Use of Capillary Blood Samples Leads to Higher Parasitemia Estimates and Higher Diagnostic Sensitivity of Microscopic and Molecular Diagnostics of Malaria Than Venous Blood Samples

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Background. Diagnosis of malaria is usually based on samples of peripheral blood. However, it is unclear whether capillary (CAP) or venous (VEN) blood samples provide better diagnostic performance. Quantitative differences of parasitemia between CAP and VEN blood and diagnostic performance characteristics were investigated.

Methods. Patients were recruited between September 2015 and February 2016 in Gabon. Light microscopy and quantitative polymerase chain reaction (qPCR) measured parasitemia of paired CAP and VEN samples. CAP and VEN performance characteristics using microscopy were evaluated against a qPCR gold standard.

Results. Microscopy revealed a median parasitemia of 495/μL in CAP and 429/μL in VEN samples, manifesting in a 16.6% (P = .04) higher CAP parasitemia compared with VEN parasitemia. Concordantly, in qPCR –0.278 (P = .006) cycles were required for signal detection in CAP samples. CAP sensitivity of microscopy relative to the gold standard was 81.5% vs VEN sensitivity of 73.4%, while specificities were 91%. CAP and VEN sensitivities dropped to 63.3% and 45.9%, respectively, for a subpopulation of low-level parasitemias, whereas specificities were 92%.

Conclusions. CAP sampling leads to higher parasitemias compared to VEN sampling and improves diagnostic sensitivity. These findings may have important implications for routine diagnostics, research, and elimination campaigns of malaria.

Keywords. malaria; sensitivity; specificity; capillary; venous.

Malaria constitutes a major global health problem. According to estimates by the World Health Organization (WHO), 216 million malaria cases had occurred in 2016, with 445 000 resulting in death [1]. Furthermore, 90% of all malaria cases and deaths worldwide occurred in the WHO African Region [1]. These figures demonstrate the high disease burden that resource-limited countries need to withstand and the consequent need for control strategies in those settings that are not only effective, but also affordable.

To date, early diagnosis is among the most important malaria control strategies and, where available, light microscopy of thick blood smears is considered the gold standard for diagnosis in clinical routine [1, 2]. Currently, no specific recommendation exists with regard to the source of blood sample that should be used for malaria diagnostics. Most commonly sampled blood stems from finger pricks (ie, capillary [CAP] blood) and venous (VEN) blood draws.

Previously, 2 studies have reported potentially superior performance of CAP blood samples for malaria diagnosis compared to VEN blood samples [3, 4]. These studies, however, were underpowered and used methodologies that do not permit to fully support these conclusions. As described above, cost-efficient optimizations of malaria diagnostics are urgently needed in resource-limited settings. Furthermore, the question whether certain blood sources may potentially influence measurements of parasitemia is also important for the clinical management of malaria as well as for research studies [5–7].
This present study used state-of-the-art methods to investigate a potential difference in parasitemia between CAP and VEN samples and to determine the diagnostic performance characteristics of CAP and VEN samples to detect malaria parasites.

**METHODS**

**Study Design and Study Population**
The study population of this prospective diagnostic study was recruited at the Centre de Recherches Médicales de Lambaréné and the Centre de Recherches de la Nguinié, 2 clinical research centers in central Gabon [8, 9]. The study region is hyperendemic for malaria and has a perennial transmission pattern. All patients presenting to the diagnostic services of the 2 health centers for malaria diagnosis were invited to participate in this study. Recruitment took place between September 2015 and February 2016.

**Malaria Diagnosis**

**Sampling**
CAP blood stemmed exclusively from finger pricks and VEN blood was obtained from puncture of the cubital vein. CAP and VEN sampling was performed in parallel within a 5-minute interval. Prior to sample preparation, blood from both sources was first collected in separate containers with ethylenediaminetetraacetic acid (EDTA) coating to avoid blood clotting (250-μL tube for CAP blood and 3-mL tube for VEN blood). After blood sampling the following steps were taken: For each patient, 2 thick smears of CAP and VEN blood were prepared using standardized quantities of 10 μL. One CAP-VEN pair was employed in analysis; the other pair was kept as backup for use in case of insufficient quality of the first pair. In addition, 2 thin smears of CAP and VEN blood using 5 μL blood were prepared and, standardized quantities of 20 μL CAP and VEN blood were applied on filter papers (Whatman 903 Protein Savers). CAP and VEN sample preparation followed the exact same methodology.

