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## Review Article

# Mounting media: An overview

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### ABSTRACT

Histological sections, which need to be examined for any length of time or to be stored must be mounted under a cover-slip. There are various types of mounting media available both commercially and also are prepared in one's own laboratory for mounting tissue sections. Some types of mounting media harden to hold the coverslip firmly in place and other types use different solvents such as water, glycerin and xylene because the stains in the sample preparation are sensitive to particular solvent. In order to prevent the of immonofluorescent slides, few mounting media contain antifade reagents. As less emphasis is given in the literature on mounting media, an attempt is made to review, venture and summarize on various types of mounting media and their uses in routine histopathologically and immunochemical staining.

**Key words:** antifade, coverslip, mounting media

### INTRODUCTION

In histology or a pathology laboratory, mounting is the last procedure in the series that ends with a permanent histological preparation on the table, well after the tissue processing and staining. Viz., (1) fixing, (2) paraffin embedding, (3) sectioning, (4) staining, (5) dehydrating, and (6) clearing operations.<sup>[1]</sup>

The mounting medium is the solution in which the specimen is embedded, generally under a cover glass. It may be liquid, gum or resinous, soluble in water, alcohol or other solvents and be sealed from the external atmosphere by non-soluble ringing media.<sup>[2]</sup> The main purpose of mounting media is to physically protect the specimen; the mounting medium bonds specimen, slide and coverslip together with a clear

durable film. The medium is important for the image formation as it affects the specimen's rendition.<sup>[3]</sup>

Mounting media should ideally have a refractive index (RI) as close as possible to that of the fixed protein (tissue) (approximately 1.53). As light passes from one medium to another, it changes speed and bends. An example of this is the apparent bending of a stick when placed in water. Light travels fastest in a vacuum and in all other media light travels more slowly. The RI of a medium is the ratio of the speed of light in a vacuum to the speed of light in the medium (it is always >1).<sup>[4]</sup>

A mounting medium with an RI close to that of the fixed tissue will therefore render it transparent, with only the stained tissue elements visible. This is where the term "clearing" comes from-xylene, for instance, has an RI very close to that of fixed tissue; therefore, inducing a certain amount of transparency.<sup>[4]</sup>

A mounting medium with an RI too far either side of 1.53 will provide poor clarity and contrast. This can be demonstrated practically by viewing a tissue section with no mounting medium since air has a RI of 1.0.<sup>[4]</sup> A mounting medium should be chosen that will not fade the particular stains used; for example, basic aniline dyes should be mounted in non-acid containing mountants. Preparations showing the Prussian blue reaction should be mounted in non-reducing media.<sup>[5]</sup>

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## PROPERTIES OF AN IDEAL MOUNTING MEDIA (MOUNTANT)

1. RI should be as close as possible to that of glass, i.e., 1.5.
2. It should be colorless and transparent.
3. It should not cause stain to diffuse or fade.
4. It should be dry to a non-stick consistency and harden relatively quickly.
5. It should not shrink back from the edge of cover-glass.
6. It should be able to completely permeate and fill tissue interstices.
7. It should have no adverse effect on tissue components.
8. It should be resistant to contamination (particularly microorganism growth).
9. It should protect the section from physical damage and chemical activity (oxidation and changes in pH).
10. It should be completely miscible with dehydrant or clearing agent.
11. 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).
12. Finally, once set, the mountant should remain stable (in terms of the features listed above).<sup>[6,7]</sup>

## CLASSIFICATION OF MOUNTING MEDIA

1. Resinous (hydrophobic/adhesives/organic/non-aqueous)
2. Aqueous media (hydrophilic/non-adhesive)

### Resinous/non-aqueous/adhesive media

These are natural or synthetic resins dissolved in benzene, toluene or xylene and are used when a permanent mount is required and frequently used in routine H and E staining procedures.<sup>[6]</sup>

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 toward 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) and they provide firm adhesion of the coverslip, these mountants are the type most frequently used.<sup>[7]</sup>

