Laboratory diagnosis of malaria in the North West Region of Cameroon: analysis of limitations

Eric Ndofor*, Tom van Goolb and Henk Gillisb

*aFaculty of Medicine and Pharmacy, Human Ecology Department, Vrije Universiteit Brussel, Belgium, Campus Jette, Laarbeeklaan 103, 1090 Brussels, Belgium; bClinical Parasitology Department, Academic Medical Centre, University of Amsterdam, Holland, Postbus 22660, 1100 DD Amsterdam, The Netherlands

*Corresponding author: Present address: 8000 Chanute Pl. #15, Falls Church, VA 22042, USA. Tel: +1 571 233 5974; E-mail: ericndofor@yahoo.fr

Received 4 October 2012; revised 4 January 2013; accepted 18 January 2013

Background: Malaria is still rife and perennial in Cameroon despite remarkable progress in controlling the disease. About 95% of the country is malaria endemic. Prompt and accurate diagnosis of malaria may lead to improved patient care and reduced morbidity. This paper analyses limitations in malaria diagnosis in the North West Region of Cameroon and opportunities for improvement.

Methods: The sample units were 40 laboratories in governmental health facilities (GHFs) selected by cluster (Health Districts) and stratified sampling. The three categories of GHFs in the Region – Hospitals, District Medical Centres (Centre Médical d’Arrondissement [CMAs]) and Integrated Health Centres (IHCs) – were strata in the sample. With pre-tested interviewer-administered structured questionnaires and visits to laboratories, mechanical and optical components of microscopes and malaria diagnostic techniques were studied systematically and in detail.

Results: The main finding was that locally prepared Giemsa-stained malaria smears were of unusable quality in 52 and 46% of GHFs for thick and thin smears respectively. Some loss of quality was observed in laboratories with good and moderate quality smears.

Conclusion: The quality of malaria diagnosis was not optimal and GHFs did not have sufficient tools and resources to overcome loss of quality. Nonetheless, limitations in malaria diagnosis in the Region can be corrected.

Keywords: Malaria, Quality, Microscopic diagnosis, Governmental health facilities, Improvement, Cameroon

Introduction

Malaria is still a serious health problem in Cameroon despite remarkable progress in reducing the disease. Moreover, current malaria control efforts look fragile. In 1901, malariologist Battista Grassi, called malaria a ‘giant with clay feet’: an obstacle that can be purged if well tackled with accurate diagnosis, the cornerstone of effective case management. In Cameroon, laboratory diagnosis of malaria (LDM) is done largely by microscopy. It may lead to improved patient care if well performed, thus leading to reduced malaria morbidity. This paper analyses limitations in the quality of LDM in governmental health facilities (GHFs) in the North West Region (NWR) of Cameroon and opportunities for improvement.

Plasmodium falciparum malaria is rife in Cameroon and its transmission follows climatic and geographic factors. About 95% of the country is malaria endemic. In the north, characterized by Sudano-Sahelian planes, malaria is seasonal (transmission lasts one to three months) featuring ten monthly infective mosquito bites per person (b/p/m). Transmission is hyper-endemic and perennial (seven to twelve months) and marked by 100 b/p/m in the south, including the NWR, characterized by vast forests and coastal areas. In endemic seasonal zones – the high inland Adamawa plateau and Savannah-forest transition zones – transmission is long, seasonal (four to six months) and features 20 b/p/m. Malaria accounts for 38 and 45% morbidity among pregnant women and in children below five respectively, 24% of deaths in GHFs out of which 67% occur in infants, 36% of medical consultations and 48% of hospitalizations. It causes 26% loss of man working days and consumes 40% of family health expenses. Accurate diagnosis and effective treatment can break the life cycle of Plasmodium and prevent it from causing malaria. This entails an early detection of malaria signs and symptoms and a parasitological confirmation by microscopic examination of thick and thin blood smears stained with Giemsa, the gold

© Royal Society of Tropical Medicine and Hygiene 2013. All rights reserved. For Permissions, please email: journals.permissions@oup.com.
standard. Microscopy is affordable, sensitive - detects densities as low as five to ten parasites/μl of blood - and helps to differentiate and quantify *Plasmodium* species. The preparation and interpretation of quality malaria smears requires microscopes with sufficient contrast, focal plane, resolution and significant expertise in microscopy, especially at low level parasitaemia. These attributes together with good quality smears may prevent false results or poor species differentiation. The importance of LDM is well-recognized yet little attention is given to quality. An improvement in the quality of LDM and standardization of diagnostic techniques are unprecedented actions against malaria.

