Smear Microscopy Examination for Malaria and Tuberculosis at Primary Health Care Unit Level of Guragae Zone, Southern Ethiopia

Teha Shumbej Gebi (shumbejt@gmail.com)
Wolkite University
Menu Sofia
Wolkite University
Teklemichael Gebru
Wolkite University
Solomon Absra
Wolkite University
Kahase Daniel
Wolkite University
Alemayehu Mihret
Wolkite University
Keyredin Nuriye
Wolkite University
Mesfin Dereje
Wolkite University
Girum Tadele
Wolkite University
Bekele Fitsum
Wolkite University

Research article

Keywords: Quality assurance, Malaria, Tuberculosis, Gurage zone, Ethiopia

Posted Date: December 11th, 2019

DOI: https://doi.org/10.21203/rs.2.18653/v1

Abstract

Background: Accurate early diagnosis and prompt treatment is one of the core strategies employed to address malaria and tuberculosis related problems. Laboratory confirmation improves disease management most efficiently within well-managed health laboratory systems. External quality assurance participation is associated with improved laboratory performance over time as it is a system for objectively checking a laboratory's performance. However, many professionals in Sub Saharan Africa countries are unable to effectively implement a quality assurance program. This study aimed to assess the quality of smear microscopy in Guragae zone primary health care unit, Southern Ethiopia.

Methods: Health institution-based cross-sectional study was conducted to recruit twenty-one primary health care units between May and August 2019. Blind rechecking was used to collect data. The sensitivity, specificity, positive predictive values, and negative predictive values were calculated by considering the final re-reading result as the gold standard. The level of agreement was measured using Kappa value. SPSS version 21 was used for data management and analysis.

Result: A total of 860 and 318 stained slides for tuberculosis and malaria were collected, respectively. From total collected slides for tuberculosis, about 13.1 % of them were reported positive and from total malaria slides collected, half were positive for Plasmodium species at the peripheral laboratory while about 36.1% and 13% were confirmed positive for Plasmodium species and tuberculosis, respectively during re-reading at Wolkite University laboratory. Referring to the final result, the surveyed health facilities achieved "moderate agreement"(K=0.6) on malaria slide detection and "almost perfect agreement" (K=0.9) on slides for acid-fast bacilli. Only 4.4% of the surveyed health facilities incorporate malaria parasite count estimation in their report as per the current guideline.

Conclusion: Now is the time to build sustainable laboratory capacity in resource-poor settings like Ethiopia that can be used to manage existing infectious diseases including malaria and tuberculosis. Malaria related technical problems were identified in this study. Thus, the authors believe that a continuous and strong malaria quality assurance schemes should be implemented at each laboratory to ensure reliable results.

Background

Both Infectious and noninfectious diseases continue to cause millions of deaths and a huge burden of disability every year mainly in developing countries(1). Vaccination, health education, use of insecticide, impregnated bed-nets, improved provision of clean water and sanitation, and mass drug administration are among strategies used for reducing the burden of diseases. Moreover, appropriate disease case management at the primary health care unit(PHCU) level has paramount importance in the prevention and control of infectious diseases(2). In order to be successful in the national elimination endeavor, the capacity of the country's health system in terms of availability of reliable laboratory results needs to be ensured.

When a laboratory test used optimally; it generates knowledge that facilitates appropriate disease case management and leads to more cost-effective healthcare(3). Laboratory confirmation based disease management may prevent; unnecessary treatments, reduce the potential development of drug resistance(4) and encourage health care providers to search for alternative causes for confirmed negative patients(5). Quality assurance consists of quality control, external quality assurance (EQA), and quality improvement components, which are essential tools to yield reliable and reproducible laboratory results(6).

Providing health care in Sub Saharan Africa(SSA) is a complex problem. Policymakers, clinicians, and the public frequently fail to understand that laboratory diagnosis is essential to the prevention and treatment of disease(7). Allocation of resources to diagnostic laboratory testing has not been a priority for SSA and access to reliable laboratory

diagnostic testing is severely limited in this region (8). Lack of access to good quality diagnostic tests for case management is a major contributor to the enormous burden of diseases in the developing world(6).