### Natural resinous media

- Canada balsam (RI = 1.52-1.54)
- Phenol balsam (variant of Canada balsam)
- Dammar balsam (RI = 1.52-1.54)
- Euparal (RI = 1.48).<sup>[2]</sup>

### Canada balsam: (RI = 1.54)<sup>[8]</sup>

Canada balsam 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. The standard mountant for histology and also for taxonomy, be it zoological or botanical is Canada balsam, a now scarce and very expensive natural resin.<sup>[1,7]</sup>

Canada balsam was first described by as a suitable mounting media by Andrew Pritchard in the 1830's. It is the most widely used mountant because of its proven archival quality, with a track record of 150 years and does not crystallize or absorb moisture. Mount and Pitkin state that Canada balsam is the only mountant not to deteriorate when kept for years in various climates. Noyes states several million years as longevity of Canada balsam, comparing it with natural fossilized amber. Walker and Crosby mention that Canada balsam is particularly recommended because it's RI (1.53) is very close to that of glass.<sup>[2]</sup>

Rawlins relegates Canada balsam as having been widely used at one time as it is strongly "autofluorescent." The harmful solvents which, constitute a health hazard such as xylene may limit its use as mountant and use of non-toxic solvents instead of xylene like histomount may solve the safety problem, but might cause other problem such as slow hardening and premature darkening. Other authors have reported problem such as "crazing." Other disadvantages include; 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.<sup>[2,7]</sup>

### Eupharal (RI = 1.48)

Eupharal is a semi-synthetic mountant. It is composed of a mixture of eucalyptol, sandarac (a resin from

the tree, *Tetraclininarticulata* grown in North West Africa), paraldehyde and camphor (camphor and phenyl salicylate). It is regarded as good permanent preservative, proven over the passage of time, of consistent quality, safe, quick and easy to use, good optically with low RI and drying quickly. It does not use the carcinogenic solvent, xylene and hence being better than Canada balsam and is used as an alternative to balsam. Some fading may occur in hematoxylin stained sections; in this situation the green (or "Vert") copper-containing form of Euparal is advocated.<sup>[2,5,7]</sup>

#### Phenol and dammar balsam

These are similar to Canada balsam and are rarely used as mountant because of dirt and impurities usually present and difficulty of filtering prepared mountant.<sup>[5]</sup>

#### Synthetic resinous media

A great number of synthetic resins either made in the laboratory or prepared commercially are available. The most commonly used are the polyesterenes, such as Kirkpatrick & Lendrum's mountant and Gurr's distrene plasticizer xylene (DePex).

1. DPX (DePeX [Distrene 80: A commercial polystyrene, a plasticizer, e.g., dibutyl phthalate and xylene]) (RI = 1.52)
2. Histomount (RI = 1.49-1.50)
3. Cover bond (RI = 1.53)
4. Gurr's neutral mounting medium (RI = 1.51)
5. Histo-clad (RI = 1.54)
6. Permount (RI = 1.526)
7. Pro-texx (RI = 1.495)
8. Technicon Resin (RI = 1.62)
9. UV-inert (RI = 1.517)
10. XAM (RI = 1.52).<sup>[6,7]</sup>

#### DPX

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). Gurr suggested that it undergoes considerable degree of shrinkage when drying and should be applied liberally to the slide. 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. Most commonly used routine mountant. It has a greater advantage over balsam

that slides can be cleaned of excess mountant simply by stripping it off after cutting around the edge of coverslip.<sup>[2,5,7]</sup>

#### Gurr's neutral mounting medium (RI 1.51)

This is a mixture of coumarone and other resins as 76% solution in cineol. This mountant is a rather viscous solution.

#### Histo-clad (RI 1.54)

Clay Adam's Histo-clad is a 60% solution of a synthetic resin in toluene.

#### Permount (RI 1.528)

Fisher's Permount is a 60% solution of naphthalene polymer in toluene.

