

TECHNICAL REPORT

STAINING OF INTESTINAL PROTOZOA WITH HEIDENHAIN'S IRON HEMATOXYLIN

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SUMMARY

Due to its unique properties, iron hematoxylin has been traditionally recommended for staining intestinal protozoa. This process can be simplified by reducing the number of steps and periods of permanence of the slides in some of the liquids used, without detriment to the quality of the results. Thus iron hematoxylin becomes adequate for routine use. Hematoxylin is a natural dye extracted from *Haematoxylon campechianum*, of the family Leguminosae. It must first be 'ripened', i.e. oxidized to hematein, which reacts with ferric ammonium sulphate to produce the ferric lake (iron hematoxylin), a basic dye. Iron hematoxylin most frequently stains regressively, i.e. the slides are first overstained and then differentiated.

KEYWORDS: Iron hematoxylin staining; Intestinal protozoa.

Iron hematoxylin staining of intestinal protozoa is often thought of as too complex and time-consuming to be used in routine fecal examinations. This process can, however, be simplified without detriment to the quality of the results. Hematoxylin is a natural dye extracted from *Haematoxylon campechianum*, a tree of the family Leguminosae. In actual fact, to be used as a dye, hematoxylin must first be 'ripened', i.e. oxidized to hematein, a slow process when its crystals are dissolved in water and exposed to atmospheric oxygen¹. Immediate ripening is produced by the addition of an oxidizing agent^{1,4}, such as hydrogen peroxide (a few drops), to the solution. This must be done cautiously, as further oxidation produces oxihematein, which is not a dye⁴. Hematoxylin solutions usually contain a mixture of hematoxylin, hematein and oxihematein. The well-known 'hematoxylin' staining properties result from the combination of hematein with a mordant to produce a lake. 'Iron hematoxylin', which is quite stable and can stain sharply the fine structures used to identify intestinal protozoa², results from the reaction of hematein with iron alum (ferric ammonium sulfate)^{1,4}. It is mostly used to stain regressively, which means that the slides are first overstained and then differentiated. By adding a weak acid to the hematoxylin solution, the iron lake can stain progressively. About 0.02 g of citric acid or a few drops of acetic acid per 100 mL of the solution will be enough.

Solutions:

Schaudinn's solution

Saturated solution of mercuric chloride in distilled water 80 mL

Ethyl alcohol (95%) 20 mL
Acetic acid 3 mL

Iodine solution

Ethyl alcohol (95%) 98 mL
Tincture of iodine (2%) 2 mL

Mordant-differentiating solution

Iron alum (ferric ammonium sulfate) 2 g
Water (distilled) 98 mL

Hematoxylin solution

Hematoxylin 0.30 g
Water (distilled) 100 mL

Staining procedure:

1. On clean slides, prepare thin smears and while they are still wet, immerse them in Schaudinn's solution for about at least one minute. Longer periods will cause no harm to the samples. If the smears do not stick to the slides, place a small amount of blood serum upon the slide and mix it with the fecal sample.
2. Rinse the slides in tap water (three changes).
3. Immerse them in alcohol (70 – 95%)
4. To remove remaining mercury residues, place the slides in the iodine solution for one minute, then in alcohol (70 – 95%) for the same period.

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5. Rinse in tap water (three changes).
6. Place in the mordant solution (about 30 seconds).
7. Rinse in tap water (four to five changes).
8. Place in the staining solution for three to five minutes (according to the proportion of 'ripe' staining substance). Staining should be controlled by inspection.
9. Rinse in water (three changes).
10. If differentiation is needed, immerse rapidly the slide in the mordant-differentiating solution, then rinse in tap water (three to five changes).
11. Dehydrate by immersing in alcohol (70–95%) for about two minutes, then in 95% alcohol, then in two changes of absolute alcohol (two minutes each); immerse in xylene before mounting.

The last step can be dispensed with when the slides are intended for immediate examination during routine diagnosis. A rapid mount is obtained by placing a drop of glycerin between the smear and the coverslip. Although optically not quite so good as the usual permanent mounts, such preparations are compatible with the use of high-power immersion objectives.

A simple test is suggested to evaluate the staining property of the hematoxylin solution: place one drop of the hematoxylin solution and one of the mordant solution side by side on the surface of a piece of filter paper. As both solutions spread out, a black spot formed in the region where they mix will be indicative of good staining property.

RESUMO

Coloração de protozoários intestinais pela hematoxilina férrica de Heidenhain

Por suas propriedades peculiares, a hematoxilina férrica tem sido tradicionalmente recomendada para a coloração de protozoários intestinais. O processo pode ser simplificado sem perda de qualidade dos resultados, tornando-se aplicável à rotina. Para isto reduzem-se o número de passagens e os tempos de permanência das lâminas nos líquidos usados. A hematoxilina é um corante natural, extraído de *Hematoxylon campechianum*, da família Leguminosae. Antes do uso ela deve "amadurecer", isto é transformar-se, por oxidação, em hemateína, que reage com o sulfato férrico-amônio para produzir a laca férrica (hematoxilina férrica), um corante básico. Habitualmente, a hematoxilina férrica é usada para corar regressivamente: as lâminas são primeiramente supercoradas e ulteriormente diferenciadas.

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