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BRIEF REPORT

# Comparison of Capillary Versus Venous Blood for the Diagnosis of *Plasmodium falciparum* Malaria Using Rapid Diagnostic Tests

**Abalinda M. Gorret,[1] Rabbison Muhindo,[2] Emma Baguma,[2] Moses Ntaro,[2] Edgar M. Mulogo,[2] Molly Deutsch-Feldman,[3] Jonathan J. Juliano,[4,5] Dan Nyehangane,[1,6] and Ross M. Boyce[2,4]**

[1]Department of Medical Laboratory Science, Faculty of Medicine, Mbarara University of Science and Technology, Mbarara, Uganda, [2]Department of Community Health, Faculty of Medicine, Mbarara University of Science and Technology, Mbarara, Uganda, [3]Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA, [4]Division of Infectious Diseases, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA, [5]Curriculum in Genetics and Microbiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA, and [6]Epicentre Mbarara Research Centre, Mbarara, Uganda

We enrolled 250 febrile children in western Uganda to compare the results of malaria rapid diagnostic tests (RDTs) when using capillary vs venous blood. Participants were tested with 4 different RDT types. Polymerase chain reaction testing was performed as the reference standard. Sensitivity and specificity were broadly similar across RDT types and sampling method. Agreement between sample type was high, ranging from 0.95 to 0.99. When following the manufacturer's recommended interpretation, only 5 tests would have resulted in a different clinical diagnosis. These results demonstrate that malaria RDTs perform similarly when using capillary or venous blood in febrile children with *Plasmodium falciparum* malaria.

**Keywords.** malaria; *Plasmodium*; diagnostic test.

While microscopy remains the reference standard for the diagnosis of malaria, rapid diagnostic tests (RDTs) have emerged as a cornerstone of the "test and treat" strategy [1]. Sales of malaria RDTs in Africa have risen almost every year since 2008 with >200 million RDTs delivered in 2019 alone, accounting for the majority of all malaria diagnostic tests performed in sub-Saharan Africa [2]. Compared to light microscopy, RDTs have many advantages, including their low cost, environmental stability, and relative ease of use, which enables a parasitological diagnosis of malaria even in settings with limited laboratory infrastructure [3].

For these same reasons, RDTs are increasingly employed in research and surveillance programs, the most notable of which are national Malaria Indicator Surveys [4]. Nearly all malaria RDTs utilize whole blood, typically from capillary (ie, finger prick) or venous sampling, the choice of which may vary with the need to perform other laboratory tests. While test results of these different sampling methods are generally considered interchangeable, *Plasmodium falciparum* parasites are known to sequester in capillaries; a biologic phenomenon that may yield greater sensitivity with the use of capillary blood. To date, however, published studies comparing the results of light microscopy have come to different conclusions regarding parasitemia levels [5–7].

Yet no study has directly compared the results of malaria RDTs when using capillary vs venous blood. Given the millions of kits that are deployed for surveillance, research, and routine care each year, this represents an important knowledge gap, especially as newer, more sensitive RDTs become available [8, 9]. Therefore, the objective of the project was to compare the results of parallel capillary and venous blood across a variety of RDT assays that utilize different antigen targets.

## METHODS

### Study Setting

Bugoye Level III Health Center (BHC) in the Kasese District of Western Uganda functions as the primary health center for the Bugoye subcounty. This rural subcounty comprises 35 villages and covers an area of approximately 55 km$^2$. The catchment area of the health center includes the >50 000 residents of the Bugoye subcounty as well as residents of neighboring villages from the Maliba subcounty immediately to the east. The health center maintains general, pediatric, and obstetric wards for inpatient services, along with a busy outpatient department that cares for 60–80 patients per day. Clinical officers, nurses, midwives, and laboratory technicians employed by the Ugandan Ministry of Health staff the health center.