Due to logistic reasons EDTA-coated tubes for CAP sampling were not available throughout the entire conduct of the study. In case of nonavailability of CAP sampling tubes, CAP blood was directly aspirated from the finger with a pipette and immediately used for sample preparation. These samples were included in the microscopic evaluation but were excluded from molecular analysis.

**Light Microscopy**
Thick and thin smears were stained with 4% Giemsa for 60 minutes. Thick smears were used for quantification of asexual parasitemia (expressed as asexual parasites/μL blood) and thin smears for malaria species determination.

Parasite quantification methods were used as recommended by WHO [10–12]. Each slide was read by 2 expert microscopists and the arithmetic mean of these 2 readings was recorded. Another reading by a third microscopist was performed if the ratio of parasite densities from the higher to the lower count was >1.5 or if there was a discrepancy in positivity. By employing this strict 2- to 3-reader approach, variability of parasite counting was reduced in accordance with standard practice for clinical trials on antimalarial chemotherapy [13, 14]. A thick smear was classified as negative if at least 100 microscopic high-power fields were judged negative. Microscopists were blinded to the blood source during parasitological analysis as well as to quantitative polymerase chain reaction (qPCR) results.

**Quantitative Polymerase Chain Reaction**
DNA purification was performed using QIAamp DNA mini kits (Qiagen) as recommended by the manufacturer [15]. qPCR was conducted employing a validated assay at the German reference center for tropical pathogens (Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany) with 10 μL of purified DNA using the RealStar Malaria PCR Kit (Altona Diagnostics) according to the manufacturer’s recommendations [16].

**Parasitological Definitions, Analyses, and Statistical Considerations**

**Comparison of Parasitemia**
Due to the skewed distribution of parasitemia (expressed as parasites/μL), a log-transformation of parasitemia estimates was performed. Difference plots of \( \log_2(CAP_{parasitemia}) - \log_2(VEN_{parasitemia}) \) were computed. In analogy to Bland–Altman analysis, this was performed over the whole range of measurements [17, 18]. However, as the objective was to determine differences rather than agreements, no limits of agreement were computed. As differences of log-transformed data correspond to ratios, the methodology allowed us to demonstrate the mean excess parasitemia in percentage of either one blood source over the other \( \log_2(CAP_{parasitemia}) - \log_2(VEN_{parasitemia}) \) [19, 20].

Patients were considered for analysis of the quantitative comparison of \( CAP_{parasitemia} \) and \( VEN_{parasitemia} \) if they were positive for malaria in both blood sources. In the case of discordance of positivity (CAP positive and VEN negative or vice versa) patients were still eligible for analysis if qPCR proved positivity for malaria in the respective blood sample that was negative in microscopy. It is a common strategy to assign a value below the limit of detection (LOD) to a nondetect if there is reason to conclude that the unit-to-detect is actually present [21, 22]. Thus, the power of a study is improved, as log-transformation of 0 or a negative number would entail a missing data pair. This concept was applied in this study by assigning half the parasitemia of the paired positive sample for the microscopically negative sample. This conservative approach ensured that no artificial outliers were produced, thus avoiding measurement bias.

Difference plots of CAP cycles – VEN cycles were computed for qPCR analysis. Patients were eligible for analysis if qPCR was positive in both CAP and VEN blood.
Comparison of Performance Characteristics

A patient was considered positive for malaria if either CAP or VEN blood was positive for malaria in qPCR. Thus, any of the permutations CAP positive and VEN positive, CAP positive and VEN negative, and CAP negative and VEN positive constituted the gold standard definition of malaria for this study, against which performance characteristics of microscopy of CAP and VEN blood were evaluated.

