**Paraffin processing and embedding of tissues**

**Purpose:** Paraffin processing provides a maximum structural support during sectioning.

**Principle:** The fixed tissue is dehydrated through graded alcohols, cleared with an antemedium and then infiltrated with paraffin.

**How to proceed:**

- Place the fixed tissue in plastic processing cassettes
- Process using the enclosed tissue processor (VIP6 form Sakura). These processors are characterised by the transfer of tissues, contained within a basket, through a series of stationary reagents arranged in-line or in a circular carousel plan. Temperature, pressure and agitation reduce the duration of tissue processing and improve the quality of infiltration:
  - 70% alcohol 45min RT Pressure & vacuum Mixing
  - 90% alcohol 45min RT Pressure & vacuum Mixing
  - 96% alcohol 45min RT Pressure & vacuum Mixing
  - Absolute alcohol 45min RT Pressure & vacuum Mixing
  - Absolute alcohol 45min RT Pressure & vacuum Mixing
  - Absolute alcohol 45min RT Pressure & vacuum Mixing
  - Xylene 45min RT Pressure & vacuum Mixing
  - Xylene 1 hour RT Pressure & vacuum Mixing
  - Xylene 1h15 RT Pressure & vacuum Mixing
  - Paraffin 30min 60°C OFF Mixing
  - Paraffin 45 min 60°C Pressure & vacuum Mixing
  - Paraffin 1 hour 60°C Pressure & vacuum Mixing
  - Paraffin 1h15 60°C Pressure & vacuum Mixing
- After the tissue is fully infiltrated with paraffin wax, it is embedded in excess wax in a square block which acts as a support during sectioning:
  - Using an embedding centre, a metal mould on a hot plate is filled with molten paraffin wax.
  - Tissue is orientated appropriately (usually tissues are embedded with the surface to be cut facing down in the mould). Making sure that there are no air bubbles trapped between the tissue and the molten wax.
  - The wax-filled mould containing the tissue is then cooled on a cold plate.
  - Once tissue begins to be caught by paraffin, the deeper part of the cassette is placed on the metallic mould. Be careful that sufficient wax covers the grid.
  - Block is now ready to section but can be stored indefinitely at 4°C.