Materials and methods

Study site

Located in the western highlands of Cameroon the NWR has a surface area of 17,812 km² and 2,149,971 people. It lies within latitudes 5°40′ and 7°N and longitudes 9°45′ and 11°10′E. The Region has many ethno-linguistic groups with urban and rural growth rates of eight and three percent respectively. Agriculture and trading are the main economic activities.

Sampling

Sample units were 40 laboratories in GHFs drawn from all 18 Health Districts (clusters) in the NWR selected based on sophistication/type, number and location of GHFs in the cluster, malaria prevalence and level of development (urban or rural area) and population size. The sample units were grouped into three strata by sophistication/type as follows: Stratum one: Hospitals: Regional and Divisional/District Hospitals (the most sophisticated GHFs); Stratum two: District Medical Centres (called Centre Médical d’Arrondissement [CMAs]): lower than Hospitals, one resident doctor and offer outpatient, preventive and limited in-patient/maternity services in suburban areas; Stratum three: Integrated Health Centres (IHCs): small GHFs lower than CMAs, managed by nurses and offer outpatient and preventive services in peripheral and rural areas.

The strata follow the classification of GHFs by the Ministry of Public Health (MPH) Cameroon. Laboratories were selected from each stratum by cluster and stratified sampling.

Data collection

Baseline data was obtained from the Malaria Unit Bamenda, the MPH and GHFs. Pre-tested interviewer-administered structured questionnaires were used to collect data on diagnostic techniques for thick and thin smears, materials used, work sites, routing of smears and storage. The mechanical and optical aspects of microscopes were studied through inspection/observation. Optical quality (oculars and objectives) was further examined under local microscopes. All data was analysed with Epi Info 6 (CDC, Atlanta, GA, USA) and comparisons were made by Pearson χ² test to calculate the p-values and odds ratios for each explanatory variable at 95% CI. Comparisons were made by Fisher's exact test for small sample data. The results were considered statistically significant for p-values <0.05.

Results

Approximately 24% of GHFs in the NWR were visited including twelve Hospitals, eight CMAs and 20 IHCs (Table 1). All 40 sample units performed both clinical and microscopic diagnosis of malaria. While thick smears were universal, only 55% (22/40) of GHFs employed thin smears.

Data from questionnaires

Giemsa-stock and glass-slides

The NW Special Fund for Health supplied good quality laboratory materials and equipment from prominent manufacturers at subsidized rates to GHFs. Giemsa was supplied as a stock solution and it was diluted with a buffer/diluent to produce Giemsa stain by laboratory staff. Ninety-five percent (38/40) of laboratories used Giemsa stain for diagnosis while the remaining 5% (2/40) preferred Field’s stain. Twenty-four percent (9/38) of the sample diluted Giemsa-stock with buffered water with a pH of 7.2 (recommended) while 71% (27/38) diluted Giemsa-stock with distilled water (18%, 7/38) and regular water (53%, 20/38). Measurements for Giemsa-stock dilution (ratio of Giemsa-stock to diluent) varied considerably among GHFs ranging from 1:2 to 1:20 (Figure 1). The most common ratios were 1:10 and 1:20 as seen in 70% (28/40) of GHFs. The MPH recommended a 1:10 dilution or 3ml of Giemsa-stock in 97ml of buffered water with a pH of 7.2. Only 11% (4/38) of laboratories, all in Hospitals met this standard out of which two produced good quality smears. The other two had moderate and poor qualities. Thus, Hospitals were more likely to follow recommendations. The demographics of laboratory personnel were not studied further but the diversity in age, gender and education/training was significant in each GHF. Glass-slides were transparent in 92% (37/40) of GHFs but were a bit scratched and greasy. Three GHFs had cloudy glass-slides. Glass-slides in all laboratories were immediately cleaned with bleach after diagnosis and reused. This explains abrasion of glass-slides and why 11 and 9 GHFs did not have stored thick and thin malaria smears respectively available for this study.

