

4. Concentration Method for Microfilariae with Formaldehyde Solution (+ Delafield's haematoxylin stain)

Swiss Tropical Institute, Basel

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4.0 Concentration Method for Microfilariae

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4.1 Concentration Method for Microfilariae

Materials:

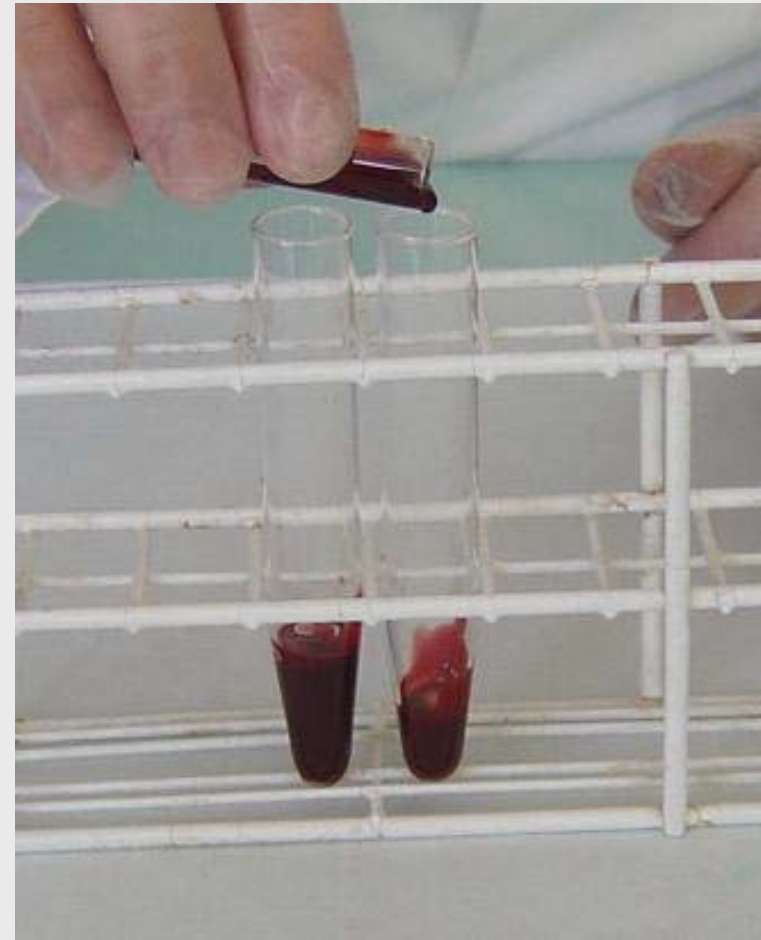
- Centrifugation tubes
- 2% Formalin solution
- Centrifuge
- Microscopic slides
- Cover slips
- Methylene blue
- Pipettes
- Gloves





4.2 Concentration Method for Microfilariae

Fill 2 centrifugation tubes with 1 ml of anti-coagulated blood



4.3 Concentration Method for Microfilariae

Add 9 ml of a 2%
Formalin solution to
each tube

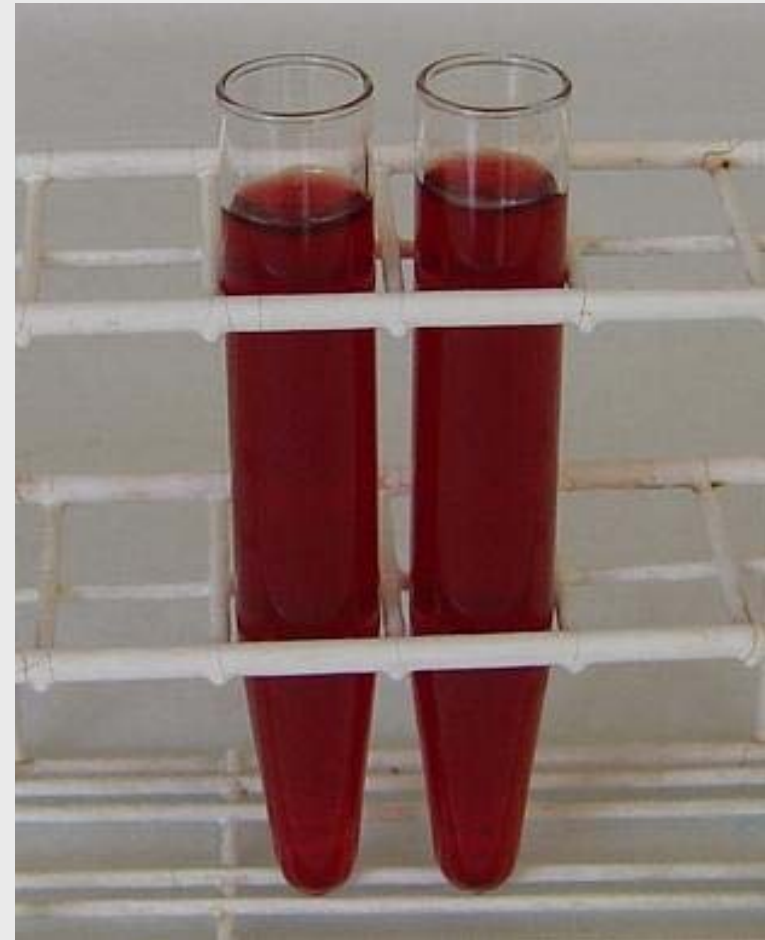
(final dilution: 0.2%)



