The Use Of Dried Tube Specimens Of *Plasmodium* Falciparum In An External Quality Assessment Program To Evaluate Health Worker Performance For Malaria Rapid Diagnostic Testing In Healthcare Centers In Togo

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Research

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Abstract

Background: The use of rapid diagnostic tests (RDTs) to diagnose malaria is common in Sub-Saharan African laboratories, remote primary health facilities and in the community. Currently, there is a lack of reliable methods to ascertain health worker competency to accurately use RDTs in the testing and diagnosis of malaria. Dried tube specimens (DTS) have been shown to be a consistent and useful method for quality control of malaria RDTs, however, its application in National Quality Management programs has been limited.

Methods: A *P. falciparum* strain was grown in culture and harvested to create DTS of varying parasite density (0, 100, 200, 500 and 1,000 parasites/µL). Using the dried tube specimens as quality control material, a proficiency testing (PT) program was carried out in 80 health centers in Togo. Health worker competency for performing malaria RDTs (mRDTs) was assessed using five blinded DTS samples, and the DTS were tested in the same manner as a patient sample would be tested by multiple testers per health center.

Results: All the DTS with 100 parasites/µl and 50% of DTS with 200 parasites/µl were classified as non-reactive during the pre-PT quality control step. Therefore, data from these parasite densities were not analyzed as part of the PT dataset. PT scores across all 80 facilities and 235 testers was 100% for 0 parasites/µl, 63% for 500 parasites/µl and 93% for 1,000 parasites/µl. Overall, 59% of the 80 healthcare centers that participated in the PT program received a score of 80% or higher on a set of 0, 500 and 1,000 parasites/ µl DTS samples. Sixty percent of health workers at these centers scored higher than 80%.

Conclusions: The use of DTS for a malaria PT program was the first of its kind ever conducted in Togo. The ease of use of the DTS illustrates that they can serve as well-characterized, stable samples to assess staff competency. The implementation of quality management systems, refresher training and expanded PT at remote testing facilities are essential elements to improve the quality of malaria diagnosis.

Background

The use of rapid diagnostic tests (RDT) contributes to achieving the World Health Organization (WHO) recommendations that all patients with suspected malaria have a confirmed diagnosis prior to antimalarial treatment initiation. Globally, 93% of malaria cases are found in Sub-Saharan Africa (SSA), with 213 million cases reported in the region in 2018 (1). Over 99% of malaria cases in Africa are attributed to *Plasmodium falciparum* parasite (1).

Unlike blood smear, which is considered the gold standard method and which has been used to diagnose malaria for decades, RDTs do not require specific equipment or highly skilled personnel and can be used in field settings by health workers without extensive laboratory training. RDTs are now the most widely used test to diagnose malaria in suspected cases, comprising 75% of all malaria tests performed (1). While testing at or near the point-of-care (POC) can accelerate potentially life-saving medical decisions, procedural challenges can result in misinterpretation and misuse of RDTs, poor accuracy and

misdiagnosis. Data from studies in the Democratic Republic of Congo have shown that only 18% of health workers correctly reported malaria RDT (mRDT) results obtained as part of an external quality assurance (EQA) program (2). Unacceptably high false-positive and false-negative results from RDT use have been shown in other studies (3) (4) (5).

When mRDTs are used and stored under optimal conditions (e.g. light, temperature and humidity levels), they can accurately and rapidly detect malaria antigens. Recognizing that optimal conditions do not exist in many POC settings, it is important to monitor RDT usage and identify opportunities to maintain high standards of patient care.

In Togo, where malaria remains a major public health problem, children and pregnant women are the disproportionately affected. The rate of malaria cases confirmed by mRDT has rapidly risen in Togo, from 0.39 million in 2010 to 0.98 million in 2018, with 2.1 million estimated cases of malaria in 2018 nationwide (1). In 2017, rates of malaria were 233 per 1,000 people with a 4% mortality rate, children under 5 years and pregnant women represented 34,6% and 3,6%, respectively, of confirmed cases (6). External Quality Assessment (EQA), an internationally recognized system, is designed to help testers monitor how well they perform a test. Proficiency testing (PT), a form of EQA, is the process of testing quality control (QC) material containing known analyte levels (e.g. malaria HRP-2 antigen), in which testers are blinded to the composition of the QC samples (7).

Currently, there is a lack of universal, field-ready malaria QC material to test the quality and performance of mRDTs (8), or ascertain staff competency. While some malaria QC products exist, data on their use and performance is limited and are not readily available. For example, QC products using recombinant antigens do not work on all brands and types of mRDTs (9). Therefore, the availability of high-quality, stable and affordable control material for mRDTs, with broad compatibility to common RDTs, would contribute to the expansion of malaria testing (9).

Dried tube specimens (DTS), comprised of dried *Plasmodium falciparum* infected blood, have been reported to be suitable for quality control and EQA in malaria testing facilities (10) (11). Our work discusses findings from the use of malaria DTS in a PT program in 80 healthcare centers across Togo and further highlights the need for proper quality management systems (QMS) in mRDT testing.

Methods

This was a nationwide proficiency testing survey for health workers at 80 health centers that use mRDTs to diagnose malaria cases representing a subset of all testers within Togo. The program was performed in Togo in October, 2018.

