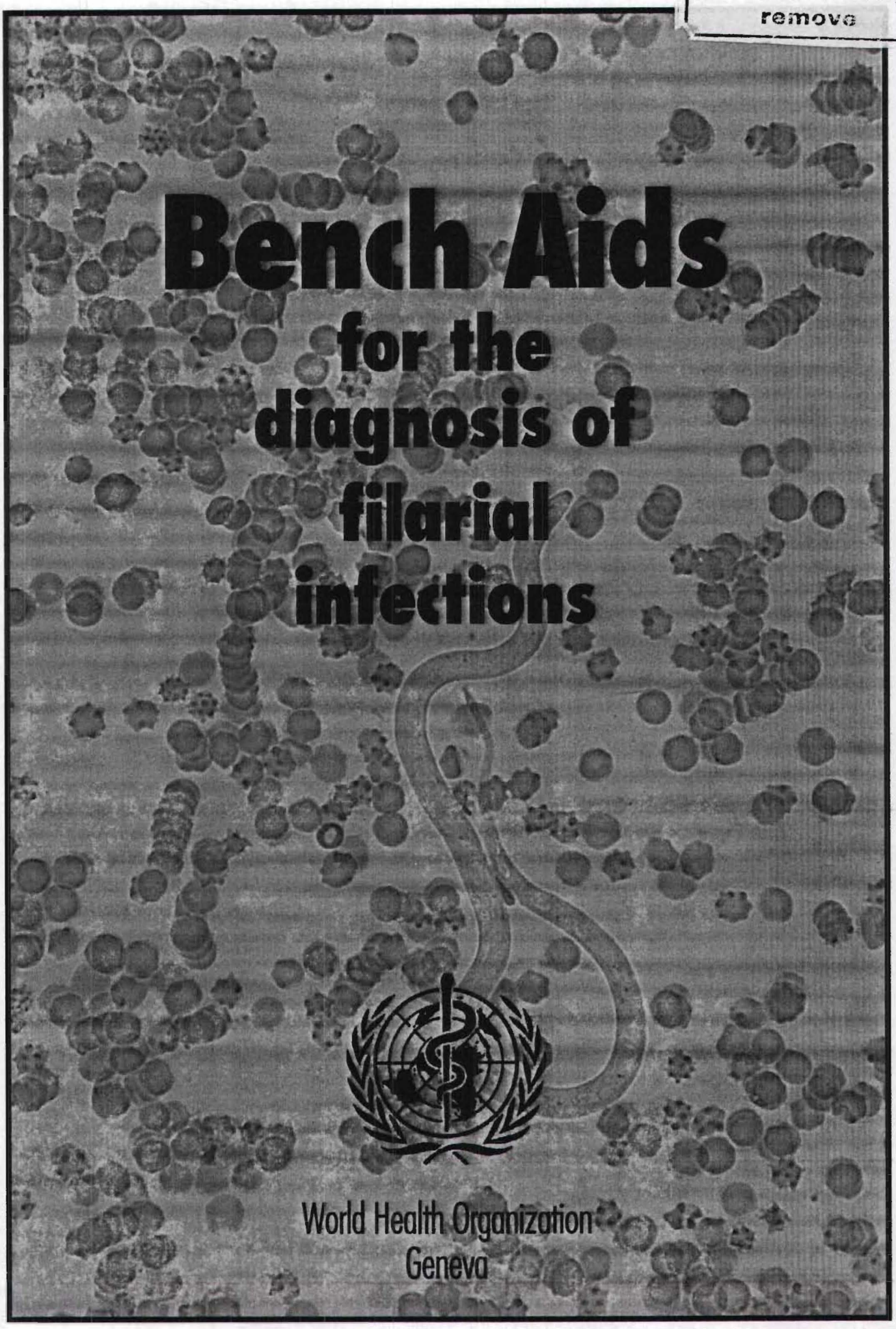

Bench Aids
for the diagnosis of filarial infections

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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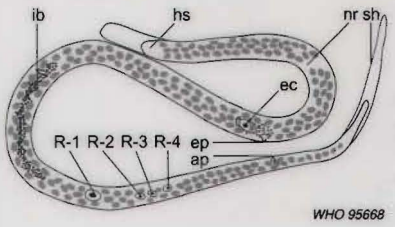
Introduction

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

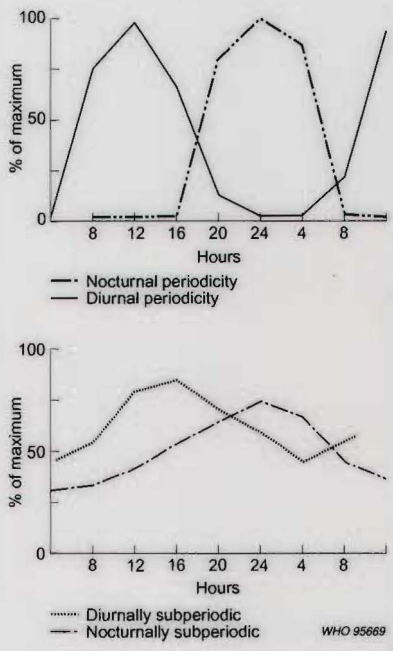


Periodicity

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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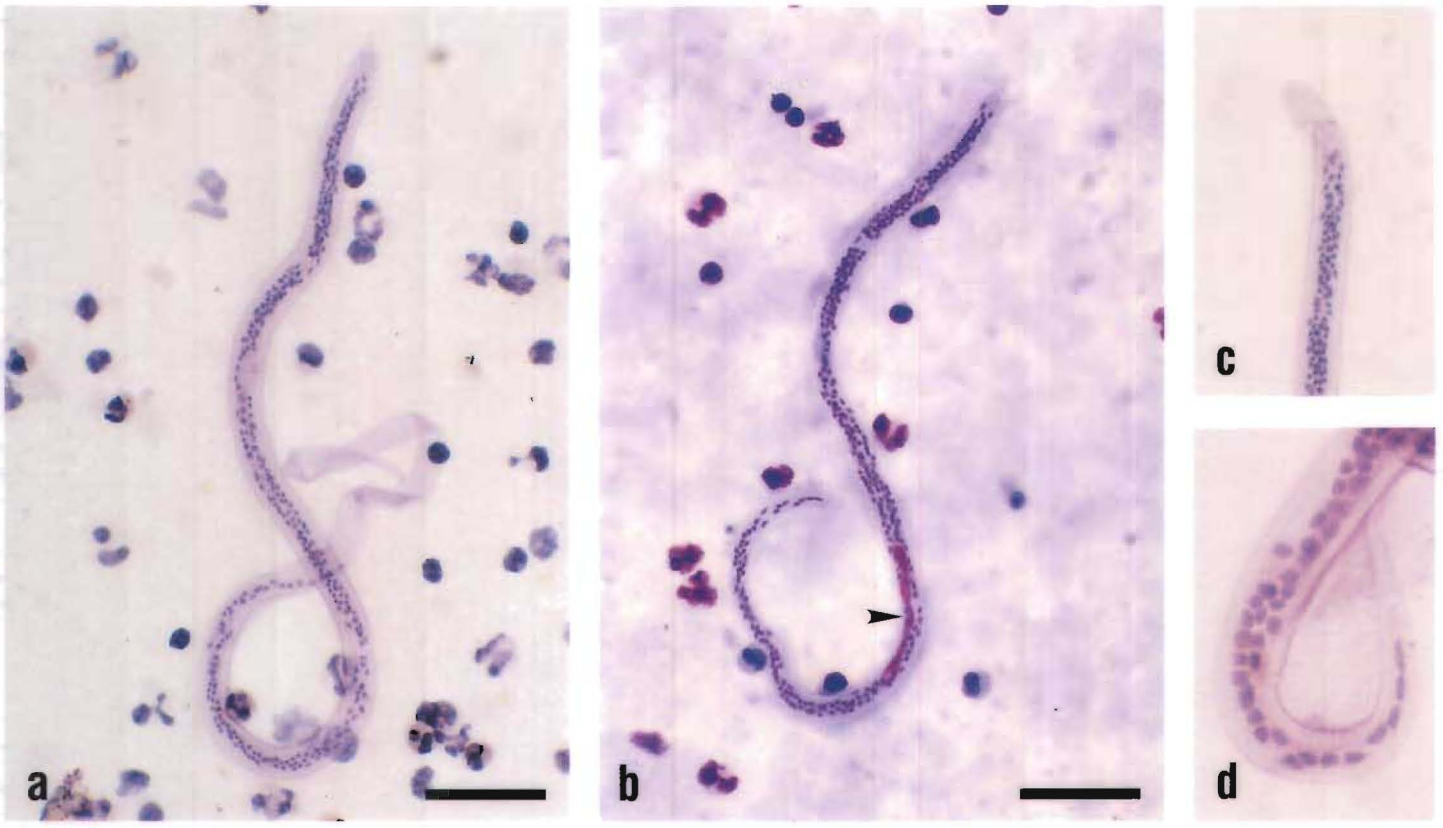
Orihel TC, Ash LR. *Parasites in human tissues.* Chicago, ASCP Press, 1995.

