

# Study of Hematoxylin and Eosin dye and it's Alternatives in Oral Cancer Detection

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**Abstract**— One of the most common and prominent stains in histology is Hematoxylin and eosin stain also spelled as haematoxylin and eosin stain and abbreviated as H&E stain or HE stain. It is used as the gold standard in staining the biopsy tissue for detection of presence of malignant cells. Use of H&E stain has been more than a century in histological cancer detection. In this paper we give the introduction on H&E stain. We also discuss the procedure of applying this stain and provide the alternative to H&E dye.

**Index Terms**— Hematoxylin and Eosin, Oral Cancer, staining, pathology.

## I. INTRODUCTION

H&E stain is comprising of two dyes viz., haematoxylin and eosin. These two stains were independently invented by Böhmer in 1865 and Fischer in 1875 respectively. In 1876, Wissowzky presented the combination of haematoxylin and eosin as a tissue staining procedure. The eosin normally stains the extracellular in red color where as hematoxylin stains nucleic acids, nuclei, and other cellular components (such as keratohyalin granules) blue in color. Haematoxylin does not stain unless it is oxidized in a process called ripening to yield haematein, which is then combined with certain chemicals called mordants [2]. The colour of the dye can be altered by mixing it with other agents such as iron alum (black), potash alum (blue) and salts of tin (red) [3]. Of these two dyes, Hematoxylin is used first and then Eosin is used as the counter stain for Hematoxylin.

## II. COMPOSITION OF DIFFERENT TYPES OF HEMATOXYLIN AND EOSIN SOLUTIONS

Depending on the mordants used the stain color differs. Specific information on the mordants[6] for Hematoxylin is provided in the table 1.

Different Hematoxylin Solutions are (A) Ehrlich's hematoxylin having the composition of hematoxylin-2g, Absolute alcohol-100ml, Distilled water-100ml, Glycerin-100ml, Glacial acetic acid-10ml and Potash alum-15g (B) Mayer's hematoxylin composing of Hematoxylin-1g, Distilled water-1000ml, Potash alum-50g, Sodium iodate-0.2g, Citric acid-1g, Chloral hydrate-50g (C) Hariss's hematoxylin, a mixture of Hematoxylin-2.5g, Absolute alcohol-25ml, Distilled water-500ml, Mercuric oxide-1.5g, Glacial acetic acid-20ml, Potash alum-50g (D) Gill's hematoxylin having the combination of Hematoxylin-2g, Sodium iodate-0.2g, Aluminium sulfate -17.6g, Distilled water -750ml, Ethylene glycol-250ml, Glacial acetic acid-20ml (E) Cole's hematoxylin which is a combination of Hematoxylin-1.5g, Saturated aqueous potash alum-700ml, 1% iodine in 95% alcohol-50ml, Distilled water-250ml (F) Delafield's hematoxylin comprising of Hematoxylin-

4g, 95% alcohol-125ml, Saturated aqueous potash alum-400ml, Glycerin-100ml (G) Carazzi's hematoxylin composing of Hematoxylin-5g, Potash alum-25g, Potassium iodate-0.1g, Distilled water-400ml, glycerol-100ml. The composition of eosin solution is mentioned in table 2.

TABLE1: MORDANTS OF HEMATOXYLIN AND THEIR RESULTANT COLORS.

Metal	Colour
Aluminum	Purple to blue
Iron	Blue-black
Chromium	Blue-black
Copper	Blue-green to purple
Nickel	Violet shades
Tin	Red
Lead	Dark brown
Osmium	Green brown

TABLE 2: COMPOSITION OF THE EOSIN SOLUTION

Yellow eosin	1 gm
Distilled water	80 ml
Ethanol	320 ml
Glacial Acetic Acid	2 drops

The procedure to apply the H&E [4] stain is as follows.

- Deparaffinize sections, 2 changes of xylene, 10 minutes each.
- Re-hydrate in 2 changes of absolute alcohol, 5 minutes each.
- 95% alcohol for 2 minutes and 70% alcohol for 2 minutes.
- Wash briefly in distilled water.
- Stain in Harris hematoxylin solution for 8 minutes.
- Wash in running tap water for 5 minutes.
- Differentiate in 1% acid alcohol for 30 seconds.
- Wash running tap water for 1 minute.
- Bluing in 0.2% ammonia water or saturated lithium carbonate solution for 30 seconds to 1 minute.
- Wash in running tap water for 5 minutes.
- Rinse in 95% alcohol, 10 dips.
- Counterstain in eosin-phloxine solution for 30 seconds to 1 minute.
- Dehydrate through 95% alcohol, 2 changes of absolute alcohol, 5 minutes each.
- Clear in 2 changes of xylene, 5 minutes each.
- Mount with xylene based mounting medium.

