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Original Research Article

MV10B Stain as a Broad Spectrum Stain in Histology - A Substitute for Haemotoxylin & Eosin Stain

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ABSTRACT

A simple cost effective, single stain, rapid method for the differential staining of structures of all the systems in histology including central nervous system applicable to paraffin sections is described.
Several human tissues were fixed in 10% buffered neutral formalin. Paraffin sections were cut at 6 microns, slides were dewaxed, hydrated, and brought to water. Slides were stained for 3 minutes in the following staining solution.
0.25 grams of MV10B stain dissolved in 100 ml of autoclaved distilled water and filtered. Stained slides were washed in 95% alcohol, dehydrated, cleared and mounted in DPX. In central nervous system slides myelinated fibres and nerve cells were stained in varying shades of violet colour with a good colour value.
In all the other systems slides the structures were well differentiated, gives an adequate contrast for observation and photography.
As such this stain can be used as a broad spectrum stain in histology for all the systems. This single stain can be used as a substitute for heamotoxylin and eosin stain.
Key words: MV10B stain, paraffin sections, central nervous system, systems in histology.

INTRODUCTION

The purpose of staining is to render prominence to the different tissue elements, particularly cells, so that they may be recognized and studied under the microscope. The usual routine histological stain is haemotoxylin and eosin stain. It is a two die staining method. Haemotoxylin for the nuclear staining and eosin for the cytoplasm. Though this method is a standard technique which gives good results, it requires lot of steps including differentiation, blueing, etc.

There has been a search for suitable dyes particularly as a replacement for allum haemotoxylin nuclear staining. For cytoplasm a separate counter stain has to be used like eosin. The use of Heidenhain's Azan stain as a broad spectrum stain in histology has been reported (1996). [1] Mona M Everette & William A Miller used this stain for embryonic and foetal material

(1973). [2] R A Schumperili used this stain for insect nervous tissue (1977). [3] A paper has been presented using this stain for male reproductive system (1999). [4]

The Azan stain gives good results. It is a triple stain- Azocarmine G, Orange G, & Anline Blue are the dyes used in this stain. It gives a very good colour value. It involves lot of steps including higher temperature staining of Azocarmine G & Mordant. The staining procedure is time consuming. Considering all this we have evaluated many dyes and identified a single stain MV10B which can be used to stain all the structures. And this can be a substitute for haemotoxylin & eosin stain.

MATERIAL & METHOD

Several human tissues including central nervous system were fixed in 10% buffered neutral formalin. Paraffin sections were cut at 6 microns.

Preparation of the stain:

MV10B stain 0.25gms,
Autoclaved distilled water-100ml
Dissolved and filtered with Whatmann filter paper & stored in a brown bottle at room temperature.

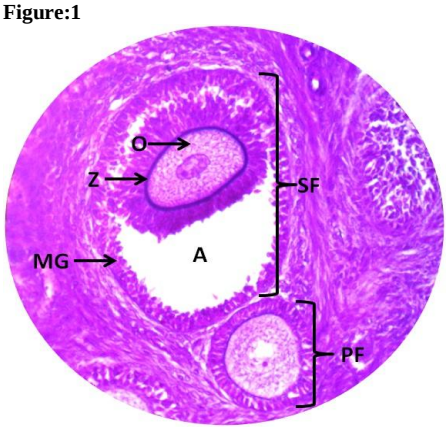
Staining Procedure:

1.Bring sections to water through xylene and alcohols.
2.Place in staining solution for 3 minutes
3.Rinse in 95% alcohol
4.Dehydrate clear & mount.

RESULTS

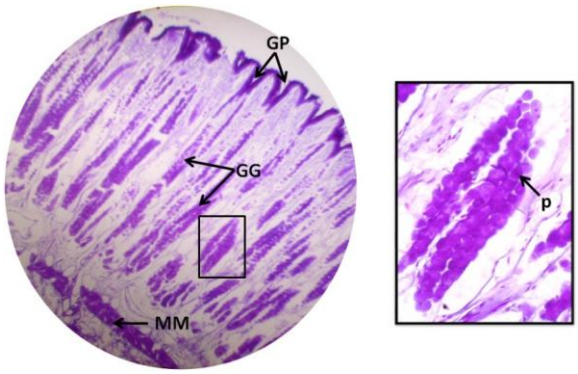
In central nervous system slides, myelinated fibres and nerve cell bodies are stained in varying shades of violet colour with a good colour value. Other systems slides structures were well differentiated gives an adequate contrast for observation. Some of them shown below are of different systems such as
Ovary-Reproductive system(shown in fig.1),
Stomach fundus – Digestive system(shown in fig.2),
Thin Skin –Intergument system(shown in fig.3) ,
Cartilage –Circulatory system(shown in fig.4),
Pituitory-CNS(shown in fig.5),
Nerve-Nervous system(shown in fig.6),
Jejunum-Immune system(shown in fig.7).

