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Opportunities and Threats in Targeting Antimalarials for the AMFm

The Role of Diagnostics

Christopher J.M. Whitty, Heidi Hopkins, Evelyn Ansah, Toby Leslie, and Hugh Reyburn

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Abstract

In malaria-endemic countries, people commonly assume they have malaria when sick and treat themselves accordingly. The Affordable Medicines Facility-malaria (AMFm) will make effective drugs more available everywhere. If longstanding problems can be successfully addressed, the use of microscopy and rapid diagnostic tests (RDTs) for malaria diagnosis alongside AMFm (though not necessarily directly part of it) could improve the management of both malaria and other febrile illness, as well as the cost-effectiveness of AMFm. In peripheral areas, RDTs are the only practical option, but available RDTs have limitations: all-or-none test results, variable heat stability, an inability to diagnose non-falciparum malaria and safety risks (especially HIV and hepatitis B) related to blood sampling. Of equal concern is that negative test results—meaning no malaria—are often ignored and patients treated anyway. R&D to solve technical problems and operational research on better ways to deploy RDTs and to make diagnosis count are needed.

Key Words: malaria, diagnosis, RDT, microscopy, cost-effectiveness, Africa

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Executive Summary

Historically and today, a large proportion of patients with febrile illness in places where malaria is common, especially in Africa, are treated with antimalarial drugs, but without specific diagnosis. The exception to the lack of diagnosis has been blood smear slides examined under the microscope for malaria parasites, a practice limited largely to hospitals and formal clinics. More recently, several rapid diagnostic tests (RDTs) for malaria have become available and the question of how they can best be deployed—and specifically, whether they should be linked to the Affordable Medicines Facility-malaria (AMFm)—arises. Targeting antimalarials to those who have malaria and identifying and treating other causes of serious febrile diseases is an undisputed long-term goal, but is far from the current state of affairs.

In rural villages in Africa, where health-care institutions are rare, chloroquine and sulfadoxine-pyrimethamine (SP) have been the main malaria drugs available, offered by shops and street vendors and purchased for patients for empirical use. Replacing those drugs with ACTs is the purpose of AMFm and a necessary component of malaria control. ACTs are relatively safe and very effective. A person with malaria is likely to be helped, and a person without malaria is not likely to be hurt (even if treating the uninfected person is not desirable).

In the peripheral, nonformal, and private sectors in Africa, where AMFm is likely to have its greatest impact, RDTs are the only realistic option. Microscopy, to be cost-effective, depends on high throughput, which is likely only in the formal sector. But are currently available RDTs ready for widespread deployment? RDTs have a number of technical limitations, including an all-or-none test result, variable heat stability, and in diagnosing non-falciparum malaria.

* Christopher J.M. Whitty, London School of Hygiene & Tropical Medicine, London, UK; Heidi Hopkins, FIND,Uganda; Evelyn Ansah, Dangme West, Ghana; Toby Leslie, London School of Hygiene & Tropical Medicine, London, UK, and Health Protection and Research Organisation, Afghanistan; and Hugh Reyburn, Joint Malaria Programme, Tanzania. This paper was commissioned for Resources for the Future's Consultative Forum on the Affordable Medicines Facility-malaria (AMFm) under a grant from the Bill & Melinda Gates Foundation.

Safety needs to be taken seriously. There is a theoretical risk of RDTs leading to true cases of malaria not being treated, and of deploying a technology that requires blood sampling by relatively untrained users in areas of high HIV and hepatitis B prevalence.

In the formal health-care sector in Africa, health-care workers are responsive to positive malaria tests, using either microscopy or RDTs, prescribing antimalarials for virtually everyone who tests positive. But about half the time, a negative test elicits the same prescription. Data from Asia, though limited, reveal a similar picture. The reasons clinicians respond irrationally to diagnostic tests are complex and may be difficult or slow to change. Data from outside the formal sector are too scant to be informative.

The bottom line is that these problems are recognized and will exist with or without AMFm. Improving diagnosis and the use of diagnostic tests alongside AMFm (though not necessarily a direct part of it) has the potential to improve management of both malaria and other febrile illness as well as the cost-effectiveness and sustainability of AMFm. The impact of improved targeting is greatest in areas where the incidence of malaria is lower and other causes of febrile disease are relatively more important. R&D to solve some of the technical problems and operational research to develop better ways to deploy RDTs and to make diagnosis count are also needed.

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Introduction

The opportunity to expand the use of effective antimalarials, in practice artemisinin combination therapies (ACTs), with the AMFm initiative is potentially a major step forward and undoubtedly very welcome, both within and outside the public sector. There is, however, a potential downside to expanding use in areas where diagnostic facilities are weak: increasing the number of febrile patients treated with an antimalarial may not translate into as large an increase in those who have malaria being treated with an antimalarial. There are many causes of febrile illness, and it is essential that antimalarial therapy is targeted as much as possible toward those who have the disease. This is important for several reasons.

First, important alternative diagnoses may be missed. Evidence is strong from many parts of Africa as well as outside it that the high burden of mortality in childhood is attributable to many treatable febrile illnesses; malaria is certainly not the only cause of death and in many settings is not even the most important (Olivar et al. 1991; Sowunmi and Akindele 1993; Ndyomugyenyi et al. 2007). Treating meningitis or pneumonia with an antimalarial, for example, is at best useless and possibly, if it leads to delays in diagnosis and appropriate treatment, even hazardous (Berkley et al. 1999; Reyburn et al. 2004; Berkley et al. 2005; Kallander et al. 2008; Orimadegun et al. 2008). The alternative to improved targeting of treatment for nonsevere febrile illness without diagnosis is syndromic management with antibiotics and antimalarials, but though this approach may have some attractions, it has very limited in-country support, with concerns about antibiotic resistance widely voiced.

