TISSUE DEHYDRATION

Principle

- Most embedding media are hydrophobic
- thus water must be removed from the tisues before further processing.

Hydrophobic liquids

There are two general methods of dehydrating tissues

- Graded dehydration series
- Rapid dehydration

Graded dehydration series

- It is the most common method
- The gradual replacement of water with an organic solvent by transferring the tissue through a series of increasingly concentrated solutions

Rapid dehydration

- Less common method
- Dehydration utilizing an organic reagent such as:
- MC: Methyl cellosolve
- DMP:Dimethoxypropane
- TEP:Triethyl phospate

Rapid dehydration

- With rapid dehydration, tissue water is replaced rapidly, without the surface tension damage (because of alcohol or acetone)

GENERAL PROTOCOL

1. Fix tissues
2. Wash with the fixative solution minus the fixative
3. Dehydrate in a graded series or use the rapid method
4. Temporarily stain tissues
5. Transfer to an intermediate solvent
6. Transfer to liquid paraffin for paraffin embedding or plastic monomer for plastic embedding
7. Embed tissue

Dehydration Using Graded Dehydration Solvent Series

- Ethanol is the most common dehydrating agent
- Dehydrate tissue by passing it through a graded EtOH series starting with 30% and ending with 100 %.
- For all but the final EtOH dilutions, commercial 95% may be used.
- Than threat 95% EtOH as 100% when calculating dilutions.
- Water scavenging substance can be used in the last EtOH solution to ensure that the final absolute ethanol treatment uses anhydrous.

Water scavenging substance

- EtOH as 100%
- absolute ethanol
- anhydrous.

Note the water concentration of fixative used and start with this concentration

- For example:
- İf we used FAA for fixation
- Since FAA is usually is 50% EtOH
- We should start with 50 % EtOH to save dehydration steps

Most fixatives requires washing step

- Wash the tissues with the fixative solution minus the fixative

- i.e. 50% Ethanol for FAA
- Buffer for gluteraldehyde

Dye

- Dehydrating tissues can render tissues transparent
- In order to see them in the blocks stain with 0.1% Safranin O, Eosin Y or Thymol Blue in the penultimate 100% EtOH or acetone step.

The length of time

- Depens on the speed at which the solvent will diffuse in to the tissue.
- Small porous tissues need only 30 min to 2 h per step
- Large and hard tissues (wood or seed) may need as much as 24 h four step

Procedure

1. Wash fixed tissues twice in the fixative solvent (50% EtOH for FAA)
2. Dehydrate in a graded ethanol

%EtOH	Time hour	Notes
30	1	
50	1	For FAA start here
70	1	
90	1	
95	1	
100-dye	2-4	
100 (absolute)	1	Use mol. sieve

Rapid Dehydration

- Usually used for plastic embedding techniques
- But they may also be used for paraffin technique
- Organic solvents such as:
- MC: Methyl cellosolve
- DMP:Dimethoxypropane
- Acetonitrile

Rapid Dehydration

- MC: Methyl cellosolve and DMP:Dimethoxypropane
- Completely replace water
- They have both hydrophilic and hydrophobic domains