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Proficiency and associated factors of laboratory professionals in sputum smear microscopy at selected peripheral public and private diagnostic laboratories in Ethiopia: cross-sectional study

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Abstract

Background In countries with a high prevalence of TB, such as Ethiopia, direct sputum smear microscopy remains the most cost-effective tool for diagnosing patients with infectious tuberculosis and monitoring their progress on treatment. However, poor-quality sputum microscopy services may lead to the failure to detect persons with active tuberculosis and may cause unnecessary anti-TB treatment for non-TB cases. Proficiency level is the percentage agreement between participants'readings and the reference panel results. The aim of this study was to assess proficiency and associated factors of laboratory professionals in sputum smear microscopy for acid-fast bacilli at selected peripheral public and private diagnostic laboratories in East Gojjam Zone, Northwest Ethiopia, in 2023.

Method An institutional-based cross-sectional study design was conducted from March 2023 to June 2023 at selected peripheral public diagnostic laboratories in East Gojjam Zone. 65 laboratory professionals were selected randomly from 41 peripheral public diagnostic laboratories in the study area. A validated questionnaire and 10 panel slides were used as data collection tools. The panel consisted of 5 pre-stained and 5 unstained slides. Data were entered and analyzed using the Statistical Package for Social Sciences software (SPSS version 20). *P* values less than 0.05 were considered statistically significant when looking for associations between dependent and independent variables.

Result The overall proficiency level of laboratory professionals in tuberculosis smear microscopy was 81.92% with 95% CI [78.46–85.38]. Previous TB smear microscopy training, work experience, and institution of education had a significant association with the overall performance of laboratory professionals in TB smear microscopy.

Conclusion The overall TB smear microscopy performance level of laboratory professionals at peripheral diagnostic laboratories in Ethiopia, was satisfactory, indicating a good level of competence. However, notable technical errors related to smear reading and reporting were observed. Thus, higher education institutions, especially private institutions, and the Zonal Health Department, should implement educational and training interventions to address the identified gaps and ultimately contribute to the national TB control program.

Keywords Tuberculosis, AFB microscopy, Proficiency testing, AFB panel slide preparation

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Background

Tuberculosis (TB) is a communicable disease and one of the top 10 causes of death worldwide. TB is caused by the bacterium Mycobacterium tuberculosis, which spreads when people with active TB expel bacteria into the air, for example, by coughing. The disease typically affects the lungs (pulmonary TB) but can also affect other sites (extra pulmonary TB). TB can affect anyone anywhere, but most people who develop the disease (about 90%) are adults; there are more cases among men than women; and of those who fell sick with TB in 2019, 87% were in 30 high TB burden countries [1].

Ethiopia remains to be among the 30 countries reported with high burden of TB for 2015 to 2020. TB related mortality is highlighted in the top ten reported causes of death among hospital admissions, with annual estimated death rate of 26 per 100,000 populations in 2015 [2]. According to the first Ethiopian national population-based tuberculosis prevalence survey (1010–2011), the prevalence of smear-positive TB among persons aged 15 years and above was 108/100, 000, whereas the prevalence of bacteriologically confirmed TB in the same age group was 277/100, 000 [3].

In countries with high prevalence of TB like Ethiopia, direct sputum smear microscopy remains the most cost-effective tool for diagnosing patients with infectious tuberculosis and for monitoring of their progress on treatment [2] Sputum microscopy is an essential component of the DOTS strategy recommended by the World Health Organization (WHO) and the International Union against Tuberculosis and Lung Disease (IUATLD) [4]. DOTS, relies on a network of laboratories to provide readily accessible and quality laboratory services to prevent the spread of infection in the community and prevent unnecessary treatment of cases who do not have TB [5].

Fluorescence microscopy (FM) using auramine staining has been shown to have 10% higher sensitivity and decreased the time required for sputum examination as compared to routine light microscopy using Ziehl–Neelsen (ZN) staining, without compromising specificity in detecting AFB in sputum smear microscopy [6, 7].