Table 1. Characteristics of common human filarial parasites

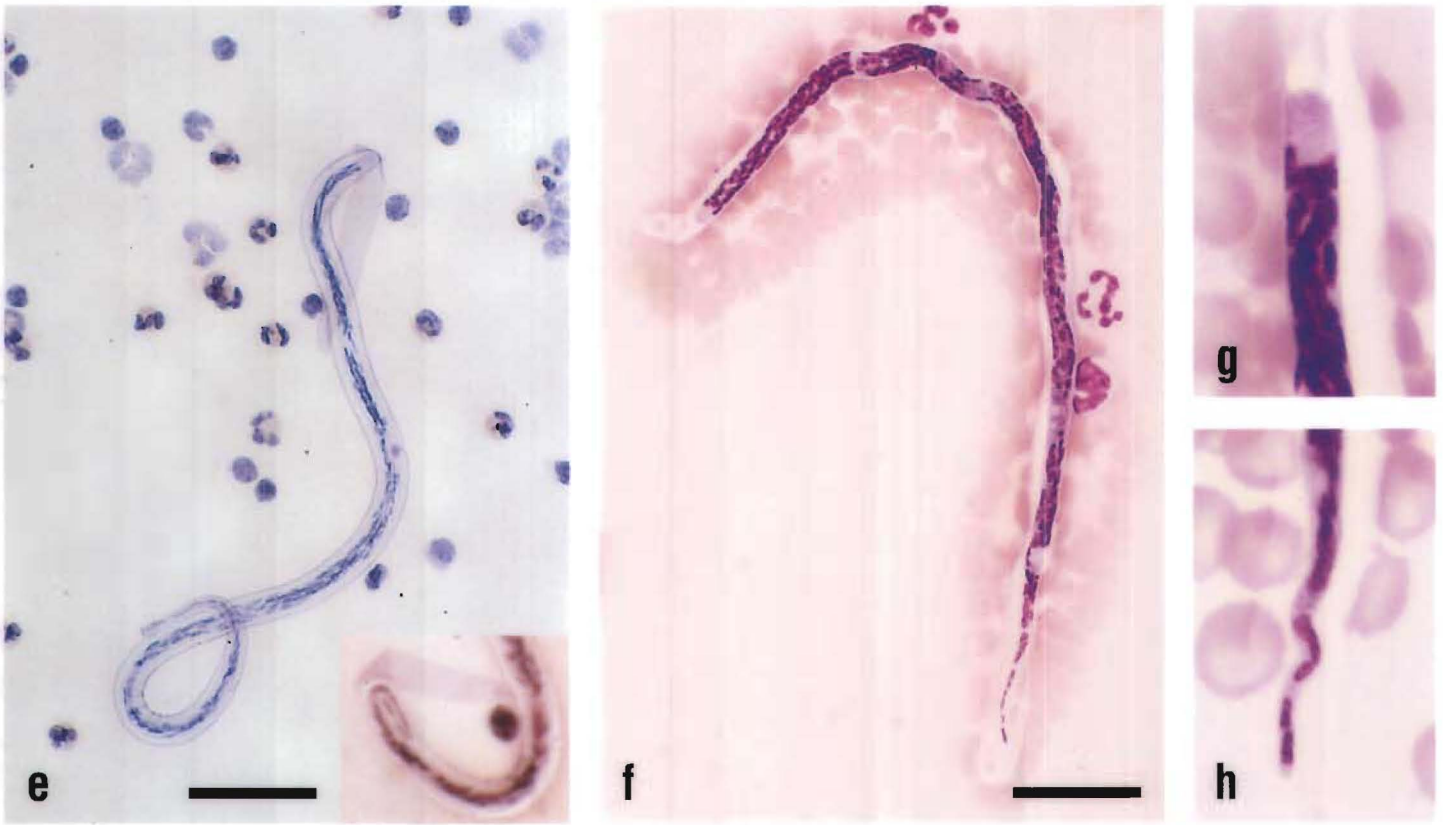
Species	*Wuchereria bancrofti*	*Brugia malayi*	*Brugia timori*	*Loa loa*	*Mansonella ozzardi*	*Mansonella perstans*	*Mansonella streptocerca*	*Onchocerca volvulus*
Geographical distribution	Tropics and subtropics worldwide	South-east Asia, Indian subcontinent	Indonesian archipelago, Timor, Lesser Sunda 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.

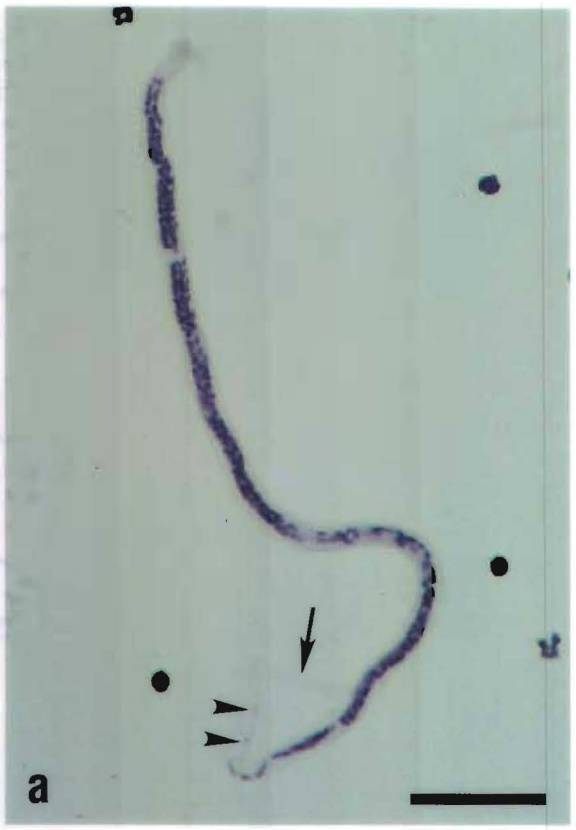
1. Thoroughly clean the microscope slides (including factory "pre-cleaned" slides) before use. Dust, grease, detergent, or cotton lint and threads may cause the blood film to lift off the slide.
2. Clean the finger tip (or ear lobe) from which the blood will be taken with a cotton ball soaked in alcohol.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