Site selection and organization of PT activity

The PT activities were planned and implemented by the National Quality Assessment Program management (NQAPM) team of the Division of Laboratories of Togo Ministry of Health. Three field teams

from the central level conducted the activity in the following health regions: Team 1: Lomé-Commune and Maritime health regions; Team 2: Plateaux health region and Team 3: Central, Kara and Savannas health regions.

Based on the information from the National Malaria Control Program (NMCP) and the number of PT samples available for use, a subset of the total number of health centers using mRDT to diagnose malaria were selected to be included in the malaria PT activity. As sites are unevenly distributed within Togo, some facilties in locations with high capacities of microscopy diagnose (e.g. Lome) were eliminated to give priority to locations with lower level facilities or without a dedicated laboratory. A second criterion was applied to this list of sites to identify those sites with higher throughput of patients and grouped geographically into the 6 regions of Togo for ease of access. Finally 10% of these sites were randomly selected as participants. The number of facilities that participated in the PT program varied across 6 regions, with Centrale Region represented by 10 facilities, Kara Region by 13 facilities, Lomé Region by 7 facilities, Maritime Region by 17 facilities, Plateaux Region by 24 facilities, and Savanes Region by 9 facilities.

Dried tube specimen preparation

DTS were prepared in the USA as previously described. [11] Briefly, a culture-adapted *P. falciparum* strain 3D7 parasite line was grown in culture and harvested when parasitemia reached 2% (~ 100,000 parasites/µL). Parasite preparation was then diluted with washed (in incomplete medium; RPMI1640, Gibco™ Thermo Fisher, Grand Island, NY, USA) parasite negative blood at a similar hematocrit to generate preparations with 0, 100, 200, 500 or 1,000 parasites/µL. Parasite negative blood was procured from a US-based blood bank and determined to be HIV and HBV negative. The diluted parasite preparations were then tested on a histidine-rich protein 2 (HRP2)-based mRDT brand with performance characteristics that met WHO procurement criteria (12). After confirmation of reactivity, the diluted parasites were distributed in 50µL aliquots in Sarstedt® Type I microtubes (Sarstedt Inc., Newton, NC, USA) and, with the caps open, air-dried overnight in a biosafety cabinet.

To check that baseline reactivity was not affected by drying, 1 vial each of DTS at each of the parasite densities was re-hydrated with a solution of PBS-Tween20 (Sigma Aldrich, St. Louis, MO, USA) and tested on the same RDTs brands as prior to drying. All DTS vials were from the same batch and stored at +4^{o}C before shipping to Togo.

mRDT procurement and storage

A batch of SD Bioline® Malaria Ag Pf RDT used in Togo was obtained from the NMCP for use in preliminary QC of DTS and in the PT. All procured kits and DTS were stored centrally until use. This allowed for the same lot number of kits to be used throughout the evaluation and provided control over kit storage prior to use. mRDTs and DTS were provided to each health center by the NQAPM team at the time of PT.

Quality control (QC) of Dried tube specimen at Malaria reference laboratory in Togo

Before distributing DTS vials to sites, the DTS panels were evaluated by the NQAPM in collaboration with the NMCP in the malaria reference lab in Lomé. Six aliquots of parasitemia (0, 100, 200, 500, and 1,000 parasites/µl), 30 vials in total, were reconstituted in 50µL of PBS-Tween rehydration buffer according to standard procedures provided by the malaria laboratory, CDC. Tubes were allowed to rehydrate for 1.5 hours, gently shaken and 5µL was then tested using SD Bioline® Malaria Ag Pf RDT (Standard Diagnostics, Inc., Abbott, Gyeonggi, Korea) in the same manner patient blood samples would be tested.

Reconstitution of the specimens

To standardize reconstitution and remove a potential source of error, all DTS were reconstituted by the NQAPM team in the field according to the procedure provided by the CDC malaria laboratory and described in previous paragraph. Each reconstituted DTS contained 50µL of sample and thus provided sufficient sample to evaluate multiple health workers at a given site.

Distribution of samples and site visits

To evaluate the performance of mRDTs, PT panels comprised of five reconstituted samples with different parasite densities (0, 100, 200, 500, 1,000 parasites/µl) were distributed to health centers and selected laboratories. One facility received only three samples containing 0, 100, and 1,000 parasites/µl due to insufficient number of samples with parasite density of 200 and 500 parasites/µl. DTS vials were rehydrated as described below. At 76 sites, each sample was tested by three health workers; in three facilities, samples were tested by two health workers; and in one facility, by one health worker. The number of participants at each site was a function of the number of staff on duty at the time of the supervisory visit. A total of 235 health workers participated in the PT activity. The NQAPM team explained the PT procedure to the health workers prior to providing reconstituted DTS.To record the observations, each evaluated health worker received results form designed by the NQAPM. Completed forms were collected by the field teams and returned to the NQTP for analysis.

Statistical methods

XLSTAT Basic statistical application for Microsoft ExcelÒ (version 2019, Addinsoft, France) was used for statistical analysis of data and Tableau Desktop (Version 2019.4, Tableau Software, Inc., USA) was used for data visualization. The difference between proportions was assessed using *Chi*-square test, followed by Marascuilo procedure to identify proportions responsible for statistically significant differences. Correlation between proportions of qualified testing personnel and regional performance was assessed using Pearson's correlation coefficient. Paired Student's *t*-test was used to compare the means of two paired samples. *P*-value of <0.05 was considered significant. For this analysis, a level of 80% correct PT result was selected as a value to group data. While 80% correct is used as a threshold for malaria microscopy testing and Stepwise Laboratory Quality Improvement Process Towards Accreditation

(SLIPTA) 5-star certification, ideally testers would achieve 100% correct results on PT when conducting malaria testing to offer appropriate patient care.