Poor quality sputum microscopy services may result in failure to detect persons with active tuberculosis and unnecessary anti-TB treatment for non-TB cases. In addition, errors in reading sputum microscopy may result in prolonged treatment, or unnecessary treatment termination which predisposes the development of drug resistant tuberculosis (MDR-TB) [8, 9].

For accurate and reliable laboratory diagnosis to be provided, an efficient, excellent and quality assured system should be maintained in the laboratory. The testing system must be monitored to ensure the quality of the overall process, so as to detect and reduce errors, and to improve laboratory performance across all laboratory testing sites [10].

Quality assurance system for the sputum microscopy is aimed at minimizing the false positive and false negative results in the laboratory. Failure of maintaining quality laboratory services would result in loss of credibility and waste of precious resources at individual level and inaccurate data and poor performance of the programme, in terms of case detection at the national level. According to the recommendations made by the national tuberculosis guideline, the quality assurance system for sputum smear microscopy includes; internal quality control, quality improvement and external quality assessment [11].

External Quality Assurance is a system designed to monitor the performance of laboratories in different methods by external body. The three components of external quality assessment (EQA) of acid-fast bacilli (AFB) smear microscopy are panel testing, blinded rechecking and on-site evaluation using a standard checklist. In panel (proficiency), testing programme, stained and unstained smear centrally prepared slides must be sent to peripheral laboratories to evaluate the ability of laboratory professionals to stain and/or read smears [12, 13].

In light of the above facts, so far there is only limited evidences or published study concerning the proficiency level of laboratory professionals on AFB smear microscopy at peripheral public and diagnostic laboratories in Ethiopia. Therefore, this study aimed to assess the proficiency and associated factors of laboratory professionals in sputum smear microscopy at selected peripheral public and private diagnostic laboratories in Ethiopia.

Methods and materials

Study area

The study was conducted at selected peripheral public and private diagnostic laboratories of East Gojjam zone, Amhara regional state, Ethiopia. East Gojjam zone is located to the northwest of Ethiopia, which is 299 km away from the capital city, Addis Ababa and 265 km from Bahir Dar, a capital city of Amhara region. The zone has about 14,010 km^2 area and has 20 administrative districts (16 rural administrative district and 4 town administrative) with an estimated population of 2,740,625. The zone has 1 comprehensive specialized hospital, 9 district hospitals, 104 health centers and 147 private health clinics with a total of about 1650 health professionals of which about 226 laboratory professionals are working at peripheral public and private diagnostic laboratories of East Gojjam zone.

Study design and period

An institutional based cross sectional study design was conducted from March 2023 to June 2023 at selected peripheral public diagnostic laboratories of East Gojjam Zone. The peripheral labs were selected randomly from a comprehensive list of public and private facilities in the East Gojjam zone.

Population

Source population

All laboratory professionals working at all peripheral public and private diagnostic laboratories of East Gojjam Zone, Northwest Ethiopia.

Study population

Laboratory professionals who were randomly selected from selected peripheral public and private diagnostic laboratories of East Gojjam Zone, Northwest Ethiopia.

Inclusion and exclusion criteria

Inclusion criteria

Laboratory professionals working in their profession and volunteering to participate in the study at selected peripheral public and private diagnostic laboratories in the East Gojjam Zone were included in the study.

Exclusion criteria

Laboratory professionals who were working at managerial level at selected peripheral public diagnostic laboratories of East Gojjam Zone were excluded from our study. Managers were excluded because the focus of the study was on laboratory professionals directly involved in routine sputum smear microscopy.

Variables of the study

Dependent variable

Proficiency level of laboratory professionals in sputum smear microscopy for acid-fast bacilli was considered as dependent variable. Proficiency level is the percentage agreement between participants' readings and the reference panel results.

Independent variables

Educational status, institution of education work experience and training status were considered as independent variables.

