Use of Rapid Diagnostic Tests in Malaria School Surveys in Kenya: Does their Under-performance Matter for Planning Malaria Control?

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Abstract. Malaria rapid diagnostic tests (RDTs) are known to yield false-positive results, and their use in epidemiologic surveys will overestimate infection prevalence and potentially hinder efficient targeting of interventions. To examine the consequences of using RDTs in school surveys, we compared three RDT brands used during a nationwide school survey in Kenya with expert microscopy and investigated the cost implications of using alternative diagnostic approaches in identifying localities with differing levels of infection. Overall, RDT sensitivity was 96.1% and specificity was 70.8%. In terms of classifying districts and schools according to prevalence categories, RDTs were most reliable for the <1% and >40% categories and least reliable in the 1–4.9% category. In low-prevalence settings, microscopy was the most expensive approach, and RDT results corrected by either microscopy or polymerase chain reaction were the cheapest. Use of polymerase chain reaction–corrected RDT results is recommended in school malaria surveys, especially in settings with low-to-moderate malaria transmission.

INTRODUCTION

Rapid diagnostic tests (RDTs) based on malaria parasite antigen detection are now a key tool in the case management of clinical malaria, especially at lower level peripheral health facilities where routine microscopy is absent or of poor quality. The RDTs are also shown to be more cost-effective in improving health outcomes than expert microscopy in most sub-Saharan African settings. In addition to their clinical use, RDTs are increasingly being used in epidemiologic surveys of Plasmodium spp. infection as part of national monitoring and evaluation efforts. For example, of the 27 recent national malaria indicator surveys conducted in sub-Saharan Africa since 2006, RDTs were used in 19 surveys, and in 3 of these surveys Plasmodium spp. infection prevalence was estimated on the basis of RDTs alone. The use of RDTs in large-scale surveys is preferable for therapeutic reasons because they provide point-of-contact diagnosis and, if required, immediate treatment. Moreover, RDTs overcome the human and technical capacity constraints faced by large-scale surveys in the use of expert microscopy in terms of quality staining and reading of thousands of blood slides and the logistics and costs associated with slide transportation, preparation, duplicate reading, and quality assurance.

A well-recognized limitation of RDTs, especially those tests that detect the parasite antigen histidine-rich protein 2 (HRP-2) specific to Plasmodium falciparum, is the occurrence of false-positive results caused by persistent antigenemia even after effective anti-malarial treatment. Although such false-positives results for RDTs may have limited relevance for clinical case management, they will overestimate the true parasite prevalence compared with expert microscopy or molecular parasite detection techniques. This overestimation principally occurs because RDTs that detect HRP-2 antigen cannot distinguish between active infections and resolved infections because of persistent antigenemia. Therefore, the observed prevalence may be indicative of prevalence over a period of time rather than point prevalence. Previous evaluations of RDTs in population-based household surveys among healthy persons in Ethiopia and Zambia reported rates of false-positivity of 1.5% and 7.9%, respectively when compared with microscopy. Such findings raise an important operational question: do false-positive results associated with RDTs matter when it comes to stratifying areas according to malaria risk in the geographic targeting of malaria intervention strategies?

The answer to this question determines whether malaria control can be guided by community or school-based surveys using RDTs alone.

To resolve the question of the usefulness of using RDTs in school malaria surveys there is a need for understanding two issues: 1) what is the occurrence of areas being misclassified in terms of intervention strategy when based on surveys using only RDTs compared with surveys using expert microscopy; and 2) what are the cost implications of different diagnostic approaches used in school malaria surveys to guide malaria control? To help address these issues, we examined the performance of three RDTs used during a nationwide school malaria survey in Kenya and investigated the cost implications of alternative diagnostic strategies, including RDTs, microscopy-corrected RDT results, expert microscopy, and polymerase chain reaction (PCR), for use in future monitoring and evaluation approaches that focus on sentinel schools.

METHODS

Malaria surveys were undertaken in 480 schools across Kenya during September 2008–March 2010, as described in detail elsewhere. In brief, 11 boys and 11 girls were randomly selected in classes 2–6 to achieve a target sample of 110 children from each school. Ethical approval for the school surveys

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was obtained from the Kenya Medical Research Institute and Scientific and Ethics Review Committees. Consent for participation was based on passive, opt-out consent by parents rather than written, opt-in consent because of the routine, low-risk nature of the surveys that were conducted under the mandate of the Ministry of Public Health and Sanitation to conduct disease surveillance. Individual assent from the students was obtained before sample collection.

