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Techniques in Histopathology & Cytopathology



Sadhana Vishwakarma

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Techniques in Histopathology and Cytopathology

A Guide for Medical Laboratory Technology Students

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CHAPTER

10

Mounting of Sections

INTRODUCTION

The final stage in the preparation of tissues for microscopy is mounting. Occasionally tissue samples are examined dry (as occurs with sections for electron microscopy and in some procedures for displaying specimens in a museum) but in almost every other situation a liquid medium or 'mountant' is applied.

Mountants with a refractive index (RI) as close as possible to that of the tissue (usually taken as the RI of fixed protein, between 1.53 and 1.54) will effectively render a section transparent. With such specimens the only features visible will be those colored by the staining method used. A medium with a much lower or higher RI than that of the tissue will increase visibility but is beneficial only if an overview of the specimen is required as the resolution can be poor (evident in the extreme if sections are cleared and examined dry; the mountant in this case, air, has a RI of 1.000). In some forms of microscopy (such as phase contrast) the use of a mountant with an RI only slightly different from that of tissue is recommended to supplement contrast enhancement.

CHARACTERISTICS OF MOUNTANT

To be effective, a mountant should possess certain characteristics. These include the following:

- It should be colorless and transparent.
- It should be able to completely permeate and fill tissue interstices.
- It should have no adverse effect on tissue components.
- It should be resistant to contamination.
- It should protect the section from physical damage and chemical activity (oxidation and changes in pH).
- It should be completely miscible with dehydrant or clearing agent.
- It should set without crystallizing, cracking or shrinking (or otherwise deform the material being mounted) and not react with, leach or induce fading in stains and reaction products (including those from enzyme histochemical, hybridization and immunohistochemical procedures).
- Finally, once set, the mountant should remain stable.

Mounted sections are often stored for many years and the use of an appropriate mountant is critical to avoid deterioration in the specimen.

TYPES OF MOUNTANT

Mounting media are either hydrophobic or hydrophilic. Methods using the former generally call for sections to be dehydrated and cleared before the mounting medium is applied. Sections are mounted in hydrophilic media directly from water.

Within each group, mountants can also be classed as adhesives or non-adhesives. In general, adhesives harden through solvent evaporation and thereby fix the accompanying coverslip to the slide. During this process, the RI of the medium alters, moving away from that of the solvent and towards that of the dry mountant. The exact RI of the applied medium cannot therefore be known. Nevertheless as the RI of hydrophobic (adhesive) mountants usually approximates that of tissue proteins (fixed), they provide firm adhesion of the coverslip, these mountants are the type most frequently used.

Hydrophilic media, although of relatively low RI, are essential for procedures in which dehydrants and hydrocarbon type clearants must be avoided. This will mostly relate to methods for demonstrating lipid, enzyme identification and immunohistochemistry. Disadvantages of hydrophilic mountants are that they may induce stains to leach from the section and many, being non-adhesive, remain soft such that the edge of the coverslip must be sealed to prevent drying out. Suitable agents (ringing media) for this purpose are nail varnish, resinous mountants or paraffin wax.

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Hydrophobic Mountants

Canada Balsam (RI = 1.52)

This is an oleoresin obtained from the bark of the fir *Abies balsamea* (of the family Pinaceae), native to North America. The dried resin is freely soluble in xylene and other organic solvents. Originally introduced in about 1832 and widely used until only recently, Canada balsam has a number of disadvantages: it yellows with age; is very slow to harden and; as it becomes increasingly acidic over time, cationic dyes are poorly preserved and the Prussian blue product of Perls' reaction is bleached.

Reagents Required

Canada balsam 55–65 g and xylene 100 mL.

Method

Prepare using the quantities indicated (greater amounts of resin will result in more viscous solutions). RI (solution) 1.523. When dissolved, the solution is filtered through soft filter paper and the desired consistency is obtained by the controlled evaporation of solvent. It should be kept in a dark glass bottle. A few crystals of calcium carbonate in the stock bottle will help to maintain neutral reaction.

