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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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 representative 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 recorded correct test results for all three samples.

**Conclusions:** The use of DTS for a malaria PT program was the first of its kind ever conducted in Togo. The ease of use and stability of the DTS illustrates that they this type of samples can be considered for the assessment of 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. Malaria RDTs (mRDT), which are lateral flow immunochromatography assays, detect the presence of circulating antigens, such as HRP2
and pLDH from different \textit{Plasmodium} species, in those who have been infected with malaria. RDTs are now the most widely used test to diagnose malaria in suspected cases, comprising 75\% of all malaria tests performed. The use of mRDTs allows for rapid diagnosis, which if acted upon accordingly, can enable a reduction in time between the onset of symptoms and treatment, thus contributing to decreased transmission, morbidity, and mortality. Especially when microscopy is not available or inconsistent, mRDTs are an effective tool for the management of malaria.

While testing at or near the point-of-care (POC) can accelerate potentially life-saving medical decisions, procedural challenges, due in part to varied competency of health workers who conduct testing, 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. Unacceptably high false-positive and false-negative results from RDT use have been shown in other studies.

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 disproportionately affected. The introduction of mRDTs has been gradual in Togo, they were first deployed into health facilities in 2006 with expansion into community settings in 2007. As this rollout has scaled up and the associated training of personnel has been expanded, 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. In 2017, rates of confirmed 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. External Quality Assurance (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.

Currently, there is a lack of universal, field-ready malaria QC material to test the quality and performance of mRDTs, 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. 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.

Dried tube specimens (DTS), comprised of dried \textit{Plasmodium falciparum} infected blood, have been reported to be suitable for quality control and EQA in malaria testing facilities. 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 across 40 districts and six regions, selected as described below, that use mRDTs to diagnose malaria cases representing a subset of all testers within Togo. These 80 health centers represent 9.6% of the total 835 health centers in Togo which use mRDTs and have been accredited by the National Malaria Control Program (NMCP). A cut off of 80 sites for this activity was necessary based on the total number of DTS vials available. 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 and Maritime health regions; Team 2: Plateaux health region and Team 3: Centrale, Kara and Savanes health regions.

Based on the information from the NMCP and the number of PT samples available for use, a subset, selected as described below, 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, facilities in locations with high capacities of microscopy diagnosis (e.g. Lomé) were eliminated to give priority to locations with lowerlevel facilities or without a dedicated laboratory. A second review of the list of sites was undertaken to identify those sites with higher throughput of patients and group them geographically to ensure representation of all six health regions of Togo while maintaining ease of access by the field teams. Finally, after applying these criteria, 80 of these sites were randomly selected as participant sites with all workers present on the day of the field team visit being evaluated. The number of facilities meeting these criteria and selected to participate 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 (Figure 1).

Dried tube specimen preparation

DTS were prepared in the USA as previously described. 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 US resident donors with no reported overseas travel and confirmed by PCR to have no malaria infections. Blood was determined to be HIV and HBV negative. Blood was also confirmed in the CDC malaria laboratory to not produce a positive test on RDTs before being used to produce DTS. 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. 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°C before shipping via commercial air service at ambient temperatures to Togo and, similarly, were stored centrally in Lomé at +4°C prior to field activities. As previously described, short term storage (up to 4 weeks) at ambient temperatures does not impact the reactivity of the DTS.

mRDT procurement and storage

A batch of SD Bioline® Malaria Ag Pf RDT (lot number 05CD094A, manufactured in Republic of Korea 09/25/2017, expiration date 09/24/2019) used in Togo was obtained from the NMCP for use in preliminary QC of DTS and in the PT. All procured kits were stored centrally until use. This allowed for the same lot number of kits to be used throughout the evaluation and provided an opportunity for baseline assessment prior to using the kits for the PT as well as eliminating the potential impact of variable storage conditions at testing sites. 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 each parasitemia level (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 were then tested using SD Bioline® Malaria Ag Pf RDT (Standard Diagnostics, Inc., Abbott, Gyeonggi, Korea) in the same manner as 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 the health workers using 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 an insufficient number of samples with parasite density of 200 and 500 parasites/µl. DTS vials were rehydrated onsite as described above. A total of 235 health workers participated in the PT activity, with three, two and one health workers testing samples in 76, three and one health facility, respectively. The number of participants at each site was a function of the number of staff on duty at the time of the supervisory visit. All personnel with a role in mRDT work and present on the day of the visit participated in the PT. 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 a results form designed by the NQAPM. Completed forms were collected by the field teams and returned to the NQAPM for analysis.

