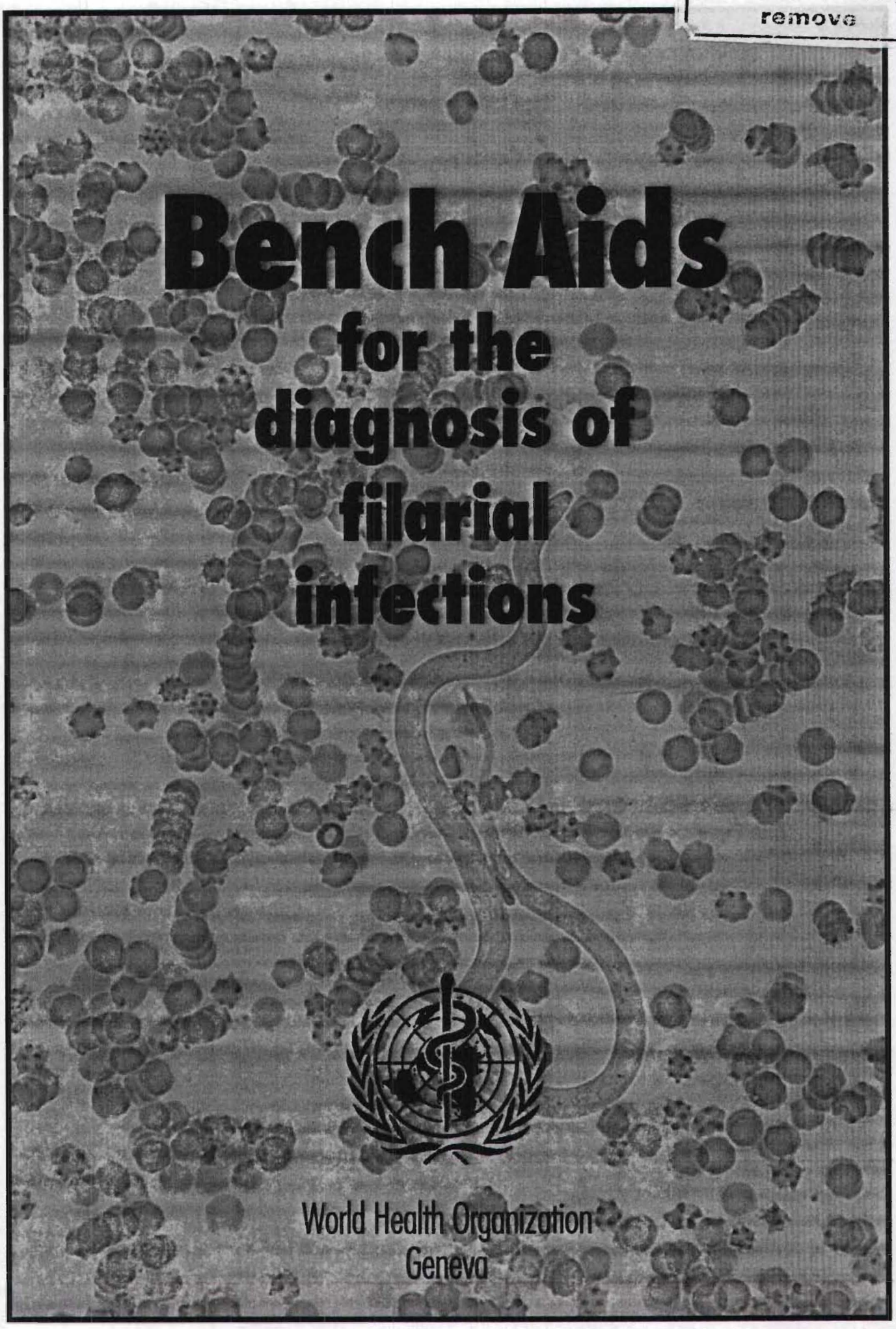

Bench Aids
for the diagnosis of filarial infections

World Health Organization
Geneva

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Front cover: Living microfilaria of *Loa loa* in a drop of fresh blood.
Back cover: Microfilariae of *Wuchereria bancrofti* in a haematoxylin-stained thick blood film.