Results

Quality control (QC) at the central laboratory

The results of the QC conducted at the malaria reference laboratory showed that all aliquots of DTS containing 0, 500 and 1,000 parasites/µl produced expected results with the SD Bioline® Malaria Ag Pf RDT used in Togo. All DTS with 100 parasites/µl and 50% of DTS with 200 parasites/µl were classified by NQAPM team as non-reactive (Figure 1). While all parasite densities were distributed and examined during the PT program, only 0, 500, and 1,000 parasites/µl DTS samples were used for evaluation of tester's performance.

Overall PT program performance

PT scores across all 80 facilities and 235 testers was 100% for 0 parasites/µl, 63% for 500 parasites/µl and 93% for 1,000 parasites/µl (Figure 2). Although health workers were not formally evaluated on their performance of testing the 100 and 200 parasites/µl DTS samples, they received those parasite densities in the PT panel and processed them. As expected and similar to the pre-study QC data, reactivity of samples with parasite density of 100 and 200 parasites/µl presented a challenge and the detection rate for samples with 200 parasites/µl was significantly lower at field sites than for the QC samples tested at malaria reference lab ($c^2(1; 238)=8.2$; $p=0.004$; effect size *phi*=0.19). Forty-seven (59%) of the 80 testing facilities that participated in the PT program received a score of 80% or higher on a set of 0, 500 and 1,000 parasites/ µl DTS samples.

Performance by healthcare worker qualification

Health workers of different levels of qualification participated in the PT program. Ninety-three percent of health workers were classified with the following qualifications (Figure 3): State nurse (26%); State auxiliary midwife (14%); State auxiliary nurse (14%); Permanent birth attendant (12%); Midwife (10%); Permanent nurse (9%); Senior health technician/ medical assistant (5%). Other qualifications made up 9% of health workers and comprised groups of five or fewer health workers and were combined into a group identified as "Other" for group performance analysis.

All qualification groups correctly identified negative DTS and performed better in the detection of malaria in samples with parasite densities of 1,000 parasites/µl than those with 500 parasites/µl (t-test for two paired samples $t=-9.36$, $DF=7$, $p<0.0001$, two-tailed test) (Figure 3). There was no significant difference between these groups of health workers in overall performance ($c^2(7; 702)=5.6$; $p=0.590$; effect size *Cramer's V*=0.03) nor when comparing only the results of tests on samples containing 500 parasites/µl ($c^2(7; 232)=3.9$; $p=0.787$; effect size *Cramer's V*=0.05) or those with 1,000 parasites/µl ($c^2(7; 235)=13.5$; $p=0.061$; effect size *Cramer's V*=0.09).

Performance by health region

The 80 health centers were distributed amongst multiple regions in the country (Figure 4). The difference in PT results between health regions of Togo was not statistically significant (c^2(5; 702)=9.9; p=0.078; *Cramer's V*=0.05) (Figure 4). There was also no difference in performance between qualification groups within regions; and there was no correlation between the percentage of top three performing groups (state nurses, midwives and senior health technicians/medical assistants) and regional PT scores (Pearson's correlation coefficient r = 0.422, p = 0.405) despite differences in the percentage of health workers drawn from these three groups between the regions (c^2(5; 235)=26.4; p<0.0001; *Cramer's V*=0.15).

When disaggregated by parasite density, the PT scores for 0 parasites/µl DTS samples were 100% correct across all regions. The difference between regions in the testing of 500 parasites/µl specimens was not statistically significant (c^2(5; 232)=6.3; p=0.278; *Cramer's V*=0.07). While a difference was detected between regions in testing 1,000 parasites/µl specimens, the effect size was negligible and the data were not sufficient to identify significantly different regions (c^2(5; 235)=15.0; p=0.010; *Cramer's V*=0.11) (Figure 5). The six health regions are further divided into 40 health districts (Figure 5) represented in this malaria PT by differing numbers of healthcare centers and health workers. Thirty-four (85%) and 12 (30%) of health districts had individual healthcare centers that scored below 80% for 500 and 1,000 parasites/ul, respectively. Overall, 142 (60%) of health workers, 47 (59%) of healthcare centers, 28 (70%) of districts and 5 (83%) of regions performed above the threshold of 80% (Figure 6).

Discussion

While health workers who conduct diagnostic testing in the laboratory or at the point-of-care strive to operate under a well-functioning QMS, such systems are not always in place. The QMS, an ISO15189:2012 requirement, is comprised of procedures and activities that contribute to a testing system, with the goal of achieving accurate and reliable results for appropriate patient management. The use of QC material for malaria rapid testing, as a part of QMS, can be used to ascertain competency for testing. The program described in this paper utilized DTS to assess health worker competency, through a PT program, for performing malaria rapid diagnostic tests.

The use of DTS for PT of malaria rapid testing in this setting allowed for the successful evaluation of testers with varying levels of education at peripheral health facilities where robust QMS may not be in place. Program administrators found that that the DTS samples were easy to disseminate and transport as cold chain was not required thus minimizing costs. Malaria DTS have been found to be stable for up to 24 weeks under ambient temperature and field conditions, easing logistical challenges of handling and testing (11).