4.4 Concentration Method for Microfilariae



Wait 5 minutes for the red cells to haemolyse



4.5 Concentration Method for Microfilariae



Mistake:

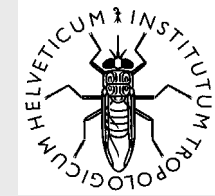
Blood coagulated
(left tube)

Correct:

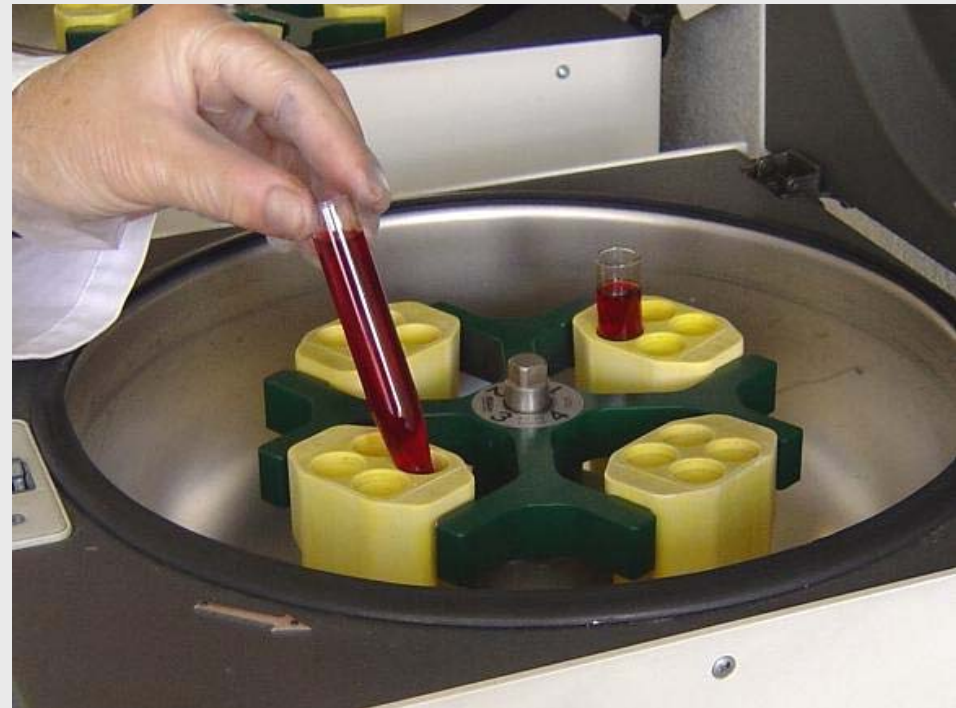
Complete haemolysis
(right tube)



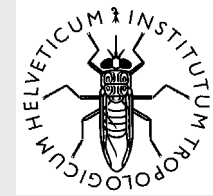
4.6 Concentration Method for Microfilariae



Centrifugation at
2000 rpm for 3 minutes



4.7 Concentration Method for Microfilariae

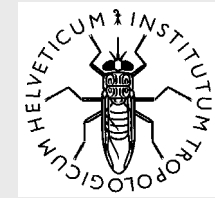


After centrifugation:

Sediment is visible



4.8 Concentration Method for Microfilariae

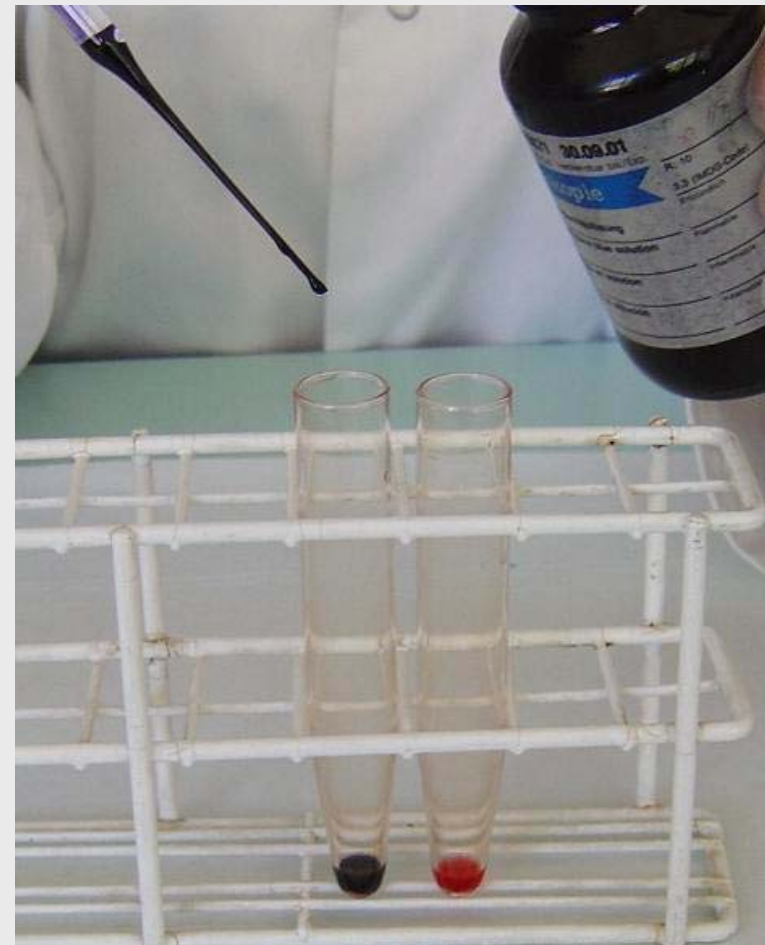


Decant the supernatant

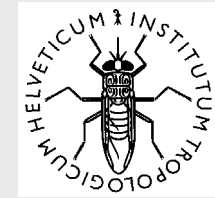


4.9 Concentration Method for Microfilariae

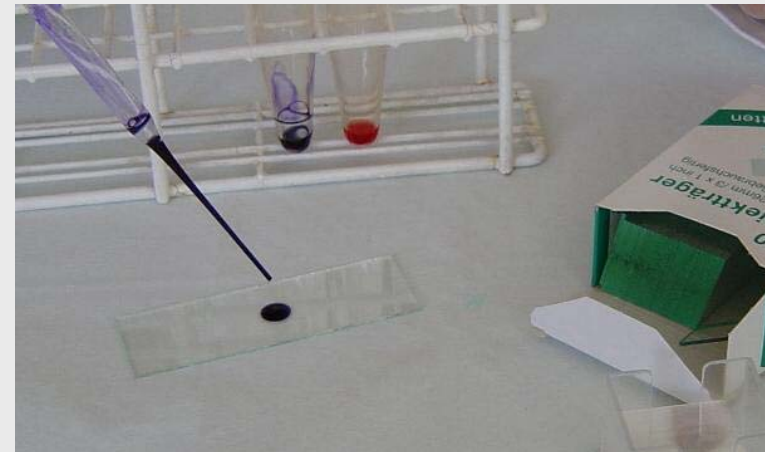
Add a drop of Methylene-blue to **one** sediment and mix **carefully**.
(for better visibility of the microfilariae)