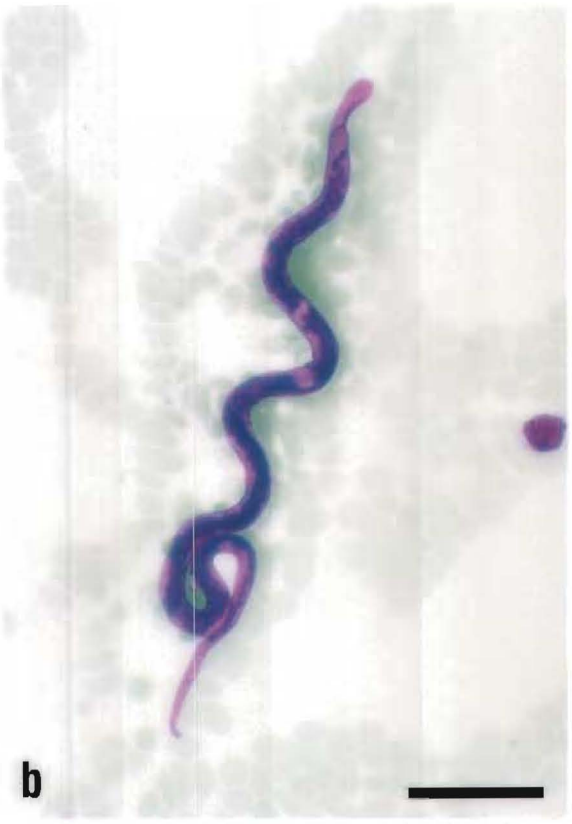
Note: Excess alcohol on the skin may partially fix the blood sample; squeezing the finger or ear lobe may dilute the sample with tissue fluids. Films that are too thick tend to lift off the slide. Blood films must be thoroughly dried before dehaemoglobinization; this may require 12–48 hours, depending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

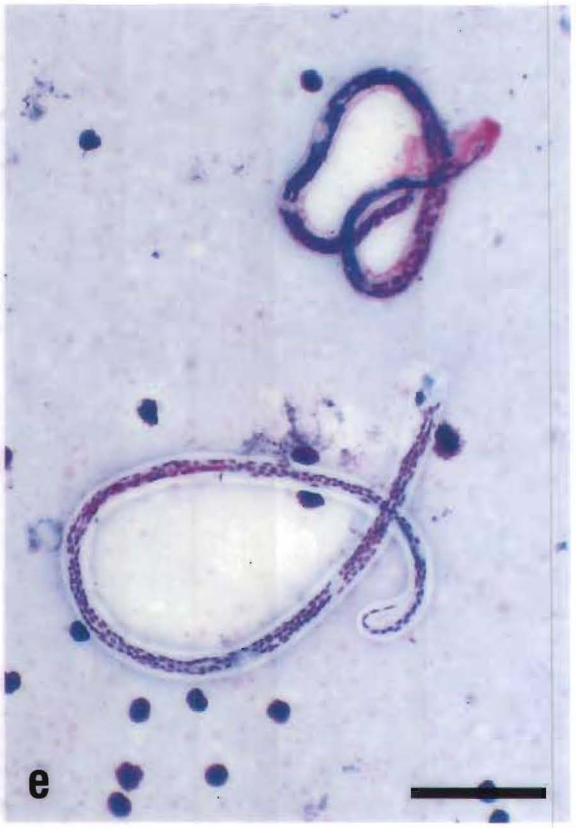
Microscopic examination of fresh blood has limited utility. It can reveal the presence of microfilariae actively moving among the red blood cells (see front cover), but species identification is not possible. However, in regions where only one species of microfilaria is found, its presence and density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

Note: All measuring bars = 30 µm

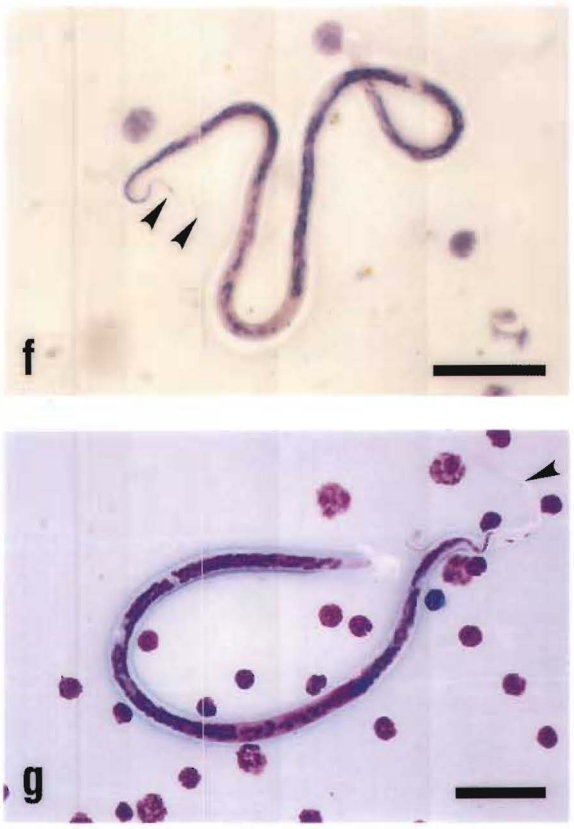




Brugia malayi microfilariae in haematoxylin (a) and Giemsa (b–d) stains. In haematoxylin, the sheath does not stain but may be faintly visible (a, *arrow*). This contrasts with the pink-stained sheath seen in Giemsa preparations (b, c). The column of nuclei is compact, and the widely separated subterminal 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

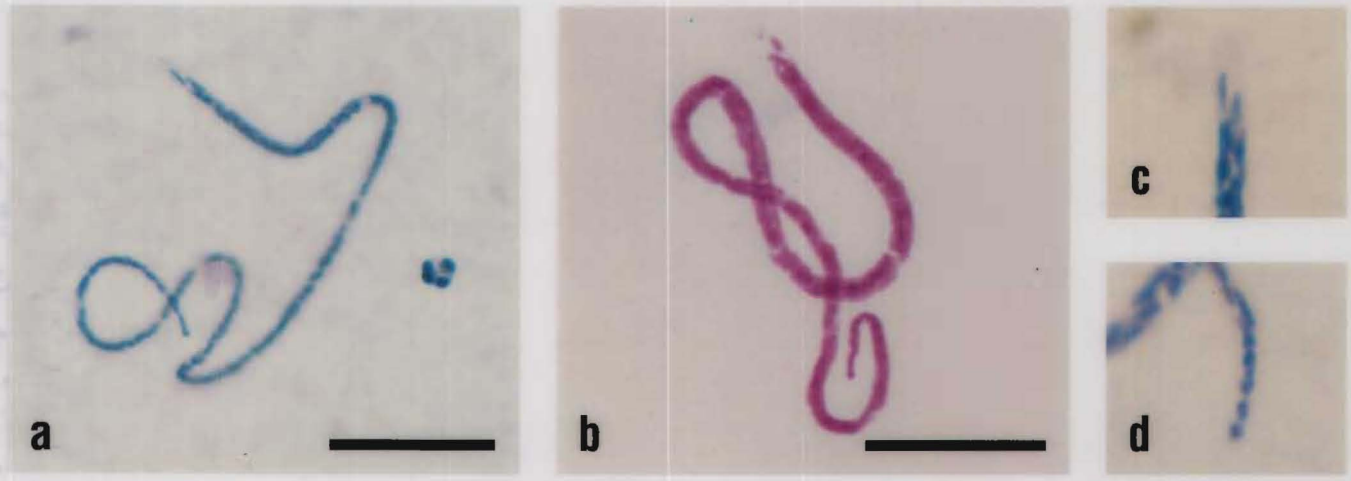
Various haematoxylin stains are used as alternatives to Giemsa stain; Delafield's haematoxylin is recommended and is widely used. It enhances nuclear detail in the microfilaria and stains the sheath, when present, a greyish-blue colour. For preparation of Delafield's haematoxylin and details of another 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

