COMPARISON OF METHODS FOR THE RAPID LABORATORY ASSESSMENT OF CHILDREN WITH MALARIA


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Abstract. Rapid diagnosis and accurate quantification of Plasmodium falciparum parasitemia are important for the management of malaria. The assessment of disease severity also depends on evaluation of metabolic indexes such as blood glucose and lactate concentrations. Here we describe an accurate and rapid alternative to conventional thick film examination (Lambaréné method). We also assess near-patient methods for measuring blood glucose (OneTouch) and lactate (Accusport). The accuracy of the Lambaréné method is similar to that of thin films. Results from the OneTouch glucose meter also are in good agreement with a YSI 2300 reference meter. Overall, the Accusport lactate meter agrees poorly with the YSI 2300 reference meter. However, the sensitivity and specificity to detect hyperlactatemia (blood lactate ≥ 5 mmol/L) are 0.94 and 0.98, respectively.

INTRODUCTION

Most deaths due to malaria in children occur shortly (< 24 hr) after admission to hospital. Early appropriate treatment of malaria and its complications may be life-saving. The objective of these studies was to optimize methods to quantify parasitemia and rapidly assess blood glucose and lactate levels.

Microscopic examination of a thick or thin blood film is essential both to diagnose malaria and to quantify parasitemia. Thick and thin blood films have advantages that vary with respect to parasitemia, ease of use, and accuracy. Thin films enumerate parasites or parasitized erythrocytes in a fixed number of red blood cells. This value is expressed as a percentage, or converted to a count per microliter of blood by means of other information (e.g., red blood cell count or hematocrit and an assumed mean corpuscular volume). Thick films can be time-consuming to read.

Thick films usually quantify parasites in relation to leucocyte numbers. These figures are converted to counts per microliter by use of a measured or assumed white blood cell count (usually 8,000 cells/μL), although this may vary up to 7-fold.1 Sometimes parasites are counted in relation to microscopic HPF, and conversion to counts per microliter then depends on an assumption of the volume of blood examined. We here report a rapid, simple, and accurate method of quantifying parasitemia that does not have the inherent approximations of conventional thick blood films.

In addition to parasitemia, we also evaluated newer near-patient methods of measuring blood glucose (OneTouch II, Lifescan, Neckargemünd, Germany) and lactate (Accusport, Roche Diagnostics, Mannheim, Germany). Hypoglycemia is an important complication of severe malaria. The ability quickly and accurately to diagnose and treat hypoglycemia in these children is essential. Hyperlactatemia (plasma or blood lactate ≥ 5 mmol/L) has been shown in several studies to be the best independent predictor of mortality in children with malaria.2–5 Blood lactate measurements are routinely performed in the management of patients on intensive care units. So far, measurement of blood lactate concentrations in children with malaria has only been conducted in a few centers because the analyzers are expensive and complex to maintain. The use of a cheap and easy-to-use handheld lactate analyzer would allow the much wider use of blood lactate concentration measurements for clinical studies and for risk assessment in clinical practice.

METHODS

These studies were approved by the ethics review committee of the Albert Schweitzer Hospital, Lambaréné, and were carried out between October 1999 and March 2000 at the Albert Schweitzer Hospital, Lambaréné, and the Centre Hospitalier de Libreville, Gabon. Thick and thin blood films were made from febrile patients and read by 4 blinded investigators (2 for thin and 2 for thick films). Thin films were read by one of the investigators (2 for thin and 2 for thick films). Thin films were scored using the Field's stain,2 and the number of parasitized red blood cells was counted per thousand erythrocytes (or per 5,000 if < 3/1,000 erythrocytes and per 10,000 if < 3/5,000 erythrocytes). Hematocrit was measured with a QBC machine (Beckton-Dickinson, Franklin Lakes, NJ), and the following equation was used to calculate the parasitemia per microliter (assumes a mean corpuscular volume of 80 fl):

Parasitemia/μL

= count per thousand red blood cells hematocrit

× 125.6

(1)

The Lambaréné method of counting slides is a variant of a method for counting thick films. Ten microliters of blood is evenly distributed on a 10- by 18-mm area of a microscope slide (drawn on paper underneath the slide) with a micropipette (Carl Roth GmbH, Karlsruhe Germany). The slide is dried and stained (20% Giemsa [Sigma Chemical, Sigma Aldrich Chemie GmbH, Taufkirchen, Germany], pH 7.2, 20 min). Each HFP on this thick smear is ~ 1/600th of a microliter (on a standard microscope at ×1,000 magnification), and a count is made per 3, 5, 10, 50, or 100 HPFs. The parasitemia per microliter is calculated by an appropriate multiplication factor that depends on the magnification and the area of the microscopic field. For most microscopes, the required multiplication factor is 400–800 for counts per HFP. This method was mentioned in brief by Kremser and colleagues6 and has the advantages of direct quantification...
of the parasitemia, speed, and higher quality compared with conventional thick blood films.

