MEASURES OF MALARIA PARASITEMIA PREVALENCE IN NATIONAL SURVEYS: AGREEMENT BETWEEN RAPID DIAGNOSTIC TESTS AND MICROSCOPY
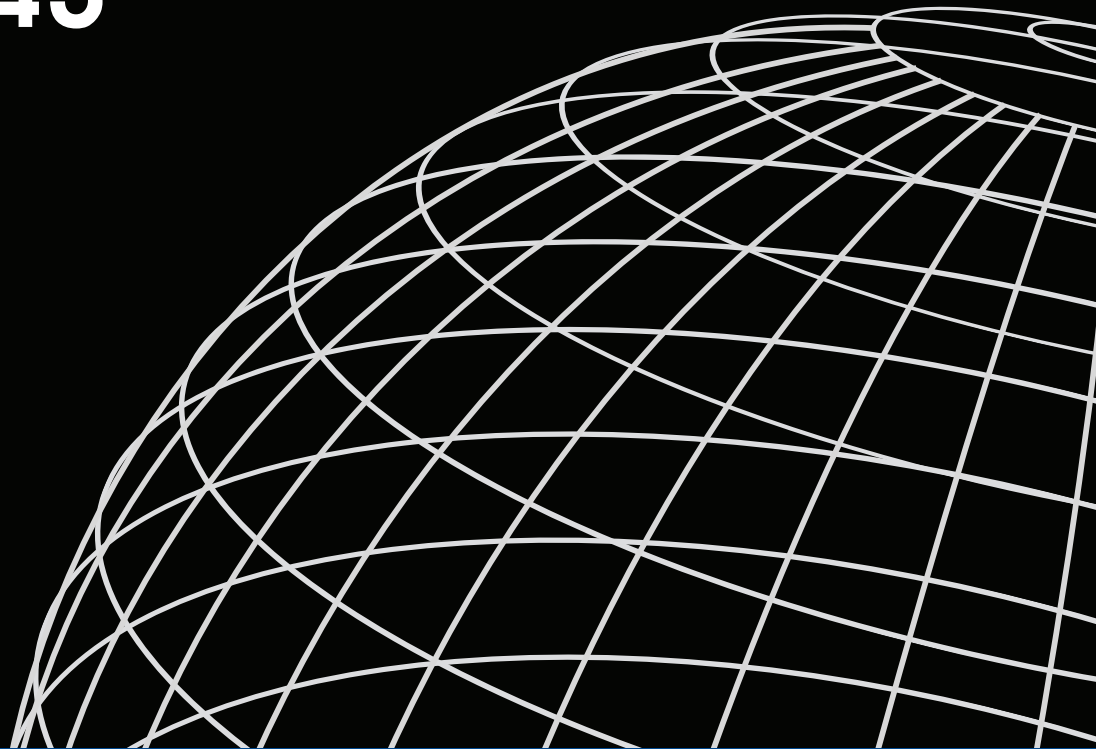
DHS ANALYTICAL STUDIES 43

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Measures of Malaria Parasitemia Prevalence in National Surveys:
Agreement between Rapid Diagnostic Tests and Microscopy

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Preface

The Demographic and Health Surveys (DHS) Program is one of the principal sources of international data on fertility, family planning, maternal and child health, nutrition, mortality, environmental health, HIV/AIDS, malaria, and provision of health services.

One of the objectives of The DHS Program is to analyze DHS data and provide findings that will be useful to policymakers and program managers in low- and middle-income countries. DHS Analytical Studies serve this objective by providing in-depth research on a wide range of topics, typically including several countries and applying multivariate statistical tools and models. These reports are also intended to illustrate research methods and applications of DHS data that may build the capacity of other researchers.

The topics in the DHS Analytical Studies series are selected by The DHS Program in consultation with the U.S. Agency for International Development.

It is hoped that the DHS Analytical Studies will be useful to researchers, policymakers, and survey specialists, particularly those engaged in work in low- and middle-income countries.

Sunita Kishor
Director, The DHS Program

Abstract

This report examines the relationship between two different diagnostic tests used in nationally-representative household surveys to measure malaria parasitemia prevalence: Rapid Diagnostic Tests (RDTs) and microscopy. Prevalence estimates from 22 surveys containing both microscopy and RDT results were compared using tests of agreement and factors that could be influencing discordance between the two tests were identified. Although prevalence estimates from the two tests were highly correlated, RDT prevalence estimates were higher than microscopy prevalence estimates in 19 of 22 surveys examined. Given the lack of a gold standard diagnostic test for malaria that is practical for use in national survey settings, discrepancies in prevalence estimates generated from microscopy testing and from RDT testing are difficult to reconcile. Although results of these tests could be useful in identification of high-risk populations for targeting of interventions, assessments of impact based on changes in malaria prevalence measured with these tests are more problematic. Development of an algorithm for program managers to use to best estimate national prevalence given the available diagnostic data would be useful for the future.

Executive Summary

This report examines the relationship between two different diagnostic tests used in nationally-representative household surveys to measure malaria parasitemia prevalence: Rapid Diagnostic Tests (RDTs) and microscopy. Substantial resources have been invested in malaria control programs in recent years, with clear goals of reducing malaria transmission. Yet measuring changes in malaria prevalence has proven challenging given the varied quality of available data from health facilities and a paucity of other data sources. As more high quality, standardized, national survey data including biological tests for malaria infection have become available in recent years, the potential uses of these data merit reexamination. Specifically, surveys in which both microscopy and RDTs were used to test for malaria infection were used in this study for comparison of prevalence estimates, examination of test agreement, and identification of factors that could be influencing discordance between the two tests.

Objectives

1) Describe the levels of malaria infection in children 6-59 months of age identified by the two diagnostic tests in all Sub-Saharan African countries in which both tests were performed;

2) Identify factors related to discordant RDT and microscopy test results for malaria infection;

3) Clarify the challenges to using national survey data on malaria prevalence for demonstrating change over time or impact of programs.

Methods

With data from the Demographic Health Surveys (DHS) and Malaria Indicator Surveys (MIS), the study examines malaria infection prevalence in children 6-59 months of age and different measures of agreement between RDT and microscopy diagnostic test results, including Kappa coefficients and Areas Under the Curve (AUC) from Receiver Operating Characteristic (ROC) analysis. Results are further stratified by fever status, by antimalarial treatment status, by level of malaria prevalence and by region. Change over time in prevalence estimates is examined for a subset of countries in which more than one survey with malaria testing is available. Additionally, the study examines the factors associated with discordance in RDT and microscopy test results in a pooled sample. Household location (urban vs. rural), household wealth, children's age and children's fever and antimalarial treatment status were all found to significantly affect the likelihood of test discordance between RDT and microscopy in pooled models.

Results

Although scatter-plots show strong correlation between national-level RDT and microscopy estimates of malaria prevalence in children 6-59 months of age in Sub-Saharan African countries, several countries have discordant prevalence results depending on the test used. RDT results were higher than microscopy results in 19 out of 22 surveys. Agreement between the two diagnostic tests were shown to vary by overall prevalence level, by recent fever status of children and by antimalarial treatment status. Regional variation was also demonstrated. Household location (urban vs. rural), household wealth, children's age and children's fever and antimalarial treatment status were all found to significantly affect the likelihood of test discordance between RDT and microscopy in pooled models. Finally, changes in malaria prevalence in the four countries with two surveys was determined to be difficult to ascertain given the level of discordance between tests and the observed sub-national variation in prevalence and in discordance.

Conclusions

Given the lack of a gold standard diagnostic test for malaria that is practical for use in national survey settings, discrepancies in prevalence estimates generated from microscopy testing and from RDT testing are difficult to reconcile. Although results of these tests could be useful in identification of high-risk populations for targeting of interventions, assessments of impact based on changes in malaria prevalence measured with these tests are more problematic and should be conducted with caution. Development of an algorithm for program managers to use to best estimate national prevalence given the available diagnostic data would be useful for the future.

1   Introduction

Malaria is a major cause of child morbidity and mortality in much of Sub-Saharan Africa. Significant resources have been allocated to prevention and control of the disease by national governments and international donors; in 2013 USD 1.97 billion international disbursements for malaria control were made and national governments contributed US$ 522 million in 2012 (WHO 2013). Monitoring the effectiveness of these investments requires data on the coverage of malaria interventions as well as information on health outcomes. Malaria-specific outcomes are notoriously difficult to measure for many reasons, including the non-specificity of malaria symptoms and challenges to data reporting common in many malaria-endemic countries. As a result, monitoring and evaluation of malaria control programs often depends on non-specific health outcomes as proxies for malaria-specific outcomes. For example, the Roll Back Malaria (RBM) consortium is currently leading a series of Malaria Impact Evaluations in countries supported by the President's Malaria Initiative (PMI) to determine if the scale-up in malaria interventions over the past decade has been effective. All-cause child mortality is being used as the major outcome variable with the assumption that a significant proportion of child mortality is caused by malaria. In order to add strength to this argument, analyses are supplemented by data on prevalence of malaria parasites (parasitemia) wherever possible.

Five species of Plasmodium parasites infect humans: *P. falciparum* (*Pf*), *P. vivax* (*Pv*), *P. malariae* (*Pm*), *P. ovale* (*Po*), and *P. knowlesi* (*Pk*). *Pf* is the most dangerous of these parasites to human health. The 2013 World Malaria Report estimates that *Pf* is responsible for 91% of all malarial infections worldwide (WHO 2013) and 90% of the deaths (WHO 2013). While *Pf* predominates in Sub-Saharan Africa, it is usually secondary to other species in other regions (WHO 2013). *Pv* is widely distributed, causing infection in Latin America, Asia, and parts of Africa, and it is responsible for up to 50% of malaria cases outside of Sub-Saharan Africa. Unlike *Pf*, *Pv* is rarely fatal although increasing evidence of severe disease and mortality associated with *Pv* infection has recently come to light, especially when infection occurs concurrently with malnutrition. *Po* and *Pm* infections are much more rare (<5-10% and 4-20%, respectively, in endemic regions (Bruce et al. 2007)) and more benign, with few infections leading to severe disease. These parasites are mostly limited to Sub-Saharan Africa, Southeast Asia, Indonesia, parts of the Western Pacific and the Amazon Basin (Westling et al. 1997). *Pk* is the most recently discovered species of human-infecting Plasmodium found in Southeast Asia. *Pk* accounts for up to 70% of malaria cases in endemic zones in Borneo and can be fatal due to a short erthyrocytic cycle (24 hours compared to an average of 48 hours for the other Plasmodium species) resulting in high density parasitemia (McCutchan, Piper, and Makler 2008).

Due to the complex nature of malaria parasite transmission and the wide array of factors that influence prevalence of infection, monitoring national trends in parasitemia is a challenging task. Two major data sources are used for this purpose: household surveys such as malaria indicator surveys (MIS) and health management information systems (HMIS). Ideally, national HMIS would provide a rich source of longitudinal data useful for monitoring trends in prevalence and incidence of malaria. Unfortunately, many countries do not have the resources, infrastructure or skilled personnel necessary to run a reliable HMIS. In this case, other data sources are required. National MIS provide a simplified snapshot of parasitemia prevalence levels in a population at the time of the survey; however, cross-sectional data are much more difficult to interpret due to the temporality of data and to the variation across surveys in other factors likely to influence infection levels.

Testing nationally representative sample populations for Plasmodium infection produces different information than monitoring trends via health information systems. First, national surveys represent the entire household-based population including those who do not access health facilities, whereas HMIS data are derived solely from people accessing care. Second, national surveys produce estimates of asymptomatic infections whereas HMIS data are typically restricted to cases of clinical disease. These differences are

important and depending on the goal of data collection, one data source may be more appropriate. Monitoring asymptomatic infection is essential when elimination of malaria parasites is the immediate goal. However, measuring patterns in clinical disease is a priority in high-prevalence areas where the primary goal is reducing malaria-associated mortality and morbidity.

The frequency of data collection is an important consideration for monitoring and evaluation of malaria control. Current evaluation efforts are challenged by a lack of baseline parasitemia prevalence data in most malaria-endemic countries. In settings with limited HMIS capacity, periodic national surveys with parasitemia testing are required in order to monitor trends. The Roll Back Malaria Monitoring and Evaluation Reference Group (RBM 2011) recommends carrying out no more than three to four surveys with parasitemia testing per decade. Meaningful changes in national prevalence estimates are difficult to detect between closely-spaced surveys which are also unlikely to be cost effective. Maximizing the value added from testing for Plasmodium parasites in national surveys requires a balance between the interpretability and utility of the data collected and the cost of the testing. As natural variations in malaria infection levels occur both annually and seasonally, annual or biannual surveys of malaria parasitemia are difficult to interpret; observed gains or failures over short time periods can be due to normal fluctuations in rainfall or temperature instead of to prevention and control interventions.

Cost is also a critical consideration in data collection for malaria M&E. In low endemic countries national-level screening is not likely to be cost effective because large sample sizes are required to generate reliable estimates of infection prevalence. Also, microscopy and rapid diagnostic tests (RDTs) are not very sensitive at detecting parasites at low parasite densities, making results more difficult to interpret. In low endemic regions, monitoring trends in malaria using HMIS data is likely to be more cost effective. In contrast, monitoring malaria morbidity and mortality take priority over monitoring asymptomatic infection in highly endemic Sub-Saharan African countries. In this case, clinical malaria would normally be the outcome of primary interest and collecting data from HMIS may be the best use of limited funds; however, a lack of reliable HMIS data in many countries leads to reliance on estimates of asymptomatic infection from survey data as a proxy measure.

Once the decision has been made to collect parasitemia prevalence data through a national survey, choosing the appropriate method for measuring malaria infection is another critical consideration. Three primary diagnostic tools are available for identifying Plasmodium species: microscopy, rapid diagnostic tests (RDTs) and polymerase chain reaction (PCR) techniques. A fourth, more recently developed tool, loop mediated isothermal amplification (LAMP), is becoming more widely available. Descriptions of these techniques and the advantages of each are provided below.

1.1   Microscopy

Microscopic techniques have been used for the identification of malaria parasites in blood samples since the French physician Charles Louis Alphonse Laveran first observed the protozoan inside red blood cells in 1880. Two microscopic techniques are commonly used in malaria diagnosis: reading of thick and thin blood smears. Thick smears are used to identify the presence or absence of malaria parasites and can be used for identification of Plasmodium species whereas thin smears are used for quantification of malaria parasites and for confirmation of Plasmodium species identification (WHO 1991).

Unfortunately, the processes required to produce high quality blood smears are complicated under field conditions. Thin smears must be fixed with methanol as soon as the smear is dry in order for the red blood cells to remain intact. As immediate transfer to laboratories is usually not practical in field settings, thin slides need to be fixed in the field. Thick smears, conversely, must remain unfixed in order to allow hemolysis of red blood cells. Staining of both thick and thin smears is also necessary for proper microscopic diagnosis of malaria infection. Staining is a process during which a dye, typically Giemsa stain (a mixture

of methylene blue, eosin, azure B and a solvent), is applied to a blood slide to differentiate between human and parasite cells for ease of detection and quantification of parasites. If slides are kept cool and dry, staining can be postponed for several days for thin smears, and for longer periods, for thick smears. Care must be taken to avoid autofixation (spontaneous fixation of red blood cells which arrests the process of hemolysis) which can occur if smears are not fixed in a timely fashion (typically 7-15 days for thick smears) or if smears are exposed to excess heat and humidity. Autofixation makes staining difficult or impossible. Staining is typically done in the lab; however staining in the field may be necessary on rare occasions.

Once slides are properly prepared and transported to laboratory facilities, microscopists may begin to stain and read the slides. The speed at which identification of malaria parasites can occur depends on multiple factors. Slides with high parasite densities typically require less time as identification of a single parasite on a thick smear is sufficient for a positive diagnosis. Identifying parasite density takes much longer. The amount of additional time required depends on the parasite density on the slide. In samples with few or no parasites, technicians must examine 200 or more fields. Similarly, identification of Plasmodium species requires more time as approximately one hundred fields of the thick slide must be examined and confirmation via thin slide is often required (WHO 1991). Reading thin smears takes 10 times as long as reading thick smears, on average (WHO 1991). A summary of World Health Organization (WHO) estimates of slide-reading capacities based on slide positivity rates is provided in Table 1.

Table 1. Slide-reading capacities, based on a 6-hour working day, not including time to collect and stain slides or report results (table from (WHO 2008)).

Slide positivity rate	10%	20%	30%	40%	50%	60%	70%	80%
Slides read/hour	7	8	8	8	9	9	10	11
Slides read/day	44	46	48	50	53	56	59	63

In practice, slide reading may require more time than the WHO recommendations suggest. In MIS surveys conducted with technical assistance from ICF International, microscopists read slides at a much slower rate, ranging from 15 to 30 slides per day. Thus, waiting for microscopy results often delays availability of survey data by several months.

Microscopy has long been considered the "gold standard" in malaria diagnosis. However, major challenges to using microscopy in national surveys in developing countries have forced specialists to reconsider this position. The time required for diagnosis for treatment purposes via microscopy can be prohibitive; it is impractical to transport slides to a laboratory for reading and to return to the household to treat any infected individuals. Since identifying infected individuals and withholding treatment is ethically unacceptable, household surveys currently use RDTs administered in the field to determine if respondents need treatment, followed by microscopy for confirmation of infection status. Blood smears must be protected from dust, wind, uneven surfaces, rain, excess heat and humidity to avoid contamination and autofixation. Smears must also be transported to a central laboratory for reading, and this must be done within 7 to 15 days depending on humidity and temperature conditions, a necessity which is often expensive. Another common challenge is lack of skilled microscopists (Mwanziva et al. 2008, Castelli and Carosi). Finding technicians only for short-term survey work is difficult, and unemployed technicians often lack expertise, which affects the quality of diagnosis. Where well-trained technicians are available, they are often engaged in other vital projects which have to stop for the survey work. The range of microscopy skills and often adverse field conditions in which blood smears are made influence the reliability and validity of parasite diagnosis by microscopy in many countries. This reality, combined with recent improvements in diagnostic capacity of RDTs, has led some experts to conclude that microscopy may not always be the gold standard in malaria diagnostics (RBM 2011).

1.2   Rapid Diagnostic Tests (RDTs)

Rapid diagnostic tests for identifying malaria infection have greatly improved over the last 20 years. In order to help rank RDT performance and guide procurement decisions, the World Health Organization (WHO)-Foundation for Innovative New Diagnostics (FIND) Malaria RDT Evaluation Programme was established (WHO 2009). FIND's website provides information on performance of specific RDTs based on rigorous testing at low and high parasite densities. RDTs are typically used to identify infection and provide immediate treatment to positive subjects in the field (or referral for follow-up treatment in a health facility) in conditions under which microscopy is impractical. RDTs have also been shown to be cost-effective in a range of settings accounting for treatment costs and alternate diagnostic options (Mosha et al. 2010, Lubell et al. 2007, Shillcutt et al. 2008).

