FACTORS ASSOCIATED WITH MALARIA MICROSCOPY DIAGNOSTIC PERFORMANCE FOLLOWING A PILOT QUALITY-ASSURANCE PROGRAMME IN PUBLIC HEALTH FACILITIES IN LOW MALARIA TRANSMISSION AREAS OF KENYA

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Factors Associated with Malaria Microscopy Diagnostic Performance Following a Pilot Quality-Assurance Programme in Public Health Facilities in Low Malaria Transmission Areas of Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

I dedicate this work to my mentor Ann Buff, my lovely wife Ruth and my beautiful little angels; Cheryl, Tatiana, Tamara and Harvey for their endurance during the entire course.

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ABBREVIATIONS AND ACRONYMS

ACT	Artemisinin-based Combined Therapy
AUC	Area under the curve
CI	Confidence interval
CPD	Continuing Professional Development
ERC	Ethical Review Committee
FBO	Faith Based Organization
FN	False negative
FP	False positive
GLP	Good Laboratory Practice
ICH-GCP	International Conference on Harmonization Guideline on Good Clinical Practice
IPT_i	Intermittent preventive treatment of malaria in infancy
IPT_p	Intermittent preventive treatment of malaria in pregnant women
IRS	Indoor residual spraying
ISO	International Standards Organization
ITN	Insecticide-treated nets
MDC	Malaria Diagnostics Centre
NMCP	National Malaria Control Programme

PI Principal Investigator

PPS Probability Proportionate to Size

PR Prevalence ratio

PT Proficiency Testing

QA Quality Assurance

QC Quality Control

RDT Rapid Diagnostic Test

SLIPTA Stepwise Laboratory Improvement Progress Towards Accreditation

SOP Standard Operating Procedure

TN True negative

TP True positive

WHO World Health Organization

DEFINITION OF TERMS

Accurate malaria diagnosis: Concordance in the presence or absence of parasites (i.e., positive or negative) between the health-facility microscopist result and the expert reference result.

Artemisinin-based combined therapy: A combination of artemisinin or one of its derivatives with an antimalarial or antimalarials of a different class.

Continuing Professional Development: The process of tracking and documenting the skills, knowledge and experience that a worker gains both formally and informally, beyond any initial training.

Drug resistance: The ability of a malaria parasite strain to survive and/ or to multiply despite the administration and absorption of a medicine given in doses equal to or higher than those usually recommended but within the tolerance of the subject, provided drug exposure at the site of action is adequate.

Endemic: Occurring frequently in a particular region or population.

Epidemic: Occurring in excess of what would be normal at a given time.

Febrile: With an increase in temperature compared with the normal.

Fever: An increase in body temperature above the normal temperature i.e. above an oral temperature of 37.5^{o} C.

Malaria Low-Transmission Areas: Zones known to have community malaria parasitaemia prevalences by microscopy of between 1 and 3% during peak malaria transmission season.

Public health facilities: Government owned health facilities.

Rapid Diagnostic Test: An antigen-based stick, cassette or card test for malaria in which a coloured line indicates that plasmodial antigens have been detected.

Severe malaria: Symptomatic infection with malaria parasitaemia with signs of severity and/ or evidence of vital organ dysfunction.

QA-pilot facilities: The health facilities that participated in the pilot quality-assurance programme.

Non-QA pilot facilities: The health facilities that did not participate in the pilot quality-assurance programme.

ABSTRACT

Malaria accounts for approximately 21% of out-patient visits annually in Kenya. Prompt and accurate malaria diagnosis is critical to ensure proper treatment. In 2013, formal malaria microscopy refresher training for microscopists and a pilot quality-assurance (QA) programme for routine malaria diagnostics (microscopy and rapid diagnostic tests) were independently implemented by the national malaria control program to improve malaria microscopy diagnosis in malaria low-transmission areas of Kenya. Malaria microscopy is tedious and is a skill that is learnt over time and through experience. In malaria low-transmission areas, community malaria parasitaemia prevalences by microscopy are between 1 and 3% during peak malaria transmission season. This study was conducted to identify factors associated with malaria microscopy performance in the same areas. From March to April 2014, a cross-sectional survey was conducted in 42 public health facilities; 21 were QA-pilot while another 21were non-QA facilities. In each facility, 18 malaria thick blood slides archived during January-February 2014 were selected by simple random sampling. January-February are not peak malaria seasons in Kenya. Each malaria slide was re-examined by two expert microscopists masked (blinded) to health-facility results. Expert results were used as the reference for microscopy performance measures. Logistic regression with specific random effects modelling was performed to identify factors associated with accurate diagnosis of malaria through microscopy technique. Of the 756 malaria slides collected, 204 (27%) were read as positive by health-facility microscopists and 103 (14%) as positive by experts. Overall, 93% of slide results from QA-pilot facilities were concordant with expert reference compared to 77% in non-QA pilot facilities ($p < 0.001$). Recently trained microscopists in QA-pilot facilities performed better on microscopy performance measures with 97% sensitivity and 100% specificity compared to those in non-QA pilot facilities (69% sensitivity; 93% specificity; $p < 0.01$). The overall inter-reader agreement between QA-pilot facilities and experts was $\kappa=0.80$ (95% CI: 0.74-0.88) compared to $\kappa=0.35$ (95% CI: 0.24-0.46) between non-QA pilot facilities and experts ($p < 0.001$). In adjusted multivariable logistic regression analysis, recent microscopy refresher training (prevalence ratio [PR]=13.8; 95% CI: 4.6-41.4), ≥5 years of work experience (PR=3.8; 95% CI: 1.5-9.9), and pilot QA programme participation (PR=4.3; 95% CI: 1.0-11.0) were significantly associated with accurate malaria diagnosis. Microscopists who had recently completed refresher training and worked in a QA-pilot facility performed the best overall. The QA programme and formal microscopy refresher training should be systematically implemented together to improve parasitological diagnosis of malaria by microscopy in Kenya.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Human malaria is caused by the five members of *Plasmodium* species, namely; *Plasmodium falciparum, P. malariae, P. ovale* and *P. knowlesi* (Antinori *et al.,* 2012). Of all these malaria species, *P. vivax* and *P. knowlesi* may not occur in Kenya (Howes *et al.,* 2015). *P. falciparum* causes the severest form of the disease, and accounts for 98% of all malaria infections in Kenya (Baliraine *et al.,* 2009). Clinically, patients with *P. falciparum* malaria who do not receive prompt and effective treatment may progress to severe malaria within a few hours to a few days (WHO, 2015a). In 2013, approximately 198 million cases of malaria and 584,000 deaths occurred globally, and 90% of the deaths were in Africa (WHO, 2014c). In 2012, Kenya had an estimated malaria mortality rate of 27.7 per 100,000 people (WHO & United Nations, 2015). Malaria accounted for almost 9 million out-patient visits in Kenya in 2012, which represented approximately 21% of all out-patient consultations (Division of Malaria Control, 2013a). Despite a number of control measures being in place in the country, the quality of malaria testing, particularly by microscopy is yet to be ascertained (Zurovac *et al.,* 2014).

Parasitological diagnosis is recommended by the World Health Organization (WHO) for all patients in whom malaria is suspected as part of the "test, treat, track" strategy (WHO, 2010; WHO, 2012b). Both microscopy and malaria rapid diagnostic tests (RDT) are the recommended malaria diagnostic methods by the Kenya National Malaria Control Programme (NMCP) (Division of Malaria Control, 2009; Division of Malaria Control, 2010; Division of Malaria Control, 2013b). Although over 90% of public health facilities in Kenya had the capacity to diagnose malaria, the proportion of health facilities performing malaria microscopy, approximately 50%, has not changed in recent years (Nyandigisi *et al.,* 2014). Despite the high proportion of health facilities offering malaria diagnostic services, only 31% of malaria cases were confirmed by parasitological diagnosis in Kenya in 2013 (Division of Malaria Control, 2013a).

Light microscopy was established over a hundred years ago and to date, is considered reference standard for clinical diagnosis of malaria (Ashraf *et al.,* 2012). Limited microscopy services in health facilities in Kenya and across sub-Saharan Africa has been attributed, in part, to limitations in the availability of equipment, supplies, working environment, training, and supervision (WHO, 2009; WHO, 2014a; Zurovac *et al.,* 2014). Increasing and sustaining access to prompt diagnosis and effective treatment for at least 80% of the population across all levels of the health care system and epidemiological zones is a key objective of the Kenya National Malaria Strategy 2009–2017 (Division of Malaria Control, 2009). Implementation of the national strategy included providing health facilities with microscopes and laboratory supplies and improving the skills of microscopists through formal microscopy refresher trainings at microscopy centers of excellence (Division of Malaria Control, 2009; Ohrt *et al.,* 2007).

From June to December 2013, the NMCP in coordination with the Malaria Diagnostics Center (MDC), Walter Reed Army Research Institute, initiated a pilot to operationalize the laboratory quality assurance (QA) policy and plan for malaria diagnostics in health facilities in malaria low-transmission areas. Malaria low-transmission areas were prioritized because of concerns surrounding over-diagnosis of malaria arising from routine data that pointed to poor microscopy practices (Division of Malaria Control, 2013b; Ministry of Health, 2015). Laboratory QA programmes have been shown to improve the diagnosis of malaria and in particular, microscopy accuracy (Ohrt *et al.,* 2007; Wafula *et al.,* 2014). Key to this programme was the pilot implementing laboratory QA officers formally trained under WHO curriculum, at the MDC, Walter Reed Army Research Institute. Laboratory personnel (herein referred to as microscopists) who had attended and excelled in the 2 weeks WHO curriculum formal refresher training in malaria microscopy, conducted at the MDC within the previous 12 months, were selected to participate as QA officers. To implement the pilot QA programme, these QA officers were further trained for 5 days on malaria diagnostics and another 5 days on formal QA/ quality control (QC) methods and laboratory management systems in accordance with ISO standard 15189.

Components of the 7-month pilot QA programme included four 1-day visits by trained QA laboratory officers, who promoted personnel training and competencies by on-job diagnostics training and supportive supervision. They provided equipment (e.g., microscopes), essential laboratory consumables and reference materials which included the national laboratory guidelines, bench and job aides. They cross-checked at least 10 malaria microscopy slides at each visit and promoted internal QA/QC processes through preparation of Standard Operating Procedures (SOPs) for equipment calibration, specimen collection, slide preparation and examination (Division of Malaria Control, 2013b). The pilot QA programme implementation is further described in detail elsewhere (Wanja *et al.,* 2017). In 2013, there were other independent laboratory-strengthening activities ongoing in Kenya, such as malaria microscopy refresher trainings and the WHO Stepwise Laboratory Improvement Progress Towards Accreditation (SLIPTA) programme. The WHO SLIPTA framework was established to improve the quality of public health laboratories in developing countries through standardized processes to meet international accreditation (WHO, 2012a). In early 2014, this survey was conducted to identify factors associated with accurate malaria diagnosis by microscopy in 42 health facilities in malaria low-transmission areas of Kenya.

1.2 Statement of the problem

Malaria imposes a heavy economic burden on individuals, saps the vitality of the workforce and causes diversion of resources needed for development to treat the sick. Microscopy is available in 50% of health facilities in Kenya and is one of the methods that NMCP greatly relies on and promotes to ensure prompt and effective disease management in febrile patients. It is the primary method for malaria diagnosis in primary and secondary or referral hospitals in Kenya.

Expert or quality-assured malaria microscopy is the ‘gold-standard’ for identifying mixed infections, treatment failures and quantifying parasite density. However, false positive and negative malaria smear results often obtained from clinical laboratories is a serious concern. Persons who are misdiagnosed as having malaria when they actually do not are at risk of not being treated for their actual disease, which could

lead to increased morbidity and mortality. According to routine health data, over-diagnosis of malaria is common in low-transmission areas, and treating people who do not have malaria with the relatively expensive artemisinin-based combination therapies (ACTs) wastes limited resources and could contribute to the development of artemisinin resistance in parasites.

1.3 Justification

Quality of malaria microscopy testing in malaria endemic and low-transmission areas of Kenya is not known, despite the ministry of health's target to reduce the 2007 figures of malaria mortality (≈ 40,000 deaths) and morbidity (≈ 15 million case) by two-thirds by 2017 through accurate diagnosis, prompt and effective treatment. Our study aimed to provide an opportunity to determine the quality of malaria microscopy diagnosis within low malaria transmission areas in Kenya.

In order to reduce the number of false positives and false negatives obtained using microscopy, there is need to determine the factors that are associated with accuracy of malaria microscopy. This study aims at interrogating institutional factors including participation in a QA programme as well as individual factors such as how recently malaria microscopy training was done and duration of work experience.

Results of the study can be used in strengthening malaria microscopy systems to ensure valid and reliable malaria testing. This would allow patients suspected of malaria the opportunity to receive appropriate treatment, potentially reducing morbidity and mortality due to malaria and other febrile illnesses. A strong malaria microscopy diagnostic system has the potential to positively impact on clinicians' confidence in the test results as well. This would restrict prescription of the relatively expensive ACTs to only those who have malaria, hence decelerating the development of artemisinin resistance by the parasites.

1.4 Research questions

1. What are the characteristics of health facilities and microscopists in low malaria transmission areas of Kenya?
2. What is the performance of measures of malaria microscopy diagnosis in low malaria transmission areas of Kenya?
3. What are the individual and institutional factors associated with accurate malaria diagnosis by microscopy in low malaria transmission areas of Kenya?

1.5 Objectives

1.5.1 Main objective

To identify factors associated with performance of malaria diagnosis through microscopy following a pilot quality-assurance (QA) program in health facilities in selected low malaria transmission areas of Kenya

1.5.2 Specific objectives

1. To describe the characteristics of QA-pilot and Non-QA pilot participating health facilities and microscopists
2. To determine performance of measures of malaria microscopy diagnosis in QA-pilot and non-QA pilot participating health facilities
3. To identify individual and institutional factors associated with accurate diagnosis of malaria by microscopy in low malaria transmission areas

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria situation

Globally, approximately 3.2 billion people are at risk of malaria (National malaria control programme, 2015). However, a WHO report indicates an estimated decrease in incidence rate of malaria by 18% globally, from 76 cases to 63 cases per 1000 population at risk, between 2010 and 2016 (WHO, 2017a). Sub-Saharan Africa accounts for about 90% of all malaria cases and malaria related deaths (National malaria control programme, 2015). In Kenya, malaria is still the leading cause of morbidity and mortality. About 70 % of country's 46 million people are at risk of the disease (National malaria control programme, 2015; WHO, 2017b). The areas around Lake Victoria and coast present the highest risk, and children under age 5 and pregnant women are the most vulnerable to infection. In Kenya, malaria prevalence is on a downward trend, thanks to the concerted efforts and effective partnerships among key players in the prevention and control. Hence, since 2013 the national prevalence of malaria has dropped from 11% to 8% (National malaria control programme, 2015).

Kenya has four divergent malaria epidemiological zones based mainly on prevalence as determined by altitude, rainfall patterns, and temperature (Division of Malaria Control, 2014b). One of the epidemiological zones is the endemic zones; which are areas of 0-1,300 meters altitude and have stable malaria. These areas are found around Lake Victoria and in the coastal regions. Rainfall, temperature, and humidity are the determinants of perennial transmission of malaria. The vector life cycle is usually short with a high survival rate due to the suitable climatic conditions. Transmission is intense throughout the year, with annual entomological inoculation rates between 30 and 100 (National malaria control programme, 2015).

The other zone is highland epidemic prone of the western highlands of Kenya. Malaria transmission in these areas is seasonal, with considerable year-to-year variation. The epidemic phenomenon is experienced when climatic conditions

favour sustainability of minimum temperatures around 18°C. This increase in minimum temperatures during periods of long rains favours and sustains vector breeding, resulting in increased intensity of malaria transmission. The whole population is vulnerable, and case fatality rates during an epidemic can be up to 10 times greater than what is experienced in regions where malaria occurs regularly (National malaria control programme, 2015).

Seasonal malaria transmission is another zone which covers arid and semi-arid areas of the northern and south-eastern parts of Kenya. This areas experience short periods of intense malaria transmission during the rainfall seasons. Temperatures are usually high, and water pools created during the rainy season provide the malaria vectors with breeding sites. Extreme climatic conditions such as the El Niño southern oscillation lead to flooding in these areas, resulting in epidemic outbreaks with high morbidity rates due to the population's low immune status (National malaria control programme, 2015).

