H&E Stain (Automated)

Purpose	H&E staining is the primary method of identifying cell structures in tissue. Pathologists rely on this stain for identification of tissue, as well as identifying disease processes. All surgical specimens fixed in 10% formalin and B+ are stained using a progressive Hematoxylin and Eosin staining method.	
Scope	Histology Technical Staff	
Materials	**Reagents**	**Equipment**
	• Dehydrant/ Alcohol (Storage: Flammable) • Xylene (Storage: Flammable) • Synthetic mounting media (Storage: Flammable)	• LEICA Autostainer XL (with containers and lids) • LEICA CV 5030 Coverslipper • 50 X 24 Coverslips • staining racks (LEICA)
Sample	Paraffin sections, most routinely cut @ 4 microns. Cytology slides: Fix in 95% Ethanol/alcohol and air-dried.	
Records/Forms/ Documents needed	Histology Requests/ QC (“Blue Sheet”) Form Leica (H&E) Autostainer- Daily Reagent Rotation Tracker Histology Quality Control Form	
Quality Control	Representative patient slides are reviewed and documented by the Histology staff in the H&E QC log before delivery to the pathologist. It is the responsibility of the pathologist to inform Histology of any defects in H&E stain quality.	
Special Safety Precautions	Xylene is an Aromatic hydrocarbon/ Petroleum solvent and should be used in well-ventilated areas, preferably under a chemical fume hood and/or the downdraft fume (coverslipping) hood. The used Xylene is collected in labeled containers for recycling. The first Alcohols/dehydrant used in the deparaffinization and clearing are NOT collected for recycling as retained paraffin causes problems with the solvent recycler; these are collected for Hazardous Waste. The Alcohols/ dehydrant used in the clearing process after the Eosin step are collected for recycling.	

Stock/ Working Solutions	**Hematoxylin 1** (Purchased from Richard Allan/ Cardinal Health) Storage: General **Eosin (alcoholic), 1%** (Purchased from Richard Allan/ Cardinal Health) Storage: Flammable **Clarifier 1** (Purchased from Richard Allan/ Cardinal Health) Storage: Flammable **Bluing** (Purchased from Richard Allan/ Cardinal Health) Storage: General **Dehydrant/ Alcohol, 95% and 80%** (May be made from recycled product (95%) or from new product (100%) as needed). Storage: Flammable	
Procedure	**Step**	**Action**
	1	The LEICA stainer oven, programmed for 15 minutes at 65°C. Slides may be dried in separate slide drying oven.
Stations 1-3	2	Xylene (X3)…….**5** minutes each. Deparaffinization
Stations 4-5	3	100% Alcohol (absolute)/ dehydrant (x2)…….**20 seconds each**.
Station 6	4	95% Alcohol/ dehydrant…….**20 seconds.**
Station 7	5	80% Alcohol/ dehydrant…….**20 seconds.**
	6	Wash 1…….**20 seconds.**
Station 8	7	Hematoxylin 1…….**3** minutes.
	8	Wash 2…….**20 seconds.**
Station 9	9	Clarifier 1…….**1** minute. .
	10	Wash 3……**20 seconds.**
Station 10	11	Bluing…….**1** minute.
	12	Wash 4…….**20 seconds.**
Station 11	13	80% Alcohol/ dehydrant…….**20 seconds.**
Station 12	14	Eosin…….4 seconds.
	15	Wash 5……..**20 seconds.**
Station 13	16	70% alcohol.....20 seconds.
Station 14	17	95% Alcohol/ dehydrant…….**20 seconds.**

