Rapid Diagnostic Modalities for Malaria

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Malaria is a global and resurging infectious disease problem causing approximately three million deaths worldwide each year, mainly in developing countries. Early diagnosis and prompt drug treatment are essential in order to minimize morbidity and mortality. For decades, microscopic examination of Giemsa-stained peripheral blood smears has been the cornerstone of malaria diagnosis. Thick smears are more useful than thin films when parasitemia is low; once parasites have been detected, thin smears can assist in Plasmodia species identification. Accurate examination of blood films depends upon technical expertise involving proper staining and handling of the slide and microscope, parasite recognition, and practice. Laboratory staff must be well-trained and should undergo refresher courses regularly.

In the search for new diagnostic modalities in malaria, the ideal rapid test should be rapid, simple to perform, easy to interpret, accurate, and inexpensive. Over the past few years there has been tremendous technologic and marketing progress in the development of a number of rapid and specific diagnostic tests to identify patients infected with malaria. As alternatives to conventional stained blood smear examination, these tests hold great promise for use in areas of the developing world where microscopes and/or trained personnel are not readily available, such as in primary health care clinics and other peripheral level facilities. These tests also have potential utility in non-endemic western countries where experience with microscopic malaria detection is limited, and they have also been evaluated for self-use by persons traveling to malaria-prone regions.

Newer, rapid diagnostic malaria tests include polymerase chain reaction (PCR)-based tests, quantitative buffy coat examination, acridine orange immunofluorescent staining, and several immunochromatographic assays. Fluorescent microscopy and nucleic acid detection techniques, however, require skills and equipment which are not universally available in many malaria-endemic countries. Recently introduced immunocapture methods, several of which are now commercially available and which can be performed in about 5 to 10 minutes, are based upon the detection of antigens derived from Plasmodia in lysed peripheral blood, and employ either a dipstick or test strip that have monoclonal antibodies directed against the target parasite antigen. These antigen-capture techniques are easy to run, and interpretation does not require complex equipment or technical support. Thus, they are important breakthroughs in the qualitative detection of malaria parasites.

The ParaSight-F assay (Becton Dickinson, Cockeysville, MD, USA), ICT Malaria Pf assay (ICT Diagnostics, Sydney, Australia), MalaQuick (ICT Diagnostics, Sydney, Australia), PATH falciparum malaria IC strip (Quorum Diagnostics, Vancouver, CA) and Determine Pf test (Abbott Diagnostics, Abbott Park, IL, USA) all detect parasite histidine-rich protein (pHRP-2), a water-soluble protein produced specifically by trophozoites and young gametocytes of Plasmodium falciparum. In field evaluations these test kits have demonstrated comparable or superior detection rates for P. falciparum than conventional microscopy, especially for smear-negative samples. However, the technique may fail to detect P. falciparum infection when blood smears contain only gametocytes, and these qualitative kits are unsuitable for monitoring the response to antimalarial treatment.

Parasite lactate dehydrogenase (pLDH) is an intracellular metabolic enzyme produced by asexual and sexual stages of malaria parasites. Another commercially-available kit, OptiMAL (Flow Inc. Portland, OR, USA), utilizes a dipstick coated with monoclonal antibodies against this enzyme, and can distinguish P. falciparum from non-P. falciparum species. Differentiation of malarial species is based
upon antigenic differences between the pLDH forms. The enzyme is produced only by live Plasmodium parasites, thus the test has the ability to differentiate live from dead organisms. In recent years, rapid non-microscopic antigen-capture tests which detect pHRP-2 and pLDH have been extensively studied in field trials in Africa and Asia\textsuperscript{1,3-9,13-20}. By comparing results from these tests with either direct microscopy or PCR, researchers have determined their accuracy and potential advantages. Diagnostic sensitivities and specificities range approximately from 84\% to 100\% and 89\% to 100\%, respectively, and positive and negative predictive values between 82\% to 100\% and 88\% to 99\%, respectively\textsuperscript{3,5,6,8,10,15-22}. The pLDH assay has the utility advantage over pHRP-2 detection in that it provides excellent correlation with traditional blood films in the identification of both P. falciparum and P. vivax malaria\textsuperscript{3}. Sensitivities of both pHRP-2 and pLDH dipstick tests appear to have direct correlation with parasite density. Lower limits of detection when false-negative results begin to appear, occur with parasitemia levels that are below 50 to 300 parasites per mL of blood\textsuperscript{4,6-9,17,19}. Rapid dipstick methods may yield positive results when peripheral blood smear staining fails to identify parasites. Such discordant results have been evaluated by re-testing using day 0 and day 1 blood samples, or by using large samples and longer incubation mes\textsuperscript{19,21}. Tests using pHRP-2 detection have shown cross-reactivil of 26\% (compared to 3\% with a pLDH kit) in blood from patients with detectable rheumatoid factor\textsuperscript{23}. False-positive results may also be explained by persistent antigenemia (occurring in pHRP-2 assays lip to 7 to 14 days after successful treatment, not seen with the pLDH assay) and the possibility of sequestered parasites from incomplete chemotherapy\textsuperscript{1,4,24}. Persistent test positivity after parasite clearance precludes the use of these tests for monitoring early therapeutic responses. Immunocapture tests have been found useful in other clinical settings including the diagnosis of placental malaria and forensic detection of malaria in postmortem blood specimens\textsuperscript{22,25}. There has also been enthusiasm in considering rapid non-microscopic dipstick tests for use by persons traveling to malaria-endemic regions\textsuperscript{11,26}. Self-interpretation of dipstick tests by travelers has frequently been associated with an unacceptably high incidence of false-negative results despite written instructions before travel; oral instruction appears to enhance performance\textsuperscript{12,26,27}. Accordingly, at the present time the self-use of dipstick tests for malaria diagnosis by travelers cannot be routinely recommended, and these kits should only be prescribed after appropriate instruction and training, and demonstration of successful performance of the test procedure.

In summary, recently introduced rapid, non-microscopic, immunocapture tests can have excellent applicability in malaria diagnosis in settings where microscopes and skilled technicians are not readily available. In general, such tests can be reliably read by field workers without any supervision, and appear to satisfy the desired criteria of being rapid, Easy to perform and interpret and extremely accurate. Making these valuable diagnostic assays affordable and widely available could contribute greatly to controlling the public health impact of this menacing disease.

References

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