



FEATURE ARTICLE – JOHN WALKER

Rapid Diagnosis of Malaria



This issue marks the retirement of **Dr John Walker**, Head of Parasitology at CIDMLS. John began his training in parasitology at the School of Public Health and Tropical Medicine at the University of Sydney in 1967. Since that time he has been involved in surveys of populations in various regions of Australia and neighbouring countries for alimentary and blood parasites, studies on the trematodes responsible for cercarial dermatitis in Australia and the molluscan hosts involved, and on the potential role of Australian freshwater molluscs as hosts for human schistosomes. Since moving to Westmead Hospital in 1987, a significant part of his work has involved the assessment of new methods of malaria diagnosis and investigations into geographic variation between isolates of *Plasmodium vivax*.

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Introduction

The incidence of imported malaria has been increasing in developed nations for several decades. The key to effective management of these infections is prompt and accurate diagnosis, usually by microscopical examination of stained thick and thin blood films. This simple procedure, the 'gold standard' for more than a century, is usually well handled by laboratories with experienced staff but, for a number of reasons, errors are common. Large teaching hospitals in urban centres usually fare well, but small laboratories that rarely see a case of malaria often have difficulty. Methods that could either replace microscopy or provide additional diagnostic support for less experienced individuals have been sought, with varying success, for decades.

Now the combination of monoclonal antibodies and molecular biology have provided diagnostic reagents of promise for point-of-care diagnostic tests that detect parasite antigens in peripheral blood. Most adopt test strips in which are embedded monoclonal antibodies specific for one or more parasite antigens. Because they can be performed in around 15 minutes, these tests have become known as rapid diagnostic tests (RDTs), the implication being that diagnosis by blood film is slow. That can be true in countries where blood films for malaria diagnosis, taken at a village health centre, are referred to a central laboratory, with the results often being reported after the patient has been treated empirically. It should not occur in urban centres in developed countries. Making, staining and examining blood films should take no longer than 30 to 45 minutes. The biggest delay in obtaining a malaria diagnosis usually occurs between taking the blood sample and getting it to the laboratory, a delay that is the same no matter what diagnostic method is used. The emphasis on rapid diagnosis once the specimen reaches the laboratory is misdirected. It is more important to accurately identify the parasites if they are present and, in the case of infections involving *Plasmodium falciparum*, make an estimate of the parasite density. Malaria is rarely life threatening in returned travellers, but in those infrequent cases where the individual is severely ill, the major issues faced by physicians involve arresting any further deterioration in the patient's condition and then eradicating the parasites.

The reliability of these tests should be of interest to clinicians caring for patients who might have malaria. There have been many assessments of their performance in a variety of situations and those involving infections in returning travellers are of most relevance here.

Studies

A recent Canadian study evaluated a test using monoclonal antibodies to histidine-rich protein II (HRP II), against a malaria specific polymerase chain reaction (PCR) and expert microscopy. HRP II is a water-soluble protein produced in the infected erythrocyte by the asexual and early sexual stages of *P. falciparum*. Because it is made at relatively high concentrations, assays using

this antigen are usually highly sensitive and specific. The 200 subjects in the study were patients seen at the Tropical Disease Unit of the Toronto General Hospital, with a history of fever and travel to a malaria-endemic region. Using PCR as the 'gold standard', the RDT had a sensitivity of 97% and a specificity of 96%. The positive predictive value was 81.2% and the negative predictive value 99.5%.

Playford and Walker also compared PCR and expert microscopy with two different RDTs, one using HRP II combined with an unspecified 'pan-malaria' antigen, and the second *Plasmodium* specific LDH antigens (one *P. falciparum* specific and the other genus specific). The PCR assay was a multiplex system that distinguished the four species. One hundred and fifty eight blood samples from 144 febrile travellers were referred to the New South Wales malaria reference laboratory at Westmead Hospital in Sydney. The sensitivity and specificity of the assay using HRP II for *P. falciparum* were 97% and 90% respectively. For the assay using LDH antigens they were 85% and 96% respectively. Perhaps more significantly, the HRP II based assay missed one *P. falciparum* infection with a parasite density of 45/μl and had positive and negative predictive values for *P. falciparum* of 95.1% and 99.1% respectively. The LDH based test failed to detect 5 *P. falciparum* infections, one with a density of 2,500/μl, and had positive and negative predictive values of 90.9% and 92.0%. The performance of the two assays in the diagnosis of non-*falciparum* malaria (*P. vivax*, *P. ovale* and *P. malariae*: neither test differentiates these species) was very different. The HRP II/pan malaria antigen assay had a sensitivity and specificity of 39.3% and 100% respectively while the LDH based test had values of 76.8% and 100%. The positive predictive values for non-*falciparum* malaria were 100% for both assays and the negative predictive values 76.1% for the HRP II/pan malaria test and 88.7% for the LDH assay. Disturbingly, both assays missed infections with densities over 5,300/μl and the HRP II/pan malaria assay missed one at 10,000/μl.

Discussion

The results of these two studies, similar to those obtained in other settings, indicate that tests using HRP II for the detection of *P. falciparum* are generally very reliable and compare well with microscopy. The high negative predictive value should provide confidence that a negative result is unlikely to be false. Good clinical practice would involve further tests if symptoms persisted. The HRP II based tests should, however remain as adjuncts to microscopy in the management of *P. falciparum* infections unless they become quantitative. They occasionally give false positive results because HRP II can persist in the blood for up to 28 days after successful eradication of parasites.