Accurate laboratory diagnosis is an essential component of control strategies and enables effective disease case management(9, 10), but it face major operational challenges in resource-limited settings like Ethiopia(11). In many SSA countries, laboratory services have suffered from inattention leading to the lack of availability of accurate laboratory results(12), this has led to the underutilization of laboratory testing for diagnosis (8). Laboratory testing may influence less than 45% of medical decision-making in SSA(13). On the other hand, a study from the USA shows laboratory testing influences 60–70% of critical decision-making in health(14).

Laboratory confirmation improves disease management most efficiently within well-managed health laboratory systems. This requires technical competence, access to good-quality reagents, and an understanding of quality control(8). EQA participation is associated with improved laboratory performance over time as it is a system for objectively checking a laboratory's performance (6).

Effective use of laboratory tests at the health facility level expected to improves disease management and patient treatment if the use of laboratory testing can be readily incorporated into routine clinical practice with effective communication between clinical and laboratory staffs. However, many professionals in SSA countries are unable to effectively implement a quality assurance program(15). Establishing, maintaining and demonstrating the accuracy of diagnostic tests is a major challenge for most laboratories in SSA(13). Inadequate quality assessment systems may contribute significantly to eroding the confidence of clinicians in applying laboratory results, which in turn leads to further neglect of laboratory services(8).

Reliable laboratory services provide a result that is consistently accurate and can be ensured through commitment to quality assurance service. Quality of laboratory diagnosis is not a guarantee despite the implementation of EQA service in all laboratories of PHCU. EQA is vital for monitoring laboratory performance and maintaining the quality of laboratory services, and is a valuable tool for identifying and assessing technology in use, identifying gaps in laboratory performance and targeting training needs. Thus, EQA at the health laboratory level is crucial to improve the role of laboratory tests on disease management. This study has the objective of assessing the quality of smear microscopy examination for malaria and tuberculosis at PHCU level in Guragae zone, Southern Ethiopia.

Methods

Study design and settings

Between May and August 2019, we conduct a health facility-based cross-sectional study. The study was conducted at the PHCU of the Gurage zone, Southern Ethiopia which is one of the zones in the Southern nation's nationalities and people's regional state of Ethiopia. Wolkite town is the capital of the zone, which is located at 155 km south from Addis Ababa. There are seven functional hospitals and sixty-four health care centers in the zone.

Sample size determination and procedure

The sample size was determined based on a suggested rule of Thumb(16), accordingly, five primary hospitals and seventeen health care centers were recruited from thirteen woredas' and two town administration of the Guragae zone using probability proportional to population size to address 30% of the source population. Each health facility was visited by a trained data collector with a standard checklist to assess the availability of laboratory consumables and major types of equipment.

On the other hand, all slides reported as 'positive for AFB(acid-fast bacilli)', 'negative for AFB' using Ziehl-Neelsen (ZN) staining technique and 'Postive for *Plasmodium species*', 'Negative for *Plasmodium species*' using Giemsa smear microscopy technique during the study period were collected from all selected PHCU laboratories. In brief, the selected health facility laboratories were informed to retain all positive and negative stained slides with their results filled in a separate form with site code (first reader). The slides were collected prospectively every two weeks during the study period, transported to and examined (second reader) in Wolkite University Microbiology and Parasitology laboratory. Slide reading results from peripheral health facility laboratories were kept confidential from the second reader. Discrepant readings between the peripheral health facility laboratory reading and the second reader were re-read and verified by a third senior medical laboratory technologist. EQA for smear microscopy guideline was used to determine the number of slides recruited for blind rechecking. Lot quality assurance sampling method was considered during collecting all necessary sample slides(17).

Data quality and analysis

Data collectors obtained training in data collection and procedure prior to data collection. The collected data were entered, cleaned and analyzed using SPSS version 21. The sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of the peripheral diagnostic laboratories was calculated by considering the final re-reading result as the gold standard. The level of agreement was measured using Kappa (K) value.

