

Doderlein bacillus	<i>L. acidophilus</i> Pap's stain: blue to lavender
<i>C. albicans</i>	Diabetic patients Sish kebab appearance
<i>T. vaginalis</i>	Pear-shaped, blue-gray to blue-green Pigs on a scruff appearance
Leptothrix	Indicates <i>T. vaginalis</i> infection
<i>G. vaginalis</i>	Clue cells
Koilocytes	Wrinkled prune appearance w/ perinuclear halo HPV (LSIL)
Ferning	Formation of salt crystals ☑ Estrogen <u>Early pregnancy</u>
Quantitative Evaluation: Cytohormonal Maturation Index (CHMI)	
CHMI	MI = P/I/S
Pregnancy	MI = 0/90/10 (☑progesterone)
Newborn (8 weeks)	MI = 0/90/10 (☑progesterone from mother)
Infancy (8 weeks-puberty)	MI = 80/20/10 (☑estrogen)
Late menopausal	MI = 100/0/0 (no estrogen)
75 y.o. woman w/ estrogen therapy	MI = 0/20/80
Quality Assurance	
3 copies/report	1. Doctor 2. Patient = original copy 3. File
Reports	Surgical pathology report Cytopathology report Autopsy report
Signatories	Request forms = patient's doctor Result forms = pathologist
Turnover of results	Surgical pathology & cytology = 24 hrs Frozen section = 5-15 mins <u>Autopsy report</u> = 1 week (<u>Autopsy procedure</u> : 24 hrs)
Storage	Specimen (tissue) = 1 month to 1 year Tissue blocks (paraffin) = 3 to 10 years Slides = indefinite
Suggested Guidelines for Record and Specimen Retention (Henry, 21st Ed.)	
Records	
Requisitions	2 years
QC	2 years
Instrument maintenance	2 years
BB QC	5 years
BB employee signatures	10 years
BB donor/recipient records	Indefinitely
Reports	
Clinical pathology lab reports	2 years
Surgical pathology (and BM) reports	10 years
Cytogenetics reports	20 years
Autopsy forensic reports	Indefinitely

Criteria for brain death	Brain death: perpetual state of deep sleep - Coma (patient will not respond) & cerebral unresponsiveness - Apnea - Absent cephalic (brainstem) reflexes - Electrocerebral silence criteria should be present for 30 mins at least 6 hrs after onset of coma & apnea
American bar association & national conference of commission of uniform state laws legislative definition of death (1980)	- irreversible cessation of circulation & respiratory functions - Irreversible cessation of all functions of the entire brain, including the brainstem is dead
American academy of neurology	Death: - Coma - Absence of the following: - Motor response - Pupillary response to light & pupils at mid-position - Corneal reflexes - Caloric responses - Gag reflexes - Coughing in response to tracheal suctioning - Sucking & rooting reflexes
Postmortem changes	<u>Algor mortis</u> 1st demonstrable change after death is cooling of the body - At room temp: 2'-2.5°F/hr (1st hr) 1.5-2°F/hr (next 12 hrs) 1°F/hr (next 12-18 hrs) - As a rule, the body cools at 1.5°F/hr (50% of cases) - Not a reliable indicator as to the time of death <u>Rigor mortis</u> - Rigidity of the body due to hardening of the skeletal muscles caused by a series of physiochemical events after death (-) ATP regeneration + ↑ acidity → formation of locking-chemical bodies between actin & myosin - This interlocking is fixed & produces rigor mortis w/o shortening of the muscles - Sets w/in 2 hrs after death (head & neck) - Complete w/ 12 hrs - Persists about 3-4 days <u>Livor mortis</u> (postmortem lividity/hypostasis) - Blood supply gravitates to the skin vessels w/c becomes toneless & dilate after circulation ceases - Becomes evident as early as 20 mins after death - Fully evident w/in 4-8 hrs - Tardien spots: petechiae <u>Postmortem clotting</u> of blood <u>Discoloration of tissue</u> - Abdomen: green - Formation of sulfur gases (bacteria) <u>Putrefaction</u> <u>Dessication</u> (mummification)
Techniques of Autopsy	
Technique of Virchow	Organs removed & dissected <u>individually</u> in the body Most widely used metohd