4.10 Concentration Method for Microfilariae



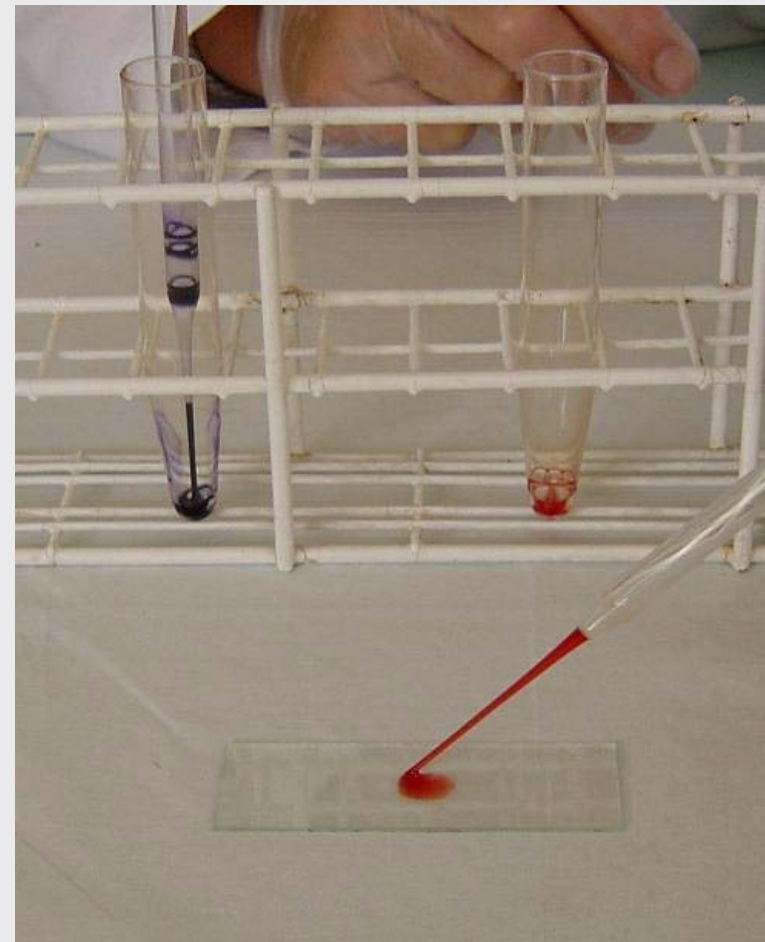
Prepare stained sediment for microscopy.
Search and count all microfilariae in the whole sediment under a cover slip
(Objective 10x)



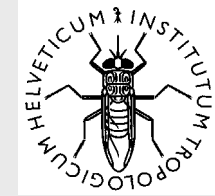
4.11 Haematoxylin stain for Microfilariae

If microfilariae are found:

Prepare thick film from the **second** sediment (containing no Methylene-blue) for identification by Delafield's haematoxylin staining



4.12 Haematoxylin stain for Microfilariae



Carefully dry the thick film



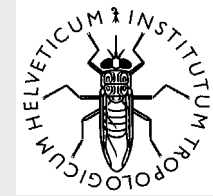
4.13 Haematoxylin stain for Microfilariae



Fix the thick smear with
Methanol



4.14 Haematoxylin stain for Microfilariae



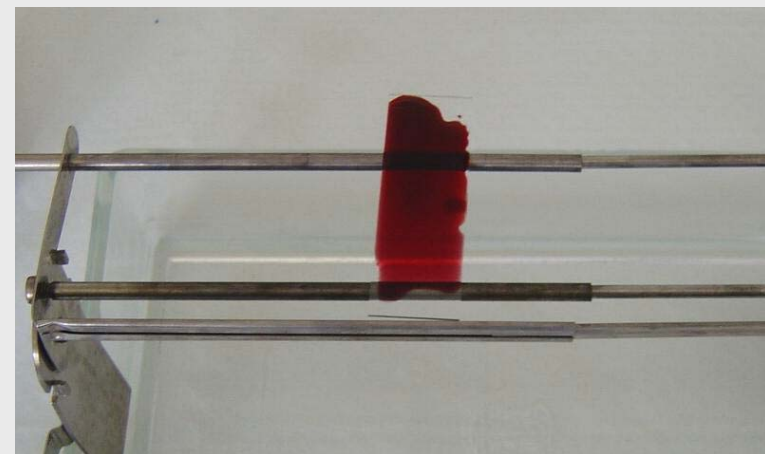
Dry the thick smear

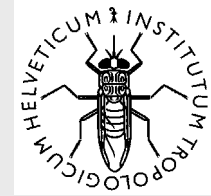


4.15 Haematoxylin stain for Microfilariae



Stain the thick film
with filtered Delafield's
haematoxylin for 30
minutes





4.16 Haematoxylin stain for Microfilariae

Intensify the blue colour by immersing the slide in tap water for 10 minutes and let the thick smear dry afterwards.

