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SHORT COMMUNICATION

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Evaluation of the ICT Malaria Pf test for rapid post-mortem diagnosis of *Plasmodium falciparum* malaria in corpses examined for forensic reasons

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Abstract To test the diagnostic value of a rapid and simple immunochromatographic test (ICT) based on the detection of *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) for post-mortem examination, blood samples from 30 consecutive corpses were analysed by ICT and Giemsa-stained blood films. Compared to microscopy, ICT had 100% sensitivity and 100% specificity even after a considerable time had passed between the presumed time of death and testing or after prolonged storage of whole blood samples. The ICT yielded positive results for four travellers who had returned from Kenya and died from *Pl. falciparum* malaria. The ICT might therefore serve as an additional tool for rapid malaria diagnosis, especially in non-endemic countries where experience with microscopic malaria detection is limited.

Key words Malaria · *Plasmodium falciparum* · ICT Malaria Pf Test · Post-mortem diagnosis · Forensic

Introduction

Imported malaria has become an increasing problem worldwide due to increased international travel and immigration. To obviate the difficulties of untrained personnel in detecting malarial parasites by microscopy especially in non-endemic countries (Chiodini 1998), a rapid and simple immunochromatographic test (ICT) has been developed (AMRAD ICT Malaria Pf test, AMRAD ICT Diagnostics, Sydney, Australia). The test is based on the detection of *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) in peripheral blood, which is secreted from infected erythrocytes and is not present in the other three human pathogenic *Plasmodium* spp. The ICT Malaria Pf test has been evaluated previously under field conditions in tropical countries (Singh et al. 1997; Durrheim et al. 1998; Garcia et al. 1996) or compared with the ParaSight-F test or PCR in endemic areas (Palmer et al. 1998; Kilian et al. 1997) and in travellers (Pieroni et al. 1998).

We report a new application of the ICT Malaria Pf Test for rapid post-mortem diagnosis of *Pl. falciparum* malaria in corpses in a forensic setting.

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Material and methods

Malaria was suspected as one possible cause of death in four tourists who had travelled to Kenya and died under unclear circumstances several days after returning to Germany. Thin blood films were prepared from peripheral blood and stained with Giemsa (BDH, Poole, UK). Biopsies were taken from brain, liver and spleen and stained with haematoxylin and eosin (H&E) for histological examination. Additionally, 10 µl of fresh or stored blood specimens was analysed using the ICT Malaria Pf Test according to the manufacturers' recommendations. This antigen-capture assay is based on the detection of PfHRP2 by two specific antibodies resulting in a pink line in a positive case after 5 min. Each test was performed twice.

To evaluate the possibility of false positive ICT test results, blood samples from 26 corpses (mean age 48.7 years, mean minimal time between death and finding 1.87 days) examined consecutively by legal autopsy were analysed by both ICT and microscopy. Thick blood films were classified as negative after examination of 200 oil immersion fields (1000 ×) before the ICT was performed by a different person without knowledge of the microscopy results.

Results and discussion

In the thin blood films of all four patients (Table 1), more than 90% of the 1000 erythrocytes counted were parasitised by *Pl. falciparum*. Very early forms of trophozoites were seen almost exclusively and no other *Plasmodium*

Table 1 Characteristics of four patients diagnosed with *Plasmodium falciparum* malaria by autopsy

Case	Age (years)	Sex	Presumed duration of disease (days)	Minimal to maximal time between death and finding (days)	Longest storage of blood at 4 °C (months)	Percentage of parasitised erythrocytes on blood films	Intensity of test line
1	23	F	18	0.5–1	24	96	+
2	26	F	7	2	5	94	+++
3	54	M	16	2–4	5	98	+
4	55	M	10	2–9	5	93	+++

species could be detected. Biopsies of brain, liver and spleen showed typical pathology features of severe malaria. In all four corpses, the ICT yielded a positive result. In two of the four, however, the pink line was obviously fainter than in the other two and in positive controls taken from living malaria patients. Interestingly, the ICT yielded positive results even after a considerable time had passed between presumed time of death and testing. Storage of blood for as long as 24 months at 4 °C did not affect the diagnostic results. In the 26 corpses analysed for evaluation of possible false ICT test results, neither the ICT nor blood smears showed a positive result. However, it should be kept in mind that false positive ICT results might be due to the prolonged persistence of HRP-2 in the blood after anti-malarial treatment. An analysis of possibly false negative ICT results which may be caused by a deletion in the HRP-2 gene, an antigenic variation, the presence of blocking antibodies, or immune complex formation could not be undertaken due to the low number of 10–20 malaria-caused deaths per year in Germany.

Thus, in our small study sample of 30 corpses, sensitivity and specificity of the ICT Malaria Pf test were both 100% as compared to the gold standard microscopy. Additionally, there was no apparent correlation between the percentage of parasitised erythrocytes and test line intensity in the ICT. Similarly, such a correlation could also not be established in a field study in India (Singh et al. 1998), while in a Uganda-based survey a linear relationship between log (parasite density) and line visualisation was found (Kilian et al. 1997).

Not much is known about immunological diagnosis of infectious diseases in post-mortem material. In our study, PfHRP2 could be detected immunochromatographically in peripheral blood drawn at least 2 days after the presumed time of death. Storage of blood at 4 °C for as long as 24 months did not alter the diagnostic outcome established by the ICT.

In summary, the ICT Malaria Pf test may serve as a first-line diagnostic tool in a forensic setting at the site where a corpse is found or as an additional laboratory test for confirmation of microscopic results. PCR may also be a laboratory method for differentiating *Pl. falciparum* from the other three human pathogenic *Plasmodium* spp. (Tham et al. 1999).

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