Distrene, Plasticizer, Xylene (DPX)

One of the most commonly used mountants, DPX is a colorless, neutral medium in which most standard stains are well preserved. It is prepared by dissolving the common plastic, polystyrene, in a suitable hydrocarbon solvent (usually xylene). A major disadvantage of polystyrene media, however, is that they set quickly and in doing so often retract from the edge of the coverslip. This can be prevented by adding a plasticizer which is thought to resist the effect by forming a mesh with the polymerized plastic.

Reagents Required

Polystyrene (Distrene 80) 18 g, dibutyl phthalate 7.5 mL and xylene 52.5 mL.

Method

Prepare using the quantities indicated. RI (solution) 1.523. Combine the dibutyl phthalate with xylene, mix and dissolve polystyrene. Label and store. It is more advantageous than Canada balsam because slides can be cleaned by excess mountant by stripping it off after cutting around the edge of the coverslip.

Euparal

Euparal is a mixture of eucalyptol, sandarac (a resin from the tree, Tetraclinis articulata grown in North West Africa), paraldehyde and camsal (camphor and phenyl salicylate). Its has relatively low RI (which is usually 1.483 but ranges from 1.478 at 20°C to 1.535 when solid) which makes it useful for mounting unstained sections. Another advantage is that slides may be transferred directly from 95% alcohol, eliminating the need for complete dehydration and clearing. Some fading may occur in hematoxylin stained sections; in this situation the green (or 'vert') copper-containing form of Euparal is advocated.

Resin-Embedded Tissue

Sections of tissue embedded in plastic compounds (such as epoxy resins) can be successfully mounted in a liquid resin of the same type. Sections should be completely dry before applying mountant, which is best set using the same conditions prescribed for tissue blocks.

Photosensitive Resins

Light polymerizing resins have the advantage of very short setting times, requiring in the order of 10–30 seconds exposure to UV light to harden completely. Once cured, however, the mountant cannot be dissolved nor the coverslip removed (as might be necessary for restraining). Acrylic based light sensitive resins are also suitable for fluorescence microscopy.

Hydrophilic (Aqueous) Mountants

Water

Although of low RI (1.333), water serves as a convenient temporary mountant for some whole specimens for examining certain microorganisms live (saline mount) and particularly when checking sections during staining procedures.

Glycerol

Glycerol is also a useful temporary mountant, but with a higher RI (1.460) and longer drying time than water. Ringing the coverslip with a hydrophobic seal will extend the life of mounted sections, although cationic dyes will diffuse into the medium over time. Phosphate buffered glycerol (RI 1.47) is commonly used to mount sections for immunofluorescence. Glycerol may be added to other agents to retard drying and cracking.

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Reagents Required
0.2 mol/L phosphate buffer-1 part and glycerol-9 parts.

Method
Prepare using the quantities indicated. The addition of gum arabic (derived from species of Acacia) to a solution of glycerol will result in an adhesive mountant (Farrants medium) with a RI of 1.436. The preparation is acidic but rendered neutral through the inclusion of potassium acetate, which also retards dye extraction from methyl violet stained sections of amyloid.

Glycerin (Glycerol) Jelly

This commonly utilized aqueous mountant is a mixture of glycerol and gelatin and has a RI of 1.47. It should set quite hard but for long-term preservation sections are best ringed and sealed. Various formulations are in use.

Apathy's Medium

Sucrose added to gum arabic preparations increases the RI and prevents overdrying. The inclusion of potassium acetate will prevent leaching of metachromatic dyes.

Reagents Required
Gum arabic 20 g, cane sugar 20 g, potassium acetate 20 g, distilled water 40 mL and thymol 0.02 g.

Method
Dissolve components in warm distilled water. Store in air tight containers.

Polyvinyl Alcohol

Polyvinyl alcohol, often used as a mountant in immunofluorescence microscopy, has been recommended as an alternative for glycerin jelly. Adding paraphenylenediamine to the preparation is effective in retarding photo fading.

