Invalid result of *Plasmodium falciparum* malaria detection with the BinaxNOW Malaria rapid diagnostic test

Malaria is endemic in many tropical countries and causes the deaths of at least 1 000 000 people per year. People who live in non-endemic countries are also concerned by fatal cases when they travel to endemic areas for vacations or business (Ryan & Kain, 2000). Mainly, malaria diagnoses are made after return from travel and require biological tests. Besides blood smears, which remain the reference for malaria diagnosis, new rapid diagnostic tests (RDTs) are available. BinaxNOW Malaria (Binax) is an immunochromatographic test (ICT) that detects malarial antigens in patient blood (Murray et al., 2003). Both pan-specific (aldolase) and *Plasmodium falciparum*-specific (histidine-rich protein 2, HRP-2) antigens can be detected by this test with high sensitivity concerning *P. falciparum* detection. Many evaluation studies comparing these RDTs with traditional (e.g. thin or thick blood smear) or accurate (e.g. PCR) methods have been published.

Sources of false positive (rheumatoid factor) or false negative (low parasitaemia) results with these test are known, but invalid cases have never been pointed out to our knowledge (Murray et al., 2003).

Each year, 200 malaria tests in blood samples are performed in our laboratory (located in a teaching hospital of a non-endemic country), and BinaxNOW Malaria ICT has now been routinely used for 2 years. We report here the case of a patient in whom the diagnosis of malaria was not possible with the BinaxNOW Malaria test. Severe *P. falciparum* malaria was diagnosed for a patient 5 days before his hospitalization in our teaching hospital. Quinine treatment was started 4 days before his admission. We received a whole blood sample of this patient in an EDTA tube on the day of his admission to control parasitaemia. In this sample, no parasite was seen by examination of thin blood smear nor with the Quantitative Buffy Coat method (QBC, Becton Dickinson). Antigen detection of plasmodia was also performed on this sample with the BinaxNOW Malaria ICT used strictly according to the manufacturer’s instructions. The HRP-2 line (T1) was positive according to the clinical history of the patient but the test could not be validated because of the non-appearance of the control line (C) even when the assay was performed with different batches of the test by different experienced practitioners (Fig. 1).

According to the manufacturer’s instructions, the control line should always appear at the top of the ICT in order to validate the assay. We hypothesize that an inhibitor is present in the blood of this patient, preventing high-quality development of the assay. In order to prove this, we diluted the blood of the patient with isotonic NaCl solution (9 ‰). We then searched for the presence of a human anti-mouse antibody (HAMA) known to potentially modify immunological tests (Thorpe et al., 2003), since mouse immunoglobulins are utilized in this ICT. The 1/8 dilution of the sample made the control line become visible with the positive HRP-2 line still present, while the 1/16 dilution made the positive HRP-2 line become undetectable with the control line still positive. This experience suggests that an inhibitor was present in the patient’s blood, but no HAMA was detected in the serum of the patient (HAMA-Elisa Medac, Medac Diagnostika).

Such a case of an invalid result with BinaxNOW Malaria ICT has never been published to our knowledge even though multiple studies have evaluated this test. Thus, patients presenting with a circulating inhibitor might not be common; however, the increase in the number of travellers from non-endemic countries to endemic areas and the increasing use of RDTs as diagnostic tools for malaria require us to consider the possibility of observing invalid results.

**Fig. 1.** BinaxNOW Malaria ICT assay performed 4 days after the beginning of the quinine treatment in a case of *P. falciparum* malaria. The HRP-2 *P. falciparum*-specific line (T1) is positive but no control line (C) becomes visible. Assays were performed three times by different experienced practitioners with different batches of the test.

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