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Protocol 008: Protocol for *L. Loa* and *M. Perstans* microfilariae diagnosis from peripheral blood

Version Number	Written by	Reviewed by	Approved by
1.0			Prof. Samuel Wanji

Collection of Sample and Slide Preparation

- (i) First disinfect the tip of the finger where blood will be collected with a gauze pad dipped in 70% alcohol and allow to air dry
- (ii) Use a sterile lancet and gently pierce (once) the disinfected area of the finger
- (iii) Collect the blood sample using an anticoagulant-free 70 µl calibrated capillary tube.
- (iv) Spread the collected blood sample on a clean and grease free slide labelled with the participant ID
- (v) Spread the blood sample while forming a rectangular shape (1 cm x 2.5 cm) on the slide. Only 50 µl of blood will be released from the capillary tube.
- (vi) Allow the slide to air dry and avoid exposure to sun light

Thick smear staining

- (i) Fix the smear by flooding with 100% methanol for 1 min, remove excess, air dry
- (ii) Stain the smear for 45 mins in 1:9 dilution of stock Giemsa stain (Use distilled water as diluent)
- (iii) Rinse gently in distilled water to remove surplus stain
- (iv) Air dry the smear

Slide examination to identify parasite microfilariae

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- (i) Examine the slide under a light microscope with x10 objective for microfilariae
- (ii) *Loa loa*: examine at medium power noting presence of sheath, it has a short head space and a compact column of nuclei that extends to the end of the tail and the last few nuclei are irregularly spaced
M. perstans: the microfilariae are tinner and thread-like with short head space, lacks a sheath and is readily recognised by the blunt tail that is filled by a column of nuclei.
- (iii) Record the results (count of microfilariae) on record forms with participant's ID.
- (iv) Calculate the average microfilariae count from 2 independent readers and multiply by 20 to express the parasitaemia as per millilitre of blood.
- (v) Store the slides in slide boxes

Reference

- WHO 1997. Bench Aids for the diagnosis of filarial infections