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Diagnostic Performance of Malaria Rapid Diagnostic Test and Microscopy Compared with PCR for Detection of *Plasmodium falciparum* Infections among Primary Schoolchildren in Kibiti District, Eastern Tanzania: An Area with Moderate Malaria Transmission

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Abstract. A substantial decline of malaria transmission intensity has been observed in sub-Saharan Africa over the past two decades and may affect the diagnostic performance of malaria rapid diagnostic test (mRDT) and microscopy. Diagnostic performance of histidine-rich protein II (HRP-II)/pan-lactate dehydrogenase (pLDH)–based mRDT and microscopy was evaluated against polymerase chain reaction (PCR) for the diagnosis of *Plasmodium falciparum* infection among 316 primary schoolchildren in Kibiti district, in 2016. Polymerase chain reaction detected more cases of *P. falciparum* infection than mRDT or microscopy. Using PCR as reference, the sensitivity and specificity of mRDT were 75.9% (95% CI = 62.8–86.1) and 96.9% (95% CI = 94.0–98.7), respectively, whereas that of microscopy were 63.8% (95% CI = 50.1–76.0) and 95.7% (95% CI = 92.5–97.9), respectively. Polymerase chain reaction and other molecular methods should be considered for use in schools and other epidemiological surveys as supplement to mRDT or microscopy.

Malaria is still a public health problem in sub-Saharan Africa despite the recent substantial gains in reduction in transmission intensity of the infection, morbidity, and mortality following the scale-up of interventions.[1] The marked reduction in malaria transmission has led to most settings that were previously categorized as high-transmission settings moving to low-transmission settings.[2–4] Consequently, children are acquiring immunity to malaria much more slowly than before.[5] Notably, there is more evidence of epidemiologic shift of malaria incidences to children aged older than 5 years and teenagers, that is, mainly in school-aged children.[6,7] Thus, the use of primary school malaria surveys can provide rapid and cheap surveillance data and complement national representative surveys.[8,9] These epidemiologic surveys, however, rely on the use of microscopy and malaria rapid diagnostic test (mRDT) for detection and identification of malaria parasites. However, diagnostic performance of microscopy and mRDT is affected by several factors including local epidemiology, choice of study population, low parasite density, skills of technicians, and quality of microscopy.[10,11] This underscores the need to investigate the diagnostic performance of these tools for surveillance of malaria parasites among asymptomatic schoolchildren, known to harbor low-density malaria parasites. On the other hand, polymerase chain reaction (PCR) is a nucleic acid–based test that can detect as low as 1–5 parasites/μL of blood (≤ 0.0001% of infected red blood cells) compared with around 50–100 parasites/μL of blood by microscopy or mRDT.[10] This study, therefore, evaluated the diagnostic performance of microscopy and mRDT as compared with PCR for the detection and identification of malaria parasites among schoolchildren in Nyamisati village, Kibiti district, eastern Tanzania.

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We conducted a cross-sectional study in Nyamisati village, Kibiti district, among schoolchildren attending Nyamisati primary school between February and March 2016. Nyamisati is a rural area located along the mangrove swamps of Rufiji River delta, in eastern Tanzania. The area has tropical climate, with annual average precipitation of 1,071 mm and temperature of 25.3°C. Malaria transmission occurs throughout the year, with seasonal fluctuations following the long and short rains between March–June and November–December, respectively.[2] The village had an estimated total population of 2,300 in 2015, of which 919 were children younger than 18 years.