Low risk malaria zone covers the central highlands of Kenya, including Nairobi. In these areas, temperatures are usually too low to allow completion of the sporogonic cycle of the malaria parasite in the vector. However, increasing temperatures and changes in the hydrological cycle associated with climate change are likely to increase the areas suitable for malaria vector breeding and introduce malaria transmission in these areas where it did not previously exist (National malaria control programme, 2015).

2.2 Medical laboratory personnel in Kenya

Technical personnel who work in clinical laboratories in Kenya are holders of certificate in medical laboratory technology (technicians), diploma in medical laboratory technology (technologists) or Bachelor of Science degree in medical laboratory sciences (scientists). In addition, there are specialist levels of training in medical laboratory sciences, which include higher diploma in medical laboratory technology, Master of Science and doctor of philosophy degrees in medical laboratory sciences (National Public Health Laboratory Service, 2006).

2.3 Health system in Kenya

Health systems in Kenya are categorized into public sector, commercial private sector, and Faith Based Organizations (FBOs). The public sector has the largest number of health facilities (Ministry of Health, 2014). Laboratory services are distributed disproportionately among the various socio-economic classes, with the rich being more favoured. There are more laboratory facilities with qualified staff in their areas (Toda *et al.*, 2012). This is despite mortality and morbidity indicators reportedly being worse among the poor, majority of who reside in slums and rural areas (Mberu *et al.,* 2016). In Kenya, there is a surplus of trained laboratory technicians and technologists, although generally their pre-service training often has knowledge and skill gaps necessary for effective diagnosis of diseases (World Bank, 2009).

Initially, the National Health Sector Strategic Plan II 2005-2010 organized healthcare system in Kenya in levels as follows: Level1;community, level 2; dispensaries, level 3; health centres, level 4; district hospitals, level 5; provincial hospitals and level 6; national hospitals. Currently, National Health Sector Strategic Plan III 2012-2017 has reorganized these levels into tiers as follows: Tier 1: Community; tier 2: Primary care level; the previous level 2 and 3, tier 3: County level; the previous level 4, and tier 4: National level; the previous level5 and 6 (Ministry of Health, 2014).

Dispensaries are the lowest level after community interventions in public health system and are often the first point of contact with patients. They are staffed by nurses, public health technicians and dressers. They hardly have a functional laboratory since there are no clinical officers or doctors to request for laboratory tests; hence they mostly depend on mRDT for malaria diagnosis (Division of Malaria Control, 13a). Those with laboratory services should maintain at least two technicians with certificate level training (National Public Health Laboratory Services, 2006). Health centers often have basic clinical laboratories and are managed by clinical officers who make laboratory requests for tests. Malaria microscopy test is expected at this level, but due to priorities and limited resources

they are affected by understaffing coupled with high workload. They augment their service coverage with outreach services and refer services and complicated cases to the district hospitals. Health centers should maintain at least two laboratory technicians; certificate level training or technologists; diploma level training (National Public Health Laboratory Services, 2006)

County (primary) hospitals are the first referral level and national (secondary) hospitals are the ultimate referral levels. Some are teaching hospitals for various cadres of healthcare personnel, including laboratory technicians and technologist. They offer specialized treatment and testing not available at the dispensary and health centers. As such, staffing is often higher at this level because they are prioritized during personnel deployment. Personnel in hospitals have mixture of skills and specialization, hence expert malaria microscopy is expected at this level. County level hospital is expected to have at least 24 laboratory technical staff that include diploma and higher diploma trained personnel in medical laboratory technology. On the other hand, the national level hospital should have, in addition to technologists, two pathologists (National Public Health Laboratory Services, 2006). Many hospitals laboratories are enrolled in the SLIPTA, which is a program that enhances quality of testing (WHO, 2012a).

2.4 Malaria diagnostics

Microscopic examination of thick and thin blood films remains the gold standard for laboratory diagnosis. It can be used to detect and quantify malaria parasites in a thick blood film, hence useful in monitoring of treatment (Mathison, 2017). When examined by knowledgeable microscopists under optimal conditions, the thick film has reported detection threshold of 10 to 50 parasites/microliter of blood; approximately 0.001% parasitemia, assuming an erythrocyte count of 5×10^6 cells/µl. Thin film is useful for species identification (Ochola, 2006). Since management of different *Plasmodiyum* species may differ, e.g. *P. vivax* requires different regimens for clinical management from the rest of the human species, it's important to identify the parasite to species level (Mathison, 2017).

Rapid antigen tests may also play a useful role in detection of acute infection. They are lateral flow assays. Commonly used antigens are *P. falciparum* histidine-rich protein-II (HRPII), *Plasmodium* spp. lactate dehydrogenase, and *Plasmodium* spp. aldolase. Depending on the format and number of antigens present, assays may detect to the *Plasmodium* genus level only, or they may detect specific species e.g., *P. falciparum*, *P. vivax* (Mathison, 2017). In some settings, increasing levels of histidine-rich protein 2 gene (HRP2) deletions threaten the ability to diagnose and appropriately treat people infected with falciparum malaria. An absence of the HRP2 gene enables parasites to evade detection by HRP2-based RDTs, resulting in a false-negative test result. Although the prevalence of HRP2 gene deletions in most high-transmission countries remains low, further monitoring is required (WHO, 2017a).

Serological tests to detect antibodies are available, but are not often used. They are sometimes used to screen blood donors, in epidemiologic surveys and research. The common serological tests (use serum or plasma) are immunofluorescence assays or enzyme immune assays (Centers for Disease Prevention and Control, 2013). The tests may be positive in cases where parasites are not seen on peripheral smears. They are not appropriate for detection of acute disease due to relatively long time it takes for human body to develop detectable antibody levels (Mathison, 2017).

Nucleic acid amplification methods may also play a useful role in detection of acute infection. *Plasmodium* spp nucleic acid can be detected using DNA/RNA hybridization, conventional and real-time PCR, loop-mediated isothermal amplification (LAMP), and nucleic acid sequence-based amplification (NASBA). This methods as listed are largely used in research (Vasoo, 2013). These are expensive high-complexity methods. They have the highest sensitivity, and just like expert microscopy they can detect mixed infections (Mathison, 2017).

2.5 Current status of malaria diagnostics in Kenya

World Health Organization recommends prompt malaria diagnosis either by microscopy or malaria rapid diagnostic test (RDT) in all patients with suspected malaria before treatment is administered (WHO, 2012b; WHO, 2015a). In Kenya, especially in low transmission areas, many febrile patients have been treated for

malaria without testing (Ye *et al.,* 2009) despite over 90% of health facilities being able to provide at least one of the recommended diagnostic methods (Zurovac *et al.*, 2014; Nyandigisi *et al.,* 2014). Moreover, only 31% of suspected malaria cases were confirmed by parasitological testing in 2013 (Division of Malaria Control, 2013a). But according to a recent survey, testing has improved following the implemetation of the 'test and treat' case-management policy in Kenya (Zurovac *et al*., 2014). In Kenya, RDT is the primary method for diagnosis of malaria in most primary care health facilities (dispensaries and health centre), while microscopy is the primary method in hospitals (Division of Malaria Control, 2014a).

2.6 Performance measures of malaria diagnostics

Malaria microscopy, the 'gold standard, is relatively labour intensive and is often associated with delays and sometimes erroneous results (WHO, 2015b; Bejon *et al.*, 2006). It involves examination of blood films (thick/thin smear) processed by staining either in Field stain or Giemsa (WHO, 2009). Although Giemsa stain is more expensive than Field stain, it is the stain of choice because of its stability during storage in powder form and it has a consistent, reproducible staining quality over a range of temperatures (WHO, 2015b; Wanja *et al.,* 2017).

Key performance measures in malaria microscopy diagnosis are validity and reliability (WHO, 2015b). Validity measures include; sensitivity, specificity, negative predictive value and positive predictive value (WHO, 2009). Reliability on the other hand is a measure of agreement levels between independent observers denoted as kappa statistic (Landis & Koch, 1977). These measures as stated had not been substantially evaluated in the study areas (Zurovac *et al.,* 2014). The lack of evaluation of performance measures may compromise the accuracy of the test results, and potentially negatively impact the quality of patient care (Isiguzo *et al.,* 2014; Ohrt *et al.,* 2007).

According to the National Guidelines for Diagnosis, Treatment and Prevention of Malaria for Health Workers in Kenya, microscopy of stained blood film has a sensitivity range of 86-98% with a lower sensitivity in parasitaemia $\leq$ 320 parasites/µl of blood (Division of Malaria Control, 2010). But a cross-sectional

study in Dar es Salaam of routine microscopy using expert microscopy with single reading as comparator, gave a sensitivity of 71.4% (CI: 35.9%, 91.8%) and a specificity of 47.3% (CI: 41.9%, 52.7%), with positive and negative predictive values of 2.8% (CI: 1.2%, 6.4%) and 98.7% (CI: 95.5%, 99.6%), respectively (Kahama-maro *et al.,* 2011). Further, in a study carried out in western Kenya, sensitivity of microscopy was found to depend on parasite density, with 100% sensitivity achieved at > 200 parasites/µl for experts and > 500 parasites/µl for qualified readers (Ohrt *et al.,* 2007).

In another recent study in Western Kenya, using expert microscopy as 'gold standard', the sensitivity of RDTs to detect malaria parasites was 90.3–94.8 %, the specificity was 73.3–79.3 %, the positive predictive value was 62.2–68.8 %, and the negative predictive value was 94.3–96.8 % (Wanja *et al.,* 2017). A number of nucleic acid amplification techniques are also available and they are more sensitive in detection of malaria compared to RDTs and microscopy (Mathison, 2017). Generally, the use of more sensitive diagnostic tools should be considered only in low transmission settings and parasite prevalence rates (e.g. < 10%) where there is already widespread implementation of malaria diagnostic testing and treatment (WHO, 2014b).

2.7 Approaches for enhancing the quality of malaria diagnosis

World Health Organization designed a manual to assist national malaria programmes, reference laboratories and other malaria technical agencies to control quality assurance of malaria microscopy test. The key areas of focus include: assessment of competence of microscopists, setting up of malaria slide bank, proficiency testing (PT), slide validation and outreach training and supportive supervision (WHO, 2015b). The manual recommends among other things, a minimum of 100 fields examination of high-power (x100) magnification of blood smear before the slide is classified as negative. This recommendation has been adopted by the NMCP in Kenya (Division of Malaria Control, 2010). Quality of laboratory confirmatory diagnosis of malaria includes external competence assessment of malaria microscopists (Ohrt *et al.,* 2007). Multi-country external

quality assurance programs are known to strengthen national malaria microscopy standards (Ashraf *et al.,* 2012).

Limitations in microscopy validity and reliability in the diagnosis of malaria, efficacy estimates in malaria drugs and vaccine malaria trials led to the establishment of Malaria Diagnostics Centre in Kisumu to enhance malaria parasite detection. This centre trains in malaria diagnostics, especially expert microscopy (Ohrt, 2007). World Health Organization supports malaria microscopy proficiency testing (PT) to determine the performance of individual laboratory for malaria microscopy. Proficiency testing focuses on *Plasmodium* species identification and distinguishing artifacts from parasites. One or more artifacts, and malaria parasites are sent to participating laboratories for examination and results reported by each laboratory are compared with reference; expert values (WHO, 2015b). This approach is already being implemented through the national malaria reference laboratory (National malaria control programme, 2015).

Microscopists gain enormously through training in Good Laboratory Practice (GLP). The training is intended to promote the quality and validity of test data. It is a managerial concept covering the organizational process and the conditions, under which laboratory studies are planned, performed, monitored, recorded and reported (WHO, 2009). Many laboratories are also enrolled and are implementing SLIPTA. These SLIPTA-empowered laboratories are making marked improvement in accurate and timely diagnosis of disease and patient care, transforming the landscape of health systems, one laboratory at a time (WHO, 2012a).

2.8 Malaria control strategies

There are a number of interventions being implemented across the world. They include Case management (diagnosis and treatment) and prevention which comprised of insecticide-treated nets (ITNs), intermittent preventive treatment of malaria in pregnant women (IPTp), intermittent prevention treatment of malaria in infancy (IPTi) and indoor residual spraying (IRS). In a recent study in Kenya, IPTp and ITN implementation through ANC was ineffective (Dellicour *et al.,* 2016). Malaria epidemiology determines the type of intervention to be implemented in a

given area; hence IPTp is not recommended in low-transmission areas (National malaria control programme, 2015). In Kenya, malaria mosquitoes have already developed some resistance to pyrethroid insecticides used in the two main malaria prevention tools; bed nets and indoor spraying (WHO, 2017b).

Effective treatment is a key malaria control measure (WHO, 2015a). But, the recommended ACT drugs for malaria treatment are facing threat of resistance. Multidrug resistances, which partially include artemisinin resistance and partner drug resistance, have been reported in five countries of the Greater Mekong sub-region. In Africa, artemisinin (partial) resistance has not been reported to date and first-line ACTs remain efficacious in all malaria endemic settings (WHO, 2017b).

2.9 Factors impacting on microscopy test performance and malaria treatment

Refresher training in malaria centers of excellence can improve the capacity of individual microscopists (Obare *et al.,* 2013). However, trained microscopists often face challenges such as heavy workloads and lack of trust in their test results by clinicians upon return to their respective health-facility laboratories (WHO, 2009; Ohrt *et al.,* 2007). Some health workers treat malaria presumptively based on assessment of signs and symptoms, whose success vary depending on the knowledge and practice of the health worker, and by the prevalence of other acute febrile illnesses (WHO, 2010). Ideally, clinicians in Kenya should rely on parasitological diagnosis of malaria before treatment (Division of Malaria Control, 2010).

Documented institutional factors with the potential of significantly impacting on quality of malaria microscopy diagnosis include equipment, laboratory supplies and well-trained technicians (Hailegiorgis *et al.,* 2010).

Working environment of the microscopists, training and supervision have also been shown to impact on the individual microscopists' performance (WHO, 2014a; Zurovac *et al.,* 2006). Public knowledge and understanding affect policy uptake, as evidence, indicates that policy change processes have often been derailed by public suspicions, personal experiences, and lack of trust (Nanyunja *et al.,* 2011).

2.10 Conceptual framework

This represents the flow of events and illustrates that independent individual and institutional factors can impact the accuracy of test result. The extent of the True (positive and negative) and false (positive and negative) test results determines the validity and reliability levels (Fig. 2.1).