#### Pro-texx (RI 1.495)

Pro-Texx from Lerner Laboratories 171 industry Pittsburghs is a mounting medium of neutral pH and with an antioxidant additive to preserve stain quality. It is soluble in both toluene and xylene.

#### Technicon resin (RI 1.62)

This mountant from the Technicon Corporation is a 60% solution of coumarone-indene polymer in benzene and xylene in equal parts. It has a tendency to form bubbles on hardening due to the high volatility of benzene.

#### UV-inert (RI 1.517)

A patented mounting medium that is non-fluorescent from Gurr.<sup>[6]</sup>

#### Resin-embedded tissue

Sections of tissue embedded in plastic compounds (such as epoxy resins) can be successfully mounted in 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.<sup>[7]</sup>

#### Photosensitive resins

Light polymerizing resins have the advantage of very short setting times, requiring in the order of 10-30 seconds exposure to ultraviolet light to harden completely. Once cured; however, the mountant cannot be dissolved nor the coverslip removed (as might be necessary for restaining). Acrylic based light sensitive resins are also suitable for fluorescence microscopy.<sup>[7]</sup>

### Aqueous Mounting Media

Aqueous mounting medium are used for mounting sections from distilled water when the stains would be decolorized or removed by alcohol and xylene as would be the case with most of the fat stains (Sudan methods). These media are of three types: The syrups, Gelatin media, and Gum Arabic media.<sup>[5]</sup>

Some of the metachromatic stains tend to diffuse from the sections into mounting media shortly after mounting: this may be prevented by using fructose syrup. Some stains, e.g., methyl violet tend to diffuse into medium after mounting. This can be avoided by using Highman's medium. Aqueous mounting media require the addition of bacteriostatic agents such as phenol, crystal of thymol or sodium merthiolate to prevent the growth of fungi.<sup>[5,6]</sup>

1. Water (RI = 1.333).
2. Glycerine jelly (RI = 1.47).
3. Glycerine-Glycerol (RI = 1.47).
4. Apathy's medium (RI = 1.52).
5. Farrant's medium (RI = 1.43).
6. Highman's medium (RI = 1.52).
7. Fructose syrup (RI = 1.47).
8. Polyvinyl alcohol.

#### Water

In spite 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 the staining procedures.<sup>[7]</sup>

#### Glycerine jelly

This is usually regarded as the standard mountant for fat stains. Dissolve the gelatin in the distilled water in a conical flask in a water bath and add glycerine and phenol mix well and store.<sup>[5]</sup>

#### Glycerine-glycerol

This commonly utilized aqueous mountant is a mixture of glycerol and gelatine 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.<sup>[7]</sup>

As with other solvents it is used because it is cheap, safe and quick to use with little preparation. 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 and glycerol may be added to other agents to retard drying and cracking.<sup>[7]</sup>

#### Apathy's medium (RI = 1.52)

It is one of the most useful aqueous mountants for fluorescent microscopy, being virtually non-fluorescent.

Dissolve the ingredients with the aid of gentle heat.

#### Farrant's medium (RI = 1.43)

Dissolve the gum Arabic in the distilled water with gentle heat, add glycerin and arsenic trioxide. It is also recommended for fat stains.<sup>[5]</sup>

#### Highman's medium (RI = 1.52)

Recommended with the metachromatic dyes especially methyl violet.<sup>[6]</sup>

#### Polyvinyl alcohol

Polyvinyl alcohol, often used as a mountant in immunofluorescence microscopy, has been recommended as an alternative for glycerine jelly. Adding paraphenylenediamine to the preparation is effective in retarding photo fading.<sup>[7]</sup> Various mounting media and their uses are discussed in Table 1.