Second, today's ACTs generally appear safe and well tolerated, but no drug is without risk (Maiteki-Sebuguzi et al. 2008). Treating malaria with an antimalarial is a balance of the

* Christopher J.M. Whitty, London School of Hygiene & Tropical Medicine, London, UK; Heidi Hopkins, FIND,Uganda; Evelyn Ansah, Dangme West, Ghana; Toby Leslie, London School of Hygiene & Tropical Medicine, London, UK, and Health Protection and Research Organisation, Afghanistan; and Hugh Reyburn, Joint Malaria Programme, Tanzania. This paper was commissioned for Resources for the Future's Consultative Forum on the Affordable Medicines Facility-malaria (AMFm) under a grant from the Bill & Melinda Gates Foundation.

relatively small risk of the drug against the very high risk of the disease. If, however, the great majority of those treated with an antimalarial do not have the disease and therefore cannot benefit, the risk-benefit changes presenting a significant safety issue for drugs, and especially newer drugs.

Third, the cost-effectiveness of any antimalarial program will be substantially reduced if most of the money spent on antimalarials goes to treat people who do not have malaria (Snow et al. 2003; Yeung et al. 2008). This is potentially an important limitation on the long-term sustainability of funding, and, in particular, of maintaining widespread political support. Because the success of upscaled malaria interventions (i.e., access to effective drugs and insecticide-treated nets) will result in reduced incidence of disease, without diagnosis, cost-effectiveness will only decrease; the proportion of febrile cases with malaria decreases though there may be no great change in the number of cases administered antimalarials. Being able to shift resources from drugs to other interventions as this change occurs will make diagnosis more important over time.

Fourth, the risk of increasing the potential for the emergence and particularly the spread of antimalarial drug-resistance, especially to the partner drugs in ACTs, and to a lesser extent, the artemisinins themselves, is small but real, dependent on endemicity. Low levels of drug circulating in many individuals in a population is an ideal situation to speed up the spread of resistance (Payne 1988; Talisuna et al. 2004). Recent data suggest that artemisinin-tolerant parasites may exist on the Thai-Cambodian border, which the WHO recognizes as a potential global emergency with serious implications for malaria control efforts (WHO 2008). Strategies to delay the spread of resistance and preserve ACTs as effective antimalarial therapies are critical to long-term malaria control goals (White and Olliaro 1996; Duong et al. 2004).

Fifth, if a large proportion of those treated with an antimalarial do not get better because they are not actually suffering from malaria, this can undermine popular support for new and effective antimalarials. Additionally, real gains (and losses) in malaria control programs can be missed when all fever cases are classified as malaria.

It is thus essential to move toward targeting antimalarials so that they go to those with malaria and not those whose fever is caused by a different infection. AMFm, by moving ACTs farther into the community, beyond the formal health-care system, could be a vehicle for better diagnostic practices—thus improving management of all causes of febrile illness. If diagnostic issues are ignored, AMFm could simply increase the amount of ACTs given to patients who do not have malaria. The diagnostic issues are certainly not a reason to delay or stop AMFm;

increasing the use of effective antimalarials in peripheral settings, where the poorest live, is essential. Technical decisions about AMFm must, however, take diagnostics and drug targeting into account if AMFm's potential is to be maximized.

There is now increasing evidence of a reduction in malaria transmission in many of the countries where malaria is a major problem (Barnes et al. 2005; Nyarango et al. 2006; Bhattarai et al. 2007; Okiro et al. 2007; Guerra et al. 2008; Sievers et al. 2008). This phenomenon is extremely welcome, and the use of artemisinins may well be contributing to the decrease, in some cases substantially. This means, however, that antimalarial targeting will become more rather than less important. A diagnostic system in which most febrile illness is treated with antimalarials is rational when most children with potentially fatal febrile illnesses have malaria. This approach is harder to justify when only a minority do. In addition when incidence decreases, the age distribution of cases widens. This observation highlights a technical limitation with the current RBM Monitoring and Evaluation Reference Group (MERG) indicators, which emphasize the need for children with febrile illnesses—rather than febrile illness with parasitemia—to be treated with an effective antimalarial within 24 hours. This empiric approach makes sense in many settings but is less appropriate when malaria is a relatively smaller problem than bacterial disease. This is the situation for much of highland and urban Africa and increasingly for some previously highly endemic parts of rural Africa as well (Barnes et al. 2005; Nyarango et al. 2006; Bhattarai et al. 2007; Ndyomugyenyi et al. 2007; Okiro et al. 2007; Sievers et al. 2008).

The rest of this paper summarizes the current knowledge about the diagnosis of malaria in the field, reviews technical aspects of malaria diagnostic tests, looks at the potential cost-effectiveness of these tests, and then examines the experience so far in trying to improve diagnostic practices within the formal health-care sector. It finishes with discussion of the potential for extending diagnostic services into the nonformal sector in Africa, which is the where AMFm is likely to have its greatest impact, and the possible role of AMFm in facilitating greater use of rapid diagnostic tests (RDTs). The discussion is somewhat speculative because data on this topic are scarce, making it a target for operational research and evaluation of different strategies.