Statistical Considerations

Stata/SE15.1 software (StataCorp) was used for statistical analysis. Stata software packages were downloaded for computation of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) [23], and likelihood ratios [24]. Area under the receiver operating characteristic (ROC) curve was computed with Stata’s “roctab” command. Pearson r was calculated for correlation analysis of CAP\textsubscript{parasitemia} and VEN\textsubscript{parasitemia} and intraclass correlation coefficients (ICCs) were created to assess the reproducibility of CAP\textsubscript{parasitemia} in VEN blood, regarding patients as random effects and the blood source as fixed effect. Absolute agreement was selected and average ICCs were reported for microscopic data (as multiple readers determined the definitive result) and individual ICCs were reported for qPCR (as only one result determined the definitive result). A significance level of 2-sided α < .05 was considered as statistically significant.

Two small and mainly descriptive studies were identified having investigated a potential CAP-VEN parasite difference and using light microscopy as the sole method for parasite detection [3, 4]. Data from these studies were extracted and, alongside microscopic data from this present study, odds ratios (ORs) for paired data were computed as described in the literature [25]. In this context, such ORs quantify the likelihood of parasite detection in one sampling site compared to the other. Odds ratios of these studies were evaluated in meta-analysis. Meta-analytical summary measures and forest plots were computed with Stata’s “metaan” command [26].

According to sample size calculations, which are demonstrated in the Supplementary Materials, and due to fluctuations of malaria prevalence among local patient populations overall, recruitment was planned to continue until reaching a sample of 375 patients. Biological samples were then shipped to Europe for further parasitological analysis.

Ethical Considerations

The study was conducted according to the Declaration of Helsinki, the applicable guidelines for Good Clinical Practice as defined by the "International Conference On Harmonization Of Technical Requirements For Registration Of Pharmaceuticals For Human Use"; and the applicable laws and regulations of Gabon. The study was approved by the independent ethics committee of the Centre de Recherches Médicales de Lambaréné (reference number: CEICERMEL, 009/2015). Upon diagnosis of malaria, an effective antimalarial treatment was provided to the participants at no cost.

RESULTS

Baseline Characteristics

Three hundred seventy-six patients were recruited into this diagnostic study. Among those, 346 participants were eligible for analysis of performance characteristics, as well as 204 and 108 participants for analysis of quantitative differences between CAP\textsubscript{parasitemia} and VEN\textsubscript{parasitemia} in microscopy and qPCR, respectively (Figure 1). The median age of participants in various analyses was between 11 and 15 years with a female/male ratio of almost 1 (Table 1).

CAP\textsubscript{parasitemia} and VEN\textsubscript{parasitemia}: Correlations, Reproducibility, and Differences

CAP\textsubscript{parasitemia} and VEN\textsubscript{parasitemia} as determined by microscopy and qPCR show good correlation with Pearson r of >0.9 for the respective analyses (Table 2). ICCs demonstrate excellent reproducibility of CAP\textsubscript{parasitemia} in VEN blood with values consistently >90% in all models (P = .0001). Median (interquartile range [IQR]) CAP\textsubscript{parasitemia} was slightly higher than median VEN\textsubscript{parasitemia}, and inspection of CAP-VEN differences over the whole range of measurements demonstrates a small but significant excess parasitemia in CAP blood (Table 2 and Figure 2). Microscopy quantified the mean CAP\textsubscript{parasitemia} to be +16.6% (95% confidence interval [CI], +7.0% to +35%) higher than VEN\textsubscript{parasitemia}. Concordantly, qPCR analysis demonstrated that fewer cycles (~0.278 [95% CI, ~0.473 to ~0.083]) were required for parasite signal detection in CAP blood than in VEN blood.

Thirty-four patients were identified as gametocyte carriers. Gametocytes were 4.2 times more likely to be detected in CAP blood than in VEN blood (odds ratio [OR], 4.2; P = .0017) and CAP gametocytemia was +55.9% (95% CI, +22.1% to +99%) higher than VEN gametocytemia (Supplementary Tables 1 and 2).

A stratified analysis per Plasmodium species indicated that this CAP-VEN difference occurred both in Plasmodium falciparum monoinfections with +17.9% (95% CI, +6.4% to +37.9%) and in nonfalciparum infections with +14.4% (95% CI, +47.0% to +146.7%) more parasites in CAP blood relative to VEN blood (Table 2). Mixed infections showed no such difference, however, being highly likely due to sampling variation (P = .95).