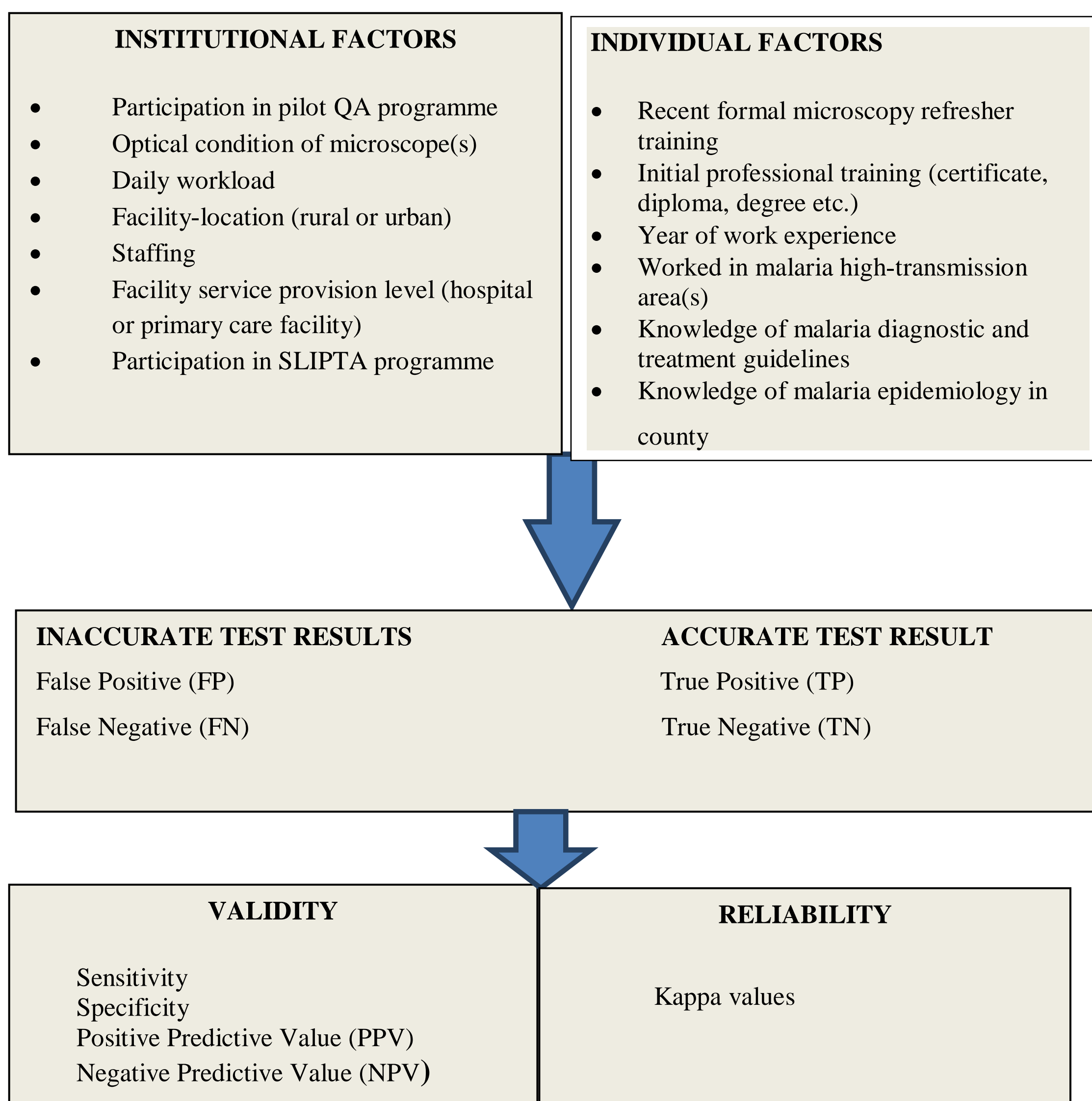

Figure 2.1: Conceptual framework showing independent institutional and individual factors impacting on malaria microscopy test accuracy, validity and reliability in low malaria transmission areas of Kenya, 2014.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

From March to April 2014, a survey was conducted in 42 public-sector health facilities that included 21 pilot-quality assurance (QA) programme facilities and 21 non-QA pilot facilities to identify factors associated with accurate malaria microscopy diagnosis in malaria low-transmission counties in Kenya (Fig. 3.1). The facilities that participated in the pilot QA programme are herein referred to as 'QA-pilot facilities'. Facilities which did not participate are referred to as 'non-QA pilot facilities'. The health facilities were widely distributed in 10 (38%) of 26 low-malaria transmission counties in the Central, Eastern and Rift Valley regions and represented approximately 4% of public-sector health facilities in the 10 counties. The counties that were surveyed are: Murang'a, Kiambu, Machakos, Nakuru, Kericho, Narok, Uasin Gishu, Nyeri, Trans Nzoia and Nandi. Five of the counties were in the highland epidemic zone where malaria transmission is seasonal (Noor *et al.,* 2012). In the 10 counties, community malaria parasitaemia prevalences by microscopy is between 1 and 3% during peak malaria transmission season (National Malaria Control Programme [NMCP], Kenya National Bureau of Statistics [KNBS] and ICF International, 2016). January-February, during which the study malaria thick blood films were archived, was not malaria peak transmission season in Kenya.

Counties in low malaria transmission under our study, herein referred to as surveyed counties are shown in figure 3.1. The various malaria epidemiological zones are shown in Figure 3.2. Normally, parasitaemia prevalences of these epidemiological zones are as follows: Coast endemic zone; 1 %-< 5% with some places having prevalence of up to 40%, Highland epidemic zone; 5 %-< 10%, Lake endemic zone; ≥40%, Low risk zone; <0.1%, Semi-Arid and Seasonal zone; 0.1 %-< 1.0% (Division of Malaria Control, 2014).

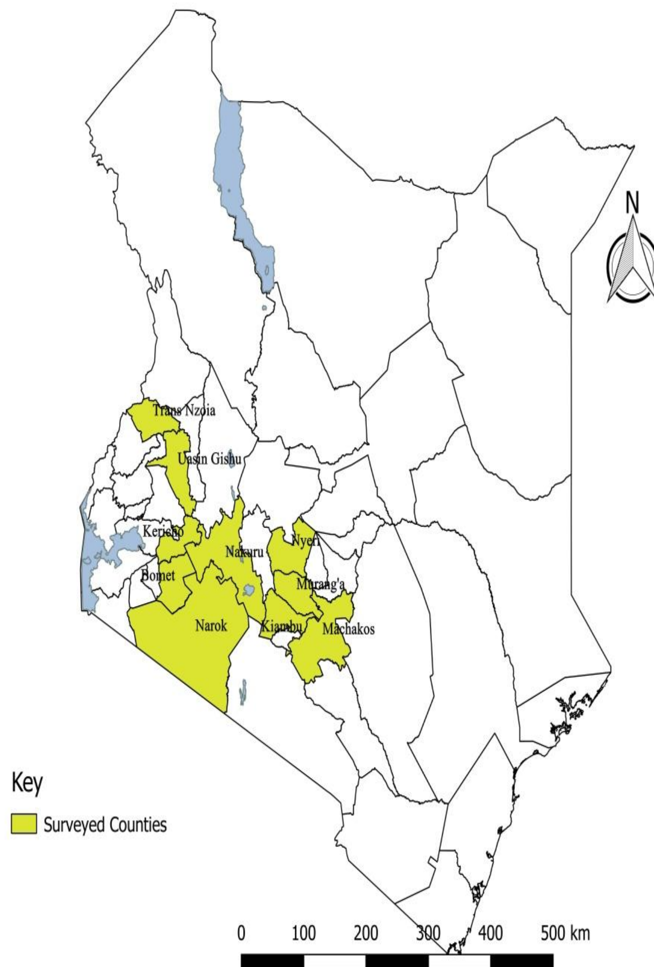

Figure 3.2: Map showing counties in low malaria transmission under our study (surveyed counties), 2014.

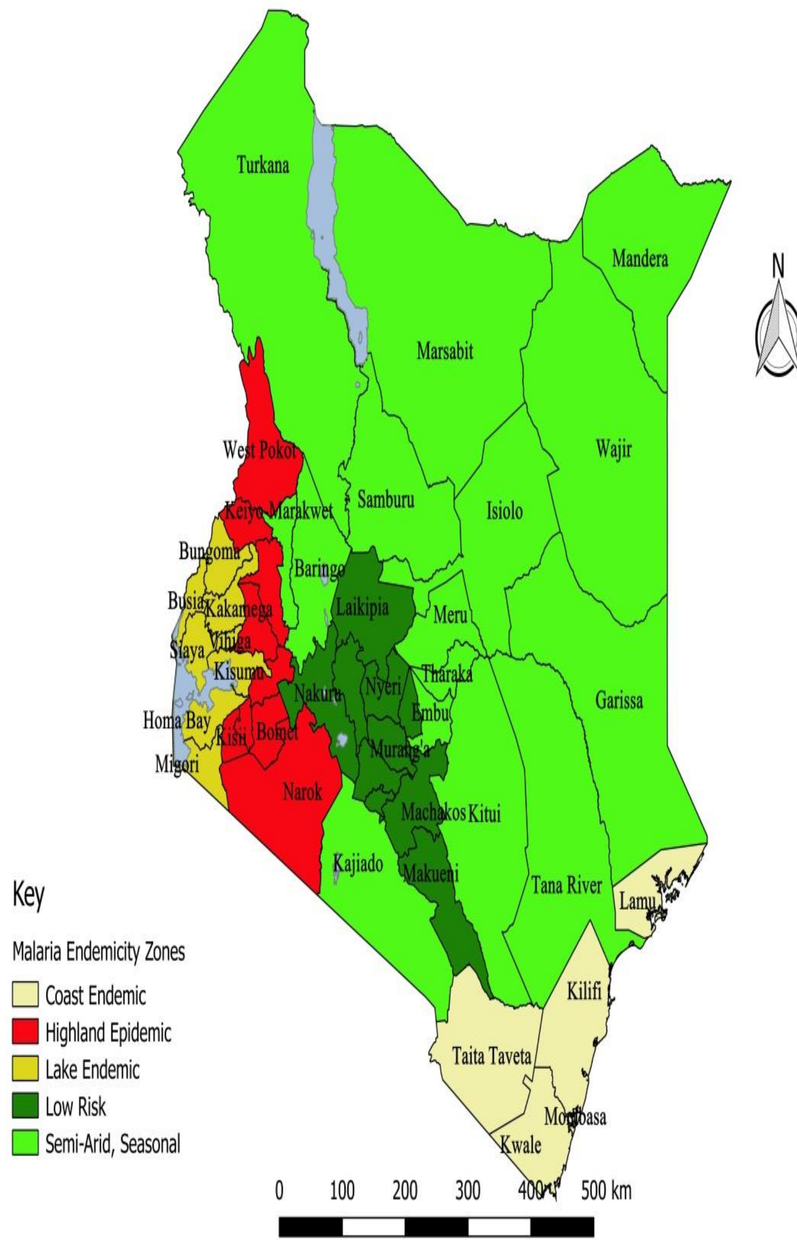

Figure 3.2: Map showing malaria epidemiological zones in Kenya

(Source: The Kenya National Malaria Indicator Survey 2015 report)

3.2 Study design and sampling units

This was a cross-sectional survey. The study sampling units consisted of public-sector health facilities in malaria low-transmission areas. The study health facilities comprised all the service provision levels; dispensary, health centre, primary hospital and secondary or referral hospital. Dispensaries and health centres together comprised primary-care health facilities.

3.3 Ethical consideration

The study was conducted according to the Declaration of Helsinki and International Conference on Harmonization Guideline on Good Clinical Practice (ICH-GCP). The protocol was reviewed and approved by the Jaramogi Oginga Odinga Teaching and Referral Hospital's Ethics and Review Committee (#01713, ref: ERC 1B/VOL.1/70; Appendix III) prior to any protocol-related procedures being conducted (Appendix V and VI). The investigator informed the Ethics and Review Committee (ERC) about the progress of the study on a regular basis per the ERC requirements, but at minimum once a year. Written consents from the hospital management and participating personnel (Appendix I and II) was sought and kept strictly confidential and anonymous. Purpose of the study, participation and withdrawal rights, risks and benefits were explained fully to the participants. Unique codes were assigned to participants, which were used to identify them for purposes of this study. All study records were maintained in a secured location. Participants' information were not obtained or released without written permission from the participant/participant's legally authorized representative except as necessary for conducting this study. Furthermore, the results of this study were presented as a group and no individual participants are identified.

3.3.1 Inclusion and exclusion criteria for health facilities

Public-sector health facilities within the defined malaria low-transmission QA-pilot implementing counties were included. Private and faith-based health facilities were excluded for not being clearly defined by service-provision levels.

3.4 Sample size determination

3.4.1 Determination of number of study health facilities

A convenient sample of 83 health facilities had been selected by the QA-officers to implement the QA-pilot program. Eighty (80) health facilities comprising 45 public-sector health facilities completed the QA-pilot programme during June to December 2013. The following formula (Yamane, 1967; Israel, 1992) was adopted to determine the number of public-sector implementing QA-pilot program to be included in this study:

$$n = \frac{N}{1 + N(e)^2} ,$$

where;

n = Desired sample size (Desired number of health facilities).

N = Population size (Total number of eligible health facilities).

e = Level of precision

N=45, e =0.05

$$n = \frac{45}{1 + 45(0.05)^2} = 40$$

Finite population correction for small sample size:

$$n = \frac{n_0}{1 + \frac{(n_0 - 1)}{N}} ,$$

where: $n_0 = 40$

Therefore, $n = 21$.

The ratio of QA-pilot public-sector laboratories to non-QA pilot laboratories was 1:1 for comparison purposes.

Twenty one public-sector QA-pilot and 21 non-QA pilot clinical laboratories were therefore targeted, making a total of 42 laboratories.

3.4.2 Proportionate sample size of health facilities by service provision levels

To ensure that health facilities across the four service provision levels (secondary or referral hospital, primary hospital, health centre and dispensary) were included in the sample, the number of health facilities selected within a service-level stratum was proportionate to the overall number of facilities within that stratum (probability proportionate to size sampling [PPS]) as follows:

$n_j = n\left(\frac{n_i}{N}\right)$, where;

n_j = Desired number of health facilities in each level.

n = Desired sample size (facilities).

N = Total number of health facilities.

n_i = Total number of facilities in each level.

N = 45, n = 21, n_i: Referral hospital= 1, Primary hospital= 17, Health Centre= 22, Dispensary= 5, n_j =?

n_j: Referral hospital = 1, Primary hospital = 8, Health Centre = 10 and Dispensary = 2.

The ratio in each service provision level between QA-pilot and non-QA pilot was 1:1, i.e. referral hospital: one QA-pilot and one non-QA pilot facility; Primary hospital: Eight QA-pilot and eight non-QA pilot facilities; health centre: Ten QA-pilot and ten non-QA pilot facilities; dispensary: Two QA-pilot and two non-QA pilot facilities.

3.4.3 Determination of number of study malaria slides

The effective sample size used in this survey was computed with the intention of comparing accuracy in diagnosis between the QA-pilot and non-QA pilot arms of the study (Hajian-Tilaki, 2014). Assuming an index of accuracy of 90% based on the area under the curve (AUC) (Kotepui *et al.,* 2015), a 0.05 level of significance and power of 0.80, the sample size required to detect a 5% difference in the accuracy index (AUC) with the non-QA arm was 756.

The sample size formula used was:

$$n = \frac{\left[Z_{\frac{\alpha}{2}}\sqrt{2V_{H0}(\widehat{AUC})} + Z_{\beta}\sqrt{V(\widehat{AUC_1}) + V(\widehat{AUC_2})}\right]^2}{[AUC_1 - AUC_2]^2}$$

Where;

- AUC_1 and AUC_2 are the AUCs for the QA-pilot and non-QA pilot arms of the study, respectively.
- $V(\widehat{AUC_1})$ and $V(\widehat{AUC_2})$ are estimates of the variance of the AUCs for the QA-pilot and non-QA arms of the study, respectively.
- The $V(AUC)$ is obtained from the expression;

$$V(\widehat{AUC}) = \left(0.0099 \times \exp(-a^2)\right) \times (6a^2 + 16),$$

Where;

$a = \Phi^{-1}(AUC) \times 1.414$ and Φ^{-1} is the inverse of standard cumulative normal distribution.

The effective sample size here was:

$$n = \frac{\left[1.96\sqrt{2 \times 0.1227} + 0.8\sqrt{0.1221 + 0.12234}\right]^2}{[0.9 - 0.85]^2} = 756$$

3.4.4 Sampling procedure

3.4.4.1 Health facilities sampling design

The pilot QA programme was implemented in conveniently selected 83 health facilities (45 [54%] public-sector and 38 [46%] private-sector). Facilities were selected to participate in the pilot-QA program based on capacity to perform malaria microscopy and distance from the QA officers' primary duty station (Wanja *et al.,* 2017). For this study, a total of 42 public health facilities were selected to participate in the survey. Twenty-one were part of the pilot QA programme (QA-pilot facilities) from June to December 2013; these facilities were randomly selected from among 45 public-sector pilot QA programme facilities across 4 service-provision levels (i.e., dispensary, health centre, primary hospital, secondary hospital). Twenty-one non-QA pilot public health facilities of the same service-provision level and located in the same county as the QA-pilot facilities, but which did not participate in the QA-pilot programme, were randomly selected to participate in the survey; by simple random sampling technique, using a random number table where the sequence boundary was the number of facilities in specified service-level in each county.

3.4.4.2 Sampling design for malaria thick films

A total sample size of 756 malaria slides was calculated to detect a 5% difference in diagnostic accuracy between the QA-pilot and non-QA pilot facilities, assuming an index of accuracy of 90%, power of 0.80, 0.05 level of significance (Hajian-Tilaki, 2014; Kotepui *et al.,* 2015). All facilities that consented to participate in the survey were provided with slides and requested to label and archive all slides prepared for malaria diagnosis between 1 January and 28 February, 2014. All thick-smear slides prepared for malaria diagnosis with a result recorded in the health-facility laboratory parasitology log-book and archived from 1 January to 28 February, 2014 were eligible for survey inclusion. Eighteen malaria slides were collected by the survey team from each health facility.