While the use of appropriate QC material is key to conducting PT, not all QC material is of high-quality or meets the needs of the testing program being evaluated. During the QC step of this PT program, it was noted that the optimal panel set consisted of 0, 500 and 1,000 parasites/µL, which provided expected

results on malaria rapid diagnostic test strips. The 100 and 200 parasite/μL DTS were not reliably detected by the RDTs during the QC step. As most mRDTs on the market have a limit of detection (LOD) at or near 200 parasite/μL (13), RDT sensitivity is reduced at low parasite densities and cases of low sensitivity of mRDTs in samples of parasite densities less than 200 parasite/μL have been reported (14).

The parasite densities of 0, 500 and 1,000 parasites/μL are consistent with those used by other groups for PT using malaria DTS (11). Because most malaria tests will be reactive at 500 and 1,000 parasites/μL density, any unexpected results (non-reactive) can reasonably be attributed to health worker performance rather than the test. Improper storage of RDTs prior to use could also impact performance. However, for the purposes of this study, kits were delivered to sites on the day of the PT visit, thus reducing the impact of potential differences in RDT storage conditions.

With the increased use of RDTs, shifting the role and location of diagnostic testing from laboratory technicians in higher-level facilities to community health workers in peripheral or point-of-care settings has become common practice. While this movement of testing to the point of care or community settings improves access to healthcare, it is important that quality and accuracy of test results are not compromised. The results of this PT program showed that 47 (59%) of the testing facilities that participated in the PT program received a score of 80% or higher. When drilling down at the data, low performing sites and groups of testers who scored below 80% could be identified and targeted for corrective actions. Examination of the data when arranged by regions shows 83% of the six health regions scored 80% or higher on PT. When broken down to testers, 60% scored ≥ 80%, with the remaining testers scoring lower. This data illustrates the need to expand PT programs to have the greatest possible level of participation in order to truly understand the situation within a health system.

Incorrect results have negative clinical implications. While no false positive results were reported by study participants, 34 (43%) of 500 parasites/μL and 13 (16%) of 1000 parasites/μL samples were incorrectly deemed negative by at least one tester at a site. At a site level, 13 (16%) of 500 parasites/μL but none of 1000 parasites/μL samples were designated as negative by all testers at a site. This creates a case for testing of samples by multiple staff members or for results to be confirmed via microsopy where personnel and reagent resources allow.

Variation in results was also seen amongst testers of different qualifications (although non-significant) and amongst different parasite density PT samples: 1,000 parasites/μL DTS were more commonly identified as reactive than the 500 parasites/μL DTS. Although most clinical malaria cases are often associated with parasite densities of 1,000p/μL or higher, clinical cases with lower densities are not uncommon. Low parasite densities are associated with low test band intensities and identification of such bands is subject to tester competency and experience with such results (2)(15), but also to variations such as light at the testing bench and visual acuity of the tester (16). This information can be useful to guide mentorship, training, and allocation of resources to maximize impact.

Many factors can impact the performance of mRDTs such as lack of adherence to the manufacturer's instruction including misreading or misinterpretation of results by health workers. Errors in procedures

can result in mismanaged care for patients with malaria. Such human errors include using the wrong amount or type of sample or buffer or incubating the test strip for an incorrect amount of time, especially shorter times can result in provision of incorrect test results (17). In many settings worldwide, healthcare workers are tasked with using mRDTs to diagnose malaria, however, they have not been given proper instructions on use, thus underscoring the importance of high-quality training and PT (18). The use of QC samples for PT offers data to guide further investigations to better understand the root causes of the observed errors.

Conclusions

Using malaria DTS, the Division of Laboratories of Togo Ministry of Health was able to conduct a nationwide proficiency testing activity across multiple health worker categories. This PT program validates the feasibility of the use of malaria DTS for PT in a field setting. The ease of preparation, dissemination, shipping and reconstitution of the DTS, along with reportable results from testers, demonstrates that DTS can serve as well-characterized, stable blinded samples for PT at parasite densities above the LOD of the rapid tests.

The PT data from Togo demonstrates that there is room for adherence to QMS and use of QC to assess health worker competency to improve malaria rapid testing across all testing settings. This program showed that regardless of education level, the challenge for RDT users lies closer to lower parasitemia levels where minor errors in technique when testing can become much more impactful.

High-quality and routine (at minimum yearly) health worker assessments, training, site supervision and monitoring visits to follow adherence to the testing protocol can contribute to higher levels of health worker performance. These practices coupled with other QMS principles can standardize testing, thus improving patient outcomes (19) (20).

List Of Abbreviations

CDC - Centers for Disease Control and Prevention

DTS - Dried Tube Specimen

EQA - External Quality Assurance

GSSHealth - Global Scientific Solutions for Health

HRP2 - Histidine-rich protein 2

ISO - International Standards Organization

LOD - Limit of Detection

MO - Missouri

MOH - Ministry of Health

mRDT - Malaria Rapid Diagnostic Test

NMCP - National Malaria Control Program

NQAPM - National Quality Assurance Program Manager

NY - New York

PBS - Phospate Buffered Saline

POC - Point of Contact

PT - Profficency Test

QC - Quality Contol

QMS - Quality Management System

RDT- Rapid Diagnostic Test

SLIPTA - Stepwise Laboratory Improvement Process Towards Accreditation

SSA - Sub-Saharan Africa

USA - United States of America

WHO - World Health Organization

µL = Microliter

Declarations

Ethics approval and consent to participate

As this activity was a routine activity of Division of laboratories, specific ethic approval was not required. However, oral agreement to participate was obtained from each health worker before completing the PT.

