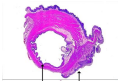


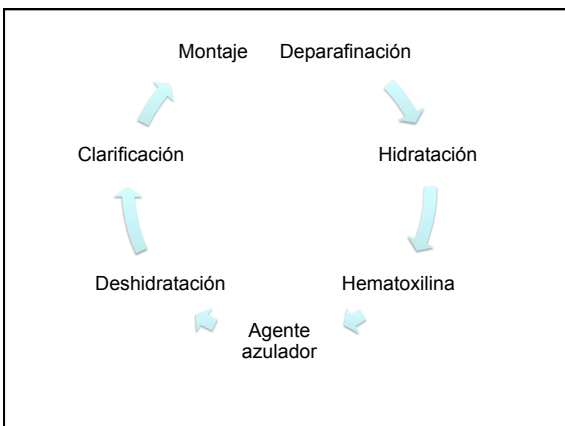
The Hematoxylin and Eosin

Luis E. Ferrer Torres, MD FCAP

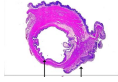
Introduction



- Most widely used
- Heartwood (logwood) of the tree *Haematoxylon campechianum*
- West Indies
- Hematoxylin not a stains per se
- The oxidative product hematein is the active (staining) component

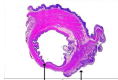


Hematoxylin



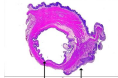
- Natural oxidation (longer shelf life)
 - Air and sun
- Chemical oxidation (shorter shelf life)
 - Sodium iodate
 - Mercuric oxide
- Anionic (negative charged), poor affinity for tissue
- Needs mordant to adhere to tissues

Hematoxylin



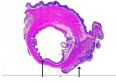
- Mordants (metal cation):
 - Aluminum
 - Iron
 - Tungsten
 - Lead

Hematoxylin



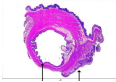
- Hematoxylin solutions are classified according to the mordant used:
 - *Alum hematoxylin*s
 - *Iron*
 - *Tungsten*
 - *Molybdenum*
 - *Lead*
 - *Without mordant*

Alum hematoxylin



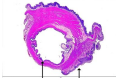
- Cole's
- Delafield's
- Ehrlich's
- Mayer's (P+R)
- Harris' (P+R)
- Carazzi's (P+R+F)
- Gill's (R)

Hematoxylin



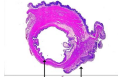
- Dedifferentiation
 - Necessary for regressive staining
 - Acid alcohol
 - 1% HCl (Mayer's)
 - 5-10% Acetic acid (Harris')
- Staining times
 - Depends in fixation, decalcification, type, age and pathologist

Hematoxylin



- Disadvantages:
 - Sensitivity to any subsequently applied acidic staining solutions, e.g. vanGieson, trichrome
 - Use an iron hematoxylin like Weigert's (acid resistant)
 - Use Celestine blue-alum hematoxylin procedure (makes alum hem more resistant to acid)

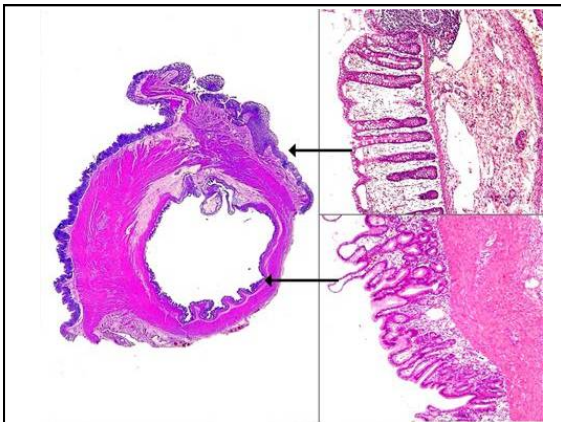
Eosin



- Best combination with hematoxylin
- Cytoplasm and stroma in different grades
- Xanthenes dye
 - **Eosin Y**, ethyl eosin, eosin B
- Add thymol to inhibit fungi
- Add acetic acid to increase sharpness
- Add Phloxine B for better details

Table 9.2 The uses of hematoxylin stains

Mordant	Oxidant	Examples	Applications
Alum	Natural	Ehrlich	Nuclear stain used with eosin. Stains some mucins
Alum	Natural	Delafield	Nuclear stain used with eosin
Alum	Sodium iodide	Mayer	Nuclear stain used with eosin. Nuclear counterstain
Alum	Mercuric oxide	Harris	Nuclear stain used with eosin
Alum	Iodine	Cole	Nuclear stain used with eosin
Alum	Potassium iodate	Carazzi	Nuclear stain used with eosin (used with frozen sections)
Alum	Sodium iodate	Gill	Nuclear stain used with eosin
Iron	Natural	Weigert	Nuclear stain used with acid dyes
Iron	Natural	Heidenhain	Intranuclear detail, muscle striations
Iron	Natural	Verhoeff	Elastic fibers
Iron	Natural	Loyez	Myelin
Tungsten	Natural	Mallory PTAH	Fibrin, muscle striations, glial fibers
Molybdenum	Hydrogen peroxide	Thomas	Collagen, endocrine cell granules
Lead		Solcia	Endocrine cell granules
Without mordant		Mallory	Iron, copper, lead
Chromium-copper		Weigert-Pal	Myelin (in block preparation)