Staining and fixation

The period of staining smears with Giemsa stain ranged from 1–30 minutes. Equal timing was observed for thick and thin smears as they were performed on the same slide (Figure 2). Seven laboratories stained Giemsa for 3–5 minutes, eight for 6–10 minutes and 24 for more than 10 minutes. A correlation between dilution and time of staining was difficult to establish as the latter varied within individual laboratories. Only...
18% (4/22) of GHFs used absolute grade methanol (recommended) as fixative for thin smears while 64% employed fixatives containing 70–100% alcohol. Another 18% were not sure of the contents of the fixative. The duration of fixation also differed among GHFs.

**Microscopes**

Although we found many brands of microscope in GHFs, Olympus and Optic/Ivy system were common and ranged in age from zero to twenty years. About 45% of them were under five years old. There was no link between age of microscope and sophistication/type of GHF. All laboratories had one functional microscope with a 100x oil objective. Up to 92% (37/40) of laboratories did not have a maintenance scheme for microscopes. About 65% (26/40) of microscopes had built-in illuminators. Power outages did not significantly affect diagnosis in 73% (19/26) of laboratories contrary to 27% (7/26) where it sometimes lasted for several hours or all day and interrupted diagnosis. About 35% (14/40) of microscopes were illuminated by daylight with the help of a mirror. Illumination was not optimal in bad weather especially during the long wet season. Clinicians resorted to clinical diagnosis in these challenging situations.

**Duration of diagnosis, speciation, negative slides and reporting**

The duration of diagnosis ranged from 20–180 minutes depending on workload, staining time, the weather and other factors. About 15% (6/40) of GHFs used 60 minutes per case to complete diagnosis while 20% (8/40), 35% (14/40) and 13% (5/40) used 45, 30 and 20 minutes respectively. Diagnostic results were usually recorded as positive (+) or negative (−). For thick smears parasitaemia was indicated by one and three plus signs for uncomplicated and severe malaria respectively in 48% (19/40) of GHFs. The remaining GHFs reported it as the number of parasites per 100 or 200 leucocytes. Thin smears were reported as a percentage of infected erythrocytes. Approximately 28% of GHFs neither identified nor reported species (assumed P. falciparum) while some 26% reported only P. falciparum. Forty-five percent of the sample reported all Plasmodium species. The number of fields examined before a smear is called negative is crucial yet it varied largely from three to two hundred. Only 5% (2/40) of GHFs examined 200 fields (the gold

---

**Table 1.** Distribution of Governmental Health Facilities (GHF) and the study sample in Administrative Divisions of the North West Region (NWR) of Cameroon

<table>
<thead>
<tr>
<th>Administrative Division (AD)</th>
<th>No. of Health Districts in AD</th>
<th>Urban/Rural</th>
<th>Number of GHFs in AD</th>
<th>Type, no. and percentage of GHFs by ADs included in the sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hospitals</td>
<td>CMAs</td>
</tr>
<tr>
<td>Bayo</td>
<td>1</td>
<td>Rural</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Bui</td>
<td>2</td>
<td>Rural</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Donga-Mantung</td>
<td>4</td>
<td>Rural</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Menchum</td>
<td>2</td>
<td>Rural</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Mezam</td>
<td>5</td>
<td>Urban</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Momo</td>
<td>3</td>
<td>Rural</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Ngohketungia</td>
<td>1</td>
<td>Rural</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total (Total %)</td>
<td>18</td>
<td></td>
<td>13</td>
<td>17</td>
</tr>
</tbody>
</table>

§Health facilities owned by the Government, run by the Ministry of Public Health. Includes Hospitals, Centre Médical d’Arrondissement (CMAs) and Integrated Health Centres (IHCs).

Geographical territories defined for administrative purposes. ADs contain 1–5 Health Districts.

Small hospitals that have one resident doctor; offer outpatient, preventive and limited inpatient/maternity services.

Small health units that offer basic outpatient and preventive services to peripheral and rural areas; managed by nurses.

---

**Figure 1.** Variation in the dilution of Giemsa-stock with diluents in Governmental Health Facilities (GHFs). The ratios and numbers on the chart describe how Giemsa-stock was diluted in GHFs, e.g. ‘1:8, 4’ means one part of Giemsa-stock was diluted with eight parts of a buffer solution (diluent) in four GHFs. N/A: Not applicable: Giemsa-stock was not diluted before use or Fields stain was the diluent of choice.