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and analyzed are not publicly available but are available from the Laboratory Division of Togo Ministry of health, by the corresponding author on reasonable request.

Competing interests

No competing interests

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Authors' contributions

MAD oversaw the entire program and provided key input and review of the manuscript. MA provided the DTS, technical input for DTS use and writing of the manuscript. EM analyzed and interpreted the data. KCK coordinated PT activities and reviewed the manuscript, AKK and GK supervised field activities, KG and KP were involved in field activities. MOM, MLA, RC, EM and KCK contributed to writing and organizing the manuscript. All authors read and approved the final manuscript.

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Figures

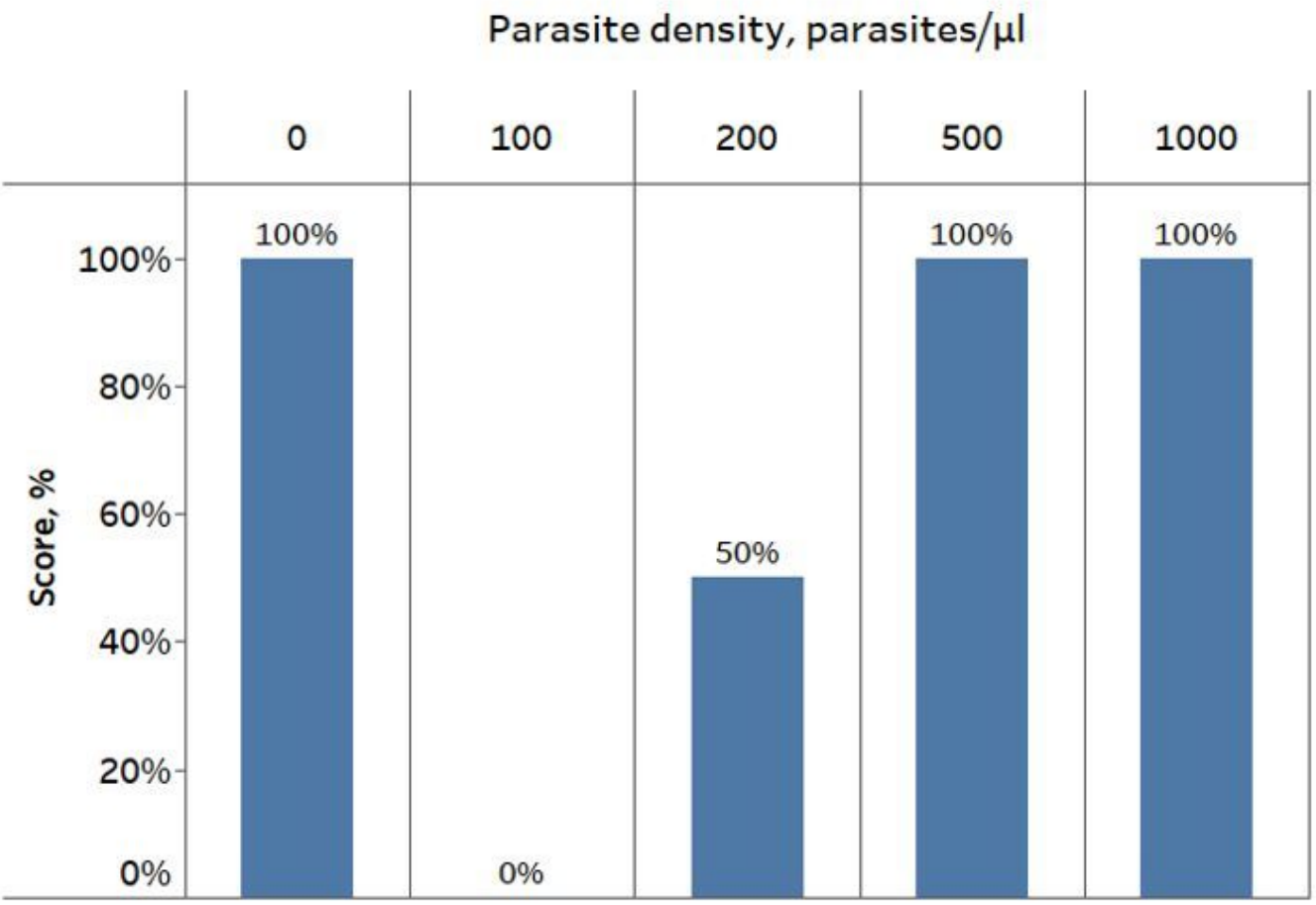

Figure 1

Results of QC testing (6 aliquots per parasite density) conducted at the Malaria reference lab prior to the initiation of proficiency testing. Percentage indicates actual results obtained for each parasite density during the QC.

