

THE FEASIBILITY OF FILTER PAPER COLLECTED BLOOD FOR THE SERODIAGNOSIS OF MALARIA

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Serological studies provide useful data to the epidemiological surveillance of the disease. However, one of the obstacles to large scale seroepidemiology is the obtention and separation of serum samples under field conditions.

Collection of blood on filter paper (FP) seems to provide a convenient approach to this problem (R. I. Anderson et al. 1961, *Exp. Parasitol.*, 11: 111; R. S. Bray 1962, *Trans. R. Soc. Trop. Med. & Hyg.*, 56: 436). Specimens gathered in this way are easy to handle and transport. In this study, we compared the sensitivity of serum and blood collected on FP for the screening of malaria antibodies. Patients were 30 young miners from Bolívar State with confirmed *Plasmodium falciparum* malaria. After the finger puncture drops containing approximately 16 μ l of blood were allowed to soak into the FP (Whatman No. 1), several drops were obtained from each patient. Samples for serum were concomitantly withdrawn by venipuncture. Antibodies to *P. falciparum* were assayed by the ELISA test. A short-term culture of a local isolate of *P. falciparum* obtained from the blood of a patient from Bolívar State was used as source of antigen. Parasites were harvested at 7% parasitemia and antigen was extracted from infected erythrocytes after treatment with Nonidet P40 (NP-40) as described by L. J. Mackey et al. 1982, *Bull. WHO*, 60: 69. Protein A coupled to horseradish peroxidase (Amersham) was used as secondary probe. The chromogenic substrate was ABTS (SIGMA) (S. Al-Kassi & Mostrato, 1987, *J. Immunological. Method.*, 58: 127). Recordings were made at 405 nm (OD) using a Titertek Multiskan II. Antibodies were eluted from a 6.2 mm \emptyset FP disc (FPD) punched out of each sample with a commercial perforator; after 2 h extraction with 400 μ l of PBS at 4 °C the sample was centrifuged at 200 xg/5 min and the supernatant used to search for antibodies to *P. falciparum*. Equivalent dilutions of extracted

blood and serum were prepared. The former was calculated by the volumetric method assuming that haematocrit ranged between 42 and 50%. Since each FPD containing 10 μ l of blood was extracted with 400 μ l of PBS, the average dilution factor for extracted blood was 1:90. Negative reference values for sera and blood collected on FP from 30 blood donors were determined. Optimal screening dilution of serum and extracted blood for the ELISA test was 1:180. Significant values ($\bar{X} + 3$ EDM) were 0.23 and 0.35 for serum and extracted blood respectively.

Results showed that the assay was much less sensitive with the FPD. Thus, whereas serum assay gave 87% seropositivity, that using FPD was only 66%. This suggests that poor elution of antibodies from blood collected on FP probably accounted for the low sensitivity herein reported. In order to improve antibody elution from FPD, non-ionic detergents, Tween-20 and NP-40 were included in the extraction buffer. It was showed that extraction of blood with 1% NP-40 (20 μ l for a 6.2 mm \emptyset FPD) for 5 min at room temperature followed by the addition of 380 μ l of PBS-0,1% (v/v) Tween-20 (PBS/T) and further incubation by 2 h at 4 °C, significantly improved antibody elution. Seropositivity increasing from 66% to 84%. It is worth of mention that most sera tested in this work showed a low *P. falciparum* antibody titer in the ELISA test (data not shown). In this situation a good elution of antibody from FPD is fundamental to the successful use of FP collected blood for seroepidemiology of malaria. It was also found that FP collected blood maintained in plastic bags could be stored at -70 °C during 3-4 months without any significant loss of the activity of the extracted blood in the ELISA test for *P. falciparum*. From this preliminary work it was concluded that elution of antibodies from FPD can be substantially

improved by including the detergents NP-40 and Tween-20 in the extraction buffer. The potential advantage of this modification for the screening of malaria antibodies is currently being evaluated in field trials.

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