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Section: Parasitology Manual	Subject Title: Laboratory Procedures for Blood and Tissue Parasites	
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Hematoxylin Stain for Microfilaria

PRINCIPLE

In order to determine the species of microfilaria isolated by other methods it is necessary to perform a hematoxylin stain on the specimen. Giemsa staining is faster and easier, however the staining of the sheath, an important feature for determining the species can be variable with Giemsa stain.

SPECIMEN

A microfilaria isolated using an isolation method.

REAGENTS

Hematoxylin A & B (commercial product)
Xylol
Methanol
Entellan (commercial product)

SAFETY

Microfilaria require passage through their insect vector before they become infective, therefore the sample cannot produce a filaria infection from a sharps stick.

PROCEDURE

This is considered to be a non-routine procedure therefore it should only be performed by experienced personnel.

1. If a thick film is being examined dehemaglobinize the slide in tap water for 10 minutes and then air dry the specimen.
2. If a thin film is being examined fix in absolute methanol for 15 minutes and then allow the specimen to air dry.
3. Place in running tap water for 5-10 minutes.
4. Place in hematoxylin-mordant solution for 10 minutes (30 minutes if *M. perstans* is suspected).
5. Rinse in tap water 1 minute
6. Rinse in 50% methanol + 10 drops of ammonia for a few seconds
7. Rinse in 70% methanol + 10 drops of ammonia for a few seconds

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8. Rinse in 85% methanol for a few seconds
9. Rinse in 95% methanol for a few seconds
10. Place in absolute methanol for 5 minutes
11. Place in Xylol for 5 minutes
12. Place in Xylol for 5 minutes
13. Mount in Entellan

PROCEDURE NOTES

1. This stain gives the best morphological details. Organisms can be missed under bright light, therefore low power observations may be preferable.
2. The most important step in the preparation of a well stained permanent smear is good fixation. If good fixation has not been successful the microfilaria will be distorted or may stain poorly.
3. Once the staining process has started the smear should not be allowed to dry at any of the subsequent steps until they are cover-slipped.
4. Slides should always be drained thoroughly between solutions, touch the end of the slide with a paper towel to remove excess fluid.
5. In the final stages of dehydration the 100% alcohol and xylol should be kept as free from water as possible. Containers must have tight fitting caps to prevent evaporation or absorption of moisture. If xylol becomes cloudy replace with fresh stock.
6. If smears peel or flake off, the specimen may have been inadequately dried on the slide or the smear may be too thick
7. Patient's stained slides must be allowed to dry thoroughly, (eg. overnight) before examination.
8. Vernier measurements should be taken for the location of any parasite with unusual morphology so that confirmation can be obtained.
9. The species of the microfilaria should be determined using reference material such as "Bench Aids to the Diagnosis of Microfilaria (WHO).

REPORT

Presence and species of microfilaria.

LIMITATIONS OF PROCEDURE

- Species determination is a desirable outcome, however sometimes the numbers or condition of the microfilaria in the sample makes this difficult.

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