Requirements for Diagnosis of Malaria at Different Levels of the Laboratory Network in Africa

Earl G. Long, PhD

Key Words: Malaria; Chloroquine; Parasitological diagnosis

Abstract

The rapid increase of resistance to cheap, reliable antimalarials, the increasing cost of effective drugs, and the low specificity of clinical diagnosis has increased the need for more reliable diagnostic methods for malaria. The most commonly used and most reliable remains microscopic examination of stained blood smears, but this technique requires skilled personnel, precision instruments, and ideally a source of electricity. Microscopy has the advantage of enabling the examiner to identify the species, stage, and density of an infection. An alternative to microscopy is the rapid diagnostic test (RDT), which uses a labeled monoclonal antibody to detect circulating parasitic antigens. This test is most commonly used to detect \textit{Plasmodium falciparum} infections and is available in a plastic cassette format. Both microscopy and RDTs should be available at all levels of laboratory service in endemic areas, but in peripheral laboratories with minimally trained staff, the RDT may be a more practical diagnostic method.

In past campaigns against malaria in the tropics, diagnosis was considered unnecessary because first-line drugs such as chloroquine were cheap and effective. As a result, patients experienced prolonged and worsening illness if the symptoms were not due to malaria, communities suffered from reduced productivity and school attendance, and there were unnecessary purchases of drugs and increased levels of drug resistance.\textsuperscript{1}

Reliance on clinical diagnosis of malaria has been shown to have a specificity of 42% when a combination of fever, splenomegaly, and nail-bed pallor was used and a specificity of 21% when fever alone was used.\textsuperscript{2} Some infections with symptoms similar to malaria are influenza, dengue fever, viral encephalitis, febrile gastroenteritis, lobar pneumonia, meningitis, septicemia, rickettsial infections, leptospirosis, and urinary tract infections.

Effective control of malaria depends on precise indicators of prevalence and incidence that can only be obtained by parasitological diagnosis: microscopy or rapid diagnostic tests (RDTs). Otherwise investigators cannot measure the impact of their interventions.

The costs of neglecting diagnosis in malaria are human and economic. Some drugs, such as the sulfadoxine-pyrimethamine combination drug (Fansidar), have serious adverse effects, such as Stevens-Johnson syndrome, and, inevitably, other etiologies will be left unidentified. Economic costs will accrue from unnecessary treatment (when another cause is involved) and from overtreatment if the prevalence of malaria is reduced by control measures. Inevitably, indiscriminate and widespread use of expensive new antimalarials will result in resistance of parasites to treatment and exposing susceptible populations to even higher risk.
A recently published study estimates that a diagnostic test with 95% sensitivity and 95% specificity requiring minimal infrastructure would avert more than 100,000 deaths and about 400 million unnecessary treatments.\(^3\)

Malaria is unique in remaining the only major infectious disease in which diagnosis is not the leading indicator for epidemiology, treatment, and control. If there are future efforts to integrate activities with tuberculosis and HIV/AIDS control, diseases that would never be treated without a definitive diagnosis, then an acceptable justification must be provided for the exemption of malaria from this requirement. The exception offered by the World Health Organization for treatment of malaria without diagnosis is in children living in hyperendemic areas who are younger than 5 years, with fever, because the disease is rapidly fatal in this population.\(^4\)

The Laboratory Network and Malaria Diagnosis

There are usually 4 to 5 levels of service for malaria diagnosis:

1. Provincial hospital laboratories, with capability for microscopy, RDTs, training, reference, quality control/assurance, data management, surveillance, and supervision of lower levels
2. District hospital laboratories, for microscopy, RDTs, reference, quality control/assurance, data management, and surveillance
3. Health center laboratories for microscopy and RDTs, and data management
4. Health posts or huts for RDTs. Microscopy is not available or practical at this level.

Some countries have a national reference laboratory with services and levels of expertise that exceed those listed. The national laboratory can provide higher levels of microscopy, RDTs, training, reference, quality control/assurance, research and evaluation, standard operating procedures, data management, surveillance, equipment maintenance, and laboratory supervision. The national reference laboratory has a central role in the delivery of diagnostic services at all levels and is responsible for planning, implementation, and monitoring of quality control/assurance. The human and financial resources are seldom available for a national reference laboratory to operate independently of a major hospital or research institute, and it should be an essential resource for the national malaria control program.