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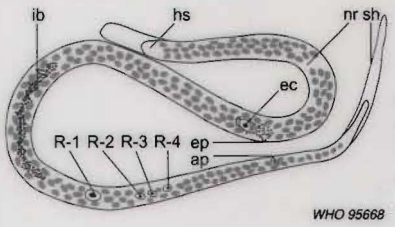
Introduction

Several species of filarial worms infect humans in the tropical and subtropical regions of the world (Table 1, overleaf). The adult worms inhabit various tissues and organs of the body and are inaccessible for identification. Consequently, diagnosis of filarial infections depends primarily on the identification of the larval stage of the parasite (microfilaria). Most species of microfilaria circulate in peripheral blood; however, some are found in the skin.

The microfilaria

At the light-microscopic level and with the aid of a variety of stains, a microfilaria appears as a primitive organism, serpentine in shape and filled with the nuclei of many cells. Figure 1 is a diagram of a typical microfilaria. In many, but not all, species, the body may be enveloped in a membrane called a sheath (**sh**). Where a sheath is present it may extend a short or long distance beyond either extremity of the microfilaria. In some species, depending on the stain used, the sheath displays a characteristic staining quality which aids in species identification. The nuclei of the cells that fill the body are usually darkly stained and may be crowded together or dispersed. The anterior extremity is typically devoid of nuclei and is called the cephalic or head space (**hs**); it may be short or long. Along the body of the microfilaria there are additional spaces and cells that serve as anatomical landmarks. These include the nerve ring (**nr**), excretory pore (**ep**), excretory cell (**ec**), and anal pore (**ap**). In some species, an amorphous mass called the innerbody (**ib**) and four small cells called the rectal cells (**R-1**, **R-2**, **R-3**, **R-4**) can be seen, usually with the aid of special stains. These structures and their positions are sometimes useful for species identification. The shape of the tail and the presence or absence and distribution of nuclei within it are also important in species identification.

Fig. 1 Typical microfilaria

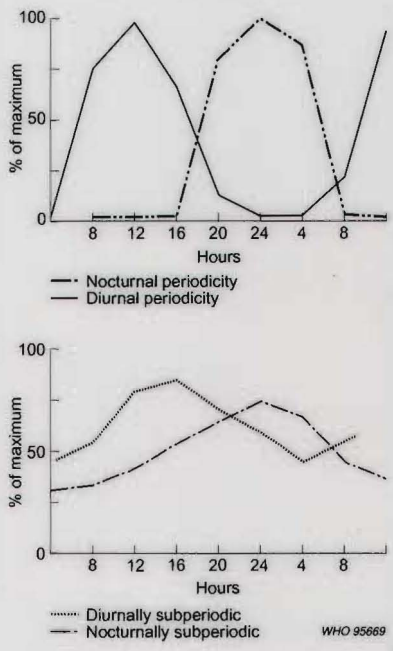


Periodicity

Some species of microfilariae circulate in peripheral blood at all hours of the day and night, while others are present only during certain periods. The fluctuation in numbers of microfilariae present in peripheral blood during a 24-hour period is referred to as periodicity (Fig. 2). Species that are found in the blood during night-time hours but are absent at other times are designated *nocturnally periodic* (e.g. *Wuchereria bancrofti*, *Brugia malayi*); those that are present only during certain daytime hours are designated *diurnally periodic* (e.g. *Loa loa*). Microfilariae that are normally present in the blood at all hours but whose density increases significantly during either the night or the day are referred to as *subperiodic*. Microfilariae that circulate in the blood throughout a 24-hour period without significant changes in their numbers are referred to as *nonperiodic* or *aperiodic* (e.g. *Mansonella* spp.).

The periodicity of a given species or geographical variant is especially useful in determining the best time of day to collect blood samples for examination. To determine microfilarial periodicity in an individual, it is necessary to examine measured quantities of peripheral blood collected at consecutive intervals of 2 or 4 hours over a period of 24–30 hours.