Figure:1



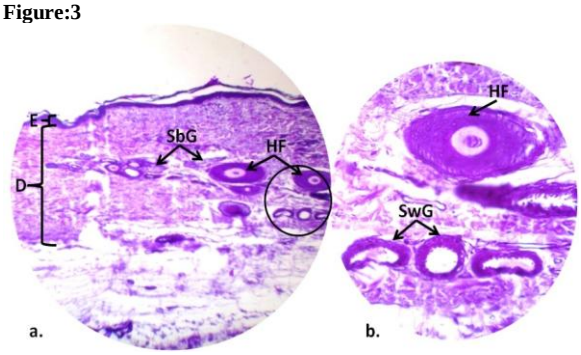
Ovary (x400): SF -Secondary follicle, PF - Primary follicle, O - Oocyte with nucleus, Z - Zona Pellucida , A - Follicular antrum, MG - Membrana Granulosa

Figure:2



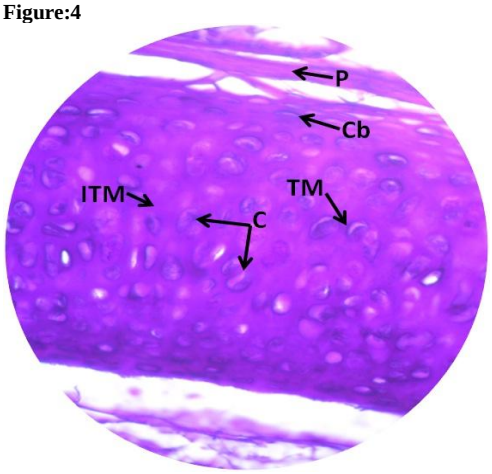
Stomach Fundus (x100): GP - Gastric Pits, GG - Gastric Glands, MM - Muscularis Mucosae. Inset (x400): High magnification of rectangular area shows P - Parietal cell

Figure:3



a.) Thin Skin (x100): E - Epidermis, D - Dermis, SbG - Sebaceous gland , HF - Hair Follicle.
b.) Inset (x400): SwG - Ducts of sweat gland , HF - Hair Follicle

Figure:4



Hyaline Cartilage (x400): P - Perichondrium, Cb - Chondroblasts, C - Chondrocytes in lacunae, TM - Territorial Matrix, ITM - Interterritorial matrix

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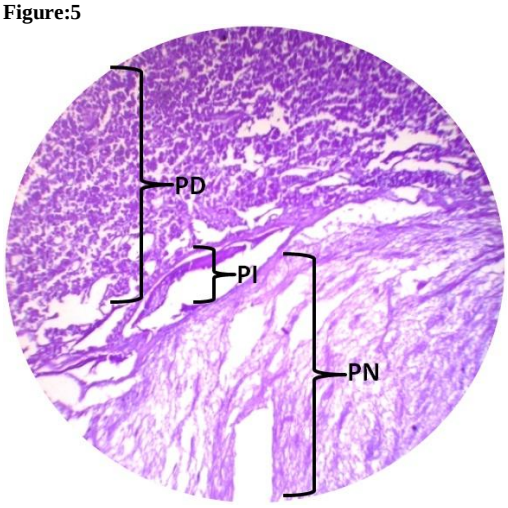
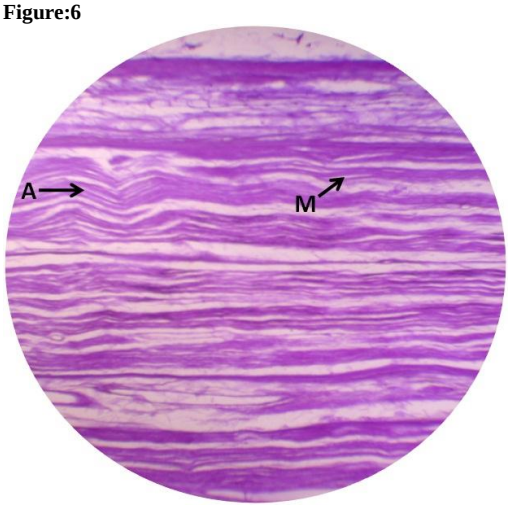
Figure:5



