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# MODIFIED MASSON'S METHOD OF STAINING TISSUES- AN EXPERIMENTAL MICROANATOMY STUDY

Anatomy

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## ABSTRACT

Staining of the the tissues is an art which gives fruitful results over a period of time. Many researchers have used various stains, some are costly and some are economical. Special stains like Mallory's trichrome and Massson's trichrome are routinely used for staining various tissues. As few ingredients of Masson's Trichrome were not available, we replaced certain ingredients which were not available by readily available ingredients on an experimental basis and a study was carried out. We used Ponceau S instead of Ponceau 2R and Ehrlich's Haematoxylin instead of Weigert's Iron Haematoxylin for staining paraffin sections of tongue, pituitary gland, colon, adrenal gland and hyaline cartilage. Tissues were collected from the cadavers allotted for first year MBBS students for dissection at Dr B R Ambedkar medical college, Bangalore. After processing the tissues, paraffin embedded blocks were prepared and the sections were taken and later washed in tap water followed by staining with special stains with certain ingredients replaced due to non availability. After that, sections were washed by water, dehydrated in graded alcohol, cleared in xylene and mounted in DPX and the Photographs of the slides were taken through the microscope by focusing both under low power and high power. In routine Masson's Trichrome stain, Ponceau S was used instead of Ponceau 2R because of it's non-availability. Further the cost of Ponceau S is very much lesser than Ponceau 2R. Also Ehrlich's Haematoxylin was used instead of Weigert's Iron Haematoxylin. We obsereved that various components like nuclei, cytoplasm, muscle, acidophil and basophil granules, collagen, cartilage, mucin were all well differentiated. On an experimental basis, we replaced the components Ponceau 2R by Ponceau S and Weigert's Iron Haematoxylin by Erhlich's Haematoxylin in Masson's Trichrome method of staining and various tissues were stained with good results.

## KEYWORDS

Masson's Trichrome, Mallory's Trichrome,Ponceau S, Ponceau 2R, Erhlich's Haematoxylin, Weigert's Iron Haematoxylin

## INTRODUCTION

Procedures for the differential staining of connective tissue fibres and muscle are an important part of histological technique and their use is often helpful in the diagnosis of pathological changes in the tissues. Because of this, many methods have been described for the demonstration of these components, some of them selectively staining different types of fibres by the use of several dyes in combination or in sequence.[1]

The most commonly employed method for demonstrating connective tissue is that of Van Gieson, either as a counter stain with Iron Haematoxylin, or following on the elastic fibre stains .Of the many methods available, Heidenhain's AZAN stain or Lillie's allochrome method probably give the best and most consistent results, but this is largely a matter of personal preference,and both Masson and Mallory Trichrome techniques are very popular.[2]

Special stains like Mallory's trichrome, Masson's trichrome were being routinely used in the histology department of our college.While staining the tissues by Masson's Trichrome, it was found that one of the ingredient ponceau 2R was not available, instead Ponceau S which is usually used for Von Gieson's stain was easily available. Just on an experimental basis, we used Ponceau S instead of Ponceau 2R. Similarly because of easy availability of Ehrlich's Haematoxylin, we replaced Weigert's Iron Haematoxylin by Ehrlich's Alum Haematoxylin. So these two methods where in Ehrlich's Alum Haematoxylin and Ponceau S in one method and Weigert's iron haematoxylin and Ponceau S in other method were used. Various tissues were stained by these two methods and are thus being presented as Modified Masson's method of staining of tissues.

## MATERIALS AND METHODS

Tissues like tongue, pituitary gland, colon, adrenal gland and hyaline cartilage were collected from the cadavers allotted for first year MBBS students for dissection at Dr B R Ambedkar medical college, Bangalore. Dehydration of the tissues was done using graded alcohol, followed by clearing with xylene before impregnation with molten paraffin wax which was subsequently allowed to solidify by cooling. Serial sections were taken using Rotary Microtome and were mounted on the slide and dried in an oven.

Materials used were - Routine Masson's Trichrome stain,where in Cytoplasmic stain(Ponceau S) was used instead of Ponceau 2R and Nuclear stain (Ehrlich's Haematoxylin) was used instead of Weigert's Iron Haematoxylin. Methodology-Formalin fixed sections were mordanted for up to three hours in saturated alcoholic picric acid containing three percent mercuric chloride followed by thorough washing to remove picric acid staining. Sections were washed by water, Nuclei were stained with Ehrlich's Haematoxylin instead of Weigert's Iron Haematoxylin, after washing well with water, nuclear stain was differentiated using 0.5 percent Hydrochloric acid in 70 percent alcohol. Later, washing well in tap water and rinsing in distilled water. Then stained in cytoplasmic stain (Ponceau S) instead of ponceau 2R.rinsed in water and counter stained with either aniline blue or light green for 2 to 5 minutes. Then washed well in one percent acetic acid for at least one minute, blot, dehydrated in absolute alcohol ,cleared in xylene and mounted in DPX and the Photographs of the slides were taken through the microscope by focusing both under low power and high power.