Karo Corn Syrup (RI = 1.47)

Karo corn syrup	:	1 volume
Distilled water	:	2 volume
Thymol (preservative)	:	1 crystal

Dilute corn syrup with distilled water and add thymol, mix, label and store at 4°C.

Levulose (Fructose) Syrup (RI = 1.47)

Levulose (fructose)	:	70 g
Distilled water	:	20 mL

Dissolve the levulose in the distilled water by heating at 37°C for 24 hours. Mix well and store.

Farrant's Medium (RI = 1.43)

Gum Arabic	:	50 g
Distilled water	:	50 mL
Glycerol		50 mL
AsO_3 (preservative)	:	1 g

Dissolve gum Arabic in the distilled water with the aid of gentle heat, add glycerol and AsO_3 mix well and label but thymol (100 mg), merthiolate (15 mg) or cresol (0.1 mL) are effective substitutes.

Kaiser's Glycerin Jelly (RI = 1.47)

Gelatin	:	10 g
Glycerol	:	70 mL
Distilled water	:	60 mL
Phenol crystals (preservative)	:	0.25 g

Add gelatin in distilled water and incubate in water bath at 60°C till the gelatin gets dissolved. Add glycerol and crystals of phenol. Mix well, label and store at 4°C.

Temporary Mountant

A mixture of equal parts of pure glycerol and distilled water may be used as a temporary mountant with fresh or watery preparations while thick cedar wood oil (RI = 1.52) is suitable with dehydrated specimens.

Methods of Mounting Sections (Cover Slipping)

First a clean coverslip of the appropriate size and wipe off the excess of xylene from the side with a dust free cloth. If xylene remain on the section then it will mix with the mounting medium and form air bubbles which become trapped beneath the coverslip. There are four methods of mounting stained sections which are as follows:

Method 1:	Place mounting medium on a coverslip Invert glass slide with stained section on the coverslip.
Method 2:	Place the mounting medium on a coverslip. Place the coverslip on the stained section on the slide.
Method 3:	Place the mounting medium on the stained section. Place coverslip over it.
Method 4:	Stained section is placed on the coverslip. Glass slide with mounting medium is placed over the coverslip (Fig. 10.1).

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Method 1

- Select the appropriately sized coverslip and place on a white paper sheet.
- Place a drop of mountant in the center of the coverslip.
- Remove surplus clearant from the back of the slide and around the section, leaving a margin of approximately 3 mm. The section should not be allowed to dry out.
- Invert the slide (section face down) over the coverslip and with one end resting on the paper sheet, gradually lower the other end until the mountant touches the section. Mountant will spread quickly over the section, between slide and coverslip. The slide, with coverslip attached, is then turned upright. Any trapped air is gently squeezed out whilst aligning the coverslip.
- The mountant is allowed to set. The time required will depend upon the particular agent used but in some cases warming the slides (37°–60°C) will hasten the process. If the result is inadequate, slides are returned to the solvent (appropriate to the mountant) to have the coverslip removed and the process repeated.

Method 2

- Select the appropriate sized coverslip.
- Remove surplus clearant from the back of the slide and around the section and place the slide on a level surface.
- Place a drop of mountant in the center of the coverslip.
- Invent the coverslip so that drop face the downward.
- Now keep the coverslip over the slide with one end resting on the paper sheet, and gradually lower the other end until mountant touches the section.
- Remove the trapped air by gentle squeezing.

Method 3

- Remove excess clearant from the back of the slide and around the section.
- Place the slide on a level surface and apply a drop of the mountant.
- Hold the coverslip at 45° angle to the surface of the slide, and allow the bottom edge to touch the drop of mountant. When the drop spread along the edge of the coverslip, let go of the coverslip and allow the mountant to spread slowly.
- Remove excess of mountant while wet with a tissue paper or with a razor blade after mountant dried sufficiently in 30 minutes.

Method 4

- Select an appropriate size coverslip, place stained section on it and place it on a leveled surface.
- On a cleaned glass slide, add a drop of mountant in the center.
- Invent the slide and slowly lower the slide on the coverslip until the mountant touches the section.
- Mountant spread quickly over the section.