Result

Overall Blind Rechecking of Malaria and AFB Slides

For this reliability assessment(rechecking), the World Health Organization(WHO) guideline for microscopy quality assurance manual version 2 for grading the performance of parasite detection and species identification was used(18). A total of 860 stained slides for AFB smear microscopy and a total of 318 stained blood film stained slides for malaria were collected from twenty-one PHCU laboratories. About 13.1% of the collected AFB slides were reported positive and half of the collected malaria slides were positive at the peripheral laboratory(first reader). Of total malaria slides collected, about 36.1% were confirmed to be positive malaria and from total AFB slide collected, 13% were confirmed to be positive for AFB at Wolkite University Microbiology and Parasitology laboratory(second reader). Referring to the final result readers, the participants achieved "moderate agreement" (agreement: 87.8%, K = 0.6) on malaria parasite detection and "almost perfect agreement" (agreement: 99.8%; K = 0.9) on AFB slide detection. About 76.1% of study health facility reports include a grading system(semi-quantification) for AFB but only 4.4% of the surveyed health facilities include malaria parasite count estimation in their report as per the current WHO guideline. Most (71.4%) health facilities claimed to have taken part in the AFB EQA scheme and received feedback while only 9.5% of surveyed facilities participated in the malaria EQA scheme over the last six months prior to the data collection (Table 1).

Table 1

Agreement in readings of malaria and acid-fast bacilli slides among selected in Guragae Zone Primary Health Care Unit laboratories and final re-reading, Southern Ethiopia, 2019

Number of collected Stained Slides	Peripheral lab. result	Final Rereading	Agreement (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	% Slides Reported with Grading	K
Negative for AFB	747	748	99.8	100	99.8	99.1	100		0.9
Positive for AFB	113	112							
Total	860	860							
1–9 AFB/100	1	3							
Grading as + 1	14	44							
Grading as + 2	38	37							
Grading +3	33	28							
Total Graded	86	112						76.1	
Negative for malaria	159	203	87.8	100	82.2	72.3	100		0.6
Positive for malaria	159	115							
Total	318	318							
P.F species Identification	75	55	73.3						
P.V species Identification	84	60	71.4						
Grading as + 1	5	58							
Grading as + 2	1	37							
Grading +3	1	20							
Total Graded	7	115						4.4	

Grade distinction for AFB = Negative-No AFB found in at least 100 fields, Exact figure – 1 to 9 AFB per 100 field, 1+-10 to 99 AFB per 100 fields, 2+-1 to 10 AFB per field (count at least 50 fields), 3+-more than 10 AFB per field (count at least 20 fields).

Grade distinction for malaria = 1–10 per 100 high power fields grade as '+1', 11–100 per 100 high power field grade as '+2', 1–10 in every high power field graded as '+3', and more than 10 in every high power field grade as '+4'.

AFB-acid-fast bacilli, EQA-External Quality Assessment, NPV- Negative Predictive Values, PPV- Positive Predictive Values, K- Kappa, P.F- Plasmodium falciparum, P.V- Plasmodium vivax.

Number of collected Stained Slides	Peripheral lab. result	Final Rereading	Agreement (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	% Slides Reported with Grading	K
EQA participation over the last quarter		Participated and feed back received		Participated but feedback not received			Not Participated at all		
EQA for AFB		15(71.4%)		6(28.6%)			-		
EQA for Malaria		-		2(9.5%)			19((90.5%)		
Grade distinction for AFB = Negative-No AFB found in at least 100 fields, Exact figure – 1 to 9 AFB per 100 field, 1+-10 to 99 AFB per 100 fields, 2+-1 to 10 AFB per field (count at least 50 fields), 3+-more than 10 AFB per field (count at least 20 fields).									
Grade distinction for malaria = 1–10 per 100 high power fields grade as '+1', 11–100 per 100 high power field grade as '+2', 1–10 in every high power field graded as '+3', and more than 10 in every high power field grade as '+4'.									
AFB-acid-fast bacilli, EQA-External Quality Assessment, NPV- Negative Predictive Values, PPV- Positive Predictive Values, K- Kappa, P.F- Plasmodium falciparum, P.V- Plasmodium vivax.									

Laboratory Supplies and Equipment over the last six months prior to data collection

Of the surveyed facilities, all PHCU had at least one electric binocular microscope, centrifuge, and refrigerator. Otherwise, all PHCU had limited availability of the major types of laboratory equipment and consumables as illustrated in Table 2. About 28.6% of health facilities use both microscopy and RDT to detect malaria parasite. On the other hand, of the surveyed facilities with laboratory services, 57.2% reported stock problems on maintaining main supplies for smear microscopy examination over the last six months prior to this study. But, the survey indicated all PHCU was with the supply of staining solution for ZN technique, and staining solution for malaria during this study. None of the facilities surveyed had buffered water and only 9.5% of the surveyed facilities were with an analytical balance(Table 2).