RDTs detect parasite antigens instead of actual parasites. The most commonly used RDTs test for histidine-rich protein- 2 (HRP2) or species-specific plasmodium lactate dehydrogenase (pLDH), which are antigens that can persist following parasite clearance causing false positive test results. This difference limits the direct comparability of microscopy and RDT results, as recently treated infections will often appear as positive by RDTs but not by microscopy. HRP2-based RDTs have been shown to be sensitive and stable diagnostic tests for *P. falciparum* infection; however, residual antigens can produce positive tests for several weeks after successful treatment of infection (Swarthout et al. 2007, Mayxay 2001). As much as 74% of patients remain HRP2 positive 35 days post clearance of infection (Swarthout et al. 2007, Houze et al. 2009, Kyabayinze et al. 2008). LDH-based RDTs may not be as sensitive or heat-stable as the HRP2 tests; however, they are more specific as LDH does not persist in the blood after successful treatment of infection (Perkins and Bell 2008, Iqbal et al. 2004). Improvements in the sensitivity and heat stability of LDH tests to the level of HRP2 tests have been recently documented (Ratsimbasoa et al. 2007, Ashley et al. 2009, Gerstl et al. 2010, WHO 2011b) and these tests are therefore likely to be more widely used in the future.

A major advantage of RDTs is their relative simplicity. The test requires only a single drop of blood from a finger or heel stick and do not require skilled technicians or access to laboratory equipment. RDTs are becoming increasingly available in rural settings in which microscopy is not a viable diagnostic option (Masanja et al. 2012). The top 10-15% of RDTs reviewed in the FIND study perform well, with sensitivity similar to that of a skilled microscopist (using Polymerase Chain Reaction diagnosis as the gold standard) (RBM 2011). MIS surveys carried out with support from The DHS Program typically use RDTs from the group with the highest levels of sensitivity and specificity. Another advantage to RDTs is that they produce results rapidly, within 15 minutes, whereas obtaining microscopy data usually requires several months after the completion of interviews.

Average costs for RDT testing in MIS surveys implemented by The DHS Program are lower than microscopy. Average cost of microscopy testing in an MIS, including materials (glass slides, methanol, Giemsa stain, etc.), and shipping costs are USD 1.50 per test compared to an average cost of USD 1.23 per test for RDT[1]. A previous comparative cost analysis of malaria diagnostic testing in MIS found RDTs to be much more cost effective than microscopy (USD 0.71 vs. USD 1.53 for materials alone, unpublished data). Part of the reason for reduced relative cost effectiveness is the increasing use of HRP2-pLDH combo RDTs which are more costly than the HRP2 only tests.

In summary, in a national household survey setting, diagnosing malaria infection using a high quality RDT is less expensive than using microscopy. Material costs alone are less expensive for RDTs than for microscopy and microscopy requires additional resources for transporting samples to laboratories and for

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[1] Assuming 5000 samples with 100% response rate, 25% added for pretest/ training and 15% for loss/breakage. Excludes costs of general supplies such as lancets, gauze, gloves, biohazard bags, etc. Also excludes salaries of microscopists and field workers and costs to transport samples to laboratory facilities.

salaries for microscopists. In addition, surveys that include microscopy testing must also use RDTs to test for infection in the field in order to enable treatment of infected individuals, an ethical obligation; whereas RDT testing can be done without microscopy. However, RDTs are limited in their ability to identify *Plasmodium* species and cannot be used for quantification of parasites; microscopy is still required in countries where those data are needed.

1.3   Polymerase Chain Reactions (PCR)

PCR is a molecular test in which Plasmodium DNA is detected from a patient's blood. Typically, blood samples are collected on a filter paper card from a finger stick or heel stick and allowed to dry. When protected from humidity, the resulting dried blood spots (DBS) can be stored at room temperature (for months if necessary) until processing in the laboratory takes place. Carrying out a PCR test requires special reagents and equipment, as well as skilled technicians. As a result, PCR is costly, between USD 0.50 and USD 2.50 per assay for the materials with an additional USD 10,000 to USD 25,000 for the PCR machine (Malhotra 2005, Bass et al. 2008). One study found quantitative PCR to be six-fold more expensive than microscopy for identification of malaria parasites with a USD 14.80 per sample cost of reagents and a one-time cost for the purchase of a thermocycler at USD 36,000 (Khairnar et al. 2009).

PCR works by amplifying parasite DNA (by orders of magnitude) to the point at which it can be detected. PCR is extremely sensitive; theoretically a single parasite can be detected from a blood sample. In practice PCR has been shown to detect five parasites or less per microliter of blood (Abba et al. 2011). This contrasts with RDTs and thick smear microscopy, which have limits of detection of 100 parasites per microliter of blood and 50 parasites per microliter blood, respectively (Moody 2002). Because of this extreme sensitivity, prevalence estimates from surveys using PCR as a diagnostic tool should not be compared with those using microscopy or RDT. For example, Taylor and colleagues compared microscopy results with a new cost-saving method of testing pooled PCR samples and found microscopy to have a 68% sensitivity and a 92% specificity as compared to PCR results (Taylor et al. 2010).

PCR has several other strengths. It can be used to determine the specific *Plasmodium* species (e.g., diagnosis of infection with *P. knowlesi* requires PCR due to morphological similarities to *P. malariae* species (Singh et al. 2004)) and for testing for drug resistance markers. Although its cost and required expertise make its use currently impractical in most malaria-endemic countries, PCR is a useful tool in countries in the elimination or pre-elimination phase of malaria control where very sensitive diagnostic tools are required. For example, both Djibouti and Swaziland have completed MIS surveys in which PCR was used to estimate prevalence of infection (Kingdom of Swaziland Ministry of Health 2011, Noor et al. 2011). As laboratory technologies are rapidly advancing, the feasibility of using PCR in MIS should be regularly revisited.

1.4   Loop mediated isothermal amplification (LAMP)

Another molecular diagnostic tool has recently been developed for malaria: Loop mediated isothermal amplification (LAMP). Highly sensitive and specific, this test produces results faster than PCR and requires less laboratory expertise and equipment (Hopkins et al. 2013, Polley et al. 2013). The LAMP assay detects sequences of mitochondrial DNA of *Plasmodium* parasites, either *Pf* specifically or sequences common to all *Plasmodium* species that infect humans (*Pf*, *Pm*, *Po*, *Pv*, or *Pk*, also known as pan-*Plasmodium*) (FIND 2012). The test is able to detect as few as one parasite per microliter of blood in one hour from fresh or frozen blood samples or from dried blood spots (FIND 2012). Although in some ways an improvement on PCR (less expensive, less technically demanding, less time-consuming), LAMP is currently best suited to detecting low density infections. As such, LAMP could be useful in screening programs in areas slated for malaria elimination, and in serving as a reference standard against which other diagnostic tools can be tested. Other potential uses include antenatal screening, diagnosis in returned travelers, antimalarial drug efficacy trials, resistance monitoring programs and vaccine trials.

Table 2. Summary of Malaria Diagnostic Tests' Characteristics

	Ease of Use	Cost	Training Required	Sensitivity†	Quantifi-cation possible	Specia-tion possible	Results Immediate
Microscopy	Low to Moderate	Moderate	Moderate to High	~ 50-100 parasites/µL	Yes	Yes	No
RDT	High	Low	Low	~100 parasites/µL	No	Some*	Yes
PCR	Low	High	Extensive	~5 parasites/µL	Yes**	Yes	No***
LAMP	Low	Moderate/High	Moderate	~1 parasite/µL	No	Some††	Within 1 hour

*Some RDTs are designed to be species specific and others will detect multiple species; however, only the following combinations of parasite detection are available: *Pf* only, *Pf*/pan, *Pf*/*Pv*, *Pf*/*Pv*om, *Pv* only and pan only (where pan= *Pf*, *Pv*, *Pm* and *Po* and *Pv*om = *Pv*, *Po* and *Pm*) (WHO 2011a).
**Nested PCR and traditional multiplex PCR do not allow for quantification of parasites. Real time PCR does.
***Innovative rapid PCR diagnostic tools are under development for use in the field(FIND).
†See reference (Moody 2002)
†† *Pf*-LAMP can specifically detect *Pf* parasites, pan-LAMP can detect the presence of any species of *Plasmodium* parasite.

1.5   Literature Comparing RDTs and Microscopy

Few formal studies have compared sensitivity or specificity of these diagnostic tests in the context of national, population-based household surveys. The few that have are plagued by challenges such as lack of a comparable gold standard across studies. Measures of sensitivity and specificity are dependent on the choice of gold standard. Typically, microscopy is considered the gold standard, but the quality of microscopy results depends heavily on the expertise of the technicians, and microscopy is less sensitive at low parasite densities (<200 parasites per microliter of blood). Thus, using microscopy as a gold standard can be misleading.

Some research has found RDTs to produce more sensitive measures of parasite infection than microscopy. Hopkins and colleagues (Hopkins 2007) used PCR-adjusted microscopy as a gold standard in a study set in seven sentinel sites of varied malaria transmission intensity in Uganda. Results from two different types of RDTs (HRP2 and pLDH) and microscopy were compared. Samples that were positive by RDT but negative by microscopy were confirmed with PCR. HRP2-based RDTs performed better than both pLDH-based RDTs and routine microscopy at detecting cases (PCR confirmed) across all study sites. Another study looking at samples from travelers returning to the United States from malaria endemic areas found RDTs to have higher sensitivity and higher negative predictive value than microscopy using PCR as the gold standard. This finding held for both *falciparum* and non-*falciparum* cases (Stauffer et al. 2009).

Other researchers believe that microscopy is preferable to RDT testing in survey settings. Keating and colleagues (Keating et al. 2009) compared microscopy and RDT results in a national-level household survey in Zambia and concluded that microscopy results should continue to be used as the gold standard. The authors argue that RDT results represent measures of prevalence over a period of approximately two weeks compared to the point prevalence estimate generated by microscopy and that comparison of national parasitemia levels over time will be complicated by changing recommended diagnostic procedures. They also posit that the multiple brands of RDTs used limits comparability of survey results, especially where trends in national parasitemia prevalence are of interest for programming, monitoring and evaluation purposes.

Comparisons of these diagnostic methods are also important in clinical settings. A review of RDTs and microscopy for the WHO guidelines for the management of common illnesses in hospitals with limited resources conducted by Allen Cheng and David Bell concluded that diagnostic recommendations should be country specific (Cheng and Bell 2006). After assessing 145 studies, Cheng and Bell concluded that uptake

of RDTs in health facilities should take into consideration local realities such as existing quality of microscopy services, availability of skilled personnel, baseline malaria prevalence, predominant *Plasmodium* species, costs and supervisory capacity. Recommendations from this review include use of RDTs in countries where cost-effectiveness has been established at the local level based on local conditions. Specific guidelines on type of RDT were also provided: HRP2-based RDTs are advised where *P. falciparum* (*Pf*) predominates whereas combination HRP2/pLDH or pLDH-based RDTs are recommended in countries endemic to multiple *Plasmodium* species as HRP2 tests will detect *Pf* only and pLDH will detect all Plasmodium species. In other settings, microscopy may still be preferred. Thus country-specific conditions play an important role in determining appropriate diagnostic recommendations.

Despite the lack of clear results in the malaria literature, experts working on the FIND study state that the highest quality RDTs are as sensitive and specific as expert microscopy with less variability in results, especially when microscopy is being conducted by newly trained technicians. Supporting this, a recent Cochrane Review article assessed the diagnostic performance of RDTs detecting HRP2 and pLDH antigens from 74 unique studies and concluded that all of the RDTs tested had sufficient sensitivity and specificity compared to microscopy to replace other diagnostic methods for detecting uncomplicated *Pf* malaria (Abba et al. 2011).

1.6 Parasitemia Testing in The DHS Program

Since 2000, The DHS Program has collected data on mosquito net ownership and use, treatment of fever in children, and intermittent preventive treatment of malaria in pregnant women. In 2006, starting with Angola, The DHS Program began incorporating parasitemia testing into some surveys. To date, The DHS Program has provided technical assistance for 32 DHS and Malaria Indicator Surveys with malaria parasitemia testing in 24 countries (Table 3).

Table 3. Summary of malaria parasitemia testing in DHS and MIS

Survey	Months of fieldwork	RDT type
Included in Analyses		
Angola 2011 MIS	Jan - May	SD Biolone Malaria AG *Pf*/*Pv*
Benin 2011-12 DHS	May - Jan	Parcheck *Pf*
Burkina Faso 2010 DHS	Dec - March	Parcheck *Pf*
Burundi 2012 MIS	Nov - Jan	SD Bioline Malaria Ag *Pf*/pan
Cote d'Ivoire 2011-12	Dec - May	SD Bioline Malaria Ag *Pf*
Ghana 2011 MICS	Sept - Dec	CareStart Malaria HRP2/pLDH (pf/pan) Combo
Guinea 2012 DHS	June - Oct	First Response Malaria Ag Combo
Kenya 2010 MIS	July - Sept	CareStart Malaria HRP2/pLDH (pf/pan) Combo
Liberia 2009 MIS	Dec - March	Paracheck *Pf*
Liberia 2011 MIS	Sept - Dec	First Response Malaria Ag HRP2
Madagascar 2011 MIS	March - June	Paracheck *Pf*
Madagascar 2013 MIS	April - June	CareStart Malaria HRP2/pLDH (pf/pan) Combo
Malawi 2012 MIS	April - May	SD Bioline Malaria Ag *Pf*
Mali 2010 A&P	Aug - Oct	Paracheck *Pf*
Mozambique 2011 DHS	May - Nov	SD Bioline Malaria Ag *Pf*/*Pv*
Nigeria 2010 MIS	Oct - Dec	Paracheck *Pf*
Rwanda 2007-08 IDHS	Dec - April	Optimal-IT
Rwanda 2010 DHS	Sept - March	First Response *Pf*
Senegal 2008-09 MIS	Dec - Jan	Paracheck *Pf*
Senegal 2010-11 DHS	Oct - April	Paracheck *Pf*
Tanzania 2011-12 AIS/MIS	Dec - May	SD Bioline Malaria Ag *Pf*/pan
Uganda 2009 MIS	Nov - Jan	Paracheck *Pf*
Not Included - No Microscopy Data		
Angola 2006 MIS	Jan - April	Paracheck *Pf*
Cameroon 2011 DHS	Jan - Aug	Malaria Ag *Pf*
Tanzania 2007-08 MIS/AIS	Oct - Feb	Paracheck *Pf*
Not Included - Data Not Available by April 2014		
DRC 2013 DHS	Aug - Dec	SD Bioline Malaria Ag *Pf*
Equatorial Guinea 2011 DHS	July - Nov	ICT Malaria Combo
Gambia 2013 DHS	Feb - April	SD Bioline Malaria Ag *Pf*/pan
Mali 2013 DHS	Nov - Feb	SD Bioline Malaria Ag *Pf*
Senegal Cont. DHS 2012-13	All Year	SD Bioline Malaria Ag *Pf*/*Pv*
Sierra Leone 2013 MIS	Feb - March	First Response Malaria Ag HRP2
Togo 2014 DHS	Oct - Feb	First Response Malaria Ag HRP2

2   Methods

2.1   Data Sources

Data included in our analysis come from DHS and MIS surveys conducted in malaria-endemic countries in Sub-Saharan Africa that tested children age 6-59 months for *Plasmodium* parasites by RDT and by microscopy. As of April 2014, 29 nationally representative household surveys implemented with technical assistance from The DHS Program have measured parasitemia in children aged 6-59 months using both RDTs and microscopy (Table 3). Of these 29 surveys, data were available from 22 at the time of data analysis (Figure 1, Figure 2). Data are nationally representative estimates generated from responses to household questionnaires and to individual questionnaires asked of women of reproductive age 15–49. Responses to child health questions were collected for all children born to interviewed women during the five years immediately preceding the survey. Malaria parasite testing was done on all (or a representative subsample of) children 6-59 months of age from interviewed households.

Figure 1. Map of countries with DHS or MIS surveys between 2007 and 2013 in which children age 6-59 months were tested for malaria parasites by both RDT and microscopy
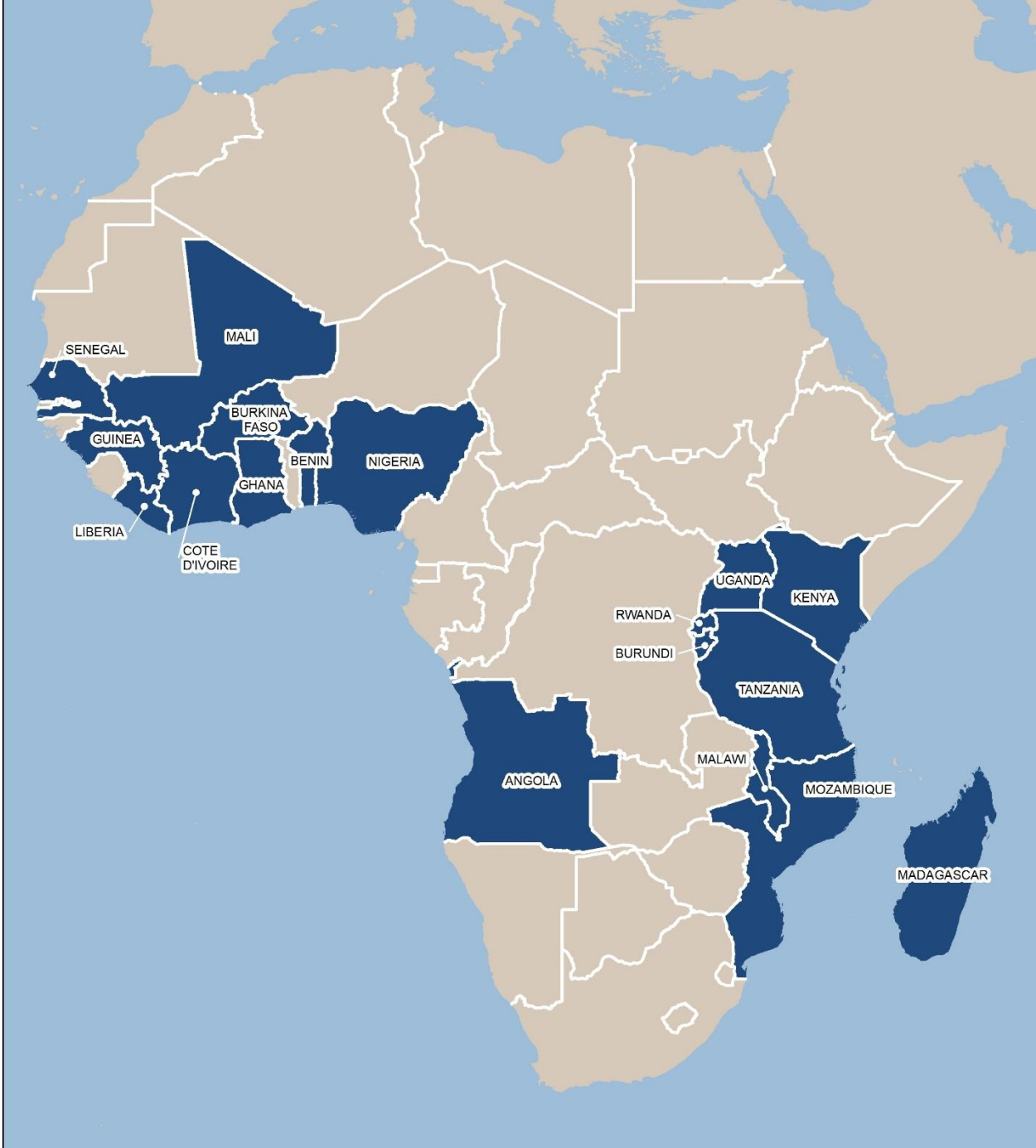
Figure 2. Timeline of available survey data

2007	2008	2009	2010	2011	2012	2013
• Rwanda Interim DHS	• Liberia MIS • Senegal MIS	• Uganda	• Angola MIS • Burkina Faso DHS • Kenya MIS • Mali A&P • Nigeria MIS • Rwanda DHS • Senegal DHS	• Benin DHS • Cote d'Ivoire DHS • Ghana MICS • Liberia MIS • Madagascar MIS • Mozambique DHS • Tanzania MIS/AIS	• Burundi MIS • Guinea DHS • Malawi MIS	• Madagascar MIS
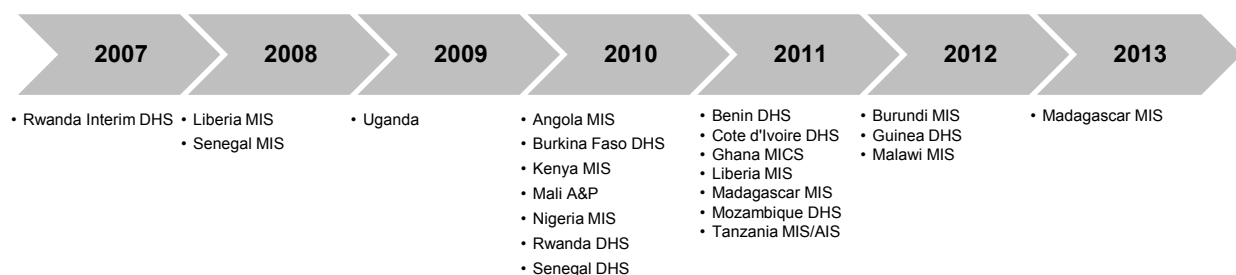
2.2 Study Population

Children age 6–59 months at the time of the survey living or staying the night before in interviewed households, who were tested for malaria parasites by both microscopy and RDT comprised the study population. Children under age 6 months were excluded because infants are protected by maternal antibodies. For some analyses the study population is further restricted to children born to interviewed women age 15-49 for whom data were collected on recent fever and on treatment seeking behavior in response to fever.