From the slide boxes, 9 positive and 9 negative slides were collected per facility via simple random sampling using a random number table where the sequence boundary was the number of slides archived at each facility. Slides which were found unlabelled (i.e., no date, laboratory number, patient age, or sex), stuck together, not entered in the log-book or with results that were not signed by the examining microscopist were excluded. At facilities with fewer than 9 positive slides, all the positive slides were selected and the balance was randomly selected from negative slides to total 18 per facility. The slides were first collected in QA-pilot health facilities in each county and the ratios matched in corresponding non-QA pilot health facilities.

3.5. Data collection

3.5.1 Training data collectors and piloting data collection tools

Data collection team comprised of the principal investigator (PI; the student writing this thesis) and two medical laboratory technologists. Each of these individuals would collect data independently during the actual study. The training of medical laboratory technologists, by the PI, on how to collect data was both theoretical and practical and took two days covering the entire data collection process. Data collectors were trained, and then participated in piloting of the data collection tools. Data collection tools included standardized forms for facility information and slide collection (Appendix IV), structured questionnaires (Appendix V), and worksheet for reporting expert microscopists' results (Appendix VI).

To minimize measurement errors, the questionnaire was piloted in four health facilities that were located in counties that were part of the survey in malaria low-transmission areas. The selected health facilities and group of respondents mirrored the intended respondents in the field. Number of pilot health facilities and respondents were 10% each of the estimated study health facilities and respondents. The research team paid attention and made notes to instances when respondents hesitated to answer or asked for clarifications, as these might have been indications that the question or answers were too vague, difficult to understand or had more than one meaning. After the respondent finished the survey, the researcher would ask one

respondent at a time how they understood each question and response choices by going over the survey again, and for each question, had the respondent explain what they thought they were being asked. They would be asked about the instances when they hesitated or needed clarification. For questions with multiple responses they would be asked if there were any other choices that should have been listed. After piloting the questionnaire on the requisite number of people, debrief with the research team to look for patterns in feedbacks was held. These data was used to revise the questionnaire. The revised questionnaire was field tested before starting actual data collection.

3.5.2 Data for characterizing health facilities and microscopists

Standardized form for laboratory and facility conditions (Appendix IV) and structured questionnaires (Appendix V) were used to collect institutional and individuals' (microscopists') data respectively. Institutional data comprised of participation in QA-pilot programme, health-facility service level, health-facility location (rural or urban), participation in SLIPTA programme, condition of microscope(s), daily workload and staffing. Individuals' data on the other hand included recent microscopy refresher training, initial professional training, years of work experience, work experience in malaria high-transmission areas, knowledge of malaria diagnostics and treatment guidelines, knowledge of malaria epidemiology in county and knowledge of malaria case importation (infection acquired from other counties). Recent training for microscopists was defined as having attended initial or refresher malaria microscopy training within the year prior to the survey.

3.5.3 Data for determining performance measures of malaria microscopy

Thick-blood smear slides were examined for the presence or absence of parasites by expert microscopists who had been certified through the WHO External Competency Assessment for Malaria Microscopy scheme. Two independent expert microscopists cross-checked each of the slides and a third independent expert microscopist was a tie-breaker when the first two expert readers disagreed. The expert microscopist results, or the tie-breaker result when necessary, were considered the reference value. Expert microscopists were masked to both the

health-facility microscopy results and the other expert microscopy results. Expert microscopists examined a minimum of 100 fields of high-power (x100) magnification before the slide was classified as negative per national and WHO guidance (WHO, 2009; Division of Malaria Control, 2013b). Each microscopist read a maximum of 20 slides per day. Accurate malaria diagnosis was defined as concordance in the presence or absence of parasites (i.e., positive or negative) between the health-facility microscopist result and the expert reference result. The health-facility results were compared to expert reference to obtain validity and reliability performance measures.

3.5.4 Data for identifying individuals and institutional factors associated with test performance

Standardized form for laboratory and facility conditions (Appendix IV) and structured questionnaire (Appendix V) were used to collect institutional and individuals data respectively as explained in section 3.5.2 above.

3.6 Data management and analysis

Data was stored in three different locations: Hard disc exclusively used for this purpose kept at the Malaria Diagnostic Centre, personal computer in a controlled folder and in yahoo e-mail account. Access to these data was secured using passwords that were only known to the PI and the Walter Reed Army Research Institute supervisor. The data was however shared with all the university supervisors during handling and analysis.

Research materials included records and thick blood films for malaria parasite detection. All the patient malaria slides collected during the data collection period were stored in the slide boxes provided by the research team. Sampled slides were then transferred in slide boxes to Malaria Diagnostics Center, Walter Reed Army Research Institute, where cross-checking took place.

Following the completion of the study, PI (author of this thesis) and Walter Reed Army Research Institute supervisor were in-charge of the secured storage of the

research materials in environment that enables continued access and ease of retrieval. Access is controlled to prevent unauthorized use, removal or destruction of the materials and unauthorized disclosure of information they contain. Research materials will be destroyed when agreed retention period of three years expires. At the end of the storage period, slide boxes containing the cross-checked blood slides will be placed in a waste bin container (a wide-mouth plastic jar) and the paper records in a another bin container, which will then be incinerated.

To describe health facilities and microscopists, data from the checklist were entered into Microsoft Excel TM 2010 (Microsoft, Seattle, WA, USA) from where counts, proportions, median and range were obtained.

To determine performance of malaria microscopy diagnostic measures, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the health-facility microscopy results were calculated with 95% confidence intervals (CI) using exact method by Graph Pad Prism version 5.01 (Graph Pad Software, La Jolla, CA, USA). Inter-reader agreement for facilities *versus* reference values was expressed as kappa (κ) values with 95% CIs using Graph Pad Prism version 5.01 (Landis & Koch, 1977).

In determining factors associated with accurate malaria microscopy, accurate malaria diagnosis was used as the outcome of interest. Multivariable logistic regression with institutional-specific random effects was performed using Stata version 12 (Stata Corp LP, College Station, TX, USA). Both individual (i.e., recent microscopy refresher training status, level of initial training, years and location of work experience, and malaria knowledge) and institutional-level factors (i.e., participation in pilot QA programme or other external QA programme, condition of microscopes, number of microscopists, service-provision level, location and daily workload) were included as independent variables in the regression model.

CHAPTER FOUR

RESULTS

4.1 Description of health facilities and microscopists in malaria low-transmission areas

All 42 selected heath facilities agreed to participate in the survey. Participating health facilities were located in 10 (38%) of 26 low-malaria transmission counties. Among surveyed facilities, 58% were primary-care facilities (i.e., dispensaries [10%] and health centres [48%]) and 42% were hospitals (i.e., primary [38%] and secondary or referral [4%]) (Table 4.1). More QA-pilot facilities were in urban settings (48% *versus* 19%), participated in an external laboratory-strengthening program (i.e., SLIPTA) (19% *versus* 14%), and had microscopes in good optical condition (95% *versus* 86%) compared to non-QA pilot facilities. The number of microscopists per facility and daily malaria slide workload were similar across surveyed facilities (Table 4.1). Overall, the daily range of malaria slides prepared was 4–28 in QA-pilot and 4–52 in non-QA pilot facilities (Table 4.1). The number of slides collected for the survey represented <5% of all malaria slides archived from 1 January to 28 February, 2014 at each facility.

Table 4.1: Characteristics of surveyed health facilities in malaria low-transmission areas of Kenya, 2014

Characteristic	QA-pilot health facilities (N=21)		Non-QA pilot health facilities (N=21)	
	Number	Percentage	Number	Percentage
Health-facility level				
Primary care facilities	12	58	12	58
Dispensary	2	10	2	10
Health centre	10	48	10	48
Hospitals	9	42	9	42
Primary hospital	8	38	8	38
Secondary or referral hospital	1	4	1	4
Urban location	10	48	4	19
Participates in SLIPTA program	4	19	3	14
Microscope(s) in good optical condition	20	95	18	86
Workload >10 malaria slides per day	14	67	13	70
	Median	**Range**	**Median**	**Range**
Number of microscopists				
Dispensary	1	—	3	2–3
Health centre	2	1–4	2	1–2
Primary hospital	7	5–12	4	2–7
Secondary or referral hospital	7	—	7	—
Malaria slide workload per day				
Dispensary	22	5–28	30	7–52
Health centre	17	5–21	15	4–19
Primary hospital	21	7–28	18	6–52
Secondary or referral hospital	9	4–16	18	7–28

QA quality assurance, *SLIPTA* Stepwise Laboratory Improvement Towards Accreditation, an external laboratory-strengthening program sponsored by World Health Organization

More microscopists in QA-pilot facilities had completed recent refresher training (68% *versus* 29%), had worked in a malaria high-transmission area (63% *versus*21%), and had knowledge of national malaria diagnostic and treatment guidelines (84% *vs* 39%) (Table 4.2).

Table 4.2: Characteristics of surveyed microscopists in malaria low-transmission areas of Kenya, 2014

Characteristic	Microscopists at QA-pilot health facilities (N=56)		Microscopists at non-QA pilot health facilities (N=82)	
	Number	Percentage	Number	Percentage
Individual level				
Training and work experience				
Recent microscopy refresher training	38	68	24	29
More than diploma-level initial training	45	80	59	72
≥5 years of work experience	49	88	66	80
Worked in malaria high-transmission area	35	63	17	21
Knowledge				
Malaria diagnostic and treatment guidelines	47	84	32	39
Malaria epidemiology in county	55	98	70	85
Malaria case importation	55	98	75	91

QA quality assurance; recent training was defined as in the year prior to the survey

4.2 Performance of malaria microscopy diagnosis measures

A total of 756 malaria slides were collected from the health facilities surveyed. Out of these, 204 (27%) slides were read as positive for malaria by health-facility microscopists and 103 (14%) as positive by expert microscopists. Expert readers disagreed on 9 (1%) slides requiring a third tie-breaker. In Figure 4.1, slides are stratified by facility QA-pilot programme participation and recent training status (i.e., formal initial or refresher microscopy training within the year prior to the survey) of the microscopists. More microscopists (68%, 38 of 56) had completed recent refresher training in the QA-pilot facilities compared to non-QA pilot

facilities (29%, 24 of 82) (p <0.01) (Table 4.2). In QA-pilot facilities, recently-trained microscopists read 285 (75%) slides compared to 176 (47%) in the non-QA pilot facilities (p <0.001). Recently-trained microscopists in QA-pilot facilities performed better on all microscopy performance measures with 97% sensitivity and 100% specificity compared to recently-trained microscopists in the non-QA pilot facilities with 69% sensitivity and 98% specificity (p <0.01). Microscopists without recent microscopy refresher training performed the same regardless of facility participation in the QA-pilot programme (Figure 4.1).

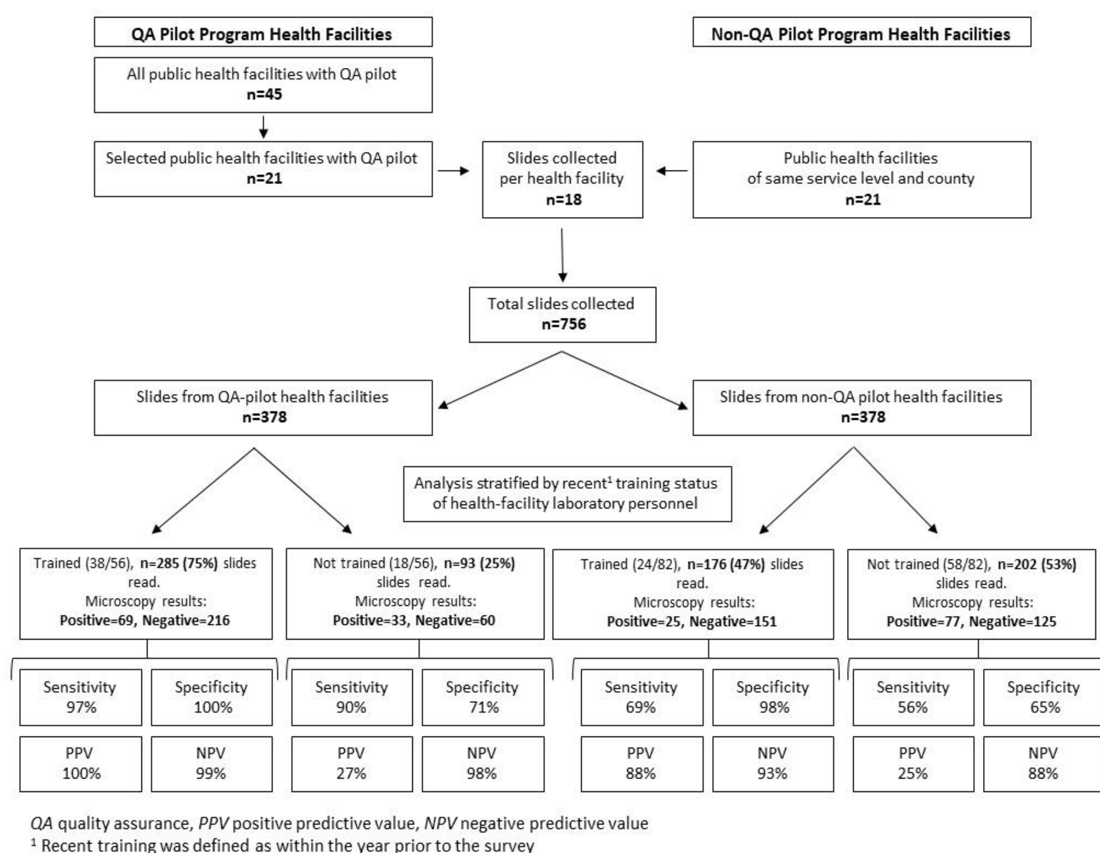

Figure 4.1: Malaria microscopy performance stratified by pilot QA programme participation and recent training status of microscopists in malaria low-transmission areas of Kenya, 2014.

Overall, QA-pilot facilities performed significantly better on measures of diagnostic accuracy (i.e., sensitivity, specificity, PPV and NPV) against expert reference compared to non-QA pilot facilities (Table 4.3). The overall inter-reader agreement between QA-pilot facilities and expert microscopy was $\kappa=0.80$ (95% CI: 0.74-0.88) compared to $\kappa=0.35$ (95% CI: 0.24-0.46) in non-QA pilot facilities ($p < 0.001$). When the diagnostic performance measures were stratified by service level; only primary hospitals participating in the pilot QA programme performed statistically better on all diagnostic accuracy measures compared to non-QA pilot facilities (Table 4.3). In total, 351 (93%) slide results were read as concordant with expert reference from QA-pilot facilities compared to 292 (77%) in the non-QA pilot facilities ($p < 0.001$) (Figure 4.1).

Table 4.3: Measures of malaria microscopy performance in surveyed health facilities in malaria low-transmission areas of Kenya, 2014

	Quality-assurance pilot Programme	Number of slides	Sensitivity		Specificity		Positive predictive value		Negative predictive value		Kappa value	
			%	95% CI	%	95% CI	%	95% CI	%	95% CI	κ	95% CI
Overall	Yes	378	96	(90–99)	92	(88–95)	76	(67–84)	99	(97–99)	0.8	(0.74–0.88)
	No	378	62	(49–74)	80	(76–85)	40	(31–50)	91	(87–94)	0.35	(0.24–0.46)
Dispensary	Yes	36	100	(59–100)	100	(88–100)	100	(59–100)	100	(88–100)	1	—
	No	36	62	(24–91)	54	(34–72)	28	(10–53)	83	(59–96)	0.11	(-0.16–0.38)
Health centre	Yes	180	88	(69–97)	90	(85–94)	60	(42–75)	98	(94–100)	0.65	(0.51–0.79)
	No	180	59	(39–76)	94	(89–97)	65	(44–84)	92	(87–96)	0.55	(0.38–0.72)
Primary hospital	Yes	144	100	(92–100)	93	(85–97)	87	(75–95)	100	(96–100)	0.89	(0.82–0.97)
	No	144	62	(41–80)	69	(59–77)	30	(18–44)	89	(81–95)	0.22	(0.06–0.37)
Secondary hospital	Yes	18	100	(40–100)	93	(66–100)	80	(28–99)	100	(75–100)	0.85	(0.58–1.00)
	No	18	100	(29–100)	87	(60–98)	60	(15–95)	100	(75–100)	0.68	(0.29–1.00)

CI Confidence interval, *italic* denotes statistical significance

4.4 Individual and institutional characteristics associated with accurate malaria microscopy diagnosis

In unadjusted logistic regression analysis shown in table 4.4, all the microscopist characteristics were positively associated with accurate malaria diagnosis except initial level of training. Only pilot QA programme participation and good optical condition of microscopes were institutional factors associated with accurate malaria diagnosis. In adjusted multivariable logistic regression analysis, recent microscopy refresher training (prevalence ratio [PR] =13.8; 95% CI: 4.6-41.4), ≥5 years of work experience (PR=3.8; 95% CI: 1.5-9.9), and pilot QA programme participation (PR=4.3; 95% CI: 1.0-11.0) were the only factors significantly associated with accurate malaria diagnosis (Table 4.4).

