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A RAPID TECHNIQUE FOR IRON-HEMATOXYLIN STAINING REQUIRING NO MICROSCOPIC CONTROL OF DECOLORIZATION

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The importance of iron-hematoxylin staining in the diagnosis of protozoa is well recognized. While it is used extensively in research institutions, and in many clinical laboratories, it has never become a routine laboratory procedure for the average technician on account of the difficult and laborious technique. The particular difficulty seems to have been the control of the decolorization process which had to be carried out under the microscope, a step requiring considerable experience in the use of the stain and more than average knowledge of protozoology.

A technique has been evolved that removes this step which has been responsible for its unsuccessful use, and which makes it possible for any laboratory technician to use the iron-hematoxylin method and obtain uniform and satisfactory results.

The only new feature of this technique is the use of a 0.25 per cent solution of iron-alum for decolorization instead of the stronger 1 and 2 per cent solutions. In this weaker solution there is considerably less danger of carrying the decolorization process too far and makes it possible to time this step and destain by the clock rather than the microscope.

In abbreviated form the technique is as follows:

	minutes
1. Fix in hot Schaudinn's solution plus 5 to 10 per cent acetic acid*	10
2. 95 per cent alcohol plus iodine (port wine color)	5
3. 70 per cent alcohol	5
4. Rinse in tap water	1–3
5. 4 per cent iron alum	15
6. Rinse in tap water	1–2

	minutes
7. Stain (0.5 per cent aqueous hematoxylin)	10
8. Decolorize in 0.25 per cent iron alum	12
9. Wash in running water	3–30†
10. Dehydrate, clear and mount	

* For Dientamoeba use 15 to 20 per cent acetic acid for best results. Flagellates also require more acid.

† If slides are for diagnosis only, a short washing period is sufficient; but if intended for permanent record they should be washed at least thirty minutes At no stage in the staining process should the smears be allowed to dry.

This schedule, in our hands, has been found to give excellent preparations of the human amoebae in stools and of *Endamoeba histolytica* in lesions of the gut.

The flagellates destain more rapidly than the amoebae, so the time of decolorization must be reduced two to four minutes for best results in staining them.

Some observations in regards to stains and solutions, used in this procedure, may be noted.

Certified hematoxylin crystals should always be used in preparation of the staining solution, and unless used, consistent results cannot be expected. Our solution is made by dissolving 0.5 gram of the certified crystals in a small amount of absolute alcohol (10 cc.) and then adding enough distilled water to make 100 cc. This stain can be used immediately, but much better results are obtained if it is allowed to ripen for at least three weeks or longer.

We have tested some eight or nine batches of stain prepared in this way, and aged for periods varying from three weeks to one and a half years and have obtained excellent results by this technique in the preparation of fecal smears and tissue sections.

Alcoholic staining solutions have not proved satisfactory and are not recommended for this technique.

The iron-alum solution is made from only the purple crystals. The destaining solution is made from the 4 per cent mordanting solution just before use. When the 4 per cent solution begins to show a cloudiness or a precipitate it is discarded and new solutions are made. This is not necessary in the older techniques, due to the fact that decolorization is controlled by microscopic observation; in this technique, however, the success of destaining

depends on solutions of constant strength and therefore fresh solutions should always be used.

The fecal smears should always be thin and even. By adding a very small drop of saline to the slide and mixing thoroughly with the fecal material on the slide this result can be obtained. Thick films are never satisfactory.

The technique just described is not a cytological procedure and is not intended for cytological studies. It has been, however, a distinct help in this laboratory in the diagnosis of the human intestinal protozoa and it is for this purpose that it is intended.