**Statistical methods**

XLSTAT Basic (version 2019, Addinsoft, France), a statistical application for Microsoft Excel®, 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 the assessment of individual tester performance, PT scores were calculated as percentage of samples identified by a tester correctly (that is, negative aliquot content was tested negative and positive aliquot content was tested positive). For the assessment of health center, district, or regional performance, correct results were aggregated as percentage of tests performed correctly at corresponding level from a total number of tests performed at corresponding level. For this analysis, a level of 80% correct PT result was selected as a value to mark acceptable proficiency. 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 to offer appropriate patient care when conducting malaria testing.

**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. All DTS with 100 parasites/µl and 50% of DTS with 200 parasites/µl were classified by NQAPM team as non-reactive (Figure 2). 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 3). 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 ($\chi^2(df=1;N=238)=8.2; p=0.004$; 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-one percent of health workers were classified into groups, with the majority of being state nurses, auxiliary midwives and auxiliary nurses (together 54%) while senior health technicians or medical assistants represented only 5% (Figure 4). Other qualifications included categories with only 5 or less health workers in each and were combined into a single 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 4). There was no significant difference between these groups of health workers in overall performance ($\chi^2(df=7; N=702)=5.6; p=0.590$; effect size Cramer’s $V=0.03$) nor when comparing only the results of tests on samples containing 500 parasites/µl ($\chi^2(df=7; N=232)=3.9; p=0.787$; effect size Cramer’s $V=0.05$) or those with 1,000 parasites/µl ($\chi^2(df=7; N=235)=13.5; p=0.061$; effect size Cramer’s $V=0.09$).

Performance by health region

The 80 health centers were distributed amongst multiple regions in the country (Figure 1). The difference in PT results between health regions of Togo was not statistically significant ($\chi^2(df=5; N=702)=9.9; p=0.078$; Cramer’s $V=0.05$) (Figure 5). There was also no difference in performance between qualification groups within regions (data not shown); 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, although with small effect size, in the percentage of health workers drawn from these three groups between the regions ($\chi^2(df=5; N=235)=26.4; p<0.0001$; 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 ($\chi^2(df=5; N=232)=6.3; p=0.278$; 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 ($\chi^2(df=5; N=235)=15.0; p=0.010; Cramer's V=0.11$) (Figure 6). The six health regions are further divided into 40 health districts (Figure 6) 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/µL, respectively. Overall, 47 (59%) of healthcare centers, 28 (70%) of districts and 5 (83%) of regions performed above the threshold of 80%, and 142 (60%) of health workers correctly identified all three samples (Figure 7).

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 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. In this study, we did not examine the impact of DTS rehydration by health workers and any errors introduced by requiring results to be returned i.e. testing without a supervisor present. While the potential impact of these activities could be relevant for large-scale program roll-out, precedent from DTS use in HIV PT has shown that these steps can be easily incorporated into a well-planned DTS-based PT program for malaria.

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 mRDTs. 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, RDT sensitivity is reduced at low parasite densities and cases of low sensitivity of mRDTs in samples of parasite densities less than 200 parasites/µL have been reported.

The parasite densities of 0, 500 and 1,000 parasites/µL are consistent with those used by other groups for PT using malaria DTS. 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. Even though the conditions of mRDT storage were not evaluated it is shown that
improper storage of RDTs prior to use can also impact performance. 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. A limitation of the study is that despite efforts to be certain DTS produced the correct results on the RDTs, the possibility remains, especially for parasites at 500 p/µL, that some RDTs could have still produced negative results. However, based on pre-study testing, we believe such instances would be rare and unlikely to affect the overall result.