18% (4/22) of GHFs used absolute grade methanol (recommended) as fixative for thin smears while 64% employed fixatives containing 70–100% alcohol. Another 18% were not sure of the contents of the fixative. The duration of fixation also differed among GHFs.

---

**Dilutions of Giemsa with buffer, distilled or regular tap water in 38 GHFs**
standard) on negative slides. Six of the 40 GHFs (15%) did not present monthly diagnostic statistics to the MPH as required.

**Organisation and training**

Overall, there were one to four laboratory staff members in GHFs. Fifty-two percent (21/40) of laboratories, mainly in rural areas, had one laboratory technician (often a microscopist) who performed LDM and all other laboratory operations/services. The rest (48%) was made up of a head technician assisted by one to three other technicians and/or microscopists. Laboratory technicians in Cameroon are trained for one to three years and microscopists for three to six months. Hospitals and some CMAs in urban and suburban areas had laboratory technologists/scientists with postgraduate degrees and certificates assisted by technicians and/or microscopists. About 90% of laboratory staff members in Hospitals and some CMAs had over 20 years of laboratory experience. However, diagnostic quality was not significantly different among Hospitals, CMAs and IHCs (Table 6). Update training and refresher courses for laboratory personnel were either not available or not well advertised.

**Stock, quality control and supervision**

Only 15% (6/40) of GHFs kept stock/inventory records. Yet supplies were regular and of good quality except for glass-slides. Roughly 52% (21/40) of GHFs had severe glass-slide shortages; as a result, thick and thin smears were performed on the same slide. Ninety-five percent of laboratories had an internal quality control and supervision scheme, yet not quite productive as feedback and follow-up was absent. Up to 45% (18/40) of laboratories had not been supervised by Regional laboratories and the MPH during the previous year. Laboratories that were supervised equally had no feedback or follow-up.

**Economic considerations**

The cost-effectiveness of microscopy including costs for organization, supervision, quality control, training and hiring was beyond the scope of this study. But the cost of individual tests to patients was lower for microscopy compared to rapid diagnostic tests (RDTs). If well performed, these tests may complement each other and reduce unnecessary purchase of expensive medication for malaria and, in the case of microscopy, other diseases as well.

**Data from laboratory visits**

*Appearance and mechanical function of local microscopes*

The quality of local microscopes was studied by visual and mechanical inspection using locally prepared smears and malaria-positive smears from the AMC. Forty-five percent (18/40) of microscopes had a moderate to poor appearance and mechanical issues with slide tables, condensers, clips, replacement parts (e.g., incompatible 100x oil objective), mirrors and fine/coarse adjustments (Table 2). These issues made 10% (4/40) of microscopes fairly unusable. Nevertheless, 75% of microscopes had a good source of illumination and clean oculars. The magnification of these oculars equally varied among microscopes and included 5x, 10x, 8x, 12.5x and 16.5x. Four IHCs in rural areas had monocular microscopes. All other GHFs used binocular microscopes.

*Optical quality of local microscopes studied with AMC smears*

Parasites on AMC thick and thin smears were not clearly visible under several local microscopes indicating defective oculars and/or objectives (Table 3). However, microscopes in 75% (9/12) of Hospitals, 38% (3/8) of CMAs and 45% (9/20) of IHCs clearly displayed *P. falciparum* on AMC thick smears. *Plasmodium vivax* on AMC thin smears was quite visible under microscopes in 67% (8/12), 38% (3/8) and 50% (10/20) of Hospitals, CMAs and IHCs respectively. Microscopes illuminated by daylight did not clearly display parasites at night or in bad weather. Tiny parasites and low parasitaemia were difficult to see under low quality microscopes especially those with low optical quality. Microscopes with good optical and mechanical capacities were more likely to be seen in Hospitals reflecting a correlation...
between sophistication/type of GHF and quality of equipment. Consequently, Hospitals were often flooded by patients leaving IHCs created to decongest the former with less workload. Heavy workload contributed to reduced output following limited supplies and effort/time spent in some procedures like examination of negative slides.