This study was part of the regular annual malaria infection surveys in the village, whereby schoolchildren from Nyamisati primary school were invited at our research unit located at the village health center to participate in the survey. A structured questionnaire was used to collect information on demographic characteristics, use of bed nets, and malaria symptoms in the past 2 weeks. Finger-prick blood samples were used to make thick and thin smears for microscopy, mRDT, and measurement of hemoglobin level, whereas venous blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes for PCR analysis. Malaria antigen (Histidine Rich Protein II (HRP-II)/pan-Lactate Dehydrogenase (pLDH) based) test was performed using the Pf/Pan immunochromatographic test kit (SD® Bioline; Standard Diagnostics Inc.,Yongin-si, Korea) according to the manufacturer's instructions. Thick and thin smears were stained using 10% Giemsa, and asexual parasites were counted against 200 white blood cells. This number was multiplied by 40, assuming 8,000 leukocytes per microliter of blood, to gain an approximate parasite density. A blood slide was considered negative if no parasites were seen after examining 100 fields. Two, independent microscopists read all the microscopy slides. A third independent reading was performed in case of disagreement on the presence of parasitemia or if the density differed by > 25%. Hemoglobin estimation was performed using HemoCue® Hb 201+ (HemoCue AB, Ängelholm, Sweden). The MUHAS (MUHAS/2016/AEC/Vol.X/224) and the Regional Ethics Committee, Stockholm, Sweden (Dnr. 00-084) approved the

study. Written informed consent was obtained from the parents/guardians of the schoolchildren, whereas assent was obtained from all the participants before their enrollment. Venous blood collected in EDTA was separated and stored as frozen plasma and packed cells. A magnetic bead separation method was used to extract genomic DNA from packed cells using Hamilton Chemagic Star Robot (Hamilton, Bonadouz, Switzerland). Real-time PCR was used to qualitatively detect *Plasmodium* infections. Species-specific PCR targeting mitochondrial genome 18S rRNA gene was performed using ABI Taqman 7500 or QuantStudio™ 5 Real-Time PCR system (Applied Biosystems, Foster City, CA), as previously described.[12]

The clinical and demographic data were summarized as frequencies and percentages, medians, and interquartile ranges (IQRs), as appropriate. The performance of each diagnostic test method was calculated by means of sensitivity, specificity, positive predictive value, and negative predictive value using conventional PCR as a reference standard, and kappa statistic was used to assess the agreement of the tests.

Three hundred sixteen schoolchildren were screened for malaria parasites, and 58.7% (186/316) of them were females. The median age of the study participants was 11 years (IQR: 6–16). Majority of the children (82.0% (259/316) were frequent users of bed nets. About 15% (46/316) of children had a history of fever 2 weeks before the survey, and 18% (56/316) of them were anemic, that is, hemoglobin level < 11 g/dL (Table 1).

Malaria parasite positivity rates using microscopy, mRDT, and PCR are presented in Table 2. Of the mRDT-positive subjects, 28.9% (15/52) showed positive bands for both HRP-II and pLDH indicating *P. falciparum* or *P. falciparum* mixed with non-*falciparum* infections. Polymerase chain reaction analysis showed that 53.4% (31/58) of the infections were *P. falciparum*, 17.2% (10/58) *Plasmodium ovale*, 3.4% (2/58) *Plasmodium malariae*, and 25.9% (15/58) were mixed infections. Of the PCR-determined mixed infections, 66.7% (10/15) were harboring *P. falciparum* and *P. malariae*, and 33.3% (5/15) were *P. falciparum* and *P. ovale*. No mixed infections were detected by microscopy.

The sensitivity, specificity, and positive and negative predictive values of mRDT against microscopy or PCR are presented in Table 2. The mRDT had good agreement with microscopy results, with a kappa value of 0.76 (Table 2). Using PCR as a reference point, both microscopy and mRDT had low sensitivity. Both microscopy and mRDT had the lowest level of agreement with PCR (kappa < 0.20). There were 12 mRDT-positive results that were negative by microscopy, and, of these, four were positive by PCR for *P. falciparum* infection. On the other hand, eight samples were microscopy positive (parasite density ranged from 200 to 560 parasites/μL of blood), but mRDT negative, and, of these, two were confirmed to be *P. falciparum* infection by PCR. All the nine subjects with indeterminate results, that is, either microscopy/mRDT positive but PCR negative, were due to *P. falciparum* infection.