Diagnostic Tests for Malaria

It is widely accepted that clinical algorithms, which use only symptoms to identify those with malaria, are difficult to use and have low sensitivity and specificity (Redd et al. 1996; Luxemburger et al. 1998; Tarimo et al. 2001; Chandramohan et al. 2002). The options therefore

are either to treat all individuals with a fever or recent history of fever, or to use a diagnostic test. For malaria, the choice of test is between microscopy, which identifies parasites directly in blood, and RDTs, which are immunochromatographic tests that detect malaria antigens in the blood and can be read visually with the naked eye. To summarize, light microscopy, provided it can be done well, remains the gold standard in settings where many patients are to be tested, but in more peripheral settings in Africa is unlikely to be either feasible or cost effective (although use of portable microscopy has been used successfully in parts of Asia). Therefore, if diagnostic tests are to be considered for use in these peripheral settings most relevant for AMFm, the choice is likely to be between RDTs and clinical diagnosis based solely on fever. Important differences between microscopy and the most prominent types of RDT are summarized in table 1.

Other approaches, such as using the polymerase chain reaction (PCR) and immunological tests for malaria are also available but are useful only for research in field contexts in Africa, Asia, and South America. There is no realistic probability of PCR becoming a useful clinical tool in low-resource health-care settings in the foreseeable future. Immunological tests, even when well performed, are unreliable for acute diagnosis of malaria, though they are useful in a number of other contexts, such as blood banks. We therefore concentrate on the two standard technologies of microscopy and RDTs and discuss possible developments to these within a five-to-ten year timeframe.

Microscopy

The mainstay of malaria diagnosis for the last 100 years has been light microscopy of blood stained with Field and Giemsa variations. A more recent development is fluorescence microscopy (QBC II), which reportedly improves sensitivity and user effectiveness (Gray et al. 1991; Gay et al. 1996; Guy et al. 2007). The vogue for fluorescent technology seems to have largely passed though fluorescence microscopes are still used in some parts, particularly in East Africa where many were donated, but those that break tend not to be replaced and, in practice, they have had relatively little additional impact over light microscopy.

It is easy to write off light microscopy as yesterday's technology. That it has survived so long is largely because it is still the best method, provided there are good microscopes, good slides and stains, and highly trained and motivated technicians with enough time. In expert hands, the lower limit of detection of malaria in thick and thin films is about 50 parasites/µl of blood (assuming a total RBC count of 5×10^6/µl of blood), which is equivalent to 0.001% of RBC infected parasites per micro-liter (Moody 2002). The detection of low-level parasitemias may not have a great bearing on diagnosis in most African settings, given that high (and

therefore relatively easily detected) parasitemia is the norm in clinical cases. It is, however, important in areas of low transmission and in pregnant women.

The microscopes used for diagnosing malaria have remained essentially unchanged for the last 50 years because they do their job effectively and are generally robust. They can be adapted to situations where there is no power, using sunlight reflected in mirrors as a light source, though sensitivity tends to decrease compared with powered light sources. A number of portable microscopes have been developed that are effective in certain situations. It is therefore premature to write an obituary for light microscopy, even though we do not anticipate any new technological developments. It suffers, however, from a number of drawbacks:

- The initial capital outlay is significant and the cost of employing trained technicians and supplying them with adequate reagents and equipment is not trivial. This cost is justified in high throughput settings, where the cost-effectiveness of microscopy tends to dominate that of any other diagnostic method (Jonkman et al. 1995; Lubell et al. 2007). Light microscopy is therefore likely to remain the ideal standard in hospital outpatient and other high-volume settings for the foreseeable future. The good examples of National Malaria programs that were successful using microscopes in the periphery, such as Thailand and Vietnam, seem unlikely to be replicated in Africa. Microscopy's cost-effectiveness decreases significantly as the throughput of true cases of malaria decreases, such as in clinics in the periphery, in pharmacist shops, and in community settings. Certain externalities, however, such as using the same staff and equipment for TB or stool tests, may increase cost-effectiveness in lower throughput settings.

- Providing new microscopes has proved relatively easy for donors and ministries of health, but maintaining them is far more challenging. Microscopes are relatively simple mechanically, but problems with fungal hyphae and physical damage (to lenses, focusing mechanisms, and spare parts such as bulbs) mean that many microscopes are either unusable or usable only at standards far below optimal.
- The quality of slides and stains is often poor and frequent power outages are a significant problem when generators are not available.

- Training and maintaining staff morale are ongoing issues. Judged by a gold-standard of double-read research slides, many studies have demonstrated that sensitivity of microscopy in operational practice, even with recently replaced equipment, often falls below 70 percent and that specificity is equally poor (Durrheim et al. 1997; Stow et al. 1999; Coleman et al. 2002). Clinicians are aware of this deficiency, which is probably largely the reason for a widespread belief in African and Asian settings that "slide-negative malaria" is a common problem. Series from Europe, the United States and Southeast Asia, where resource constraints are fewer and equipment is of better quality, demonstrate that slide-negative malaria is in fact exceptionally rare, even in settings where most people are not immune and very low parasitemia can thus cause disease.

> Much more often, malaria is called slide-negative when the microscopy result is in fact false-negative because of poor slide preparation or examination technique. A negative cycle therefore tends to occur when clinicians ignore slide results, slide readers learn that clinicians ignore their results, slide reader motivation drops still further, and diagnostic accuracy decreases even more. This has mainly to do with the attitude of clinicians and may not be true at other levels of care.

Various attempts have been made to improve microscopy training and equipment (Ssekabira submitted for publication). The general experience is that such efforts begin well but deteriorate rapidly. Maintaining an excellent microscopy service in high throughput settings is therefore an ideal, but often unrealized.

