Mounting Media

Objectives

At the end of the lecture, you must be able to:

- Define mounting media
- Describe the reasons for mounting tissues
- State the criteria of an acceptable mounting media
- Outline the types of mounting media

Mounting Media

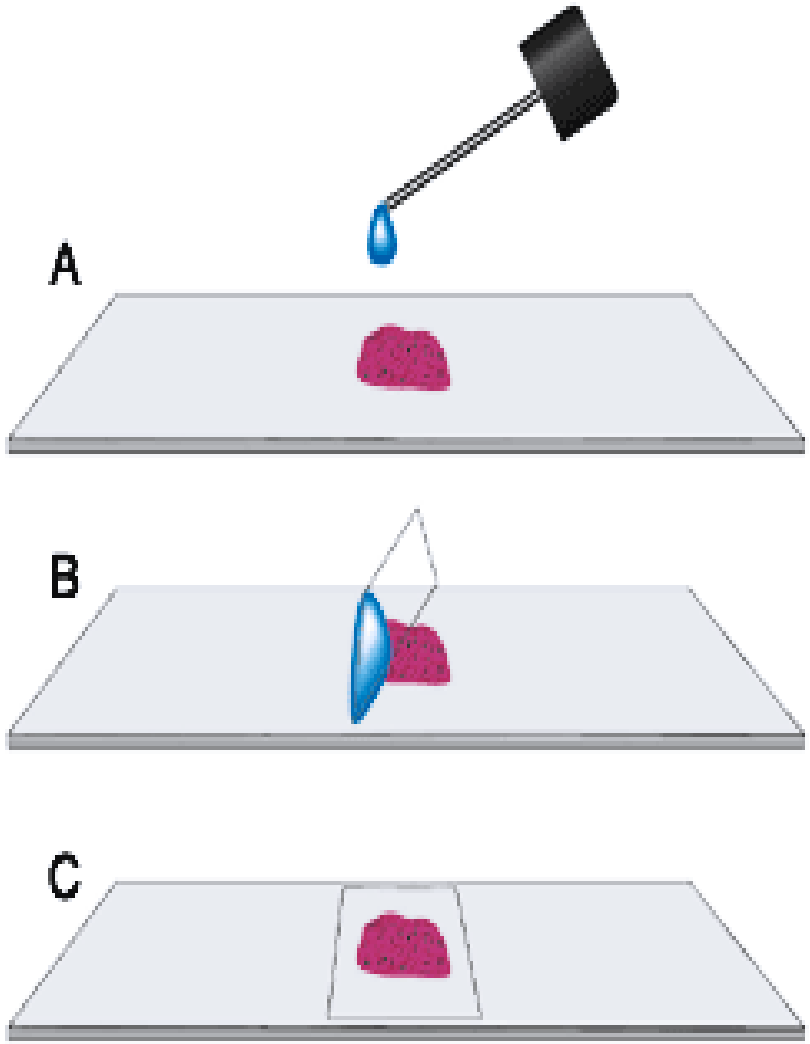
- The mounting medium is the solution in which the specimen is embedded, generally under a cover glass.
- Essential to get the best and clearest view of the specimen.

- Uses:

i. Holds the specimens in place between the cover slip and the slide (protect specimen).

ii. For making permanent slides.

iii. To maintain a high refractive index.



Mounting Media

- Most tissues have an RI of between 1.5 and 1.55, so a mounting medium with an RI in this range will give maximum clarity.
- There are two major types of mounting media used and the difference is in the solvent.

Criteria of Acceptable Mounting Media

- RI should be as close as possible to that of glass, i.e., 1.5
- It should be colourless and transparent
- Inability to cause stain to diffuse or fade
- It should be dried to a non-stick consistency and harden relatively quickly
- Inability to shrink back from edge of cover-glass
- It should be free flowing and free bubbles

Resinous Mounting Media

- Commonest types
- Are based on hydrophobic organic solvents, usually xylene
- Need the section to be dehydrated and cleared before mounting. eg
    - Canada balsam - Natural resin (R.I. - 1.52)
    - D.P.X. (R.I. 1.52)
    - Eukitt
        -Eupharal
        -Clear nail polish

Canada Balsam

- Natural mounting medium obtained from Canadian fir tree
- Refractive index 1.54

Advantage	**Disadvantages**
Permanent slides can be stored for many years	Specimen must be placed into xylene before embedding (because it is hydrophobic)
	Darkens with age
	Slowly becomes acidic
	Fading of many stains
	Not cheap

DPX

- A synthetic polystyrene resin that is dissolved in xylene and has some plasticizer added (Distrene 80, plasticizer and xylene).

- Colourless, neutral medium in which most standard stains are well preserved (RI 1.52).