**Survey procedures.** In all schools, students were asked to provide a finger prick blood sample, which was used to assess *Plasmodium* spp. infection in the peripheral blood using RDTs. Four types (three brands) of RDTs were used in the surveys, depending on availability. The first test was the OptiMal-IT (DiaMed, AG, Cressier, Switzerland), which uses monoclonal antibodies against the metabolic parasitic enzyme lactate dehydrogenase of *Plasmodium* spp., one specific for *P. falciparum* and another pan-specific for *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. The second and third tests were the Paracheck-Pf device and Paracheck-Pf dipstick (Ochrid Biomedical Systems, Goa, India), which detect the *P. falciparum* HRP-2. The fourth test was the CareStart Pf/Pv combo (Access Bio, Boyce, VA), which uses monoclonal antibodies specific for *P. vivax* lactate dehydrogenase and *P. falciparum* HRP-2.

The number of children examined by using each of the different RDT types by prevalence category is shown in Table 1. However, CareStart RDTs were found to have very poor specificity (38.1%) compared with microscopy; results were200 leukocytes), assuming a leukocyte count of 8,000 cells/mL. A smear was considered negative after reviewing 100 high-powered fields. Thin blood smears were reviewed for species identification. Two independent microscopists read the slides, and a third microscopist resolved discrepant results. A total of 612 (10.2%) slides had to be re-stained before a third reading was performed because of poor-quality staining. The poorly stained slides were immersed in xylene to remove immersion oil, discolored using acetone, and re-stained with 3% Giemsa stain for 45 minutes.

**Diagnostic performance of RDTs among persons.** The diagnostic performance of the three different RDTs (OptiMal-IT, Paracheck-Pf device, and Paracheck-Pf dipstick) in detecting infection was compared with the assumed gold standard of expert microscopy, which is the approach commonly adopted by national malaria control programs. At the individual level, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and false-positive rate (FPR), and their 95% confidence intervals (CIs) were calculated by using the `diagt` command in Stata version 11.0 (StataCorp LP, College Station, TX).

**Classification of districts and schools by using RDTs.** We compared the use of RDTs alone in classifying districts and schools according to specified prevalence categories against RDT results corrected by expert microscopy. Currently, the Kenya national malaria control program categorizes districts into one of four malaria zones: stable transmission, seasonal transmission, epidemic-prone, and low risk.15 We used a slightly different classification based on prevalence of *Plasmodium* spp. infection: < 1%, 1–4.9%, 5–39%, and ≥ 40%. These categories reflect underlying differences in the population dynamics and intensity of malaria transmission useful for selecting control strategies16,17 and the mixture of interventions estimated to have maximal impact.17,18 Such control-related endemicity classifications are important for malaria control programs because they determine the expected impact of intervention (because of the underlying basic reproductive number) and influence the choice of control interventions. For example in areas where infection prevalence is ≥ 40%, studies suggest that a combination of universal coverage with insecticide-treated nets and complementary control interventions is necessary to interrupt transmission17 and in areas of low transmission settings a single intervention strategy may suffice. For simplicity, we assume that the schools surveyed in each district provide a representative sample to estimate prevalence in each district, and that district-level prevalence is calculated as follows: total number of children found to be positive in the district/total number children examined in the district. On this basis, sensitivity was calculated as the percentage of districts in a given prevalence category that were correctly classified as such, and specificity was calculated as the proportion of districts not in a given

<table>
<thead>
<tr>
<th><em>Plasmodium</em> spp. prevalence (%) category</th>
<th>Paracheck <em>Pf</em> device</th>
<th>Paracheck <em>Pf</em> dipstick</th>
<th>OptiMal-IT <em>Pf</em>/PAN</th>
<th>CareStart <em>Pf</em>/Pv</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–0.9</td>
<td>16,549 (48.4)</td>
<td>5,303 (15.5)</td>
<td>3,924 (11.5)</td>
<td>8,436 (24.7)</td>
<td>34,212</td>
</tr>
<tr>
<td>1–4.9</td>
<td>8,436 (47.8)</td>
<td>1,073 (16.9)</td>
<td>1,833 (28.9)</td>
<td>408 (6.4)</td>
<td>6,344</td>
</tr>
<tr>
<td>5–39.9</td>
<td>5,538 (68.2)</td>
<td>324 (4.0)</td>
<td>2,044 (25.2)</td>
<td>220 (2.7)</td>
<td>8,126</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>1,209 (100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1,209</td>
</tr>
</tbody>
</table>