Rapid Diagnostic Tests

Rapid diagnostic tests (RDTs), commercially available as cassettes or dipsticks, are increasingly common and have been shown effective in a variety of clinical settings. RDTs are performed by placing a drop of blood on a cassette or dipstick and applying a buffer solution to diffuse the blood across a membrane, which then shows control and test lines, similar to rapid HIV tests or pregnancy tests. RDTs detect parasite antigens (proteins) in whole blood. RDTs today are designed to detect one or more of three target antigens: histidine-rich protein 2 (HRP2), parasite lactate dehydrogenase (pLDH), and aldolase. The majority of RDTs on the market detect either HRP2 or LDH. More than 80 RDTs, mainly of these two types, are currently on the market, (WHO/WPRO 2008) with a relatively rapid turnover of manufacturers.

Currently available RDTs have a number of advantages and disadvantages compared with conventional microscopy. Several advantages are clear cut:

- RDTs are relatively simple to use, requiring minimal training to master the mechanics of test preparation and interpretation (Premji et al. 1994; Mayxay et al. 2004; Rennie et al. 2007; Harvey et al. 2008).

- RDT sensitivity and specificity in detecting parasitemia is comparable to good-quality microscopy (Craig et al. 2002; Guthmann et al. 2002; Moody 2002; Hopkins et al. 2007) and therefore better than an underskilled microscopist or a microscopist using poor equipment.

- In low throughput settings, when only a few patients who might have malaria are seen per day RDTs are more cost-effective than microscopy(Bualombai et al. 2003; Shillcutt et al. 2008).

- RDTs do not require electricity or special laboratory equipment.

- RDTs are portable.

- RDT are good for quality control because they can be verified days after being performed.

- Results of RDTs are understandable by the patients so that, in principle, they are less likely to be ignored by the prescribers, although evidence for this in practice is lacking.

These last two advantages make them very useful for rapid screening, use in refugee settings and, of particular importance for the current discussions, use in remote or rural settings.

RDTs do have a number of limitations as well:

- Current tests are all-or-none, that is, results are either positive or negative, and do not allow for quantification of parasitemia. This is not a limitation in settings where people are not immune because any degree of parasitemia may signify a clinically significant infection. It is more of a problem where transmission is high (malaria is meso- to holo-endemic), and many children and adults (20 percent of the population might be typical) carry parasites at any given time, but remain asymptomatic and essentially well (these are not seen in the dispensaries). If they present with another cause of febrile illness, they may well be correctly diagnosed as having malaria parasitemia, but the diagnosis may be misleading about the real cause of their illness if the parasite count is very low. Microscopy allows a quantitative assessment of parasitemia, which is more useful to clinicians in this setting (although this advantage is lost where clinicians do not trust the results of the microscopist). A patient with a low parasite count will still benefit from malaria treatment, but other potentially serious diseases may be left undiagnosed.

- Current tests are not heat stable (Jorgensen et al. 2006; Chiodini et al. 2007). This varies by test type, but all RDTs are at risk of deterioration and reduced sensitivity when they are exposed to heat or humidity for prolonged periods, some becoming essentially useless over the course of just a few days when exposed to temperatures above 40° C. RDTs are commonly subject to high temperatures between leaving the factory gate and use in the field. Therefore, unless they can be deployed using a cold chain or made substantially more heat stable, they are poorly suited for use in Africa and other tropical areas where malaria is a serious issue. This is less of a problem for heavy users (such as medical NGOs) because they are used quickly in clinics and the NGOs are aware of temperature vulnerabilities.

- In high throughput settings, the cost of RDTs, most of which retail at around 60 cents per test, is substantially higher than light microscopy and therefore less cost effective than microscopy (assuming a level of accuracy of microscopy).

- Sensitivity and specificity in placental malaria in pregnancy has not been determined (Mockenhaupt et al. 2006; Uneke 2008). Very sensitive tests, i.e., tests that detect very nearly all cases (accepting a higher level of false positives), are needed during pregnancy.

- HRP2-based RDTs may show positive results for days to weeks after an effectively treated episode of malaria because of persistent circulating antigen. This reduces their specificity in settings where malaria is common and could lead to other febrile illnesses being misdiagnosed as malaria on the basis of a genuinely positive test but a false-positive one in clinical practice (B. Nadjm, personal communication) (Mayxay et al. 2001; Tjitra et al. 2001; Singh and Shukla 2002; Swarthout et al. 2007).

Three of these disadvantages may be overcome by technological advances. Heat stability has the potential to improve, and attempts by WHO to provide a systematic lot-testing system is a financial incentive for companies to maintain their manufacturing standards beyond the period when the tests are being assessed for initial deployment (http://www.wpro.who.int/sites/rdt). Second, it seems likely that quantitative tests will be deployed in due course, which will allow differentiation between high and low parasite counts, but this is still some years away. Such technological advances are unlikely to reduce costs and may well increase them. Third, it seems probable that, as the technology matures, costs will decrease over time, but it is improbable that in high transmission, high throughput settings RDTs will ever exceed the cost-effectiveness of accurate microscopy.

Without doubt, however, the existence of RDTs has revolutionized the possibilities for parasite-based diagnosis beyond the hospital (or even clinic) setting. They may even have a role in hospitals and other higher-level facilities where light microscopy is not reliable or during off-hours.

One fact frequently forgotten as a practical issue is that RDTs still require blood to be drawn. The safety of deploying lancets for repeated fingerpricks in nonformal health-care settings where HIV prevalence is high (much of Africa, particularly Southern Africa) or hepatitis B is common (also Africa, particularly West Africa, and many parts of Asia) is an important consideration in discussions of RDT deployment outside the formal health-care sector. Noninvasive diagnostic tests, such as using urine or saliva, have been proposed but are not currently near deployment. They may never reach that stage and plans should not be made on the assumption that they will.