Sample size and sampling technique

The number of peripheral public and private diagnostic laboratories was determined with according to suggested rule of Thumb [13] According to this rule, if number of units/facilities is large (500–1000), take a 10% sample. If it is medium size (100–500), take 20–30% sample and if it is very small (< 50), take 31–50% of the sample. There are about 138 peripheral diagnostic laboratories (85 health center and 53 private medium and specialty laboratories) excluding hospital, EQA center health center laboratories and lower private clinics in the study area. Therefore, the maximum sample size of peripheral diagnostic laboratories in the study area was 41(30% of 138) since 138 is within 100–500. Then, 41 peripheral diagnostic laboratories and 65 laboratory professionals were selected randomly from the study area.

Data collection and laboratory methods

Data collection

The questionnaire used in our study was developed for this study (annex v). Short-term training or orientation was provided to data collectors about the objectives of the study, participants'rights &confidentiality, preparation of proficiency testing slide, transportation, storage and how to approach and interview participants before the actual data collection. Study participants who agreed to give written consent after being informed about the purpose of the study were interviewed and finally 10 PT (5 stained and 5 un stained) validated slides were given to examine with microscopy.

Laboratory methods

Panel testing slide preparation

Specimens with known positive and negative results from leftover sputum samples, which had been tested using the GeneXpert machine, were collected from the Debre Markos Comprehensive Specialized Referral Hospital TB laboratory and transported to the Amhara Public Health Institute for culture to confirm TB positive and TB negative before being used for PT slide preparation. Then, panel slides were prepared using negative and positive stock according to the PT manual (annex-V) by trained laboratory professionals on panel slide preparation.

Panel testing smears were prepared using sodium hydroxide (NaOH) method described by international procedures [14]. To prepare positive panel smears, 3 ml of AFB positive specimen (greater or equal to 2+ AFB ZN direct smear) was placed into a 50 ml screw cap plastic tube, one drop of 40% Formaldehyde was added per 1 ml of sputum and vortexed well, incubated for one hour at room temperature(25–30 ^{0}C), then 1 ml of 4% NaOH was added vortexed thoroughly for 4–5 min, up to 20 ml of distill water was added and mixed well, incubated in a water bath for 30 min at 56–60 ^{0}C, occasionally it was mixed by inverting the tube during incubation, distill water was added to a total volume of 40 ml and mixed by inversion; centrifuged at 3000 g for 20 min at room temperature (25- 30 ^{0}C),then the supernatant was decanted

carefully; 0.5–1 ml of distill water was added to suspend pellets.

To prepare negative panel smears, negative specimens (fresh specimens, no more than 2 days) which was 5 ml or above, white to green, an AFB negative specimen with 20 or more white blood cells per field and the thickness of sputum which was less mucous specimens were preferred to increase consistency. 3–4 ml aliquots of AFB-negative sputum was distributed into 50 ml screw cap tubes, several good quality negative sputa were pooled together and then divided into 3 ml aliquots, then 1 drop (approx. 50 µl) of 40% formaldehyde was added per 1 ml of sputum, vortexed well, incubated for 1 h at room temperature (25–30 ^{0}C), 1 ml of 4% NaOH (if the sputum was too thick, up to 2 ml of NaOH) solution was added that the final concentration of NaOH was always 1–2%), Vortexed for 2–3 min, up to 20 ml of distilled water was added mixed well, and incubated in a water bath for 10 min. at 55–60 ^{0}C.

A set of 10 panel slides (5 positive and 5 negative) for each study participant(10 ×65 =650) were prepared from the sediment by taking1 - 3 drops with a wire loop, and smearing was made on a microscope slide and dried in the air. The positive panel slide smears were prepared by dilution technique as 1 slide 3+, 1 slide 2+, 1 slide 1+ and 2 slides scanty. Then the total 650 prepared panel slides (325 stained and 325 unstained) were validated by senior and qualified laboratory professionals before distributing to the peripheral diagnostic laboratory.

Panel slide reading, interpretation and score grading

Ten validated panel slides were distributed to each study participant along with a prepared reporting format for reading. All technicians received identical sets of slides with a standard range of smear grades (e.g., 3+, 2+, 1+, scanty, and negative slides). The results of the study participants were classified as correct, minor error, or major error (Table 1).