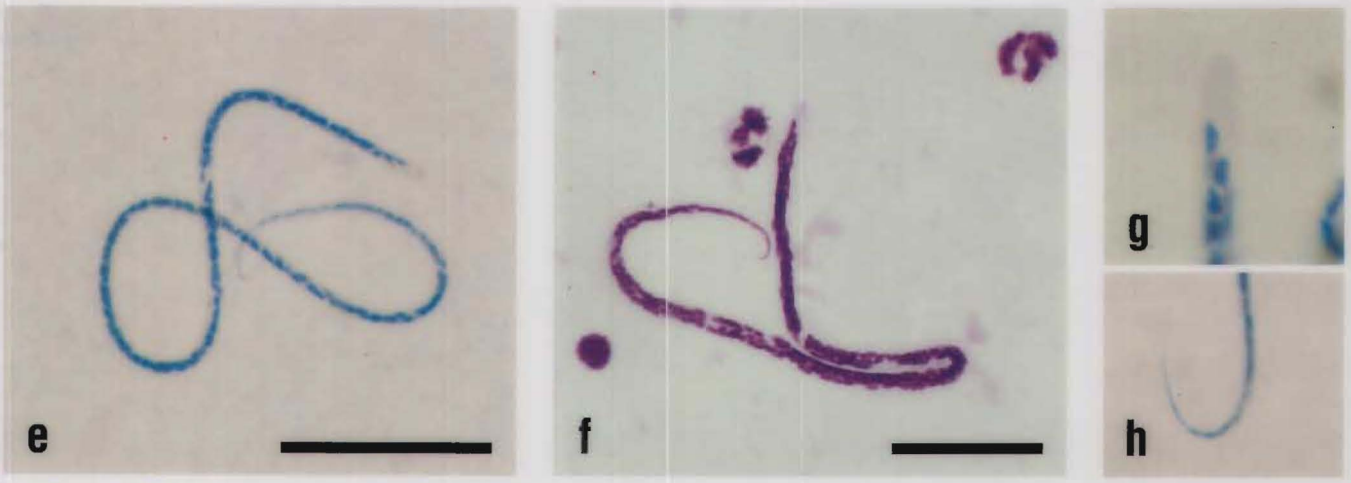
1. Thick blood films should be dried thoroughly, dehaemoglobinized, and fixed as described above. If films are prepared from sedimented Knott concentration material, dehaemoglobinization and fixation are omitted.
2. Stain slides for 10–15 minutes in Delafield's haematoxylin solution. Rinse in distilled water to remove excess stain.
3. Destain in 0.1% (1 g/l) aqueous hydrochloric or acetic acid for approximately 1 minute. Rinse slides in distilled water for 1 minute.
4. Place the slides in tap water containing several drops of ammonia for 3–5 minutes. The films will become dark blue in colour.
5. Rinse in tap water for 2–5 minutes and allow to dry.

Note: Films may be made permanent by adding a synthetic mounting medium and a coverslip. Alternatively, simply clarify with a drop of immersion oil, add a coverslip, and examine under low magnification.

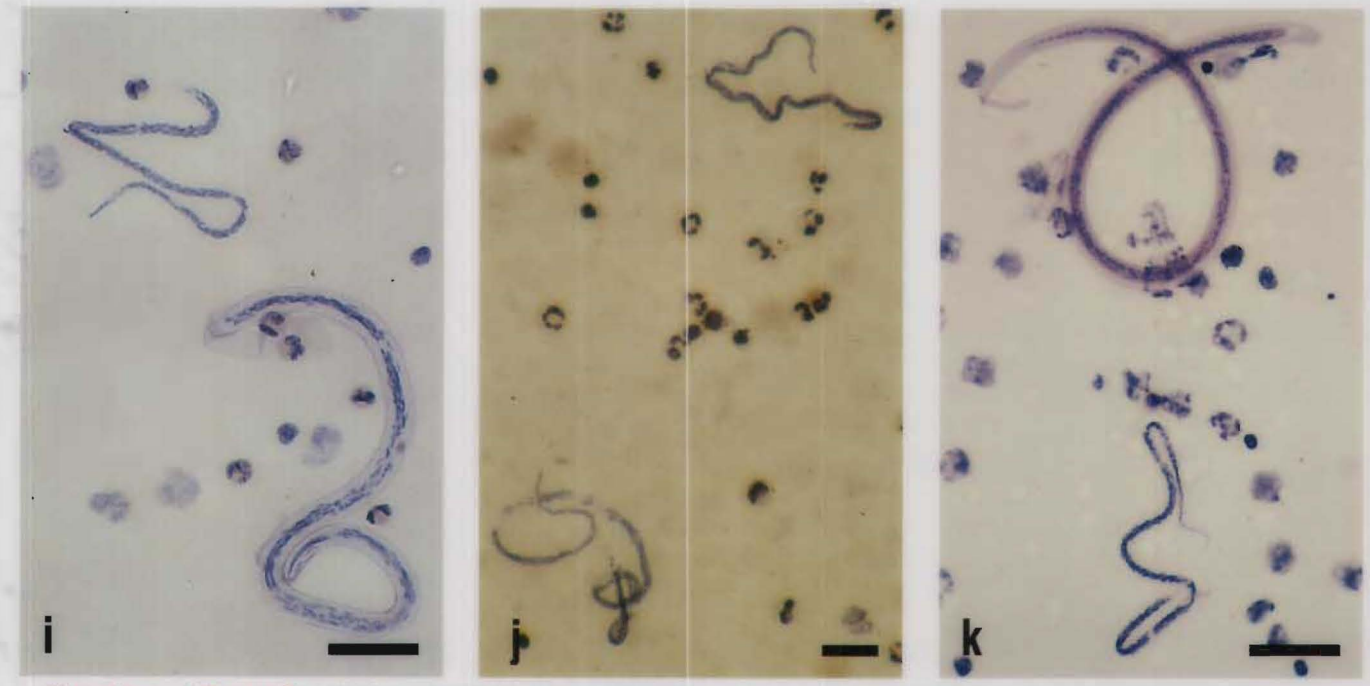
Note: All measuring bars = 30 µm



Mansonella perstans microfilariae in haematoxylin (a, c, d) and Giemsa-stained (b) blood films. ***M. perstans*** is small, has a short head space (c), lacks a sheath, and is readily recognized by the blunt tail that is filled by the column of nuclei (a, b, d). In thick blood films stained with Giemsa stain without fixation, the body usually appears thickened, and individual nuclei may be indistinct (b).



Mansonella ozzardi microfilariae in haematoxylin (e, g, h) and Giemsa (f) stains. Key features of this small, unsheathed microfilaria include a compact column of nuclei, a head space that is slightly longer than it is wide (g) and, most importantly, a tail that is long, slender, and devoid of nuclei (h). The appearance is the same in haematoxylin and Giemsa stains (e, f).



M. perstans and ***M. ozzardi*** are often found in individuals infected with other filariae in areas where species overlap. It is not uncommon to see, as in (i), ***M. perstans*** (upper) with ***L. loa*** or, more rarely, as in (j), ***M. perstans*** with Microfilaria ***semiclarum*** (lower).[1] In the Americas, as shown in (k), mixed infections of ***W. bancrofti*** (upper) and ***M. ozzardi*** are often seen. Mixes of ***M. perstans*** and ***M. ozzardi*** are also common. Microfilariae (i–k) stained in haematoxylin.