Preparation Giemsa stain (working solution) from Giemsa-stock

Good quality thick smears were produced by laboratories that stained them for about 10 minutes with Giemsa stain obtained by diluting Giemsa-stock with a buffer in the ratio of either 1:10 or 1:20. A lower quality was observed when the smears were stained for less than five minutes. The staining of thin smears was more critical as they needed more processing time (10–15 minutes of staining time) to ensure good quality. As a control test glass-slides, Giemsa stain and methanol from the AMC and the NW Special Fund for Health were used to prepare thick and thin smears following Giemsa-diluent ratios and staining techniques employed in GHFs visited. The results showed great agreement between products from the AMC and Cameroon. Glass-slides and methanol were equally of great quality.

Overall quality of locally stained malaria smears

Two thick and two thin smears collected from respectively 73% (29/40) and 33% (13/40) of GHFs were taken to the AMC in Holland to re-assess the quality of staining. The results were recorded as good, moderate or low/unusable. Thin smears

### Table 2. Optical and mechanical characteristics of microscopes

<table>
<thead>
<tr>
<th>Description</th>
<th>Total and percentage (%)</th>
<th>12 Hospitals</th>
<th>8 CMAs&lt;sup&gt;a&lt;/sup&gt;</th>
<th>20 IHCs&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Total 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>General outside impression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>8/12 (68)</td>
<td>4/8 (50)</td>
<td>11/20 (55)</td>
<td>22/40 (55)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>3/12 (25)</td>
<td>2/8 (25)</td>
<td>3/20 (15)</td>
<td>8/40 (20)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>1/12 (8)</td>
<td>2/8 (25)</td>
<td>7/20 (35)</td>
<td>10/40 (25)</td>
<td></td>
</tr>
<tr>
<td>Oculars</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clean</td>
<td>12/12 (100)</td>
<td>7/8 (88)</td>
<td>11/20 (60)</td>
<td>30/40 (75)</td>
<td></td>
</tr>
<tr>
<td>Dirty</td>
<td>0/12 (0)</td>
<td>1/8 (12)</td>
<td>9/20 (45)</td>
<td>10/40 (25)</td>
<td></td>
</tr>
<tr>
<td>Working condition of microscope</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 Hospitals</td>
<td>8 CMAs</td>
<td>20 IHCs</td>
<td>Total (40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slide Table Vertical</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functions correctly</td>
<td>8/12 (67)</td>
<td>6/8 (75)</td>
<td>11/20 (55)</td>
<td>25/40 (63)</td>
<td></td>
</tr>
<tr>
<td>Functions moderately</td>
<td>4/12 (33)</td>
<td>2/8 (25)</td>
<td>7/20 (35)</td>
<td>13/40 (32)</td>
<td></td>
</tr>
<tr>
<td>Doesn’t function</td>
<td>0/12 (0)</td>
<td>0/8 (0)</td>
<td>2/20 (10)</td>
<td>2/40 (5)</td>
<td></td>
</tr>
<tr>
<td>Slide Table Horizontal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functions correctly</td>
<td>11/12 (91.7)</td>
<td>7/8 (87.5)</td>
<td>12/20 (55)</td>
<td>29/40 (72)</td>
<td></td>
</tr>
<tr>
<td>Functions moderate</td>
<td>1/12 (8.3)</td>
<td>1/8 (12.5)</td>
<td>7/20 (35)</td>
<td>9/40 (23)</td>
<td></td>
</tr>
<tr>
<td>Doesn’t function</td>
<td>0/12 (0)</td>
<td>0/8 (0)</td>
<td>2/20 (10)</td>
<td>2/40 (5)</td>
<td></td>
</tr>
<tr>
<td>Clip</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functions correctly</td>
<td>9/12 (75)</td>
<td>7/8 (87.5)</td>
<td>9/20 (45)</td>
<td>25/40 (63)</td>
<td></td>
</tr>
<tr>
<td>Functions moderate</td>
<td>2/12 (16.7)</td>
<td>1/8 (12.5)</td>
<td>9/20 (45)</td>
<td>12/40 (30)</td>
<td></td>
</tr>
<tr>
<td>Doesn’t function</td>
<td>1/12 (8.3)</td>
<td>0/8 (0)</td>
<td>2/20 (10)</td>
<td>3/40 (7)</td>
<td></td>
</tr>
<tr>
<td>Brightness of microscope and quality of immersion oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 Hospitals</td>
<td>8 CMAs</td>
<td>20 IHCs</td>
<td>Total (40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White light source on microscope</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/12 (100)</td>
<td>5/8 (63)</td>
<td>13/20 (65)</td>
<td>30/40 (75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good quality immersion oil</td>
<td>11/12 (92)</td>
<td>8/8 (100)</td>
<td>20/20 (100)</td>
<td>39/40 (97)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>CMA: Centre Médical d’Arrondissement: Small hospitals with one resident medical doctor; offer outpatient, preventive and limited inpatient/maternity services.

