Review Article

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Mounting media used in Histopathology and Laboratory : Roles and Functions : A Short Review.

Abstract:
Mounting is considered as very important step. Various mounting media are there like aqueous mounting media and resinous mounting media. Various researches are done to check which mounting media is best for mounting the histopathological tissues and suitable for different types of microscopy. The mounting media helps in holding the tissue sample in between the slide and the cover slip. The refractive index of the mounting media is also an important factor. In this review article, we are trying to review the various types of the mounting media with special emphasis on their .The data was acquired from earlier published literature and also from searching the different database like Pubmed and Google Scholar.

Key words: Mounting medium, Aqueous mounting medium, Resinous mounting medium

Introduction:

After the biopsy of the tissue, the biopsied sample is sent to histopathological lab. In histopathologial lab, this biopsied tissue underwent various steps like grossing of sample, fixation and processing of the sample[1]. In the last step, the tissue is mounted onto the glass slide with the help of media which is known as mounting media. This step of mounting the specimen onto the slide is considered as crucial as well as important because mounting step is important for seeing a nice and clear image of the biopsied sample under the microscope. Also it helps in preservation of the sample for long time[2].

Mounting media lies in between the coverslip and tissue section. So it is important to select the ideal mounting media which gives the best result when used. Using inappropriate mounting media may lead to abnormal image formation, which may lead to wrong or poor histopathological diagnosis. Mounting media which have refractive index closed to the glass is considered as best mounting media[3].

Representative or ideal properties of mounting media[4]:

A. Mounting media should have refractive index equal to or near to 1.5.

B. It should be transparent as well as colourless.

C. It should not cause's fadeness to stain.

D. It should harden quickly.

E. It should be resistant to contamination.

F. It should have capability to permeate the tissue easily.

G. It should not cause any adverse effect on tissues.

H. It should not shrink from the side or edge of the coverslips.

I. It should set without shrinking and cracking.

J. It should be stable.

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Composition of Mountants[5]:

The mountant is made up of various contituents. It includes

A. Base

B. Antifade agents

Various base constituent of mounting media are aqueous, natural oil, gylcerol, plastic

Various antifade constituent are NPG (N-propyl gallate), 4-POBN (4-Pirydyl-1-oxide)- N- tert-butyl nitrone), DABCO (1,4-diazabicyclo[2.2.2]octane, PPD (P-phenylanediamine).

Classification of Mounting Media[2] :

The mounting media is classified into 2 types :

A. Aqueous mounting media

B. Resinous mounting media

A. Aqueous mounting media[2] :Most of the aqueous mounting media have low refractive index i.e. 1.4 to 1.42.

The aqueous mounting media are of 3 types :

A. Gum arabic media

B. Gelatin media

C. Syrups

It is important to note that in gum arabic media and gelating media type of aqueous mounting media, glycerine is homogenize so as to prevent the cracking as well as splitting of the aqueous mounting media[2]. For prevention of fungal growth in aqueous mounting media,bacteriostatic agents are added like thymol crystal, phenol, sodium merthiolate.

B. Resinous mounting media :

Resinous mounting media are natural resins or synthetic resins which is dissolved in benzene, xylene or toluene. When long lasting mount is needed, resinous mounting media is used. Also, resnious mounting media are regularly used during regular hematoxylin and eosin staining procedures[6].

Various resinous mounting media are :

Natural resinous media : Canada balsam, Euparal, Phenol and dammar balsam, Castor oil,

Synthetic resinous media : DPX, Eukitt, Permount, Cytoseal 60, Polyglass, Plastic UV mount mounting media, CMCP macro invertebrate medi, Photosensitive resins, Resin-embedded tissue

AQUEOUS MOUNTING MEDIA::

A. Water:

Refractive index of water is 1.33. For some specimens (for demonstrating live microorganisms), water can act as temporary mountant media .

B. Glycerine-Glycerol:

It is one of the commonly used aqueous mounting media. It is a combination of glycerol and gelatine. The refractive index of glycerine-glycerol is 1.47.This mounting media is safe and economical[4].

C. Glycerine Jelly:

Glycerine jelly is known as one of the standard mounting media for fat stains. For preparing the glycerine jelly mounting media, gelatin is dissolved in the distilled water in conical flask and then mix glycerine and phenol and stored it[2].

