

INFILTRATION

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Tissues must be encased in a solid matrix to add structural support before sectioning

All support media start out liquid, are introduced into the tissues via a slow diffusion process, then subsequently are solidified.

The process of uniformly distributing the support medium throughout the tissue is termed infiltration.

INFILTRATION

- Is the saturation of tissue cavities and cells by a supporting substance which is generally, but not always, the medium in which they are finally embedded.
- Is the process of replacing clearing agent by embedding media.
- Tissues are infiltrated by immersion in a substance such as a wax, which is fluid when hot and solid when cold.

INFILTRATION

- Alternatively, tissues can be infiltrated with a solution of a substance dissolved in a solvent, for example nitrocellulose in alcohol-ether, which solidifies on evaporation of the solvent to provide a firm mass suitable for sectioning.

IDEALLY AN INFILTRATING AND EMBEDDING MEDIUM SHOULD BE

- soluble in processing fluids
- suitable for sectioning and ribboning
- molten between 30°C and 60°C
- translucent or transparent; colourless
- stable
- homogeneous
- capable of flattening after ribboning
- non-toxic
- odourless
- easy to handle
- inexpensive

PARAFFIN WAX

- **PROPERTIES**

Paraffin wax is a polycrystalline mixture of solid hydrocarbons produced during the refining of coal and mineral oils.

PARAFFIN WAX

- Wax hardness (viscosity) depends upon the molecular weight of the components and the ambient temperature.
- High molecular weight mixtures melt at higher temperatures than waxes comprised of lower molecular weight fractions.
- Paraffin wax is traditionally marketed by its melting points which range from 39°C to 68°C.

PARAFFIN WAX

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- Tissue-wax adhesion depends upon crystal morphology of the embedding medium.
- Small, uniform sized crystals provide better physical support for specimens through close packing.
- Crystalline morphology of paraffin wax can be altered by incorporating additives which result in a less brittle, more homogeneous wax with good cutting characteristics.

PARAFFIN INFILTRATION

PROPERTIES OF PARAFFIN

- Is rapidly converted from solid to liquid form on heating.
- permeates the tissue in a liquid state.
- solidifies relatively quickly on cooling.
- When the paraffin solidifies it becomes firm enough to section at room temperature.

MODIFIED PARAFFIN WAXES

- The properties of paraffin wax are improved for histological purposes by the inclusion of substances added alone or in combination to the wax:
 - Improve ribboning: prolong heating of paraffin wax at high temperatures or use micro-crystalline wax.
 - Increase hardness: add stearic acid.
 - Decrease melting point.
 - Improve adhesion between specimen and wax.

PARAFFIN INFILTRATION...CON

- Once the liquid paraffin has infiltrated hardened, it maintains the components of the tissue in proper relation to each other; otherwise these components would be compressed and distorted during sectioning.

ADVANTAGE OF PARAFFIN INFILTRATION

- Time of infiltration and embedding are short.
- Thin sections can be cut with the rotary microtome and sections will adhere to each to form a ribbon.
- Tissue once infiltrated and embedded can be stored in a dry condition indefinitely without damage to the tissue.

Ribbon

PARAFFIN INFILTRATION

- Infiltration proceeds by placing the tissue in at least two or three changes of liquid paraffin in the paraffin oven (58-60°C) for a total of 2-4hr.

VACUUM INFILTRATION

- is the impregnation of tissues by a molten medium under reduced pressure.
- The procedure assists the complete and rapid impregnation of tissues with wax, reduces the time tissues are subjected to high temperatures thus minimising heat-induced tissue hardening, facilitates complete removal of transition solvents, and prolongs the life of wax by reducing solvent contamination. Vacuum infiltration requires a vacuum infiltrator or embedding oven

MANUAL TECHNIQUE

- The usual method for processing tissue is to pass the tissue from one fluid to another. It needs the person physical appearance.
- To avoid handling of the tissue with forceps use the method of decantation.
- In this method the tissue is in one beaker only and the fluids are added to and poured off from the beaker.

ROUTINE TIMING SCHEDULE FOR MANUAL TECHNIQUE

1-fixation : 12-24 hr

2-Wash : uses running tap water (6-8 hr)over night.

3-Dehydration: 70% alcohol-----1hr

80%alcohol-----1hr

95%alcohol-----1hr

100%alcohol I-----1hr

100%alcohol II-----1hr

4-Clearing

a-clearing agent I-----1hr

b-clearing agent II-----1hr

ROUTINE TIMING SCHEDULE FOR MANUAL TECHNIQUE

5-Infiltration(in paraffin Oven)

a-Paraffin-I(56°-58°C) ----- 1 ½ hr

b-Paraffin-II ----- 1 ½ hr.

6-Embed

AUTOMATIC TISSUE PROCESSING

- Tissue can be rapidly processed without any attention on the part of the technician from fixative through infiltration by automatic tissue processors (**histokinetic machine**). In the machine the tissue is forwarded automatically to the next step after specified period of time.
- The most recent automatic tissue processor use vacuum, vertical oscillation and heat to rapidly promote chemical activity, penetration and interchange of the various solutions used in processing of the tissue. Every step is adjusted for specific period of time by using a time device.