Microscopic Diagnosis

Microscopic examination of Giemsa-stained blood films is still the mainstay of diagnosis worldwide. It is inexpensive if the infrastructure is available, sensitive, permits differentiation among the 4 species of malarial organisms, can be used for determining parasite densities, and can be used for diagnosing other diseases and detecting other blood-borne pathogens. The limitations of microscopy are the requirements for high-quality microscopes that require a source of electricity for illumination and highly skilled and experienced microscopists.

Supplies and Equipment

The needed supplies and equipment are as follows:

1. Binocular microscope with \( \times10 \) oculars and \( \times10, \times40, \) and \( \times100 \) objectives (The major manufacturers offer instruments that are specially treated to resist growth of molds in hot, humid climates and are sufficiently rugged for daily use.)
2. Microscope slides and slide boxes for storage
3. Staining trays
4. Plastic measuring cylinders (100 and 500 mL)
5. Plastic serologic pipettes (1, 5, and 10 mL)
6. Wash bottles
7. Lens cleaning tissues
8. Handheld electric hair dryer or drying rack
9. Battery-powered LED (light-emitting diode) illuminators for laboratories without electricity
10. Stock Giemsa stain
11. Stock buffer solution (municipal tap water usually suitable)
12. Methanol
13. Immersion oil
14. Lens cleaning fluid
15. Lancets
16. Alcohol disinfectant swabs
17. Sharps container
18. Nonflammable xylene substitute: Citrosolve or limonene
19. 2-Channel cell counters
20. Timer
21. Gloves
22. Register

Rapid Diagnostic Tests

These alternatives to microscopy are an increasingly important component of a diagnostic strategy but cannot be considered a standard for diagnosis. Advantages of RDTs are that they can be used where reliable microscopic diagnosis is not available; they do not require laboratory facilities or special equipment; results are available in about 20 minutes; and users such as community health workers with little or no laboratory experience can be quickly trained in their use. Nevertheless, very careful training and continual supervision
are necessary for the best use of RDTs. The instructions on the package inserts do not always provide instructions accurately, especially with the introduction of blood into the cassettes; and incubation times may need to be slightly longer than recommended.

RDTs are immunochromatographic tests that are usually available in a plastic cassette format. They use gold-labeled antibodies that capture 1 of 2 groups of parasite antigens from small quantities of peripheral blood. The 2 most targeted antigens are the histidine-rich protein 2 of \textit{Plasmodium falciparum}—HRP2 produced by asexual and sexual stages; and the parasite lactate dehydrogenase—pLDH. RDTs should not be used without quality control using expert microscopy on a representative sample of blood specimens.

HRP2-based RDTs have higher positive and negative predictive values than pLDH RDTs and are a better choice for areas of high transmission in Africa. In areas of low or intermittent transmission, pLDH-based RDTs may be more suitable.\footnote{5}

Disadvantages of RDTs include their unreliability in detecting parasitemias lower than 200 parasites per microliter; their higher cost per patient compared with microscopy; inability to differentiate between species; false-positive reading after cure because of the persistence of circulating antigens; and rapid deterioration of test reagents when exposed to high temperatures and humidity. There are also anecdotal reports that very high parasitemia levels gave false-negative results, probably due to a prozone effect.

Supplies and Equipment

The needed supplies and equipment are as follows:

1. RDT cassettes (stored in unopened foil packages at temperatures less than 30°C)
2. Gloves
3. Alcohol disinfectant swabs
4. Lancets
5. Timer
6. Register

Finally, there must be a clear definition of the role and importance of the laboratory during the planning and management of malaria control activities, and there should be the establishment of a national reference core group of expert microscopists with adequate financial, material, and administrative support. A plan of action should include continuous interaction among national laboratory experts, clinicians, and epidemiologists.

\textit{From the Malaria Branch, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA.}

\textit{Address reprint requests to Dr Long: F 22, Malaria Branch, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Atlanta, GA 30333.}

References