2.3 Study Variables

2.3.1 Outcome: Agreement between microscopy and RDT results

National-level summaries of each survey were compiled to look at agreement between microscopy and RDT test results. Several measures of agreement were used including Kappa coefficients and Area Under the Curve (AUC) estimates from Receiver Operating Characteristic (ROC) analyses. Models of RDT-microscopy discordance were also run.

2.3.2 Explanatory Variables

Agreement between microscopy and RDT results was also assessed in several stratified analyses. Separate analyses were run for children with a fever in the two weeks preceding each survey and for children without a recent fever. This stratification was done due to the likely correlation between a positive malaria parasitemia test result at the time of data collection and fever status, as fever is a common symptom of malaria. However, questions on fever are asked about children 0-59 months of age of interviewed women for a period of two weeks before the survey:

525	Has (NAME) been ill with a fever at any time in the last 2 weeks?	YES .... 1 NO .... 2 (SKIP TO 527)◄─┤ DON'T KNOW 8	YES .... 1 NO .... 2 (SKIP TO 527)◄─┤ DON'T KNOW 8	YES .... 1 NO .... 2 (SKIP TO 527)◄─┤ DON'T KNOW 8

whereas malaria parasitemia testing is done on all eligible, consented children in the household whether or not his/her mother is interviewed. Thus analyses including fever status were limited to children born to interviewed mothers who had valid test results for both microscopy and RDT.

As HRP2-based RDTs detect *Plasmodium* spp. antigens for a period of up to several weeks post clearance of infection (Mayxay 2001, Swarthout et al. 2007), discrepancies in results between HRP2-based RDT and microscopy are likely to be higher in settings of moderate to high malaria transmission with high levels of care seeking and appropriate antimalarial treatment. In order to determine if antimalarial treatment could

be influencing the concordance of test results, analyses were further stratified by child's recent antimalarial treatment status. The standard DHS/MIS Women's questionnaires ask:

537	At any time during the illness, did (NAME) take any drugs for the illness?	YES . . . . 1 NO . . . . 2 (GO BACK TO 503 IN NEXT COLUMN; OR, IF NO MORE BIRTHS, GO TO 553) DON'T KNOW 8	YES . . . . 1 NO . . . . 2 (GO BACK TO 503 IN NEXT COLUMN; OR, IF NO MORE BIRTHS, GO TO 553) DON'T KNOW 8	YES . . . . 1 NO . . . . 2 (GO TO 503 IN NEXT-TO-LAST COLUMN OF NEW QUESTIONNAIRE; OR, IF NO MORE BIRTHS, GO TO 553) DON'T KNOW 8
538	What drugs did (NAME) take? (13) Any other drugs? RECORD ALL MENTIONED.	ANTIMALARIAL DRUGS SP/FANSIDAR . . . A CHLOROQUINE B AMODIAQUINE C QUININE . . . . D COMBINATION WITH ARTEMISININ E OTHER ANTI-MALARIAL _____ . . . F (SPECIFY) ANTIBIOTIC DRUGS PILL/SYRUP . . . G INJECTION . . . H OTHER DRUGS ASPIRIN . . . . I ACETA-MINOPHEN . . . J IBUPROFEN . . . K OTHER _____ X (SPECIFY) DON'T KNOW . . . . . Z	ANTIMALARIAL DRUGS SP/FANSIDAR . . . A CHLOROQUINE . B AMODIAQUINE C QUININE . . . . D COMBINATION WITH ARTEMISININ E OTHER ANTI-MALARIAL _____ . . . F (SPECIFY) ANTIBIOTIC DRUGS PILL/SYRUP . . . G INJECTION . . . H OTHER DRUGS ASPIRIN . . . . I ACETA-MINOPHEN . . . J IBUPROFEN . . . K OTHER _____ X (SPECIFY) DON'T KNOW . . . . . Z	ANTIMALARIAL DRUGS SP/FANSIDAR . . . A CHLOROQUINE B AMODIAQUINE C QUININE . . . . D COMBINATION WITH ARTEMISININ E OTHER ANTI-MALARIAL _____ . . . F (SPECIFY) ANTIBIOTIC DRUGS PILL/SYRUP . . . G INJECTION . . . H OTHER DRUGS ASPIRIN . . . . I ACETA-MINOPHEN . . . J IBUPROFEN . . . K OTHER _____ X (SPECIFY) DON'T KNOW . . . . . Z

of children of interviewed women who were reported to have fever in the two weeks directly preceding the survey. For this analysis, any of the response options under "Antimalarial Drugs" were considered valid antimalarials. As these questions were only asked of children with fever, the sample size in this stratified analysis is smaller than those of the previous sections.

Other variables that might influence agreement in malaria diagnostic test results were used in analyses. These variables included household wealth, location of the household in an urban or rural setting, age of the child in months, sex of the child, in addition to recent fever status, and self-reported treatment with antimalarials in response to recent fever that have been previously discussed. Table 4 contains a summary of these variables.

Table 4. Summary of explanatory variables

Variable	Type	Details of Measurement
Wealth Index	Categorical with 5 ordinal categories	Asset-based principal component analysis
Residence	Categorical with 2 categories	Household classified as being in an urban or rural setting
Child's Age	Categorical with 3 categories: 6-11 months; 12-17 months, and 18-23 months	Based on date of birth and date of interview
Child's Sex	Categorical with 2 categories: male, female	From women's interview
Recent Fever	Categorical with 2 categories: yes/no	Whether or not the child had a fever in the 2 weeks preceding interview by mother's report.
Antimalarial Treatment	Categorical with 2 categories: yes/no	Whether or not a child with a reported recent fever took antimalarial medication by mother's report.

2.4   Analytical Procedures

Separate, national-level estimates of malaria parasitemia prevalence in children 6-59 months of age were generated for the twenty-two surveys that collected both microscopy data and RDT data using the subset of records containing results for both tests. Estimates were weighted to adjust for the sample design of each survey. Agreement between the two estimates generated by RDT testing and by microscopy was further examined using Kappa coefficients and ROC analysis.

Cohen's Kappa coefficient is used as a measure of rater agreement, which, although controversial for quantifying the level of agreement, is appropriate for evaluation of whether or not agreement exceeds levels expected by chance for binary ratings (tests of rater independence). Kappa coefficients are affected by the distribution of outcomes in the study population and are thus not comparable across populations (Thompson and Walter 1988, Feinstein and Cicchetti 1990). Kappa coefficients may be low even with high levels of agreement and despite accurate individual ratings. This depends on what assumptions are made about raters' decision-making. In short, while Kappa can be used to measure a departure from levels of agreement between tests that could be expected by chance alone, it does not determine whether or not the raters correctly classify the cases (Uebersax 2010). Kappa values are calculated using estimates of the observed and expected agreement among raters.

Table 5. Standard two-by-two table

	Test A +	Test A -	TOTAL
Test B +	a	b	a+b
Test B -	c	d	c+d
TOTAL	a+c	b+d	N = a+b+c+d

From the standard two-by-two table (Table 5), *Observed agreement* is:

$$Pr(a) = (a+d)/N$$

and *Expected agreement* based on chance is:

$$Pr(e) = ((a+b)*(a+c))+((c+d)*(b+d))/ N^2$$

Cohen's Kappa is calculated using both of these estimates:

$$\kappa = (Pr(a) - Pr(e))/(1-Pr(e))$$

Unlike the previous tests, using ROC analysis to look at interrater reliability requires determination of a gold standard. Results of a second diagnostic test are compared to the gold standard and the ***Area Under the Curve (AUC)*** of the sensitivity versus 1-specificity for various cutpoints can be generated:

$$Z = a_1 - a_0/SE\ a_1 - a_0 \sim N(0,1)$$

Where a_1 = area under the diagnostic test curve, a_0 = 0.5, SE a_1 = standard error of the area, and SE a_0 = 0.00

AUC can be interpreted as the probability that a randomly selected abnormal individual will produce a diagnostic test result greater than the result of the same diagnostic test from a randomly selected normal subject. Thus, higher AUC values are indicative of better diagnostic tests. A value of 0.5 for the AUC implies no agreement between tests and poor predictive power. A high value (significantly higher than 0.5) implies a good test for predicting the outcome and a value significantly lower than 0.5 implies that the test identifies inverse predictors of the outcome. The N(0,1) distribution is used as a reference for an individual curve. Figure 3 provides an example of an AUC from ROC analysis.

Figure 3. Example of an AUC from ROC analysis
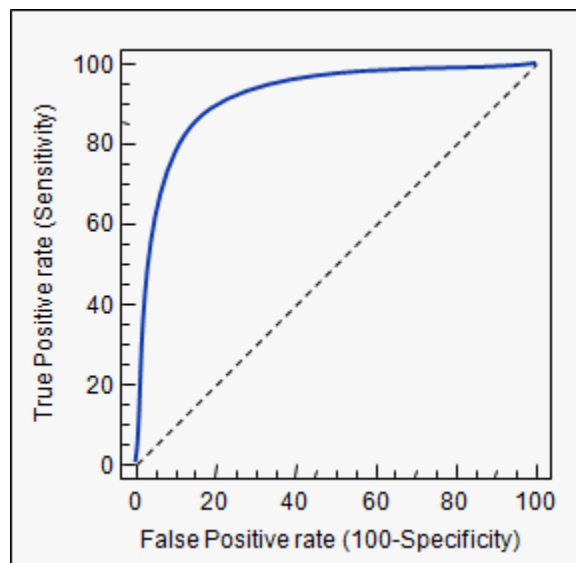
The AUC is a measure of the discriminating ability of the test and is equal to the probability that the test correctly classifies patients as true positives or true negatives. In the case of an imperfect gold standard, the measure gives an indication on the similarities or dissimilarities between the two tests.

Following these descriptive analyses, additional agreement tests were generated stratified by recent fever history in the study population (children 6-59 months) and by use of antimalarials in response to recent fever as reported by mothers during interview. This was done to investigate whether measures of interrater reliability varied between strata. As HRP2-based RDTs measure antigen that can persist for several weeks following effective treatment of malaria parasites and cause positive test results, some discrepancies

between RDT and microscopy would be expected (higher RDT prevalence). It should be noted that the type of RDT used was not consistent across surveys. The specific RDT used in each survey is noted in Table 3.

Scatter-plots were made of national malaria parasitemia prevalence as measured by microscopy on the x-axis and RDT on the y-axis for each survey. Best-fit lines were plotted and used to compare correlation between the two measures across surveys with equality lines. This was done for the population of children 6-59 months of age. In this figure, each dot represents a survey. An additional pair of scatter-plots were constructed stratified into lower and higher levels of parasitemia prevalence (30% as measured by microscopy was used as the cut-off). Another pair of scatter-plots were constructed stratified by recent fever status in children, and a final pair of plots were constructed stratified by antimalarial treatment status in children tested for malaria during the survey who had reported recent fever. Scatter-plots comparing RDT and microscopy estimates of malaria prevalence were also produced for survey data stratified by WHO regions within Sub-Saharan Africa. West African, Central African and East African countries were represented.

Sub-national trends were examined using a map of discordance in RDT and microscopy estimates of malaria parasitemia prevalence which was generated at the regional level for the most recent survey in each country. The regional stratifications defined in each survey were used.

Differences in malaria prevalence as measured by the two diagnostic tests were also compared over time in the four countries with more than one survey containing the necessary data. Data from the two surveys in Liberia were used to illustrate regional changes over time in the prevalence estimates generated from the two diagnostic tests.

Datasets were pooled and multivariable logistic models were run to investigate predictors of discordance in test results. Discordance was defined as either a positive RDT result and a negative microscopy result or a negative RDT result and a positive microscopy result. Models were adjusted for the household residence (urban/rural) of the child, the household wealth quintile, the child's age categorized into 6-23 months of age or 24-59 months, and the child's sex. In addition, the first set of models were adjusted for the child's history of fever in the two weeks preceding the survey (Y/N). The second set of models were limited to children who had reported recent fever and were adjusted for self-reported treatment with antimalarials (Y/N). Dummy variables for survey were included to control for country-level variation.

3 Results

3.1 Section 1: Survey specific results

3.1.1 Malaria parasitemia prevalence and test agreement

In surveys conducted in malarious countries in Sub-Saharan Africa between 2007 and 2013 in which malaria parasitemia was measured by both RDT and by microscopy, prevalence of parasitemia in children age 6-59 months ranged from 1.4% in Rwanda in 2010 to 66% in Burkina Faso in 2010 (Figure 4; Table 6). Prevalence as measured by RDT ranged from 2.2% in Rwanda in 2007/8 to 72% in Burkina Faso in 2010. In almost every survey, RDT estimates of malaria parasitemia prevalence were higher than microscopy estimates. The exceptions were the Benin 2011-12 DHS, the Rwanda 2007-08 IDHS and the Senegal 2010-11 DHS.

Table 6. Malaria parasitemia prevalence in children 6-59 months from national surveys and measures of agreement between microscopy and RDT test results

Survey	Microscopy Prevalence	RDT Prevalence	% Agreement	Kappa	ROC	Sensitivity	Specificity
Angola 2011 MIS	10.1	13.5	0.93	0.65	0.87	94.6	93.1
Burkina Faso 2010 DHS	65.9	71.8	0.76	0.43	0.70	89.7	50.6
Benin 2011-12 DHS	28.5	24.7	0.72	0.31	0.65	48.5	82.0
Burundi 2012 MIS	17.3	22.0	0.93	0.76	0.92	91.2	93.1
Cote d'Ivoire 2011-12	18.0	41.5	0.66	0.26	0.72	81.1	62.2
Ghana 2011 MICS	27.5	47.5	0.75	0.51	0.79	95.9	62.2
Guinea 2012 DHS	43.9	46.9	0.74	0.51	0.74	72.9	75.4
Kenya 2010 MIS	8.2	12.5	0.94	0.69	0.93	91.7	93.8
Liberia 2009 MIS	31.7	36.5	0.85	0.65	0.84	82.3	85.5
Liberia 2011 MIS	27.7	44.7	0.75	0.50	0.79	91.0	67.2
Madagascar 2011 MIS	6.3	8.7	0.96	0.61	0.88	78.4	96.9
Madagascar 2013 MIS	9.0	10.0	0.96	0.72	0.88	78.8	97.6
Malawi 2012 MIS	37.5	42.5	0.82	0.65	0.86	91.8	79.4
Mali 2010 A&P	27.7	43.3	0.84	0.60	0.84	82.6	84.8
Mozambique 2011 DHS	35.1	38.2	0.84	0.64	0.83	80.6	85.8
Nigeria 2010 MIS	42.0	51.5	0.74	0.48	0.75	78.1	71.9
Rwanda 2007-08 IDHS	2.5	2.2	1.00	0.89	0.91	81.0	100.0
Rwanda 2010 DHS	1.4	2.6	0.98	0.54	0.89	78.4	98.6
Senegal 2008-09 MIS	5.7	11.8	0.92	0.56	0.88	82.7	93.2
Senegal 2010-11 DHS	2.9	2.7	0.96	0.44	0.71	43.3	98.3
Tanzania 2011-12 AIS/MIS	4.1	9.2	0.93	0.46	0.85	76.1	93.4
Uganda 2009 MIS	44.7	54.9	0.82	0.65	0.83	92.2	74.1

Figure 4. Prevalence of malaria parasitemia in children 6-59 months as measured by A) microscopy and by B) RDT in national surveys.
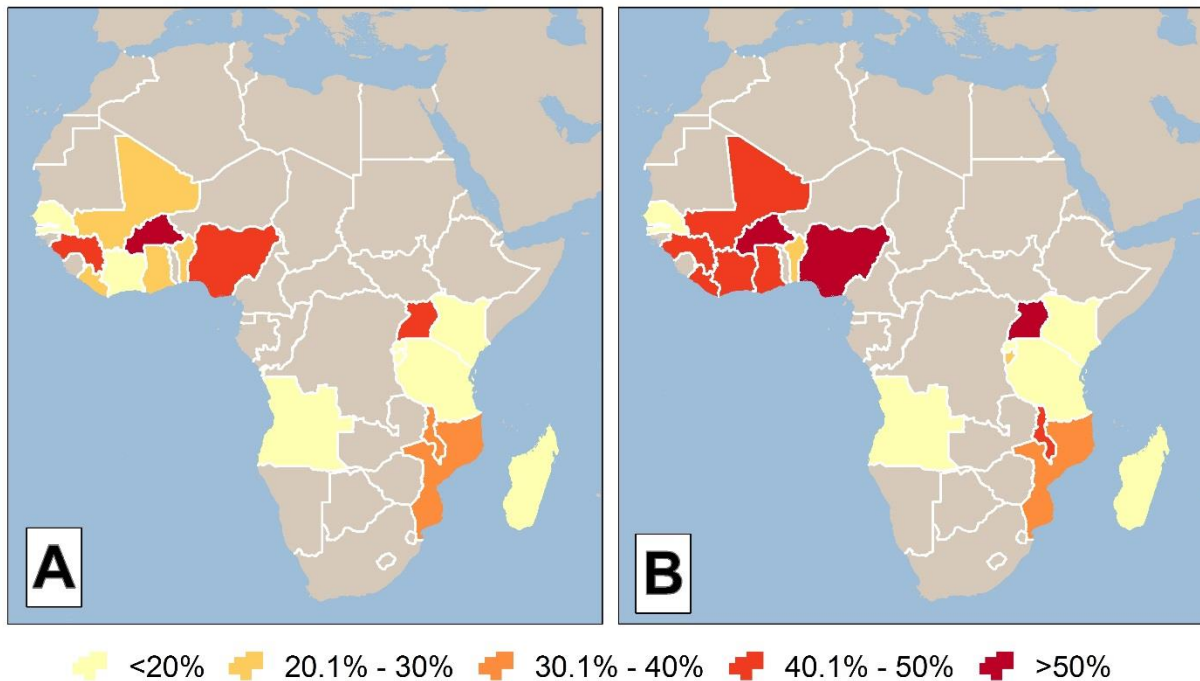
Note: Data from the most recent survey is presented in countries with more than one national survey (Liberia, Madagascar, Rwanda, and Senegal)

The maps in Figure 4 show microscopy (map A) and RDT (map B) estimates of malaria parasitemia for the study populations in each survey. The darker colors indicate higher malaria prevalence. The higher RDT estimates are evident comparing map B to map A, especially in West Africa**.**

A scatter-plot of national microscopy estimates versus national RDT estimates among the study population shows fairly strong agreement between the two measures with an R^2 of 0.89 (Figure 5). The best-fit line is above the equality line, indicating that RDT results reported higher prevalence than did microscopy results. The slope of the best-fit line is greater than 1.0 suggesting that the difference between the RDT and microscopy results is larger in countries with higher malaria prevalence.