### Ringing media/sealant media

Liquid, glycerin, and gum chloral mount need to be ringed around the edge of the coverslip to seal the mountant and prevent escape, loss through evaporation or oxidation through contact with air. Excess mountant from round the edge should be removed by scalpel before ringing. If the mountant is prone to shrinkage, ringing may not stop the ingress of air as any stress applied to the ring or coverslip may fail. The resin mountants usually do not need ringing as the solvent in the resin needs to evaporate in order to harden. Though, Gray suggests that Canada balsam be ringed to stop it darkening from atmospheric oxidation. One can use solid vaseline, paraffin wax or beeswax, Valap or nail polish as ringing media or sealant.<sup>[1,3]</sup>

### Ringing the coverslip

The term "ringing" originated because round coverslips were initially used and the coating applied in the form of a circle or "ring." A ringing iron is needed to spread the solid type of medium around

**TABLE 1: COMMONLY USED MOUNTING MEDIA AND ITS USES**

Mounting medium	RI	Implications
Resinous DPX	1.52	Very commonly used. Sets quickly
Canada balsam	1.52-1.54	Most widely used previously. Turns yellow with age. Sets slowly and due to use of xylene as solvent (carcinogen) hence preferred less
Euparal	1.48	Best alternative for Canada balsam and Xylene free. Can fade hematoxylin counterstaining of cell nuclei hence use green copper containing euparal
Aqueous glycerol	1.46	Can be added to other media to slow drying and cracking
Glycerine-glycerol	1.47	Commonly used for fluorescent labels e.g.: Phosphate buffered glycerol
Apathy's medium	1.52	Most useful aqueous mountants for fluorescent microscopy, being virtually non-fluorescent
Farrant's medium	1.43	Recommended for fat stains
Polyvinyl alcohol	1.5	Alternative for glycerine jelly commonly used for fluorescent labels with paraphenylenediamine as antifading agent

DPX = Distrene, plasticizer, xylene/dibutylpyhlate; RI = Refractive index

the coverslip. A T-shaped piece of copper, which is about one-eighth of an inch in thickness with the bottom of the upright piece embedded in a rubber bung or wooden handle is used. During use the cross piece is heated in the flame of Bunsen burner, touched onto the surface of the ringing agent and then applied to the edges of the coverslip. Nail polish varnish can be applied with a brush gently. With practice ringing may be simply, quickly and cleanly completed.<sup>[1,5]</sup>

1. Paraffin wax is applied with a ringing iron and is satisfactory as a temporary ringing agent.
2. Du noyer's wax-colophonium resin mixture:

It is prepared by heating 10 parts of paraffin wax in evaporating dish and dissolving in it 40 parts of colophonium resin. It is a more permanent mount.

3. Ladies nail varnish:

Nail varnish with or without color can be used as ringing media. A liquid preparation sealed well with nail polish could last some months.

4. Cement:

Asphalt based cement can be applied as ringing media direct from collapsible tubes making it a permanent mount.<sup>[1,2,5]</sup>

### Adhesives

Various laboratories use synthetic adhesives to glue sections and small specimens on the microscopic slides. RI and permanency are important as also are the pH and chemical constituents of glue or mountant as such might erode or even destroy the specimen. Adhesive media presently used are araldite epoxy resin, cellofas, crystalbond thermoplastic resin, etc.<sup>[2]</sup>

### Mountants for immunochemical staining (immunofluorescence and enzymatic labeling)

The choice of mounting medium following immunochemical staining is largely dictated by the label (and in the case of enzymatic labels, the chromogen) used to visualize the antigen. Aqueous mounting medium is generally suitable for all enzymatic label/chromogen combinations and fluorescent labels. Specimens mounted in such media are mounted straight from the aqueous phase (with no dehydration or clearing).<sup>[4]</sup>

Fluorescence emission of fluorescein isothiocyanate from labeled antibody in microscopical preparations may be influenced by the characteristics of the mounting medium, in particular, its pH, its ionic strength, its viscosity and the presence of quenching agents (Cherry, 1970). Fluorescence emission is greater at alkaline than at acid pH (Hiramoto, Bernecky, Jurand and Hamlin, 1964). A semi-permanent medium containing polyvinylalcohol grade 51-05 Elvanol1 and glycerol was described by Rodriguez and Deinhardt in 1960.<sup>[9]</sup>

