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JOHNS HOPKINS MEDICINE

Hackam Lab
H&E staining (regressive method)

1. Dewax and hydrate paraffin embedded tissue sections:

   Warm slides at 60°C for 30' in vacuum incubator

   - Xylene (I & II) 2x5'(dips in and out for all steps for best results)
   - 100% ETOH (I & II) 2x5'
   - 95% (I) 1x5'
   - 70% 1x5'
   - Water 2x5'

2. Hematoxylin 15 minutes
   (Modified Harris Hematoxylin, Sigma, HHS32-1L)
3. Rinse in running tap water 5 minutes
4. Differentiation solution 4 dips
   (1% HCl in 70% ethyl alcohol)
5. Ammonia water, 0.25% to make blue color 45 seconds
   (DO NOT agitate; section loss may occur)
6. Rinse in running tap water 2 minutes
   (Check staining under microscope, return to HCl step 4 if see over staining)
7. Rinse in Alcohol, 95% (II) 10 dips
8. Eosin Y counter stain *(Sigma HT110132)* 25 seconds
9. 95% alcohol (III) 2x5' (dips in and out for best results)
10. 100% alcohol (I&II) 2x5' (dips in and out for best results)
11. Xylene (III&IV) 2x5' (dips in and out for best results)
12. Mount with xylene based mounting medium (Slides should be in xylene while waiting to mount)

Reagents:

Ammonia water (1L) 2 ml of 20% Ammonium hydroxide +998 ml water
(Fisher scientific cat# A470-250)

Differentiation sol. (1L) 27 ml of 36.5-38% HCl + 973ml of 70% ethanol
(Fisher scientific cat# 194054)