## RESULTS

We observed that various components like nuclei, cytoplasm, muscle, acidophil and basophil granules, collagen, cartilage, mucin,were all well differentiated. In routine Masson's stain, Ponceau S was used instead of Ponceau 2R because of it's non-availability. Further the cost of Ponceau S is very much lesser than Ponceau 2R. Further Ehrlich's Haematoxylin was used instead of Weigert's Iron Haematoxylin.[Table 1] We came across various tissues like tongue,pituitary gland, colon, adrenal gland and hyaline cartilage, wherein nuclei appeared bluish purple and blue black. Cytoplasm, muscle, acidophil granules stained red. Collagen fibre, cartilage, mucin and basophil granules appeared blue or green depending on the counter stain.

**TABLE – 1**
**Weigert's Iron Haematoxylin Versus Ehrlich's Alum Haematoxylin**

| Tissue Components | Mod. Masson's Trichrome (Weigert's Fe. H) | Mod. Masson's Trichrome (Ehrlich's Alum. H) |
|---|---|---|
| Nuclei | Blue black | Bluish purple |
| Cytoplasm, muscle, acidophil granules, RBC's | Red | Red |
| Mucin, collagen, cartilage, basophil granules | Blue | Blue |

## DISCUSSION

Weigert's Iron haematoxylin is employed for Masson's Trichrome method of staining. Since it stains nuclei black, resists removal by counter stains containing differentiating agents such as picric acid, and will not be decolourized by light, it is therefore a more permanent stain than the Alum Haematoxylins. Ehrlich's Alum Haematoxylin retains its staining power for years and lasts for months in a coplin jar.[2]

In the present study, we replaced Weigert's Iron Haematoxylin with Ehrlich's Alum haematoxylin, because of its easy availability. Ponceau 2R is a red azo dye soluble in water, insoluble in ethanol, contrasting stain usually used in Masson's Trichrome method of staining. Ponceau S [$C_{22}H_{12}N_4Na_4O_{13}S_4$] is a polyazide, acid used for Von Geisson's stain and used for electrophoresis.[3]

In the present study, we replaced Ponceau 2R with Ponceau S, as Ponceau S was easily available and economical compared to Ponceau 2R. Cost Effectiveness of replacing Ponceau 2R by Ponceau S is as follows: Ponceau 2R – 25gms – Rs.2,500.00 and Ponceau S – 25gms – Rs.450.00.

Masson-Fontana method utilizes Fontana's silver solution does not employ a reducing bath and will thus only demonstrate substances capable of Silver-salt reduction. Whilst it is not specific for argentaffin cells, it does gives a clearer background than the Bodian technique.[4]

Nuclei are demonstrated using preformed metal complex stains,such as Weigert's Iron Haematoxylin or Iron Celestine blue-Aluminium haematoxylin. Alternatives for the first acid dyeing step include the following commercially available dyes-Acid Fuchsin, Biebrich scarlet, Bordeaux Red, Chromotrope 2R and Nitrozine Yellow.[5]

Masson's Trichrome proved to be a very useful stain to delinate the follicular units. The distribution of hair follicles in follicular units was only present above the insertions of arrector pili muscles at the infundibulum and isthmus.[6]

The spectral fingerprints of Masson's Trichrome stain distinguished the nucleolus from the rest of the nuclear chromatin enabling the demarcation and calculation of the nucleolar area. Spectrally resolved imaging of human hepatocytes stained by Masson's Trichrome stain revealed marked differences between the nucleolar area in human hepatocytes compared with hepatocellular carcinoma.Masson's Trichrome stain also distinguished the nucleolar area in human breast carcinoma cells and keratinocytes.[7]

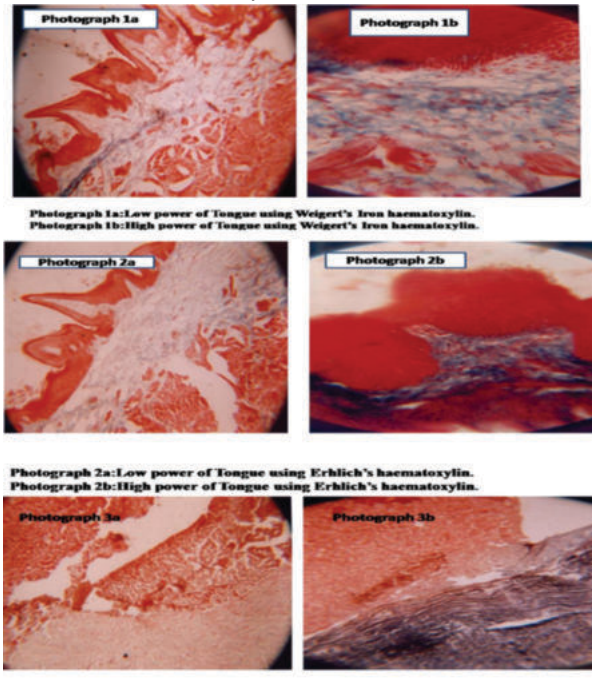


**Figure 1: Microscopic features in tongue using Erhlich haematoxylin & pituitary gland using both Haematoxylin**

Immunohistochemical techniques employing anti-S-100,HMB-45 antimelanoma antibody and Fontana-Masson's staining revealed selective photothermal damage of basal melanin granules, pigmented basal melanocytes and epidermal keratinocytes as well as pigmented superficial dermal melanocytes.[8]