The climate in Bugoye permits year-round malaria transmission marked by semiannual transmission peaks typically following the end of the wet seasons, traditionally occurring March–May and September–November. *Plasmodium falciparum* accounts for the vast majority (>95%) of infections, although *Plasmodium malariae* and *Plasmodium ovale* are identified both as isolated infections and coinfections with *P. falciparum* [10]. The most recent malaria indicator survey undertaken in the region found a parasitemia prevalence of 17.6% in 2014 [4]. There is, however, substantial local

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Correspondence: Ross M. Boyce, MD, MSc, Division of Infectious Diseases, University of North Carolina at Chapel Hill, 130 Mason Farm Road, Chapel Hill, NC 27599 (roboyce@med.unc.edu).

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variation in transmission intensity with many of the villages around BHC having parasitemia prevalence of 20%–30% (unpublished data).

### Study Design

The study was a prospective, observational cohort of febrile children presenting to a peripheral health center in a malaria-endemic region of western Uganda. Study staff screened all children for eligibility according to the predefined criteria as outlined below:

Inclusion criteria:

- Child between the ages of 5 and 12 years
- Febrile (axillary temperature >38°C)

Exclusion criteria:

- Child without a parent or guardian
- Inability to obtain required blood samples after 3 attempts

After written consent was provided by a parent or guardian, participating children were moved to the laboratory to undergo testing for malaria. All participants were tested using 4 different RDTs types, each with blood drawn via finger prick and venous phlebotomy (ie, 8 total RDTs). Capillary blood was collected in a capillary tube and placed directly onto the RDT and filter paper to create 2 dried blood spots (DBSs), while approximately 3 mL of venous blood was collected into an ethylenediaminetetraacetic acid tube before being placed onto the RDTs and filter paper.

The RDT tested are shown in Table 1. Each RDT was performed in accordance with the manufacturers' specific instructions for sample collection, application, and test interpretation. Two staff members recorded the RDT results. A third, senior staff member adjudicated any discrepancies between the first 2 reads. Given the number of tests performed, invalid tests were not repeated in order to minimize the number of needle sticks. After diagnostic testing was complete, results of the SD BIOLINE Malaria Ag P.f were provided to the responsible provider for clinical management purposes as this histidine-rich protein II (HRP-II)–based RDT is similar to the assays currently utilized for the diagnosis of malaria. Patients with test results positive for malaria were treated at the discretion of the attendant provider.

Molecular diagnosis was completed at the Epicentre Mbarara Research Centre using capillary and venous DBSs. DNA from DBSs was extracted using a previously described Chelex extraction protocol in a final volume of 100 µL [11]. In brief, punched spots were placed in deep 96-well plates and incubated overnight at 4°C with 1 mL of phosphate-buffered saline (PBS) and 10% saponin. Plates were centrifuged for 30 seconds and the resultant supernatant was discarded, after which another 1 mL of PBS was added to each well. The plate was vortexed and incubated at 4°C for 30 minutes, after which time the plate was centrifuged again and supernatant discarded. Chelex-100 Resin (Bio-Rad, Hercules, California) and 100 µL of sterile water was added to each well. Fifty microliters of 20% Chelex was added to each well. Plates were incubated in a water bath at 95°C–99°C for 12 minutes with the plate being vortexed every 3 minutes. Following incubation, the plate was centrifuged at 1500 rpm for 5 minutes. The solution was transferred to a new plate and re-centrifuged. Resulting supernatant, without beads, was pipetted to a new 96-well plate, labeled, and stored at –20°C until use.

After extraction was complete, we used a high-resolution melt (HRM) master mix (Qiagen, Hilden, Germany) and primers targeting the 18S ribosomal RNA (rRNA) gene as previously described [12]. In brief, we prepared reagents on a cooled sample rack, and each 25-µL polymerase chain reaction (PCR) mixture included 12.5 µL of Rotor-Gene Probe PCR master mix, 0.7 µM (final concentrations) of the forward and reverse primers (PL1473 F18 and PL1679 R18, respectively), 3 µL of template DNA, and 6 µL of RNase-free water in the final reaction mixture. We performed PCR cycling using the following conditions: 95°C for 5 minutes, followed by 40 cycles of 95°C for 10, 57°C for 30, and 72°C for 10 seconds. We performed HRM analysis of the resulting PCR product using a ramp from 65°C to 95°C, increasing 0.1°C in each step. Thermocycling, fluorescent

