

TROUBLESHOOTING THE PAP STAIN  
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| <b>PROBLEM</b>   | <b>POSSIBLE REASON</b>  | <b>REMEDY</b>   |
|--|---|---|
| <b>DARK NUCLEI</b>   | Too much time in Harris' Hematoxylin.<br><br>Not enough time in HCl or HCl concentration less than recommended.   | Reduce time in HTX by 10,15,20,30 sec intervals<br><br>Increase time in acid by 5,10 sec.   |
| <b>PALE NUCLEI</b>   | Polyethylene glycol coating not removed from cells prior to Hematoxylin.<br><br>Concentration of HCl greater than recommended or too much time in HCl.<br><br>Not enough time in Hematoxylin.<br><br>Hematoxylin diluted by water (if water not properly drained from slides).<br><br>Stain not changed frequently enough resulting in Hematoxylin exhausted  | Extend prestaining soak with aqueous ethanol<br><br>Reduce time in acid by 5,10 sec and ensure correct amount of acid is added to the solution.<br><br>Increase time in HTX by 10,15,20,30 sec intervals<br><br>Ensure the arm of the staining machine is operating correctly.<br><br>Ensure a set amount of slides are stained and then stains are changed.  |
| <b>CYTOPLASMIC COLOUR NOT CONSISTENT</b>                     | Air drying prior to fixation.<br><br>Polyethylene coating inadequately removed from cells.<br><br>Solutions not at proper level within staining dishes.<br><br>Excessive time in Hematoxylin or Hematoxylin not removed prior to OG and EA dyes.<br><br>Slides left too long in ethanol rinses or clearing solutions following OG and EA.<br><br>Inadequate rinsing of slides between solutions.<br><br>Insufficient rinsing following staining solutions.<br><br>pH of tap and distilled water not sufficiently alkaline.<br><br>pH of EA needs to be controlled (pH 4.5 to 5 achieves maximum results).<br><br>EA dye exhausted | Report the findings to the referring clinician<br><br>Extend prestaining soak in aqueous ethanol<br><br>Check staining solution level<br><br>Reduce time in HTX by 10,15,20,30 sec intervals<br><br>Reduce ethanol rinse time<br><br>Check if ethanol is changed regularly<br><br>Increase ethanol rinse time<br><br>Check pH<br><br>Check pH<br><br>Ensure a set amount of slides are stained and then stains are changed. |
| <b>MACROSCOPICALLY ALL SLIDES ARE PINK, ORANGE OR YELLOW</b> | Slide drying oven temperature too high  | If this happens there is nothing that can be done to obtain a well-stained sample.  |
| <b>DULL PINK AND DEGENERATE APPEARANCE</b>                   | This usually occurs to smears that accompany histology specimens. It is usually due to formalin fixation.   | Ensure formalin pot and smear is transported in separate bags.  |
| <b>DULL, GREYISH APPEARANCE OF CELLS</b>                     | Water contamination of dehydrating and clearing solutions.<br><br>Polyethylene glycol coating not removed from cells prior to staining of filter.   | Ensure dehydrating and clearing solutions are changed regularly.<br><br>Extend the prestaining soak time.   |
| <b>OPAQUE/WHITE COLOUR ON BACK OF SLIDE</b>                  | Bluing agent not rinsed from slides.  | Use two separate but thorough water rinses following Scott's Tap Water substitute. (For progressive Pap staining)   |
| <b>STAIN DEPOSIT</b>   | Staining dyes not changed or filtered properly  | Ensure staining dyes changed or filtered regularly  |
| <b>FUNGAL CONTAMINATION</b>                                  | Slides contaminated by fungus during the staining process   | Change staining solutions regularly and ensure the staining containers are disinfected with a dilute bleach solution.   |