Table 4.4: Individual and institutional characteristics associated with accurate malaria microscopy diagnosis in surveyed health facilities in malaria low-transmission areas of Kenya, 2014

				Unadjusted		Adjusted	
Characteristic	**Response**	**Slides n (%)**	**Accurate diagnosis n (%)**	**Prevalence ratio**	**95% Confidence interval**	**Prevalence ratio**	**95% Confidence interval**
Individual							
Recent microscopy refresher training	No	295 (39.0)	197 (66.8)	1.00 (Ref)		1.00 (Ref)	
	Yes	461 (61.0)	446 (96.7)	*40.5*	*15.1–108.6*	*13.8*	*4.6–41.4*
More than diploma-level initial training	No	141 (18.7)	109 (77.3)	1.00 (Ref)			
	Yes	615 (81.3)	534 (86.8)	2.1	0.9–4.6		
≥5 years of work experience	No	137 (18.1)	78 (56.9)	1.00 (Ref)		1.00 (Ref)	
	Yes	619 (81.9)	565 (91.3)	*23.7*	*9.7–57.6*	*3.8*	*1.5–9.9*
Worked in malaria high-transmission area	No	383 (50.7)	290 (75.7)	1.00 (Ref)			
	Yes	373 (49.3)	353 (94.6)	12.1	5.2–28.3		
Knowledge of malaria diagnostic and treatment guidelines	No	194 (25.7)	119 (61.3)	1.00 (Ref)			
	Yes	562 (74.3)	524 (93.2)	25.6	10.4–62.9		
Knowledge of malaria epidemiology in county	No	94 (12.4)	45 (47.9)	1.00 (Ref)			
	Yes	662 (87.6)	598 (90.3)	22.8	8.7–60.3		
Knowledge of malaria cases	No	63 (8.3)	20 (31.7)	1.00 (Ref)			

importation	Yes	693 (91.7)	623 (89.9)	21.1	7.2–62.1		
Institutional							
Quality-assurance pilot programme	No	378 (50.0)	292 (77.2)	1.00 (Ref)		1.00 (Ref)	
	Yes	378 (50.0)	351 (92.8)	*6.0*	*1.9–18.9*	*4.3*	*1.0–11.0*
Good optical condition of microscope(s)	No	72 (9.5)	46 (63.9)	1.00 (Ref)			
	Yes	684 (90.5)	597 (87.3)	7.6	1.1–51.4		
Rural location	No	505 (66.8)	415 (82.2)	1.00 (Ref)			
	Yes	251 (33.2)	228 (90.8)	2.7	0.7–10.1		
Participation in SLIPTA program	No	647 (85.6)	539 (83.3)	1.00 (Ref)			
	Yes	109 (14.4)	104 (95.4)	4.8	0.8–28.8		
>3 laboratory staff	No	361 (47.8)	308 (85.3)	1.00 (Ref)			
	Yes	395 (52.2)	335 (84.8)	1.1	0.3–3.3		
Hospital-level facility	No	432 (57.1)	376 (87.0)	1.00 (Ref)			
	Yes	324 (42.9)	267 (82.4)	0.8	0.2–2.5		
Workload >10 slides per day	No	269 (35.6)	239 (88.8)	1.00 (Ref)			
	Yes	487 (64.4)	404 (83.0)	0.6	0.2–2.1		

SLIPTA Stepwise Laboratory Improvement Towards Accreditation, sponsored by World Health Organization; *Ref* reference; *italic* denotes statistical significance; recent training was defined as in the year prior to the survey

CHAPTER FIVE

DISCUSSION, CONCLUSION, RECOMMENDATIONS

5.1 Characteristics of health facilities and microscopists in malaria low-transmission areas

We established that the surveyed health facilities had laboratory human resource and equipment challenges, although at individual level, substantial number of microscopists had considerable experience and training. There was inadequate number of laboratory staff across all service provision levels in the surveyed areas (National Public Health Laboratory Services, 2006; Netherlands enterprise Agency, 2016). Despite this, a surplus of trained laboratory technicians and technologists has been reported in Kenya (World Bank, 2009). First step to addressing laboratory staffing challenge in Kenya may be to focus on health systems financing and laboratory personnel recruitment to bridge the gap and ensure adequate staffing at all health care service-provision level. The laboratory human resource problem is however not unique to Kenya, since its experienced across sub-Saharan Africa (Zurovac *et al.,* 2006; WHO, 2009; Hailegiorgis *et al*., 2010; WHO, 2014a).

Laboratory staff with sound background in malaria microscopy is an extremely important resource in the accurate diagnosis of malaria (Payne, 1988). Hence, this survey assessed a number of individual level characteristics necessary in the diagnosis of malaria. It was found that a substantial number of microscopists had ≥5 years of work experience. This level of experience is beneficial in diagnostic and testing services (Obare *et al.,* 2013). Additionally, the study demonstrated that most of the microscopists had higher than diploma (≥degree) level initial training. However, knowledge and skill gaps necessary for effective diagnosis of diseases have been reported among new pre-service trained laboratory staff in Kenya (World Bank, 2009). Initial training alone may, therefore, not be sufficient individual-level factor in achieving accurate diagnosis of malaria. Many microscopists in the non-QA pilot facilities in the surveyed areas had not recently undertaken microscopy refresher training, which should be supplemental to the initial training. More

microscopist in the QA pilot programme had recent microscopy refresher training and had worked in malaria high-transmission areas, which could have given them the advantage they had in performance measures over their counterparts in the non-QA facilities since parasite identification is a skill learned over time and though practice (Obare *et al.,* 2013). The microscopists' knowledge on malaria case importation and malaria epidemiology in county was largely similar between microscopists in the QA pilot and non-QA pilot facilities. The QA pilot microscopists however had a huge advantage in knowledge on malaria diagnostic and treatment guidelines, which might have sensitized them to perform better (Mayo, 1933; Roethlisberger & Dickson, 1939).

Hospitals require expert microscopists for the management of complicated patients with severe malaria and co-morbidities. Expert microscopy is the gold standard for identifying mixed infections and treatment failures, and quantifying parasite density (WHO, 2009; Division of Malaria Control, 2013b; WHO, 2015b). Hospitals generally have more substantial laboratories and resources available to maintain at least adequate, if not expert, diagnostic microscopy programmes compared to out-patient health centres and dispensaries (Netherlands enterprise Agency, 2016). In contrast, in this survey, hospitals performed no better than the primary care facilities in test accuracy. Out-patient health centres and dispensaries generally have high patient workloads, which makes labour-intensive diagnostics, such as malaria microscopy, challenging. Despite having the high workloads for malaria, dispensaries had the fewest staff. Moreover, staff in dispensaries in Kenya generally have the minimum level of pre-service and least access to refresher training (National Public Health Laboratory Services, 2006).

Historically in Kenya, programmes and training cascaded from the highest service-provision levels to the lowest and often did not reach dispensaries due to limited resources and lower prioritization. In 2010, Kenya prioritized dispensaries to receive malaria RDTs for parasitological diagnosis, since expert microscopy services were not expected at this level (Division of Malaria Control, 2013a). Dispensaries do not provide in-patient services and refer patients with severe malaria (Division of

Malaria Control, 2010; National Public Health Laboratory Services, 2006). However, due to convenient sampling, two surveyed QA-pilot and non-QA pilot facilities with microscopy were dispensaries.

The challenge of proper equipment, particularly the condition of microscopes used for malaria microscopy, is not unique to malaria low-transmission areas of Kenya (Hailegiorgis *et al.,* 2010). Ideally all equipment, more so microscopes used for the diagnosis of malaria require thorough evaluation to ensure the whole unit, including the optics or lenses are in good working conditions. It is known that poor optical condition of a microscope could lead to unreliable diagnosis of malaria (WHO, 2009). It was therefore least expected, that some surveyed health facilities would be participating in malaria diagnosis using microscopes with poor optical conditions.

Parasitaemia prevalence of malaria is known to differ between urban and rural settings. Urban areas generally have a substantially lower parasitemia prevalence compared to rural areas and a greater percentage of QA-pilot facilities were located in urban areas (Landis & Koch, 1977; Kotepui *et al.,* 2015;). Therefore, it's possible that persons who presented to QA-pilot facilities in urban areas would have had lower parasite densities overall, underestimating the test accuracy association with participation in pilot-QA.

The WHO SLIPTA was being implemented independently across the country during this survey. It has broader scope than the malaria diagnostics QA programme. The SLIPTA frameworks was established to generally improve the quality of disease diagnosis in public health laboratories in developing countries through standardized processes that would help them meet international accreditation (WHO, 2012a). Evidently, the international accreditation by the surveyed health facilities is still far from being achieved, since very few of even the hospital-level facilities had been enrolled.

5.2 Performance of malaria microscopy diagnostic measures in malaria low-transmission areas

QA-pilot facilities out-performed the non-QA pilot facilities in all the validity and reliability measures. Dispensaries, followed by the primary hospitals, had the best overall performance. The largest overall performance differences measured at the dispensaries level might have been due to having only one microscopist at this service-provision level and subsequently more of the QA officer's time and attention. Overall in QA-pilot facilities, inter-reader agreement measure of reliability was substantial to almost perfect at kappa, $\kappa=0.8$ compared to fair at $\kappa=0.35$ in non-QA facilities (Landis & Koch, 1977). Likewise, the sensitivity, specificity and Negative Predictive Value (NPV) were very high at over 90% in QA-pilot facilities. These findings are consistent with a recent study in the same area (Wanja *et al.,* 2017).

The Positive Predictive Value (PPV) was much lower, but lower PPVs and higher NPVs would be expected because all the surveyed facilities were located in malaria low-transmission counties. Predictive values are dependent on the prevalence of the disease in the population, with PPV increasing and NPV decreasing with increase in prevalence. These counties have community malaria parasitaemia prevalences by microscopy of between 1 and 3% during peak malaria transmission season (National Malaria Control Programme [NMCP], Kenya National Bureau of Statistics [KNBS] and ICF International, 2016). Positive and negative predictive values obtained in this study were comparable to those obtained in a recent study in Tanzania (Allen *et al.,* 2013). A 2014 national health-facility survey for malaria infection found that 3.4% of outpatients who reported a history of fever within the last 48 hours had positive malaria RDT in seasonal low-transmission counties in Kenya (Githinji *et al.,* 2016). Since the survey was designed to assess malaria microscopy performance during low malaria transmission, and routine health data was indicating over diagnosis of malaria in low transmission areas (Division of Malaria Control, 2013b; Ministry of Health, 2015), malaria slides were collected in January and February, which is not the peak malaria transmission season in Kenya (Wanja *et al.,* 2017). Therefore, most

persons presenting to health facilities, even if febrile, were unlikely to have malaria at the time of the survey.

In malaria low-transmission settings, the low PPV findings translate into a large number of false-positive results. Persons misdiagnosed as having malaria when they do not are at risk of not being treated for their actual illness, which can lead to increased morbidity and potentially mortality. In addition, treating people who do not have malaria with the relatively expensive artemisinin-based combination therapy wastes limited resources and could contribute to the development of artemisinin resistance (WHO, 2009; WHO, 2010; Division of Malaria Control, 2010; WHO, 2015b). Improved performance in malaria microscopy may increase clinicians' confidence in malaria microscopy test and refine their suspicion indices in considering other causes of fever and disease (Zurovac *et al.*, 2006; Ohrt *et al.,* 2007).

5.3 Individual and institutional factors associated with accuracy of malaria microscopy diagnosis in malaria low-transmission areas

This observational study demonstrated that diagnostic accuracy of malaria microscopy was positively associated with recent microscopy refresher training and ≥5 years of experience for microscopists and health facility participation in the pilot QA programme. The findings are consistent with other studies from Kenya and elsewhere that have shown both laboratory QA programmes and microscopy refresher trainings improve malaria microscopy performance (Ohrt *et al.,* 2007; Ngasala *et al.,* 2008; Kiggundu *et al.,* 2011; Obare *et al.,* 2013; Wafula *et al.,* 2014). In 2013, the NMCP independently started both formal refresher trainings for microscopists at a malaria microscopy centre of excellence and the pilot QA programme for malaria diagnostics at 83 health facilities; both the refresher trainings and pilot QA programme were intended to improve malaria diagnosis by microscopy. However, implementation of the two diagnostic strengthening components was not coordinated or systematic in health facilities, across service-

provision levels or administrative zones, which hindered independent evaluation of the pilot QA programme.

Refresher trainings in malaria microscopy is known to improve malaria diagnosis through its key components such as quality of thick and thin smear preparation and parasite detection (Kiggundu *et al.,* 2011; Leslie *et al.,* 2012; Nateghpour *et al.,* 2012; Moura *et al.,* 2014). In this survey, recent microscopy refresher training at the individual level was more strongly associated with accurate malaria diagnosis than health facility participation in the pilot QA programme. However, microscopists who had recently completed refresher training and worked in a facility that was part of the pilot QA programme had the best performance for all measures of diagnostic accuracy. These findings suggest that synergies exist between formal microscopy refresher training and the pilot QA programme. Implementation of both diagnostic strengthening components together appears to produce the best performance results.

Malaria microscopy refresher training for individuals was an important confounder in this study. The study was powered to detect differences at the health-facility level rather than at the individual microscopist level, and malaria microscopy refresher training was not uniform across surveyed facilities. Twice as many microscopists from QA-pilot facilities had recent refresher training compared to non-QA pilot facilities. Three-quarters of the malaria slides from QA-pilot facilities were read by microscopists who had recently completed malaria microscopy refresher training compared to less than half of the slides from non-QA pilot facilities. In addition, there were other general laboratory-strengthening activities ongoing, such as SLIPTA, in a minority of facilities that were included in the survey. Although participation in the WHO SLIPTA programme was not significantly associated with accurate malaria microscopy diagnosis, the programme might have contributed to overall laboratory improvements that were not specifically measured in this study (WHO, 2012a).

5.4 Limitations of the study

This study had a number of limitations as highlighted below. Although health facilities were randomly selected for the survey, the facilities selected to participate in the pilot QA programme were a convenience sample. Thus, the surveyed facilities are not representative of all public health facilities in Kenya, which limits the generalizability of the findings.

A baseline evaluation of microscopy performance was not conducted prior to the start of the refresher trainings or the pilot QA programme. Microscopists and facilities selected for participation in the diagnostic strengthening components might have performed better at baseline compared to those not selected. Therefore, the association between microscopy performance and refresher training and the pilot QA programme might have been overestimated. Additionally, when health facilities consented to participate in the survey, they were asked to store slides during a specific time interval for later retrieval. Facilities might have preferentially stored slides for which they felt confident about the results, and microscopists might have performed better during this period because they were aware of the survey i.e., Hawthorne effect (Mayo, 1933; Roethlisberger & Dickson, 1939). Both situations would have resulted in an overestimation of diagnostic accuracy, but the potential bias should be non-differential across all facilities.

Another important limitation was that slide preparation quality, including the stain type and adequacy, was not evaluated. Although both NMCP and WHO recommend Giemsa preferentially for malaria microscopy, the use of both Giemsa and Field stains was common in health facilities (WHO, 2009; Division of Malaria Control, 2013b; Wanja *et al.,* 2017). Slides were not matched on parasite density either. Thick films were examined for the presence or absence of parasites; no thin films were examined for parasite density or speciation (WHO, 2009; Division of Malaria Control, 2013b). Slides from QA-pilot facilities might have had higher parasite densities, which would make malaria easier to identify correctly. However, urban areas generally have a substantially lower parasitaemia prevalence compared to rural

areas and a greater percentage of QA-pilot facilities were located in urban areas (Landis & Koch, 1977; Kotepui *et al.,* 2015). Therefore, it is possible that persons who presented to QA-pilot facilities in urban areas would have had lower parasite densities overall; if this represented the true situation, then QA-pilot enrolled facilities would have performed better than estimated (underestimation of association) compared to non-QA pilot facilities.