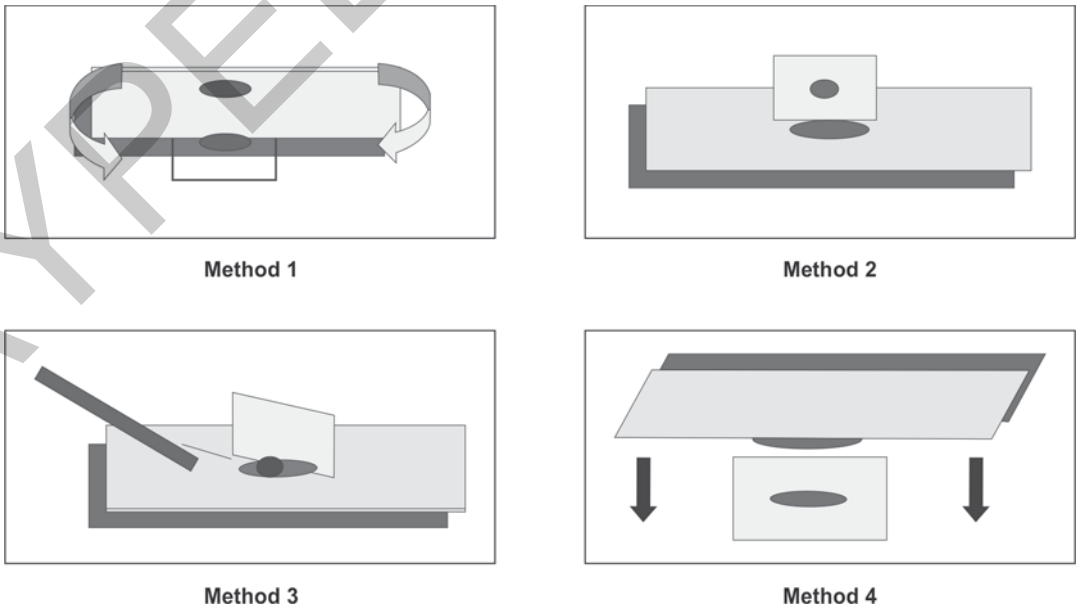

Fig 10.1: Techniques of mounting

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- Now, turn the slide upside down.
- Remove the air trapped by gently squeezing.
- Remove excess mountant.

RESTAINING

In some instances, it becomes necessary to gain access to a section previously set in mountant to restain specimens that has faded appreciably, remove the original stain and apply a new technique or to superimpose an additional stain. Mounted tissue may also need to be retrieved for additional assessment such as by electron microscopy or X-ray analysis. In this case, coverslips can be removed by soaking in the solvent appropriate to the mountant. This may require one to two days after which the coverslip is gently removed and sections are rehydrated and treated as required. Alternatively, slides can be left at –20°C for up to one hour then a sharp blade used to carefully prize off the coverslip.

LABELING AND STORAGE OF SLIDES

Slide mounted sections are identified during preparation by inscribing the slide with the tissue accession number or suitable code using a diamond marker or pencil (frosted slides). Care should be exercised when using characters such as A, I, O, T, V and the like as these appear the same when viewed from either side of the slide; incorrectly identifying the side upon which the section is mounted may lead to poor staining or section damage. After staining and mounting it is common practice to affix to the slide a paper label upon which the laboratory number, staining method and date are usually written. This may be supplemented with a bar code or other symbol which can be recognized by a computer supported image scanner. It is important when attaching identifying labels to be aware of the slide storage system to be utilized. Standard 7.5 × 2.5 (3" × 1") slides are usually stored, standing on their short side, in metal or plastic drawers. Labels need to be fixed to the opposite end and orientated so the details can be read with the slide in this upright position (Fig. 10.2).