	c. Chromic acid = preserves CHO d. K_2CrO_4 = mitochondria (if acidified, fixes chromatin bodies & chromosomes but destroys mitochondria)
Chromate pigments	Fine, yellow brown
Lead fixatives	Used in 4% aqueous solution of basic <u>lead acetate</u> For acid MPS and mucin
Picric acid fixatives	Highly <u>explosive</u> when dry Excessive <u>yellow staining</u> of tissues Picrates $\rightarrow$ Protein $\rightarrow$ Ppt. (H_2O soluble) $\rightarrow$ Add 70% ETOH $\rightarrow$ Insoluble Never wash in H_2O before dehydration For glycogen (excellent) a. Bouin's = for embryos, Masson's trichrome stain, glycogen b. Brasil's alcoholic picroformol = less messy than Bouin's, glycogen (excellent)
Glacial acetic acid	Solidifies at 17°C Fixes & precipitates nucleoproteins, chromosomes, & chromatin material Most commonly combined w/ other fixatives
Alcoholic fixatives	Disadvantage: polarization (glycogen granules $\rightarrow$ poles/ends of the cells) "MEICAN" a. Methanol = BM & blood smears b. Ethanol = preserves but does not fix glycogen (Disadv: polarization) c. Isopropanol = for touch preparations d. Carnoy's = most rapid (1-3 hrs) for chromosomes Decalcifies (acetone) e. Alcoholic formalin (Gendre's) = sputum f. Newcomer's = for MPS nuclear & histochemical fixative
Osmium tetroxide (Osmic acid)	Inhibits hematoxylin Produce black precipitate crystals (osmium oxide) For lipids a. Flemming's = permanently fixes fat, for nuclear structures (excellent) Fixative & decalcifying agent (chromic acid) b. Flemming's w/ acetic acid = for mitochondria
Trichloroacetic acid	Precipitates proteins Swelling effect $\rightarrow$ counteract shrinkage by other fixatives Weak decalcifying agent (softening effect)
Acetone	Recommended for H_2O -diffusible enzymes (phosphatases, lipases) Rabies
Heat fixation	Bacteriologic smears Microwave: 45-55°C Underheating: poor sectioning Overheating (>65°C): vacuolation, overstained cytoplasm
2' fixation	Placing an already fixed tissue in a 2 nd fixative
Post-chromatization	Primarily fixed tissue $\rightarrow$ 2.5-3% K_2CrO_4 (mordant)
Washing out	Removing excess fixative a. Tap H_2O = remove excess chromates, formalin, osmic acid (NOT Bouin's) b. 50-70% alcohol = wash out excess picric acid (Bouin's) c. Alcoholic I_2 = remove excess mercuric fixatives
EM fixatives	Glutaraldehyde $PtCl_3$ $PtCl_3$ - formalin (Zamboni's) $AuCl$ Osmium tetroxide 10% NBF = acceptable but not recommended
Stains (EM)	"PUL"

Removal of substances soluble in fixing agent	Wrong choice of fixative
Presence of artifact pigments on tissue sections	Incomplete washing of fixative
Tissues are soft & feather-like in consistency	Incomplete fixation
Loss/inactivation of enzymes needed for study	Wrong choice of fixative
Shrinkage & swelling of cells & tissue structure	Overfixation
Tissue blocks are brittle & hard	Prolonged fixation
♪ An incompletely fixed tissue may lead to improper & incomplete clearing & impregnation, and may later prove to be a hindrance to normal sectioning & staining of specimen	