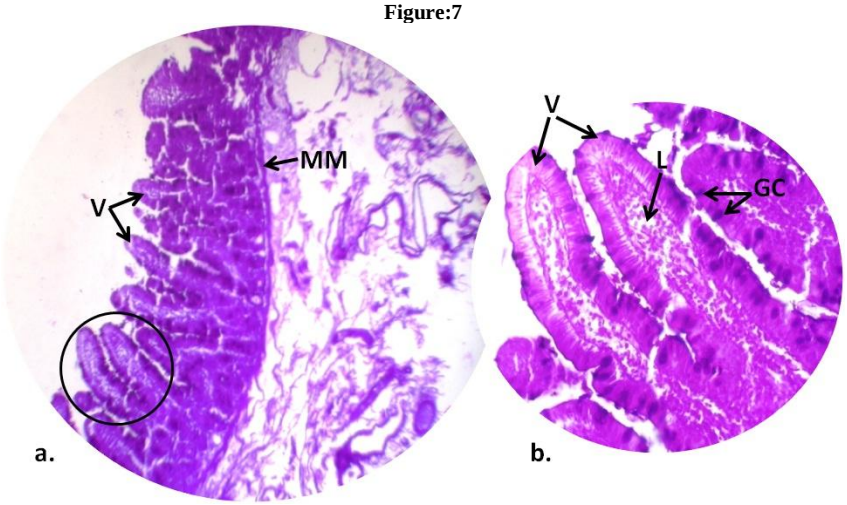
Pituitary Gland (x100): PD - Pars Distalis, PI - Pars Intermedia, PN - Pars Nervosa

Figure:6



Peripheral nerve LS (x100): A - Axon, M – Myelin

Figure:7



a.) Jejunum(x100): V - Villi, MM - Muscularis Mucosae.
b.) Inset (x400): V - Villi, L - lacteal, GC - Goblet cells

DISCUSSION

There has been a search for suitable substitute dyes particularly as a replacement for haemotoxylin. Lillie et al (1973) recommended Celestin blue B, Gallocyanin, and Gallatin as suitable. [5] Llewellyn [6] (1974) & Hogg & Simpson[7] (1975) suggested Mordant Blue 3 (Chromoxane cyanin, eriochrome cyanin RS, Solochrome cyanin R, Alizarol Cyanin R –C.I 43820). Lillie, Pizzolato & Donaldson (1976) evaluated many dyes and said that Celestin blue B, Phenocyanin TC, Gallain, Fluorone black, Alizarin cyanin BB, and Alizarin blue S were all suitable. [8]

Trichrome stains are staining methods in which three anionic dyes are used, in conjunction with either phosphomolybdic acid (PMA), phosphotungstic acid (PTA), or a mixture of these heteropolyacids. Probably the first trichrome method was that of Frank B Mallory, an American pathologist, first published in 1900. [1] Unfortunately, none of Mallory's publications (they go from 1891 to 1938) provide any explanation of the rationales of either his trichrome or his phosphotungstic acid-haematoxylin (PTAH) method. Nobody knows why Mallory introduced heteropolyacids into microtechnique.Mallory's trichrome method, using acid fuchsine followed by a solution containing PTA, orange G and aniline blue, provides dark red nuclei, orange erythrocytes, and blue collagen fibres, cartilage matrix and mucus.. In 1915, M. Heidenhain introduced azocarmine G in place of the acid fuchsine of Mallory's method. Heidenhain also introduced visually controlled destaining to provide for different colours in cell nuclei (dark red), collagen (blue) and a variety of colours in cytoplasm. [9]

The present technique is very simple, highly cost effective single stain which stains all the structures, gives adequate results. This can be used as a broad spectrum stains and a substitute for haemotoxylin and eosin for routine and research work.

Methyl violet is the family of organic compound that are mainly used as dyes. Depending on the amount of attached methyl groups the colour of the dye can be altered. Its main use is as the purple dye for textiles and to give deep violet colours in paint and ink. Methyl violet 10B is also known as crystal violet. (and many other names) and has medical uses.

Methyl violet 10B has six methyl groups. It is known in medicine as gentian violet (or crystal violet or pyoctanin (e) & is the active ingredient in gram stain used to classify bacteria. It is used as a Ph indicator with a range between 0 and 1.6. The protonated form (found in acidic conditions) is yellow terming blue violet above Ph levels of 1.6. Gential violet destroys cells and can be used as a disinfectant. Compounds related to methyl violet are potential carcinogens. Methyl violet 10B also inhibits the growth of many gram positive bacteria except streptococci, when used in conjuction with nalidixic acid (which destroys gram negative bacteria). It can be used to isolate the streptococci bacteria for the diagnosis of an infection.

Methyl violet 10B also binds to DNA. This means it can be used in all viability assays in biochemistry. However this binding to DNA will cause replication errors in living tissue, possibly leading to mutations and cancer. [10]

CONCLUSION

We have identified MV10B stain as broad spectrum stain in histology and a substitute for haemotoxyln and eosin stain after evaluating many dyes. The technique is very simple, highly cost effective, rapid giving consistent results. This can be used in routine and research work.

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