Fig. 2 Patterns of periodicity



Further reading

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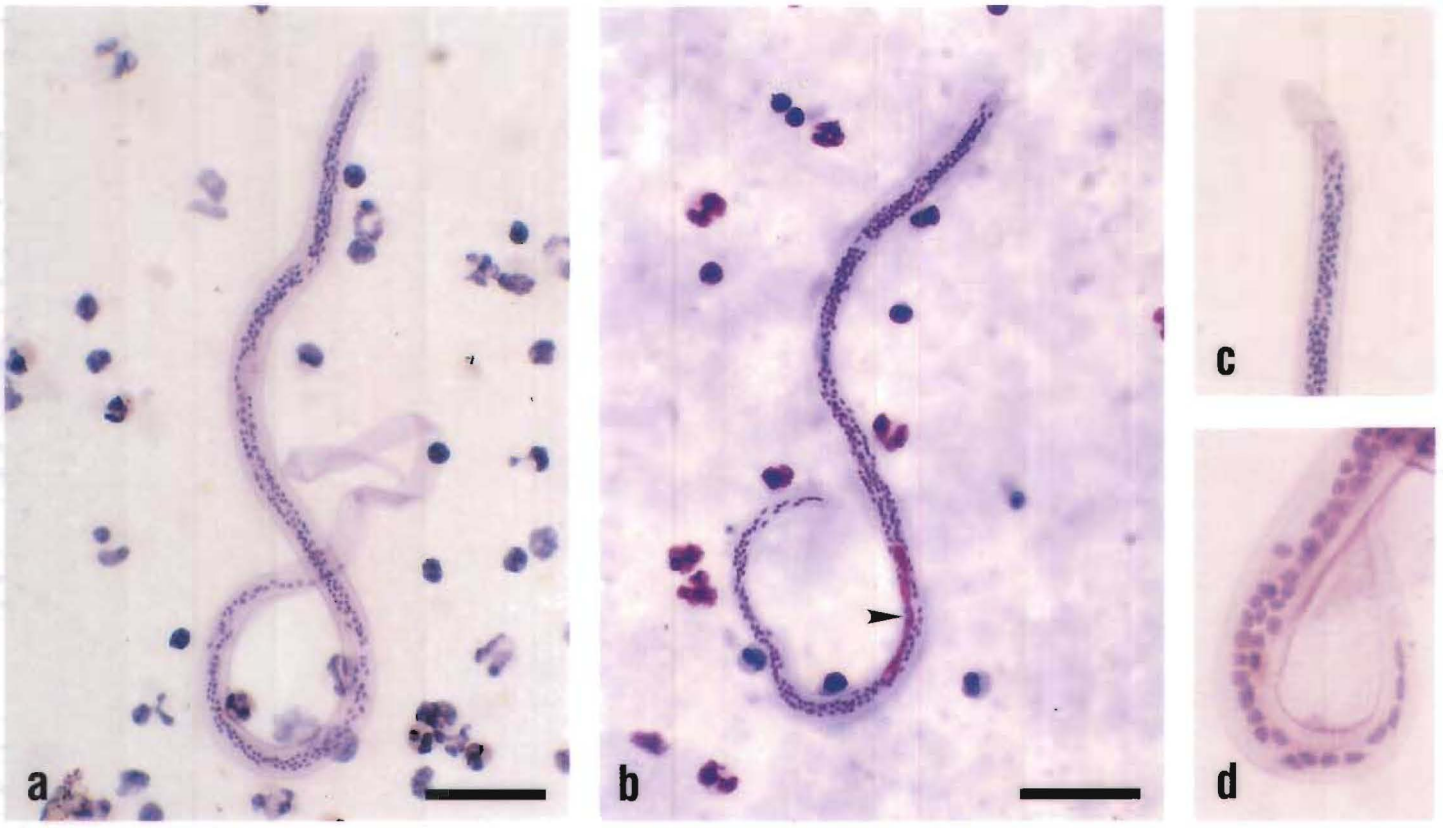
Table 1. Characteristics of common human filarial parasites