The Trichrome method of staining undecalcified tissues according to Masson's is adjusted for staining decalcified bone sections. Based on preservation of the affinity to staining of the calciphylaxis zones after their decalcification. Adapted Masson's method stains mineralized bone (blue) and an osteoid (red).[9]

Anticollagen monoclonal antibodies to collagen types I, III and IV were used in control ureters and refluxing megaureters. Additionally, all were stained with Masson's Trichrome to further define the extracellular matrix.[10]

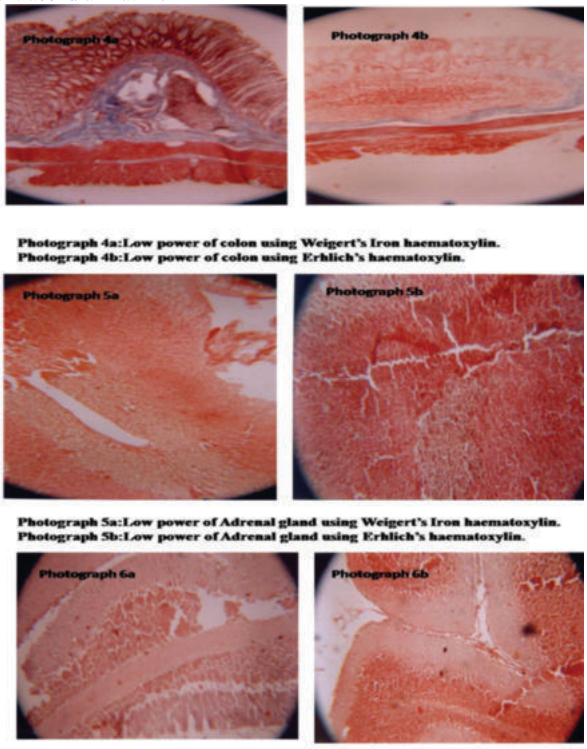


**Figure 2: Microscopic features in colon, adrenal and cerebellum using Weigert's Iron Haematoxylin and Ehrlich's Alum Haematoxylin.**

Cross sections of upper, middle and lower ureteral segments from human autopsy specimens and animals were evaluated after preparation with Masson's Trichrome stain. The epithelium was remarkably consistent across all species, both morphologically and in terms of absolute size.[11]

Although the histology of Duputyren's tissue is well documented, conventional stains do not distinguish between the different types of collagen which biochemistry and immunochemistry suggest are present. Duputyren's specimens were stained with haematoxylin and eosin, Van Gieson's, Mallory's, Masson's and Herovici's picropolychrome stain and examined for both cellularity and collagen staining characteristics. All stains illustrated the marked cellularity of the nodules, contrasting with a paucity of cells within the cords.[12] Collagenous material identified by Masson's Trichrome stain was distributed mainly in the invasive area of human colon cancer, where type I and type III collagens and chondroitin 4-sulfate stained intensely in the periacinar area, and further fibronectin stained.[13]

The accuracy of Masson's Trichrome stain to predict the presence of immune complexes was determined in 63 renal biopsies. The commonest error in their assessment of the Masson's Trichrome stained specimens was the erroneous interpretation of specimens having minimal change nephropathy or ischemic glomerulopathy by the immunofluorescence and electron microscopy.[14]

Masson's Trichrome stain [Modified Mallory:Masson's 1929]Biebrich Scarlet –Acid Fuchsin solution, phosphomolybdic-Phosphotungstic acid solution, Counter stain with either light green or Aniline blue. Mature collagen appears blue or green depending on counter stain and newly formed collagen appears red. Muscle and keratin appears red and nuclei appears blue.[15]

Masson's triple stain was used to stain the interscapular brown adipose tissue in male, white wistar rats in the 1,3,6,15,30 and 60th day of regeneration of adrenals after their enucleation was investigated.The initiation of the endocrine activity of the brown adipose tissue in the course of regeneration of the adrenals proceeds probably without the direct participation of catecholamines originating from the adrenals.[16]

## CONCLUSION

On an experimental basis, we changed the components used for Masson's Trichrome, we replaced Ponceau 2R by Ponceau S and Weigert's Iron Haematoxlin by Erhlich's Haematoxylin. Various tissues were stained by this modified method and better differentiation of various components in a tissue is appreciated. If all the tissues are better differentiated by this method, then it can be used as a Standardized procedure and this study will be continued in our laboratory.

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