What Is Relevant for AMFm?

If AMFm is approved, the ACT expansion it will lead to will occur in the peripheral, informal, and private sector (meaning small unregulated shops). Microscopy will, for reasons

outlined, continue to play little role in this sector in Africa. The choice will therefore be between continuing with clinical diagnosis (as with chloroquine), and deploying RDTs as well as the ACTs. Here we outline behavioral issues on how diagnostic tests are used in clinical practice. First, though, we need to answer three questions about the technology.

- Could deploying RDTs lead to a reduction in correct treatment of true malaria cases because of false-negative test results? This depends, critically, on consistent manufacturing quality, test robustness under typical transport and storage conditions, and correct test performance by end users. In principle, syndromic treatment should be almost 100 percent sensitive but very nonspecific. In practice, however, it is as often applied unsystematically. The 100 percent sensitivity is thus theoretical, but diagnostic tests may improve its specificity.

- Can deploying RDTs be made safe from the risk of increasing transmission of bloodborne infections, especially HIV and hepatitis B? This will depend on adequate training in safe blood taking, a free supply of gloves and lancets (eliminating the incentive to use either twice), and a reliable method for safe disposal of sharps.

- Is there a financial incentive to shopkeepers and others to use RDTs, if they are available, and, if not, can incentives be devised if it is thought desirable?

If the answer to the first question is yes, or the answer to the second or the third is no, the deployment of rapid tests alongside AMFm is unlikely to be advisable even if the considerable behavioral challenges outlined can be addressed.

How Diagnostic Tests Are Used in Africa and Asia

It is well recognized that the majority of children and pregnant women with malaria do not access formal health care, (Breman 2001) and typically are either not treated with an antimalarial or treated with an ineffective one. It is the second problem that the AMFm seeks to address. The probability is high that before children and adults receive formal health care they will not—despite having malaria—receive an antimalarial. The probability is equally high, however, that once they do receive the care, they will receive an antimalarial regardless.

The majority of the information we have on diagnostic targeting of antimalarials to people with malaria comes from the formal public sector (hospitals and clinics), rather than peripheral dispensaries and the various elements of the private and informal sector where the AMFm is likely to have greatest impact. Some degree of extrapolation is therefore necessary. Extrapolation, however, carries risks: behavior in one segment of the health-care system (broadly defined) may be very different from others. With this caveat in mind, a number of studies in

Africa have looked at variations on the question of what proportion of children and adults given an antimalarial has actually had malaria. The answer has been consistent: overtreatment of children and especially adults with antimalarials is substantial. Overdiagnosis is defined as giving an antimalarial to a patient who does not have any malaria parasites detected on a malaria test the clinician has requested. This is true for severe disease as well as for nonsevere disease, but this review concentrates on nonsevere disease because it is most relevant to AMFm.

The picture that emerges from the literature across Africa is clear: clinicians almost invariably respond to positive malaria tests by prescribing antimalarials, but often respond to negative tests by ignoring them and prescribing antimalarials anyway. When diagnostic facilities are available, half or more of those with negative test results are still treated for malaria (Hamer et al. 2007; Reyburn et al. 2007; Zurovac et al. 2008). When diagnostic facilities are not present, the proportion is even higher. Where malaria is common, most of those given an antimalarial actually have malaria parasites but a significant minority does not. In the many settings when malaria is not the predominant cause of febrile illness, that is, fewer than 10 percent of children presenting with fever have malaria parasites, the proportion of negative tests treated stays the same but the absolute numbers of those with a negative test treated with an antimalarial increase substantially. In low transmission settings more than 90 percent, and in very low transmission settings more than 99 percent, of children treated with an antimalarial do not have malaria, despite tests being available (Ndyomugyenyi et al. 2007).

There is little doubt that this situation has not been helped by the ambivalent and potentially confusing message from WHO, and subsequently from national malaria control programs, that it is important to perform diagnostic tests for malaria in children under five in high transmission settings, and that if the clinician thinks a child has malaria, the child should be treated for malaria regardless of the test result (WHO 2006). This has also been interpreted as a need for blanket treatment of malaria in all children with fever. It must be noted that the term *high transmission* is not further defined in these guidelines. In true high transmission settings, when the majority of febrile illness in young children is malaria, this approach is safe, provided antibiotics are also considered. However, empiric treatment becomes problematic when this logic, which is explicitly meant only for high-transmission settings, is applied across the continent regardless of malaria incidence, almost all clinicians supposing their region to be a high malaria transmission area. Nursing and medical curricula may contribute to this impression. The true epidemiological situation in Africa is of course much more complex than this. In many areas, such as highland and some urban areas, malaria transmission is low or even nonexistent

and has been so since records began. In other areas, transmission appears to be dropping significantly. The issue, then, is far from trivial.

Recent data from African settings without facilities for diagnosing malaria are currently sparse, but unsurprisingly find the same pattern of overdiagnosis (Olivar et al. 1991; Sowunmi and Akindele 1993). Inevitably, however, evidence of misdiagnosis in both directions is also greater, with true cases of malaria being missed. Because malaria symptoms are nonspecific, especially in the early stages, only a diagnostic strategy that treated every unwell child as for malaria could be guaranteed to miss no cases.