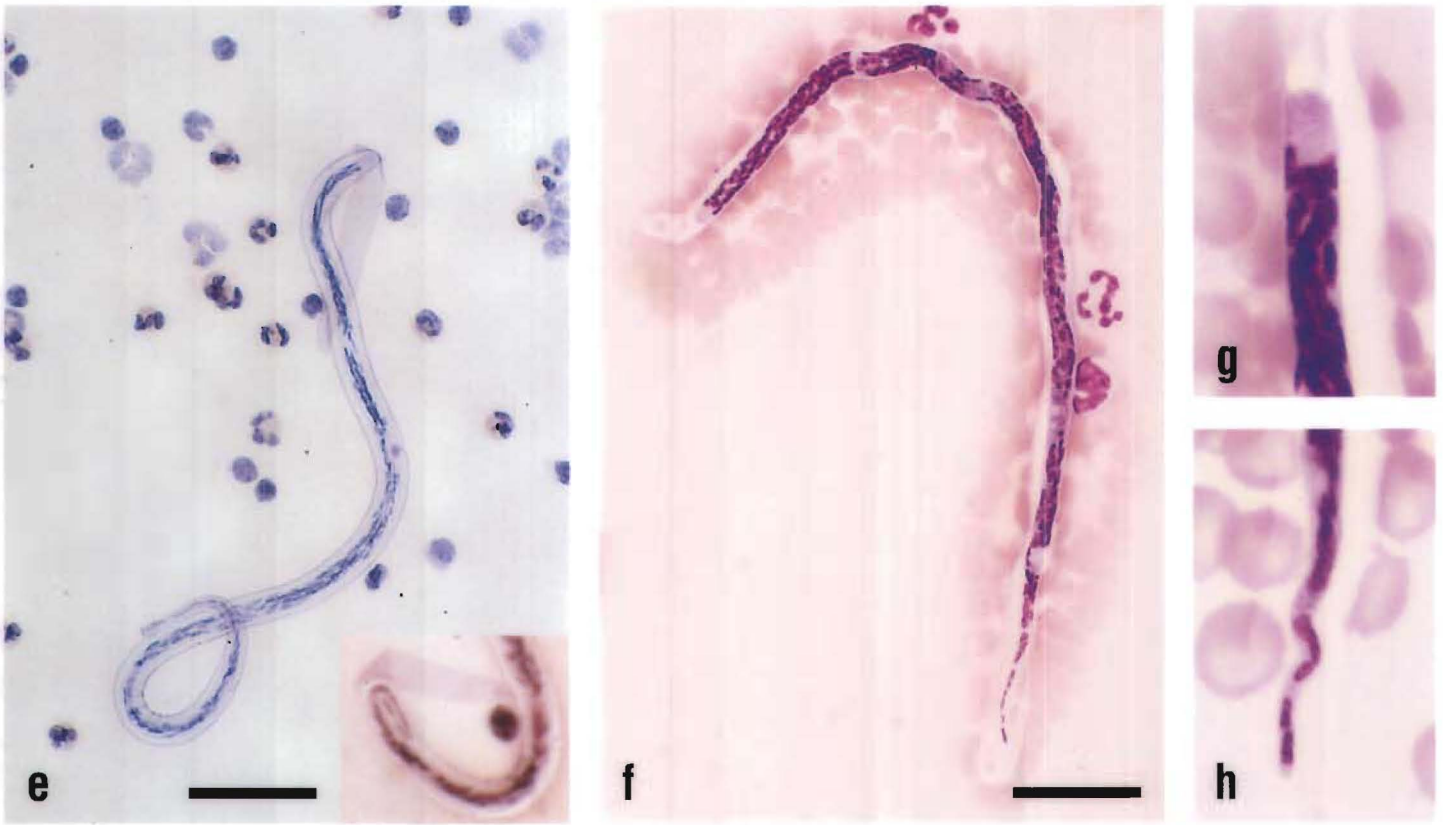
Species	*Wuchereria bancrofti*	*Brugia malayi*	*Brugia timori*	*Loa loa*	*Mansonella ozzardi*	*Mansonella perstans*	*Mansonella streptocerca*	*Onchocerca volvulus*
Geographical distribution	Tropics and subtropics worldwide	South-east Asia, Indian subcontinent	Indonesian archipelago, Timor, Lesser Sunda Islands	West and Central Africa	Caribbean, Central and South America	Africa and South America	West and Central Africa	Africa, Yemen, Central and South America
Vectors	Mosquitos: *Culex, Aedes, Anopheles, Mansonia*	Mosquitos: *Mansonia, Anopheles, Aedes*	Mosquitos: *Anopheles*	Tabanid flies: *Chrysops*	Biting midges: *Culicoides* Black flies: *Simulium*[a]	Biting midges: *Culicoides*	Biting midges: *Culicoides*	Black flies: *Simulium*
Adult habitat	Lymphatic system	Lymphatic system	Lymphatic system	Subcutaneous tissues, conjunctivae	Subcutaneous tissues	Mesenteries, connective tissues of abdominal organs	Dermis	Subcutaneous and deeper tissues
Habitat of microfilaria	Blood	Blood	Blood	Blood	Blood	Blood	Skin	Skin
Periodicity	Nocturnal[b]	Nocturnal[c]	Nocturnal	Diurnal	Aperiodic	Aperiodic	—	—
Sheath	Present	Present	Present	Present	Absent	Absent	Absent	Absent
Length (μm)[d]								
smears	244–296 (260)	177–230 (220)	265–323 (287)	231–250 (238)	163–203 (183)	190–200 (195)	—	—
2% formalin	275–317 (298)	240–298 (270)	332–383 (358)	270–300 (281)	203–254 (224)	183–225 (203)	—	—
skin snips	—	—	—	—	—	—	180–240 (210)	304–315 (309)
Width (μm)	7.5–10.0	5.0–6.0	4.4–6.8	5.0–7.0	3.0–5.0	4.0–5.0	5.0–6.0	5.0–9.0
Tail	Tapered; anucleate	Tapered; subterminal and terminal nuclei widely separated	Tapered; subterminal and terminal nuclei widely separated	Tapered; nuclei irregularly spaced to end of tail	Long, slender, pointed; anucleate	Bluntly rounded; nuclei to end of tail	Bluntly rounded; bent into hook; nuclei to end of tail	Typically flexed; tapered to a point; anucleate
Key features of microfilaria	Short head space; dispersed nuclei; sheath unstained in Giemsa; body in smooth curves	Long head space; sheath stains pink in Giemsa; terminal and subterminal nuclei	Long head space; sheath unstained in Giemsa; terminal and subterminal nuclei	Single row of nuclei to end of tail; sheath unstained in Giemsa	Small size; long slender tail; aperiodic	Small size; blunt tail filled with nuclei; aperiodic	Slender shape; hooked tail filled with nuclei; occurs in skin	Flexed tail; occurs in skin, occasionally in urine or blood after treatment