*Values are no. (%). RDT = rapid diagnostic test; *Pf* = *Plasmodium falciparum*; *Pv* = *P. vivax.*
prevalence category correctly classified as such. The PPV was calculated as the proportion of districts in a given category by RDTs that were correctly identified, and NPV was calculated as the proportion of districts not to be in a given category by RDTs that were correctly identified as such. Ninety five percent exact binomial CIs were calculated.

The performance of RDTs at the school-level was first investigated by plotting a cumulative plot of school prevalences based on RDTs alone and on microscopy-corrected results. Sensitivity, specificity, PPV, and NPV at the school-level were calculated on the same basis as the district-level analysis.

Cost analysis of alternative diagnostic strategies. Six alternative diagnostic strategies were evaluated. These methods were 1) use of RDTs alone, 2) expert microscopy alone, 3) slide-corrected RDT results based on microscopy of all RDT-positive results and an equal sample of RDT-negative results, 4) PCR-corrected RDT results based on PCR of all RDT-positive results and an equal sample of RDT-negative samples, 5) RDT and expert microscopy of all samples, and 6) RDT and PCR of all samples.

The financial costs associated with the RDT and microscopy diagnostic strategies were based on our experience of conducting the school surveys in Kenya. The PCR costs were estimated based on the assumption that outsourcing PCR readings would cost US $5 per sample examined (Drakeley C, unpublished data). In estimating the costs of microscopy, it was assumed that 18% of the slide readings would be discrepant and therefore need to be examined by a third reader, as observed in the present study. For the PCR-corrected RDT approach, it was assumed that all RDT-positive samples would be examined individually by using PCR, and RDT-negative samples would be combined into pools of five samples to help reduce costs.\textsuperscript{18} For simplicity, the costs of RDTs were based on the average cost of Paracheck-Pf RDTs (US $1.40) from our procurement experience during the school surveys.

To calculate the costs associated with PCR-corrected RDT and RDT plus PCR for all samples, we adopted a conservative estimate of RDT sensitivity (80%) and specificity (60%) estimates based on results from published studies that have compared Paracheck-Pf RDTs and PCR.\textsuperscript{20,21} The number of true-positive (TP) results, false-positive (FP) results, true-negative (TN) results, and false-negative (FN) results at different levels of infection prevalence were calculated according to the equations $TP = SpN$, where $Se$ is the sensitivity, $p$ is prevalence, and $N$ is the number of children per school; $FP = (1 - Sp) (1 - p)N$, where $Sp$ is specificity; $TN = (1 - Se)SpN$; and $FN = (1 - p)SpN$. As a simplification, we assume that a sample of 110 children per school would be included in the survey, and the sensitivity and specificity remained constant at all prevalence levels. The costs of the microscopy-corrected RDT and PCR-related diagnostic strategy are assumed to vary with the proportion of children who are RDT positive.

Relevant unit costs of the different diagnostic approaches were identified according to a standard ingredients-based approach to costing.\textsuperscript{22} The quantity and cost of each ingredient was identified from the project accounting systems and interviews with survey staff. Ingredient items were divided into staff, capital, and consumables. Capital costs such as the costs of netbook computers and freezer, were annualized over the estimated useful life of the survey equipment using a discount rate of 3%, in line with World Health Organization (WHO) recommendations (Supplemental Table 1).\textsuperscript{23} Useful lives for the capital items were taken from either the WHO-CHOICE initiative estimates for Kenya or interviews with survey staff. To estimate the cost of the items per school, it was assumed that all capital items, with the exception of the freezer, would be used on a per team basis and each team would be able to visit 40 schools per term or survey phase. Thus, capital costs were divided across 40 schools. For the freezer, it was assumed that it could store samples from 200 schools and therefore the cost was divided across 200 schools. An average travel cost of 10,000 Kenya shillings (US$ 134.05) per day was assumed based on the cost of hiring a 10-seat vehicle for a day in Kenya in 2010. A 10% contingency allowance was also included. Costs were estimated in local currency and their current values were converted into equivalent US dollars by using an average exchange rate for the period between September 1, 2008 and February 20, 2010 of 74.6 Kenya shillings = US $1 (www.oanda.com). The unit costs are presented in Supplemental Table 1, assuming 7.6% infection prevalence, as observed in this study using RDTs alone.

