HISTOCHEMISTRY

Dept.of Oral Pathology
& Microbiology

Purpose Statement

At the end of the lecture student should be able to describe the

- Processing Technique
- Procedure For Ground Sections
- Advantages and procedure of frozen sections
- Maintenance of laboratories
- Advantages and procedure of microwave fixation

Learning Objectives

- At the end of the lecture the student should be able to

S.N.	Learning Objectives	Domain	Level	Criteria	Condition
1	Enumerate Processing Technique	Cognitive	Desirable to Know	All	
2	Enumerate steps in procedure For Ground Sections	Cognitive & Psychomotor	Desirable to Know	All	
3	Enumerate Advantages and procedure of frozen sections	Cognitive	Nice to Know	All	
4	Give maintenance of laboratories	Cognitive	Nice to Know	All	
5	Give advantages and procedure of microwave fixation	Cognitive	Nice to Know	All	

PROCESSING TECHNIQUE

WAX EMBEDDING

- Formalin fixation. - 24 Hr
- 70% alcohol - 30 mins
- 80% alcohol - 30 mins
- 95% alcohol - 30 mins
- 95% alcohol - 1 hr
- 100% alcohol - 1 hr
- 100% alcohol - 1 hr
- 100% alcohol - 1 hr
- Xylene - 1 hr
- Xylene - 1 hr
- Wax bath - 2 hr
- Wax bath - 2 hr
- Embedding

WAX EMBEDDING (RAPID TECHNIQUE)

- Carnoy's fluid - 45 mins
- 100% alcohol x6 - 15 mins each
- Xylene - 10 mins
- Xylene - 15 mins
- Wax - 20 mins
- Wax - 45 mins

ACRYLIC EMBEDDING

- Fixation
- Rinse in buffer.
- Dehydration with increasing conc of alcohol, for 15-30 mins each.
- Impregnating solution for 1 hour
- Embedding in medium.

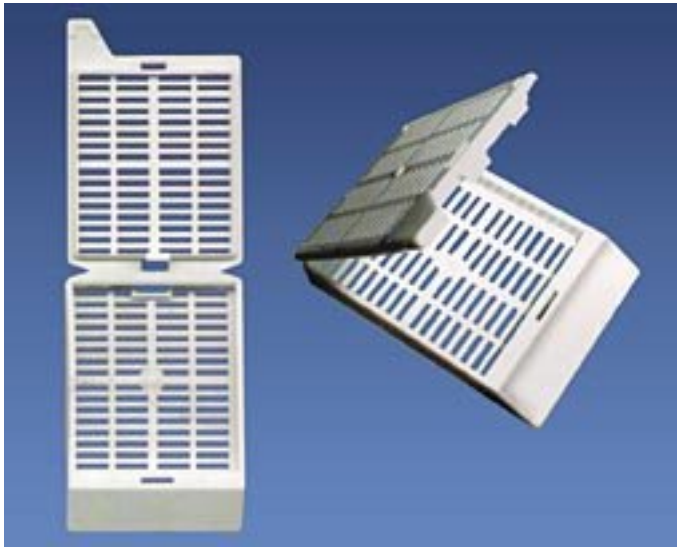
EPOXY EMBEDDING

- Dehydration: Ethanol or acetone.
- Infiltration with transitional solvent.
- Gentle agitation.
- Embedding and curing.

BLOCK FORMING

- Mold prepared.
- Wax poured.
- Tissue placement and orientation.
- Labeling.
- Immersion in cold water.

AUTOMATIC PROCESSOR



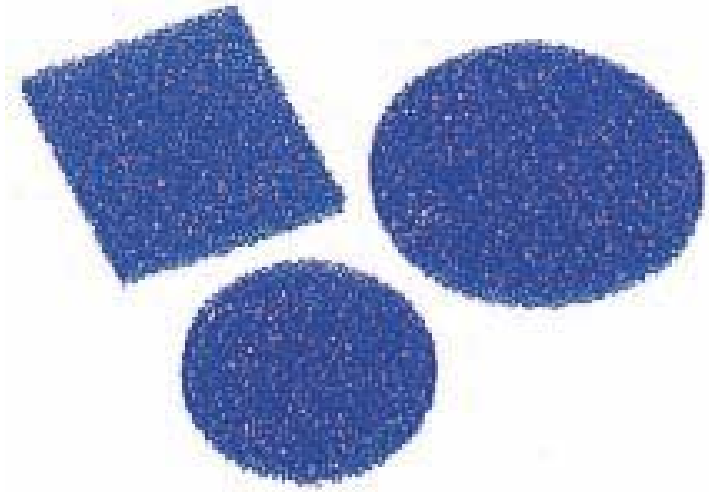
Processing cassette
Made of Acetyl polymer