[a] Reported in Brazil, Guyana, and the Amazon region of Colombia.
[b] Diurnally subperiodic in New Caledonian and Polynesian regions; nocturnally subperiodic in rural areas of Thailand.
[c] Nocturnally subperiodic in parts of Indonesia, Malaysia, Philippines, and Thailand.
[d] Mean values given in parentheses.

Note: All measuring bars = 30 µm



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 µl and preferably 60 µl in volume are recommended.

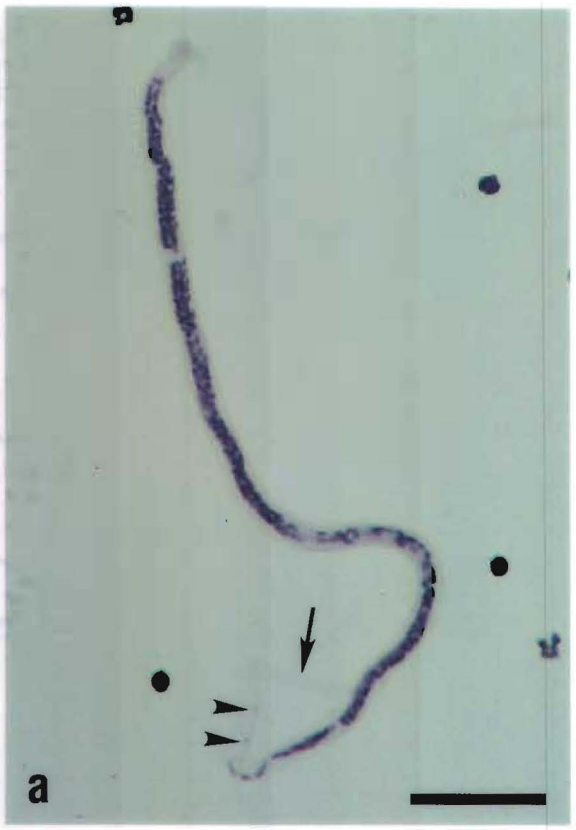
1. Thoroughly clean the microscope slides (including factory "pre-cleaned" slides) before use. Dust, grease, detergent, or cotton lint and threads may cause the blood film to lift off the slide.
2. Clean the finger tip (or ear lobe) from which the blood will be taken with a cotton ball soaked in alcohol.
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.