Table 2

Major laboratory equipment and Supply for laboratory consumable in Guragae Zone Primary Health Care Unit, Southern Ethiopia, 2019

Equipment	Number of facilities with functional equipment	Number of facilities with non-functional equipment	Number of facilities without equipment
Light Microscope	21	-	-
Glass slides and Cover slides not reused	15(71.4%)	6(28.6%)	-
Refrigerator	21		
Glucometer	21		
Haemoglobinometer	2(9.5%)	9(42.9%)	10(47.6%)
Heamacue	2(9.5%)	-	19(90.5%)
Incubator	6(28.6%)	6(28.6%)	9(42.9%)
Fluorescent Microscope	2(9.5%)	-	19(90.5%)
Xpert machine	1(4.8%)	-	20(90.5%)
Centrifuge	21	-	-
Haematology analyzer	3(14.3%)	-	18(85.7%)
CD4 Counter	1(4.8%)	-	20(90.5%)
WBC Chamber	4(19%)	14(66.7%)	3(14.3%)
Vortex Mixer	2(9.5%)	-	19(90.5%)
Analytical balance	2(9.5%)		19(90.5%)
Laboratory consumables	Facilities with at least one available	Facilities with at least available but expired	Not available
Malaria rapid diagnostic test Kit	6(28.6%)	-	15(71.4%)
buffered water	-	-	21
Slide staining rack	21	-	-
Slide drying rack	21	-	-
DBS test KIT	21	-	-
Slide box	21		
Staining solution for malaria	21		
Staining solution for AFB	21		
HIV-1/2 antibody test Kit	20(95.2%)	1(4.8%)	
Cartridge	1(4.8%)	-	20(95.2%)

Equipment	Number of facilities with functional equipment	Number of facilities with non-functional equipment	Number of facilities without equipment
Funnels	3(14.3%)	2(9.5%)	16(76.2%)
Timer	3(14.3%)	2(9.5%)	16(76.2%)
Stains Solution for CBC and differential	7(33.3%)	-	14(66.7%)
Filter Paper	1(4.8%)	-	20(95.2%)
Glove	21	-	-
Gram staining Solution	8(38.1%)	5(23.8%)	8(38.1%)
Lancet	21	-	-
Hematocrit tube	21	-	-
Pastor Pipettes(Micro)	21	-	-
	Less than 7 days	7–14 days	Greater than 14 days
Stock out over the last six months	12(57.2%)	5(23.8%)	4(19%)

Discussion

There have been changes in laboratory diagnostic techniques that have been used for the diagnosis of different diseases. Direct smear microscopy is the most cost-effective and routinely implemented tool for diagnosing patients with tuberculosis and malaria and for monitoring their progress on treatment(19, 20). The sensitivity of the smear microscopy technique has been reported to be variable ranging from 20 to 60%(18). The lack of time and laboratory experts to make thorough searches of each field under the microscope is in part related to the low sensitivity of this method.

Errors in smear reading may result in failure to detect persons with tuberculosis and malaria(21, 22). Studies done in Pakistan and Congo showed that external quality assessment methods were found to be feasible and acceptable to improve the reliability of smear microscopy (23). EQA is essential to determine the source of performance problems and take remedial actions(6). EQA for smear microscopy is a process that assesses peripheral laboratory performance by higher-level laboratory and it includes blinded rechecking, on-site evaluation and panel testing(24).

Reliable laboratory services in resource-poor settings like Ethiopia are critical for meeting the health related goals. The development and execution of quality assured laboratory services at each tier of health care provision, from primary health centers to referral centers, are the very underpinnings to successful care and treatment of infectious diseases like malaria and tuberclosis (25). EQA program ensures reliable diagnosis that recognized as an important component of effective case management and control. However, an EQA program for malaria not recognized as an important method to improve the quality of smear microscopy services in study area with most surveyed peripheral laboratories failed to participate in the malaria EQA scheme.