Classification of district-level *Plasmodium* spp. infection prevalence by RDTs compared with microscopy-corrected RDT results according to prevalence category in school malaria surveys is shown in Supplemental Table 2. Classification of school-level *Plasmodium* infection prevalence by RDTs compared with microscopy-corrected RDT results according to prevalence category in school malaria surveys is shown in Supplemental Table 3.

**RESULTS**

A total of 49,891 school children, aged 5–18 years, in 480 schools participated in the surveys.\textsuperscript{14} Of these children, blood slides were examined by using microscopy for 6,017 children: 3,117 children who were RDT positive and 2,900 children who were RDT-negative. All slides were read twice, 1,125 slides (18.7%) were read by a third microscopist to resolve discrepancies, and 612 (10.2%) slides were re-stained because

### Table 2

<table>
<thead>
<tr>
<th>RDT type</th>
<th>No.†</th>
<th>RDT positive</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>All tests excluding CareStart</td>
<td>6,017</td>
<td>3,117</td>
<td>96.1 (95.6–96.6)</td>
<td>70.8 (69.7–72.0)</td>
<td>62.7 (61.5–63.9)</td>
<td>97.2 (96.8–97.7)</td>
</tr>
<tr>
<td>Paracheck <em>Pf</em> device</td>
<td>4,708</td>
<td>2,595</td>
<td>96.3 (95.7–96.8)</td>
<td>68.8 (67.5–70.1)</td>
<td>64.2 (62.9–65.6)</td>
<td>96.9 (96.4–97.4)</td>
</tr>
<tr>
<td>OptiMal</td>
<td>736</td>
<td>365</td>
<td>94.9 (93.3–96.5)</td>
<td>77.4 (74.4–80.5)</td>
<td>71.5 (68.3–74.8)</td>
<td>96.2 (94.9–97.6)</td>
</tr>
<tr>
<td>Paracheck <em>Pf</em> dipstick</td>
<td>573</td>
<td>157</td>
<td>96.3 (94.8–97.8)</td>
<td>76.0 (72.5–79.5)</td>
<td>16.6 (13.5–19.6)</td>
<td>99.8 (99.4–100)</td>
</tr>
</tbody>
</table>

*Values in parentheses are 95% confidence intervals. RDT = rapid diagnostic test; PPV = positive predictive value; NPV = negative predictive value.
†No. children tested for malaria.
of poor initial staining in the field. Of 6,017 slides microscopically examined, 2,034 (33.8%) were *Plasmodium* spp. positive.

**Performance of RDT at the individual level.** The overall prevalence of *Plasmodium* spp. infection was 7.6% (95% CI = 6.3–8.9%) on the basis of RDT results alone and 4.3% (95% CI = 3.3–5.2%) by microscopy-corrected RDT results. Diagnostic performance of RDTs, overall and by RDT type, is shown in Table 2. The overall sensitivity of RDTs alone was 96.1% (95% CI = 95.7–96.6%) and ranged from 94.9 to 96.3% according to RDT type. Overall specificity was 70.8% (95% CI = 69.7–72.0%). In terms of differences by RDT type, the Paracheck *Pf* device had the highest FPR (31.2%), and Opti-Mal had the lowest FPR (22.6%). Overall, the PPV was 62.7%; the PPV was lowest for the Paracheck *Pf* dipstick (16.6%) and NPV overall was 96%. A total of 80 (1.3%) RDT readings yielded false-negative results compared with microscopy; just more than half (52.5%) of the false-negative results showed a parasite density < 200 parasites/μL.

**Classification of districts and schools by prevalence class.** The proportion of districts correctly classified according to prevalence category based on RDT results compared with microscopy-corrected RDT results is shown in Table 3. Across all prevalence categories, 87.0% (60 of 69) of the districts were correctly classified by using results of RDTs alone. Correct classification was highest for districts in the < 1% and > 40% categories and lowest in the 1–4.9% category. Similarly, levels of sensitivity were highest in the < 1% and > 40% categories and lowest in the 1–4.9% category. Specificity was consistently high across all prevalence categories. The occurrence of false-negative results (estimated as 1 – sensitivity) was greatest in the 1–4.9% and 5–39.9% categories, and false-positive results were highest in the 5–39.9% category.

