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Parasitology

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Staining of Blood parasites other than malaria parasites

Species of microfilariae

Method

a. Slides are fixed in methanol for 2 minutes
b. If microfilariae of *Loa loa*, follow steps iii, iv, v and vi because the sheath of *Loa loa* does not stain with Giemsa. For all other sheathed microfilariae, proceed only to step iv. since their sheaths stain with Giemsa..
c. Stain with a 1 in 10 dilution of Giemsa stain in pH 7.2 buffered water for 25 minutes (this stage stains the nuclei).
d. Gently wash in running water
e. The sheath can be stained by using a 1 in 10 dilution of Delafield's haematoxylin in distilled water for 25 minutes (used only for microfilariae of *Loa loa*).

NOTE. The timings and the concentration of Delafield's could differ depending on the stain manufacturer and batch of stain. Timings and concentration must first be established using a small number of slides before staining the whole batch.

f. Gently wash in running water and leave to air dry.

Result

Nuclei stain blue and the sheath stains pale grey/blue.

- The sheath of *Wuchereria bancrofti* stains pink with Giemsa.
- The sheath of *Loa loa* **does not** stain with Giemsa therefore Delafields haematoxylin must be used in order to make the sheath visible for microscopy.
- The sheath of *Brugia* sp. stains dark pink with Giemsa.

Leishmania and Trypanosoma species

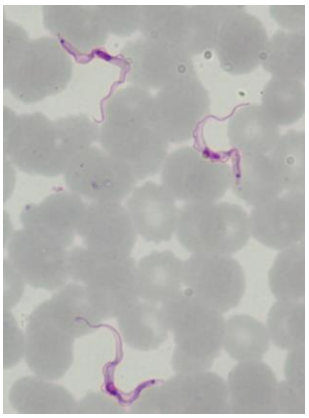
Method

This method applies to both thin blood films and tissue films

a. Fix in methanol for 2 minute
b. Stain with Giemsa 1 in 10 in buffered distilled water pH 6.8 for 30
c. Wash the slide in running water and drain dry

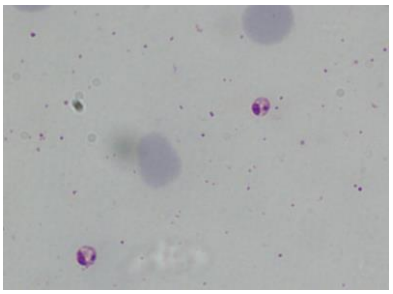
Result

- Amastigotes of *Leishmania* should be seen in positive smears. They are approximately 2-4 µm in size, oval and are frequently seen within the cytoplasm of the macrophage. The amastigotes possess a nucleus and a rod - shaped kinetoplast within the cytoplasm.
- Trypomastigotes of *Trypanosoma* species is an elongated cell with single nucleus which usually lies near the centre of the cell. Each cell bears a single flagellum which appears to arise from a small granule - the kinetoplast. The length and position of the trypanosome's flagellum is variable. In trypanosomes from the blood of a host the flagellum originates near the posterior end of the cell and passes forward over the cell surface, its sheath is expanded and forms a wavy flange called an undulating membrane.



African trypanosomes

Microfilariae of *Loa loa*



Amastotes of *Leishmania* spp.