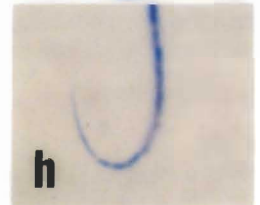
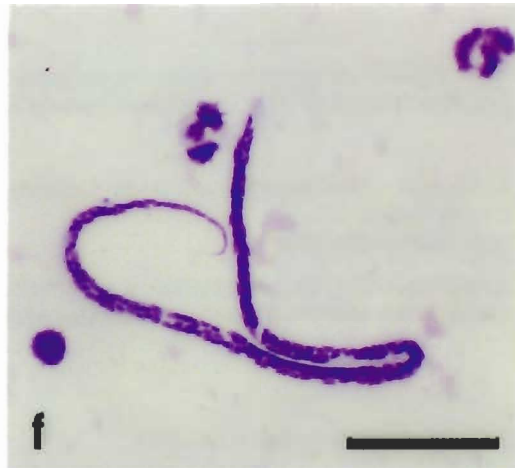
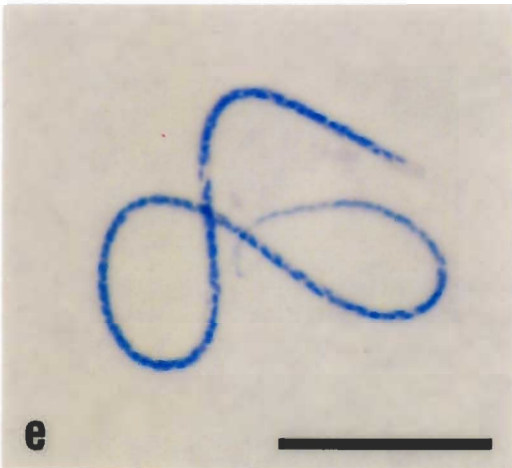


Mansonella perstans microfilariae in haematoxylin (a, c, d) and Giemsa-stained (b) blood films. *M. perstans* is small, has a short head space (c), lacks a sheath, and is readily recognized by the blunt tail that is filled by the column of nuclei (a, b, d). In thick blood films stained with Giemsa stain 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.

¹Microfilaria ***semiclarum*** (†) has been found in the blood of people in Zaire (Faim 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 (7). 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

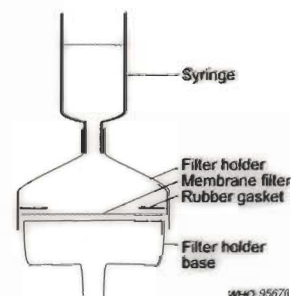


Fig. 4