Metal processing cassette

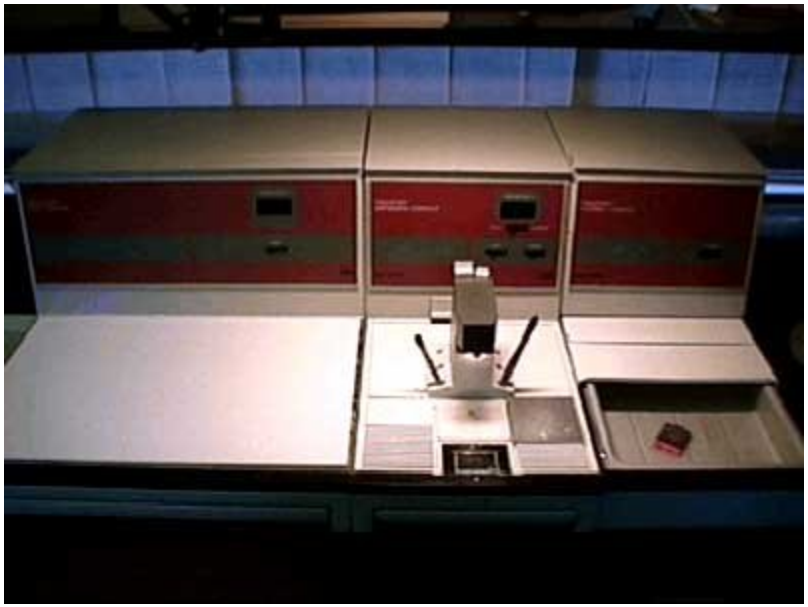
Embedding rings
Made of Polystyrene

Embedding cassette
Made of Polypropylene

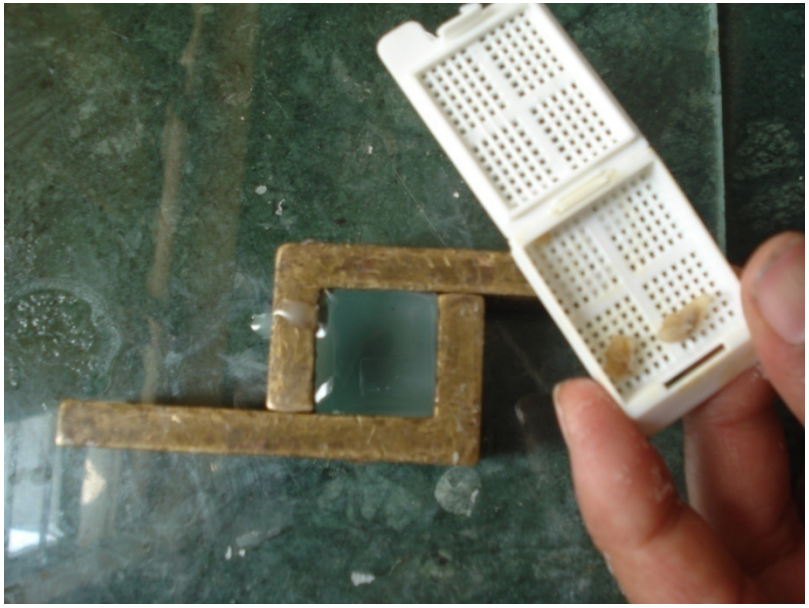
Micro processing cassette



Polyester urethane foam pads

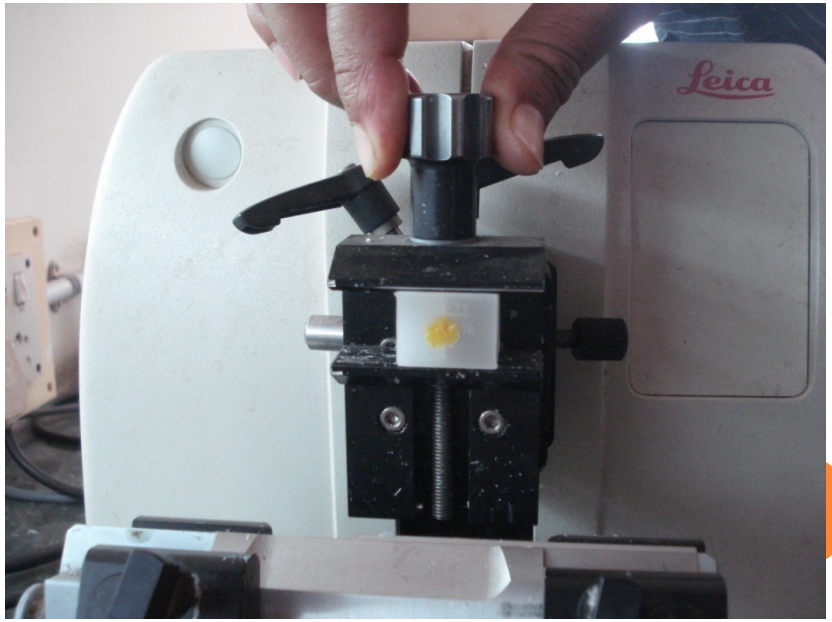



ALALCHOL
ALCOHOL
XYLENE




FREEZER CONTROL
LOW COOL
HIGH COOL


Leica


Leica

Procedure For Ground Sections

- Equipment – Lathe

  Coarse & Fine

  Spray of water,

  Wooden block & adhesive tape.

- Hold tooth adjacent to a rotating coarse-abrasive wheel.
- Reduce half of the tooth surface
- On a wooden block wrap adhesive tape with the adhesive end facing outward.

- Stick the ground surface of the tooth over the wooden block & grind the opposite surface.
- Grind the tooth to approx. thickness of 0.5mm.
- Use the fine abrasive wheel for further grinding the section till it becomes as thin as desired. (approx. 30-50µm.)

- Ground section is soaked in ether/alcohol to remove water.
- Mounted on a slide & viewed under the microscope

Frozen sections

- Rapid diagnosis.
- Study of tissues lost during conventional processing.
- Tissue components that are heat liable.
- Immunoflourescent study.
- SEM
- IHC

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PROCEDURE

- Fresh unfixed tissues are cut into 10-15µm sections by freezing the block of tissue with liquid/solid carbon dioxide
- Quench tissue at -160 Deg C
