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Malaria Surveillance in the Democratic Republic of the Congo: Comparison of Microscopy, PCR, and Rapid Diagnostic Test

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Abstract

Malaria surveillance is critical for control efforts, but diagnostic methods frequently disagree. Here we compare microscopy, PCR, and a Rapid Diagnostic Test in 7,137 samples from children in the Democratic Republic of the Congo using Latent Class Analysis. PCR had the highest sensitivity (94.6%) and microscopy had the lowest (76.7%).

Keywords

malaria; surveillance; diagnostics; microscopy; PCR; rapid diagnostic test; RDT; latent class analysis; LCA

Malaria is a particularly severe problem in the Democratic Republic of the Congo (DRC) (WHO, 2015). Both surveillance and case management of malaria depend on accurate

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diagnosis, but different methods of parasite detection vary in ease of use, reliability, and cost.

Three commonly used methods of parasite detection are light microscopy, PCR, and Rapid Diagnostic Tests (RDTs). Microscopy, the traditional diagnostic method, has a limit of detection of about 50 parasites/ μL of whole blood (Kilian *et al.*, 2000; Mwingira *et al.*, 2014). PCR can detect as few as 0.002 parasites/ μL (Kamau *et al.*, 2011), but is more costly and often unavailable in endemic areas. RDTs are inexpensive and easy to use but perform variably below 200 parasites/ μL (WHO, 2014).

Here, in an effort to improve malaria surveillance, we compare microscopy, PCR, and RDT on a large common set of samples. We determined the sensitivity and specificity of microscopy and RDT compared to PCR. Additionally, we used Latent Class Analysis (LCA) to estimate an alloyed gold standard and then computed the sensitivity and specificity of each method.

Samples from children under five years old were obtained during a Demographic and Health Survey (DHS), a population-representative survey conducted between November 2013 and February 2014 (MPSMRM, 2014). Finger- or heel-prick blood was obtained from each participant. Giemsa-stained slides were read by two independent microscopists who conferred upon discordances. RDTs targeting the *Plasmodium falciparum* histidine-rich protein II (*PfHRP2*) (SD BIOLINE Malaria Ag P.f, Standard Diagnostics, Gyeonggi-do, Republic of Korea) were performed on-site. Blood was also applied to filter papers as dried blood spots (DBS). These DBS were transported to the University of North Carolina, where from each DBS one ¼" diameter hole was punched into a well of a 96-well deep-well plate (Life Technologies, Carlsbad, CA).

From these DBS, genomic DNA (gDNA) was extracted as previously described (Plowe *et al.*, 1995) and stored at -20°C . Each gDNA sample was tested in duplicate in a duplex real-time PCR assay targeting the *P. falciparum* lactate dehydrogenase gene (*pfdh*) and, as a control, the human β -tubulin gene. Primer and probe sequences used to amplify *pfdh* (Taylor *et al.*, 2010) and human β -tubulin (Beshir *et al.*, 2010) were previously published, with the exception that a VIC-TAMRA probe was used for the human β -tubulin assay. Primer and probe sequences, reaction components, and cycling conditions are given in Table S1.

Cycle threshold (C_T) values were imported into Microsoft Excel 2007 (Microsoft, Redmond, WA) and were compiled and analyzed in R (version 3.0.3, www.R-project.org). Samples in which human β -tubulin did not amplify or had a C_T value above 35 were discarded. Samples were considered positive for *pfdh* if both replicates amplified or if one amplified with a C_T value of 38 or lower. This assay had a limit of detection between 10 and 50 parasites/ μL .

DBS were collected from 9,790 children; of these, 978 were unavailable for parasite testing. From the remaining 8,812 samples, four samples did not amplify human DNA in any replicates, 1,558 were from children aged five or older, and 113 samples lacked data for microscopy and/or RDT; these were excluded. The remaining 7,137 samples constitute the analyzable population.

The overall prevalence of *P. falciparum* parasites was 38.6% by PCR, 26.8% by microscopy, and 36.2% by RDT. The malaria prevalence data and maps are available in the DHS Final Report (MPSMRM, 2014). Of 6,301 children for whom responses were available, 2,094 (33.2%) were reported to have had a fever in the previous two weeks (40.9% of microscopy-positive, 42.4% of RDT-positive, and 39.4% of PCR-positive children).

Of the 7,137 samples analyzed here, 3,652 (51.2%) were negative by all three parasite detection methods and 1,461 (20.5%) were positive by all three tests. 234 (3.3%) were positive by PCR and microscopy only, 508 (7.1%) by PCR and RDT only, and 98 (1.4%) by microscopy and RDT only. Additionally, 549 (7.7%) were positive by PCR only, 118 (1.7%) by microscopy only, and 517 (7.2%) by RDT only. The distribution is illustrated in Figure 1.

PCR detected the greatest number of infections overall, most likely owing to its lower limit of detection and a high prevalence of low-density infections. Using PCR as the reference standard, the sensitivity of microscopy was 61.6%, similar to sensitivities reported in other studies [reviewed in Okell *et al.* (2009)]. The specificity of microscopy compared to PCR was 95.1% (Table 1). The DHS microscopists did not report different parasite species, so the “false positive” microscopy diagnoses could have been infections with *P. ovale* or *P. malariae*, which are endemic at a low prevalence across the DRC (Taylor *et al.*, 2011).