Performance Characteristics

The proportion of false-negative microscopic results was higher in VEN samples (26.6%) than in CAP samples (18.5%) (Table 3) and microscopically determined parasitemias of false-negatives were low with a median of 27 (IQR, 16–256) and 43.5 (IQR, 32–86) parasites/μL, respectively. This indicates a difference in sensitivity of approximately 8% (sensitivity, 81.5% for CAP vs 73.4% for VEN), whereas specificities remained roughly equal at 91%. This results in a more favorable positive likelihood ratio for CAP samples, which is approximately 2 units higher than for VEN samples (10.1 vs 8.3), as well as a more favorable negative likelihood ratio (0.2 vs 0.29). Both area under the ROC curves
are >0.8; although 95% CIs did not go below 0.8 for CAP samples (.83–.9), they did so in VEN samples (.78–.86) (Table 4). Investigation of a subpopulation of low-level parasitemias (<250 parasites/μL) decreased CAP sensitivity and VEN sensitivity; however, simultaneously increasing the above-mentioned sensitivity difference (sensitivity, 63.3% for CAP vs 45.9% for VEN).

Specificities remained high at approximately 92% (Table 4). A cross-tabulation of quantitative polymerase chain reaction (qPCR) results shows that malaria DNA was twice more likely to be detected in CAP blood than in VEN blood (odds ratio, 2.17; \(P = .11\); Supplementary Table 3).

Meta-analytical summary measures demonstrate that malaria parasites were 2.63 (95% CI, 1.68–4.08) times more likely to be detected by light microscopy in CAP blood than in VEN blood (Figure 3 and Supplementary Table 4). Measures of heterogeneity did not indicate heterogeneity (\(P = .96\); Supplementary Table 5).

**DISCUSSION**

This study investigated the diagnostic performance characteristics of 2 easy-to-access blood sources, capillary and venous blood. CAP blood was shown to be of diagnostic superiority, manifesting in an 8% sensitivity gap between CAP and VEN blood in the overall study population and widening by >2-fold in a subpopulation of low-level parasitemias (sensitivity gap of 17%).

This CAP superiority in sensitivity can be explained by the comparatively higher parasite abundance in CAP blood as diagnosis of malaria in microscopy is based on the demonstration of asexual parasites. Microscopy has a theoretical LOD of approximately 10 parasites/μL [10–12], whereas under real-life settings in endemic areas, a LOD of approximately 90 parasites/μL is typical [27]. Particularly in cases of poorly trained personnel, LODs may rise considerably above these limits. In the study presented here, blood smears were investigated by expert microscopists, who had been assessed by dedicated examinations for malaria diagnostics according to WHO standards. However, it is concluded that even a small CAP-VEN difference in parasitemia of approximately 15% may facilitate diagnosis in CAP blood (as just above the LOD), but not in VEN blood (as not yet surpassed the LOD). The diagnostic superiority in favor of CAP blood could potentially rise inversely with the degree of microscopist training. As LODs would accordingly rise to higher parasitemias (eg, 100 parasites/μL vs 10 parasites/μL) for less well-trained microscopists, an approximate 15% difference would result in a higher absolute value of parasite difference (15 parasites/μL vs 1.5 parasites/μL), thereby potentially increasing the sensitivity gap. Given the lack of microscopists with adequate training and time to read many fields in resource-limited, malaria-endemic settings, this is a potential topic of concern. However, in high-resource settings where malaria is not endemic, increased LODs are also likely, as uncommon or rare diseases may not receive sufficient resources and training.

Irrespective of blood source, sensitivity of microscopy can be increased by investigating larger sample volumes. In our
experience, finger pricks were more accepted than venous punctures, and sampling CAP blood quantities of up to 100 μL was well tolerated. Such quantities allow the preparation of multiple thick smears facilitating the microscopic investigation of >1000 fields, thereby further lowering LODs.

Sensitivity and specificity are fixed assay-specific properties. However, PPVs and NPVs combine these assay properties with characteristics of the respective target population and constitute the degree to which one can trust in the correctness of the respective positive or negative test result [28]. While PPVs are affected by low disease prevalence, NPVs are affected by high prevalence. Examples of changing PPVs and NPVs for different prevalence settings were modeled and are depicted in the Supplementary Materials. CAP blood may be of particular diagnostic benefit in low-prevalence settings of malaria to rule in malaria as differential diagnosis (by higher PPV) and help in high-prevalence settings to rule out malaria as differential diagnosis (by higher NPV).