Note the use of tap water in the washing steps—tap water provides the alkalinity necessary for the "bluing" process.

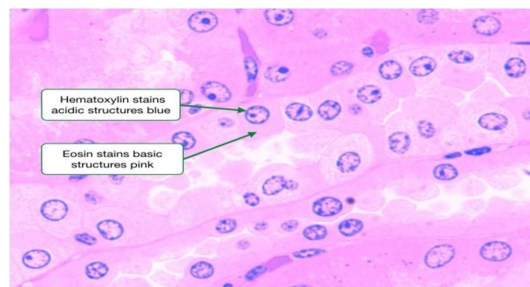


Figure1: Interpretation of the colors of an HE stained image

### III. INTERPRETATION OF HE STAINED TISSUE IMAGE

The interpretation[5] can be better understood with the help of an image shown in figure 1. The nuclei, protein generating part of the tissue which is acidic in nature is stained with blue color by Hematoxyline whereas the basic in nature is stained in pink by the Eosin dye.

### IV. LIMITATIONS OF H&E STAINING AND ITS ALTERNATIVES

One important limitation of H&E stain is when it is used to differentiate between non-living and morphologically intact cells. It is easy to determine where the gross microscopic damage ends and seemingly normal tissue starts with routine histological stains. However, morphological changes which can be observed routine histological methods cannot be observed if there is cell death because of tissue damage. This short coming of the H&E stain can be overcome by using 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) technique[1]. The mitochondrial dehydrogenase enzyme in living cell of the tissue is able to convert the yellow MTT solution to a purple formazan precipitate. This facilitates to differentiate between the actual living and morphologically intact dead cells. The shortcoming of MTT procedure is the time window within which it should be completed. This window to perform MTT procedure is only of 12 hours after the surgery.

Keratin Pearl is one of the important features which helps in the detection of the analysed tissue being benign or malignant. Keratin is a protein which is found in the pattern of pearl in the tissue analysed. The area, its distance from the epithelium and the degree are very important parameters. To stain the tissue in such a way that the pearls are clearly distinguishable is more preferred one. Figure 2 gives an example of how the keratin pearl looks like in the H&E stained image[7]. Arrows point to the Keratin Pearls of an Oral (Lip) biopsy image stained with H&E procedure.

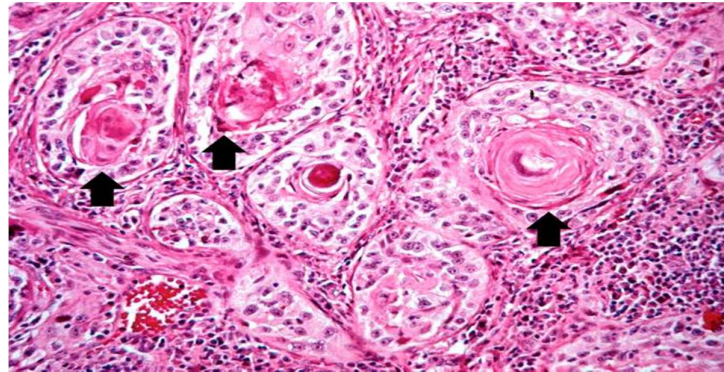


Figure1: Interpretation of the colors of an HE stained image

Ref [8]., analyses some of the staining methods which are comparable with the h&e staining methods, special stains like ayoub-shklar (as) method, dane-herman (dh) method, alcian blue –periodic acid schiff 's (ab-pas), rapid papanicolaou (pap) and gram's stain are been compared and concluded as d-h, a-s and ab-pas demonstrated overall staining quality comparable to h and e, suggestive of their potential use as alternative stains for keratin. Also, all the special stains were able to successfully identify the type of keratinization and distinctly stain keratin distinguishing it from the other connective tissue components with a higher intensity, however, h and e showed a higher specificity on an overall basis.

### V. CONCLUSION

In this paper we have discussed the use of Hematoxylin and Eosin as a staining material which is the most widely used method to stain the biopsy images to differentiate the tissue as a cancerous one or the benign one. We have also discussed the limitations of H&E staining method and the different alternative methods available to do the same task as that of H&E stain. The importance of detecting the Keratin pearl is also being discussed in brief in this paper.

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