Because of low sensitivity, the RDTs can not be recommended as alternatives to microscopy for the diagnosis of non-*falciparum* malaria in developed countries where even moderately competent microscopists would have little trouble finding *P. vivax* parasites at the densities missed by both tests in the Sydney study. Microscopists experienced in the diagnosis of malaria are able to detect parasites at densities of 10-

20/μl in well made and stained thick films. Densities of 50-100/ μl should present no problem to competent laboratory scientists examining good films with well-maintained microscopes. However, not all scientists called on to examine a film for malaria parasites have sufficient experience, particularly those working the 'graveyard shift' in the middle of the night, and for these individuals the immunochromatographic tests can provide much needed support.

An issue of concern involving these tests is a concomitant deterioration in capability in malaria microscopy. Laboratories, once quite capable of detecting and identifying malaria parasites now frequently refer blood films of poor quality to the reference laboratory at Westmead Hospital, for species identification. A tentative, non-specific diagnosis has been obtained by RDT, but the staff, once capable of specific identification of malaria parasites by microscopy, have lost confidence in their previous skills. Much of this stems from poor laboratory practice; the RDT is done first and the blood films for microscopic diagnosis prepared later (often much later).

The delay in blood film preparation is easily detected by the significant alteration to parasite morphology in referred films, and it is this that makes the essential identification of the species difficult for inexperienced microscopists. If laboratories still used microscopy as the 'gold standard' and the RDTs as adjuncts to diagnosis, much of this problem would disappear. Blood films should be made at the time the specimen is received, even if they are not looked at immediately (the preferred option).

Ironically, the RDTs are of less value at present in countries with hyperendemic malaria, where most malaria deaths occur. This is because most people in these countries have protective immunity acquired and maintained through constant re-infection. They frequently have parasitaemias that are clinically irrelevant and any form of parasite detection, blood film PCR or RDT, will be positive. The RDTs are more useful in regions of lower endemicity where there is little immunity and most infections are symptomatic. These are often regions with serious problems of multi-drug resistance where identification of the parasites is important before expensive, alternative drugs are used.

ROGAN LEE TALKS ABOUT THE DIAGNOSIS OF CHRONIC STRONGYLOIDIASIS



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Strongyloides stercoralis is a parasitic nematode with a free living and a parasitic stage in its life cycle. The adult worm lives in the small intestine of humans and lays eggs, which quickly develop as they move down the gastrointestinal tract. All adult worms in the human host are females who produce their own eggs by a mitotic process (parthenogenesis) and therefore do not require the presence of a male worm for fertilisation. Some larvae hatch as they move down to the lower bowel. If movement of the larvae is slowed sufficiently, some of these larvae can develop in to infective 3rd stage larvae before they are passed in the stools. Infective larvae can therefore re-infect the same host by penetrating back into the gut wall thus maintaining a continual infection for decades. War veterans who were infected with this parasite have been known to carry the infection for over 30 years after the war. Migrants and indigenous populations are other groups at risk of being infected. In immunocompetent hosts the infection is suppressed to low numbers of worms but this balance can change when immunosuppression either develops from pathological causes or is induced by steroids. When the host immunity is suppressed, hyperinfection with *Strongyloides* can ensue. Larvae burrow through the gut wall and travel to other tissues of the host causing a gram-negative septicemia.

Other nematode infections such as hookworm and ascarids resolve without treatment, if the host exposure to infection stops. *Strongyloides*, however, remains in the host for a long time. It is not known whether the host can eventually develop complete immunity to this infection. Diagnosis of chronic strongyloidiasis is difficult because larvae are not regularly passed in the stools in immunocompetent hosts. The most sensitive test available is

detection of antibodies to the worm by ELISA. A crude antigen extract of *Strongyloides* larvae is used to bind to the ELISA wells and human antibody to this antigen is detected by a labelled anti-human antibody. A commercial kit has recently been made available. A preliminary evaluation of this kit by PathCentre, WA showed that antibodies from two of 28 patients, known to be infected with *Strongyloides*, were not detected in this test (Figure 1).

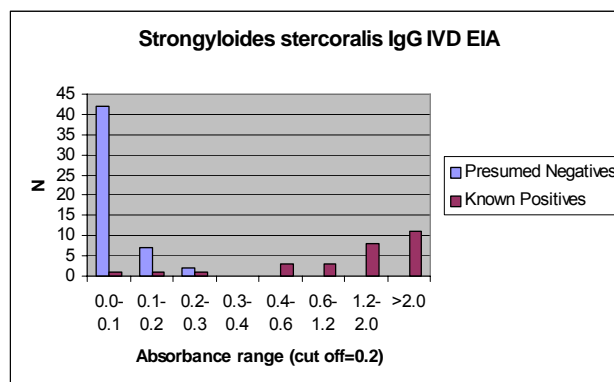


Figure 1: Antibody detection of *Strongyloides* infected patients using *S. stercoralis* commercial kit. Ian Sampson *et al*, PathCentre, 2005

Cross-reactive antibodies may be present which can lower the specificity of our assay. Our research interest is to improve the sensitivity and specificity of current serological tests.

CIDM PUBLIC HEALTH – EDUCATION PROGRAMME

04 November 2005: The Salmonella Journey – From diarrhoea to database.

Salmonella continues to play a major role as the causative agent of gastroenteritis. Each year around 1800 – 2000 salmonella isolates are submitted to the Enteric Reference Laboratory at the Centre for Infectious Diseases and Microbiology (CIDM) at Westmead for serotyping and identification. Database information generated from the identifications is distributed daily to Public Health Units (PHUs) throughout NSW, to aid in the tracing of salmonella outbreaks.

To receive further information & join our e-list, please email judithh@icpmr.wsahs.nsw.gov.au