Free HTL Guide – Stain Cheat Sheet (Preview)

Quick-reference summaries for common histology stains. For study only; verify procedures with your lab's SOPs.

Modules covered: H&E, PAS/PAS-D, GMS (fungi), Ziehl-Neelsen (AFB), Masson's Trichrome, Prussian Blue, Reticulin, Oil Red O, QC & Troubleshooting.

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Hematoxylin & Eosin (H&E)

Target/principle: Hematoxylin (after oxidation/mordant) stains basophilic nuclei blue-purple; eosin stains acidophilic cytoplasm and ECM pink.
Critical reagents: Hematoxylin (ripened/oxidized), bluing reagent (alkaline, e.g., ammonia water), Eosin Y/Phloxine.
Key steps: Deparaffinize -> Hematoxylin -> Rinse -> Blue -> Eosin -> Dehydrate -> Clear -> Mount.
Controls: Any routine tissue with good nuclear detail (e.g., tonsil).
Expected: Crisp blue nuclei; pink cytoplasm/collagen; RBCs bright orange-red depending on eosin.
Pitfalls: Poor deparaffinization -> patchy stain; under-blueing -> muddy nuclei; over-differentiation -> pale nuclei; old eosin -> flat appearance.
Troubleshooting: Check xylene/alcohols, renew hematoxylin, extend blueing, refresh eosin/differentiation.

Periodic Acid-Schiff (PAS) and PAS with Diastase (PAS-D)

Target/principle: Oxidation creates aldehydes in carbohydrates; Schiff reagent yields magenta color.
PAS-D: Diastase digests glycogen - loss of staining confirms glycogen.
Controls: Liver with glycogen (PAS positive, PAS-D negative for glycogen), kidney basement membranes.
Expected: Magenta for glycogen, neutral mucins, basement membranes; nuclei counterstained blue.
Pitfalls: Over-oxidation -> weak stain; contaminated Schiff -> poor color; inadequate diastase digestion -> false positive.
Troubleshooting: Check periodic acid time, Schiff freshness (sulfurous smell gone), verify diastase activity and temperature.

Gomori Methenamine Silver (GMS) - Fungi

Target/principle: Carbohydrate-rich fungal walls oxidized to aldehydes; silver impregnation reduces to black deposits.
Critical steps: Oxidation (chromic/periodic acid) -> Methenamine silver -> Gold chloride tone (optional) -> Sodium thiosulfate.
Controls: Known fungal-positive tissue (e.g., Candida).
Expected: Fungi black on light green background (with light green counterstain).
Pitfalls: Under-oxidation -> weak fungi; non-specific background silver; overheated solution -> precipitate.
Troubleshooting: Verify oxidizer time, keep glassware clean, filter silver, monitor temperature carefully.

Ziehl-Neelsen / Kinyoun – Acid-Fast Bacteria (AFB)

Target/principle: Mycolic acids bind basic fuchsin; acid-alcohol decolorizes non-acid-fast organisms.
Kinyoun is a cold method; Ziehl-Neelsen uses heat to drive dye in.
Controls: Known AFB-positive tissue (e.g., Mycobacterium).
Expected: AFB bright red; background blue (methylene blue).
Pitfalls: Over-decolorization -> false negatives; old carbol fuchsin; contamination.
Troubleshooting: Shorten decolorization, verify reagent potency, include positive/negative controls each run.

Masson's Trichrome – Collagen vs. Muscle

Principle: Acid dyes differentiate muscle (red) from collagen (blue/green) after plasma staining and differentiation.
Controls: Uterus, appendix, or other tissue with both muscle and collagen.
Expected: Collagen blue/green; muscle and cytoplasm red; nuclei dark.
Pitfalls: Inadequate fixation → weak staining; over-differentiation → pale collagen; old PMA/PTA → poor contrast.
Troubleshooting: Ensure NBF fixation; check differentiation times; refresh aniline blue or light green solutions.

Perls' Prussian Blue – Iron

Target/principle: Ferric iron reacts with potassium ferrocyanide in acid to form ferric ferrocyanide (Prussian blue).
Controls: Spleen or liver with known hemosiderin deposits.
Expected: Iron deposits blue; backgroumd pink (nuclear fast red).
Pitfalls: Acid decalcification can remove iron; poor fixation reduces sensitivity.
Troubleshooting: Avoid strong acid decalcifiers; verify fresh reagents; ensure 4–5 um sections.

Reticulin (Gomori) – Reticular Fibers

Principle: Silver impregnation demonstrates argyrophilic reticulin fibers after oxidation, sensitization, and development.
Controls: Liver or lymph node.
Expected: Reticulin fibers black; background pale.
Pitfalls: Dirty glassware causes background silvering; incorrect timing during ammonia/silver steps.
Troubleshooting: Clean glassware, strict timing, fresh ammoniacal silver; avoid over-toning.

Oil Red O – Lipids (Frozen)

Principle: Lipid-soluble dye stains neutral lipids in frozen sections; paraffin processing removes lipids – use frozen tissue.
Controls: Fatty tissue (e.g., adipose).
Expected: Lipids red; nuclei blue (hematoxylin).
Pitfalls: Using paraffin sections → false negative; sections too thin → faint staining.
Troubleshooting: Use 8–10 µm frozen sections; avoid alcohol dehydration steps that dissolve lipids.

QC & Troubleshooting - Quick Reference

Fixation: 10:1 volume; adequate time; NBF standard. Over-fixation may mask antigens.
Sectioning: 3-5 um for most paraffin H&E; check knife angle and water bath temp.
Deparaffinization: Incomplete clearing -> patchy stains; refresh xylene and alcohols.
Controls: Use appropriate positive controls each run; verify storage and expiry.
Reagents: Label, date, rotate stock; document QC logs.
Artifacts: Chatter/compression (microtomy), formalin pigment (use NBF; removal with alcoholic picric acid if needed).
Documentation: Record lot numbers, times, temps; investigate outliers with a change log.