Advantages	**Disadvantage**
Little tendency to fade dyes	Not recommended for use with thick sections
Hardens in about 24hrs	
Specimens do not need ringing (sealing the edges of the coverslip)	

Clear Nail Polish

- Can be used to seal the sides of the coverslip when using aqueous mounting media or directly as a mounting medium.
- The specimens must first be dehydrated in alcohol and can then be directly mounted (without xylene) in nail polish

Advantage	**Disadvantage**
Readily available	Shrinks a lot when making very thick mounts i.e. whole insects
Avoids use of toxic organic solvents to treat specimens	

Aqueous Mounting Media

- Used for mounting sections from distilled water when the stains would be decolorized or removed by alcohol and xylene.
- Most are best considered as **temporary mounts** and need **ringing** to hold the coverslip in place and prevent drying out.
- Tissues do not need any treatment before mounting and can be mounted directly from water or buffer.
- Require addition of bacteriostatic agents such as phenol, crystal of thymol or sodium merthiolate to prevent the growth of fungi.

Glycerol

- A trihydric alcohol with a high RI.
- Can be used alone or with the addition of a buffer to control the pH.

Advantage	**Disadvantage**
Water based avoids use of alcohol dehydration which possibly deforms specimens and toxic organic solvents	Difficult to prepare slides which are permanent
Does not shrink	

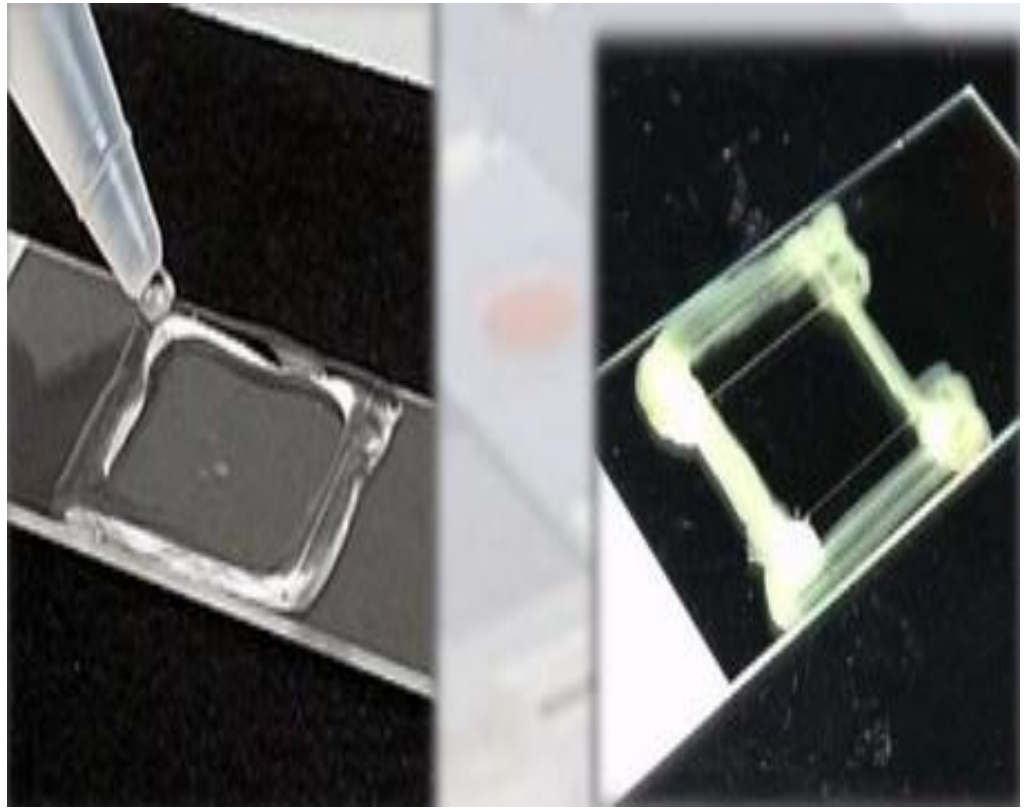
Glycerol Jelly

- Useful for making mounts of water organisms, algae, protozoa etc.
- Commonly used to preserve pollen samples.

Advantage	**Disadvantage**
Avoids use of alcohol dehydration which possibly deforms specimens and toxic organic solvents	Difficulty of mounting the sections (bubbles)
Does not shrink	Need to seal coverslip with nail polish

Temporary Mounts Need Ringing

- Ringing is the term used for sealing the edges of a coverslip when the mounting medium does not set.
- Good temporary ringing can be achieved in a number of ways using ordinary nail varnish, Many styrene-based cements, Paraffin wax, etc.



Storage of Slides

- Mounted slides should always be carefully labelled and stored horizontally until fully dry and set when they can be stored on their edge or end.

- Stained slides should be stored away from light as the dyes will fade even in the best mountant.

Summary

- Mounting media are needed for making permanent slides for microscopy and storage.

Aqueous media	Temporary medias	Useful for immunoflourescent techniques, mounting sections of fat stains
Resinous media	Permanent medias	Routinely used- DPX