<sup>b</sup>IHC: Integrated Health Centre: Small health units with limited outpatient and preventive services managed by nurses.

### Table 3. Quality of local microscopes based on studies with a thick smear containing *Plasmodium falciparum* and a thin smear containing *P. vivax*

<table>
<thead>
<tr>
<th>Technique examined</th>
<th>No. of microscopes (laboratories)</th>
<th>Good quality (%)</th>
<th>Moderate quality (%)</th>
<th>Poor quality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thick film</td>
<td>39</td>
<td>23 (59)</td>
<td>13 (33)</td>
<td>3 (8)</td>
</tr>
<tr>
<td>Thin film</td>
<td>39</td>
<td>18 (46)</td>
<td>12 (31)</td>
<td>9 (23)</td>
</tr>
</tbody>
</table>

Academic Medical Center Amsterdam (AMC), Holland supplied smears for control tests.
without parasites from the AMC but stained by laboratory staff in GHFs were also taken back to the AMC to assess total quality. The overall quality of locally stained slides was not optimal (Tables 4 and 5). However, 34% (10/29) and 66% (19/29) of GHFs produced moderate to good and low to unusable quality thick smears respectively. And 47% (6/13) produced moderate to good quality thin films. About 67% (27/40) of AMC smears stained locally were of low to unusable quality.

For thick and thin smears there was no significant difference in ‘unusable’ quality among GHFs (p > 0.05) (Table 6). Also, when grouped under ‘sufficient’ (i.e., good and moderate) and ‘insufficient’ (i.e., low and unusable) qualities there was equally no significant difference among GHFs (p > 0.05).

Based on studies using *P. vivax* on AMC thin smears good microscopes were found in 67% (8/12), 38% (3/8) and 37% (7/19) of Hospitals, CMAs and IHCs respectively. Again, the differences were statistically insignificant (p > 0.05).

**Discussion**

This is the first systematic and detailed study of the quality of laboratory equipment, supplies and techniques for microscopic diagnosis of malaria in all types of GHFs (Hospitals, CMAs and IHCs) drawn from all Health Districts and Administrative Divisions in the NWR of Cameroon. The main finding was that locally prepared Giemsa stained thick and thin smears were of unusable quality in 52 and 46% of GHFs respectively. Good quality thick and thin smears were observed in, respectively, 17 and 8% of GHFs. Some loss of quality was observed in the remaining GHFs with moderate quality thick and thin smears. Laboratory personnel did not have the necessary tools and resources to overcome the loss of quality.

Mechanical and optical defects of microscopes due to age and poor maintenance contributed to low quality diagnostic results. These defects can be overcome by synchronized...
instruction of laboratory personnel on maintenance and repair/replacement schemes and an operational supervision scheme. Hybrid microscopes with built-in and external sources of illumination and available replacement parts should be acquired. A generator may add value to such hybrid equipment at night and in bad weather in areas that depend solely on daylight for illumination.

The origin of a significant number of defective smears was intriguing. The quality of glass-slides, buffer including the pH, Giemsa stain, fixative and the duration of fixation and staining determined the quality of malaria smears. Interestingly, data from questionnaires showed all GHFs used good quality Giemsa-stock and 74% properly diluted it. Seventy-one percent supposedly fixed and stained smears long enough. But, we observed that the actual dilution of Giemsa-stock, fixation and staining duration was not accurately reported. This lack of precise information was reflected by significant variations in the preparation of Giemsa stain, fixative, duration of fixation and staining and an important percentage of GHFs with defective smears. Standard operative procedures for malaria diagnosis were not actually followed.