Figure 5. Prevalence of malaria parasitemia in children 6-59 months as measured by RDT and by microscopy in national surveys
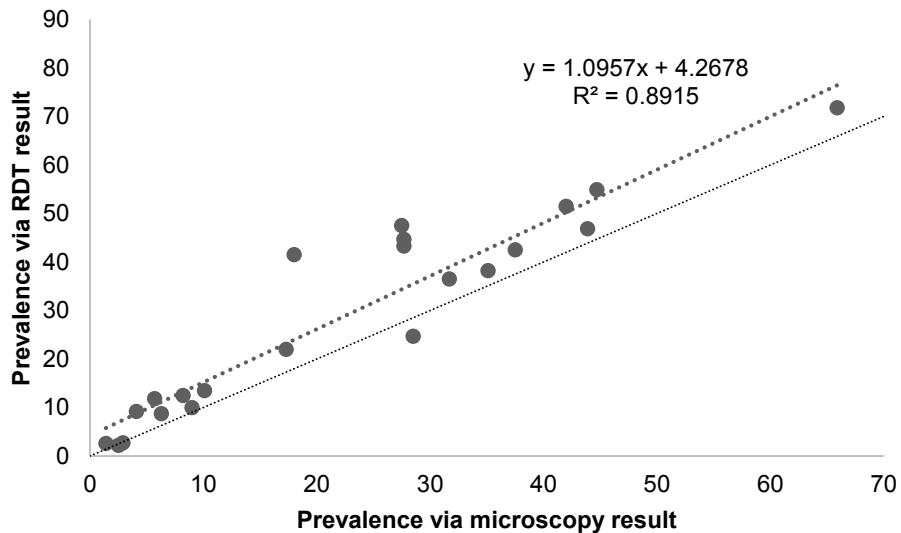
Note: Small dotted line is the equity line (microscopy prevalence = RDT prevalence) and the larger dotted line is the regression line whose equation is specified.

Another set of scatter-plots showing national malaria prevalence estimates from microscopy versus RDT among the study population were made, stratified by level of malaria parasitemia ($<30\%$, $\geq 30\%$) (Figure 6). The Malaria Atlas Project uses a cut-off of 40% Plasmodium falciparum prevalence rate in children 2-10 years of age to define high transmission areas. Thirty percent prevalence was used as a cut-off in this analysis as too few of the surveys included in these analyses had parasitemia prevalence greater than 40%. Results show that the correlation between microscopy and RDT results is higher in high malaria prevalence countries than in low malaria prevalence countries (low: $R^2 = 0.83$, high: $R^2 = 0.95$). The slope of the best-fit line in low prevalence surveys is positive (m=1.5) indicating that the difference between prevalence estimates measured by RDT and microscopy increases as the prevalence increases, with RDT values being higher than microscopy estimates. In high prevalence surveys, the slope of the best-fit line is positive but very close to one (m=1.05) indicating that the linear correlation between the two diagnostic tests is very high. RDT prevalence estimates are higher than microscopy estimates in both low prevalence and high prevlance surveys with the exception of Benin 2011 DHS. It should be noted that these results are sensitive to the prevalence cut-off chosen, especially since four surveys in the mid-prevalence range have very discordant results (Cote d'Ivoire 2011-12 DHS, Ghana 2011 MICS, Liberia 2011 MIS, and Mali 2009 A&P).

Figure 6. Prevalence of malaria parasitemia in children 6-59 months as measured by RDT and by microscopy in: A) national surveys with <30% malaria parasitemia and B) national surveys with ≥30% malaria parasitemia
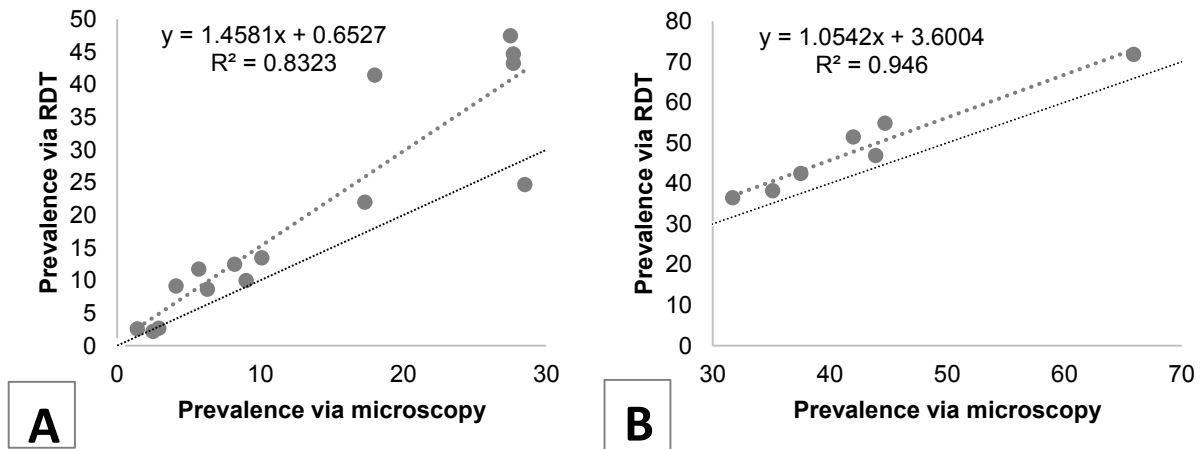

Note: Small dotted line is the equity line (microscopy prevalence = RDT prevalence) and the larger dotted line is the regression line whose equation is specified.

Interrater reliability measures such as the observed percent agreement and the Cohen's Kappa coefficient comparing results of the two diagnostic tests are summarized in Figure 7 and Table 6. The observed percent agreement between the tests ranged from a low of 65.5% in Cote d'Ivoire 2011-12 to a high of 99.5% in Rwanda 2007-08. Cohen's Kappa coefficient adjusts the observed agreement by a level of agreement that would be expected based on chance and is therefore lower than the observed agreement, ranging from 26% in Cote d'Ivoire 2011-12 to 89% in Rwanda 2007-08. Kappa statistics are affected by baseline prevalence levels which accounts for the greater variation in values as compared to the observed percent agreement.

Figure 7. Interrater reliability measures (percent agreement and Cohen's Kappa coefficient) between microscopy and RDT prevalence in children 6-59 months of age from national surveys
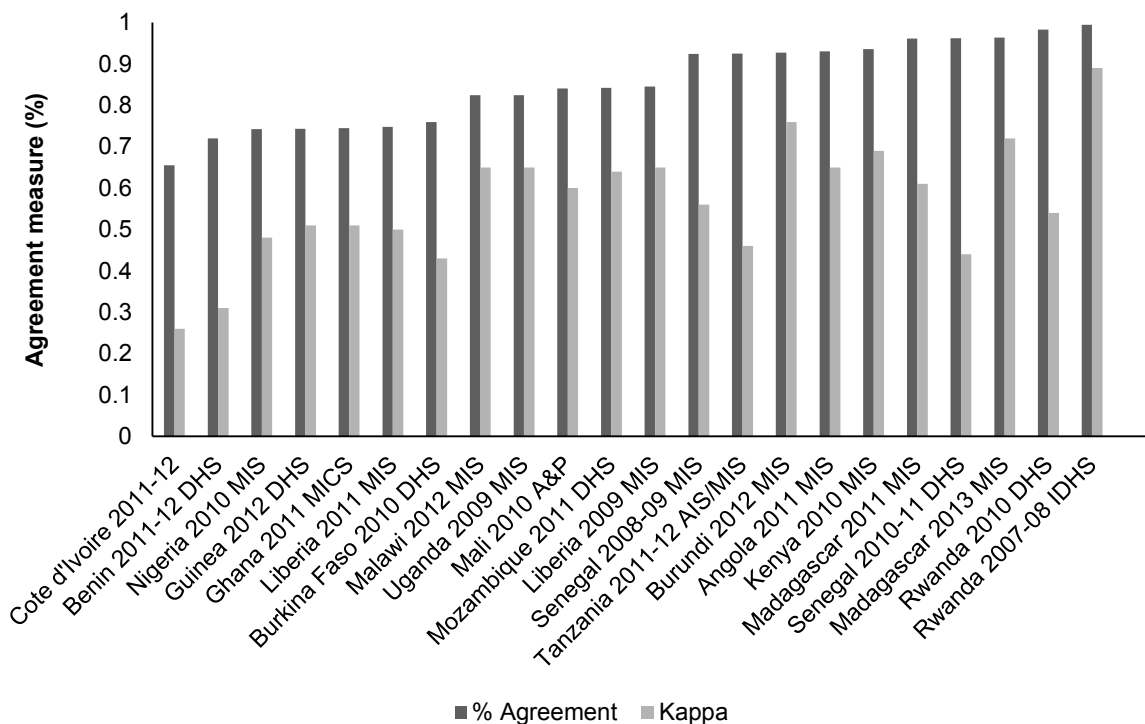
ROC analyses produced values of the area under the curve (AUC) for each survey which ranged from 65% in Benin 2011 to 93% in Kenya 2010. The AUC is a product of both the sensitivity and the specificity of the RDT test for detecting the same result as the microscopy test (gold standard). Figure 8 presents surveys in which the specificity is higher than the sensitivity on the left hand side and surveys in which the sensitivity is higher than the specificity on the right hand side.

Figure 8. AUC from ROC analysis: Sensitivity and specificity of RDT tests as compared to microscopy for diagnosing malaria parasitemia in children 6-59 months from national surveys
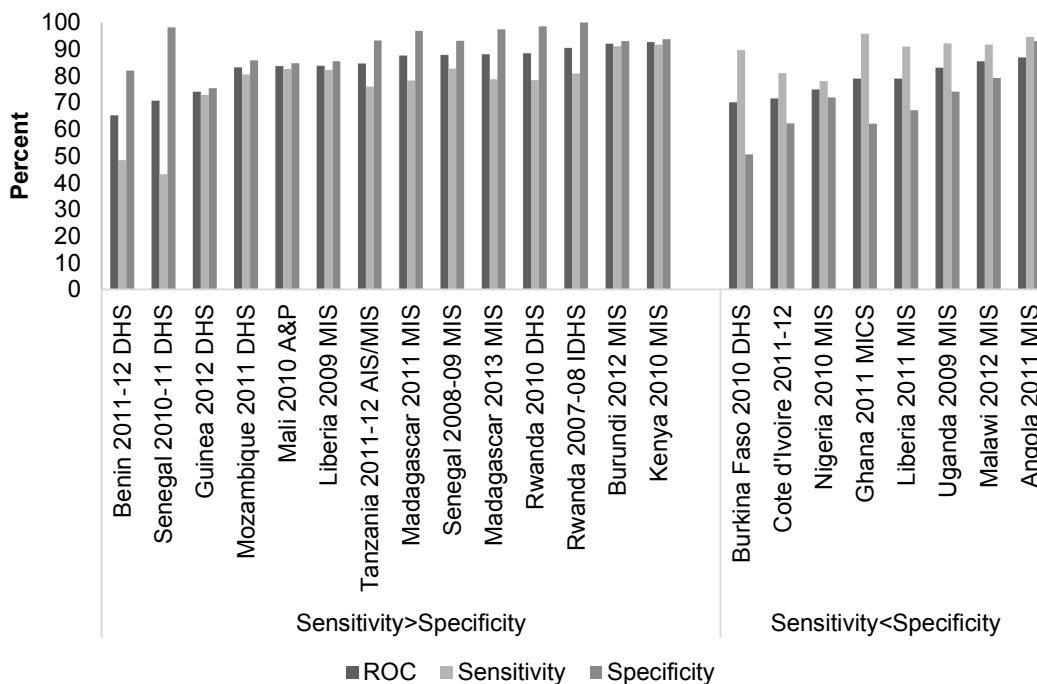
Note: Left side bars represent countries in which specificity is higher than sensitivity and right side bars represent countries in which sensitivity is higher than specifcity.

3.1.2   Stratified Analyses: Recent Fever

The prevalence of fever in children in the study population varied from 9% in Benin 2011 DHS to 55% in the Liberia 2011 MIS (Figure 9).

Figure 9. Fever prevalence in children 6-59 months of age with both RDT and microscopy results available from national surveys
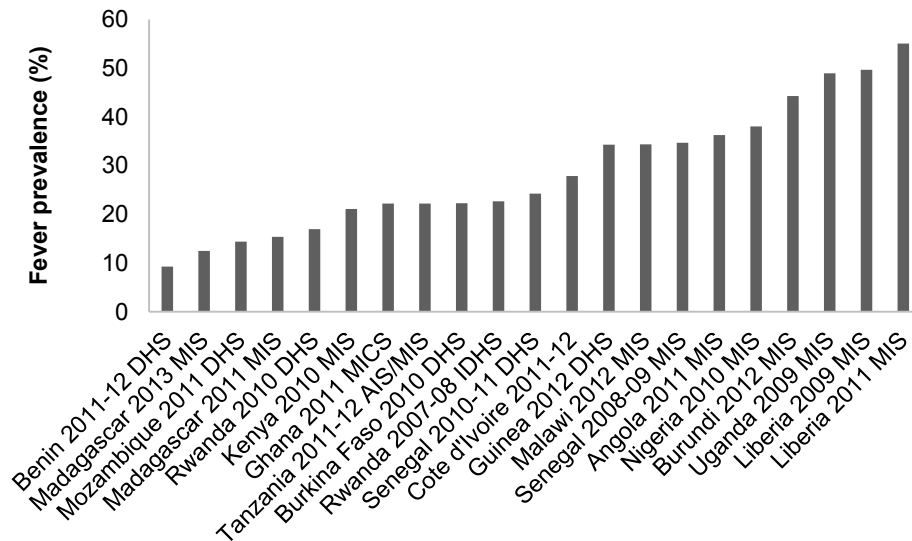
Malaria parasitemia prevalence and agreement statistics were generated for children who were reported to have had fever in the two weeks preceding the interview and for those without (Table 7, Figure 12). In almost all surveys, microscopy prevalence estimates were higher in children who reported a recent fever than in those who did not (Figure 10). Burkina Faso 2011-12 DHS, Liberia 2009 MIS and Liberia 2011 MIS are the exceptions. In all cases, RDT prevalence estimates were higher in children who reported a recent fever than in those who did not (Figure 11), and the difference between the RDT prevalence estimate and the microscopy prevalence estimate was greater in children with recent fever than in those without in all surveys (Table 7, Figure 12).

Figure 10. Malaria parasitemia prevalence as measured by microscopy in children 6-59 months of age with and without fever in the past two weeks
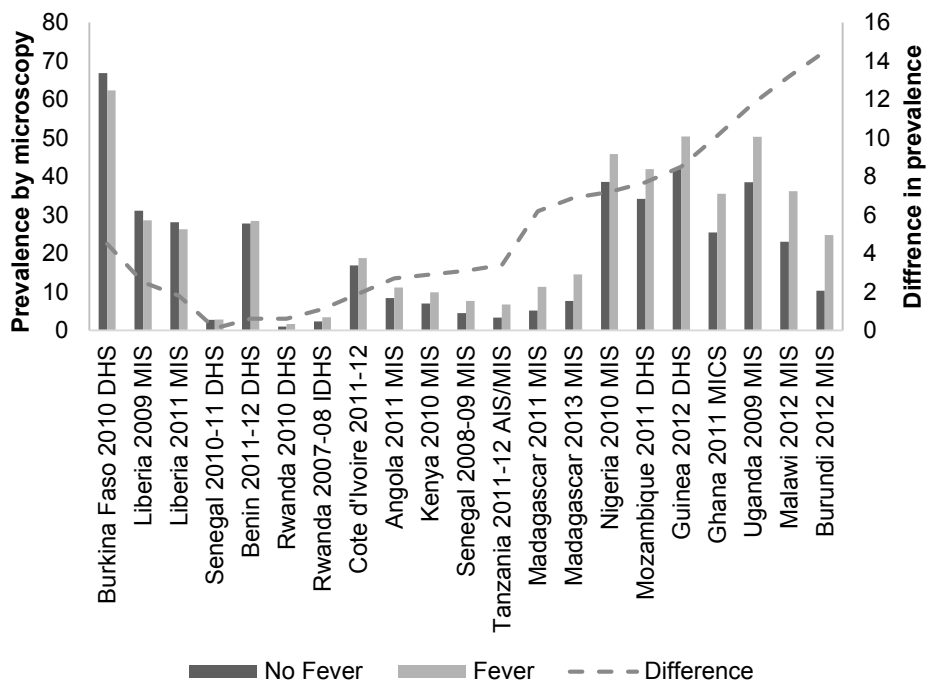
Note: Sorted by difference in microscopy prevalence with higher prevalence in the non-fever strata on the left to higher prevalence in the fever strata on the right.

Figure 11. Malaria parasitemia prevalence as measured by RDT in children 6-59 months of age with and without fever in the past two weeks

Note: Sorted by difference in RDT prevalence between no fever and fever strata, from smaller to larger, left to right.