The major problems in malaria diagnosis were reported to be low detection of smear-positive and over-diagnosis of smear-negative slides(26). In line with this, the present study found an overall false malaria smear reading of 12.2% with moderate agreement(87.8, K-0.6) between the first reader and controller. This may due to poor participation in malaria EQA scheme as only 9.5% claimed to have taken part in malaria EQA scheme. As an ISO 15189(27) document

requirement for quality and competence recommends, the urgent implementation of a mentoring program using short courses and work-based improvement projects could require for laboratory quality improvement.

Moreover, as Ethiopia has got closer to malaria elimination, malaria parasite species identification and detection should be sharpened to ensure accurate diagnosis and treatment. In contrast to this fact, the overall agreements of health facility laboratory in the present study on detection and identification of Plasmodium falciparum(P.F) and Plasmodium vivax(P.V) with reference readers were 73.3 and 71.4%, respectively, which was less than the national guideline recommendation(28). The result was also less than the study conducted in Africa which reported 82% parasite identification rate(29). However, similar parasite species identification rate was reported with the study conducted in North Gondar (77%)(30).

The reliability in blind rechecking of sputum smear microscopy is expected to be near 95%(31). In line with this fact AFB slides blinded rechecking in this study has found an overall agreement of 98.8%, which is in line with the one set by the WHO guideline. The excellent agreement with AFB sides the present study may be due to a strong AFB EQA scheme in the study area. The distinction indicated by the plus signs is is important for the treatment follow-up to the effectiveness of the medications prescribed by health professionals(32) while only 76.8% of study health facilities include the grading system for AFB in their report that requires urgent alteration.

In the Southern part of Ethiopia, the blinded rechecking study shows an overall false reading of 3.2% (33), and the major challenge found in the stated study was poor equipment and poor reagents (34). High-quality laboratory equipment and laboratory supplies are very important to provide accurate and reliable laboratory results. The physical infrastructure needed at each level for the laboratory to provide a safe and efficient work environment in which the physical space matches the equipment needed for laboratory assays(8), the supply chain of laboratory cosumables to prevent stock depletion(25). This aspect is often overlooked in SSA including Ethiopia, in agreement with this fact more than half surveyed PHCU in this study were reported stock problems on maintaining main supplies for essential laboratory services.

The limitations of this study include laboratory professionals at peripheral laboratories who may tend to retain slides with good quality staining regardless of the instruction before data collection which leads to slide selection bias. Moreover, due to budget limitations, the study could not evaluate the performance of health facilities regarding the quality of staining solutions preparation and staining procedures using panel slides.

Conclusions

Now is the time to build sustainable laboratory capacity in resource poor settings like Ethiopia that can be used to manage existing infectious diseases including malaria and tuberculosis(35). The main goal of the current malaria National Strategic Plan 2014–2020 in Ethiopia(36) is to eliminate malaria in selected low transmission areas by 2020. To achieve this goal, quality assured smear microscopy for malaria is crucial but malaria related technical problems were identified in this study. Thus, a continuous and strong malaria EQA schemes should be implemented at each laboratory to avoid reporting errors and produce quality results in study area. Only 9.5% of surveyed facilities participated in the malaria EQA scheme. Thus, the authors also believe that the responsible body needs to be aware that EQA is not a one-off expenditure, but is rather a recurring cost that must be factored in any discussion of quality and standards.

List Of Abbreviations

AFB-Acid Fast Bacilli, EQA-External Quality Assurance, K- Kappa, NPV- Negative Predictive Value, PPV-Positive Predictive Value, P.F- *Plasmodium falciparum*, P.V- *Plasmodium vivax*, PHCU-Primary Health Care Unit, SSA- Sub Saharan Africa, WHO-World Health Organization.

Declarations

Ethical Considerations

The study protocols were reviewed and approved by the Ethical Review Committee of Wolkite University (*Ref.No.156/2019*). Letters of permission were obtained from Guragea zone health department and each district's health office. The objectives, as well as the nature of the study, explained to each director of the selected PHCU.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

Funding: The study was sponsored by Wolkite University. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors Contributions: TS conceived the study, participated in the study design and coordinated the laboratory work. All authors contributed towards data analysis, drafting and critically revising the paper, and gave final approval of the version to be published. **Acknowledgments:** The authors would like to sincerely thank the head of the zonal and each district's health offices, health facility laboratory directors, data collectors, and health facility laboratory staff.