D. Aqua-poly mount :

It is non-fluorescing water: soluble mounting media which is made for mounting the sections from aqueous solutions. As Aqua-poly mount mounting medium intensifies the fluorescent stains, this stain is very advantageous for immunofluoroscent techniques. The refractive index of aqua-polu mount mounting media is 1.45 to 1.46

E. Farrant's Medium:

The farrant's medium is prepared by mixing the gum arabic in distilled water with soft heat. After that add glycerine as well as arsenic trioxide[2].The refractive index of Farrant's medium is 1.43

F. Fluorsave:

The Fluorsave mounting media have very good antifade properties, that's why it is highly endorsed in immunofluoroscent microscopy[7].

G. Highman's Medium:

The refractive index of Highman's mounting media is 1.52. This mounting media is used in metachromatic dyes like methyl violet.

H. Apathy's medium:

The refractive index of apathy's medium is 1.52. It is used for mounting of fluorescent microscopy sections.

I. Polyvinyl Alcohol:

As an substitute for glycerine jelly, polyvinyl alcohol is favored. This mounting media is used in immunofluoroscence microscopy.

J. Thiodiglycol (2,2'-thiodiethanol, TDE):

It is recent water soluble mounting medium. They are used in high resolution optical microscopy[8] .

RESINOUS MOUNTING MEDIA:

I. Natural resinous media:

A. Canada balsam :

Canada balsam is an oleoresin which is obtained from bark of Abies Balsamea (belongs to family of pinaceae). The dried resin is easily soluble in xylene and some other organic solvents. Earlier the Canada balsam were used as medicine for common cold. But later it is using as mounting medium[9,10].

Andrew pritchard in the year 1830 was the first who described the canada balsam as satisfactory and convinient mounting medium, which is used in transmitted light microscopy. It is most widely used mounting media and it does not crystallise or absorbs the moisture.

Eastop noticed that canada balsam slides of aphids which is made by Francis walker in year 1847 displayed no signs of degradation except for yellowing[11]. According to Mound and Pitkin, canada balsam is only mounting medium which is known not to disintegrate even if kept for number of years in different climate conditions[12]. Noyes also advices that canada balsam has longevity of several million years[13].

Hood,who have experience of mounting more than 50,000 sides also states that canada balsam mounting media was the only mountant to use[14]. According to some workers, canada balsam deteriotes in the form of crazing, which may be due to inappropriate preparation of specimens or either by absolute issue in minorty of balsam mount[15].

Raw canada balsam is made up of various unsaturated compounds. These compounds are supposed to bleach various stains like prussian blue[16,17]. The acids that are present in the canada balsam causes cationic stains to fade. Also the acids leads to dissolution of the carbonate structure[18,19]. One of the biggest advantage of canada balsam is that it lasts for atleast 150 years on slides. Also, it does not deteriorate in different types of climates[20,12].

It is important to note that canada balsam yellows as well as darkens with age. The darkening starts from periphery, which suggest that there is impact of oxygen diffusion[21,22]. Clearing in clove oil may also causes darkening. Extra darkening as well as blackening may result from the presence of phenol in resin[23,24,20].

B. Euparal:

In 1904, Prof.G.Gilson made up mounting medium which is known as Euparal. This mounting medium contains sandarc, camphor, paraldehyde, eucalyptol and phenyl salicylate. Because of the presence of the natural oils in Euparal, it smells nice. Euparal is safe, easy to use, optically good and have low refractive index i.e.1.48

The main advantage of using Euparal is that tissue specimens can directly be transferred from alcohol to Euparal. On the other hand, the major disadvantage is that it takes very long time for dry and sometimes fading may be seen in hematoxylin stained sections. In case of fading, use of green (or vert) copper-containing form of Euparal is recommended[25].

Euparal is considered as better or alternate option of mounting medium than canada balsam because euparal does not use xylene,a carcinogenic solvent[20]. According to Rawlins, Euparal is more popular than canada balsam as permanent mounting media[26].

According to Imms, Euparal will not yellowing with time as canada balsam and have low refractive index[27]. According to Hood, Euparal mounting medium is unacceptable as Euparal instantly developed a meniscus, which further deteriorate the fine structure when specimen is placed in the mountant. Bubbles take long time to clear. Moreover the slides were susceptible to crystallization[14].

C. Castor oil:

In the medical field, pure castor oil is used for its beneficial healing properties. Also, castor oil is used in manufacturing of dyes, lubricants, paints and resins. Castor oil has good refractive index i.e. 1.47 -1.48.based on the accuracy of cellular characteristics, castor oil exhibit remarkable quality as a mounting media. It is important to note that the castor oil did not adhere to glass slide[28].

D. Phenol and Dammar balsam:

Because of presence of impurities and dirts, phenol and dammar balsam are rarely used. The refractive index of phenol and dammar balsam is 1.52-1.54[2].