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. Other studies show that community healthcare workers can successfully follow the mRDTS procedural steps to diagnose malaria using mRDTs when they are provided with adequate training and job materials. \textsuperscript{16,17} While this shift 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 probing further, 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, while at an individual level about 60\% of testers scored 80\% or more. Sites participated in the current study represent only a small subset of the nationwide testing points and these data, therefore, illustrate 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 and incorrect interpretation of 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 1,000 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 1,000 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 microscopy 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: as expected, 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 densities of 1,000 parasites/µL or higher, clinical cases with lower densities are not uncommon. \textsuperscript{18,19,20} 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 \textsuperscript{3,21} but also to variations such as light at the testing bench and visual acuity of the tester. \textsuperscript{22} This information can be useful to guide mentorship, training, and allocation of resources to maximize impact. With such information training and other corrective actions could be appropriately targeted. This study shows the feasibility of a DTS-based PT activity for malaria. The final implementation could be as described here
with supervisors taking DTS to sites or as described for HIV and syphilis where DTS are shipped to sites and results returned to coordinating laboratory.\textsuperscript{23,24}

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.\textsuperscript{25} In many settings worldwide, healthcare workers are tasked with using mRDTs to diagnose malaria without receiving proper instructions on RDT use, thus underscoring the importance of high-quality training and PT.\textsuperscript{26} 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 during 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.\textsuperscript{27,28}

**List Of Abbreviations**

CDC - Centers for Disease Control and Prevention

DTS - Dried Tube Specimen

DF - Degrees of freedom

EQA - External Quality Assurance

GA - Georgia
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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References


**Figures**
Figure 1

A map of Togo showing health regions and locations of the sites participating in the PT program (blue dots).
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Figure 2

Results of QC testing (6 aliquots per parasite density) conducted at the Malaria reference lab prior to the initiation of proficiency testing. Data labels indicate percentage of aliquots tested correctly for each parasite density during the QC; that is, all six aliquots (100%) with parasite density 0 parasites/µl were tested negative, none of six aliquots (0%) with parasite density 100 parasites/µl were tested positive, only three aliquots (50%) with parasite density 200 parasites/µl were tested positive, all six aliquots (100%) with parasite density 500 parasites/µl were tested positive, and all six aliquots (100%) with parasite density 1,000 parasites/µl were tested positive.
Figure 2

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Figure 3

Average PT results for 235 testers across 80 participating facilities. Data labels indicate percentage of testers who correctly identified aliquot content for each parasite density during the PT; that is, all testers (100%) correctly identified aliquots with parasite density 0 parasites/µl as negative, 1% of testers correctly identified aliquots with parasite density 100 parasites/µl as positive, 11% of testers correctly identified aliquots with parasite density 200 parasites/µl as positive, 63% of testers correctly identified aliquots with parasite density 500 parasites/µl as positive, and 93% of testers correctly identified aliquots with parasite density 1,000 parasites/µl as positive.
Average PT results for 235 testers across 80 participating facilities. Data labels indicate percentage of testers who correctly identified aliquot content for each parasite density during the PT; that is, all testers (100%) correctly identified aliquots with parasite density 0 parasites/µl as negative, 1% of testers correctly identified aliquots with parasite density 100 parasites/µl as positive, 11% of testers correctly identified aliquots with parasite density 200 parasites/µl as positive, 63% of testers correctly identified aliquots with parasite density 500 parasites/µl as positive, and 93% of testers correctly identified aliquots with parasite density 1,000 parasites/µl as positive.

Figure 4

Results of PT disaggregated by health worker qualifications and DTS sample parasite density.
Figure 4

Results of PT disaggregated by health worker qualifications and DTS sample parasite density.
Figure 5

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

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 6

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%. District numbers are arbitrary and were assigned during the study in lieu of district names to anonymize sites during data analysis and to simplify the presentation of results. The difference between regions in the testing of 500 parasites/µl specimens was not statistically significant. 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.
Figure 6

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%. District numbers are arbitrary and were assigned during the study in lieu of district names to anonymize sites during data analysis and to simplify the presentation of results. The difference between regions in the testing of 500 parasites/µl specimens was not statistically significant. 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.
Figure 7

Summary of proficiency testing results across health workers, healthcare centers, districts and regions. Data labels indicate number and percentage of health workers (panel 7a), healthcare centers (panel 7b), districts (panel 7c) and regions (panel 7d).