Short Report

# An improved Knott's concentration test for the detection of microfilariae

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The simplest method of demonstrating microfilariae (mf) is the microscope examination of a film made from capillary blood (KHAMBOONRUANG *et al.*, 1987; SCHULTZ, 1988; SCHUURKAMP *et al.*, 1990; SABRY, 1992). The amount of blood used for fingerprick films varies from 20 to 60 μL and low levels of mf may be missed by this method. Loss of mf during fixation and staining can also lead to erroneous results (PANICKER *et al.*, 1991). To increase the sensitivity, concentration techniques are used. The membrane filter technique using 1–5 mL of blood (CHULEREK & DESOWITZ, 1970) is widely used but the method is slow when large numbers of samples have to be processed, and the disassembly of the filters to retrieve the membrane exposes the investigator to infection with blood-borne pathogens. The filter also tends to block when mf counts are high, and unless the filter holders are carefully washed and dried between samples carry-over of mf between samples is a problem. We find the Knott's test (KNOTT, 1935) to be very sensitive and convenient. One millilitre of blood is mixed with 9 mL of 1% formalin in water. The red cells are lysed and the mf are preserved by the formalin. Samples can be preserved in this state for many weeks without loss of mf, making the method very convenient for working in remote areas. In the laboratory the tubes are centrifuged at 120 ***g*** for 5 min, the supernatant is removed, a drop of 1% methylene blue is added to the deposit, the deposit is transferred to a slide and the mf are counted by microscopy. The theoretical sensitivity of the method is 1 mf/mL. The accuracy and ease of use of the Knott's test are reduced when screening people with hyperproteinaemia, a common finding in tropical areas, because the gamma globulin in the sample is precipitated by the formalin—a phenomenon utilized in the formol–gel test for leishmaniasis (DAVIES & BECK, 1981). The resulting precipitate increases the amount of deposit that has to be examined and can make identification of mf very difficult. In an attempt to overcome this problem we conducted the following experiment.

Twenty blood samples collected, after informed consent, from microfilaraemic subjects in Papua New Guinea were tested by the standard Knott's test and by a modification as follows. One millilitre of blood was mixed with 9 mL of 2% Triton X-100 (Sigma Biochemicals) in water. The mixture was left to stand at room temperature for 30 min and then centrifuged at 120 ***g*** for 5 min. The amount of precipitate was measured with a pipette. It averaged 820 μL in the standard test and 125 μL in the modified test, a reduction of 84·8%. The supernatant was removed from the modified test and the deposit resuspended in 100 μL of 1% formalin in water. Mf in deposits from both the traditional and modified tests were counted by microscopy after the addition of methylene blue and results were compared by Student's *t* test. Counts ranged from 16 to 690 per mL in the unmodified test and there was no significant variation with the modified test ($P = 0{\cdot}9638$). The major benefit of the modified test is the saving in time due to the small amount of deposit that needs to be examined. For example, with a deposit of 800 μL and a 22 × 44-mm coverslip approximately 6 slides are required to examine the whole deposit and each slide requires about 2 min examination time. A 100-μL deposit can be examined on a single slide—a considerable saving in time and effort. Mf are easy to see and subsequent Giemsa staining, if it is required, is not affected by the modified procedure. Mf were still well preserved after the deposits from the modified tests had been stored for 3 months in a tightly stoppered tube.

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