Table 7. Summary of malaria parasitemia prevalence in children 6-59 months of age and agreement statistics from national surveys, stratified by recent fever status

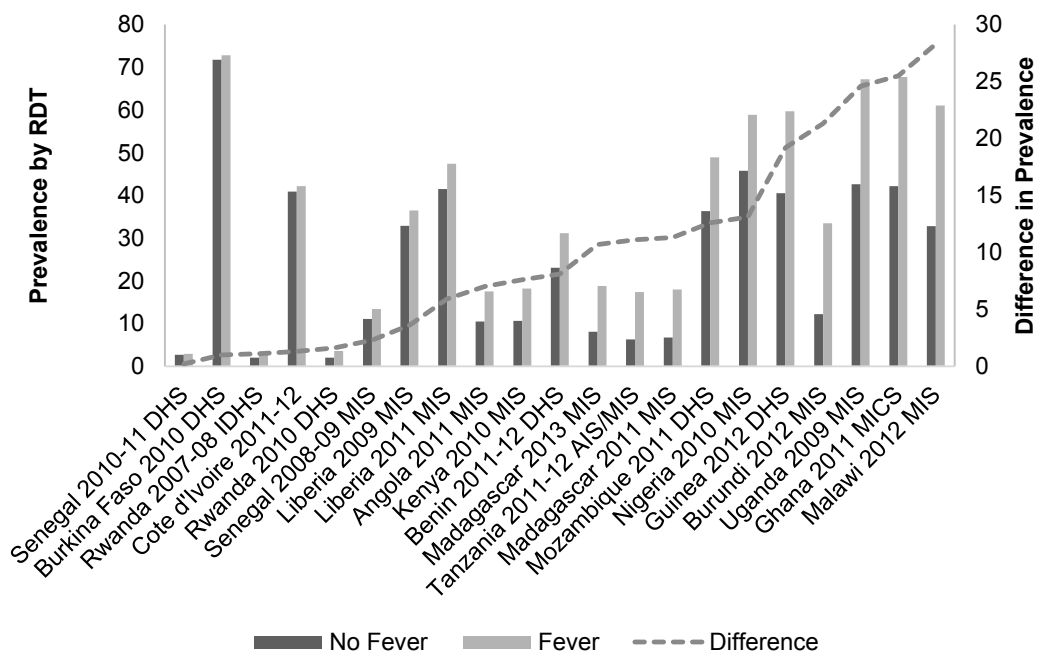|	Microscopy Prevalence		RDT Prevalence		Difference		% Agreement		Kappa		ROC	
	No Fever	Fever	No Fever	Fever	No Fever	Fever	No Fever	Fever	No Fever	Fever	No Fever	Fever
Angola 2011 MIS	8.4	11.1	10.5	17.5	2.1	6.4	94.0	91.4	0.64	0.63	0.85	0.89
Benin 2011-12 DHS	27.8	28.4	23.1	31.2	-4.7	2.8	72.4	72.2	0.61	0.37	0.64	0.69
Burkina Faso 2010 DHS	66.9	62.4	71.8	72.8	4.9	10.4	76.5	73.8	0.44	0.40	0.71	0.69
Burundi 2012 MIS	10.3	24.8	12.2	33.5	1.9	8.7	95.9	88.3	0.79	0.71	0.92	0.90
Cote d'Ivoire 2011-12	16.9	18.8	40.9	42.2	24.0	23.4	67.0	62.3	0.27	0.23	0.73	0.69
Ghana 2011 MICS	25.4	35.5	42.2	67.7	16.8	32.2	77.7	65.1	0.56	0.35	0.82	0.69
Guinea 2012 DHS	41.9	50.4	40.5	59.7	-1.4	9.3	76.1	71.6	0.51	0.43	0.72	0.75
Kenya 2010 MIS	7.0	9.9	10.6	18.2	3.6	8.3	94.6	89.6	0.71	0.61	0.93	0.91
Liberia 2009 MIS	31.1	28.6	32.9	36.5	1.8	7.9	85.2	84.4	0.66	0.65	0.83	0.85
Liberia 2011 MIS	28.1	26.3	41.5	47.4	13.4	21.1	77.7	72.7	0.55	0.46	0.81	0.78
Madagascar 2011 MIS	5.1	11.3	6.7	18.0	1.6	6.7	96.7	93.6	0.58	0.66	0.86	0.94
Madagascar 2013 MIS	7.6	14.5	8.1	18.8	0.5	4.3	97.1	93.4	0.73	0.68	0.88	0.90
Malawi 2012 MIS	23.0	36.2	32.8	61.1	9.8	24.9	86.8	74.0	0.64	0.50	0.87	0.79
Mozambique 2011 DHS	34.2	41.9	36.3	48.9	2.1	7.0	84.6	81.6	0.64	0.62	0.83	0.83
Nigeria 2010 MIS	38.6	45.8	45.8	58.9	7.2	13.1	76.1	71.5	0.50	0.44	0.76	0.73
Rwanda 2007-08 IDHS	2.3	3.4	2.0	3.1	-0.3	-0.3	99.5	99.5	0.89	0.92	0.91	0.93
Rwanda 2010 DHS	1.0	1.6	2.0	3.6	1.0	2.0	98.6	97.1	0.52	0.39	0.88	0.82
Senegal 2008-09 MIS	4.5	7.6	11.1	13.4	6.6	5.8	92.9	91.0	0.51	0.59	0.90	0.86
Senegal 2010-11 DHS	2.7	2.8	2.7	2.9	0.0	0.1	96.2	96.6	0.40	0.51	0.70	0.76
Tanzania 2011-12 AIS/MIS	3.3	6.7	6.3	17.4	3.0	10.7	94.6	86.3	0.48	0.40	0.83	0.84
Uganda 2009 MIS	38.5	50.3	42.6	67.2	4.1	16.9	86.5	77.2	0.73	0.54	0.87	0.77

Figure 12. Malaria parasitemia prevalence in children 6-59 months of age with recent fever as measured by A) microscopy and B) RDT and in children without recent fever as measured by C) microscopy and D) RDT
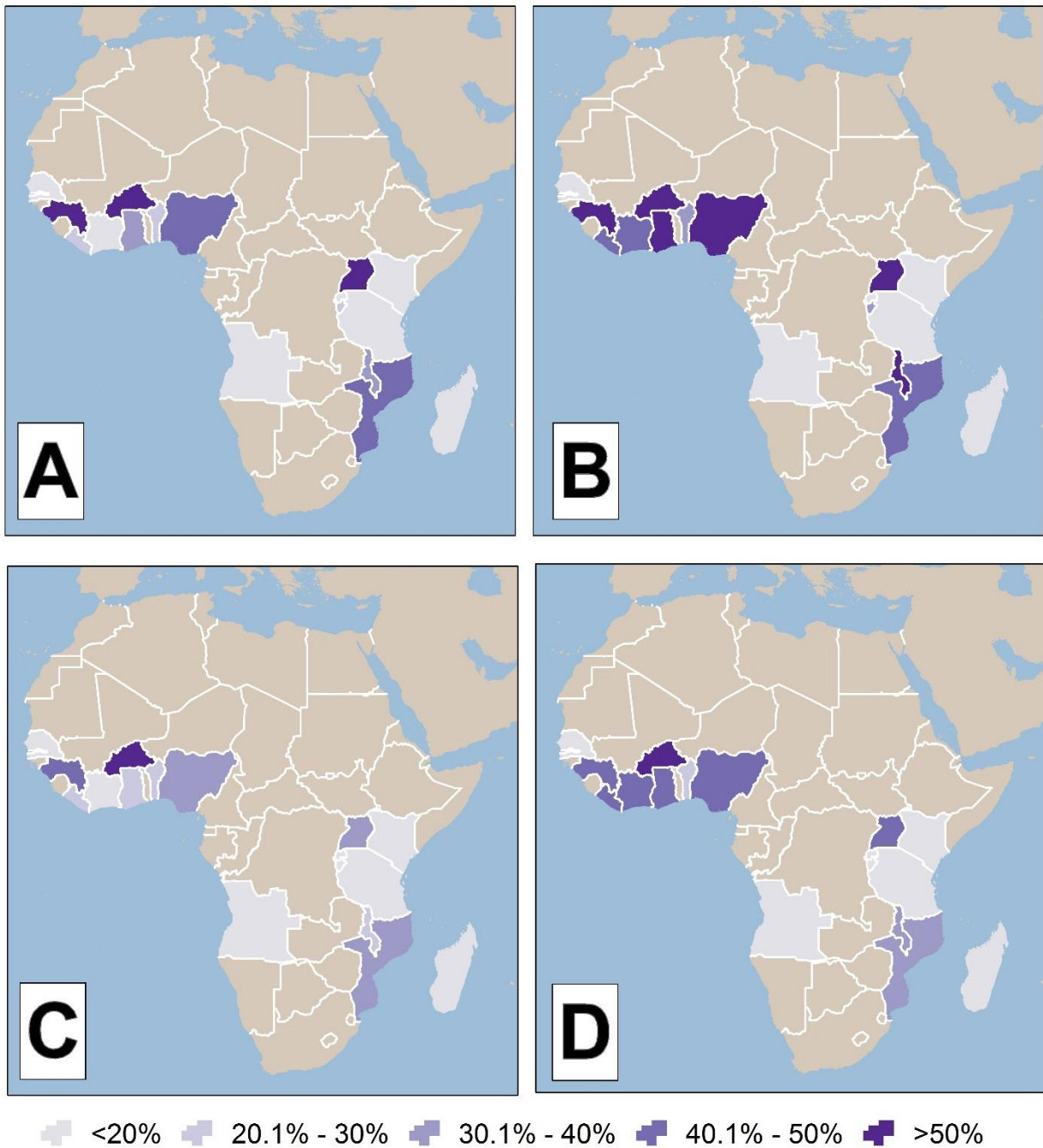
Note: Data from the most recent survey is presented in countries with more than one national survey (Liberia, Madagascar, Rwanda, and Senegal)

The maps in Figure 12 compare malaria prevalence estimates by diagnostic test and by fever status in children 6-59 months of age. Darker colors indicated higher prevalence estiamtes. Comparing map A and map B shows spatially the countries in which microscopy estimates were lower than RDT estimates for malaria prevalence in children with fever. Map C compared to Map D shows the difference in microscopy and RDT estimates for malaria prevalence in children without recent fever. Looking at Map A and Map C, comparisons of microscopy estimates of malaria prevalence in children with and without recent fever is possible. A similar comparison can be made for RDT estimated malaria prevalence using Map B and Map D.

A scatter-plot of national microscopy estimates versus national RDT estimates among the study population stratified by recent fever status shows that the diagnostic tests were more similar among children without recent fever than among those with recent fever (Figure 13). The best-fit lines in the figures show that RDT results reported higher prevalence than did microscopy in all children (i.e.the best-fit lines are above the equality line), but that the difference was more extreme in children who were recently febrile. At a national level, the correlation between microscopy prevalence and RDT prevalence was similarly strong with R^2 estimates of 0.89 for both.

Figure 13. Malaria parasitemia prevalence in children 6-59 months in national surveys as estimated by RDT and by microscopy stratified by recent fever status
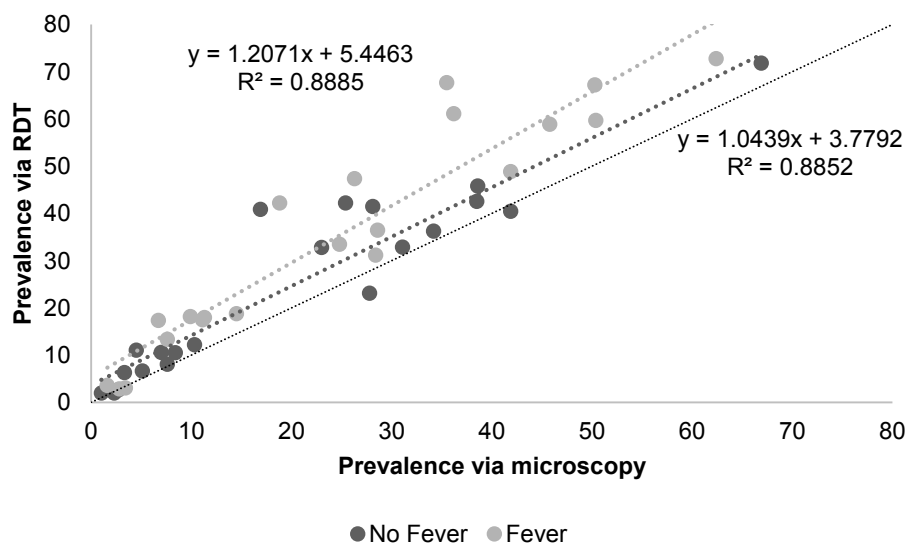
Note: Thin dotted line represents equality in RDT and microscopy results

Observed percent agreement was lower in all surveys in children with a recent fever than in those without in all but a few surveys (Table 2, Figure 14). Kappa coefficients were also lower in children with a recent fever than in those without (Table 2, Figure 15). The largest differences in kappa statistics between children with recent fever and those without were in Benin 2011-12 DHS (0.606 vs. 0.366), and in Ghana 2011 MICS (0.564 vs. 0.350) and Uganda 2009 MIS (0.7252 vs. 0.544). Patterns in AUC from ROC analysis are more difficult to determine. The largest differences between children with and without recent fever were observed in the Ghana 2011 MICS and the Uganda 2009 MIS (Figure 16). The dotted lines in Figures 14-16 show the difference between the various agreement measures in children without recent fever compared to those who had fever within the past two weeks.

Figure 14. Percent agreement between estimates of malaria parasitemia prevalence as measured by RDT and by microscopy in children 6-59 months who had recent fever compared to those who did not and the difference in percent agreement between the two strata
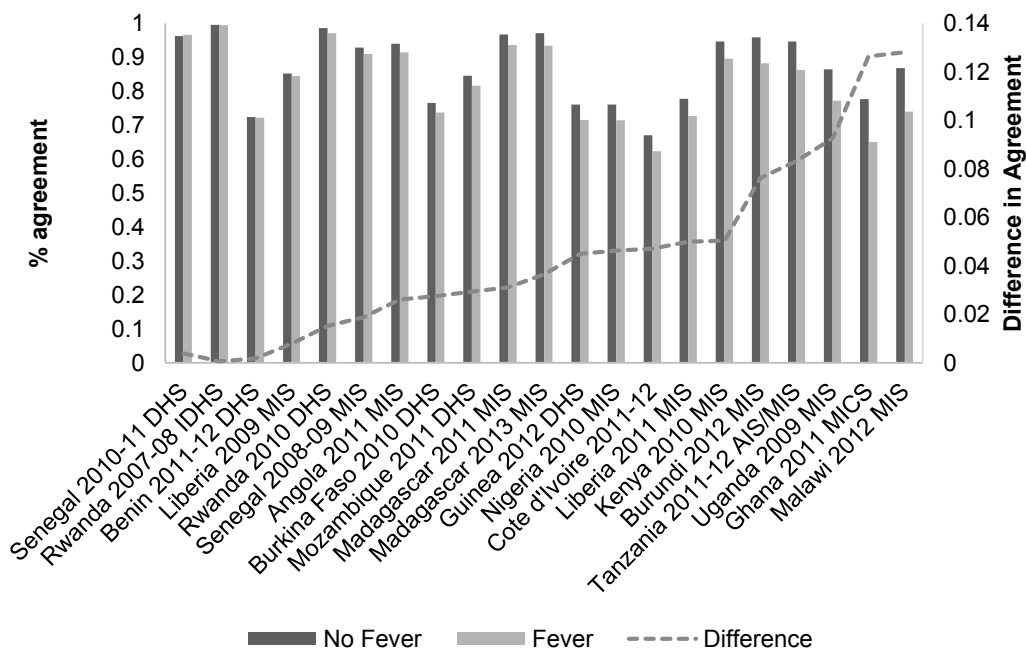
Figure 15. Kappa statistics between estimates of malaria parasitemia prevalence as measured by RDT and by microscopy in children 6-59 months who had recent fever compared to those who did not and the difference in percent agreement between the two strata
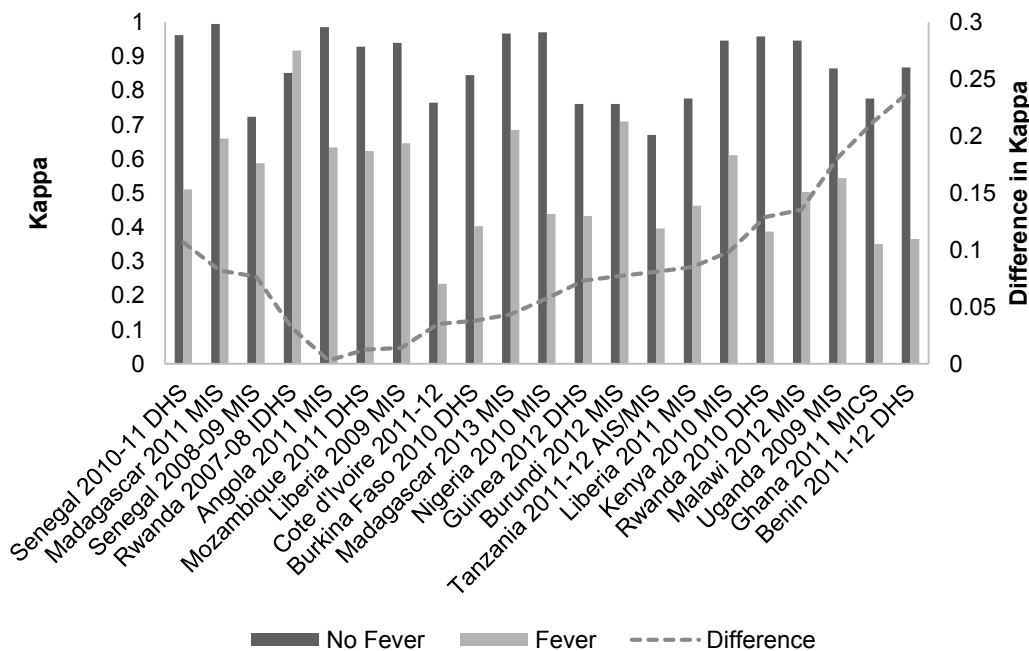
Figure 16. Area under the curve from ROC analysis between estimates of malaria parasitemia prevalence as measured by RDT and by microscopy in children 6-59 months who had recent fever compared to those who did not and the difference in percent agreement between the two strata
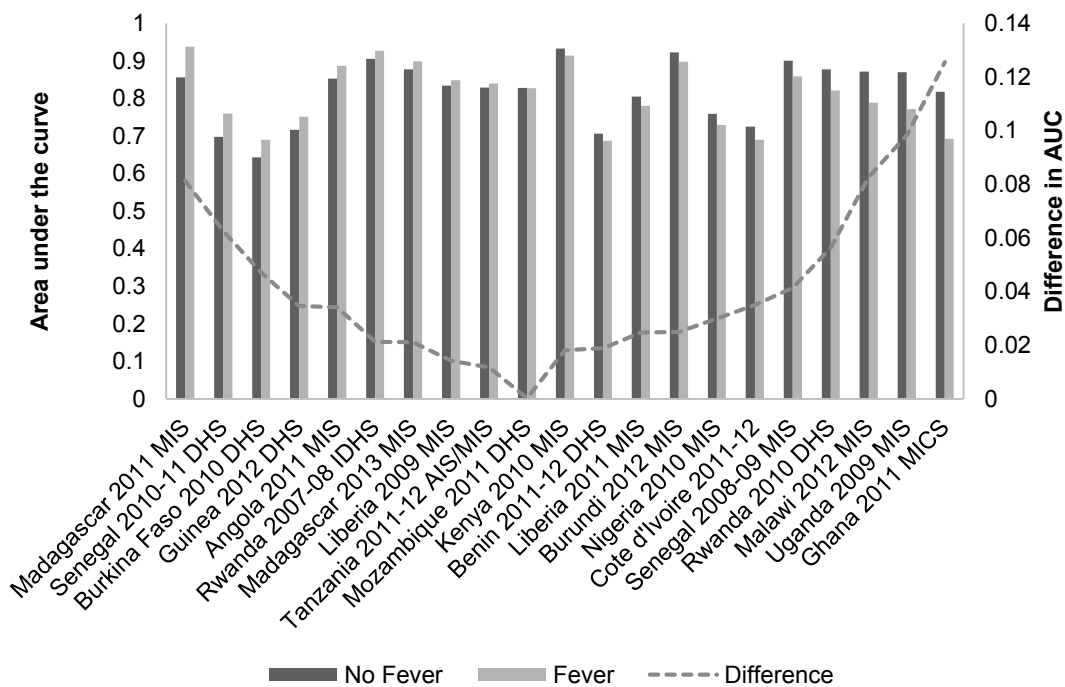
3.1.3   *Stratified Analysis: Antimalarial Treatment*

The prevalence of antimalarial treatment of fever in children in the study population varied from 6% in Rwanda in the 2007/08 Interim DHS to 67% in the Liberia 2009 MIS (Figure 17).