**Table 1. Characteristics of Malaria Rapid Diagnostic Tests Included in the Evaluation**

| RDT | Antigen(s) | Lot Number(s) | Expiration Date |
|---|---|---|---|
| SD BIOLINE Malaria Ag P.f | HRP-II (P.f) | 05BDDC069 | 14 Jun 2020 |
| SD BIOLINE Malaria Ag P.f/Pan | HRP-II (P.f)<br>pLDH (Pan) | 05BDD077A<br>05EDD077A | 14 Oct 2020 |
| SD BIOLINE Malaria Ag P.f/P.f/P.v | HRP-II (P.f)<br>pLDH (P.f)<br>pLDH (P.v) | 05GDD003A | 5 Nov 2020 |
| Alere Malaria Ag P.f | HRP-II (P.f) | 05LDE001A | 20 Feb 2020 |

Abbreviations: HRP-II, histidine-rich protein II; pLDH, parasite lactate dehydrogenase; P.f, *Plasmodium falciparum*; P.v, *Plasmodium vivax*; RDT, rapid diagnostic test; SD, Standard Diagnostics, Inc.

detection, and HRM steps were performed in a Rotor-Gene Q real-time PCR instrument, using a 72-well rotor (Qiagen).

We used *Plasmodium* species plasmid controls from the American Type Culture Collection (ATCC, Manassas, Virginia). The plasmids included *P. falciparum* (MRA-177-Pf ssu rRNA nest 1 PCR plasmid clone 8, lot number 5946054), *P. malariae* (MRA-179-Pm ssu rRNA nest 1 PCR plasmid clone 34, lot number 61909614), *P. ovale* (MRA-180-Po ssu rRNA nest 1 PCR plasmid clone 54, lot 59467055), and *Plasmodium vivax* (MRA-178-Pv ssu rRNA nest 1 PCR plasmid clone 16, lot number 58067149). Samples with either a positive capillary or venous PCR result were considered positive for the determination of RDT performance (ie, sensitivity, specificity).

### Data Analysis

Study staff recorded demographic characteristics (ie, age, sex, village of residence), axillary temperature, and test results in an electronic database [13]. Data were analyzed with Stata 15.1 (StataCorp, College Station, Texas). We first described the study cohort using summary statistics. We determined the test performance of each RDT compared to PCR. Isolated positive pan-LDH or Pv-LDH bands were not considered in the calculation of sensitivity and specificity. We then estimated the level of agreement between results obtained from capillary as compared to venous blood for each assay using Cohen κ coefficient [14].

### Ethical Approvals

Ethical approval of the study was provided by the institutional review boards of the University of North Carolina at Chapel Hill (18–0880), the Mbarara University of Science and Technology (06/05-18), and the Uganda National Council for Science and Technology (HS 2482). Written informed consent for participation in the study was obtained from a parent or guardian, and assent was requested from children ≥8 years of age.

## RESULTS

From 13 February to 29 April 2019, a total of 250 children underwent malaria RDT with blood samples drawn from both capillary (ie, finger prick) and venous blood. The median age was 10 years (interquartile range [IQR], 7–12 years). There were more female participants (n = 150 [60%]) than male (n = 99 [40%]). A total of 109 (44.3%) participants were positive by the HRP-II–based SD BIOLINE Malaria Ag P.f test, which is similar to the existing standard of care used for routine diagnosis.

The results of each RDT type, stratified by blood sample source, are shown in Table 2, and the results of PCR testing are available in Supplementary Table 1. Sensitivity and specificity were broadly similar across RDT types and sampling method, with sensitivity ranging from 92.7% to 95.2% and specificity ranging from 79.5% to 83.1%. Agreement between sample type was high, ranging from 0.95 to 0.99. Across all tests, there were only 11 discordant band results, most of which were attributable to differing pan- or *P. falciparum*–specific LDH positivity (Supplementary Tables 2–5). When following the manufacturer's recommended interpretation, only 5 tests would have resulted in a different clinical diagnosis. Three of these discordant results were due to positive capillary but negative venous results, whereas 2 were due to positive venous but negative capillary results. Three of the 5 discordant results were negative by PCR, suggesting HRP-II antigen persistence or false positivity.