Set of 10 panel testing slides, each slide carries 10 points, total possible score =100 (for 10 slides). Committing major error (HFP and HFN) result in a score of 0 whereas minor error (LFP, LFN and QE) result in a scores of 5 points. The overall passing score was 80 points and poor performance was <80% [15, 16].

Sensitivity and specificity were also calculated using results from reference readers as the gold standard.

Data quality assurance

Appropriate training was given for data collectors regarding socio-demographic, laboratory test procedures, the quality control and reporting of panel slide results. Data quality was assured through use of standardized data collection materials, pretesting of the questionnaires, checking for its completeness, accuracy and consistency with intensive supervision during data collection by the principal investigator.

Generally, the quality of sputum sample collection for panel slide, smear preparation, staining and reagents were assured according to national guideline [17].

Table 1 Evaluation and interpretation of errors between the study participant and controller

Result of study participant	Result of controller				
	Negative	**Scanty**	**1+**	**2+**	**3+**
Negative	Correct	LFN	HFN	HFN	HFN
Scanty	LFP	Correct	Correct	QE	QE
1+	HFP	Correct	Correct	Correct	QE
2+	HFP	QE	Correct	Correct	Correct
3+	HFP	QE	QE	Correct	Correct

Data analysis and interpretation

Data were be entered and analyzed using the Statistical Package for Social Sciences software (SPSS version 20). Each laboratory professional was evaluated with percentage of major errors (HFP&HEN) and minor errors (LFP, LFN &QE). Chi square test was used to see any association with different variable. *P* values less than 0.05 will be taken as statistically significant when looking for association between dependent and independent variables.

Result

Socio-demographic characteristics of study participants

In this study, 9 laboratory professionals from nine private health institutions and 56 laboratory professionals from 32 governmental health institutions with a total of 65 study participants were included. From the study participants, 46.2% were males and 53.8% were females. 86.2% of the study participants had educational level of diploma where as 13.8% of study participants had educational level of degree. 44.6% and 55.4% of laboratory professionals were graduated from governmental and private institution of education respectively. 41.5% of study participants were taking training on TB microscopy. Majority (44.6%) of study participants had work experience of 2–5 years (Table 2).

Performance of laboratory professionals in panel testing

A total of 650 validated proficiency panel testing slides (n= 325 positive slides and n= 325 negative slides) with different grade level of bacilli were read by 65 laboratory professionals who were working at governmental and private peripheral laboratories. The overall proficiency level of laboratory professionals in tuberculosis smear

Table 2 Socio-demographic characteristics of study participants working at peripheral laboratories of East Gojjam Zone, North West Ethiopia, 2023

Variables	Categories	Frequency (n)	Percentage (%)
Sex	Male	30	46.2
	Female	35	53.8
Educational level	Diploma	56	86.2
	Degree	9	13.8
	Master	0	0
Institution of education	Government	29	44.6
	Private	36	55.4
Working institution	Government	56	86.2
	Private	9	13.8
Work experience	< 2 years	22	33.8
	2–5 years	29	44.6
	> 5 years	14	21.5
TB microscopy training	Yes	27	41.5
	No	38	58.5

Table 3 Over all proficiency level of laboratory professionals in sputum smear microscopy for acid fast bacilli working at peripheral laboratories of East Gojjam Zone, North West Ethiopia, 2023