Figure 17. Frequency of antimalarial treamtent for recent fever in children 6-59 months of age with both RDT and microscopy results available from national surveys
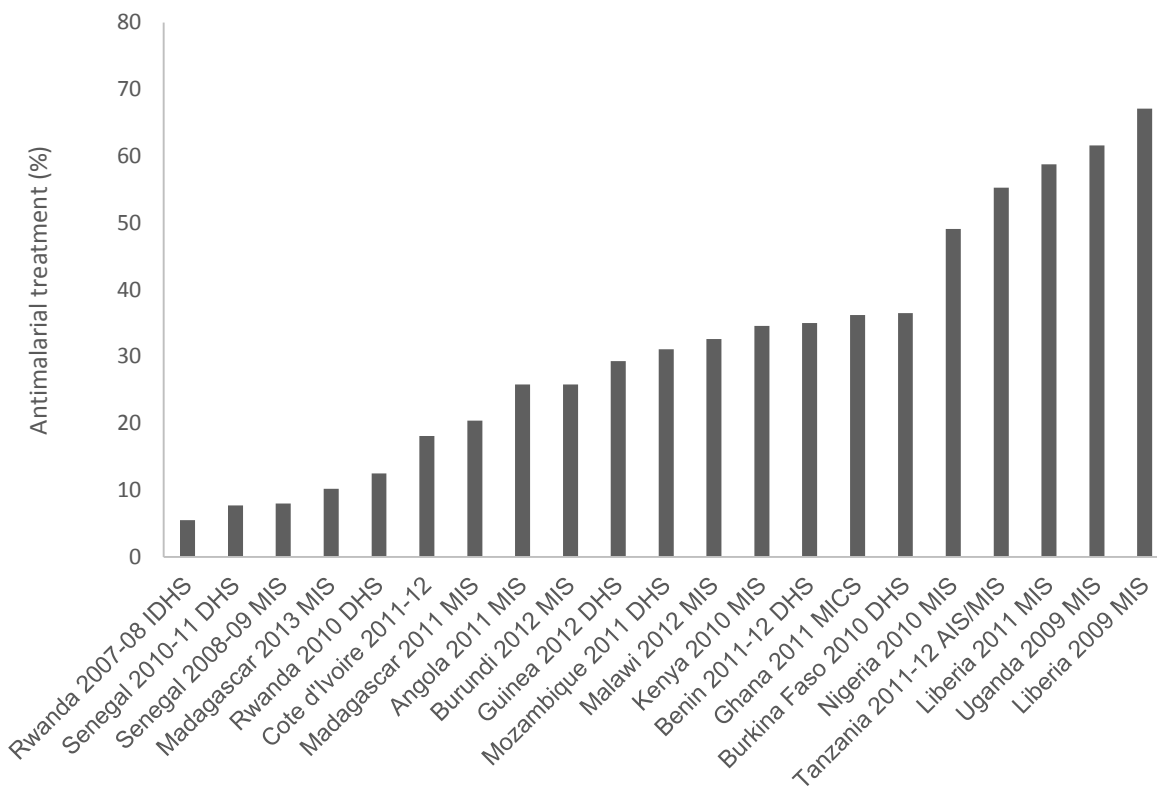
Malaria parasitemia prevalence and agreement statistics were generated for children who had fever in the two weeks preceding the interview who took any antimalarial and for those who did not (Table 8). In almost all surveys, microscopy prevalence estimates were higher in children with a recent fever who did not report treatment with an antimalarial compared to those who did report treatment (Figure 18, Table 8). Madagascar 2013 MIS and 2011 MIS, Burundi 2012 MIS, Mozambique 2011 DHS and Rwanda 2007-08 Interim DHS are the exceptions. RDT prevalence estimates varied substantially between children with a recent fever who reported antimalarial treatment and those who did not (Figure 19, Table 8), with some surveys reporting higher prevalence in the treated children and others with higher prevalence in the untreated children.

Figure 18. Malaria parasitemia prevalence as measured by microscopy in children 6-59 months of age with fever in the past two weeks by antimalarial treatment status
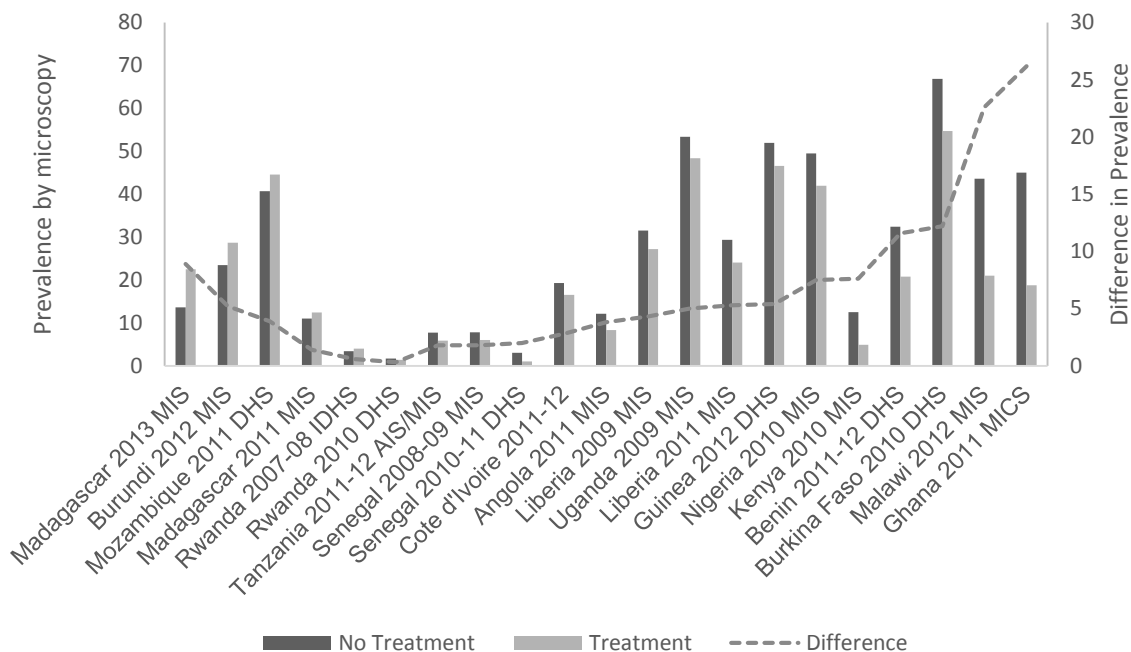
Figure 19. Malaria parasitemia prevalence as measured by RDT in children 6-59 months of age with fever in the past two weeks by antimalarial treatment status
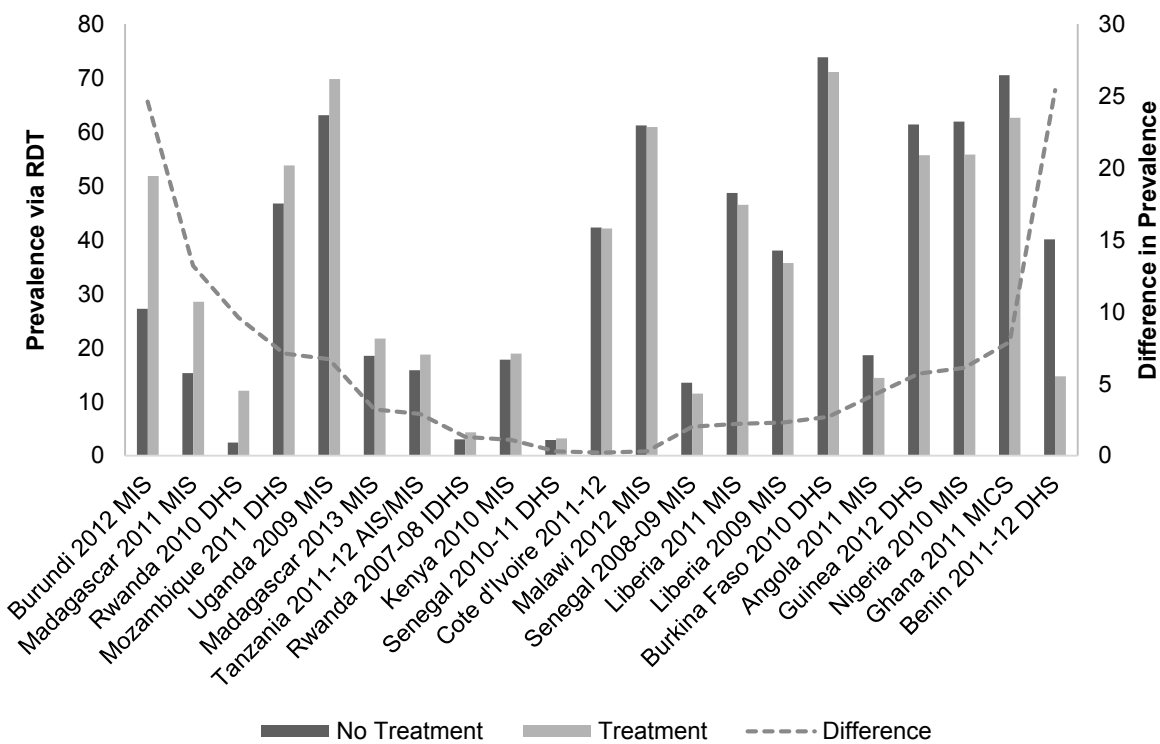
Table 8. Summary of malaria parasitemia prevalence in children 6-59 months of age and agreement statistics from national surveys, stratified by recent treatment with antimalarials

	Microscopy Prevalence		RDT Prevalence		Difference		% Agreement		Kappa		ROC	
	No Treatment	Treatment	No Treatment	Treatment	No Treatment	Treatment	No Treatment	Treatment	No Treatment	Treatment	No Treatment	Treatment
Angola 2011 MIS	12.1	8.3	18.6	14.4	6.5	6.1	91.6	91.0	0.67	0.48	0.90	0.83
Benin 2011-12 DHS	32.4	20.8	40.1	14.7	7.7	-6.1	70.4	75.8	0.38	0.23	0.70	0.60
Burkina Faso 2010 DHS	66.9	54.7	73.8	71.1	6.9	16.4	76.2	69.6	0.42	0.37	0.69	0.68
Burundi 2012 MIS	23.5	28.7	27.2	51.8	3.7	23.1	92.6	75.6	0.80	0.51	0.92	0.81
Cote d'Ivoire 2011-12	19.3	16.5	42.3	42.1	23.0	25.6	62.5	61.3	0.25	0.16	0.70	0.63
Ghana 2011 MICS	45.0	18.8	70.5	62.6	25.5	43.8	72.0	51.7	0.43	0.21	0.71	0.67
Guinea 2012 DHS	52.0	46.6	61.4	55.7	9.4	9.1	72.4	69.4	0.45	0.39	0.72	0.70
Kenya 2010 MIS	12.5	4.9	17.8	18.9	5.3	14.0	92.8	83.4	0.74	0.34	0.93	0.88
Liberia 2009 MIS	31.5	27.2	38.0	35.7	6.5	8.5	85.2	84.0	0.68	0.62	0.85	0.85
Liberia 2011 MIS	29.4	24.1	48.7	46.5	19.3	22.4	73.5	72.2	0.48	0.45	0.78	0.79
Madagascar 2011 MIS	11.0	12.4	15.3	28.5	4.3	16.1	94.6	89.4	0.67	0.62	0.94	0.92
Madagascar 2013 MIS	13.6	22.5	18.5	21.7	4.9	-0.8	93.7	90.2	0.69	0.61	0.91	0.82
Malawi 2012 MIS	43.6	21.0	61.2	60.9	17.6	39.9	81.5	57.9	0.64	0.25	0.84	0.71
Mozambique 2011 DHS	40.7	44.6	46.7	53.8	6.0	9.2	84.2	75.6	0.66	0.52	0.85	0.77
Nigeria 2010 MIS	49.5	42.0	61.9	55.8	12.4	13.8	72.8	70.1	0.46	0.41	0.74	0.72
Rwanda 2007-08 IDHS	3.4	4.0	3.0	4.3	-0.4	0.3	99.4	100.0	0.91	1.00	0.92	1.00
Rwanda 2010 DHS	1.7	1.4	2.4	12.0	0.7	10.6	98.2	89.2	0.49	0.18	0.81	0.95
Senegal 2008-09 MIS	7.8	6.0	13.5	11.5	5.7	5.5	91.0	91.3	0.58	0.67	0.85	0.95
Senegal 2010-11 DHS	3.0	1.0	2.9	3.2	-0.1	2.2	96.8	94.6	0.52	0.38	0.75	0.97
Tanzania 2011-12 AIS/MIS	7.7	5.9	15.8	18.7	8.1	12.8	90.2	82.8	0.53	0.30	0.87	0.82
Uganda 2009 MIS	53.4	48.4	63.1	69.8	9.7	21.4	84.5	72.8	0.68	0.47	0.83	0.74

Figure 20. Malaria parasitemia prevalence in children 6-59 months of age with fever in the past two weeks who took antimalarials as measured by A) microscopy and B) RDT; and in children who did not take antimalarials as measured by C) microscopy and D) RDT
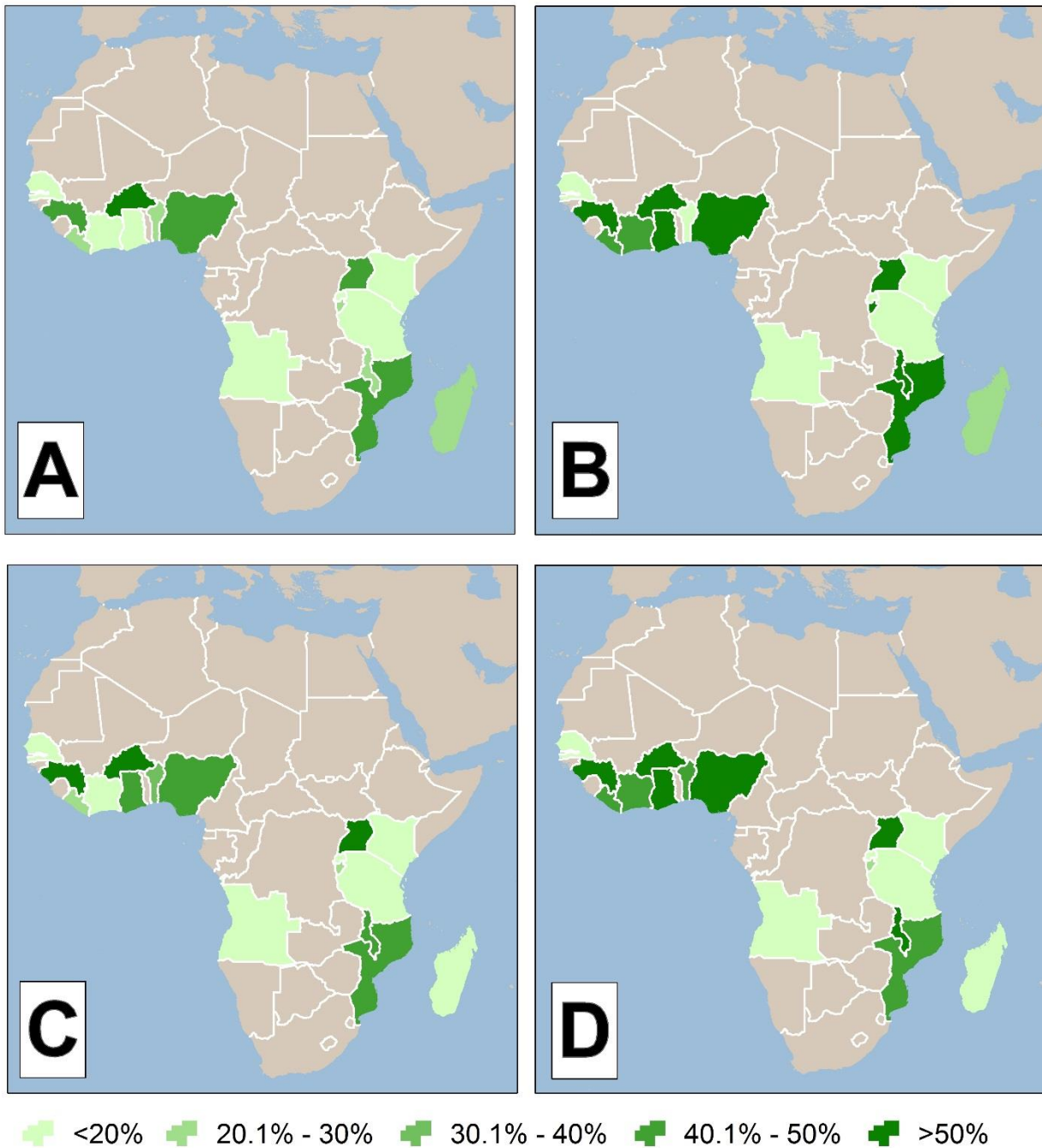

Note: Data from the most recent survey is presented in countries with more than one national survey (Liberia, Madagascar, Rwanda, and Senegal)

The maps in Figure 20 compare malaria prevalence estimates by diagnostic test and by antimalarial treatment history in children 6-59 months of age with recent fever. Darker colors indicated higher prevalence estiamtes. Comparing map A and map B shows spatially the countries in which microscopy estimates were lower than RDT estimates for malaria prevalence in children with recent fever who took antimalarials. Map C compared to Map D shows the difference in microscopy and RDT estimates for malaria prevalence in children with recent fever who did not take antimalarials. Looking at Map A and Map C, comparisons of microscopy estimates of malaria prevalence in children with recent fever who took antimalarials and those who did not is possible. A similar comparison can be made for RDT estimated malaria prevalence using Map B and Map D.

A scatter-plot of national malaria parasitemia estimates as diagnosed by microscopy versus by RDT among the study population stratified by antimalarial treatment status among those with recent fever shows that the diagnostic tests were more similar among children who had not received antimalarials than among those with who did (Figure 21). The best-fit lines in the figures show that RDT results reported higher malaria parasitemia prevalence than did microscopy in all children (shown by best-fit lines being above the equality line), but that the difference was more extreme in children who recently took antimalarials for fever than in those who did not. The correlation between microscopy prevalence and RDT prevalence was lower in treated children than in untreated children at the national level (R2 =0.71 vs. R2 =0.93).