Importantly, microscopic findings were further supported by molecular detection of parasite DNA. Results of microscopy are concordant to findings of qPCR, in that fewer cycles were necessary to detect parasite DNA in CAP blood compared to VEN blood. The employed qPCR protocol has a LOD of approximately 1 parasite/μL blood [16, E. Tannich, personal communication, 2017]. Thus, the CAP-VEN difference can be extrapolated to roughly one-tenth of the LOD of microscopy while it remains uncertain how this effect would behave for

![Figure 2. A, Microscopic comparison of capillary blood (CAP) parasitemia and venous blood (VEN) parasitemia. B, Quantitative polymerase chain reaction (qPCR) comparison of CAP cycles and VEN cycles. Solid lines indicate mean differences; long dashed lines indicate 95% confidence intervals for mean differences; short dashed line indicates line of equality.](https://academic.oup.com/jid/article-abstract/218/8/1296/5003449)
much lower parasite densities. Interestingly, qPCR detected parasite DNA only in CAP blood in twice as many patients than only in VEN blood (OR, 2.17; \( P = .11 \); Supplementary Table 3).

Erythrocytes infected by \( P. falciparum \) are known to contain a number of highly variable parasite proteins on their surface, such as \( P. falciparum \) erythrocyte membrane protein 1, repetitive interspersed family, or sub-telomeric variable open reading frame [29–31]. These molecules show affinity to certain vascular receptors, such as intercellular adhesion molecule 1 (ICAM-1) [32, 33]. ICAM-1 is expressed in CAP blood vessels in response to proinflammatory cytokines whose production is triggered by malarial pathogen-associated molecular patterns during a malaria infection [29, 34, 35]. This pathophysiological phenomenon could thus serve as explanation for the higher parasitemia of CAP blood relative to VEN blood. Furthermore, infected erythrocytes that do not bind to vascular endothelium have a higher chance to become eliminated in the spleen, thereby also contributing to a relatively lower parasitemia in VEN blood.

This study demonstrates a significant association between gametocyte carrier status and CAP blood sampling (OR, 4.2; \( P = .0017 \)), potentially owing to a higher CAP gametocytemia (+55.9%; \( P = .0008 \)) compared with VEN gametocytemia. This seems plausible as CAP blood is used by \( Anopheles \) mosquitos during blood meals and gametocytes are parasite stages aiming...
### Table 2. Comparison of Capillary Blood Parasitemia and Venous Blood Parasitemia Using Microscopy and Quantitative Polymerase Chain Reaction

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Infection</th>
<th>No.</th>
<th>CAP Median Parasitemia (IQR)</th>
<th>VEN Median Parasitemia (IQR)</th>
<th>Light Microscopy, % (95% CI)</th>
<th>qPCR*, Average Cycles (95% CI)</th>
<th>ICC, % (95% CI)</th>
<th>ICC, % (95% CI)</th>
<th>PValuef</th>
<th>Pearson r</th>
<th>ICC, % (95% CI)</th>
<th>PValuef</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopic analysis; totals</td>
<td>Plasmodium falciparum monoinfection</td>
<td>183</td>
<td>512 (79–4800)</td>
<td>433 (46–4750)</td>
<td>+17.9 (+6.4 to +37.9)</td>
<td>.04</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.92</td>
<td>–</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Mixed infection (P. falciparum + nonfalciparum spp)</td>
<td>10</td>
<td>699 (215–1114)</td>
<td>797 (282–10306)</td>
<td>−1 (−2.9 to +38.5)</td>
<td>.95</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.97</td>
<td>99 (96.8–99.8)</td>
<td>.0001</td>
</tr>
<tr>
<td></td>
<td>Nonfalciparum infection (Only Plasmodium ovale + Plasmodium malariae)</td>
<td>11</td>
<td>193 (27–760)</td>
<td>101 (21–888)</td>
<td>+14.4 (−4.7 to +146.7)</td>
<td>.71</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.8</td>
<td>97 (90.7–99.3)</td>
<td>.0001</td>
</tr>
<tr>
<td></td>
<td>Microscopy analysis; when qPCR gold standard was positive</td>
<td>187</td>
<td>519 (85–4349)</td>
<td>448 (55–4091)</td>
<td>+16.7 (−8 to +37.3)</td>
<td>.06</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.91</td>
<td>95 (93.5–96.3)</td>
<td>.0001</td>
</tr>
<tr>
<td>qPCR analysis</td>
<td>Totals</td>
<td>108</td>
<td>25.735 (23.27–27.35)</td>
<td>25.95 (23.585–27.325)</td>
<td>–</td>
<td>–</td>
<td>−0.278 (−.473 to −.083)</td>
<td>.006</td>
<td>.94</td>
<td>93 (90.1–95.2)</td>
<td>.0001</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CAP, capillary blood; CI, confidence interval; ICC, intraclass correlation coefficient; IQR, interquartile range; qPCR, quantitative polymerase chain reaction; VEN, venous blood.