A YSI 2300 (YSI, Yellow Springs, OH) glucose/lactate analyzer was used as a reference for blood glucose and lactate concentration measurements. This machine uses an electrode to measure current generated by reactions catalyzed by glucose or lactate oxidase. The analyzer was checked against an external standard daily and calibrated every 6 samples according to the manufacturer’s instructions.

Portable analyzers were used to measure glucose (OneTouch II) and lactate (Accusport). These analyzers use strips impregnated with lactate oxidase or glucose oxidase and a colorimetric method to determine lactate and glucose concentrations. Measurements were performed on anticoagulated venous whole blood (lithium heparin, 15 IU; Sigma). Measurements were performed within 5 min of sampling and simultaneously with measurements with the YSI analyzer.

Statistical analyses used STATA 6.0 (Stata Corp., College Station, TX) and SYSTAT 5.2 (SYSTAT, Elvaston, IL). Agreement was assessed by Bland-Altman analysis. Data from parasitemias was log$_{10}$ transformed. The mean of the log$_{10}$ transformed parasitemias from the 2 blinded observers was used to compare counts on thick films with counts on thin films. Proportions were compared by Fisher’s exact test and correlation with Pearson’s correlation coefficient. Variances of correlated variables were compared with Pittman’s test.

RESULTS

Parasitemia. Eighty children had paired thin film parasite counts and hematocrit measurements. One hundred one children had paired thick films. Parasite counts ranged from 0–524,000 (thin film) and 0–600,000 (thick film).

Paired thin film counts were well correlated ($r = 0.89$, $n = 80$, $P < 0.0001$). Bland-Altman analysis showed almost no mean difference between observers (difference mean $-0.235$ per 1,000 red blood cells and 95% limits of agreement [LOA] of $-11$ and 11 per thousand red blood cells [1%]). There was high interobserver correlation between the counts per microliter on thin films ($r = 0.94$, $n = 101$, $P < 0.0001$) and on thick films ($r = 0.94$, $n = 101$, $P < 0.0001$).

Parasite counts were compared after conversion to counts per microliter. The difference mean (95% LOA) for the log$_{10}$-transformed parasite counts for the thin film was $-0.035$ ($-1.316$, 1.385, $n = 78$) (Figure 1a) and thick films was $-0.030$ ($-1.112$, 1.052, $n = 101$) (Figure 1b), and variances were not significantly different ($P = 0.85$).

To compare the agreement of thick and thin film counting methods, the mean of log$_{10}$-transformed thick film counts was compared with the mean of thin film counts, giving a mean difference (95% LOA) of 0.115 ($-1.199$, 1.430, $n = 78$). Thus, comparisons made between thick and thin film methods of counting give similar difference means and 95% LOA to comparisons of counts by the same method of assessment.

Because thick films may be less accurate at higher levels of parasitemia, Bland-Altman analysis was performed separately for parasite counts above and below 4 log$_{10}$ units (10,000/μL). There was better agreement between counts of thick and thin films at higher counts, difference mean (95% LOA) for the parasite counts $< 4$ log$_{10}$ units was 0.177 ($-1.744$, 2.097, $n = 31$) and $> 4$ log$_{10}$ units was 0.075 ($-0.611$, 0.761, $n = 47$) (Figure 1d). Standard deviations were not significantly different between thick and thin film for parasitemias $< 4$ log$_{10}$ units (Pittman’s test, ratio of standard deviations 0.90, $P = 0.44$, $n = 31$). The standard deviations for thick film counts was significantly larger than for thin film counts for parasitemias $> 4$ log$_{10}$ units (ratio of standard deviations 1.49, $P = 0.004$, $n = 47$).

Time for counting. Time taken to count the thin films
was measured in a subset of 38 thin film counts (23 counts per 1,000, 5 per 5,000, and 10 per 10,000 red blood cells) and 29 thick films. The mean (standard deviation) time to count thin films was 10.9 (0.20) min and was significantly longer than for thick films, with mean (standard deviation) of 3.0 (0.9) \( P < 0.001 \).

Glucose measurements. There were 48 paired glucose measurements made, with a median (range) of 5.1 (1.8–9.0) mmol/L. The measurements for the YSI and the OneTouch analyzers were well correlated \( r = 0.95, P < 0.001 \). Bland-Altman analysis gave a difference mean (95% LOA) of 0.13 (−0.93, −0.67, \( n = 48 \)) mmol/L for the 2 methods. There was only one episode of hypoglycemia in a child during this study, and this was detected by both machines.

Lactate measurements. Lactate was measured on 2 handheld Accusport analyzers of the same model, designated A and B (one at Libreville and one at Lambaréné), and each value was compared with a YSI analyzer. There were 114 pairs of measurements made, with a median (range) of 2.1 (0.8–19.6) mmol/L. The YSI and Accusport machine A lactate measurements were highly correlated \( r = 0.95, P < 0.001, n = 51 \). Agreement, however, was poor, with a difference mean (95% LOA) of −1.131 (−2.269, 0.006, \( n = 51 \)) mmol/L (Figure 2a). Above the blood lactate concentration of 5 mmol/L, the Accusport was less accurate.