Pigment	Color	Removed by:
Acid formaldehyde hematin	Brown/black granules	“SAKaL” a. Saturated picric acid b. Alcoholic KOH c. Kardasewitsch method d. Lillie’s method
Mercuric chloride pigment	Black granules	Alcoholic iodine
Chromate pigment	Fine, yellow brown	Acid-alcohol
Osmium tetroxide pigment	Black precipitate crystals	Cold H ₂ O
Crush artifact	Intense eosinophilic staining at the center of the tissue (H & E) Due to partial coagulation of partially fixed protein	
Decalcification		
20:1	Ratio of decalcifying agent to tissue	
37°C	Impaired nuclear stain by Van Gieson’s stain	
55°C	Tissue → Digestion (24-48 hrs)	
RT (18-30°C)	Optimum temperature	
24-48 hrs	Time	
Decalcifying agents	Acids Chelating agents (EDTA/versene) Ion exchange resins Elec. ionization (electrophoresis)	
HNO ₃	Most common a. Perenyi’s = tissue softener & decalcifying agent b. Phloroglucin-HNO ₃ = most rapid - Disadvantage: Yellow color on tissue (neutralize w/ sodium thiosulfate)	
5% Formic acid	Both fixative & decalcifying agent Best general decalcifying agent For small pcs of bones & teeth	
HCl (Von Ebner’s)	For small pcs of bones & teeth For surface decalcification (HCl)	
EDTA	For EM, IHC, & enzyme staining	
Ion exchange resins	Hastens decalcification by removing calcium ions from formic acid-containing decalcifying solutions	
Electrophoresis	Ca ²⁺ are attracted to negative electrode (cathode)	
Measuring extent of decalcification	Physical method Chemical method = CaOx test (routine) Turbidity = (+) Ca ²⁺ X-ray = most ideal, most sensitive, most reliable but very expensive - X-ray paper = Kodak X-omat or Faxitron	
Post-Decalcification	Removal/neutralization of acid from the tissues after decalcification Lithium carbonate or sodium bicarbonate solution	
Tissue softeners	4% phenol Molliflex = tissues appear swollen & soapy 2% HCl	

	Hematoxylin --- (Ripening) ---> Hematein (active coloring substance)
Lake	Tissue-Mordant-Dye complex
Oxidizing agents	H ₂ O ₂ HgO ₂ = Harris' K ₂ MnO ₄ Na perborate Na iodate = Mayer's, Ehrlich's, Gill's
Alum hematoxylin	<u>Routine</u> H & E = Red Mordant: K Alum "MEGDH" Mayer's = Na iodate (ripening agent) Ehrlich's = Na iodate (ripening agent) Gill's Delafield's Harris' = HgO ₂ (ripening agent)
Iron hematoxylin	Mordant = oxidizing/ripening agent = Iron a. Weigert's - Mordant: FeCl ₃ - Weigert's + Van Gieson's = CT & <i>E. histolytica</i> b. Heidenhain's - Mordant: Ferric ammonium sulfate
Tungsten hematoxylin	a. Mallory's PTAH - Mordant = sunlight/K ⁺ - Stain fibrin
Copper hematoxylin	Spermatogenesis
Eosin (Eosin Y)	Cytoplasmic/acidic/2 nd stain Counterstain a. Eosin Y (Yellowish) = most commonly used b. Eosin B (Bluish) = deep red c. Eosin S/Eosin S/Eosin alcohol soluble
Coplin jar	Holds 5-9 slides
Slotted staining dishes	Holds 5-19 slides
Metal/glass staining racks/ carriers	Holds 10-30 slides
H & E staining steps	1. Xylol (2) = deparaffinization 2. Descending grade of alcohol = rehydration 3. H ₂ O 4. Remove fixative artifact pigments <u>after</u> rehydration & <u>before</u> staining 5. Stain: Nucleus = light blue 6. H ₂ O 7. Acid alcohol (differentiator): Nucleus = light blue 8. Ammonia water (blueing agent): Nucleus = blue - NH ₄ OH - LiCO ₃ - Scott's tap H ₂ O 9. Wash 10. Stain: Eosin Y 11. Ascending grade of alcohol = dehydration 12. Xylene = dealcoholization/clearing 13. Mount & label Nuclei: blue to blue black Cytoplasm: pale pink