[1] Microfilaria ***semiclarum*** (j) has been found in the blood of people in Zaire (Fain A. Dipetalonema semiclarum sp. nov. from the blood of man in the Republic of Zaire (Nematoda: Filarioidea). *Annales de la Société belge de Médecine tropicale*,1974, 54:195–207.). A valid genus name has not been assigned to this species of filarial worm.

Concentration procedures

The detection of microfilariae in peripheral blood when few are present is best accomplished by concentration procedures, which allow for the examination of a larger volume of blood. The use of membrane filtration and the Knott concentration method are the most widely used procedures.

Membrane filtration

Membrane filtration allows for removal of elements in the blood by filtration through a membrane of desired pore size. Membrane filtration is more effectively used to determine microfilarial density than as a means of microfilaria identification. Cellulose–mixed-ester filters (e.g. Millipore filters) and polycarbonate filters (e.g. Nuclepore filters) are the most common membrane filters used. Formerly, fresh blood samples required processing soon after they were obtained. Recently, however, a procedure for membrane filtration of preserved blood has been published (*1*). Both are described below.

Filtration of fresh whole blood

Materials and reagents

1. Sodium citrate solution, 3.8% (38 g/l) or EDTA (ethylenediaminetetraacetic acid) solution, 7.5% (75 g/l).
2. Teepol–saline solution, 10% (prepare by adding 50 g Teepol concentrate to 450 ml saline).
3. Saline, 0.85% (8.5 g/l).
4. Giemsa stain.
5. Syringe (disposable polypropylene with rubber plunger tip), 20-ml capacity.
6. Membrane filter holder (e.g. Swinnex type).
7. Membrane filter, 3–5-µm porosity, 25-mm diameter.

   Note: Although a pore size of 5 µm is ideal for *L. loa* microfilariae, 4 µm is more efficient for filtration of *W. bancrofti* and other smaller species of microfilariae such as *Mansonella perstans*.

8. Absolute methanol.

Procedure

1. Collect a fresh blood sample in sodium citrate or EDTA solution.
2. Add 1 ml of citrated or EDTA-preserved blood to 10 ml of Teepol–saline solution.
3. Place moistened membrane filter, secured with a rubber gasket, into filter holder (Fig. 3).
4. Remove plunger from barrel of 20-ml syringe and connect barrel of syringe to filter holder.
5. Pour the blood–Teepol mixture (from step 2) into barrel of syringe, replace plunger in syringe and, by applying gentle, even pressure, force solution through filter (Fig. 4). Discard blood into disinfectant for disposal.

   Note: Some workers prefer to push a 1-ml blood sample directly through the filter followed by 20–35 ml of water or saline to wash out the remaining blood. Others suspend the blood in 10 ml of water, agitate, and allow the mixture to stand for several minutes before passage through the filter.

6. Remove syringe from filter holder, draw up 10 ml of water into syringe, reattach filter holder, and gently wash filter by flushing the solution through it.
7. Force two syringe-volumes of air through filter to expel excess water and make microfilariae more adherent to filter.

Note: Procedures may be modified at this point depending on the type of preparation desired.

Microfilariae may be fixed and stained on the filter as follows:

8. For permanent, stained preparations:
   a. Pass 3 ml of methanol through filter to fix microfilariae.
   b. Pass air through filter to expel residual methanol.
   c. Remove filter from the holder and place on a glass slide; allow it to dry thoroughly.
   d. Stain the preparation in Giemsa stain as for a blood film.
   e. Rinse in tap water and allow to dry.
   f. Dip the slide in toluene to avoid bubbles in or under the filter. Add a drop of synthetic mounting medium and a coverslip. The slide may be examined in the same manner as any blood film and stored as a permanent preparation for future reference.

Microfilariae may be examined alive as follows:

9. Alternatively, following step 7, remove syringe from filter holder, carefully unscrew top from filter, and, using forceps, remove rubber gasket.
10. Use fine forceps to transfer wet filter to a slide, with the residue on the membrane facing upwards.
11. Add a drop of saline to the membrane and cover with a coverslip. Examine under the microscope with 10x objective; microfilariae will be seen actively moving.

Fig. 3

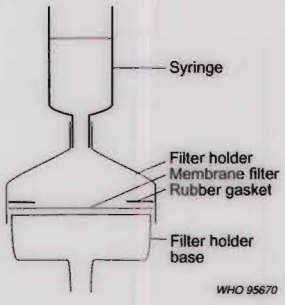
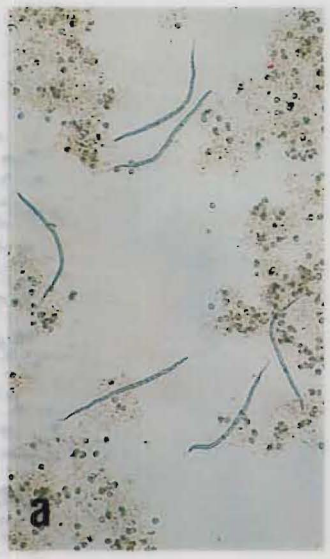
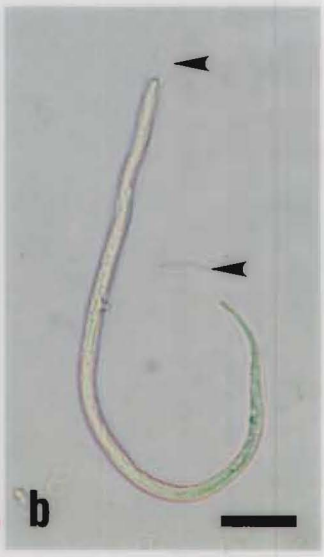


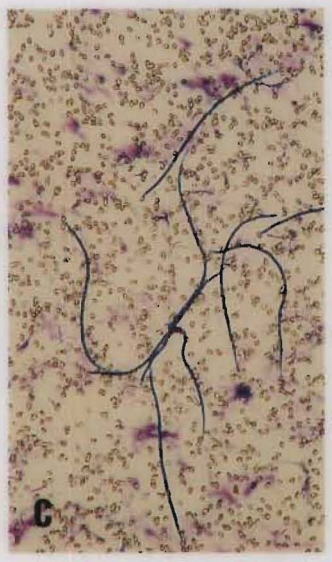
Fig. 4

Note: All measuring bars = 30 µm

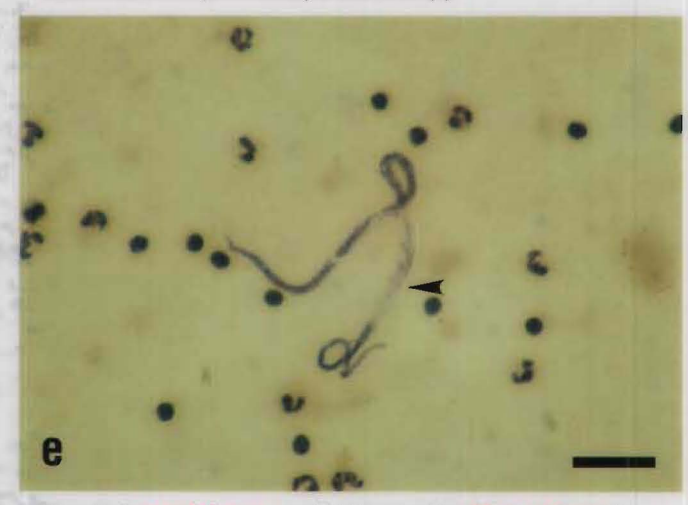




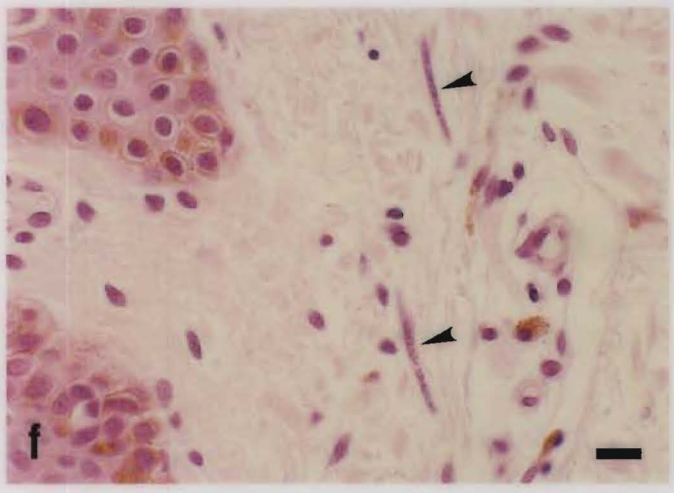
L. loa microfilariae in a Knott concentration. They are easily enumerated at low magnification (a). At high magnification, features such as size, shape, and the presence or absence of a sheath are evident (b). Note the sheath extensions (*arrowheads*) at both ends (b).



