SUBJECT: HEMATOXYLIN & EOSIN STAIN

PURPOSE: The most widely used Histologic stain is the H & E. It is a simple stain given to all tissues processed for diagnosis. Hematoxylin is a natural dye extracted from the logwood of the tree *Haematoxylon Campechianum*. This material does not act as a dye until it is oxidized to hematein. It is a nuclear stain, and as characteristic of such requires a metal salt (such as aluminum potassium sulfate or ferric ammonium sulfate) to augment the attachment of the dye molecule to the substrate material. This process, known as mordanting, forms a dye complex called a "lake". The dye-mordant lake is acidophilic in nature thus giving it an affinity for the acid DNA. The exact nature of the binding of dye lake to substrate is very complex and as yet not completely understood (Carson). Nuclei stain blue and detail should be crisp.

Eosin is the most common counterstain to Hematoxylin used in Histologic staining. Eosin is a synthetic (artificially produced) dye. It is anionic enabling to bind to the cationic or positively charged tissue groups found in the cytoplasm of cells. The optimum pH range for Eosin stain is 4.6 to 6.0, hence acetic acid may be added to enhance the reaction (Carson). Three shades of red to pink may be achieved when this dye is used properly. Erythrocytes stain a bright red-orange, collagen a pale pink, and the cytoplasm of muscle and epithelial cells orange-pink (salmon).

Tissue sections from paraffin blocks must be deparaffinized and hydrated before either of these water-based stains are used. This is accomplished by first removing paraffin with several changes of Xylene. Slides are then hydrated by immersion in successive Ethanol/water solutions of decreasing Ethanol concentration, starting with absolute Ethanol and ending with water. If the original tissue was fixed in Zenkers or other Mercury based fixatives the Mercury must then be removed before staining. This process, known as de-Zenkeriation, removes the darkly pigmented, mercury based granules which would otherwise interfere with staining. The hydrated tissue sections can then be stained with the aqueous H & E stains.

At UVM Medical Center the bulk of the H & E staining is done with progressive Hematoxylin stains. The intensity of "Progressive Stains" is meant to vary in direct proportion to the time in contact with the staining solution, no decolorization is needed. Occasionally tissues are resistant to progressive Hematoxylin stain, for example, decalcified specimens. In these cases a "regressive" stain is used. Regressive stains overstain the tissues, which must then be decolorized (differentiated) to the desired stain intensity.

Subsequent to the Hematoxylin stain, sections are immersed in an alkaline solution in order to "Blue" the sections. An alkaline pH strengthens the bond of dye-mordant to tissue and shifts the color from red to blue. The bluing agent used may be a weak base or prolonged washing (20 minutes) in plain tap water. After washing, the final step in the H & E process is application of the Eosin counterstain. Excess Eosin is washed out with water. Sections are then dehydrated by immersion in aqueous solutions of successively increasing concentration of Ethanol, ending with absolute Ethanol.

The tissue sections must then be washed with water to remove any excess ammonia and results in sharper contrast of nuclear staining. The Eosin dye solution, also aqueous in the UVM Medical Center H & E procedure is applied to stain cytoplasmic components. Excess Eosin is then quickly washed away with water and the sections are dehydrated to remove any water. Improper dehydration will result in a "muddy" section so one must be sure all water is removed before the sections are cleared in xylene. If the sections cause a "milky" reaction once they are placed in xylene it indicates that there is more than a 2%concentration of water still in the sections and they must be dehydrated further using absolute ethanol. After dehydration the xylenes serve to clear the sections of ethanol and prepare them for coverslipping, the final step in the preparation of permanent sections/

Coverslipping involves placing a thin (150um)-glass cover over the tissue section. This glass coverslip is adhered to the slide using a synthetic mounting medium that is of a low refractive index, will not yellow with age and is compatible the clearant used (i.e. xylene).

Mayer's Hematoxylin Stain:

Component	Amount	Function
Hematoxylin	0.9 gm Sigma Hematoxylin 1.6 gm Dark Hematoxylin (Gesellschaft)	dyes for nuclear components

H_20	1500 cc	diluent
Sodium iodate	0.43 gms	oxidizer
Aluminum potassium Sulfate	71 gms	mordant
Chloral hydrate	71 gms	prevents precipitate
Citric acid	1.7 gm	pH adjustment

EOSIN:

Component	Amount	Function
2% Eosin Y	200 cc	dye for cytoplasmic components
H_20	800 cc	diluent
Glacial acetic acid	50 ul	pH adjustment/stain enhancer

REGERENCES:

Carson, FL: *Histotechnology, a Self-Instructional Text.* Chicago, ASCP Press, 1997