Identificando Errores

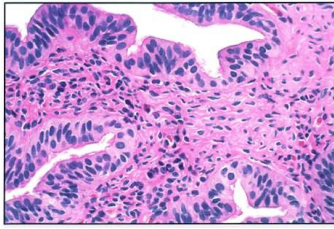
Observación	Causa
Puntos sin teñir	Deparafinación incompleta
Poco detalle nuclear	Fijación incompleta Exceso de calor durante procesamiento Se secaron las secciones durante el procesamiento
Núcleo pálido	Tiempo incompleto en hematoxilina Hematoxilina oxidada Sobre diferenciación (ácido)
Núcleo sobre teñido	Tiempo en exceso en hematoxilina Secciones gruesas Poca diferenciación
Núcleos rojos o rojo-marrón	Hematoxilina oxidada Poco tiempo en azulado

Identificando Errores

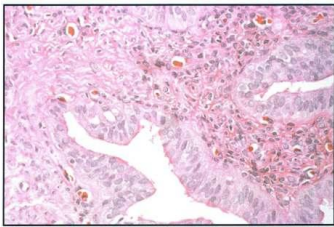
Observación	Causa
Citoplasma pálido	pH eosina >5 (se esta arrastrando el agente azulador) Secciones demasiado finas
Citoplasma Oscuro	Concentración de la solución de eosina Tiempo en exceso Deshidratación disminuida
Precipitado Azul-negro	Hematoxilina no se filtró
Laminillas opacas, blancas	Al finalizar deparafinación: contaminado con xileno Antes de montar: contaminado con agua
Orilla de la sección basofílicas	Efecto de cauterio Sobre deshidratación



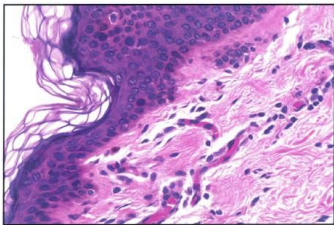
Deparafinación incompleta



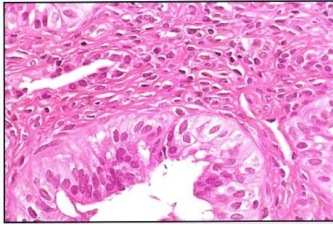
Pobre fijación
Poco detalle nuclear



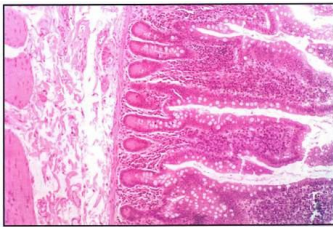
Núcleos pálidos



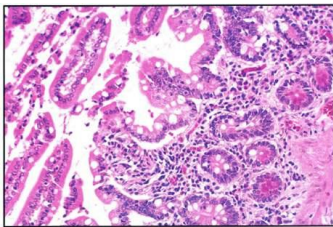
Núcleos oscuros



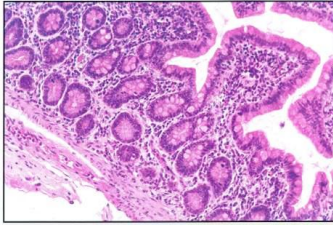
Núcleos rojo-marrón
No se puso en el agente azulador o la formalina esta oxidada



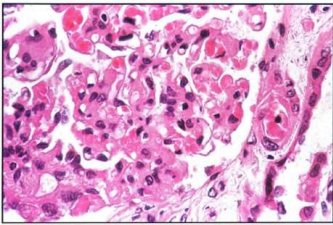
Núcleos rojo-marrón
No se puso en el agente azulador o la formalina esta oxidada



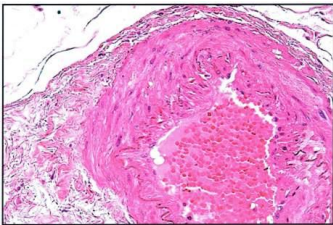
Eosina pálida



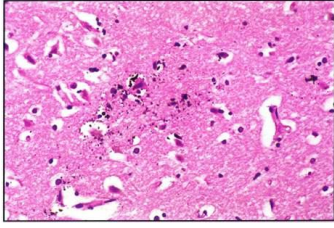
Exceso eosina/Phloxine



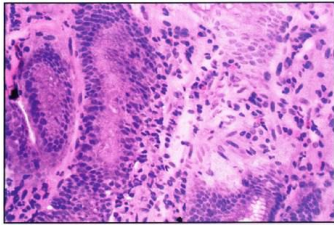
Falta clarificación



Falta clarificación



No se filtro la hematoxilina
Precipitado



Deshidratación Incompleta