5.5 Conclusion

Based on the findings of this study the following conclusions can be drawn:

1. Surveyed health facilities had laboratory human resource and equipment challenges, although at individual level, substantial number of microscopists had ≥5 years of work experience and more than diploma initial training. Unlike in the non-QA pilot facilities, most microscopists in the QA-pilot facilities had completed recent refresher training, had worked in a malaria high-transmission area and had knowledge of national malaria diagnostic and treatment guidelines. Likewise, most malaria slides in the QA-pilot health facilities were examined by microscopists with recent refresher training.
2. QA-pilot facilities out-performed the non-QA pilot facilities in all the validity and reliability measures. In our survey, dispensaries were the best overall performers, followed by the primary hospitals.
3. Diagnostic accuracy of malaria microscopy was positively associated with recent microscopy refresher training and ≥5 years of experience for microscopists at the individual level and pilot QA programme participation at the health facility level. Microscopists who had recently completed refresher training and worked in a facility that was part of the pilot QA programme had the best performance for all measures of diagnostic accuracy.

5.6 Recommendations

1. Kenya could benefit from a clear strategy to optimize laboratory human resource and equipment policy. This should focus on adequate staffing of health facilities, proper allocation of duties to ensure only competent staff perform malaria microscopy, continuing professional development (CPD) and service contracting for microscope maintenance.

2. The QA programme once implemented needs to be evaluated regularly to consolidate best practices and curb discrepancies between implementing facilities. Incorporation of PT panels of malaria slides into the QA programme should be considered to enable the implementation of targeted comprehensive individual and institutional corrective actions.

3. The NMCP should consider systematically implementing formal microscopy refresher training and the QA programme together as a package of interventions to improve parasitological diagnosis of malaria by microscopy in Kenya.

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APPENDICES

Appendix I: Informed Consent for the hospital management

Informed Consent for the Hospital Management	
Title of the study	Factors associated with Malaria Microscopy Diagnostic Performance following a Pilot Quality-Assurance Programme in Health Facilities in Malaria Low-Transmission Areas of Kenya
Researchers's name	
Phone number	
e-mail	
Introduction	
I am a post graduate student at Jomo Kenyatta University of Agriculture and Technology. As part of my masters thesis, I am conducting research under the supervision of **Dr. Jesca Okwara Wesongah** and **Dr. Elizabeth Wanja**. I invite your institution to take part in my study. Taking part in this study is voluntary . We'll share the findings of this study with you and you may benefit from the recommendations made. Information might also be gained that will benefit other institutions like yours in the long run. You may withdraw from the study at any time. My performance evaluation as a student will not be affected by your desire not to participate. The study is described below. This description tells you about the risks,	

inconveniences or discomfort that you may experience. You should discuss any questions you have about this study with the people who explain it to you.
Purpose of the study
The purpose of this study is to find out if there are any factors associated with malaria microscopy diagnostic performance in this health facility and others in similar malaria epidemiological zones.**This study involves asking your laboratory personnel questions relatedto malaria diagnosis and treatment. It also involves rechecking of malaria slides from this facility by experts to establish test accuracy and abstracting data from laboratory registers.**
Participation and withdrawal
Your participation is completely voluntary.**You may withdraw from this study at any time without penalty.** An estimated 5 members of laboratory staff will participate in this study. The duration of this study will be 01 March-30 April 2014. If you agree to participate in this study, your involvement will last for 10minutes.
Risks and benefits
For your participation in this study, you will not receive any formal compensation. **You will not suffer any risk participating in this study. We'll share the findings of this study with you and you may benefit from the recommendations made. Information might also be gained that will benefit other institutions like yours in the long run**; this study might be used by the ministry of health to strengthen national laboratory systems and to improve malaria case management.

Confidentiality
All information obtained in this study will be kept strictly **confidential and anonymous**. All softcopy information will be directed to a personal computer that is password protected; hard copies will be sealed in envelopes and securely locked up in secured location with limited access. **Participants' information will not be obtained or released without written permission from the participant/participant's legally authorized representative except as necessary for conducting this study**. Participants identifying information will not be put on any of the forms. To further protect individual identities of participants, their consent forms will be sealed in an envelope and stored separately. Furthermore, the results of this study will be presented as a group and no individual participants will be identified.
If you have any questions, please contact the student researcher (**Mr. Fredrick Odhiambo at**   **+254722390020, odhiambof@yahoo.com**) or the university supervisor (**Dr. Jesca Okwara Wesongah at +254723958983**)
Ethics review
This research has been reviewed and approved by the JOOTRH Ethics Review Committee. If you have any questions or concerns about the study, you may contact myself-------------------------------- (Telephone no. **+254----------------)** or the Secretary, JOOTRH (+254720766550)
By signing this consent form, you are indicating that you fully understand the

above information and agree that your institution participates in this study	
Respondent's full name:	
Position in the institution:	
Telephone number: +254	
E-mail address:	
Signature:	**Date**

Appendix II: Informed consent for the participating microscopists

Informed Consent for Participating Microscopists **(To be administered after all the data has been abstracted from records and requisite blood slides have been collected)**	
Title of the study	Factors associated with Malaria Microscopy Diagnostic Performance following a Pilot Quality-Assurance Programme in Health Facilities in Malaria Low-Transmission Areas of Kenya
Researchers's name	
Phone number	
e-mail	
Introduction	
I am a post graduate student at Jomo Kenyatta University of Agriculture and Technology. As part of my masters thesis, I am conducting research under the supervision of **Dr. Jesca Okwara Wesongah** and **Dr. Elizabeth Wanja**. I invite you to take part in my study. Taking part in this study is voluntary . Your terms of employment will not be affected by whether you participate or not. We'll share the findings of this study with your institution and you may benefit from the recommendations made. Information might also be gained that will benefit other institutions like yours in the long run. You may withdraw from the study at any time	

without affecting your terms of employment. My performance evaluation as a student will not be affected by your desire not to participate. The study is described below. This description tells you about the risks, inconveniences or discomfort that you may experience. You should discuss any questions you have about this study with the people who explain it to you.
Purpose of the study
The purpose of this study is to find out if there are any factors associated with malaria microscopy diagnostic performance in this health facility and others in similar malaria epidemiological zones.**This study involves asking youquestions related to malaria diagnosis and treatment. It also involves rechecking of malaria slides from this facility by experts to establish test accuracy and abstracting data from laboratory registers.**
Participation and withdrawal
You have the opportunity to participate in this study because you have been involved in diagnosis of malaria.Your participation is completely voluntary.**You may withdraw from this study at any time without penalty.** An estimated 756 patients and 100 members of laboratory staff will participate in this study. The duration of this study will be 01 March-30 April 2014. If you agree to participate in this study, your involvement will last for 8 minutes.
Risks and benefits
For your participation in this study, you will not receive any formal compensation. **You will not suffer any risk participating in this study. We'll share the findings**

of this study with your institution and you may benefit from the recommendations made. Information might also be gained that will benefit other institutions like yours in the long run; this study may be used by ministry of health to strengthen national laboratory systems and to improve malaria case management.
Confidentiality
All information obtained in this study will be kept strictly **confidential and anonymous**. All softcopy information will be directed to a personal computer that is password protected; hard copies will be sealed in envelopes and securely locked up in secured location with limited access. **Your information will not be obtained or released without written permission from yourself/your legally authorized representative except as necessary for conducting the study**. Please do not put any of your identifying information on any of the forms. To further protect individual identities, this consent form will be sealed in an envelope and stored separately. Furthermore, the results of this study will be presented as a group and no individual participants will be identified.
If you have any questions, please contact the student researcher (**Mr. Fredrick Odhiambo at +254722390020, odhiambof@yahoo.com**) or the university supervisor (**Dr. Jesca Okwara Wesongah at +254723958983**)
Ethics review
This research has been reviewed and approved by the JOOTRH Ethics Review Committee. If you have any questions or concerns about the study, you may contact myself-------------------------------- (Telephone no. +**254----------------)** or the

<table>
<tr><td colspan="2">Secretary, JOOTRH (+254720766550)</td></tr>
<tr><td colspan="2">By signing this consent form, you are indicating that you fully understand the above information and agree that your institution participates in this study</td></tr>
<tr><td>Participant’s signature</td><td>Date</td></tr>
<tr><td>Researcher’s signature</td><td>Date</td></tr>
</table>

Appendix III: Research Authorization Letter

MINISTRY OF HEALTH

Telegrams: "MEDICAL", Kisumu
Telephone: 057-2020801/2020803/2020321
Fax: 057-2024337
E-mail: ercjootrh@gmail.com
When replying please quote

JARAMOGI OGINGA ODINGA TEACHING & REFERRAL HOSPITAL
P.O. BOX 849
KISUMU

Ref: ERC 1B/VOL.1/70

Date: 14th November, 2013

Fredrick Odhiambo,
JKUAT.

FORMAL APPROVAL TO CONDUCT RESEARCH TITLED:
"COMPARISON OF MALARIA MICOSCOPY RESULTS BETWEEN PUBLIC HEALTH FACILITIES AND WITHOUT A FUNCTIONAL QUALITY ASSURANCE SYSTEM IN KENYA, AND ELUCIDATION OF FACTORS ASSOCIATED WITH PROFICIENCY LEVELS."

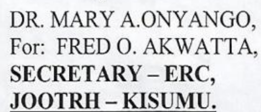
The JOOTRH ERC (ACCREDITATION NO. 01713) has reviewed your protocol and found it ethically satisfactory. You are, therefore, permitted to commence your study immediately. Note that this approval is granted for a period of one year (14th November, 2013 to 14th November 2014). If it is necessary to proceed with this research beyond the approved period, you will be required to apply for further extension.

Also note that you will be required to notify the committee of any protocol amendment(s), serious or unexpected outcomes related to the conduct of the study or termination for any reason.

Finally, note that you will also be required to share the findings of the study in both hard and soft copies upon completion.

The JOOTRH ERC takes this opportunity to thank you for choosing this institution and wishes you the best in your endeavours.

Yours sincerely,



DR. MARY A.ONYANGO,
For: FRED O. AKWATTA,
SECRETARY – ERC,
JOOTRH – KISUMU.

/bao

Appendix IV: Standardized form for facility information and slide collections

1. **HEALTH FACILITY INFORMATION**

Health facility identification number (*use the assigned unique code*) **--------------------------------------**

Health facility service level *(observe the service charter and confirm with management: 2, 3, 4, 5)* **------**

Address: P.O.Box --

***E-mail*--**

QA-pilot implementation status *(Yes/No)* **--**

Region *(Central, Eastern, Rift Valley)* **---**

***County*---**

Epidemiological zone*---*Location *(Urban/Rural)* **----------------------**

Head of health facility (*name facility in-charge or medical superintendent*) **--------------------------------**

E-mail **--Tel ---**

Head of Laboratory ---

E-mail **--Tel ---SLAMTA/SLIPTA/ WHO-AFRO Registration** (*Yes/No*) ------ Other accreditation body (*specify*) -----

Number of practicing Lab. techs in the health facility laboratory---

Date of visit/ data collection ---

Data collected by --

2. DOCUMENTS AND RECORDS

Confirm availability of the following:

Quality essentials	Y	P	N
1. Malaria blood film (MBF) sample collection SOP present.			
2. MBF preparation SOP present.			
3. MBF reading and quantification SOP present.			
4. MBF staining SOP present.			
5. Species reference charts (micrographs).			
6. Staining procedure job aid.			
7. Parasite quantification job aid.			
8. Giemsa stain preparation SOP present.			
9. PH 7.0-7.2 buffer preparation SOP present.			
10. Internal quality control for stains documented.			
11. Microscope maintenance logs complete.			
12. Test results logbook complete.			
13. Occurrence book available and complete.			
14. Slide easily traceable			

3. SLIDE INFORMATION

1. Microscope brand in-use--- -----------------------------

i. Optical condition of microscope in-use (*team to examine high-power magnification fields of stained malaria slide sample to confirm: Good=1, Bad=2*) ---

2. Turnaround time (*as stated in the Public service charter*) ------------------------- ----------------------------

3. Average workload (*total for Jan & Feb 2014 divided by total number of working days*) ---------/day

Slide collection form:

Slide no.	**Date exam**	**Health facility microscopist's result** **(positive, Negative)**	**Assigned code for microscopist/slide examiner (*use the assigned unique code*)**

Appendix V: Questionnaire for microscopist level information

Health facility identification number (*use the assigned unique code*) **-----------**

Microscopist ID (*use the assigned unique code in the slide collection form)* **------**

Age *(yy)* --

Sex *(M/F)* **---**

Date of interview--

Interviewer's name ---

Education level

1. Are you professionally trained in medical laboratory science (*Yes/No*)? -----------
----
   i. If *YES,* what was your initial training (Tick all that applies to you)?
   a. Certificate--------- ☐ -----------Date of completion (*mm/yy*)--------------------
   ----------- ☐
   b. Diploma-----------------------------Date of completion (*mm/yy*) -------------------
   ---------- ☐
   c. Higher Diploma------------------Date of completion (*mm/yy*) --------------------
   ---------- ☐
   d. Degree -----------------------------Date of completion (*mm/yy*) -------------------
   ----------- ☐
   e. Others (Specify)--- --------------Date of completion (*mm/yy*) -----------------
   ----

Work experience

1. When were you first employed to practice as a medical laboratory scientist (*mm/yy*)? -------
2. In your opinion, is it possible that *SOME* of the malaria cases reported in this health facility are from infections acquired from other malaria epidemiological areas (*Yes/No*)? ------------
   i. If Yes, and giving reasons, which of the 47 counties would the infection have been imported from (*at most five counties*)?
   ii. County / Reason(tick if applicable) / Other reasons

	County	Reason(tick if applicable)	Other reasons
a.		High-transmission, endemic	
b.		High-transmission, endemic	
c.		High-transmission, endemic	
d.		High-transmission, endemic	
e.		High-transmission, endemic	

3. In your duty, how many years have you been using microscopy in the diagnosis of malaria (yy)? --
4. Have you used methods other than microscopy in the diagnosis of malaria in your duty (Yes/No)? --
   i. If Yes, which of the following methods (*tick all that apply to you*):
   a. Rapid diagnostic tests (RDTs) to detect specific parasite antigens ☐
   b. Detection of parasite DNA by polymerase chain reaction (PCR) ☐
   c. Rapid diagnostic tests (RDTs) to detect specific parasite antibodies ☐
   d. Others (*specify*) --- ☐

ii. State the approximate duration you have used the above declared methods (mm/yyyy-mm/yyyy).

a.

b.

c.

d.