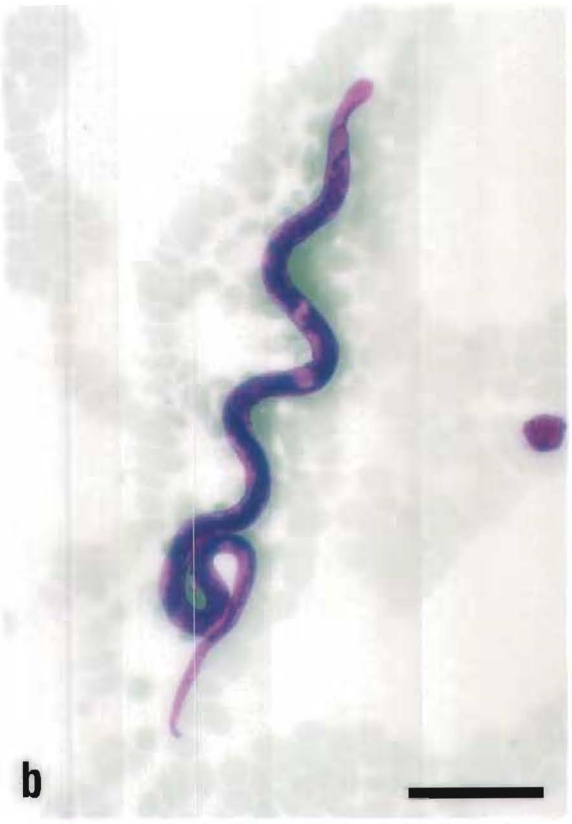
Note: Excess alcohol on the skin may partially fix the blood sample; squeezing the finger or ear lobe may dilute the sample with tissue fluids. Films that are too thick tend to lift off the slide. Blood films must be thoroughly dried before dehaemoglobinization; this may require 12–48 hours, depending 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

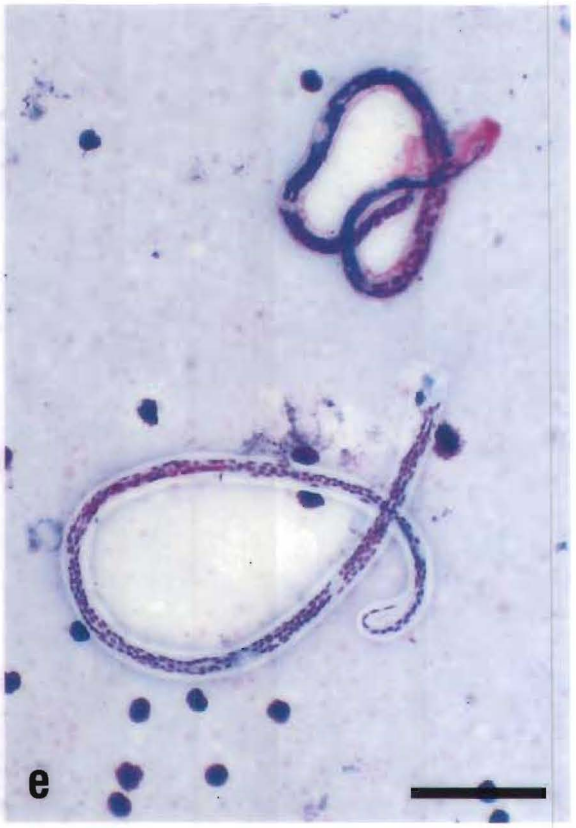
Microscopic examination of fresh blood has limited utility. It can reveal the presence of microfilariae actively moving among the red blood cells (see front cover), but species identification is not possible. However, in regions where only one species of microfilaria is found, its presence and density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