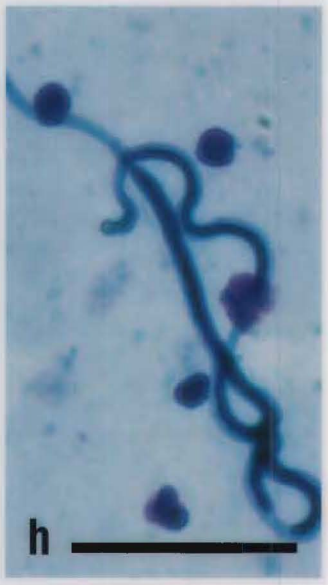
L. loa microfilariae collected on a polycarbonate filter and stained with Giemsa stain. Microfilariae are easily enumerated at low magnification (c); the distribution of nuclei in the tail allows the identification of the microfilaria at high magnification (d).

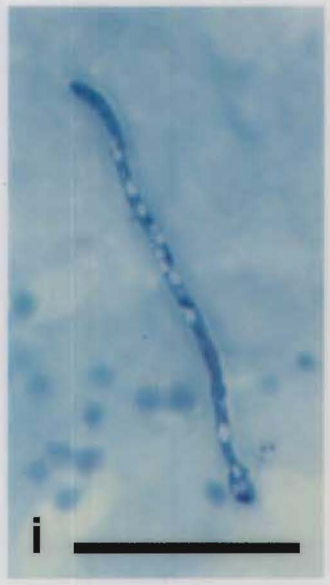


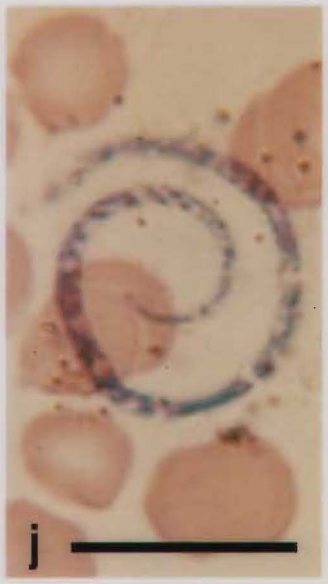
Microfilaria ***semiclarum*** superficially resembles ***M. perstans***; it is similar in size but has a sparsely nucleated area (*arrowhead*) in the posterior half of the body. The adult worms and vectors have not been identified. Preparation stained in haematoxylin.



O. volvulus microfilariae in a section of skin stained with haematoxylin and eosin. Only portions of the microfilariae are visible (*arrowheads*).







Fibres (g, h), unidentified elements (i), and fungi (***Helicosporium***) (j) found on Giemsa-stained blood films are often confused with microfilariae. In spite of similar size, the presence of a darkly stained core and/or vacuoles, the absence of nuclei, and jagged or broken ends rule out identification as microfilariae.

Concentration procedures *(continued)*

Filtration of preserved blood

If it is not possible to process fresh blood immediately, the following procedure may be used.

Materials and reagents

Materials required are the same as for processing whole blood, except that 2 ml of 37% (370 g/l) formaldehyde solution is added to 10 ml of Teepol concentrate and 88 ml of distilled water to make 100 ml of Teepol–formalin solution.

Procedure

1. Blood specimens preserved in the Teepol–formalin solution (1 ml of blood should be added to 10 ml of Teepol–formalin solution) are filtered through a membrane filter in the same manner as described in steps 3–7, above.
2. Filters can be examined wet, with or without the addition of Giemsa, haematoxylin, or other stains, to allow for enumeration of the microfilariae or study of their morphology.
3. Alternatively, the wet filter can be placed on a slide, allowed to dry, and stained as desired. A drop of synthetic mounting medium and a coverslip can be added to make a permanent preparation.

Note: Teepol lyses blood and formaldehyde preserves the morphological features of microfilariae. Blood specimens in the Teepol–formalin solution can be retained for 9 months or longer before examination, without marked deterioration of the microfilariae. Blood specimens in Teepol only, or in a Teepol–sodium azide solution, are not useful for long-term storage since microfilariae undergo degenerative changes within a week or less.

Knott concentration method

The Knott concentration method is very sensitive and relatively inexpensive to perform.

Materials and reagents

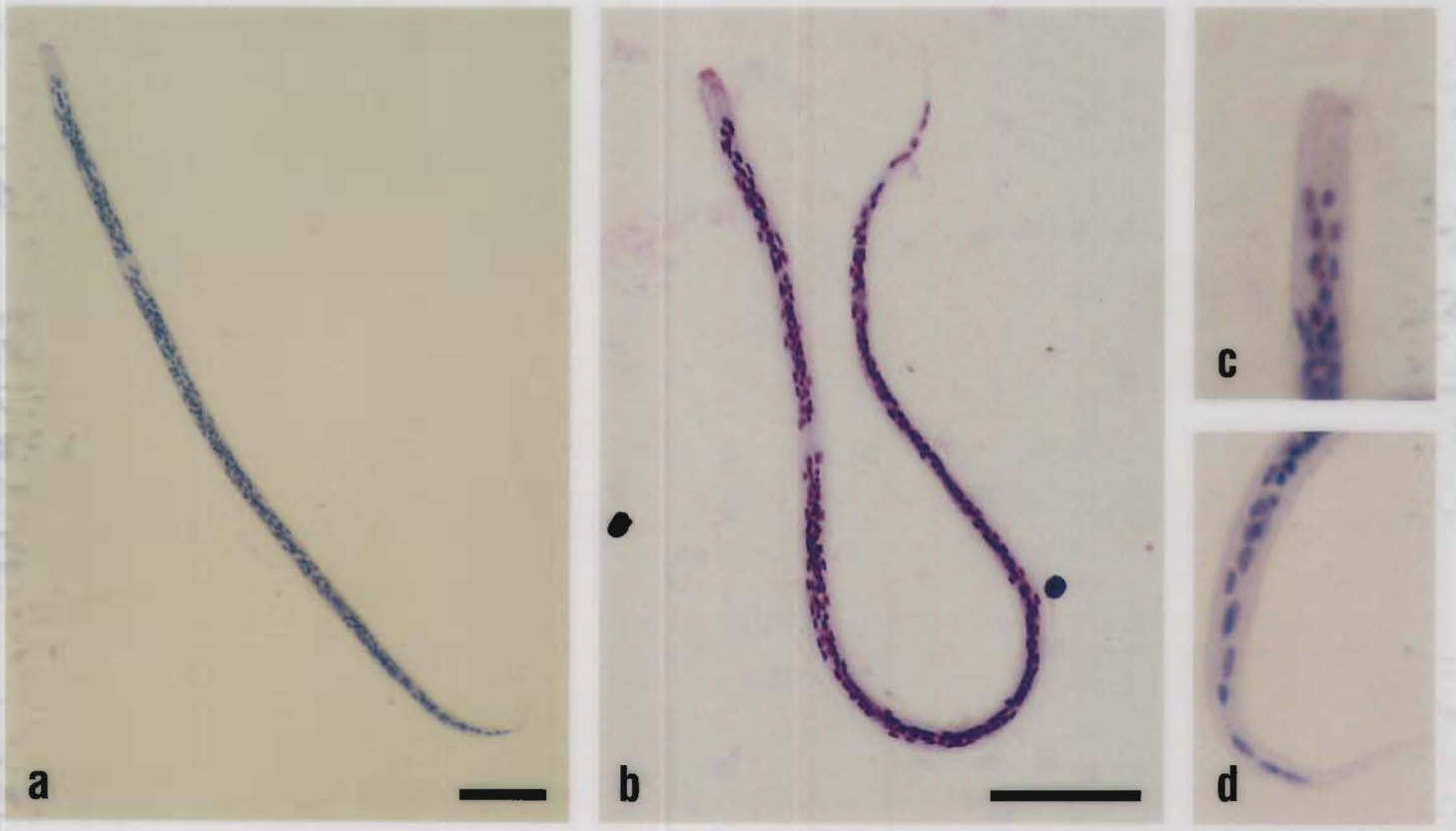
1. Centrifuge tubes, 15-ml capacity.
2. Formalin, 2% (2 ml of 37% (370 g/l) formaldehyde solution + 98 ml of distilled water).
3. Slides and coverslips.
4. Needles and syringes.
5. Centrifuge (hand- or electric-powered).