5. Have you ever worked in malaria high-transmission area(s) (*Yes/No*)? ----------- -----------

i. If *Yes* provide the following

Health Facility name	County/District	Period (*mm/yy-mm/yy*)	Comment

Refresher training

6. Have you attended any malaria microscopy refresher training between 01January-31 December 2013(*Yes/No*) --- ---------------------------

i. If *YES* provide the following:

A.

a. Name or certification of the course attended ------------------------------------- -----------

b. Date of the training (mm/yy)--- ----------

c. Duration in hours of the training ---

B.

a. Name or certification of the course attended --
b. Date of the training (mm/yy)---
c. Duration in hours of the training ---
d. Provider of the training ---
e. Venue/centre of the training ---

C.

a. Name or certification of the course attended --
b. Date of the training (mm/yy)---
c. Duration in hours of the training ---
d. Provider of the training ---
e. Venue/centre of the training ---

ii. If *No*, have you ever attended a malaria microscopy refresher training in your life (*Yes/No*) ---

If *YES* provide the following:

A.

a. Name or certification of the course attended --
b. Date of the training (mm/yy)--
c. Duration in hours of the training ---
d. Provider of the training ---

B.

a. Name or certification of the course attended --
b. Date of the training (mm/yy)--
c. Duration in hours of the training ---
d. Provider of the training ---

Guideline Knowledge

7. Have you seen this current ‘NATIONAL GUIDELINES FOR THE DAIGNOSIS, TREATMENT AND PREVENTION OF MALARIA IN KENYA, Fourth Edition”(*show a copy*) (*Yes/No*) -----------------------------------
----
8. Have you read this current ‘NATIONAL GUIDELINES FOR THE DAIGNOSIS, TREATMENT AND PREVENTION OF MALARIA IN KENYA, Fourth Edition” (*Yes/No*)--
----
9. The current ‘NATIONAL GUIDELINES FOR THE DAIGNOSIS, TREATMENT AND PREVENTION OF MALARIA IN KENYA, Fourth Edition” recommends the following regarding parasitological diagnosis and treatment of uncomplicated malaria (*Indicate FALSE or TRUE in the boxes against each statement*):
   a. Patients presenting with signs and symptoms of uncomplicated malaria should be tested for ma
   b. Only those who test positive should be treated for malaria
   c. Patients should also be assessed for other conditions that may cause fever and be managed accordi
   d. Children aged below 5 years presenting with signs and symptoms should be treated regardless of the test resul
   e. Appropriate treatment should never be delayed or denied due to inability to test for malaria
10. In your understanding, what are the commonly used routine ‘parasitological diagnostic’ methods of malaria in Kenya?

a. Microscopy, Rapid diagnostic tests (RDTs) to detect specific parasite antigens
b. Microscopy, Rapid diagnostic tests (RDTs) to detect specific parasite antibodies
c. Detection of parasite DNA by polymerase chain reaction (PCR), Rapid diagnostic tests (RDTs) to detect specific parasite antigens

d. Detection of parasite DNA by polymerase chain reaction (PCR), Rapid diagnostic tests (RDTs) to detect specific parasite antibodies

e. Rapid diagnostic tests (RDTs) to detect specific parasite antigens, Rapid diagnostic tests (RDTs) to detect specific parasite antibodies

Epidemiology knowledge

11. Kenya has how many malaria epidemiological zones (*tick one*)?
    a. Three
    b. Four
    c. Five
    d. Six
    e. Seven
12. In which of the following epidemiological zones does this county belong (*tick one*)?
    a. Endemic
    b. Seasonal
    c. Epidemic prone areas of western highlands of Kenya
    d. Low risk malaria areas
13. What is the community prevalence of *Plasmodium falciparum* in this county (*tick one*)?
    a. <0.1%
    b. 0.1%-<1.0%
    c. 1.0%-<5.0%
    d. 5.0%-<10.0%
    e. 10.0%-<20.0%
    f. 20.0%-<40.0%
    g. >=40.0%

Appendix VI: Laboratory worksheet

Slide No.	Date Exam	Result (Pos/Neg)	Examiner (initials)	Comment

Appendix VII: Publication

Malaria Journal

RESEARCH Open Access

Factors associated with malaria microscopy diagnostic performance following a pilot quality-assurance programme in health facilities in malaria low-transmission areas of Kenya, 2014

Fredrick Odhiambo[1,2*], Ann M. Buff[3,4], Collins Moranga[1], Caroline M. Moseti[1], Jesca Okwara Wesongah[2], Sara A. Lowther[3], Wences Arvelo[3], Tura Galgalo[1,3], Thomas O. Achia[3], Zeinab G. Roka[1], Waqo Boru[1], Lily Chepkurui[1], Bernhards Ogutu[5,6] and Elizabeth Wanja[5]

Abstract

Background: Malaria accounts for ~27% of outpatient visits annually in Kenya; prompt and accurate malaria diagnosis is critical to ensure proper treatment. In 2013, formal malaria microscopy refresher training for microscopists and a pilot quality-assurance (QA) programme for malaria diagnostics were independently implemented to improve malaria microscopy diagnosis in malaria low-transmission areas of Kenya. A study was conducted to identify factors associated with malaria microscopy performance in the same areas.

Methods: From March to April 2014, a cross-sectional survey was conducted in 42 public health facilities; 21 were QA-pilot facilities. In each facility, 18 malaria thick blood slides archived during January–February 2014 were selected by simple random sampling. Each malaria slide was re-examined by two expert microscopists masked to health-facility results. Expert results were used as the reference for microscopy performance measures. Logistic regression with specific random effects modelling was performed to identify factors associated with accurate malaria microscopy diagnosis.

Results: Of 756 malaria slides collected, 204 (27%) were read as positive by health-facility microscopists and 103 (14%) as positive by experts. Overall, 93% of slide results from QA-pilot facilities were concordant with expert reference compared to 77% in non-QA pilot facilities ($p < 0.001$). Recently trained microscopists in QA-pilot facilities performed better on microscopy performance measures with 97% sensitivity and 100% specificity compared to those in non-QA pilot facilities (69% sensitivity; 93% specificity; $p < 0.01$). The overall inter-reader agreement between QA-pilot facilities and experts was $\kappa = 0.80$ (95% CI 0.74–0.88) compared to $\kappa = 0.35$ (95% CI 0.24–0.46) between non-QA pilot facilities and experts ($p < 0.001$). In adjusted multivariable logistic regression analysis, recent microscopy refresher training (prevalence ratio [PR] = 13.8; 95% CI 4.6–41.4), ≥5 years of work experience (PR = 3.8; 95% CI 1.5–9.9), and pilot QA programme participation (PR = 4.3; 95% CI 1.0–11.0) were significantly associated with accurate malaria diagnosis.

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Full list of author information is available at the end of the article

Conclusions: Microscopists who had recently completed refresher training and worked in a QA-pilot facility performed the best overall. The QA programme and formal microscopy refresher training should be systematically implemented together to improve parasitological diagnosis of malaria by microscopy in Kenya.

Keywords: Malaria, Microscopy, Quality assurance, Interpretation, Validity, Reliability, Laboratory, Kenya

Background

In 2013, approximately 198 million cases of malaria and 584,000 deaths occurred globally, and 90% of the deaths were in Africa [1]. In 2012, Kenya had an estimated malaria mortality rate of 27.7 per 100,000 people [2]. Malaria accounted for almost 9 million outpatient visits in Kenya in 2012, which represented approximately 21% of all outpatient consultations [3].

Parasitological diagnosis is recommended by the World Health Organization (WHO) for all patients in whom malaria is suspected as part of the 'test, treat, track' strategy [4, 5]. Both microscopy and malaria rapid diagnostic tests (RDT) are recommended malaria diagnostic methods by the Kenya National Malaria Control Programme (NMCP) [6–8]. Although over 90% of public health facilities in Kenya had the capacity to diagnosis malaria, the proportion of facilities performing malaria microscopy, approximately 50%, has not changed in recent years [9]. Despite the high proportion of health facilities offering malaria diagnostic services, only 31% of malaria cases were confirmed by parasitological diagnosis in Kenya in 2013 [3].

Limited microscopy services in health facilities in Kenya and across sub-Saharan Africa have been attributed, in part, to limitations in the availability of equipment, supplies, working environment, training, and supervision [10–12]. Increasing and sustaining access to prompt diagnosis and effective treatment for at least 80% of the population across all levels of the health care system and epidemiological zones is a key objective of the Kenya National Malaria Strategy 2009–2017 [6]. Implementation of the national strategy included providing health facilities with microscopes and laboratory supplies and improving the skills of microscopists through formal microscopy refresher trainings at microscopy centres of excellence [6, 13].

From June to December 2013, the NMCP in coordination with the Malaria Diagnostics Center, Walter Reed Army Research Institute, initiated a pilot to operationalize the laboratory quality assurance (QA) policy and plan for malaria diagnostics in health facilities in malaria low-transmission areas [8, 14]. Malaria low-transmission areas were prioritized because of concerns surrounding over-diagnosis of malaria due to poor microscopy practices. Laboratory QA programmes have been shown to improve the diagnosis of malaria and, in particular, microscopy accuracy [13, 15]. Components of the 7-month pilot QA programme included 4 1-day visits by trained QA laboratory officers, who promoted internal QA/quality control (QC) processes, provided supportive supervision and on-job training, and cross-checked at least 10 malaria microscopy slides at each visit [8, 14, 16]. The pilot QA programme implementation is described in detail elsewhere [16]. Independently in 2013, there were other laboratory-strengthening activities ongoing in Kenya, such as malaria microscopy refresher trainings and the WHO Stepwise Laboratory Improvement Progress Towards Accreditation (SLIPTA) programme. The WHO SLIPTA framework was established to improve the quality of public health laboratories in developing countries through standardized processes to meet international accreditation [17]. In early 2014, a survey was conducted to identify factors associated with accurate malaria diagnosis by microscopy in 42 health facilities in malaria low-transmission areas of Kenya.

Methods

Study design and area

From March to April 2014, a cross-sectional survey was conducted in public-sector health facilities that included pilot QA programme facilities to identify factors associated with accurate malaria microscopy diagnosis in low-malaria transmission counties in Kenya. The health facilities were widely distributed in 10 (38%) of 26 low-malaria transmission counties in the Central, Eastern and Rift Valley regions and represented approximately 4% of public-sector health facilities in the 10 counties. In these counties, malaria transmission is seasonal with an estimated population-adjusted parasitaemia prevalence of <5% [18].

Sample size and sampling procedure

A total of 42 public health facilities were selected to participate in the survey. Twenty-one facilities were part of the pilot QA programme from June to December 2013; these facilities were randomly selected from among 45 public-sector pilot QA programme facilities across 4 service-provision levels (i.e., dispensary, health centre, primary hospital, secondary hospital). The pilot QA programme was implemented in 83 health facilities (45 [54%] public-sector and 38 [46%] private-sector); facilities were selected to participate based on capacity to

perform malaria microscopy and distance from the QA officers' primary duty stations [16]. These facilities are referred to as 'QA-pilot facilities.' Twenty-one public health facilities of the same service-provision level and located in the same county as the QA-pilot facilities, but which did not participate in the QA-pilot programme, were also randomly selected to participate in the survey. These facilities are referred to as 'non-QA pilot facilities.'

A total sample size of 756 malaria slides was calculated to detect a 5% difference in diagnostic accuracy between the QA-pilot and non-QA pilot facilities, assuming an index of accuracy of 90%, power of 0.80, 0.05 level of significance and finite population correction [19–21]. All facilities that consented to participate in the survey were provided with slides and requested to label and archive all slides prepared for malaria diagnosis between 1 January and 28 February, 2014. All thick-smear slides prepared for malaria diagnosis with a result recorded in the health-facility laboratory parasitology log-book and archived from 1 January to 28 February, 2014 were eligible for survey inclusion. Eighteen malaria slides were collected by the survey team from each health facility. Overall, the daily range of malaria slides prepared was 4–28 in QA-pilot and 4–52 in non-QA pilot facilities (Table 1). The number of slides collected for the survey represented <5% of all malaria slides archived from 1 January to 28 February, 2014 at each facility.

From the slide boxes, 9 positive and 9 negative slides were collected per facility via simple random sampling using a random number table where the sequence boundary was the number of slides archived at each facility. Slides which were found unlabelled (i.e., no date, laboratory number, patient age, or sex), stuck together, not entered in the log-book or with results that were not signed by the examining microscopist were excluded. At facilities with fewer than 9 positive slides, all the positive slides were selected and the balance was randomly selected from negative slides to total 18 per facility.

Table 1 Characteristics of surveyed health facilities in malaria low-transmission areas of Kenya, 2014

Characteristic	QA-pilot health facilities (N = 21)		Non-QA pilot health facilities (N = 21)	
	Number	Percentage	Number	Percentage
Health-facility level				
Primary care facilities	12	58	12	58
Dispensary	2	10	2	10
Health centre	10	48	10	48
Hospitals	9	42	9	42
Primary hospital	8	38	8	38
Secondary or referral hospital	1	4	1	4
Urban location	10	48	4	19
Participates in SLIPTA program	4	19	3	14
Microscope(s) in good optical condition	20	95	18	86
Workload >10 malaria slides per day	14	67	13	70
Characteristic	**QA-pilot health facilities (N = 21)**		**Non-QA pilot health facilities (N = 21)**	
	Median	**Range**	**Median**	**Range**
Number of microscopists				
Dispensary	1	–	3	2–3
Health centre	2	1–4	2	1–2
Primary hospital	7	5–12	4	2–7
Secondary or referral hospital	7	–	7	–
Malaria slide workload per day				
Dispensary	22	5–28	30	7–52
Health centre	17	5–21	15	4–19
Primary hospital	21	7–28	18	6–52
Secondary or referral hospital	9	4–16	18	7–28

QA quality assurance, SLIPTA stepwise laboratory improvement towards accreditation, an external laboratory-strengthening program sponsored by World Health Organization

Data collection
Each microscopist who had examined the selected slides was interviewed by trained survey staff using a standardized, pilot-tested structured questionnaire (Additional file 1). Laboratory and facility conditions were collected via a standardized form (Additional file 2). Recent training for microscopists was defined as having attended initial or refresher malaria microscopy training within the year prior to the survey.

Thick-blood smear slides were examined for the presence or absence of parasites by expert microscopists who had been certified through the WHO External Competency Assessment for Malaria Microscopy scheme. Two independent expert microscopists cross-checked each of the slides and a third independent expert microscopist was a tie-breaker when the first two expert readers disagreed. Expert readers disagreed on 9 (1%) slides requiring a third tie-breaker. The expert microscopist results, or the tie-breaker result when necessary, were considered the reference value. Expert microscopists were masked to both the health-facility microscopy results and the other expert microscopy results. Expert microscopists examined a minimum of 100 high-power magnification fields before the slide was classified as negative per national and WHO guidance [8, 10]. Each microscopist read a maximum of 20 slides per day. Accurate malaria diagnosis was defined as concordance in the presence or absence of parasites (i.e., positive or negative) between the health-facility microscopist result and the expert reference result. The health-facility results were compared to expert reference to obtain validity and reliability performance measures.

Data management and analysis
Data were entered into Excel 2010 (Microsoft, Seattle, WA, USA). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the health-facility microscopy results were calculated with 95% confidence intervals (CI) using exact method by Graph Pad Prism version 5.01 (GraphPad Software, La Jolla, CA, USA). Inter-reader agreement for facilities versus reference values was expressed as kappa (κ) values with 95% CIs using Graph Pad Prism version 5.01 [22]. Using accurate malaria diagnosis as the outcome of interest, multivariable logistic regression with institutional-specific random effects was performed using Stata version 12 (StataCorp LP, College Station, TX, USA). Both individual (i.e., recent microscopy refresher training status, level of initial training, years and location of work experience, and malaria knowledge) and institutional-level factors (i.e., participation in pilot QA programme or other external QA programme, condition of microscopes, number of microscopists, service-provision level, location and daily workload) were included as independent variables in the regression model.

Ethical review
The study was approved by the ethical review committee of the Jaramogi Oginga Odinga Teaching and Referral Hospital (#01713, ref: ERC 1B/VOL.1/70) in collaboration with the Ministry of Health. The study underwent human subject review at CDC and was approved as non-engagement in human subject research. The management official at each health facility and each microscopist provided written consent. No personal identifiers were collected from microscopists or extracted from laboratory or clinical records.

Results
All selected heath facilities agreed to participate in the survey. Participating health facilities were located in 10 (38%) of 26 low-malaria transmission counties. Among surveyed facilities, 58% were primary care facilities (i.e., dispensaries [10%] and health centres [48%]) and 42% were hospitals (i.e., primary [38%] and secondary or referral [4%]) (Table 1). More QA-pilot facilities were in urban settings (48 vs 19%), participated in an external laboratory-strengthening program (i.e., SLIPTA) (19 vs 14%), and had microscopes in good optical condition (95 vs 86%) compared to non-QA pilot facilities. The number of microscopists per facility and daily malaria slide workloads were similar across surveyed facilities (Table 1). As shown in Table 2, more microscopists in QA-pilot facilities had completed recent refresher training (68 vs 29%), had worked in a malaria high-transmission area (63 vs 21%), and had knowledge of national malaria diagnostic and treatment guidelines (84 vs 39%).