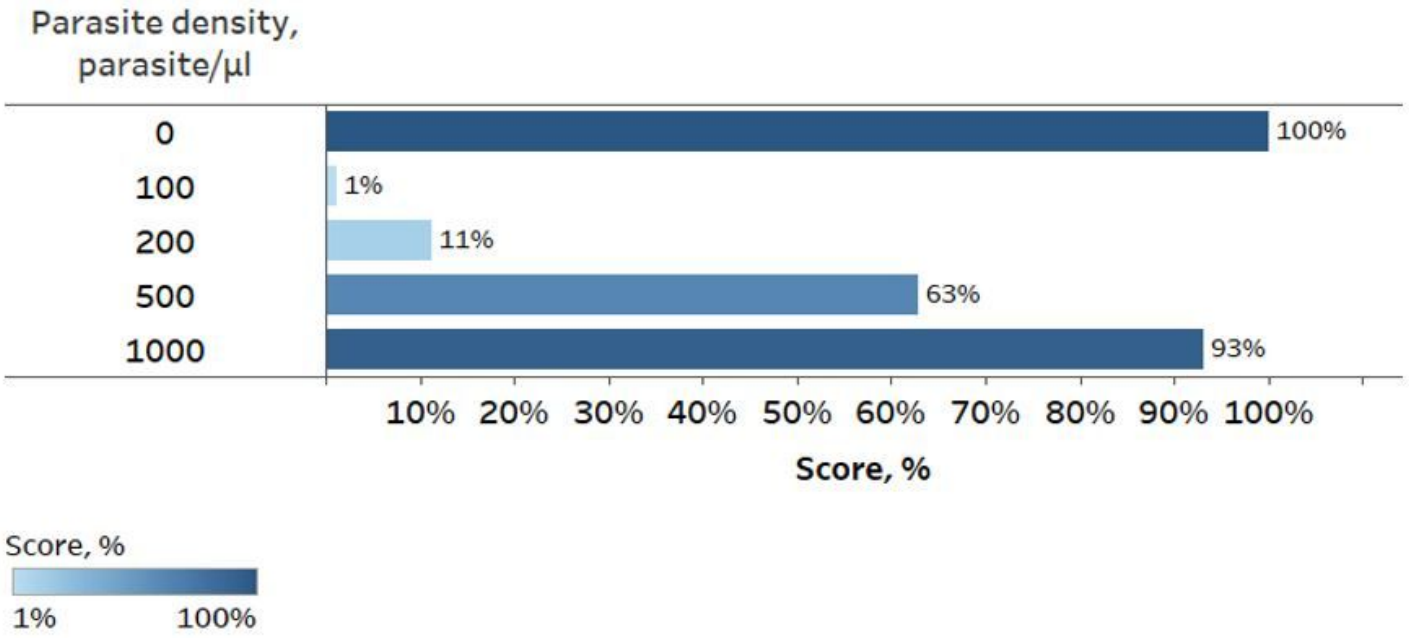

Figure 2

Average PT results for 235 testers across 80 participating facilities.

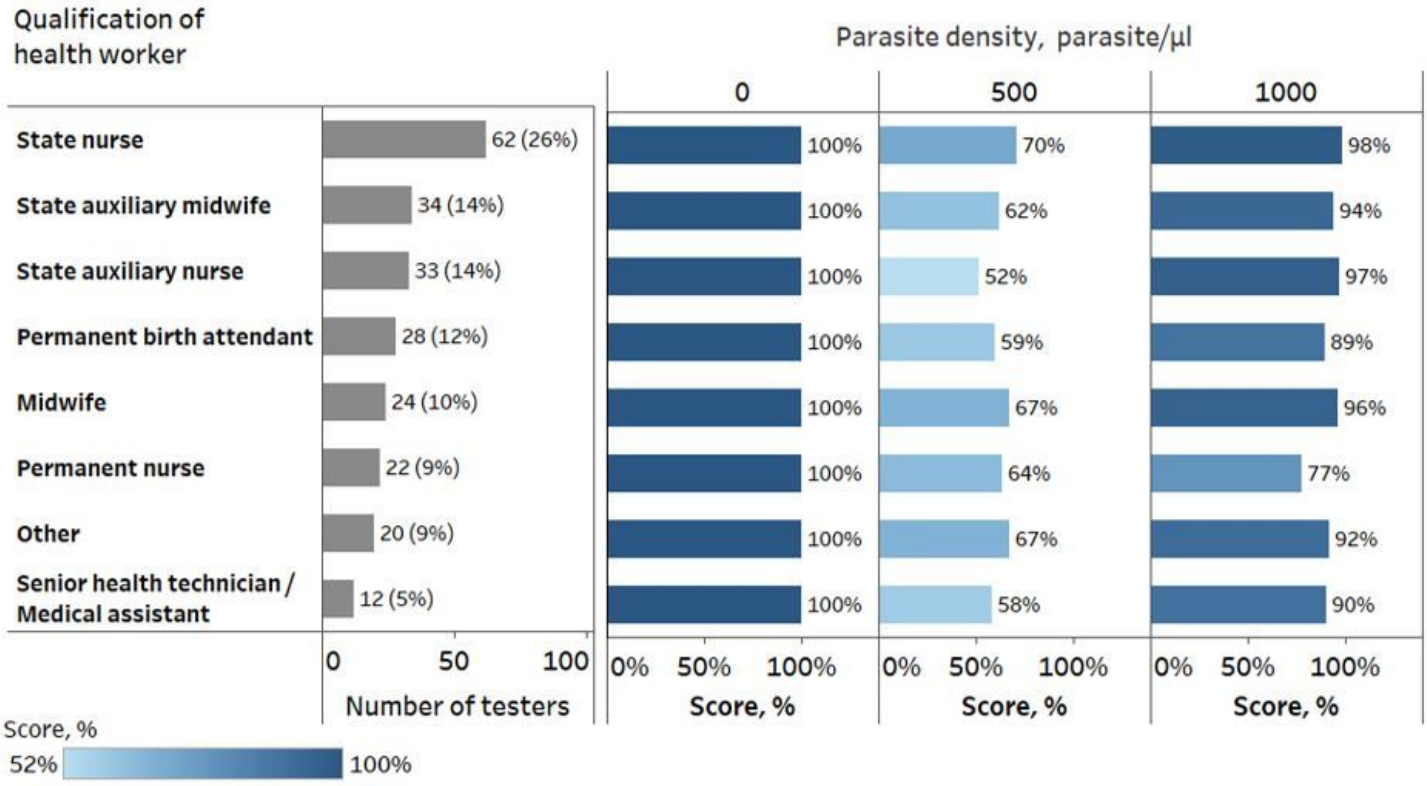

Figure 3

Results of PT disaggregated by health worker qualifications and DTS sample parasite density.

