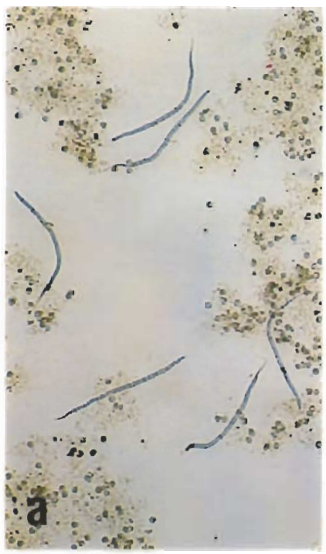
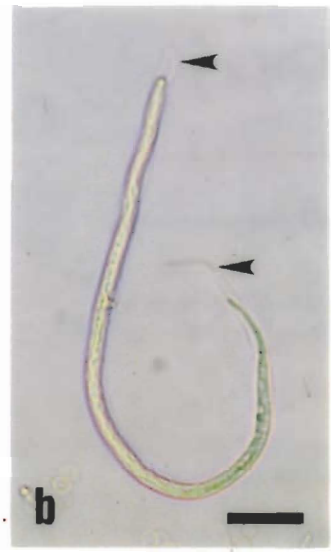
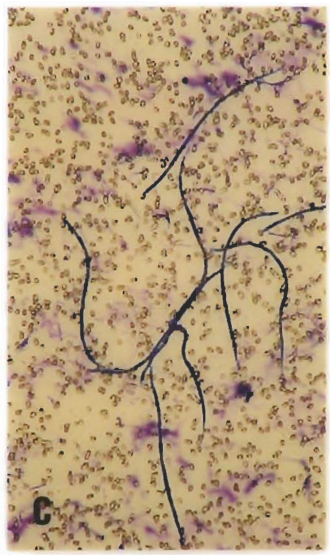
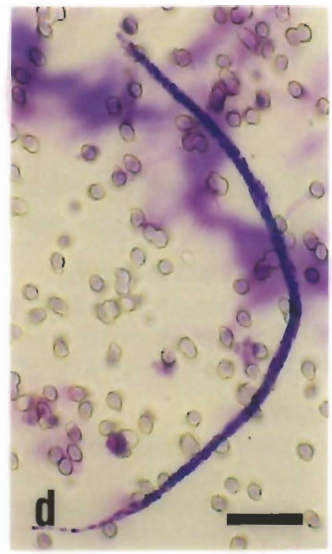
Note: All measuring bars = 30 µm



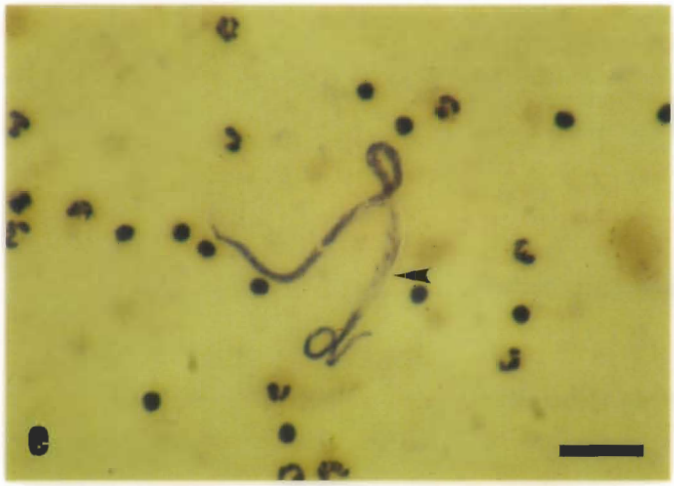


L. loa microfilariae in a Knott concentration. They are easily enumerated at low magnification (a). At high magnification, features such as size, shape, and the presence or absence of a sheath are evident (b). Note the sheath extensions (*arrowheads*) at both ends (b).

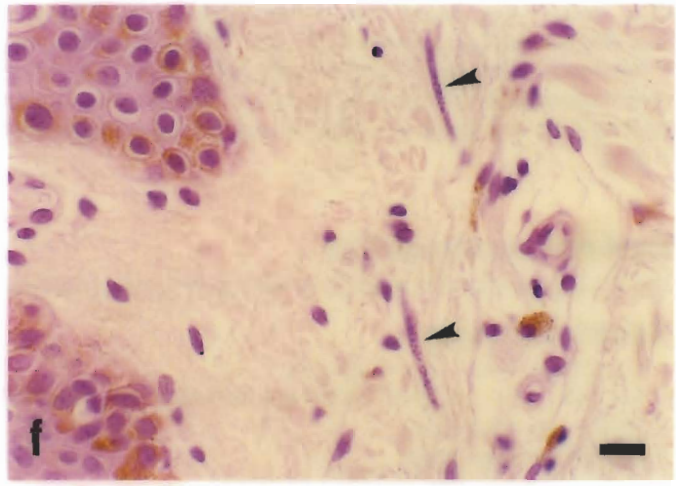




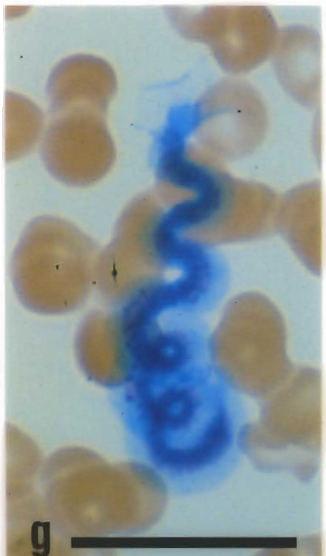
L. loa microfilariae collected on a polycarbonate filter and stained with Giemsa stain. Microfilariae are easily enumerated at low magnification (c); the distribution of nuclei in the tail allows the identification of the microfilaria at high magnification (d).

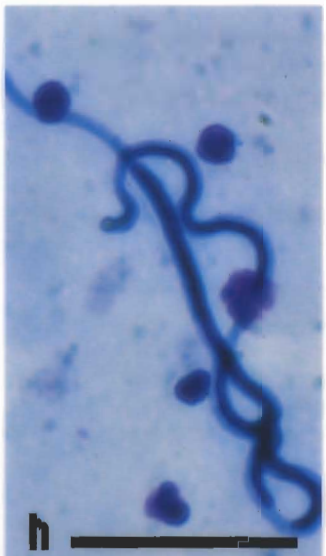


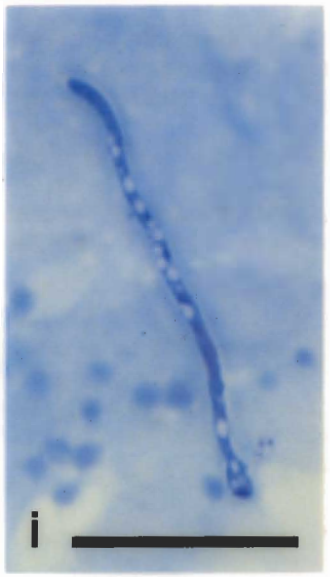
Microfilaria ***semiclarum*** superficially resembles ***M. perstans***; it is similar in size but has a sparsely nucleated area (*arrowhead*) in the posterior half of the body. The adult worms and vectors have not been identified. Preparation stained in haematoxylin.



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.