Aqueous mounting media for phycobiliprotein fluorescent labels (phycoerythrin, phycocyanin) must not contain glycerol as this quenches the staining intensity. Similarly, exposure to excitation light of most fluorescent labels results in diminished staining, a process known as photo bleaching.<sup>[4]</sup>

Fluorescent mounting media commonly contain antifade agents that slow down the photo bleaching of such labels, such as 1,4-diazabicyclo[2.2.2]octane (DABCO) and paraphenylenediamine, which act as free-radical scavengers.<sup>[4]</sup>

Resinous media can only be used for enzymatic labels where the precipitate formed between the enzyme,

and the chromogen is not soluble in the alcohols used during dehydration of the tissue.<sup>[4]</sup>

Both quality and intensity of the fluorescence signal in most immunolabeled preparations after aqueous mounting such as polyvinyl alcohol-based solutions (e.g., Mowiol) slowly diminish with time and finally, samples become unsuitable for examination. To avoid this, Espada *et al.* described a very simple and rapid non-aqueous mounting procedure for cultured cells and tissue sections, which preserves the fluorescent signal in an excellent way after immunodetection. After fluorescence labeling, preparations are dehydrated in ethanol, cleared in xylene and mounted in DePeX. Using this non-aqueous mounting medium, the fluorescent signal remains high and stable, allowing a suitable and permanent preservation of labeled and counterstained microscopical preparations.<sup>[10]</sup>

Franklin and Filion used a new technique for retarding fading of fluorescence. The antioxidant beta-mercaptoethanol (BME) was used in conjunction with the permanent mountant DePeX (DePeX-BME) retarded fluorescent fading of mithramycin, acridine orange and Hoechst 33258 stained chicken erythrocytes, each to a varying degree. The initial fluorescence of all dyes examined was more intense with DePeX-BME than with DePeX alone. Specimens mounted in DePeX-BME showed strong fluorescence and excellent morphology. They also concluded that if kept in the dark, they could be stored indefinitely without deterioration. Retarding fading of fluorescence with DePeX-BME facilitated quantitation of deoxyribonucleic acid by using fluorescence cytophotometry.<sup>[11]</sup>

### Mounting media and fading

Fading of the images is one of the key factors in diagnosis difficulties and storage of slides for long duration with few of the mountants. Various mounting media tend to fade with time. Humphrey and Pittman used a simple cytophotometric technique to quantitate stain fading of basic aniline dye-stained epoxy-embedded tissues mounted in six different commonly used mountants. Significant fading was detected with all six mountants, although rates varied. The lowest rate of fading was observed with immersion oil and the highest rate of fading with Canada balsam.<sup>[12]</sup>

Schmolke conducted the study to find the most suitable medium for durable mounting of Araldite embedded

semithin sections of rabbit cerebral cortex stained with toluidine blue and pyronin G. Among four synthetic mounting media tested, only DePeX prevented fading of the sections during the 1<sup>st</sup> month. The average optical density of sections after 1 year was higher in preparations mounted with DePeX than in sections treated with the other mounting techniques tested in the study.<sup>[13]</sup>

Preventing the fading of fluorescence intensity caused by the excitation light is very important for obtaining stable and accurate images. Several types of mounting media are available (Gill, 1979; Johnson *et al.* 1981,1982; Giloh and Sedat 1982; Harris 1986; Krenik *et al.* 1989; Longin *et al.*1993), such as *p*-phenylenediamine (Johnson *et al.* 1981,1982; Platt and Michael 1983), *N*-propyl gallate (Giloh and Sedat 1982) and 1-4 DABCO (Johnson *et al.* 1982; Langanger *et al.* 1983).<sup>[14]</sup>