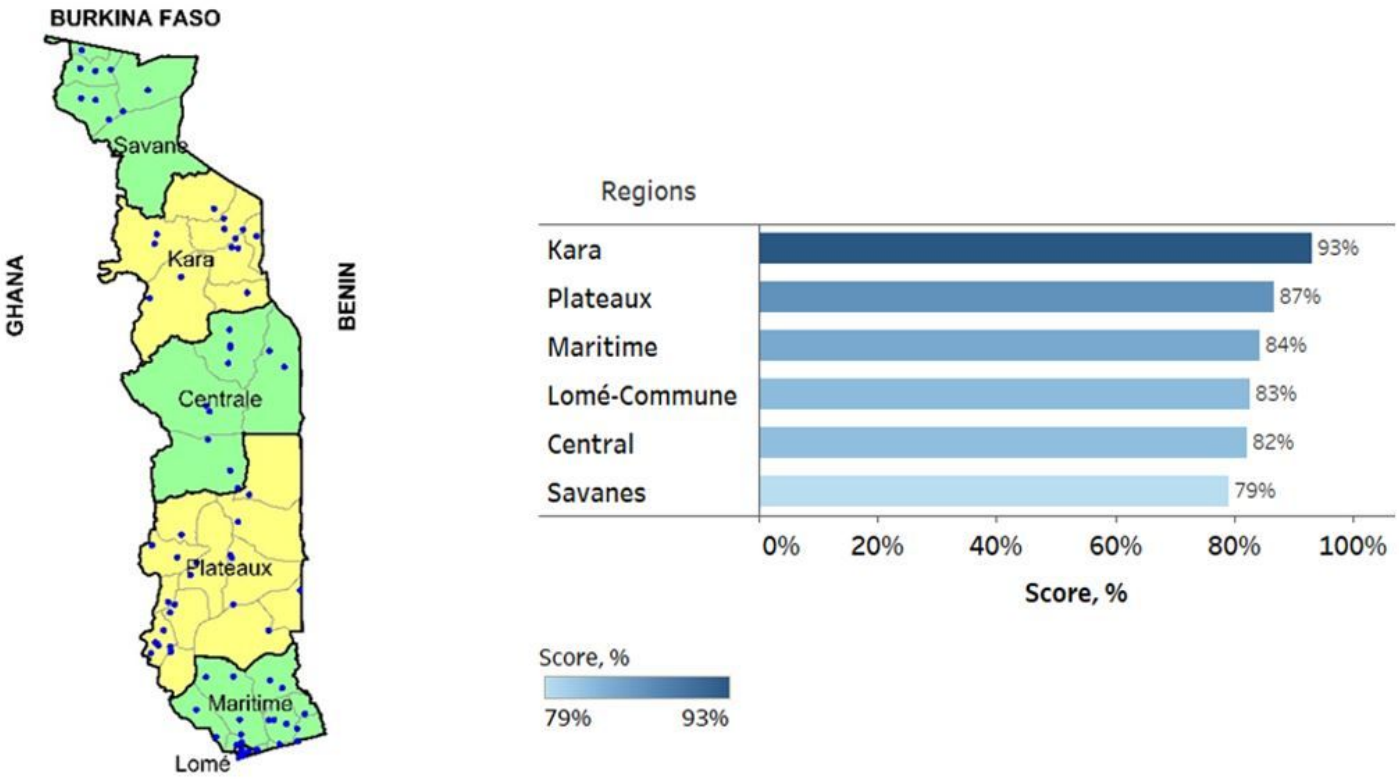

Figure 4

Left panel: A map of Togo showing country regions and locations of the sites participating in the PT program (blue dots). Right panel: PT scores disaggregated by region. Data labels indicate regional performance score (average of all testers in that region). Performance scores were calculated using data from parasite densities of 0, 500, 1,000 parasites/µl.