The sensitivity of RDT was 71.6% (Table 1), similar or slightly lower than studies done in pregnant women (Kattenberg *et al.*, 2012) and febrile patients (Faye *et al.*, 2013; Nicastrì *et al.*, 2009; Shakely *et al.*, 2013) but higher than a study in Zambia which found an RDT sensitivity of only 17% compared to nested PCR (Laban *et al.*, 2015). The specificity of RDT was 86.0% (Table 1). The lower specificity found here compared to previous studies, especially those in febrile patients, may be attributed to the well-known persistence of HRP2 antigen after parasite clearance (Abeku *et al.*, 2008).

Because each parasite detection method is imperfect, we performed Latent Class Analysis (LCA) in order to estimate an “alloyed” reference standard against which to estimate the operating characteristics of each detection method (Hui & Walter, 1980). This was calculated using the poLCA package for R (version 1.4.1, <https://cran.r-project.org/package=poLCA>), and was corroborated using the SAS Proc LCA procedure (version 1.3.2, <http://methodology.psu.edu/>). Using the LCA-generated alloyed gold standard we computed the sensitivities of PCR (94.6%), microscopy (76.7%), and RDT (86.9%), as well as the specificities of PCR (88.3%), microscopy (97.2%), and RDT (88.1%) (Table 1). Thus, using LCA, PCR was the most sensitive method and microscopy was the most specific. This is generally consistent with the findings of an LCA study of febrile patients from Tanzania (Schachterle *et al.*, 2011), although different protocols for PCR and microscopy were used.

This study demonstrates considerable discordances between malaria surveillance methods. While PCR is consistently the most sensitive, concerns remain about its utility in field settings where resources are limited. There has been a large shift toward RDT use for diagnosis and surveillance. Here, RDT missed 13% of infections. However, newer and more sensitive RDTs are under development (<http://sites.path.org/dx/malaria/>). Improved field-

forward methods of malaria parasite detection will greatly facilitate surveillance and control programs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- 7,137 samples from children in the DRC were tested by microscopy, RDT, and PCR
- Latent Class Analysis was used to compare the three diagnostic methods
- PCR was the most sensitive (94.6%), microscopy was the least sensitive (76.7%)
- Microscopy was the most specific (97.2%)

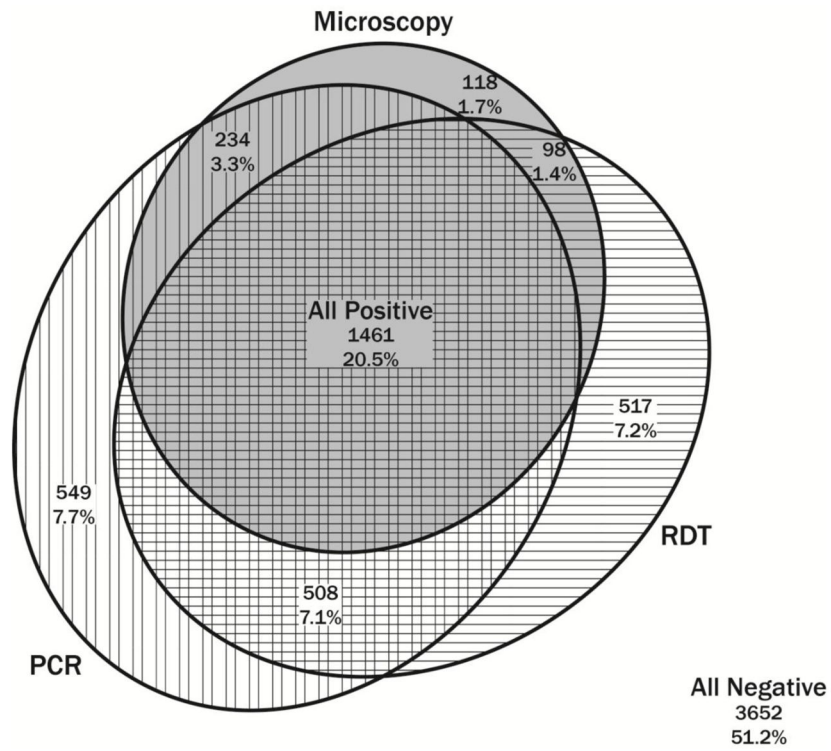


Figure 1. Proportional Venn diagram showing discordance of malaria parasite detection by PCR, microscopy, and RDT (eulerAPE, version 3.0.0, <http://www.eulardiagrams.org/eulerAPE/>). Samples falling within a given circle indicate positivity by that method of detection.

Table 1

Sensitivity and specificity of diagnostic methods vs. PCR and LCA.

	vs. PCR		vs. LCA	
	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
PCR	-	-	94.6 (93.3–95.8)	88.3 (87.2–89.4)
Microscopy	61.6 (59.7–63.4)	95.1 (94.4–95.7)	76.7 (74.6–78.8)	97.2 (96.6–97.8)
RDT	71.6 (69.8–73.2)	86.0 (84.9–87.0)	86.9 (85.2–88.6)	88.1 (87.1–89.2)

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