Procedure

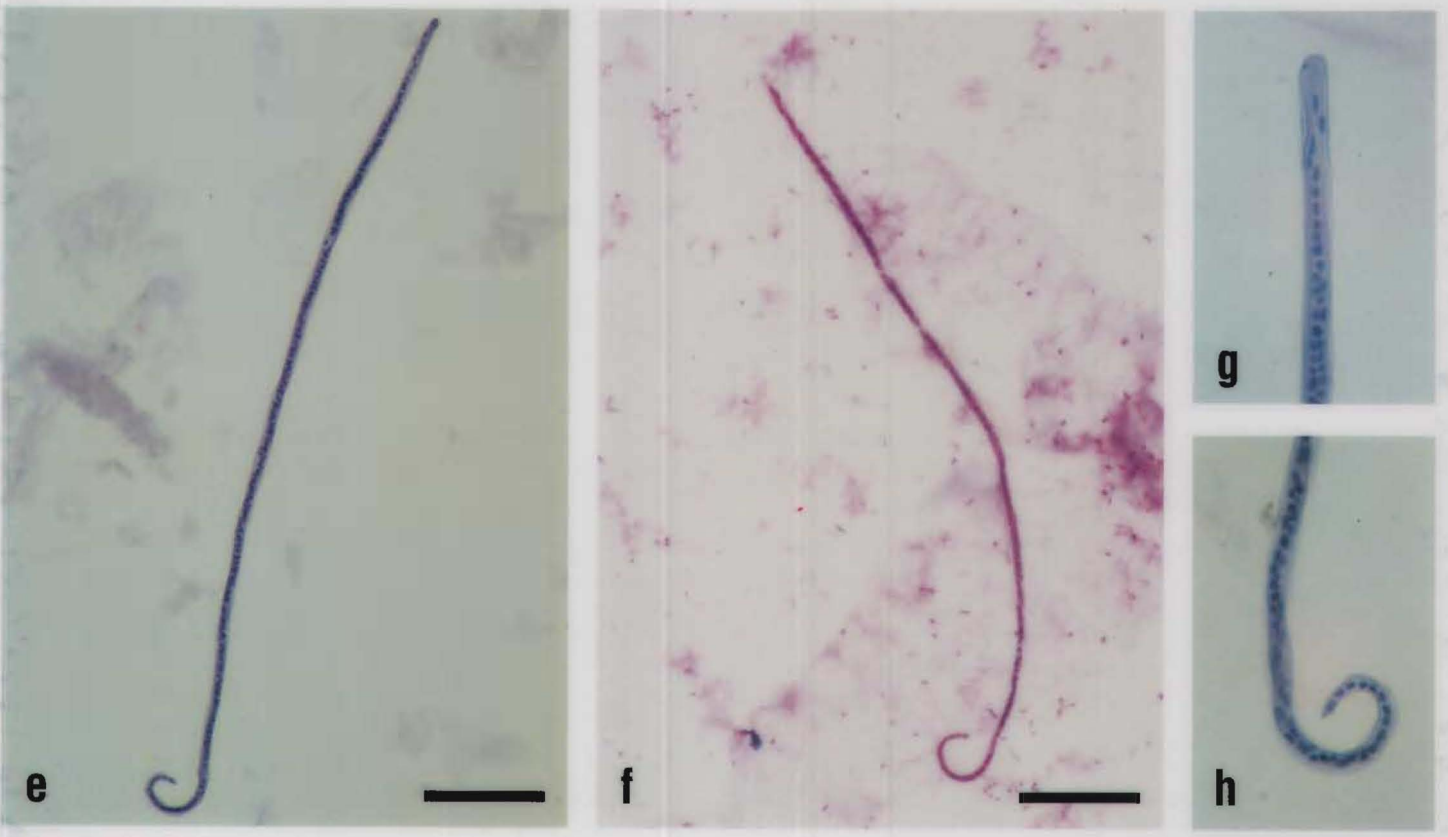
1. Collect 1 ml of blood (whole or citrated) by venepuncture and place in a 15-ml centrifuge tube containing at least 10 ml of formalin; shake vigorously. Red cells are lysed by the formalin solution.
2. Centrifuge at approximately 300*g* for 2 minutes. If a centrifuge is not available, place the tube in an upright position for 12 hours for gravitational sedimentation.
3. Decant the supernatant fluid (the small amount remaining in the tube is allowed to flow back on to the sediment).
4. Examine a drop of the sediment on a slide under a coverslip with the low-power objective of the microscope.
5. A portion of the sediment may be spread on a slide as a thick film and allowed to dry thoroughly. Stain the film with Giemsa or haematoxylin stain.

Note: Avoid adding more than 1 ml of blood to 10 ml of formalin; as much as 12–14 ml of formalin is desirable for each 1 ml of blood. Only microfilariae and white blood cells are found in the sediment; microfilariae are fixed without significant shrinkage and are easy to count accurately. A sheath, if present, is also easy to see. The technique is useful for quantification of microfilaraemia. Samples need not be examined immediately and can be stored in the laboratory for several weeks. Microfilariae present in the stained sediment will show details of internal structure.

Note: All measuring bars = 30 µm



Onchocerca volvulus microfilariae from skin snips in haematoxylin (a, c, d) and Giemsa stains (b). This microfilaria is large and has no sheath, a long head space (c) and, typically, a flexed tail (d). The column of body nuclei is only moderately compact. The most important diagnostic feature is that ***O. volvulus*** is found in the skin and only rarely in the blood.



Mansonella streptocerca microfilariae from skin snips in haematoxylin (e,g,h) and Giemsa stains (f). ***M. streptocerca*** is readily distinguished from ***O. volvulus*** by its very slender shape and "crooked" tail (e, f, h). Note that the column of nuclei starts in the anterior extremity as a single row of 10–12 (or more) nuclei (g) and extends to the end of the tail (e,f,h).