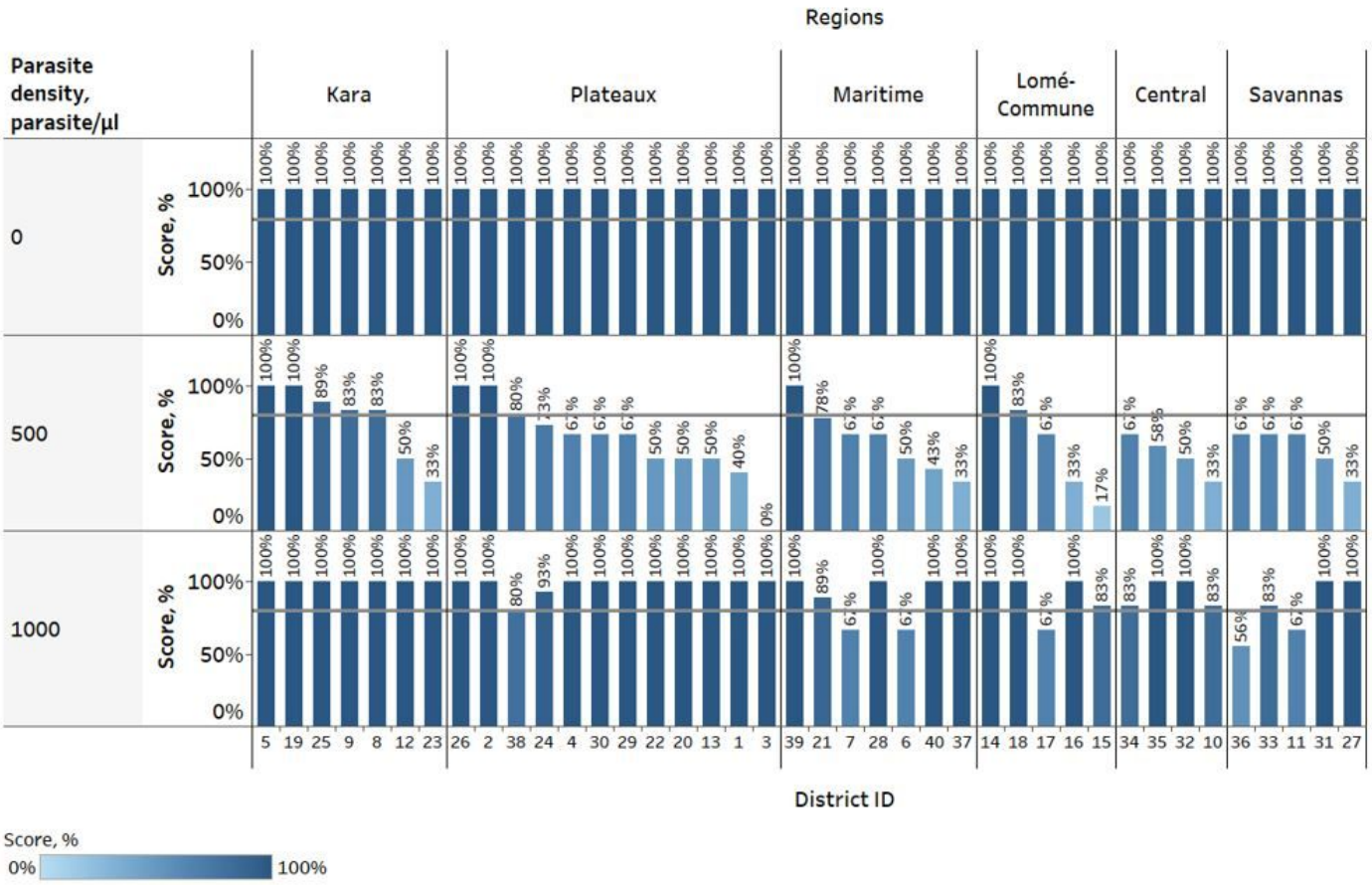

Figure 5

Average PT scores disaggregated by region, district, and parasite density. Data labels indicate average performance of all health workers in a given district. Horizontal grey lines indicate PT score of 80%.

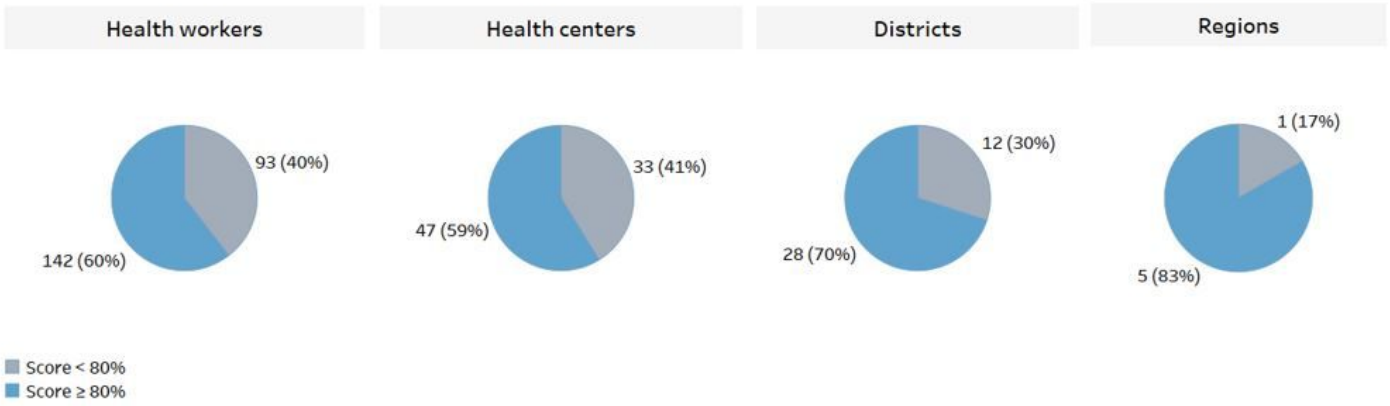

Figure 6

Summary of proficiency testing results across health workers, healthcare centers, districts and regions. Data labels indicate number and percentage of health workers (left panel), healthcare centers (middle-left

panel), districts (middle-right panel) and regions (right panel).