II. Synthetic resinous media:

A. DPX:

DPX is a neutral mounting medium which is colorless and most of the basic as well as common stain are well preserved in the DPX mounting medium. DPX is known as a admixture of distyrene, plasticizer and xylene. DPX mounting media sets quickly. DPX is mostly used as a routine mountant. The excess DPX mountant on the slides can be easily cleaned off by stripping it around the coverslip edge[2,4,20].

B. Eukitt :

Eukitt is resin based mounting medium which dries very fast. It will amalgamate within 20 minutes. Prior to the mounting with Eukitt mounting medium, the specimen sample should be free from water and should be placed in alcohol then in xylene . For diluting the Eukitt mounting medium, xylene can be added to maintain its viscosity[25].

C. Permount:

Permount mounting medium is toluene based synthetic resin medium. Permount mounting medium has lower viscosity and it sets faster than DPX[7].

D. Cytoseal 60:

Cytoseal 60 is toluene based mounting medium which is economical and have low viscosity.The base ingredient is Polymethylmethacrylate. But this mounting medium when dries forms the bubbles[7].

E.Polyglass:

Poly glass mounting medium is liquid acrylic resin. The refractive index of poly glass is 1.48 . By soking the slide in toluene or xylene, it can be easily removed[29].

F. Photosensitive resins:

Photosensitive resins mounting medium is suitable for fluorescence microscopy.

OTHER MOUNTING MEDIA:

A. Formaldehyde:

It have been used in 19th and 20th century as mounting media for mounting various specimen like rotifers. Formaldehyde have high vapour pressure[30,31]. Formaldehyde can be counterbalance by using calcium carbonate, sodium bicarbonate or pyridine. But as per Gray and Sanderson, they suggested that formaldehyde should not be buffered because buffer salts might preceipitate[32,33,34]

B. Lactophenol:

Lactophenol is used as a mounting media, but is less appropriate because its components i.e. Lactic acid and phenol have huge vapour pressure[35,36,37].

C. Celodal :

The Celodal mounting medium is introduced in the year 1938 for mounting the macroscopic specimens. This is based on urea-formaldehyde polymer[38]. Celodal is converted to celochloral by addition of glacial acetic acid, chloral hydrate and glucose. It has been observed that rhomboid shape crystals will form in storage bottle in few months. Milky cloudiness may occur because of excess water in medium.

The microscopic slides which is mounted with Celodal lasts for minimum 4 years. By putting the slides for some hours in hot water, the cross-linking of the polymer will dissolved. Also, by using concentrated acids as well as alkalis and molten phenol, the cross-linking will dissolved[38,39].

D. C-M Medium

C-M mounting medium or Clark and Morishita medium was introduced in 1950. This medium is made up of methocellulose, ethanol, diethylene glycol, carbowax, water and lactic acid[40].

E. Coumarone:

Coumarone was introduced in the year 1933 as mounting medium by Perruche. Coumarone mounting mediun was composed of naphthalene, which with time become yellow in color[41,42].

F. Hyrax:

Hyrax was named originally as A.F.S. Because it was manufacture from aniline, formaldehyde as well as sulfur. The Hyrax mounting medium is easily soluble in toluene, benzene and xylene. On drying, Hyrax becomes brittle which results in easy detaching of cover slip. With time, Hyrax may get blacken[43,44].

G. Naphrax™ :

It is one of the mounting medium which is appropriate for diatoms study. The refractive index of Naphrax™ is 1.65. On drying, Naphrax™ becomes very brittle, which results in easy detach of coverslip. With time crystallization also occurs. Due to moisture, precipitations as well as cloudiness of Naphrax™ occurs[20,44].

H. Styrax

This mounting media was introduced by Van Heurck in 1883. It is prepared from raw resin. On heating, Styrax mounting medium becomes yellow in color[45].

Discoloration in mounting media:

Discoloration of mounting medium occurs because of residual preservatives. These residual preservatives includes formaldehyde and phenols. So it highly recommended that slide should be washed thoroughly after fixation and before mounting[46].

Various agents like phenol present in canada balsam, clearing agent like clove oil are answerable for blackening. Chloral hydrate also causes blackening[24]. Degradation of phenol balsam begins at the periphery of cover slip. It indicates involvement of oxygen. A mixture of ethanol, phenol and canada balsam becomes dark in three weeks[47].

CONCLUSION :

Mounting medium is important for mounting the samples and also to stable the samples on the slide. Refractive index also plays an important role in selecting the mounting media.

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