Note: All measuring bars = 30 µm

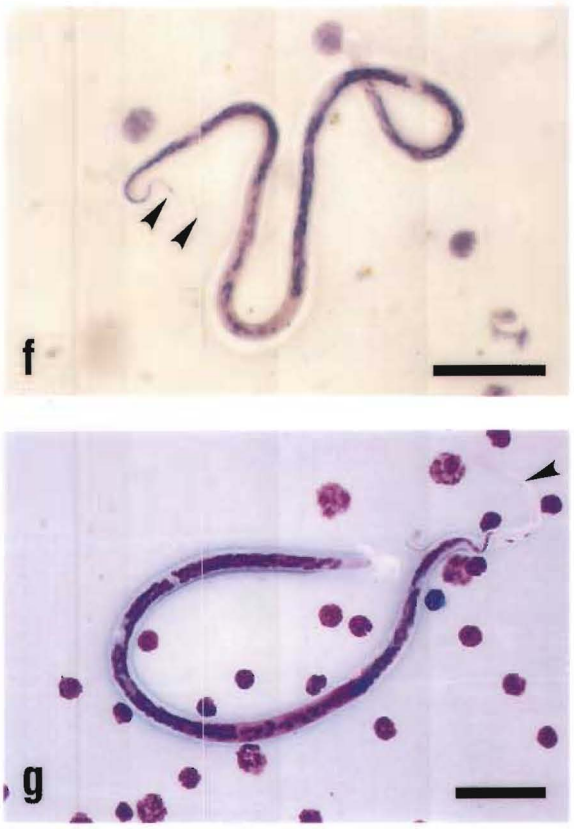




Brugia malayi microfilariae in haematoxylin (a) and Giemsa (b–d) stains. In haematoxylin, the sheath does not stain but may be faintly visible (a, *arrow*). This contrasts with the pink-stained sheath seen in Giemsa preparations (b, c). The column of nuclei is compact, and the widely separated subterminal and terminal nuclei in the tail are key diagnostic features (a, *arrowheads*; d). Nuclei are sparse in the region of the innerbody (a).



B. malayi (upper) and ***W. bancrofti*** (lower) microfilariae in the same field of a Giemsa-stained blood film (e). The pink-stained sheath and the darkly stained, compact column of nuclei identify ***B. malayi*** and distinguish it from ***W. bancrofti***



Brugia timori microfilariae in haematoxylin (f) and Giemsa (g–i) stains. ***B. timori*** is larger than ***B. malayi*** and the sheath does not stain pink (g, *arrowhead*) with Giemsa stain. The long head space and the subterminal and terminal nuclei are conspicuous features (f–i).

Staining of thick blood films

Giemsa and haematoxylin are the preferred and most widely used stains for preparing permanently stained blood films. Each has its advantages, but Giemsa stain is used most often. Slides can be processed in either small or large numbers using stainless steel, glass, or plastic staining racks and dishes.

Before staining, thoroughly dried films must be dehaemoglobinized and fixed. Immerse slides in tap or distilled water until the haemoglobin leaches out of the film, which becomes whitish in colour; this requires about 3–5 minutes. Films that are prepared from blood containing an anticoagulant and that have dried for more than a few days will dehaemoglobinize slowly, usually in 8–10 minutes. Allow dehaemoglobinized films to air-dry thoroughly. Fix the films in methanol for 30–60 seconds and air-dry.

Note: In the event that the same films are being used for malaria surveys, they should be stained without dehaemoglobinization or fixation. Microfilariae found in these preparations usually appear slightly swollen, and the nuclei are not sharply demarcated (Plate 3b).

Giemsa stain

Stain blood films for 45 minutes in a 1:50 dilution of Giemsa stain (or 20 minutes in a 1:20 dilution) at a pH of 6.8–7.2; wash films for 3–5 minutes in neutral buffered water or under running tap water. Dry films in a vertical position.

Note: The staining dilution and procedure used for processing malaria films can be used here with the expectation of acceptable results. Nuclei of microfilariae will stain blue to purple in colour. A sheath, if present, will stain pink (*B. malayi*) or not at all. The innerbody of *W. bancrofti* will stain a bright pink colour, but that of most other species does not stain.

Haematoxylin stain

Various haematoxylin stains are used as alternatives to Giemsa stain; Delafield's haematoxylin is recommended and is widely used. It enhances nuclear detail in the microfilaria and stains the sheath, when present, a greyish-blue colour. For preparation of Delafield's haematoxylin and details of another useful staining procedure, consult the WHO publication *Basic laboratory methods in medical parasitology* (1991). It is also acceptable to use other available stains and procedures.

Procedure

1. Thick blood films should be dried thoroughly, dehaemoglobinized, and fixed as described above. If films are prepared from sedimented Knott concentration material, dehaemoglobinization and fixation are omitted.
2. Stain slides for 10–15 minutes in Delafield's haematoxylin solution. Rinse in distilled water to remove excess stain.
3. Destain in 0.1% (1 g/l) aqueous hydrochloric or acetic acid for approximately 1 minute