Remarkably few equivalent data from the formal health-care sector in Asia are available, possibly in part because of an assumption that, because malaria is much less prevalent, it is much less likely that it is overdiagnosed. Such an assumption may well be false. A recent study in India, in an area with a relatively low endemicity for malaria, for example, demonstrated substantial overdiagnosis. This may well not be typical in other areas of Asia, and especially Southeast Asia, where experience from Thailand suggests RDTs have played a very useful role. Because the population of India is greater than that of malaria-endemic Africa, this finding is not trivial if it is consistent with practice elsewhere in India. Our empirical observations suggest that the same may well be true in Pakistan and Afghanistan at least, but we are aware of no published or unpublished data to support or refute this.

In summary, evidence of overdiagnosis of malaria in the formal health-care sector in many parts of Africa is clear, and some evidence from South Asia suggests a problem there as well, although this may well not be representative. Even when clinicians have tests and choose to use them, they often ignore the results. We are not aware of comparable data from Central or South America. The cost-effectiveness of RDTs erodes rapidly if negative tests for malaria are ignored (Lubell et al. 2008; Lubell et al. 2008).

Do RDTs Change Prescribing Behavior?

The purpose of a test is to change diagnostic and treatment practice. With RDTs and microscopy, we now have tools that provide accurate and relatively rapid results to guide case management. Do prescribers respond to the provision of RDTs by changing diagnostic decision making? The answer from a number of recent trials in the formal sector in Africa would appear to be either no or not very much.

Two studies from Africa, one a randomized control trial and the other a major observational study, have recently been published (Hamer et al. 2007; Reyburn et al. 2007). We

are aware of at least two other trials for which abstracts are available. These data are backed up by a number of observational studies (Chandler et al. 2008; Zurovac et al. 2008). All seem to suggest, first, that clinicians often fail to request a diagnostic test when it is clinically appropriate, and, second, that when a test result is obtained, clinicians ignore both microscopy results and RDTs.

In the two published studies, which were conducted in several epidemiological settings (one in Tanzania and the other in Zambia), approximately 99 percent of patients with a positive diagnostic test (either microscopy or RDT) and approximately 50 percent of those with a negative RDT were prescribed an antimalarial. When only 1 percent of all febrile illness is malaria, the great majority (more than 90 percent) of all antimalarials prescribed were given to patients for whom the clinician had chosen to undertake a test, had received a negative result, and had prescribed an antimalarial regardless. The impact of negative RDTs on increasing antibiotic prescribing was slightly greater but certainly not startling. Comparing microscopy with RDTs in this randomized trial demonstrated that the two tests were treated almost exactly the same, with no greater credence given to RDT results.

Of the studies reported at meetings in abstract form only, an observational study in Zambia found a similar pattern, though negative RDT results were respected more often than negative microscopy results.

All the studies reported here provided a half-day or one-day training package, designed to be realistic in terms of what a national malaria control program could deliver across a country, focusing on details of RDT performance, and typically with a brief review of national case management guidelines. The results of more intensive training efforts on health worker prescribing behavior in Uganda are encouraging. A one-week integrated course for health workers at facilities with microscopy significantly decreased unnecessary ACT prescriptions (Ssekabira submitted for publication). In addition, preliminary data from an evaluation of an RDT training course targeted to workers at peripheral health facilities, which included clear guidelines on management of patients with positive and negative RDT results, dramatically decreased unnecessary antimalarial prescriptions while maintaining satisfactory patient outcomes (Heidi Hopkins, unpublished data). It remains to be seen whether these more intensive training programs can be taken to national scale in endemic countries in Africa. Anecdotal evidence from Southeast Asia suggests community workers may be more responsive to RDTs than trained clinical staff.

To improve the use of diagnostic tests, we need to know a great deal more about why clinicians prescribe in the patterns described. Investigation is only just beginning, but initial data from Tanzania provide fairly clear indications (Chandler et al. 2008). One reason Tanzanian clinicians frequently gave was that patients expected this overprescription, but observational and anthropological studies demonstrate that this belief is incorrect (Chandler et al. 2008). Patients in outpatient settings stated fairly clearly that if they wanted an antimalarial they would simply buy one, and that they come to formal health-care settings for a diagnosis. Clinicians appeared to make malaria treatment decisions on the basis of complex mind-lines involving a mixture of conventional clinical logic and diagnostic algorithms on the one hand, and social factors with no obvious basis in clinical logic on the other. They also use tests to confirm their suspicions, rather than as a way to make a diagnosis or allocate treatment. Because malaria is the most common diagnosis outpatient clinicians in Africa make, leading to an ingrained process reinforced every working day, evidence from other settings (e.g., antibiotic prescribing in the UK and United States) suggests that changing behavior will not be straightforward.

What Is Relevant for AMFm?

Are these data helpful in assessing the potential impact of RDTs in the settings where AMFm would expand ACT access? Yes and no. The key points are that just because tests are available and used does not mean that they will change prescribing behavior, and that the theoretical effectiveness and cost-effectiveness of RDTs may be much higher than their actual effectiveness and cost-effectiveness. The data cannot be used directly to predict how shopkeepers and peripheral dispensers would use RDTs. It would not be sensible to deploy RDTs as part of AMFm except with a strong operational research component in pilot studies to assess the impact in practice. There is at least a possibility that shopkeepers and others may be more likely than doctors and clinical officers to respond to the results of diagnostic tests.

The Situation in Asia

The situation on diagnosis in Asia is even more complex than in Africa. The vastly different epidemiological settings across the continent, the co-endemicity of two species of malaria (vivax and falciparum) in varying proportions, and the scarcity of data on which to base policy contribute to this complexity. The number of settings where malaria endemicity is as high as Africa is relatively small but includes Papua New Guinea and parts of Indonesia, Assam in India, Yemen and possibly Burma. Elsewhere the incidence is generally far lower, though the

large total populations make the sheer numbers of malaria cases substantial and the numbers of febrile cases in malaria-endemic areas vast.