Estimated *Plasmodium* spp. infection prevalence in each school based on RDT results alone and on microscopy-corrected RDTs results is shown in Figure 1. The RDT-based *Plasmodium* spp. infection prevalence was systematically higher than the microscopy-corrected RDT prevalence. The degree of over-estimation was greatest in schools with a high prevalence of malaria, in which estimates based on the different diagnostic approaches span different prevalence categories. In 11 schools, estimated RDT-based *Plasmodium* prevalence was lower than estimates of prevalence based on microscopy-corrected RDT results. Overall, 81.6% of schools were correctly classified by RDTs (Table 4). Importantly, in terms of identifying schools with high (> 40%) prevalence, 100% (11 of 11) of schools were classified correctly.

### Table 3

<table>
<thead>
<tr>
<th><em>Plasmodium</em> spp. prevalence (%) category</th>
<th>Districts correctly classified (%)</th>
<th>RDT sensitivity (95% CI)</th>
<th>RDT specificity (95% CI)</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–0.9</td>
<td>42/44 (95.5)</td>
<td>95.5 (90.5–100)</td>
<td>100 (100–100)</td>
<td>100 (100–100)</td>
<td>92.6 (86.4–98.8)</td>
</tr>
<tr>
<td>1–4.9</td>
<td>5/10 (50.0)</td>
<td>50.0 (38.5–61.8)</td>
<td>96.6 (92.3–100)</td>
<td>71.4 (60.8–82.1)</td>
<td>91.9 (85.5–98.4)</td>
</tr>
<tr>
<td>5–39.9</td>
<td>11/13 (84.6)</td>
<td>84.6 (76.1–93.1)</td>
<td>91.1 (84.3–97.8)</td>
<td>68.8 (57.8–79.7)</td>
<td>96.2 (91.7–100)</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>2/2 (100)</td>
<td>100 (100–100)</td>
<td>97.0 (93.0–100)</td>
<td>50.0 (38.2–61.8)</td>
<td>100 (100–100)</td>
</tr>
</tbody>
</table>

* RDT = rapid diagnostic test; CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value.
Consistent with the district-level results, correct classification was worst in the 1–4.9% prevalence category. The false-negative rate (1 – sensitivity) was zero in the > 40% category and next lowest in the < 1% category; it was highest in the 1–4.9% category.

**Cost implications of using alternative diagnostic methods.** The cost of surveying one school using RDTs alone, microscopy-corrected RDT results, microscopy alone, and RDT and microscopy on all samples is shown in Figure 2A. Despite RDTs yielding a FPR of 29.2%, they were, unsurprisingly, the cheapest diagnostic strategy (US $660.89 per 110 children sampled per school) across all prevalence levels. The cost of microscopy-corrected RDTs (examining all RDT-positive results and an equal random selection of negative results by expert microscopy) was lower than using only microscopy where prevalence was ≤ 39%. After this point, it became more expensive than using microscopy alone. Costs of PCR-based approaches across the prevalence range is shown in Figure 2B, which shows that at low prevalence levels (< 11%), PCR-corrected RDT results and PCR plus RDT for all samples are cheaper diagnostic approaches than using microscopy only and microscopy plus RDTs for all samples.

**DISCUSSION**

The usefulness of malaria RDTs to estimate the prevalence of *Plasmodium* spp. infection in malariometric surveys will depend on their diagnostic performance, ability to correctly classify localities according to intensity of malaria transmission, and their costs relative to other diagnostic approaches. At the individual level, the current study found RDTs to have a sensitivity of 96.1% and a specificity of 70.8%, which is consistent with previous studies conducted among school-aged children.24,25 In terms of classifying localities according to infection prevalence, RDTs used in school-based surveys performed well in defining where prevalence was < 1%, characteristic of areas with low stable endemic control.26 In such areas of low transmission of malaria, malaria control programs may be more interested in ascertaining true absence of local transmission to support elimination strategies.27 This finding would require different population-based sampling strategies such as passive and active case detection, and combinations of diagnostic methods such as PCR or serologic analysis.