- Drying: Vacuum 133mPa
- Fixation: Vapors
- Embedding: Wax

LAB MAINTAINANCE

- Changing of solutions.
  - Depends on No. & Vol. of tissue.
  - Changed at least every week.
  - Downgrade solutions.
- Storage & Disposal of solutions.

General advice

- If at any stage it is felt that the processing has been done inadequately or erroneously tissue is to be transferred to a sealed container containing,

  70% alcohol - 70 ml
  Glycerol - 30 ml
  Dithionite - 1 gm

  And kept overnight. Processing to be started afresh.

MICROWAVE PROCESSING

PRINCIPLE

- The usage of microwave energy to speed up the process of diffusion of liquids in and out of the specimen.

PROCEDURE

- Dehydration done in one step.
  - 67 Deg C - 5 mins
- Addition of intermedium.
  - 74 Deg C - 3 mins
- Paraffin added in liquid form.
- Paraffin brought to boiling point of intermedium to flash evaporate the same.
  - 60 Deg C – 2 mins
  - 85 Deg C - 5 mins
- Embedding in paraffin.

ADVANTAGES

- Time and material saviour:
  - 100% ethyl alcohol for dehydration.
  - Isopropanol for inter-medium.
  - Liquid paraffin at 67 Deg C.
  - Liquid paraffin at 82 Deg C.
- Eliminates the usage of Xylene.

Summary

- Processing Technique
- Procedure For Ground Sections
- Advantages and procedure of frozen sections
- Maintenance of laboratories
- Advantages and procedure of microwave fixation

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Thank You