*aUnits: parasites per microliter for light microscopy; cycles for qPCR.

*bLight microscopy; +, more parasites in capillary blood; −, more parasites in venous blood.

*cUnlogged parasitemia was used to assess ICCs.

*dPaired t test: H0: log2(CAPparasitemia) = log2(VENparasitemia).

*eqPCR: −, more parasite DNA in CAP blood; +, more parasite DNA in VEN blood.

*fF test.
The present study is in discordance with a previously published study with similar objectives though with different methodology. This study concluded that there was no difference in sexual parasitemia between CAP and VEN blood (null hypothesis: \( \text{CAP}_{\text{parasitemia}} = \text{VEN}_{\text{parasitemia}} \); \( P = .47 \)) [36]. The authors recategorized the continuous outcome parasitemia into an ordinal response variable of 3 categories—“0 parasites,” “1–5000 parasites,” “>5000 parasites”—and used a cumulative linked mixed model to assess whether either CAP or VEN blood exposure had an influence on the ordinal outcome. It is possible that the somewhat arbitrarily chosen bounds may not have been sensitive enough to detect small CAP-VEN differences (~15%), as were demonstrated in the present study. Also, with a sample size of 137, the study was likely underpowered to detect small effects, as indicated by sample size calculations of the present study. Performing power calculations in analogy to sample size calculations as described in the Supplementary Materials, our study has an observed power >95% for the comparison of parasitemia of CAP-VEN blood in both microscopy and qPCR analyses, and analyses of performance characteristics have an observed power between 80% and 90% [37]. Last, different observations may also be explained by a biological effect, as the above-cited study recruited asymptomatic carriers, whereas our study included symptomatic malaria patients. Sensitivity analyses, however, should also apply to asymptomatic carriers. In line with this assumption, 2 smaller studies on this topic demonstrated concordant results to the present study [3, 4]. Neither of the studies performed qPCR or sensitivity analyses but demonstrate that malaria parasites were approximately 2.8 times more likely to be detected in CAP samples than in VEN samples (Figure 3).

Strengths of this study include the high methodological quality under which blood sampling and parasitological analysis were performed. Importantly, while sensitivities differed between CAP and VEN blood samples, specificities were approximately equal at 91%. Thus, the resulting similar false-positivity rate is indicative that assessor blinding was not infringed. Yet, it is a limitation of this study that CAP blood sampling tubes were not always available throughout the study period.

This study reports important evidence for research studies of various kinds: (1) clinical trials, particularly on antimalarial chemotherapy, for which reduction of parasitemia is often an important endpoint; (2) clinical trials that are associated with low parasitemias such as malaria vaccine trials and may be affected by the source of blood taken for malaria diagnostics; (3) epidemiological studies aiming at determining the true prevalence of malaria in a given context. To allow generalization of study results in a broad and multinational context, future epidemiological and clinical studies should mention whether CAP

<table>
<thead>
<tr>
<th>Microscopic Sample (n = 346)</th>
<th>qPCR (CAP or VEN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive*</td>
<td>Negativeb</td>
</tr>
<tr>
<td>CAPc</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>181 (81.5)</td>
</tr>
<tr>
<td>Negative</td>
<td>41 (18.5)</td>
</tr>
<tr>
<td>VENc</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>163 (73.4)</td>
</tr>
<tr>
<td>Negative</td>
<td>59 (26.6)</td>
</tr>
</tbody>
</table>

Data are presented as No. (column %).
Abbreviations: CAP, capillary blood; qPCR, quantitative polymerase chain reaction; VEN, venous blood.
*Positive = CAP positive and VEN positive, CAP positive and VEN negative, or CAP negative and VEN positive.
bNegative = CAP negative and VEN negative.
χ² test: \( P < .0001 \).

