# Performance and accuracy of an immunodiagnostic antigen detection test in diagnosing *Plasmodium falciparum* among Yemeni patients

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The traditional diagnosis of malaria based on the examination of thick and thin blood films is inappropriate in many locales because of the lack of facilities as well as a trained microscopist to read and interpret the blood films. The development of a rapid and specific diagnostic test to identify individuals infected with malaria is of paramount importance. Immunochromatographic antigen detection tests are promising tools for diagnosis of malaria. They have several advantages over traditional methods for diagnosis. They save time, are very stable, and require no electricity and only minimal expertise. Various experimental tests have been developed targeting a variety of parasite components.[1-3] These components are histidine-rich protein and lactate dehydrogenase. In Yemen, the predominant species of malaria is *Plasmodium falciparum*[4,5] and in all clinical settings diagnosis depends mainly on light microscope parasite detection, which suffers from many limitations. This prompted us to evaluate the accuracy and performance of a rapid dipstick antigen detection method with the aim of applying the test in the diagnosis of *P. falciparum* infection in the country. This study describes the performance and accuracy of the test for diagnosis of *P. falciparum* among Yemeni patients who were initially identified by microscopial examination.

## Subjects and Methods

This study was conducted between June 2000 and October 2002 in two different clinical centers in Yemen, one in Asshark town, a malarious area in Damar Province, and the other in Sana'a City. During the study period, 434 blood samples were collected from suspected cases of malaria. In addition, 73 blood samples were collected from healthy individuals who acted as controls. To assess the specificity of the test, blood samples were also collected from patients with heterologous infections, such as toxoplasmosis (n=8), typhoid fever (n=12) and visceral leishmaniasis (n=10). Each subject or subject's guardian was clearly informed on the purpose of blood sample collection. Blood samples were collected from those who agreed to cooperate. Thick and thin blood films were prepared from all suspected malaria patients, and for each case, a thin film was prepared, fixed with absolute methanol and stained with 3% Giemsa, (diluted with buffer water pH 7.2), while the thick film was stained unfixed.

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Positive blood films were examined by an immunochromatographic dipstick antigen detection test, OptiMAL (Flow Inc. Portland OR USA).[6] Blood samples from healthy individuals and those with heterologous infections were also examined by the dipstick test to assess the sensitivity and specificity of the test. We performed the test according to the manufacturer's instructions. Briefly, the blood sample tested (10 µL) is mixed with 30 µL lysing buffer containing monoclonal antibodies. This mixture was allowed to soak into the dipstick. After eight minutes, 100 µL of clearing buffer was added. If *P. falciparum* were present in the blood samples, three bands (including one at the top of each stick representing the controls) develop on the dipstick.

## Results

Of 434 stained blood films, 161 were positive for *P. falciparum* (Table 1). Of 161 positive blood samples on light microscope examination, 152 were found positive by the dipstick antigen detection test, giving 94% sensitivity. The specificity of the test was 100%, as cross-reactivity with heterologous infections was not observed.

## Discussion

The dipstick antigen detection test OptiMAL uses two monoclonal antibodies specific for plasmodium species infecting humans, including falciparum malaria. Various studies of the OptiMAL test[7,8] in different areas found a sensitivity between 88% and 91% and a specificity between 92% and 98%. In our study the direct traditional diagnosis was performed for the purpose of identifying cases with malaria to evaluate the performance and accuracy of the rapid dipstick antigen detection system. The results confirm that the predominant species of malaria in the country is *P. falciparum*, a finding that coincides with previous data.[4,5] Of 434 blood samples, 161 were positive for *P. falciparum* by demonstration of rings and gametocytes on microscopical examination. Of 161 parasitologically confirmed cases, 152

**Table 1.** Detection of *P. falciparum* by Giemsa-stained blood films and by the dipstick immunochromatographic antigen detection test.

| Test (group) | Number examined | Number positive | Number negative |
|---|---|---|---|
| Microscopy (suspected malaria cases) | 434 | 161 | 273 |
| Dipstick antigen (confirmed malaria cases) | 161 | 152 | 9 |
| Dipstick antigen (healthy individuals and heterologous infections)* | 103 | 0 | 103 |

* Healthy individuals (n=73), heterologous infections (n=30)

were positive by the dipstick rapid test OptiMAL, giving 94% sensitivity.

The 100% specificity of the dipstick test was obtained, as none of the heterologous infections was positive. Nine blood samples from parasitologically confirmed cases were classified as negative in the present study. Those cases may have had low parasitaemia. This problem has been described previously. Van den[9] found that the sensitivity of the dipstick antigen detection system depends on parasite count. The sensitivity was less with decreasing parasitaemia. It might be interesting to monitor parasitaemia and antigenaemia, but neither the objective nor the setting allowed for a systematic follow-up of cases in our study. Evaluation of two other commercial tests (ICT and paraSight), both of which detect histidine-rich protein-2 (HRP-2) was not attempted in the present study. HRP-2 is a water-soluble antigen expressed by the trophozoite of *P. falciparum* and also by immature gametocytes, but not by mature circulating gametocytes. Data on the rapid tests that detect HRP-2 are available in the literature. The ICT test is reported to have a high sensitivity when compared with microscopy. Of 100 samples that were slide-positive, 98 also tested positive in the ICT test, giving 98% sensitivity.[10] Among 99 malaria negative samples, 82 tested negative and 17 were positive in the ICT test, giving a specificity of 83%.[10]

Reduced sensitivity (65%) has been reported for the ICT and paraSight tests.[7] The point to be stressed is that HRP-2 can persist for days after adequate treatment and cure. Therefore, the test cannot adequately distinguish a resolving infection from treatment failure.[2] In contrast, a test based on lactate dehydrogenase detects viable parasites, which eliminates a prolonged period of false positivity post-treatment.[7] Although this rapid antigen detection test seems to be very useful and can be considered an alternative for diagnosis of *P. falciparum* in Yemen, further investigation to evaluate the test on a large population and in various endemic areas is still needed.

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