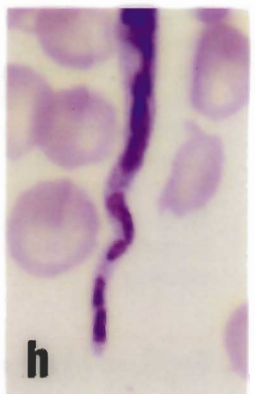
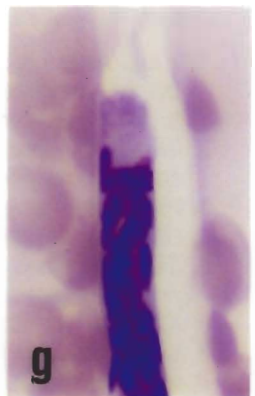
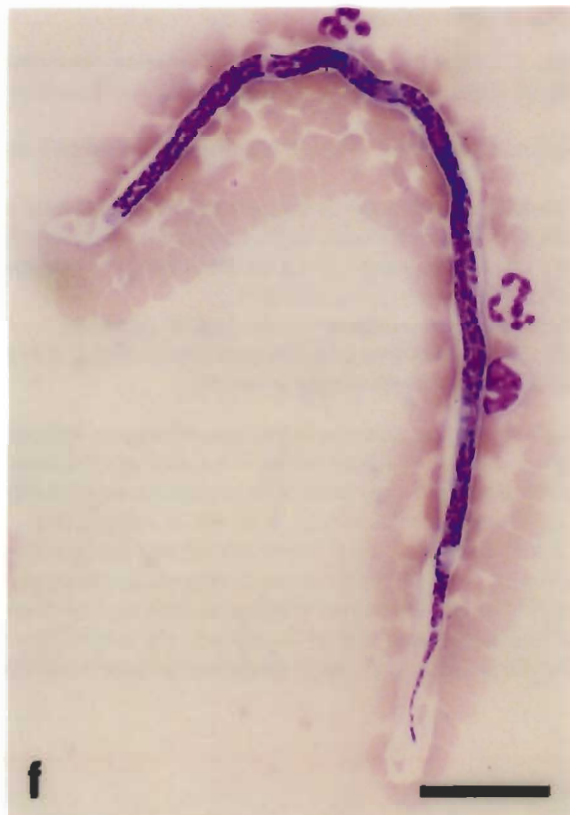




Wuchereria bancrofti microfilariae in haematoxylin (a, c, d) and Giemsa (b) stains. Characteristically, the sheath stains lightly with haematoxylin (a, c) but not with Giemsa stain (b). Key morphological features include a short head space (a, b, c) and discrete nuclei in the body. The column of nuclei does not extend to the end of the tail (d). The innerbody stains pink with Giemsa stain (b, *arrowhead*) but not with haematoxylin stain.



Loa loa microfilariae in haematoxylin (e) and Giemsa (f–h) stains. The sheath is clearly evident in haematoxylin (e) but not in Giemsa stain; however, in Giemsa stain, its presence is often demarcated by red blood cells that lie along the margin of the sheath (f). Key features of *L. loa* include a short head space (g) and a compact column of nuclei that extends to the end of the tail; the last few nuclei are irregularly spaced (h). Very frequently, the tail is flexed or coiled within the sheath (e, *inset*). There is no easily identifiable innerbody in stained *L. loa* microfilariae.



Diagnosis of filarial infections

As well as in blood and skin, microfilariae may occasionally be found in bone marrow preparations, fine-needle biopsy aspirates, cervical smears contaminated with blood, hydrocele fluid, chylous urine, and normal urine following treatment with diethylcarbamazine. Methods commonly used for the detection of microfilariae include:

Blood examination

- stained thick blood films
- direct examination of capillary blood
- membrane filtration (fresh or preserved blood)
- haemolysed venous blood concentration (Knott concentration method)

Tissue examination

- skin snips

Other body fluid examination

- urine
- hydrocele fluid

Caution: Standard biosafety guidelines should be followed in obtaining blood and tissue samples. Disposable or sterile lancets, syringes, and needles should be used for all laboratory procedures. These guidelines are summarized in *Biosafety guidelines for diagnostic and research laboratories working with HIV* (Geneva, World Health Organization, 1991; WHO AIDS Series, No. 9).

Preparation of thick blood films

The examination of thick blood films is the most widely used method in field surveys of filarial infection. Properly done, it is a reliable procedure for both identification of microfilariae and enumeration studies. Carefully measured samples of at least 20 μ l and preferably 60 μ l in volume are recommended.

1. Thoroughly clean the microscope slides (including factory "pre-cleaned" slides) before use. Dust, grease, detergent, or cotton lint and threads may cause the blood film to lift off the slide.
2. Clean the finger tip (or ear lobe) from which the blood will be taken with a cotton ball soaked in alcohol.
3. Prick the finger tip or ear lobe with a sterile lancet and allow the blood to ooze freely.
4. Draw the required volume of blood into a disposable or sterile calibrated capillary pipette.
5. Expel the blood onto a microscope slide and smear the sample uniformly in a circular or rectangular shape; avoid creating any bubbles.
6. Allow the slide to dry at room temperature in a horizontal position.
7. Label and store the slide in a dust-free environment until staining. It is also important to protect unfixed blood films from damage by insects.

Note: Excess alcohol on the skin may partially fix the blood sample; squeezing the finger or ear lobe may dilute the sample with tissue fluids. Films that are too thick tend to lift off the slide. Blood films must be thoroughly dried before dehaemoglobinization; this may require 12–48 hours, depending on humidity. If blood is collected in a heparinized capillary pipette, or if the film is made from blood containing an anticoagulant, drying requires at least 48–72 hours. Thin blood films are of little value because the volume of blood examined is small. However, when microfilariae are found in thin films they tend to be concentrated at the "feathered" end and at the margins of the film. The morphology of microfilariae found in thin films tends to be good since the films are routinely fixed before staining.

Capillary blood examination

Microscopic examination of fresh blood has limited utility. It can reveal the presence of microfilariae actively moving among the red blood cells (see front cover), but species identification is not possible. However, in regions where only one species of microfilaria is found, its presence and density in the blood can be determined with reasonable accuracy by this means.