Tissue examination

Skin snips

The microfilariae of *Onchocerca volvulus* and *Mansonella streptocerca* that reside in the skin are best detected by looking for their presence in skin snips. Intensity of infection is reflected in the numbers of microfilariae emerging from the snips. Skin snips are obtained in one of two ways:

1. Skin snips can be standardized in both size and weight through the use of sclerocorneal punches of either the Holth or Walser type. These instruments take snips of uniform diameter (approximately 2.3–2.5 mm). This is the preferred method.
2. A needle can be used to raise the skin and a razor blade to cut off the raised area; forceps and curved scissors can also be used. Such skin snips vary in size, shape, and the depth of the cut. When snips are cut too deeply, small capillaries may be lacerated and the snip may be contaminated by microfilariae that might be present in the patient's blood.

Caution: **It is of the utmost importance that all instruments used for each patient are sterile in order to avoid transmission of viral hepatitis B and HIV infections.**

Procedure

1. Skin snips should be taken from selected sites on the body. In Africa, the preferred site is the iliac crest; in Central and South America, the iliac crest or the scapular area; and in Yemen, the lower calf. In surveys, ideally two snips should be taken from all three of these sites on each side of the body of the individual.
2. Transfer skin snips from each site to a drop of normal saline, distilled water, or tissue culture medium in a well of a 96-well, flat-bottom, tissue culture tray; or place snips on a microscope slide in one of the fluids. It is not necessary to tease the snips.
3. Examine after 30 minutes to 3 hours. (Tissue culture trays may be covered with plastic wrap or similar material, and slides placed in a covered Petri dish, to retard evaporation.) If the wells or slides are negative for microfilariae, allow the snips to remain overnight in an incubator at 37 °C or at room temperature and examine them again. If microfilariae are present they will be apparent in the fluid. The morphological features of *O. volvulus* and *M. streptocerca* are so distinct that differentiation of microfilariae is quite easy.
4. To make permanent preparations of microfilariae, remove the skin snips, transfer the fluid to a slide if necessary, and allow the fluid to evaporate. When the slide is thoroughly dry, fix the microfilariae in methanol and stain with Giemsa or haematoxylin stain.

Urine and hydrocele fluid

Pour 15 ml of urine or hydrocele fluid into a conical centrifuge tube and centrifuge for 5 minutes at 350*g* or more. Pour off supernatant and examine sediment for microfilariae. Slides can be stained and/or fixed as described for blood samples.

Other diagnostic methods

Microhaematocrit

Originally used for diagnosis of trypanosomiasis, the microhaematocrit procedure is equally useful for the diagnosis of filarial infections, especially when the numbers of microfilariae present are too small for efficient detection by thick blood films. Only a small amount of blood is needed, so that one or two drops obtained by finger-prick can be used when venepuncture cannot be performed (*2*).

Quantitative buffy coat

The utilization of the quantitative buffy coat tube (microhaematocrit tube recoated with acridine orange) has been reported to be an acceptable rapid diagnostic test for the detection of microfilariae, with a sensitivity equivalent to that of the thick blood film (*3*).

Microfilariae counts

Accurate counts of microfilariae can be made from stained thick blood films of measured volume. Counting requires careful systematic scanning of the blood film with the low-power objective of the microscope. The stained slides can be kept as a permanent record. Equally reliable counts can be made from membrane filters which, if mounted with a coverslip, can be retained as a permanent record. Some investigators prefer using a counting-chamber technique, which is very reliable but does not lend itself to species identification or permanence (*4*).

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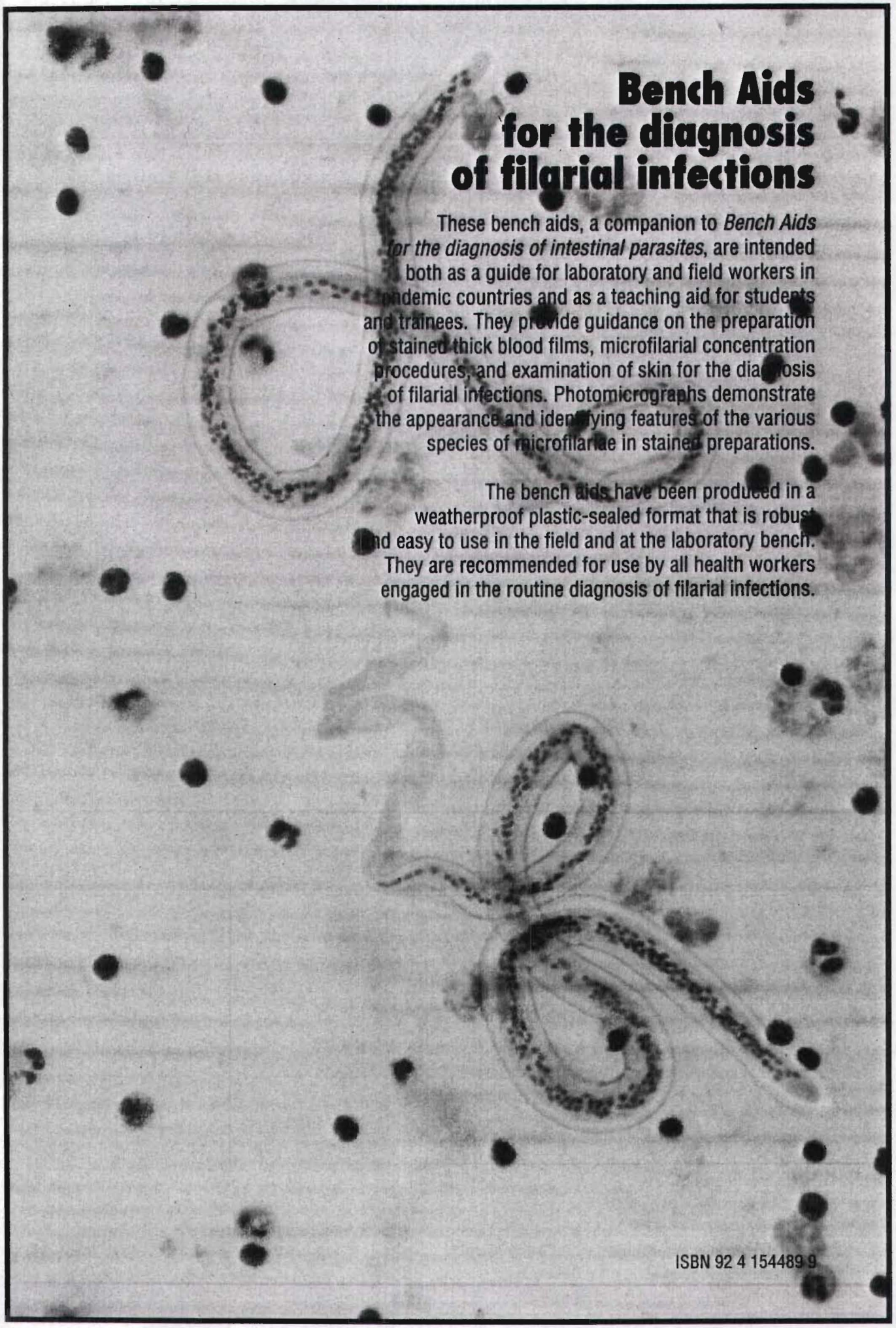

Bench Aids for the diagnosis of filarial infections

These bench aids, a companion to *Bench Aids for the diagnosis of intestinal parasites*, are intended both as a guide for laboratory and field workers in endemic countries and as a teaching aid for students and trainees. They provide guidance on the preparation of stained thick blood films, microfilarial concentration procedures, and examination of skin for the diagnosis of filarial infections. Photomicrographs demonstrate the appearance and identifying features of the various species of microfilariae in stained preparations.

The bench aids have been produced in a weatherproof plastic-sealed format that is robust and easy to use in the field and at the laboratory bench. They are recommended for use by all health workers engaged in the routine diagnosis of filarial infections.

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