There are concerns about the availability of ACTs in most of Asia, where access remains low. Where they are available, ACTs are not rationally prescribed (Joshi et al. 2008). Worryingly, recent evidence from Cambodia indicates that empiric over-the-counter treatment with artemisinin monotherapies is common and of long standing (Yeung et al. 2008) and that this is associated with detection of reduced parasite susceptibility to artemisinin drugs. In addition, the level of counterfeit and substandard drugs is high, which reduces treatment effectiveness, may propagate resistance, and undermines public confidence in the treatments. In these areas, the attributable fraction of febrile illness due to malaria is low, and the importance of targeting ACTs to the right cases is considerable.

In many parts of Asia, and in particular in South Asia, the dominant parasite species is vivax malaria. The cost-effectiveness of RDTs as estimated for falciparum malaria is likely to decrease substantially for vivax malaria, though there are fewer models for vivax. This is partly because vivax malaria is less likely to lead to loss of life, partly because the drugs effective against vivax are themselves much cheaper, and partly because the tests that reliably diagnose vivax malaria are generally more expensive than those that reliably diagnose only falciparum malaria. Sensitivity to nonfalciparum malaria remains a challenge for RDTs.

Light microscopy has an advantage when vivax and falciparum co-exist, but the advantage is realized only with greater training for microscopists, in that they need to be able to differentiate between the species as well as simply to diagnose malaria and then to quantify it. One approach is to treat all malaria with ACTs but when only a small proportion of febrile cases are malaria, and of these 90 percent are vivax malaria, this is an expensive approach. In most areas, ACTs will provide no additional benefit over cheaper drugs in terms of curing acute vivax malaria, reducing vivax gametocyte carriage (Kolaczinski et al. 2007), and are unlikely to eliminate hypnozoites. Emergent drug resistance to chloroquine may change this dichotomy to a unitary treatment with ACTs, though the switch, if it occurs, is some time away outside Southeast Asia.

When falciparum is the major species, treatment of all malaria cases with ACT will be more cost-effective. However, when vivax is the major species, falciparum will be treated more frequently with (potentially ineffective) drugs unless accurate diagnosis is available.

Clearly there is a central role for diagnosis in Asia, but what form this could and should take requires careful evaluation. Prescriber practices and the role of diagnostics in Asia receive

less attention than the sensitivity and specificity of RDTs (Kolaczinski et al. 2004; Bharti et al. 2008). The role of the private sector in Asia is understudied as well and at best speculative. This lack of data, coupled with the complex array of epidemiological settings, makes it more difficult to reach conclusions on the role of RDTs in the region. Logically, of course, RDTs do have a role, and many governments are seeking to bring RDT use to scale despite the lack of evidence of any advantage over microscopy or even presumptive treatment.

Our view is that further research in South Asia is necessary before RDTs are deployed in general health services, and before any part of any AMFm is extended there. We have no way of predicting reliably what the impact of either would be on prescribing practices, and the number of tests that could potentially be used in this setting is vast, with consequentially significant costs. Evidence from Southeast Asia has demonstrated the potential utility of RDTs, and of microscopy used in the periphery, but is unlikely to be translatable into the very different settings of AMFm in Africa.

Conclusions

1. Improving diagnosis of febrile illness so that effective antimalarials are targeted to those with malaria should be a priority, but there are no quick fixes.

2. Microscopy remains an excellent technology, but is unlikely to be relevant to the peripheral settings where AMFm would make a difference, although experience in Asia suggests that microscopy can be useful at the periphery in some settings.

3. There are sensitive and specific RDTs, and these could be used in the periphery, but technical questions over heat stability, operational safety, and financial incentives for use are significant concerns.

4. RDTs can be cost effective over a wide range of epidemiological settings—but only if their use leads to changes in prescribing.

5. Overdiagnosis of malaria is substantial in the formal health-care sector throughout Africa, and very possibly in Asia, based on clinical symptoms alone.

6. Evidence that deploying RDTs with a limited training package will change the prescribing behavior in the formal health-care sector is disappointing. Changing prescribing behavior will be challenging. This may or may not be relevant to the sectors where AMFm will have impact.

7. The low incidence of malaria and high proportion of vivax malaria complicates the diagnostic picture in South Asia, and we have relatively little data on diagnostic and prescribing practices there.

8. The importance of diagnostics increases as the incidence of malaria, and thus the prevalence of malaria in febrile children, decreases. This is likely to be particularly important for the later phases of the roll-out of AMFm.

We believe that AMFm will have to take diagnosis of malaria, and febrile illness more generally, into account if it is to have the maximum impact and remain sustainable and acceptably cost effective, but that this is likely to be more important in later stages as the roll-out occurs in areas with lower transmission (or as transmission in first-phase countries drops). In Africa, a number of actions can be taken now, and in the wings are a number of interventions such randomised trials of community education on which data will soon be available to help guide policy. In Asia, substantially more information is needed before any recommendations can be made.

What would need to be undertaken depends on the setting, which we divide broadly into formal health-care outpatient settings where microscopy is available; formal health-care settings where microscopy is not available (most clinics in Africa); the private sector, subdivided into the formal health-care sector and small unregulated shops; and community drug distributors, such as in home-based management of fever (HBMF) programs.

