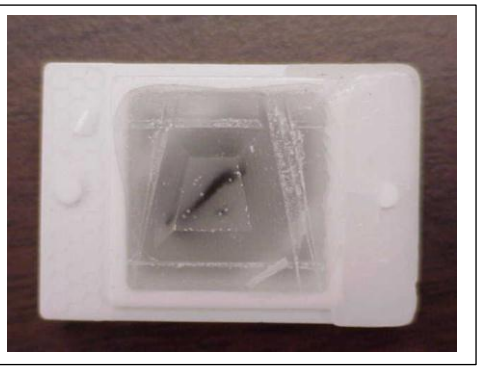
A. Orient tissue
1. cross section
2. longitudinal section
B. Dissection orientation
C. Avoid bubbles
D. Procedure
1. Place tissue cassette in melted paraffin
2. Fill mold with paraffin
3. Place tissue in mold
4. Allow to cool



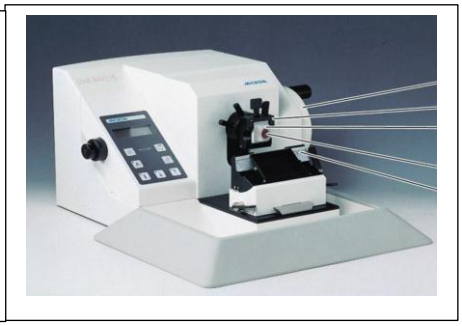
VII. Sectioning –Trimming the Block

Untrimmed tissue block

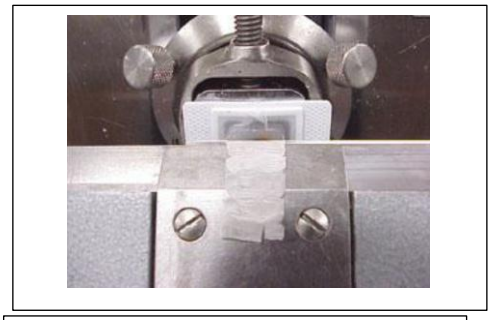
Trimmed block with excess paraffin removed and block face in a trapezoid shape



VII. Sectioning

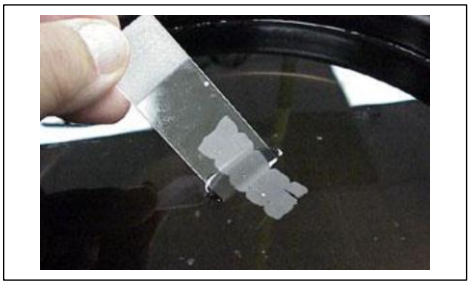


A. Rotary microtome
1. 5-10 mm
2. resolution vs. staining
B. Cryostat
C. Freezing microtome
D. Vibratome
E. Procedure
1. Place tissue block in microtome with wide edge of trapezoid lowest, and parallel to knife
2. Advance blade toward block



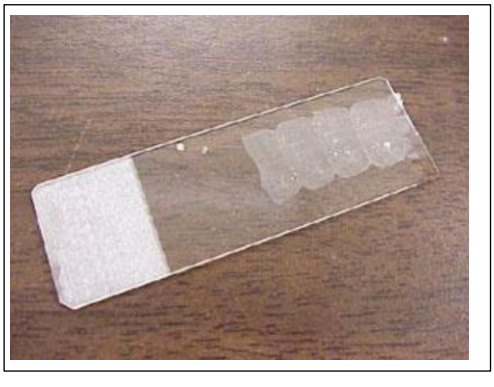
VIII. Mounting sections

A. 40oC water bath
1. Flattens paraffin section
2. Permits mounting on slide
B. Gelatin & albumin
C. Glass slides
D. Oven / air dry



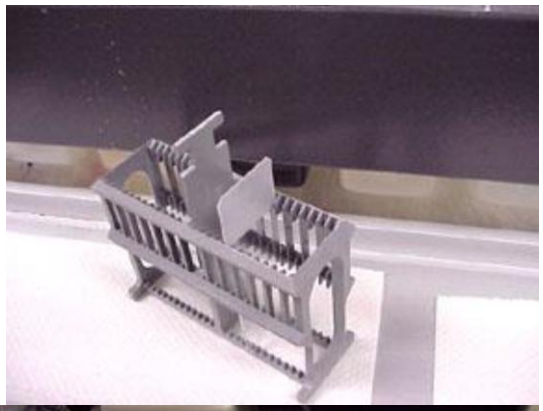
IX. Staining

A. Basic dye: hematoxylin
1. basophilic structures: DNA, RNA
2. differentiation: sodium bicarbonate
B. Acid dye: eosin
1. acidophilic (eosinophilic) structures
a. mitochondria, collagen
C. Water soluble dyes (paraffin sections)
D. Clearing agent (remove paraffin)
E. Rehydrate
F. Stain (trial & error timing)



Most of the stains are water soluble and don't mix with paraffin , so staining should started with de-waxing by using solvents (Xylene). Then rehydration of tissue using descending concentrations of alcohol (100%, 90%,70%, water)

G. Procedure
1. Slide rack
2. Solutions
a. rehydration
b. stain
c. dehydration

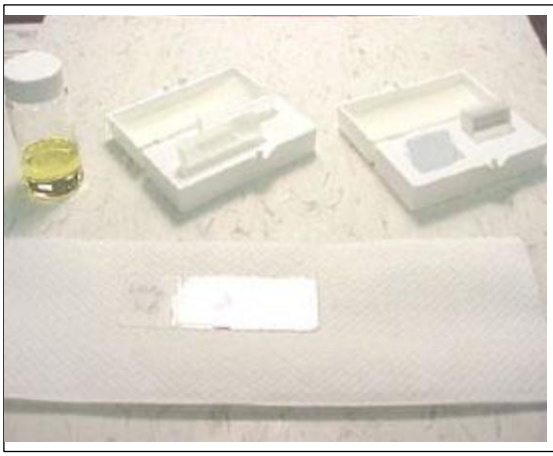


X. Coverslipping

A. Coverslip & mounting medium (not miscible with water)
B. Dehydrate
C. Clearing agent
D. Permount

XI. Pitfalls

A. Poor fixation (poor structural details)
B. Inadequate dehydration
C. Contaminated xylene (milky)
D. Poor infiltration (bubbles, poor support)
E. Embedding: orientation, bubbles
F. Poor sectioning
1. knife marks (scratches perpendicular to knife edge)
2. compression (waves parallel to knife edge)



G. Mounting sections
1. folds & tears
2. excess albumin (stain)
H. Staining
1. inadequate rehydration (uneven staining)
2. too dark or too light (timing off)
3. inadequate agitation
I. Coverslipping
1. Bubbles
I. Coverslipping
2. excess Permount
3. two coverslip