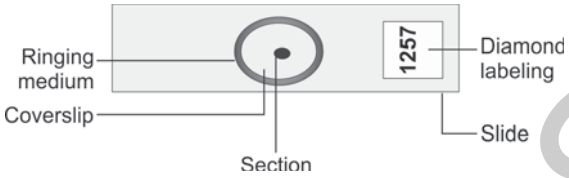

Fig. 10.2: Labeling of slide

COVERSLIPS

Coverslips are made up of borosilicate or silicate glass and it holds the samples in place and protect them from inadvertent movement and contamination. To be compatible with the standard slide, coverslips are 22 mm or 24 mm wide, but vary in length to suit the size of the section. In general a selection of 22 mm, 30 mm, 50 mm and possibly 60 mm (if preparing cell smears) will accommodate most purposes. Round coverslips of different diameters are also available. The coverslips are manufactured to a specified thickness to complement the optical specifications of microscope objectives lenses (the recommended thickness is indicated on the barrel of the lens and is normally 0.17 mm). Various thicknesses are available (with some variation between manufacturers) and are designated No. 1 (0.13–0.17 mm), No. 1½ (0.16–0.19 mm) and No. 2 (0.19–0.25 mm).

RINGING MEDIA

Fluid and semi fluid mountants, including which fail to set completely hard should be sealed at the margin of the coverslip to prevent evaporation of mountant, to immobilize the coverslip, and to prevent shrinkage or sticking of slides on storage. Ringing media may be solid, being melted for use and applied with a bent wire, or fluid being applied with a fine brush, the medium hardening by evaporation. For a solid medium, paraffin wax may be used or Kronig's cement which is composed of 2 parts wax and 7 part powdered colophonium resin. Several liquids ringing media are available commercially such as nail varnish, durofix, etc.

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EXERCISE

1. What are mountants? Classify with suitable example.
2. Enlist various characteristics of mountants.
3. Write short note on following:
   i. Canada balsam
   ii. DPX
   iii. Glycerol
4. Give composition of following mounting media:
   i. Karo Corn syrup
   ii. Levulose (fructose) syrup
   iii. Farrant's medium
   iv. Kaiser's glycerin jelly
5. Describe methods of mounting sections with the help of figure.
6. What is restaining? How restaining can be done?
7. Describe method of labeling and storage of slides.
8. Write a note on coveslips and method of coverslipping.
9. What do you mean by ringing media?

OBJECTIVE QUESTIONS

1. The mountants which are used in histology should have refractive index (RI) of _____.
   a. 1.54 b. 2.54
   c. 0.54 d. 3.54

2. A good mounting medium should be _____.
   a. Colorless and transparent
   b. Colored and transparent
   c. Colorless and opaque
   d. Colored and opaque

3. A good mounting media should not set with _____.
   a. Crystallization b. Cracking
   c. All d. Shrinking

4. A medium with much lower or higher RI than tissue provides _____.
   a. Good resolution b. Poor resolution
   c. Average resolution d. No resolution

5. _____ is not an example of hydrophobic mounting media.
   a. Canada balsam b. DPX
   c. Eupharal d. Glycerol

6. _____ is not an example of hydrophilic mounting media.
   a. Canada balsam b. Polyvinyl alcohol
   c. Apathy's medium d. Glycerol

7. DPX is an _____mounting media.
   a. Hydrophilic b. Hydrophobic
   c. Amphoteric d. None

8. Glycerol is an example of _____ mounting media.
   a. Hydrophilic b. Hydrophobic
   c. Amphoteric d. None

9. Canada balsam is an _____mounting media.
   a. Natural b. Synthetic
   c. Artificial d. None

10. _____is an resinous mounting media.
    a. Eupharal b. Glycerol
    c. Polyvinyl alcohol d. None

10. DPX is a mixture of _____.
    a. Distyrene, phenol, xylene
    b. Dibutyl, phenol, xylene
    c. Diphenyl amine, phenol, xylene
    d. Distyrene, plasticizer, xylene

ANSWERS

1-a, 2-a, 3-c, 4-b, 5-d, 6-a, 7-b, 8-a, 9-a, 10-a, 11-d.

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