Abrasion, grease and scratches on glass-slides undermined quality. Quality slides should be transparent and void of lesions. Low quality smears were equally produced because Giemsa-stock solution was diluted with buffers that had the wrong pH. These smears appeared all blue or purple. A good buffer has a stable pH of 7.2 and contributes to fine colour separation of the nucleus and cytoplasm of Plasmodium. This helps to detect and quantify parasites and provide information for better treatment decisions. Good fixatives like methanol can separate erythrocytes making it possible to detect parasite species, densities and cell abnormalities. Fixatives that were not of absolute grade did not produce optimum results. Plus, thick smears were accidentally fixed when placed upon the same slide as thin smears.

The ability to read and report smears accurately including Plasmodium species was further hampered by poorly prepared films. For best results smears should be tapered, thin, uniform, and feathered at the end. Again, the inability to examine at least 200 fields on negative slides to make certain that they are negative was an important limitation. False negatives can result in untreated malaria, misdiagnosis of patients with fever not caused by malaria and potentially severe consequences, including death. It can significantly undermine both clinical confidence in diagnostic results and credibility in the community.20 The ‘plus system’ for reporting parasitaemia on thick smears was somewhat subjective and did sway effective treatment. The ‘plus system’ is less precise as variation in the thickness of the film results in false variation in parasite count. And malaria cases were not accurately recorded in registers and bench work sheets for monthly reports.

The mean number of technicians per GHF was two. This somewhat affected efficiency as malaria and other diseases were diagnosed on a single microscope by the same technician/microscopist all day. Laboratories opened for approximately eight hours a day and malaria diagnosis lasted about 37 minutes per case. We assume that laboratories were not properly financed given the overall quality of equipment, supplies and diagnostic results. And income from laboratory services was not sufficient to help cover laboratory running costs. These financial and material constraints had a negative impact on quality.

Limitations

A good coverage was obtained for Hospitals (92%) and CMAs (47%) but due to inaccessibility a small number of IHCs was visited in some clusters. However, the overall coverage (15%) for IHCs was reasonable to good. Microscopy was the only malaria diagnostic technique and a comparison with RDTs was unrealistic. However, RDTs are an ideal complement to microscopy,21 especially in areas with outpatient services. But, the deployment of RDTs must follow comprehensive instruction on operation and quality.22 Also, the skills of laboratory personnel and financing of laboratory operations was not studied in detail.

Conclusion

The quality of malaria diagnosis was not optimal in GHFs regardless of sophistication/type. Important limitations were found in all aspects of diagnosis. But, these limitations are easy to correct. Going forward, GHFs should have quality equipment and materials and a manual of standard procedures for thick and thin films. Good and harmonized instruction and periodic refresher training for laboratory staff and managers of GHFs may cover specific limitations. The quality of diagnosis also depends on the skills and abilities of technicians/microscopists (not studied in a standard way). Training must cover the best measurements that produce quality stains, cleaning agents and methods for glass-slides that are reused, attributes of good buffers, stains, smears (such as uniformity and feathered end) and fixatives, optimum time of fixation and staining. Parasite species, density, mixed/co-infection and cell anomalies and other details are equally important specifics that need to be reported to ensure better treatment decisions. The MPH need to assess the performance of GHFs more often through quality assurance and diagnostic reports from GHFs and provide feedback and follow-up. In all, better staffing and financing, standardization of equipment, materials and procedures, good instruction and refresher training and quality assurance may benefit all laboratory services and procedures in Cameroon.

Authors’ contributions: EN and HG collected data in the field. EN, TvG and HG contributed to the study design, data analysis and review. EN did the final write-up. All the authors read through and approved the paper. EN is guarantor of the paper.

Acknowledgements: Special thanks go to the following for their support and participation: Dr. Ndifor Chu Victor, Provincial Delegate of Public Health, Bamenda, Cameroon; Staff of the North West Regional Malaria Unit, Bamenda, Cameroon; Chiefs of Centres/Governmental Health Facilities; Heads of Laboratories visited in the North West Region of Cameroon; Laboratory technicians/scientists and microscopists.

Funding: This work was supported by Prof. Tom van Gool (TvG), MD, Clinical Parasitology Department, University of Amsterdam.
Competing interests: None declared

Ethical approval: The study obtained ethical approval from the ethics committee at the NW Regional Delegation of Public Health. The approval was signed by the Regional Delegate of Public Health, who is equally the head of the ethics committee and represents the Minister of Public Health in the Region. We also got verbal consents from all heads of GHFs and laboratories after explaining the purpose and procedures of the study and presenting the ethical approval from the Minister of Public Health in the Region.

References