Figure 21. Malaria parasitemia prevalence in children 6-59 months with recent fever in national surveys as estimated by RDT and by microscopy stratified by antimalarial treatment status
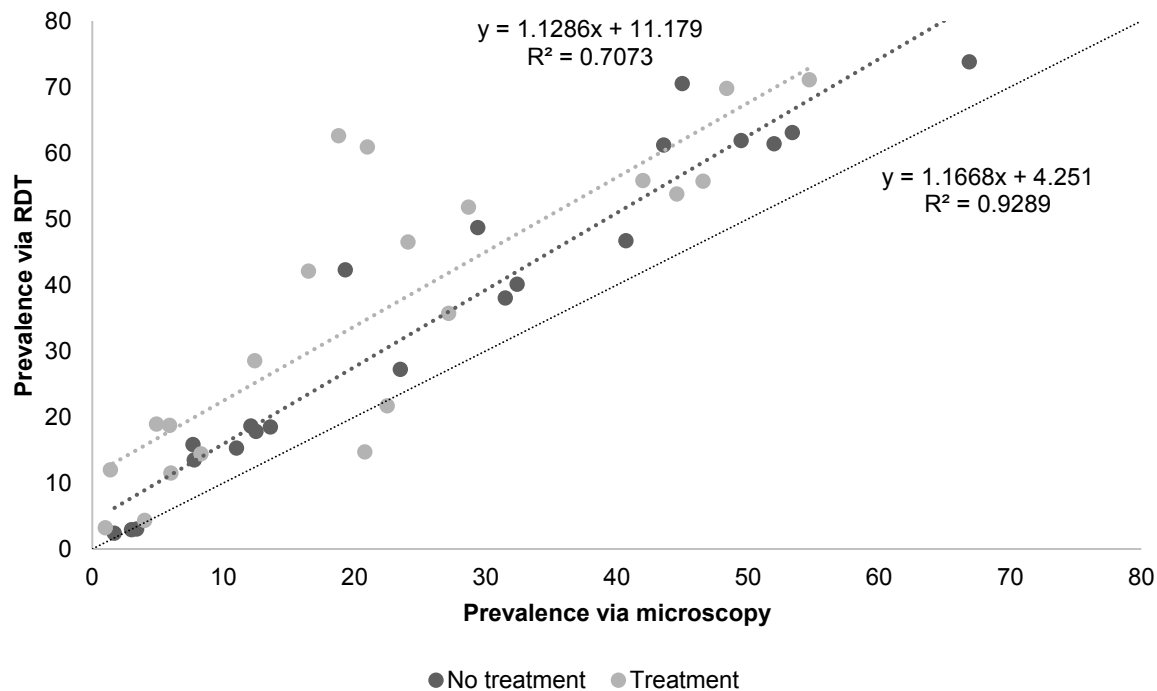
Note: Thin dotted line represents equality in RDT and microscopy results

Observed percent agreement was lower in all but three surveys in children with a recent fever who took antimalarial treatment than in those who did not (Figure 22, Table 8): Benin 2011-12 DHS, Rwanda 2007-08 IDHS, and Senegal 2008-09 MIS. Kappa statistics were also lower in children with a recent fever who took antimalarial treatment than in those who did not in all but Senegal 2008-09 MIS and Rwanda 2007-08 IDHS (Figure 23, Table 8). The largest differences in Kappa statistics between children with recent fever

who took antimalarials and those who did not were observed in Burundi 2012 MIS (0.29), Rwanda 2010 DHS (0.31), Malawi 2012 MIS (0.39) and Kenya 2010 MIS (0.39). Patterns in AUC from ROC analysis are more difficult to determine (Figure 24, Table 8). The largest differences between children with recent fever who took antimalarials and those who did not were observed in the Senegal 2010-11 DHS (0.22) and the Rwanda 2010 DHS (0.14); however, in this case the treated children had higher AUC than the untreated children. In the majority of surveys, AUC was larger in the untreated strata than in the treated strata, with the greatest differences in Malawi 2012 MIS (0.13) and Burundi 2012 MIS (0.11).

Figure 22. Percent agreement between estimates of malaria parasitemia prevalence as measured by RDT and by microscopy in children 6-59 months with recent fever who reported antimalarial treatment compared to those who did not and the difference in percent agreement between the two strata
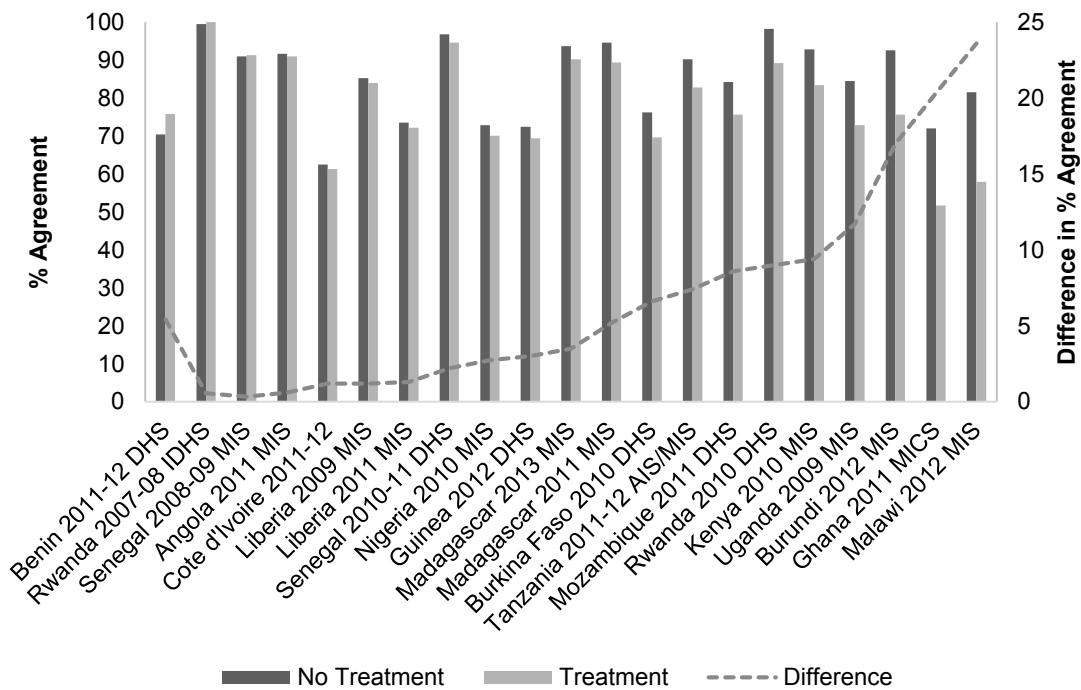
Figure 23. Kappa statistics of estimates of malaria parasitemia prevalence as measured by RDT and by microscopy in children 6-59 months with recent fever who reported antimalarial treatment compared to those who did not and the difference in Kappa statistics between the two strata
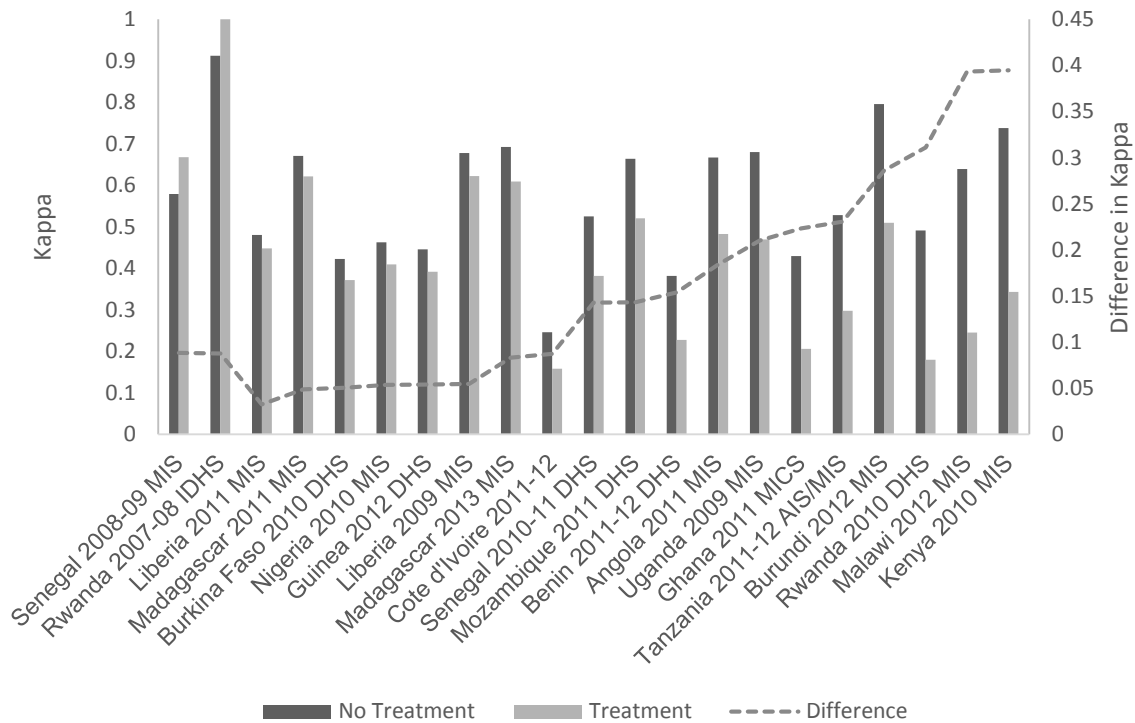
Figure 24. Area Under the Curve from ROC analysis of estimates of malaria parasitemia prevalence as measured by RDT and by microscopy (gold standard) in children 6-59 months with recent fever who reported antimalarial treatment compared to those who did not and the difference in AUC between the two strata
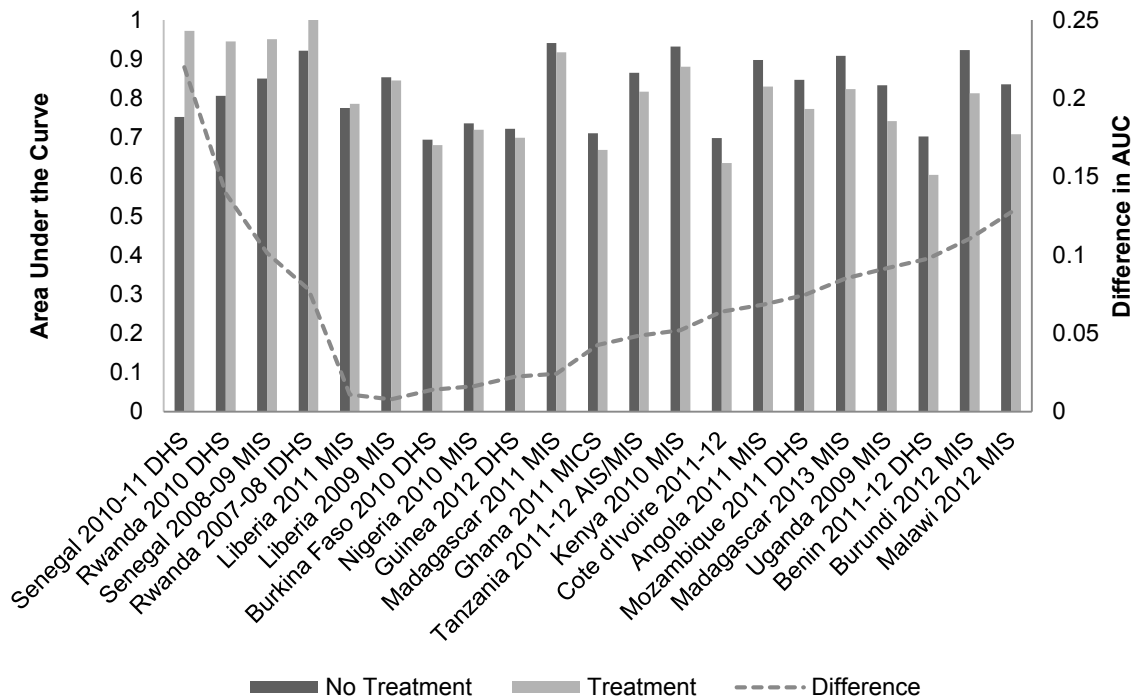
3.1.4   Stratified Analysis: WHO regional trends

Regional differences in agreement between diagnostic tests were also examined. Each survey was categorized by WHO region (WHO 2014). In this analysis surveys from the Central (2), the East (9) and the West (11) regions of Sub-Saharan Africa (SSA) were included. Due to the small number of Central African surveys the Central and East African surveys were combined. Figure 25 shows the correlation between microscopy results and RDT results by region. The diagnostic test results from the Central and East regions were highly correletated ($R^2 = 0.96$), whereas the two tests were less strongly correleted in the West African surveys ($R^2 = 0.80$).

Figure 25. Malaria parasitemia prevalence as estimated by RDT and microscopy by region of survey within Sub-Saharan Africa
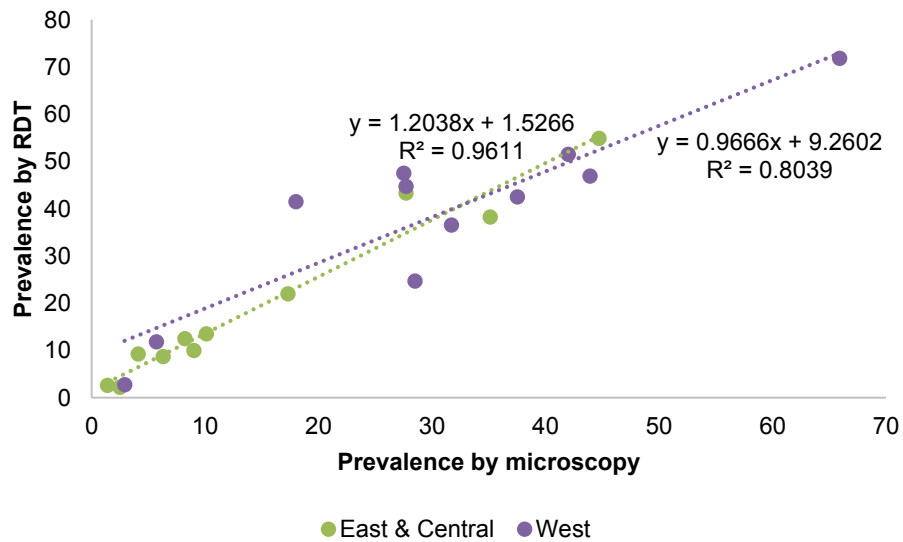
3.1.5   Stratified Analysis: subnational regional trends

Although the datasets used for these analyses come from nationally-representative surveys, most are sampled to produce representative estimates at the regional level as well. Figure 26 presents a visual depiction of the difference between RDT and microscopy malaria prevalence estimates at the regional level. As regional sample sizes are smaller than national samples, the estimates of malaria prevalence are less precise; however the map highlights some interesting trends. In general, the difference in RDT and microscopy estimates is highest in West Africa, in areas along the coast. Large differences are also seen in one coastal region in Tanzania. Similarly, the regions in which microscopy estimates of malaria prevalence are greater than RDT estimates are focused in West Africa. It should be noted that these are absolute differences and are thus likely to be smaller in areas with low malaria prevalence. This map helps to highlight areas in which further quality assurance of test results may be necessary.

Figure 26. Difference in malaria parasitemia prevalence as estimated by RDT and microscopy by subnational region
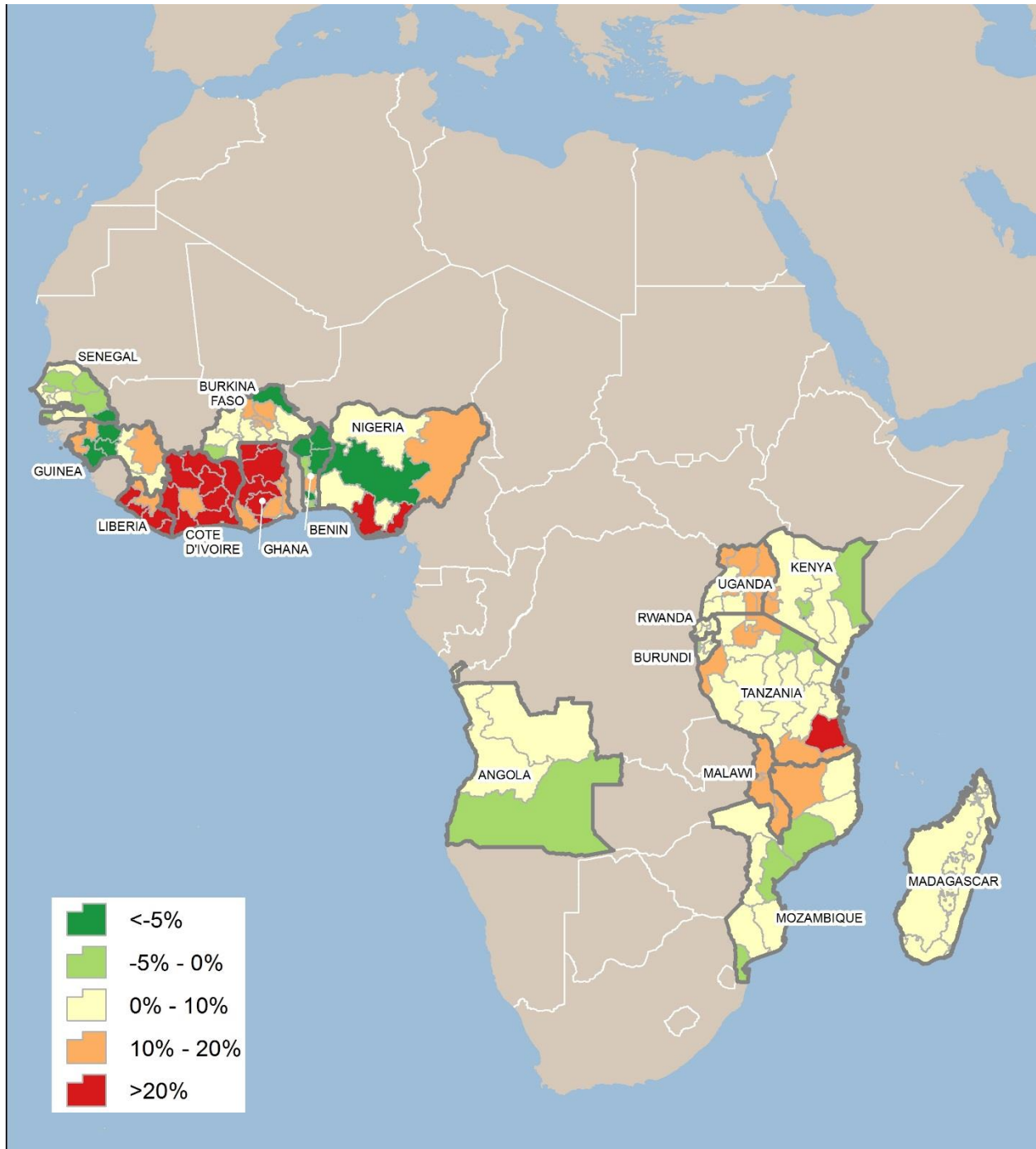
3.2 Change over time

3.2.1 *National-level change*

Four of the countries included in these analyses have had two surveys in which both microscopy and RDT have been used to test for malaria parasitemia. It is therefore possible to look at trends over time in these four countries. Figure 27 compares the microscopy estimates and the RDT estimates of malaria prevalence in two surveys from Liberia, Madagascar, Rwanda and Senegal.