A total of 756 malaria slides were collected from the health facilities surveyed; 204 (27%) slides were read as positive for malaria by health-facility microscopists and 103 (14%) as positive by expert microscopists. In Fig. 1, slides are stratified by facility QA-pilot programme participation and recent training status (i.e., formal initial or refresher microscopy training within the year prior to the survey) of the microscopists. More microscopists (68%, 38 of 56) had completed recent refresher training in the QA-pilot facilities compared to non-QA pilot facilities (29%, 24 of 82) ($p < 0.01$). In QA-pilot facilities, recently-trained microscopists read 285 (75%) slides compared to 176 (47%) in the non-QA pilot facilities ($p < 0.001$). Recently-trained microscopists in QA-pilot facilities performed better on all microscopy performance measures with 97% sensitivity and 100% specificity compared to recently-trained microscopists in the non-QA pilot facilities with 69% sensitivity and 98% specificity ($p < 0.01$). Microscopists without recent microscopy refresher

Table 2 Characteristics of surveyed microscopists in malaria low-transmission areas of Kenya, 2014

Characteristic	Microscopists at QA-pilot health facilities (N = 56)		Microscopists at non-QA pilot health facilities (N = 82)	
	Number	Percentage	Number	Percentage
Individual level				
Training and work experience				
Recent microscopy refresher training	38	68	24	29
More than diploma-level initial training	45	80	59	72
≥5 years of work experience	49	88	66	80
Worked in malaria high-transmission area	35	63	17	21
Knowledge				
Malaria diagnostic and treatment guidelines	47	84	32	39
Malaria epidemiology in county	55	98	70	85
Malaria case importation	55	98	75	91

QA quality assurance; recent training was defined as in the year prior to the survey

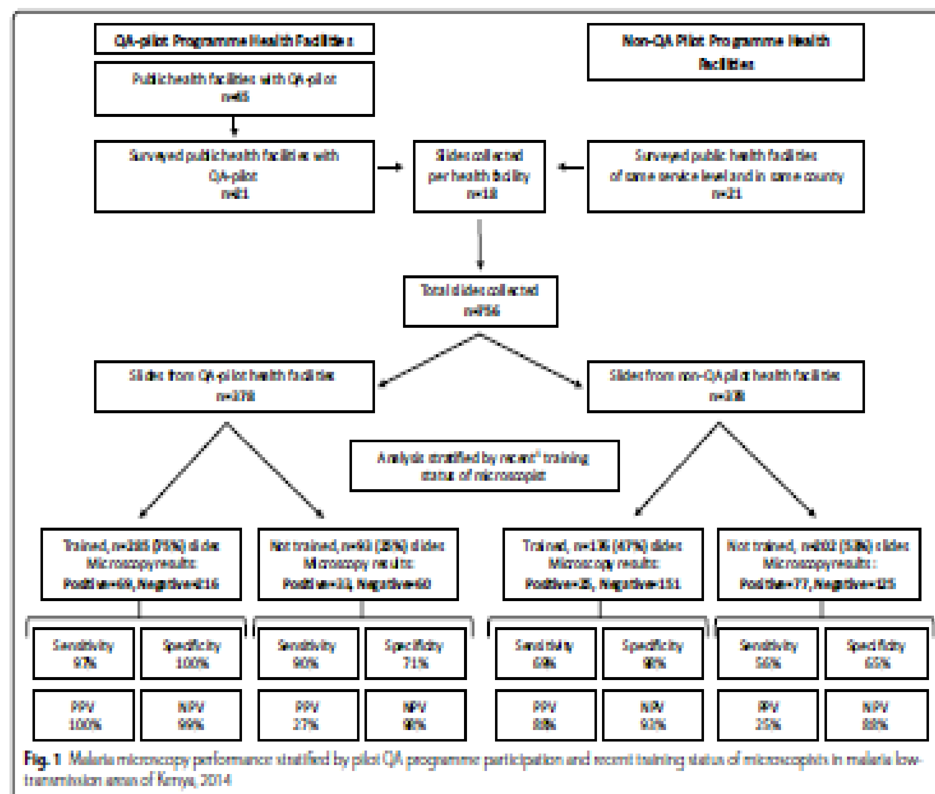

Fig. 1 Malaria microscopy performance stratified by pilot QA programme participation and recent training status of microscopists in malaria low-transmission areas of Kenya, 2014

training performed the same regardless of facility participation in the QA-pilot programme.

Overall as shown in Table 3, QA-pilot facilities performed significantly better on measures of diagnostic accuracy (i.e., sensitivity, specificity, PPV and NPV) against expert reference compared to non-QA pilot facilities. The overall inter-reader agreement between QA-pilot facilities and expert microscopy was κ = 0.80 (95%

Table 3 Measures of malaria microscopy performance in surveyed health facilities in malaria low-transmission areas of Kenya, 2014

Quality-assurance pilot programme	Number of slides	Sensitivity		Specificity		Positive predictive value		Negative predictive value		Kappa value	
		%	95% CI	%	95% CI	%	95% CI	%	95% CI	κ	95% CI
Overall											
Yes	378	96	(90–99)	92	(88–95)	76	(67–84)	99	(97–99)	0.80	(0.74–0.88)
No	378	62	(49–74)	80	(76–85)	40	(31–50)	91	(87–94)	0.35	(0.24–0.46)
Dispensary											
Yes	36	100	(59–100)	100	(88–100)	100	(59–100)	100	(88–100)	1.00	–
No	36	62	(24–91)	54	(34–72)	28	(10–53)	83	(59–96)	0.11	(−0.16 to 0.38)
Health centre											
Yes	180	88	(69–97)	90	(85–94)	60	(42–75)	98	(94–100)	0.65	(0.51–0.79)
No	180	59	(39–76)	94	(89–97)	65	(44–84)	92	(87–96)	0.55	(0.38–0.72)
Primary hospital											
Yes	144	100	(87–100)	93	(85–97)	87	(75–95)	100	(96–100)	0.89	(0.82–0.97)
No	144	62	(41–80)	69	(59–77)	30	(18–44)	89	(81–95)	0.22	(0.06–0.37)
Secondary hospital											
Yes	18	100	(40–100)	93	(66–100)	80	(28–99)	100	(75–100)	0.85	(0.58–1.00)
No	18	100	(29–100)	87	(60–98)	60	(15–95)	100	(75–100)	0.68	(0.29–1.00)

Italic denotes statistical significance

CI Confidence interval

CI 0.74–0.88) compared to κ = 0.35 (95% CI 0.24–0.46) in non-QA pilot facilities ($p < 0.001$). Table 3 also shows the diagnostic performance measures stratified by service level; only primary hospitals participating in the pilot QA programme performed statistically better on all diagnostic accuracy measures compared to non-QA pilot facilities. In total, 351 (93%) slide results were read as concordant with expert reference from QA-pilot facilities compared to 292 (77%) in the non-QA pilot facilities ($p < 0.001$) (Table 3).

In unadjusted logistic regression analysis shown in Table 4, all the microscopist characteristics were associated with accurate malaria diagnosis except initial level of training, but only pilot QA programme participation and good optical condition of microscopes were institutional factors associated with accurate malaria diagnosis. In adjusted multivariable logistic regression analysis, recent microscopy refresher training (prevalence ratio [PR] = 13.8; 95% CI 4.6–41.4), ≥5 years of work experience (PR = 3.8; 95% CI 1.5–9.9), and pilot QA programme participation (PR = 4.3; 95% CI 1.0–11.0) were the only factors significantly associated with accurate malaria diagnosis.

Discussion

This observational study demonstrated that diagnostic accuracy of malaria microscopy was positively associated with recent microscopy refresher training and ≥5 years of experience for microscopists and health facility participation in the pilot QA programme. The findings are consistent with other studies from Kenya and elsewhere that have shown both laboratory QA programmes and microscopy refresher trainings improve malaria microscopy performance [13, 15, 23–25]. In 2013, the NMCP independently started both formal refresher trainings for microscopists at a malaria microscopy centre of excellence and the pilot QA programme for malaria diagnostics at 83 health facilities; both the refresher trainings and pilot QA programme were intended to improve malaria diagnosis by microscopy. However, implementation of the two diagnostic strengthening components was not coordinated or systematic in health facilities, across service-provision levels or administrative zones, which hindered independent evaluation of the pilot QA programme.

Recent microscopy refresher training at the individual level was more strongly associated with accurate malaria diagnosis than health facility participation in the pilot QA programme. However, microscopists who had recently completed refresher training and worked in a facility that was part of the pilot QA programme had the best performance for all measures of diagnostic accuracy. These findings suggest that synergies exist between formal microscopy refresher training and the pilot QA programme. Implementation of both diagnostic strengthening components together appear to produce the best

Table 4 Individual and institutional characteristics associated with accurate malaria microscopy diagnosis in surveyed health facilities in malaria low-transmission areas of Kenya, 2014

Characteristic		Slides n (%)	Accurate diagnosis n (%)	Unadjusted		Adjusted	
				Prevalence ratio	95% confidence interval	Prevalence ratio	95% confidence interval
Individual							
Recent microscopy refresher training	No	295 (39.0)	197 (66.8)	1.00 (Ref)		1.00 (Ref)	
	Yes	461 (61.0)	446 (96.7)	40.5	*15.1–108.6*	*13.8*	*4.6–41.4*
More than diploma-level initial training	No	141 (18.7)	109 (77.3)	1.00 (Ref)			
	Yes	615 (81.3)	534 (86.8)	2.1	0.9–4.6		
≥5 years of work experience	No	137 (18.1)	78 (56.9)	1.00 (Ref)		1.00 (Ref)	
	Yes	619 (81.9)	565 (91.3)	*23.7*	*9.7–57.6*	*3.8*	*1.5–9.9*
Worked in malaria high-transmission area	No	383 (50.7)	290 (75.7)	1.00 (Ref)			
	Yes	373 (49.3)	353 (94.6)	12.1	5.2–28.3		
Knowledge of malaria diagnostic and treatment guidelines	No	194 (25.7)	119 (61.3)	1.00 (Ref)			
	Yes	562 (74.3)	524 (93.2)	25.6	10.4–62.9		
Knowledge of malaria epidemiology in county	No	94 (12.4)	45 (47.9)	1.00 (Ref)			
	Yes	662 (87.6)	598 (90.3)	22.8	8.7–60.3		
Knowledge of malaria cases importation	No	63 (8.3)	20 (31.7)	1.00 (Ref)			
	Yes	693 (91.7)	623 (89.9)	21.1	7.2–62.1		
Institutional							
Quality-assurance pilot programme	No	378 (50.0)	292 (77.2)	1.00 (Ref)		1.00 (Ref)	
	Yes	378 (50.0)	351 (92.8)	*6.0*	*1.9–18.9*	*4.3*	*1.0–11.0*
Good optical condition of microscope(s)	No	72 (9.5)	46 (63.9)	1.00 (Ref)			
	Yes	684 (90.5)	597 (87.3)	7.6	1.1–51.4		
Rural location	No	505 (66.8)	415 (82.2)	1.00 (Ref)			
	Yes	251 (33.2)	228 (90.8)	2.7	0.7–10.1		
Participation in SLIPTA program	No	647 (85.6)	539 (83.3)	1.00 (Ref)			
	Yes	109 (14.4)	104 (95.4)	4.8	0.8–28.8		
>3 laboratory staff	No	361 (47.8)	308 (85.3)	1.00 (Ref)			
	Yes	395 (52.2)	335 (84.8)	1.1	0.3–3.3		
Hospital-level facility	No	432 (57.1)	376 (87.0)	1.00 (Ref)			
	Yes	324 (42.9)	267 (82.4)	0.8	0.2–2.5		
Workload >10 slides per day	No	269 (35.6)	239 (88.8)	1.00 (Ref)			
	Yes	487 (64.4)	404 (83.0)	0.6	0.2–2.1		

Italic denotes statistical significance; recent training was defined as in the year prior to the survey

SLIPTA stepwise laboratory improvement towards accreditation, sponsored by World Health Organization; Ref reference

performance results. Therefore, the NMCP and partners should consider systematically implementing formal microscopy refresher training and the QA programme together as a package of interventions to improve parasitological diagnosis of malaria by microscopy in accordance with national and WHO guidance [8, 10, 14, 26].

Malaria microscopy refresher training was an important confounder in the study. The study was powered to detect differences at the health-facility level rather than at the individual microscopist level, and malaria microscopy refresher training was not uniform across surveyed facilities. Twice as many microscopists from QA-pilot facilities had recent refresher training compared to non-QA pilot facilities. Three-quarters of the malaria slides from QA-pilot facilities were read by microscopists who had recently completed malaria microscopy refresher training compared to less than half of the slides from non-QA pilot facilities. In addition, there were other general

laboratory-strengthening activities ongoing, such as SLIPTA, in a minority of facilities that were included in the survey. Although participation in the WHO SLIPTA programme was not significantly associated with accurate malaria microscopy diagnosis, the programme might have contributed to overall laboratory improvements that were not specifically measured [17].

Overall in QA-pilot facilities, the sensitivity, specificity and NPV were very high at over 90%. The PPV was much lower, but lower PPVs and higher NPVs would be expected because all the surveyed facilities were located in malaria low-transmission counties. These counties have community malaria parasitaemia prevalences by microscopy of between 1 and 3% during peak malaria transmission season [27]. A 2014 national health-facility survey for malaria infection found that 3.4% of outpatients who reported a history of fever within the last 48 h had a positive malaria RDT in seasonal low-transmission counties in Kenya [28]. Malaria slides were collected in January and February for the survey, which is not the peak malaria transmission season in Kenya. Therefore, most persons presenting to health facilities, even if febrile, were unlikely to have malaria at the time of the survey. In malaria low-transmission settings, the low PPV findings translate into a large number of false-positive results. Persons misdiagnosed as having malaria when they do not are at risk of not being treated for their actual illness, which can lead to increased morbidity and potentially mortality. In addition, treating people who do not have malaria with relatively expensive artemisinin-based combination therapy wastes limited resources and can contribute to the development of artemisinin resistance [4, 7, 10, 26].

Hospitals require expert microscopy for the management of complicated patients with severe malaria and co-morbidities. Expert microscopy is the gold standard for identifying mixed infections, treatment failures, and quantifying parasitic density [8, 10, 26]. Hospitals generally have more substantial laboratories and resources available to maintain at least adequate, if not expert, diagnostic microscopy programmes compared to outpatient health centres and dispensaries. Outpatient health centres and dispensaries generally have high patient workloads, which makes labour-intensive diagnostics, such as malaria microscopy, challenging. Historically in Kenya, programmes and training cascaded from the highest service-provision levels to the lowest and often did not reach dispensaries due to limited resources and lower prioritization. In 2010, Kenya prioritized dispensaries to receive malaria RDTs for parasitological diagnosis since expert microscopy services were not expected at this level [3].

However, the strategy for utilizing malaria RDTs and microscopy concurrently to improve diagnostic performance across service levels and malaria epidemiologic zones is not clear in the national diagnostic and treatment guidelines [6–8].

This study has a number of limitations. Although health facilities were randomly selected for the survey, the facilities selected to participate in the pilot QA programme were a convenience sample. Thus, the surveyed facilities are not representative of all public health facilities in Kenya, which limits the generalizability of the findings. A baseline evaluation of microscopy performance was not conducted prior to the start of the refresher trainings or the pilot QA programme. Microscopists and facilities selected for participation in the diagnostic strengthening components might have performed better at baseline compared to those not selected. Therefore, the association between microscopy performance and refresher training and the pilot QA programme might have been overestimated. Additionally, when health facilities consented to participate in the survey, they were asked to store slides during a specific time interval for later retrieval. Facilities might have preferentially stored slides for which they felt confident about the results, and microscopists might have performed better during this period because they were aware of the survey (i.e., Hawthorne effect) [29, 30]. Both situations would have resulted in an overestimation of diagnostic accuracy, but the potential bias should be non-differential across all facilities.

Another important limitation was that slide preparation quality, including the stain type and adequacy, was not evaluated. Although both NMCP and WHO recommend Giemsa preferentially for malaria microscopy, the use of both Giemsa and Field stains was common in health facilities [8, 10, 16]. Slides were not matched on parasite density either. Thick films were examined for the presence or absence of parasites; no thin films were examined for parasite density or speciation [8, 10]. Slides from QA-pilot facilities might have had higher parasite densities, which would make malaria easier to identify correctly. However, urban areas generally have a substantially lower parasitaemia prevalence compared to rural areas and a greater percentage of QA-pilot facilities were located in urban areas [21, 22]. Therefore, it is possible that persons who presented to QA-pilot facilities in urban areas would have had lower parasite densities overall; if this represented the true situation, then QA-pilot enrolled facilities would have performed better than estimated compared to non-QA pilot facilities.

Conclusions

Diagnostic accuracy of malaria microscopy was positively associated with recent microscopy refresher training and ≥5 years of experience for microscopists at the

individual level and pilot QA programme participation at the health-facility level. Microscopists who had recently completed refresher training and worked in a QA-pilot facility had the best performance for all measures of diagnostic accuracy. Therefore, formal microscopy refresher training and the QA programme should be systematically implemented together to improve parasitological diagnosis of malaria by microscopy in Kenya.

Additional files

Additional file 1. Microscopist questionnaire.

Additional file 2. Health facility information and slide collection form.

Authors' contributions

FO, BO and EW conceived and designed the study; FO, JOW, TG, and EW coordinated and performed the study; FO, CM, SL, WA, and TA analyzed the data: FO, AMB, JOW, SAL, WA, CMM, TG, ZG, WB, LC, and EW drafted manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Disclaimer

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