Ready-to-use anti-fading kits are also commercially available, such as Slow Fade Light Antifade Kit (Molecular Probes; Eugene, OR), Perma-Fluor (Lipshaw/Immunon; Pittsburgh, PA), FluoroGuard Antifade Reagent (Bio-Rad Laboratories; Hercules, CA) and ProLong Antifade Kit (Molecular Probes).<sup>[14]</sup>

Ono *et al.* compared several commercial and homemade antifade media, using a confocal laser scanning microscope coupled to a computer. They quantitatively measured fading of fluorescence to formulate an equation, evaluated the antifading ability of several antifading media and restored the faded images to the original level according to this equation. ProLong showed the highest antifading factor (*A*) value. It's *A* value remained high even under strong excitation. ProLong, however, has a low initial intensity of fluorescence (*EM1*) value. On the other hand, FluoroGuard showed the second highest *A* value and a relatively high *EM1* value.<sup>[14]</sup>

Staudt *et al.* demonstrate that a solution of 97% thiodiethanol (TDE) gives a perfect match for the  $n = 1.515$  RI of glass and immersion oil. Fixed biological specimens can be easily embedded in 97% TDE solutions, giving a uniform RI from the lens too deep in the specimen.<sup>[15]</sup>

### Combined coverslip and mountant

Several manufacturers supply a medium of a varnish-like nature, which may be used to coat the section

surface, by dipping, pouring or spraying. This type of medium obviates the use of coverslip. For low power microscopy, combined mountant and coverslip may prove quite satisfactory although little protection of the section to abrasion is given.<sup>[8]</sup>

### Mounting the sections

There are many ways to mount the coverslip on slides, but whatever way works for one is fine as long as there are no air bubbles formed.

- A. Slide method
- B. Coverslip method

#### Slide method

1. An appropriate size of coverslip for mounting is selected and laid on the blotting paper.
2. One or two drop of mountant is placed on the slide containing section preferably in the middle to avoid trapping of air bubbles.
3. The slide is quickly inverted over the coverslip, one end is placed on the blotting paper and the other end slowly lowered until the mountant touches the coverslip.
4. The mountant spreads under the coverslip and slides and with the coverslip attached, is quickly inverted and the coverslip guided into place with a dissecting needle.

Alternatively add the mountant on the slide as described,

Place one end of the coverslip on the slide and with the aid of a dissecting needle; slowly lower the coverslip into position.

#### Coverslip method

1. Add the mountant on the coverslip in the center.
2. Bring the slide down (invert) to the coverslip and let the surface tension pull the coverslip.

Use only enough mountant to fill the space on the coverslip/slide and not excess and this assessment comes with experience.

- Too little mounting media will cause air bubble at the edges of coverslip and one will be tempted to press down on the coverslip to ensure a tight seal. This pressure can crush or distort the three-dimension structures in the sample.
- Too much mounting media will make it messy and move the samples around and it can make the sample impossible to image at  $\times 100$  due to the

very short working distance of high magnification oil immersion objective lens.

Air bubbles: If there is one odd air bubble it may be removed with gentle pressure but if there are many, instead of chasing with a dissecting needle and wasting time, put the slide back in xylene so that coverslip is separated and remount the section without air bubbles.<sup>[5]</sup>

### Which mountant is the best one?

Gutierrez states that “No mounting media are fully satisfactory.” Most workers who wish their slide mounts to be permanent have prepared their own mountants and given “pet names” to it and not bothering to publish the recipe.

Many brands of mountants are and have been made to secret recipes whose names or recipes have been changed and have been copied by others. The conservator may be faced with the problem of not knowing what the deteriorating mountant consists of even if the name of the mountant is written on the slide as it may not be the published recipe.<sup>[2]</sup>

## CONCLUSION

To conclude various commercial manufacturers have various mounting media and fluorescent-free mounting media both in aqueous and resin forms. RI also plays an equal role in choosing the good mountant. Hence one should choose a mounting media that suits the viewing and preserving the required sections for further research.

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