References

1. Bryce J, Boschi-Pinto C, Shibuya K, Black RE, WHO Child Health Epidemiology Reference Group. WHO estimates of the causes of death in children. *The Lancet*. 2005 Mar 26;365(9465):1147-52.
2. Weigl BH, Gaydos CA, Kost G, Beyette Jr FR, Sabourin S, Rompalo A, De Los Santos T, McMullan JT, Haller J. The value of clinical needs assessments for point-of-care diagnostics. Point of care. 2012 Jun;11(2):108.
3. Beastall GH. Adding value to laboratory medicine: a professional responsibility. Clinical chemistry and laboratory medicine. 2013 Jan 1;51(1):221-7.
4. Carter JY. External quality assessment in resource–limited countries. Biochemia medica. 2017 Feb 15;27(1):97-109.
5. Van Lerberghe W. The world health report 2008: primary health care: now more than ever. World Health Organization; 2008.
6. Petrose LG, Fisher AM, Douglas GP, Terry MA, Muula A, Chawani MS, Limula H, Driessen J. Assessing perceived challenges to laboratory testing at a Malawian referral hospital. *AM J TROP MED*. 2016 Jun 1;94(6):1426-32.
7. Petti CA, Polage CR, Quinn TC, Ronald AR, Sande MA. Laboratory medicine in Africa: a barrier to effective health care. *Clin Infect Dis*. 2006 Feb 1;42(3):377-82.
8. Mboera LE, Makundi EA, Kitua AY. Uncertainty in malaria control in Tanzania: crossroads and challenges for future interventions. *AM J TROP MED*. 2007 Dec 1;77(6_Suppl):112-8.

9. Hamer DH, Ndhlovu M, Zurovac D, Fox M, Yeboah-Antwi K, Chanda P, Sipilinyambe N, Simon JL, Snow RW. Improved diagnostic testing and malaria treatment practices in Zambia. *JAMA*. 2007 May 23;297(20):2227-31.
10. WHO. The role of laboratory diagnosis to support malaria disease management: Focus on the use of rapid diagnostic tests in areas of high transmission.World Health Organization; 2006.
11. D'Acremont V, Lengeler C, Mshinda H, Mtasiwa D, Tanner M, Genton B. Time to move from presumptive malaria treatment to laboratory-confirmed diagnosis and treatment in African children with fever. *PLoS Med*. 2009 Jan 6;6(1):e252.
12. Yao K, McKinney B, Murphy A, Rotz P, Wafula W, Sendagire H, Okui S, Nkengasong JN. Improving quality management systems of laboratories in developing countries: An innovative training approach to accelerate laboratory accreditation. *Am J Clin Pathol*. 2010 Sep 1;134(3):401-9.
13. Carter JY, Lema OE, Wangai MW, Munafu CG, Rees PH, Nyamongo JA. Laboratory testing improves diagnosis and treatment outcomes in primary health care facilities. *ASLM*. 2012;1(1).
14. Forsman RW. Why is the laboratory an afterthought for managed care organizations?. Clinical Chemistry. 1996 May 1;42(5):813-6.
15. Mesfin EA, Taye B, Belay G, Ashenafi A. The status of medical laboratory towards of AFRO-WHO accreditation process in government and private health facilities in Addis Ababa, Ethiopia. *PAMJ.* 2015;22(1).
16. VanVoorhis CW, Morgan BL. Understanding power and rules of thumb for determining sample sizes. Tutorials in quantitative methods for psychology. 2007 Nov;3(2):43-50.
17. Sarkinfada F, Aliyu Y, Chavasse C, Bates I. Impact of introducing integrated quality assessment for tuberculosis and malaria microscopy in Kano, Nigeria. *J INFECT DEV COUNTR*. 2009;3(1):20-7.
18. WHO. Malaria microscopy quality assurance manual-version 2. World Health Organization; 2016 Apr 5.
19. Perkins MD. New diagnostic tools for tuberculosis [The Eddie O'Brien Lecture]. *Int J Tuberc Lung Dis*. 2000 Dec 1;4(12):S182-8.
20. Pai M, Kalantri S, Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis: Part I. Latent tuberculosis. Expert review of molecular diagnostics. 2006 May 1;6(3):413-22.
21. Abreha T, Alemayehu B, Tadesse Y, Gebresillassie S, Tadesse A, Demeke L, Zewde F, Habtamu M, Tadesse M, Yadeta D, Teshome D. Malaria diagnostic capacity in health facilities in Ethiopia. *Malar. J*. 2014 Dec;13(1):292.
22. Mekonnen A. Smear-positive pulmonary tuberculosis and AFB examination practices according to the standard checklist of WHO's tuberculosis laboratory assessment tool in three governmental hospitals, Eastern Ethiopia. *BMC Res. Notes*. 2014 Dec;7(1):295.
23. Mukadi P, Gillet P, Lukuka A, Atua B, Sheshe N, Kanza A, Mayunda JB, Mongita B, Senga R, Ngoyi J, Muyembe JJ. External quality assessment of Giemsa-stained blood film microscopy for the diagnosis of malaria and sleeping sickness in the Democratic Republic of the Congo. Bulletin of the World Health Organization. 2013;91:441-8.
24. Bailey JW, Williams J, Bain BJ, Parker-Williams J, Chiodini PL, General Haematology Task Force of the British Committee for Standards in Haematology. Guideline: the laboratory diagnosis of malaria. *Br J Haematol*. 2013 Dec;163(5):573-80.
25. Bates, Imelda, and Kathryn Maitland. "Are laboratory services coming of age in sub-Saharan Africa?." (2006): 383-384.
26. Ayalew F, Tilahun B, Taye B. Performance evaluation of laboratory professionals on malaria microscopy in Hawassa Town, Southern Ethiopia. *BMC Res. Notes*. 2014 Dec;7(1):839.
27. Guzel O, Guner EI. ISO 15189 accreditation: Requirements for quality and competence of medical laboratories, experience of a laboratory I. Clinical biochemistry. 2009 Mar 1;42(4-5):274-8.