Table 3. Two-Way Tables of Capillary and Venous Blood as Assessed by Light Microscopy Cross-Tabulated Against a Quantitative Polymerase Chain Reaction Gold Standard

Table 4. Comparison of Performance Characteristics of Microscopically Analyzed Capillary and Venous Blood Samples Against Quantitative Polymerase Chain Reaction Gold Standard

<table>
<thead>
<tr>
<th>Performance Characteristic</th>
<th>Totals (All Parasitemias) (n = 346)</th>
<th>Low-Level Parasitemias (&lt;250 Parasites/µL) (n = 231)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAP</td>
<td>VEN</td>
</tr>
<tr>
<td>Sensitivity, %</td>
<td>81.5 (77.4–85.6)</td>
<td>73.4 (68.8–78.1)</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>91.9 (89.1–94.8)</td>
<td>91.1 (88.1–94.1)</td>
</tr>
<tr>
<td>PPV, %</td>
<td>94.8 (92.4–971)</td>
<td>93.7 (91.1–96.2)</td>
</tr>
<tr>
<td>NPV, %</td>
<td>73.6 (68.9–78.2)</td>
<td>65.7 (60.7–70.7)</td>
</tr>
<tr>
<td>Positive LR</td>
<td>10.11 (5.56–18.38)</td>
<td>8.28 (4.68–14.63)</td>
</tr>
<tr>
<td>Negative LR</td>
<td>0.2 (1.15–27)</td>
<td>0.29 (23–37)</td>
</tr>
<tr>
<td>Area under ROC curve</td>
<td>0.87 (1.33–9)</td>
<td>0.82 (.78–86)</td>
</tr>
<tr>
<td>Prevalence*, %</td>
<td>64.2 (59.1–69.2)</td>
<td>64.2 (59.1–69.2)</td>
</tr>
</tbody>
</table>

Data in parentheses indicate the 95% confidence interval.
Abbreviations: CAP, capillary blood; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic; VEN, venous blood.
*Prevalence of malaria as determined by qPCR.
or VEN blood was used for determination of malaria parasitemia and what sample volume was investigated. If both CAP and VEN blood samples are used to determine parasitemia, a correction factor should be applied especially in the context of repeated measurements. If CAP samples constitute the minority in a series of repeated measurements, then 15% of the respective CAP parasitemia should be subtracted from its value (CAP parasitemia corrected = CAP parasitemia uncorrected − 0.15 × CAP parasitemia uncorrected). Concordantly, if VEN samples constitute the minority, then 15% should be added (VEN parasitemia corrected = VEN parasitemia uncorrected + 0.15 × VEN parasitemia uncorrected). Last, this study underlines the importance of CAP sampling in the context of malaria elimination campaigns particularly in settings of perennial endemicity where people with low-level parasitemias constitute an important reservoir of malaria parasites [38]. Approaches aiming at this target group should use CAP blood for sampling purposes.

Currently no distinction is being made with regard to the blood source from the sample that was used for malaria diagnostics. Considering an 8% increase in sensitivity by using capillary blood, an important proportion of patients may be misdiagnosed if venous blood is used for laboratory analysis. Due to the global burden of malaria, a programmatic recommendation of CAP blood for diagnosis of malaria could entail a considerable global public health benefit if these findings are reproduced and confirmed in diverse epidemiological settings.

**CONCLUSIONS**

This study investigated the diagnostic performance characteristics of microscopy and qPCR using CAP and VEN blood samples. A CAP-VEN sensitivity gap of 8% was revealed that increases to 17% for low-level parasitemias. CAP blood sampling is simple and cost-efficient, thus being potentially beneficial to optimize routine diagnostics in both low- and high-resource settings. Thus, using the adequate source of blood samples for malaria diagnostics may be of high importance.

**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**


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