A systematic difference between lactate measurements on lactate machine A and the YSI analyzer was noted for the first 35 paired measures of lactate, described by the following equation:

\[
\text{YSI lactate} = 1.01 \times \text{Accusport A lactate} – 1.22 \quad (2)
\]

This formula was used to generate a corrected value for the Accusport measurement that was then compared with the YSI analyzer. The corrected value improved agreement considerably between methods, with a difference mean (95% LOA) of 0.05 (−1.19, 1.09, \( n = 51 \)) mmol/L.

For a second Accusport lactate analyzer (Accusport B), measurements were highly correlated with the YSI machine \( r = 0.99, P < 0.001, n = 78 \). Agreement was reasonable, with a difference mean (95% LOA) of −0.34 (−1.89, 1.22, \( n = 78 \)) mmol/L (Figure 2b). The Accusport B machine tended to underestimate the blood lactate concentration at blood lactate concentrations above 8 mmol/L.

There were 16 episodes of hyperlactatemia (lactate ≥ 5 mmol/L). The sensitivity and specificity for Accusport analyzer (Accusport B) to detect hyperlactatemia were 0.94 (95% confidence interval, 88–99) and 0.98 (95% confidence interval, 93–100), respectively.

**DISCUSSION**

The best care of a child with a suspected diagnosis of malaria requires accurate diagnosis and assessment of risk factors and complications. Our study describes methods that may be useful for near-patient testing and an alternative method of thick film counting that is rapid and accurate and that avoids approximation.

The most commonly used methods for parasite counting are thin film counts carried out per 500 or 1,000 erythrocytes or a thick film count per fixed number of white blood cells. Methods of thick film quantification that used wire loops to place fixed amounts of blood to fixed areas of slides have been described before and were used by the Australian army in Papua New Guinea during World War II although these methods are no longer in general use. The Lambaréné method is a variant that uses a micropipette to accurately place a known volume of blood on a known area of a slide. Thin film counts were chosen as the reference method.

Our results showed a good agreement between the 2 methods, with a difference mean of zero and 95% LOA of −1 log₁₀ unit. The agreement was better at parasitemias > 10,000/μL, perhaps because the thin film counts are more variable when fewer parasites are counted.

The Lambaréné method of preparing thick films has the advantages of being quick, easy to perform, and sensitive. The Lambaréné method also has comparable accuracy to counts made on thin films and may therefore be considered preferable to conventional thick film counts on grounds of accuracy and reliability. The Lambaréné method of counting is also superior to thin film counts because it is quicker and easier to perform and produces good-quality blood smears, even when performed by inexperienced staff.

Two unblinded studies have compared thick and thin film counting methods of counting malaria films for hospitalized patients. These studies compared different methods for
counting thick and thin films (either per 10,000 or 100,000 red blood cells), and both found thick films were not as accurate as thin films. They also recommended a thick film counting method that quantified the count per HPF and converted to a count per microliter assuming a volume of a HPF for a “well prepared thick film” (0.02 µL). Unfortunately, neither study used a fixed volume of blood to make thick films.

Near-patient blood testing is potentially important for the management of severe malaria. Glucose strips have been used for years to detect hypoglycemia. The availability of near-patient testing for lactate and glucose and to diagnose *P. falciparum* in addition to a clinical examination allows the possibility of assessment of the severity of malaria within 2–3 min from a small sample of blood. Because handheld analyzers are cheaper and easier to quality control than laboratory-based machines, lactate measurements may become more widely available.

An assessment of a glucose measurement strips in malaria (BMsticks, Boehringer Mannheim, Mannheim, Germany) has been completed; results showed reasonable accuracy and good sensitivity for the detection of hypoglycemia.12 The OneTouch analyzer has been assessed for glucose measurement of pregnant women and found a coefficient of variation for venous blood of 2.7%.13 The agreement between the handheld glucose (OneTouch) analyzer was good, and it could be used for near-patient testing in malaria. The numbers of patients in this study were small, and further testing would be necessary to give an estimate of the sensitivity and specificity for the detection of hypoglycemia.

A number of other studies have examined the accuracy of the Accusport lactate analyzer in athletes,14,15 neonates,16,17 and critically ill patients.18,19 Studies that have used Bland-Altman analysis showed that mean differences (95% LOA) were similar to our findings, −0.2 (−1.2 to 0.8),16 −0.3 (−1.33 to 0.69),18 and for a study that tested 2 Accusport machines with machine A −0.4 (−1.8 to 1.1) and machine B −0.4 (−1.5 to 0.7).19 These published limits of agreement were also similar to the values for our Accusport B, although different for those of Accusport A.

The Accusport lacate meter had poor agreement with the YSI 2300, and there was considerable difference between the 2 machines tested. We believe that this handheld analyzer is unreliable for the measurement of blood lactate in people with malaria. However, the analyzer was sensitive and specific for the detection of hyperlactatemia (lactate ≥ 5 mmol/L); it could be used for screening patients for hyperlactatemia, then a better test performed later if necessary.

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