A Staining Technique for the Preparation of Routine Microscope Slides for Biology

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The technique described here is a rather simple variation of the conventional hematoxylin routine, which the writers devised during the fall of 1955 when preparing slides of various tissues and organs in a standard undergraduate course in microtechnique.

METHOD

1. Fix tissues in Bouin's or Zenker's fluids.
2. Wash, dehydrate, infiltrate, embed, and section tissues in the usual fashion.
3. Affix sections to slides with Mayer's albumen.
4. Deparaffin in xylene or toluene.
5. Hydrate through a descending alcohol series to water. The process is hastened considerably if three or four drops of a ten per cent Tergitol, or other wetting agent, is added to each 50cc of all grades of alcohol, water solutions, and stains, except absolute alcohol. Do not add a wetting agent to xylene or toluene.
6. Dip in Harris' Hematoxylin for about thirty seconds; the time will vary with the batch of stain and the kind of tissue.
7. Differentiate in water made acid with six drops of concentrated HCl to each 50cc. Differentiation is complete when the sections are a faint pink. Cytoplasm should be completely destained.
8. Neutralize in water made quite alkaline with .5cc of a saturated solution of sodium carbonate to fifty cc. The sections are neutralized when they are colored a rather dirty blue.
9. Pass the slides through an ascending alcohol series to fifty per cent.
10. From a dropping bottle place a few drops of aniline blue staining solution on the section. Let react thirty seconds or less, or until the section is stained a bright blue. Aniline blue staining solution is prepared by taking two parts of a .5 per cent aqueous solution to one part of absolute alcohol.
11. Rinse off the excess stain in 70 per cent alcohol and differentiate in this solution until the section is a light blue.
12. From a dropping bottle place on the section one or two drops of a .5 per cent solution of Orange G in 50 or 70 per cent alcohol. Let react for three to five seconds.
13. Rinse in 70 per cent alcohol.
14. From a dropping bottle place one or two drops of 1 per cent Rose Bengal in 70 per cent alcohol on the section. Let react for one or two seconds. This solution stains very rapidly.
15. Rinse in 70 per cent alcohol, pass rapidly to 95, and absolute alcohol, and mount in Euparol or Diaphane. If Damar, Balsam, or Clarite is preferred, pass into toluene or xylene from absolute alcohol.

If the technique is properly followed, cross sections of amphibian stomach and intestine have the visceral peritoneum stained a purplish pink, smooth muscle pink, connective tissue a golden yellow, columnar epithelium dark pink, goblet cell cytoplasm pink, and the secretory inclusions slaty blue. Nuclei are dark blue to black. In ciliated epithelium, the cilia are blue, and the distal third of the cells slaty blue to green. The remainder of the cytoplasm is pink, and the nuclei are purplish black. Endothelia of blood vessels are pink, the remainder of the vessels blue to green. Nuclei of amphibian erythrocytes are bright blue-green, and the cytoplasm is a yellowish pink. In amphibian cells undergoing mitosis chromosomes are blue-black, cytoplasm pink, and spindle fibers are varying shades of blue-green.

The advantage of this process is its ease of manipulation. Only two dropping bottles of stain are required in addition to the reagents normally used in the hematoxylin and eosin routine. Sections of tissue prepared by this triple counterstaining method have been found to mitigate considerably the task of teaching beginning students the regional microanatomy of organs. It is hoped that the method will aid those who prepare their own material for use in courses of freshman zoology and biology.

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