28. Federal Ministry of Health (FMoH). Guidelines for quality assurance of smear microscopy for tuberculosis diagnosis. Addis Ababa: Ethiopia.2009;1:23–30.
29. Frean J, Perovic O, Fensham V, McCarthy K, Gottberg AV, Gouveia LD, Poonsamy B, Dini L, Rossouw J, Keddy K, Alemu W. External quality assessment of national public health laboratories in Africa, 2002-2009. Bulletin of the World Health Organization. 2012;90:191-9.
30. Mitiku K, Mengistu G, Gelaw B. The reliability of blood film examination for malaria at the peripheral health unit. *Ethiop J Health Dev*. 2003;17(3):197-204.
31. Van Rie A, Fitzgerald D, Kabuya G, Van Deun A, Tabala M, Jarret N, Behets F, Bahati E. Sputum smear microscopy: evaluation of impact of training, microscope distribution, and use of external quality assessment guidelines for resource-poor settings. *Clin Microbiol Infect*. 2008 Mar 1;46(3):897-901.
32. Paramasivan CN, Venkataraman P, Vasanthan JS, Rahman F, Narayanan PR. Quality assurance studies in eight state tuberculosis laboratories in India. *Int J Tuberc Lung Dis*. 2003 Jun 1;7(6):522-7.
33. Shargie EB, Yassin MA, Lindtjørn B. Quality control of sputum microscopic examinations for acid fast bacilli in southern Ethiopia. *Ethiop J Health Dev*. 2005;19(2):104-8.
34. Khan MA, Walley JD, Munir MA, Khan MA, Khokar NG, Tahir Z, Nazir A, Shams N. District level external quality assurance (EQA) of malaria microscopy in Pakistan: pilot implementation and feasibility. *Malar. J*. 2011 Dec;10(1):45.
35. Birx D, de Souza M, Nkengasong JN. Laboratory challenges in the scaling up of HIV, TB, and malaria programs: the interaction of health and laboratory systems, clinical research, and service delivery. *Am J Clin Pathol*. 2009 Jun 1;131(6):849-51.
36. Bayissa, Gudissa Assefa. "ACCELERATE MALARIA CONTROL PROGRAM IMPLEMENTATION IN ETHIOPIA; STRENGTHS AND WEAKNESSES." (2016).