TABLE 1
Baseline characteristics of schoolchildren in Nyamisati village

Variable name	Values
Gender (female), *n* (%)	186 (58.7)
Age (years), median (interquartile range)	11 (6–16)
History of fever in the past 2 weeks, *n* (%)	46 (14.6)
Use of antimalarial drugs 2 weeks before survey, *n* (%)	21 (6.6)
Use of bed nets, *n* (%)	259 (82.0)
Anemia (hemoglobin < 11 g/dL), *n* (%)	56 (17.8)
Prevalence of *Plasmodium falciparum* infection by:	
Polymerase chain reaction, *n* (%)	58 (18.4)
Malaria rapid diagnostic test, *n* (%)	52 (16.5)
Microscopy, *n* (%)	48 (15.2)
Geometric mean parasite density (range)/μL	693.16 (160–88,580)

TABLE 2
Comparison of diagnostic performance of *Plasmodium falciparum* detection tests

	Polymerase chain reaction	
Reference	Positive	Negative
Malaria rapid diagnostic test		
Positive	44	8
Negative	14	250
Total	58	258
Sensitivity (95% CI)	75.9 (62.8–86.1)	
Specificity (95% CI)	96.9 (94.0–98.7)	
PPV (95% CI)	84.6 (71.9–93.1)	
NPV (95% CI)	94.7 (91.3–97.1)	
Microscopy		
Positive	37	11
Negative	21	247
Total	58	258
Sensitivity (95% CI)	63.8 (50.1–76.0)	
Specificity (95% CI)	95.7 (92.5–97.9)	
PPV (95% CI)	77.1 (62.7–88.0)	
NPV (95% CI)	92.2 (88.3–95.1)	

PPV = positive predictive value; NPV = negative predictive value.

The scale-up of control measures including insecticide-treated bed nets, indoor residual spraying, and artemisinin-based combination therapy has led to a decline of malaria prevalence in Africa.[1,2] Furthermore, in malaria-endemic settings, usually most individuals with partial immunity against malaria tend to harbor asymptomatic infection at the submicroscopic level that may affect the diagnostic performance of the presently available point of care and epidemiological survey diagnostic tools.[10,13] Both microscopy and mRDT have a detection limit in a range of 100–200 parasites per microliter of blood, thus may fail to detect parasite densities below this level.[10,14] There is, however, limited information on diagnostic performance of microscopy and mRDT in asymptomatic individuals, who frequently harbor submicroscopic infections. We report the diagnostic performance of mRDT and microscopy for detection of malaria parasites compared with PCR.[15]

The overall prevalence of malaria in our study population was moderate as detected by mRDT, microscopy, or PCR. Contrary to our findings, previous findings in the same study area showed a much lower prevalence of microscopy-determined malaria of 4.8%.[2] The observed differences might be attributed by the shift in patterns of malaria infection from young ages to older ages.

The mRDT had moderate sensitivity in detecting asymptomatic malaria infection among schoolchildren and had good agreement with microscopy (kappa = 0.740). These findings are comparable with our previous findings in Korogwe district.[16] However, the mRDT sensitivity in our study was higher than that reported in Zanzibar and Malawi.[13,17] Nonetheless, similar to the findings in Malawi,[17] there was poor agreement between mRDT/microscopy and PCR. This might be

attributed to by the very low-density malaria infections and non-*falciparum* infections detected by PCR.[10] On the other hand, both mRDT and microscopy showed high specificities. Similar findings have been reported in Zanzibar and Malawi.[13,17] Conversely, PCR analysis confirmed a number of mRDT and microscopy false-positive results. The indeterminate results by mRDT may be due to the persistent HRP-II in the blood circulation after the infection has been cleared by antimalarial chemotherapy.[10,16] We do not have clear explanation for microscopy indeterminate results in our study; however, these infections with low parasite counts could be detected by ultrasensitive PCR methods.[17] On the other hand, the presence of eight samples with microscopy positive but mRDT negative results could be due to point mutation of HRP-II gene in the parasite that has been recently reported in Tanzania and Kenya.[18,19] The point mutation causes the deletion of the gene that encodes for the *P. falciparum* HRP-II protein of the parasite, leading to false-negative mRDT results, and is common in asymptomatic infections.[20]

Microscopy and mRDT had low sensitivity than PCR for detection of malaria infections among asymptomatic schoolchildren in this low/moderate transmission setting, and also underestimated the burden of non-*falciparum* infections, thus should probably be supplemented with molecular tools.

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