The results also suggest that RDTs performed poorest in the 1–4.9% prevalence category where half of the districts were classified to be in the 5–39.9% prevalence category. The poor performance of RDTs in the 1–4.9% prevalence category might reflect a statistical artifact of the narrow prevalence interval and the small numbers of districts/schools in the prevalence category, such that slight differences in prevalence may result in misclassification. However in such low-to-moderate transmission settings that characterize most of eastern and southern Africa,28 the results suggest that correction of RDT results by using pooled PCR is cheaper than using microscopy as is routinely done in population-based surveys in these regions.29 The use of RDTs to detect infection and pooled PCR to validate infection status has been used in various field based surveys, including the 2010 malaria indicator survey (MIS) in Swaziland,30,31 and has been shown to be reliable in detecting infections and having cost saving.

In the 2010 Swaziland MIS, in which the overall prevalence by RDTs was 0.2%, PCR pools of 25 samples each were used and only 2 of 162 pools tested positive, thereby greatly reducing diagnostic costs (> 95%) and providing reliable prevalence estimates.31 In a cohort study of children in Uganda in...
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a low-endemicity setting, pools of 49 samples resulted in 95% cost and labor savings in settings where the prevalence was 0.01%. The optimal pool sizes required to achieve a balance between cost saving and accuracy are likely to depend on the underlying prevalence of infection.

In high-prevalence settings, current results indicate that correction of RDT results by using PCR is at least twice more expensive than using RDTs alone. To reduce costs, lot quality assurance sampling (LQAS) may help reduce required sample sizes. For example, a study in Malawi on the utility of LQAS in estimating mosquito net use found that LQAS provided similar estimates as the standard MIS, but at lower cost. The use of LQAS has previously been used for estimating the prevalence of *Schistosoma mansoni* infection to target mass treatment. A major limitation of LQAS surveys is that they do not provide precise estimates of prevalence, but rather classify schools/communities into predefined categories of infection prevalence.

This study has several limitations. First, RDT performance was compared with microscopy and only a sample of the RDT-negative slides was examined. The presence of sub-microscopic infections when microscopy is used as the comparator may result in false-positive results as a result of infections below the threshold of detection by microscopy. Recent studies have highlighted the extent of sub-microscopic infections, indicating that microscopy misses more than half of the infections detected by PCR. This finding could lead to an under-estimation of RDT performance because of sub-microscopic infections commonly harbored by school age children. Persistent antigenemia in HRP-2–based RDTs has also been suggested as the cause of false-positive results after treatment or after a recent resolved illness. In the absence of a reliable gold standard such as PCR to validate the RDT results, statistical methods such as latent class models may be useful in estimating the diagnostic performance.

A second limitation is that the cost analysis did not enable sensitivity and specificity of RDTs to vary with infection prevalence and RDT type, whereas it is known that the performance of RDTs crucially depends on the underlying prevalence and the type of RDT used. Such variation may cause an underestimation of costs associated with the different diagnostic strategies. A third limitation is that cost estimates were based on a conservative RDT performance of 80% sensitivity and 60% specificity, which is lower than what has been observed by the WHO-FIND malaria diagnostics program for most of the commonly used RDTs. Therefore, the costs of re-examining misclassified samples may have been overestimated. In addition to misclassification caused by poor diagnostic performance, school-based surveillance has a number of limitations, including differentials in enrollment, absenteeism, types of schools sampled, and the ages of children sampled, which might produce different prevalence estimates compared with community-based surveillance.

A striking implication of the current results is the poor performance of microscopy, even in the hands of well-trained technologists with adequate quality assurance. This performance critically depends on the quality of slide preparation and storage. It was noteworthy that 10.2% of slides required re-staining and that 18.7% of slide readings were discrepant between microscopists. The technical difficulties of microscopy should not be underestimated. During MISs conducted during 2009 in Sudan, South Sudan, and Namibia, blood slides and RDTs were collected but it was found that slides were so poorly stained or stored and microscopy was ultimately abandoned (Snow RW, Noor AM, unpublished data). In addition to the reasonable performance of RDTs in classifying localities and their low cost, RDTs offer real technical advantages for point-of-care diagnosis and immediate treatment during surveys.

In conclusion, our results indicate that RDTs represent a cheap diagnostic approach in school malariometric surveys and can be used to reliably estimate infection prevalence at low and high prevalence categories. The results also demonstrate that RDTs were least specific at moderate transmission settings but at such transmission levels, RDTs in combination with more accurate diagnostic tools such as pooled PCR still offered an affordable alternative.

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