Within the formal health-care sector, in hospital settings where microscopy is available, the priorities are clearest because the evidence base is best. The need to increase the proportion of febrile children and adults tested for malaria, and then to reduce the overprescription of antimalarials to those with negative tests, is clear. This can only happen, however, when diagnostic facilities have been improved. This may be achieved by maintaining microscopy standards and materials or, if this proves impractical, by supplying RDTs. The key, however, is not in technology, but in attitude and behavior change amongst clinical prescribers. The formal health-care sector is however not one likely to be important for AMFm; the Global Fund already provides subsidized ACTs in this setting to most endemic countries.

In the formal sector where microscopy is not available, such as in clinics, it is clear that some diagnostic facilities would be an improvement on no diagnostic facilities and that deploying RDTs is likely to be the only practical solution in most settings. Ongoing research is needed to assess the impact of RDT implementation on actual prescribing behavior and, in all probability, complex behavioral interventions as well.

Using RDTs in the formal private sector seems an entirely rational choice, but a relatively small proportion of the target beneficiaries of AMFm also use the formal private sector. Those

who do are at relatively lower risk of malaria than other groups given that, in most African and Asian countries, malaria is primarily a disease of the poor and of those in rural communities.

The largest unknown area, which offers the greatest potential gains, is the informal private sector, such as general shops, specialized pharmacies, and chemical sellers. Users of this sector are often the poorest and the most likely to be affected positively by the AMFm. Massive overprescription of antimalarials in this sector (which has long been tolerated with cheaper antimalarials) could significantly reduce sustainability of the AMFm system. We do not yet have information about how RDTs would be used in this sector in Africa, though some studies are under way. It is essential that deploying RDTs neither decreases the number of people with true malaria treated with an antimalarial (e.g., false-negative results), nor increases the proportion of people treated for malaria who do not have it (meaning they might die of other causes), significantly increases the risk of transmission of blood-borne viruses. This last element is particularly important. It would be a serious setback from a public health point of view if, in promoting better diagnosis of malaria, we also promote increased transmission of HIV or hepatitis B. This is not a trivial risk: shops working on small profit margins may try to make a little more money by re-using lancets. The safety of the shopkeepers should also be considered, given the small but real risk of needle-stick injuries. How to provide proper incineration of shop-used lancets would be another important operational question. Changing shopkeeper behavior is possible but requires intensive piloting and adapting to local conditions (Marsh et al. 1999). In our view, therefore, deploying RDTs in this setting without thoughtful operational assessment of safety and effectiveness would be premature and potentially even dangerous.

In considering RDT use by community volunteers, such as in HBMF programs, the same concerns apply. Some promising early work has been done to evaluate the implementation of RDTs in Zambia's HBMF program, and evidence should be forthcoming within the next year or two (Harvey et al. 2008). It remains to be demonstrated that successes with RDTs in Southeast Asia in this setting can be replicated in Africa.

In the longer term, and as malaria decreases in some settings, leading to a reduction in the proportion of children with febrile illness presenting with malaria, we may need to consider, at least in some settings, syndromic management of fever with a combination of antimalarials and antibiotics. Although clinicians already use syndromic treatment to some extent in individual cases where the diagnosis is in doubt, deploying it on policy to all febrile children is some way in the future and would meet substantial resistance. Concerns about drug safety and the promotion of antibiotic resistance are legitimate. For the moment, however, the choices are between improving and not improving diagnosis, and in that choice the overall goal must be clear. We

now have the technology to make a proper malaria diagnosis, and though technology is continuing to improve, the biggest stumbling block continues to be behavioral change rather than technological problems.

The AMFm provides a remarkable opportunity to improve the whole management of febrile illness in Africa. This will require operational research alongside deployment but, if undertaken properly, could have positive ramifications for all the other causes of febrile illness and thus reduce mortality beyond the malaria-attributable fraction that the AMFm is addressing directly.

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Table 1. Advantages and disadvantages of different diagnostic test methods

Diagnostic Method	Advantages	Disadvantages
Light microscopy	• Provides additional diagnostic information: quantification of parasite density, identification of parasite species, assessment of hematological abnormalities, identification of certain other infectious agents if present • Useful for monitoring response to treatment • If quality maintained, likely cost advantage over other diagnostic methods (RDTs and empiric treatment) where patient volume is high	• Reliable results require well-maintained microscopes, reagents and supplies, as well as electricity (or strong sunlight) • Need for skilled and motivated laboratory personnel • More time-consuming and labor-intensive than other diagnostic methods (RDTs and empiric treatment)
Rapid diagnostic tests (RDTs)	• Relatively simple to use, can be performed by health workers with limited formal training • Do not require special equipment or electricity • Likely cost advantage over other diagnostic methods (microscopy and empiric treatment) where patient volume is relatively low	• Give only "yes or no" information on parasitemia; not quantitative • Susceptible to degradation and loss of sensitivity when exposed to high temperatures and humidity •Require fingerstick for blood, with some risk of transmission of bloodborne diseases, including HIV and hepatitis B
RDT: HRP2 (histidine-rich protein 2)	• Sensitivity maintained at lower parasite densities • Relatively stable in typical storage conditions in endemic areas (though may vary by manufacturer, etc)	• Detects *P. falciparum* only (some tests combine HRP2 detection with pLDH or aldolase detection to allow diagnosis of non-falciparum species as well) • Antigenemia persists post-treatment, which precludes use to monitor treatment response, and may lead to false-positive results in areas of intense transmission
RDT: pLDH (plasmodium lactate dehydrogenase)	• Detects *P. falciparum, P. vivax, P. ovale,* and *P. malariae* (some pLDH-based tests can distinguish *P. falciparum* and *P. vivax* from other species) • Consistently mirrors parasitemia, so can be used to monitor treatment response	• Sensitivity drops at lower parasite densities • Less stable at typical storage conditions in endemic areas