Station 15-16	18	100% Alcohol (absolute)/ dehydrant (x2)..... **20 seconds each.**
Station 17-18	19	Xylene…….....**20 seconds each.**
Station 19	20	Xylene (hold/exit station). Coverslipped (automated) out of Xylene by using either the Automated coverslipper or by manual coverslipping technique.
	21	Automated coverslipper or manual coverslipping
Results		Nuclei …….....…….blue Erythrocytes and Eosinophilic granules…….bright pink to red Cytoplasm and other tissue elements …….....various shades of pink
Notes		Occasionally, the reagents used in the H&E are known to be working satisfactorily, yet some sections show a lack of nuclear staining. This can be attributable to (a) autolysis of that tissue section, (b) prolonged storage of the wet tissue in nonbuffered formalin, (c) overexposure to decalcifying fluids, (d) insufficient washing of the tissue after decalcification and (3) dried or burned tissue. Basophilic staining properties may sometimes be restored by treatment of the hydrated section with one of the following solutions: 5% aqueous Sodium Bicarbonate for an hour followed by a 5 minute wash in tap water prior to staining *or* 5% aqueous Periodic Acid for an hour, followed by three changes of distilled water prior to staining. Solution staining intensity is checked microscopically after each rack is run and coverslipped. Should staining be uneven or uneven in intensity, check the running water rinse(s) to ensure there are no clogs in the mechanisms and water is running freely. Uneven staining can be caused by incomplete deparaffinization and clearing. Be sure slides are dried well before staining and procedures are followed in solution rotation. This also applies to dehydration and clearing after the stain process. "Streaking" of the slides can indicate water contamination in the absolute alcohols and/or xylene containers. If levels of the Eosin drop or evaporate slightly, add 95% alcohol to raise the level. Adding larger amounts of new stain consistently to an evaporating solution will result in intensifying the stain on the tissue sections. When in doubt, empty the container and replace with new, noting the change on the Daily Preventative Maintenence Log. Should overall staining quality become compromised, empty the stain(s), blueing and clarifier(s) in the designated Hazardous Waste Satellite collection container. Wash out the empty container and rinse well with distilled water, dry and refill with new stain. On occasion, purchased stain may have been exposed to either extreme low temperatures (frozen) or high heat during shipping.

Procedure Notes	**Leica Automated Stainer** See Leica stain programs and attached schedules on Leica stainer. Program 1: Routine H&E *with* oven Program 2: Routine H&E *without* oven Program 5: H&E for cytology (starts in Hematoxylin) **XYLENE (Stations 1, 2 & 3) and (Stations 17, 18 & 19):** Change/ rotate every 200 slides OR once a day. Collect waste for recycling. (Empty solvent in station 1 and move the container with solvent from station 2 to station 1 and from station 3 to station 2. Wipe out empty container and refill; place the new/ fresh solvent in station 3. (Empty solvent in station 17 and move container with solvent from station 18 to station 17, and station 19 to station 18. Wipe out empty container and refill; place the new/ fresh solvent in station 19). Collect waste for recycling. **ABSOLUTE ALCOHOL (Station 4 & 5):** Change/ rotate every 200 slides OR once a day. *DO NOT RECYCLE - WASTE ONLY.* This alcohol is contaminated with xylene substitute and trace amounts of paraffin which will adversely affect the CBG Solvent Recycler). **(Stations 15 & 16):** Change/ rotate every 200 slides OR once a day. Collect waste for recycling. **95% & 80% (Stations 6 & 7):** Change every 200 slides OR once a day. *DO NOT COLLECT FOR RECYCLING - WASTE ONLY.* **70% ALCOHOL (Station 13):** Change after EACH FULL RACK (30 slides). **95% ALCOHOL (Station 14):** Change after EVERY OTHER FULL RACK (60 slides). Collect waste for recycling. **80% ALCOHOL (Station 11):** Changed weekly unless it becomes contaminated. **HEMATOXYLIN & EOSIN:** Changed weekly unless there are staining issues. **BLUING & CLARIFIER:** Changed twice a week; may be changed more frequently if very heavy usage demands or if solutions are contaminated. It is important that the sections/ slides clear and dehydrate well to ensure staining quality and provide for long term slide storage/maintenance.
Result Reporting	By Pathologist
References	Histotechnology : A Self-Instructional Text 2nd edition, Carson Frieda Leica Users Guide

Authorization

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Medical Director	Peter Helseth, MD	5/1/12
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Annual Review	**Designee**	**Signature**	**Date**
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