## DISCUSSION

Our evaluation demonstrated a high level of agreement between malaria RDTs performed with capillary and venous blood among febrile children in a rural area of high *P. falciparum* transmission intensity. To our knowledge, this is the first evaluation comparing the level of agreement between malaria RDTs using both capillary and venous blood. These results are consistent with similar studies evaluating microscopy among both uncomplicated and asymptomatic patients [5–7].

Of the RDTs tested, the SD BIOLINE Malaria Ag P.f/P.f/P.v assay had the most discrepant results, although we note that differences in the *P. falciparum*–specific LDH antigen band in the presence of a positive HRP-II antigen band would not have impacted test interpretation or treatment recommendations. The majority of these discrepancies were likely due to variation in parasitemia—as the parasite lactate dehydrogenase antigen band has somewhat lower sensitivity than the HRP-II antigen band—or HRP-II persistence, which could account for the lower specificity of the RDTs, both of which we have observed previously [15].

Our evaluation has many strengths including the large sample size, high rate of completion, and high prevalence of

**Table 2. Test Performance of Malaria Rapid Diagnostic Tests Compared to Polymerase Chain Reaction, Stratified by Sample Source and Agreement as Determined by Cohen κ Coefficient**

| | Capillary Blood | | Venous Blood | | Agreement |
|---|---|---|---|---|---|
| RDT Type | Sensitivity, % | Specificity, % | Sensitivity, % | Specificity, % | κ |
| Malaria Ag P.f. | 95.2 (88.1–98.7) | 81.6 (74.8–87.2) | 95.2 (88.3–98.7) | 81.2 (74.4–86.9) | 0.99 (.98–1.00) |
| Malaria Ag P.f/Pan | 95.2 (88.1–98.7) | 83.0 (76.4–88.4) | 95.2 (88.1–98.7) | 83.1 (76.6–88.5) | 0.98 (.96–1.00) |
| Malaria Ag P.f/P.f/P.v | 95.2 (88.2–98.7) | 81.9 (75.2–87.5) | 92.9 (85.1–97.3) | 81.3 (74.6–87.0) | 0.99 (.98–1.00) |
| Alere Malaria Ag P.f | 94.0 (86.5–98.0) | 79.5 (72.6–85.4) | 92.7 (84.8–97.3) | 80.1 (73.2–85.9) | 0.95 (.92–.99) |

malaria among eligible patients. However, the study also has a number of limitations. First, *P. vivax* is not endemic in western Uganda. Thus, we were not able to assess the performance of the *P. vivax*–specific LDH antigen band on the SD BIOLINE Malaria Ag P.f/P.f/P.v. Second, we did not perform parallel microscopy or quantitative PCR on samples and thus were not able to assess the correlation between discrepant results and parasitemia. Given the relatively high level of agreement between sampling sources, however, this is unlikely to have a large public health impact. Last, our study population was limited to febrile children between the ages of 5 and 12 years. Therefore, we advise against generalizing these results to a semi-immune, adult population that may harbor a higher prevalence of low-density, asymptomatic infections.

Our results demonstrate that malaria RDTs perform similarly when using capillary or venous blood in febrile children with *P. falciparum* malaria. These findings provide evidence to compare results from both types of samples and suggest that requirements for other laboratory testing should drive the method of blood collection.

## Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

***Author contributions.*** Study conception and design: R. M. B., A. M. G., D. N., E. M. M. Funding: R. M. B., E. M. M. Study implementation: A. M. G., D. N., R. M., E. B., M. N., E. M. M. Laboratory testing: A. M. G., D. N., M. D.-F., J. J. J. First draft of manuscript: R. M. B., A. M. G., D. N. Revisions: All authors.

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***Data availability.*** Deidentified individual data that support the results will be shared beginning 9–36 months after publication provided the investigator who proposes to use the data has approval from an institutional review board, independent ethics committee, or research ethics board, as applicable, and executes a data use/sharing agreement with the University of North Carolina at Chapel Hill.

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***Potential conflicts of interest.*** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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