Figure 27. Change in malaria parasitemia prevalence over time as estimated by RDT and microscopy
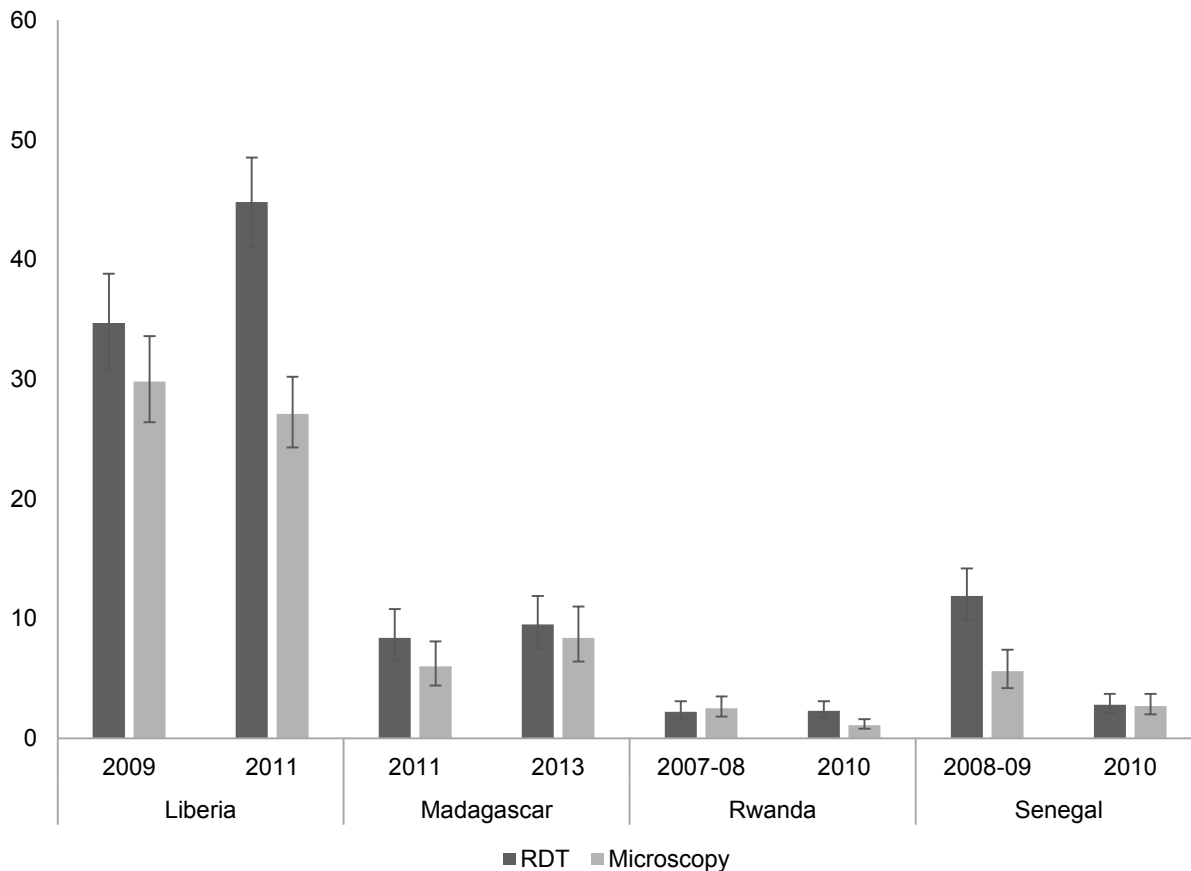
These trends are difficult to interpret. In Senegal, it appears that malaria parasitemia has declined significantly between the two surveys (despite a very short time period between the two). However, these two surveys were conducted during different periods in the malaria transmission cycle; the 2008-09 MIS was fielded between December and January whereas the 2010 DHS was conducted from October to April. In Liberia, different conclusions can be drawn depending on whether RDT or microscopy data are used to estimate malaria parasitemia. From RDT results it appears that parasitemia prevalence has increased significantly between 2009 and 2011; however microscopy results do not indicate any significant change. The Liberia surveys were also conducted during different periods, with the 2009 survey occuring between December and March and the 2011 survey between September and December. No change in parasitemia is apparent in Madagascar, where the two surveys were fielded during the same months (April-June and March-June). Rwanda also did not show any significant changes in parasitemia between the two surveys; however, the malaria prevalence is so low in Rwanda that a very large sample size would be required in

order to detect meaningful change. For this reason the Rwanda 2013 MIS did not collect blood for malaria parasitemia testing. Another factor that may influence the difference in prevalence estimates is the specific RDT used in each survey. In both Liberia and both Senegal surveys HRP2-based tests were used. In Madagascar, an HRP2-based test was used in 2011 and a combo test in 2013. In Rwanda an LDH-based test was used in 2007-08 followed by an HRP2 test in 2010.

3.2.2   *Regional-level change*

Looking at patterns in regional RDT and microscopy test results in one country over the course of two surveys permits a more nuanced examination. Comparing the 2009 MIS and the 2011 MIS in Liberia we see that patterns in test results varied significantly by region (Figure 28). Malaria parasitemia prevalence as estimated by RDT increased significantly between surveys in the South regions but nowhere else. Significant increases in microscopy prevalence were only seen in South eastern B, whereas declines in microscopy prevalence were seen in Monrovia. A much greater discrepancy in test results occurred in 2011 as compared to 2009; none of the regions had RDT and microscopy prevalence results that differed significantly in 2009 but all regions except Monrovia had significant discrepancies in 2011.

Figure 28. Regional RDT and microscopy estimates of malaria prevalence in Liberia from 2009 and 2011 MIS
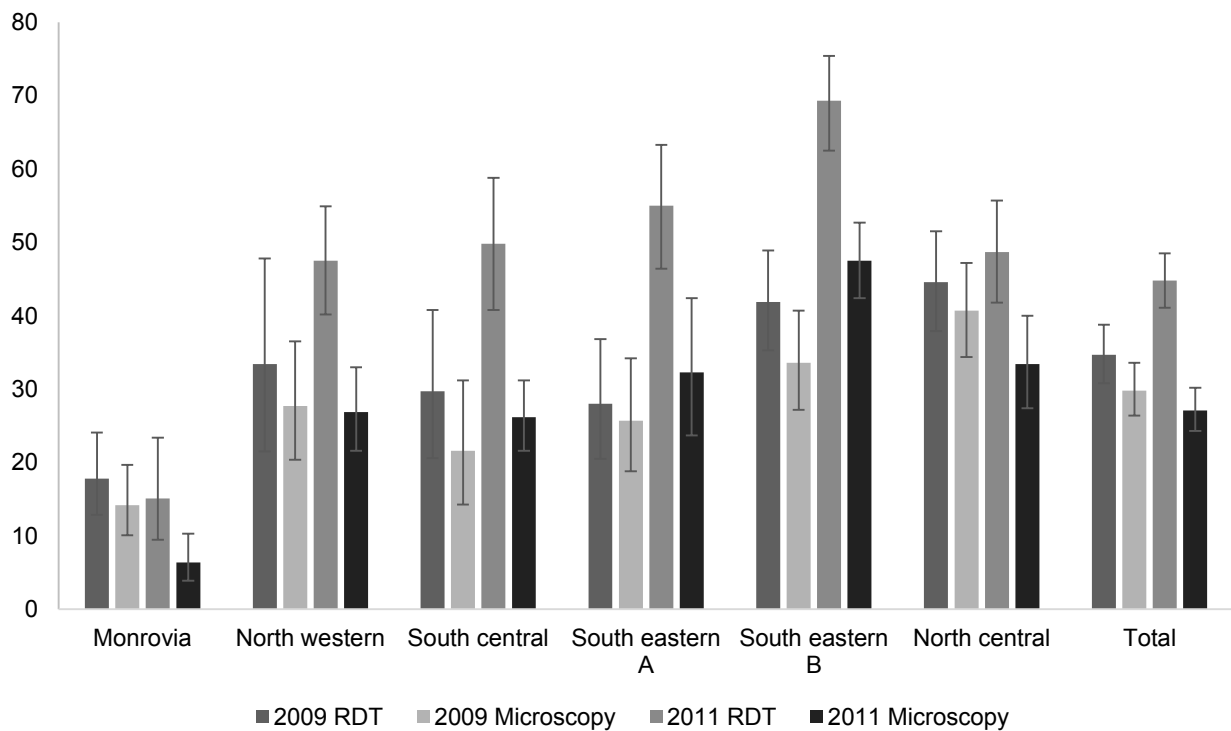
3.3 Pooled Analyses

3.3.1 Summary statistics

Survey data were pooled and summary statistics were run on the pooled sample. This included estimates of malaria parasitemia prevalence as measured by RDT and by microscopy as well as agreement measures, such as percent agreement and kappa statistics. Assuming microscopy to be the gold standard, senstivity, specificity and positive predictive values were calculated as was the AUC from ROC analysis. Results are summarized in Table 9.

Table 9. Pooled, multivariable regression model of discordant test results in children age 6-59 months who were tested for malaria via microscopy and RDT

Survey	Microscopy Prevalence	% Agree-ment	Kappa	AUC	Sensi-tivity	Speci-ficity	PPV	NPV
Fever	27%	81.9	0.59	0.84	88.0%	79.7%	61.1%	94.8%
No Fever	19%	87.9	0.65	0.85	80.8%	89.6%	65.2%	95.1%
Antimalarials	28%	75.4	0.49	0.79	87.9%	70.5%	53.7%	93.7%
No antimalarials	26%	85.4	0.65	0.86	88.1%	84.4%	66.3%	95.3%
Total	22%	86.2	0.63	0.85	83.2%	87.1%	64.0%	95.0%

The pooled dataset showed malaria prevalence as measured by microscopy to be higher in children with recent fever compared to children without recent fever (27% vs. 19%) but similar in children who had taken antimalarials compared to those who had not (28% vs. 26%). Test agreement was lower in children who had a recent fever than in those who did not (percent agreement 82% vs. 88%; kappa 0.59 vs. 0.65), but AUC values were similar (0.84 vs. 0.85). Agreement was also lower in children who took antimalarials than in children who did not (percent agreement 75% vs. 85%; Kappa 0.49 vs 0.65; AUC 0.79 vs. 0.86). RDTs were found to be more sensitive to true infection as determined via microscopy in children with recent fever than in those without (88% vs. 81%) but less specific (80% vs. 90%). The positive predictive value of RDTs was 61% in children with recent fever compared to 65% in children without. The nagtive predictive value was similar for both groups (95%). Interestingly the sensitivity of the RDT results compared to microscopy for children who had recently taken antimalarials and those who had not was similar (88% for both) but the specificity was significantly lower in the children who had recently taken antimalarials (71% vs. 84%). The positive predictive value was lower in this group as well (54% vs. 66%) although the negative predictive value was similar (94% vs. 95%).

3.3.2 Multivariable regression

The pooled dataset was also used to run an individual-level regression model with test discordance as an outcome variable (Table 10). The model revealed that discordant test results were more likely in children from rural households than those from urban households (OR = 1.2, p=0.001). The odds of a child having discordant test results were significantly lower in children from the highest wealth quintile compared to the lowest (OR = 0.46, p<0.001). Children 24-59 months of age had higher odds of discordant test results than did younger children (OR = 1.2, p<0.001). The sex of the children did not have a significant effect on discordant microscopy and RDT test results. Children who had a fever in the two weeks immediately preceding the survey were significantly more likely to have discordant test results than were children with no recent history of fever (OR=1.5, p<0.001).

Table 10. Pooled, multivariable regression model of discordant test results in children age 6-59 months who were tested for malaria via microscopy and RDT

	Odds Ratio	LCI	UCI	p-value
Residence				
Urban	ref			
Rural	1.20	1.08	1.35	0.001
Wealth Quintile				
Poorest	ref			
Poorer	0.86	0.80	0.93	<0.001
Middle	0.78	0.71	0.85	<0.001
Richer	0.72	0.65	0.79	<0.001
Richest	0.46	0.40	0.53	<0.001
Age				
6-23 months	ref			
24-59 months	1.20	1.14	1.27	<0.001
Sex				
Male	ref			
Female	0.95	0.91	1.01	0.084
Recent Fever Status				
No	ref			
Yes	1.54	1.45	1.63	<0.001
Total N				**78388**

* Adjusted for a dummy variable for survey. LCI = lower confidence interval; UCI = upper confidence interval; N = weighted sample size. Mali is not included as questions permitting calculation of a wealth index were not asked.

Another model was run for the subset of children 6-59 months of age who were tested for malaria parasitemia by both diagnostic tests and who had reported a history of fever within the two weeks preceding the survey (Table 11). This was done in order to look at the affect of antimalarial treatment on discordance of test restults. Similar results were found in this model as in the previously described model; discordant test results were more likely in children from rural households than those from urban households although this affect was only marginally significant (OR = 1.2, p=0.075). The odds of a child having discordant test results were significantly lower in children from the highest wealth quintile compared to the lowest (OR = 0.55, p<0.001). Children 24-59 months of age had higher odds of discordant test results than did younger children (OR = 1.2, p<0.001). The sex of the children did not have a significant effect on discordant microscopy and RDT test results. Children who received antimalarial treatment in the two weeks immediately preceding the survey were significantly more likely to have discordant test results than were children with no recent history of fever (OR=1.6, p<0.001).

Table 11. Pooled, multivariable regression model of discordant test results in children age 6-59 months who were tested for malaria via microscopy and RDT.

	Odds Ratio	LCI	UCI	p-value
Residence				
Urban	ref			
Rural	1.16	0.98	1.38	0.075
Wealth Quintile				
Poorest	ref			
Poorer	0.89	0.78	1.00	0.059
Middle	0.83	0.72	0.96	0.015
Richer	0.80	0.69	0.95	0.008
Richest	0.55	0.43	0.69	<0.001
Age				
6-23 months	ref			
24-59 months	1.21	1.10	1.33	<0.001
Sex				
Male	ref			
Female	0.96	0.88	1.05	0.378
Antimalarial Treatment Status				
No	ref			
Yes	1.57	1.42	1.73	<0.001
Total N				**21323**

4   Discussion

This study clarifies the challenges to using estimates of malaria parasitemia prevalence from national surveys for demonstrating impact and identifies factors related to discordant RDT and microscopy test results.

The choice of RDT has implications for levels of expected agreement. HRP2-based RDTs detect residual antigenemia and can produce false positive results after effective antimalarial treatment. Several studies have shown HRP2 RDTs to overestimate malaria parasite prevalence by 1.5% to 10% (Endeshaw et al. 2008, Keating et al. 2009, Hawkes et al. 2014). Interpretation of these estimates as well as PPV, NPV, AUC and other such agreement statistics depends on the chosen gold standard. While microscopy can be the more sensitive and specific of the two tests given skilled microscopists, good equipment and high quality smears, many studies have found the skills of local microscopists under field conditions to be varied or subpar (Stow, Torrens, and Walker 1999, Wongsrichanalai et al. 2007, Maguire et al. 2006). Using potentially inaccurate test results as gold standards makes interpretation of discordance and other measures of agreement challenging to interpret.

Agreement between microscopy and RDT results varied by prevalence of infection, by recent fever status, and by history of antimalarial treatment. Low prevalence settings are challenging environments for detection of *Plasmodium* spp. parasites for both microscopy and RDT tests. Other tools, such as PCR or LAMP may be more useful in these settings (Aydin-Schmidt et al. 2014). Recent fever is a non-specific symptom of malaria infection. Thus children reporting fever would be more likely than those without fever to have suffered from malaria in the previous two weeks. The pooled dataset confirmed this with malaria prevalence as measured by microscopy to be 27% in children with recent fever and 19% in children without recent fever. Presumably, children with malaria are more likely than those without malaria to be given antimalarial treatment but this cannot be tested with survey data as questions on antimalarial treatment are only asked about children with recent fever. Test agreement was lower in children who took antimalarials than in children who did not. This is expected due to the residual antigenicity that can cause positive HRP2-based RDT test results following successful antimalarial treatment. Aydin-Schmidt and colleagues found the median antigen clearance time to be 28 days for HRP2-based RDTs and 7 days for LDH-based RDTs (Aydin-Schmidt et al. 2013). Similarly, specificity was found to be affected by time after treatment, with HRP2 tests reaching 87% only after 42 days (Aydin-Schmidt et al. 2013). This could explain the relatively low specificity values observed in children who had recently taken antimalarials given that the time frame for fever and antimalarial treatment captured in the survey data is 14 days and most surveys used HRP2-based RDTs.

Similar to our results, in an analysis of malaria parasitemia diagnostic testing in Luangwa District of Zambia, RDT discordance was found to be highest among older children, and in those who reported having received antimalarial treatment. Regional variation was also observed (Keating et al. 2009) supporting our findings that prevalence and discordance varied substantially on a regional scale. Although Keating and colleagues interpreted their study results to mean that RDTs should not be used in place of microscopy for surveillance in survey settings, they assume microscopy to produce more accurate measures of true infection status, an assumption that may not be true in many survey settings. For example, de Oliveira and colleagues found RDTs to be more sensitive and specific than microscopy performed by health facility staff as part of routine malaria case management activities (sensitivity: 92% vs. 53%; specificity: 97% vs. 77%) (de Oliveira et al. 2009).

Interpreting results in the absence of a true gold standard is challenging. To the extent that microscopy results and RDT results agree, the true disease state is more certain. To the extent that the two tests disagree, it becomes challenging to decipher which represents the truth. That discordance is affected by many factors

including the type of RDT, the level of malaria transmission, the age of the study subjects, as well as the underlying quality of the training and equipment of the personnel further complicates interpretation. In countries with two national surveys collecting RDT and microscopy data, conclusions about whether significant change in malaria prevalence had occurred over time depended on which test results were used. Even at the regional level in Liberia, the change in malaria parasitemia prevalence depended on the diagnostic test used. In the four countries examined in this study, the time between surveys was two to three years. It is unlikely that substantial change in national malaria prevalence would be expected over this time interval. Given these results, the use of malaria parasitemia data from national surveys should be reexamined and a goal clearly defined. Perhaps on a global scale, these data can help assess the overall level of malaria infection and could be used at the national level to target resources and interventions to high risk areas or populations. However, attempts to use these data to measure the impact of malaria control programs at the national level should consider the level of uncertainty and discordance in the prevalence estimates.

This study was constrained by some limitations. Although The DHS Program uses standardized tools for training and data collection, some differences across surveys could affect the results of this study. For example, there was a range of RDTs used, some HRP2-based and some HRP2/LDH combo tests. In addition, the quality of microscopy likely varied between countries. Data were collected from surveys fielded during different seasons, which could have an effect on prevalence estimates and on levels of fever and antimalarial treatment. Ideally, data would be collected during the high malaria transmission season in order to avoid bias from seasonal variation. The magnitude of seasonal variation in parasite prevalence within a year can be significant, as was shown in a study in Chikhwawa District in Malawi (Roca-Feltrer et al. 2010). Finally, the analyses did not consider the effect of level of malaria transmission. Future analyses might include measures of malaria transmission indices to determine the effect of malaria endemicity on discordance between different malaria diagnostic tests. These types of analyses would likely need to be done on a small geographic scale, due to the often heterogeneous endemicity levels of malaria within many countries. Future work might focus on developing decision-making tools for program managers who may have data collected with a variety of diagnostic tests or during different seasons.

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