Variables	Categories	Performance Score (%)	95% CI
Sex	Male	82.33	77.56—87.11
	Female	81.57	76.39–86.75
Educational level	Diploma	81.61	77.64–85.57
	Degree	83.89	78.53–89.25
Institution of education	Government	88.62	84.49–92.75
	Private	76.67	71.93–84.40
Working institution	Government	81.96	78.16–85.77
	Private	82.22	72.22–92.23
Work experience	< 2 years	70.91	64.36–77.46
	2–5 years	86.38	82.94–89.82
	> 5 years	90.36	84.53–96.18
TB microscopy training	Yes	90.19	86.45–93.93
	No	76.05	71.53–80.58
Total performance score in TB microscopy		81.92	78.46–85.38

microscopy was 81.92% with 95% CI [78.46–85.38]. laboratory professional graduated from governmental institution scored 88.62% with 95% CI [84.49–92.75] while laboratory professionals graduated from private institutions scored 76.67% with 95% CI [71.93–84.40]. While laboratory professionals with above 5 years work experiences scored 90.36% with 95% CI [84.53–96.18], laboratory professionals with less than 2 years' work experience scored 70.91% with CI [64.36–77.46]. Laboratory professionals who were taking training on TB smear microscopy scored 90.19% with 95% CI [86.45–93.93]. However, laboratory professionals who did not take training on TB smear microscopy scored 76.05% with 95% CI [78.46–85.38] (Table 3).

The overall sensitivity, specificity, positive predictive value and negative predictive value in detecting tuberculosis bacilli was 73.23%, 92.92%, 91.26% and 77.74% respectively. The overall percent agreement of study participants with the reference readers was 83.08% (kappa = 0.66) (Table 4).

Of the total 650 panel slides, 173 slides were miss read with study participants, which means the total error rate committed by study participants was 26.61%. Overall, there was 18((2.77%) HFP, 43(6.61%) HFN, 5(0.77%) LFP, 44(6.77%) LFN and 63(9.69%) quantification errors committed by study participants (Table 5).

Factors associated with performance of study participants in TB smear microscopy

Of the total 65 study participants, 46(70.8%) laboratory professionals got pass score (≥ 80%). The Chi-square test showed that institution of education, work experience and TB microscopy training had statistically significant association with TB smear microscopy performance($P < 0.05$). However, sex, educational level and working institutions had no statistically significant association with TB smear microscopy performance (Table 6).

Discussion

Although other diagnostic tools have been available, sputum smear microscopy is still widely used in Ethiopia to diagnose a person infected with pulmonary tuberculosis. Thus, having and maintaining an acceptable level of proficiency in sputum smear microscopy is very important for the successful national tuberculosis control program.

In this study, the overall proficiency level of laboratory professionals in TB smear microscopy was found to be 81.92%, indicating a relatively good level of competence [16]. How ever, this finding is relatively lower than reports(87.1%) from another study conducted at the three earliest universities of Ethiopia [18].

The sensitivity and specificity of laboratory professionals in detecting TB bacilli were 73.23% and 92.92%, respectively. These figures indicate a reasonably high level of specificity but suggest room for improvement in sensitivity. Eventhough the specificity of TB smear microscopy observed in this study is consistent with different previous study reports [19–21], efforts should be made to enhance sensitivity to ensure accurate detection, especially considering the potential consequences of false negatives.

Table 4 Overall sensitivity, specificity, predictive value and percent agreement in detecting TB bacilli at selected peripheral public and private diagnostic laboratories of East Gojjam Zone, Northwest Ethiopia, 2023

Health institution	Participant reader	Reference reader		Total	Sensitivity	Specificity	PPV*	NPV**	% Agreement	kappa
		Positive	Negative							
Government	Positive	205	19	224	73.2	93.2	91.5	77.7	83.2	0.67
	Negative	75	261	336						
	Total	280	280	560						
Private	Positive	33	4	37	73.3	91.1	89.2	77.4	82.2	0.64
	Negative	12	41	53						
	Total	45	45	90						
Total	Positive	238	23	261	73.23	92.92	91.26	77.74	83.08	0.66
	Negative	87	302	389						
	Total	325	325	650						

Table 5 Type and quantity of errors committed by laboratory professionals in TB smear microscopy at selected peripheral public and private diagnostic laboratories of East Gojjam Zone, North west Ethiopia, 2023

Institution	Type of errors					Total errors N (%)
	Major errors		Minor errors			
	HFP N (%)	HFN N (%)	LFP N (%)	LFN N (%)	QE N (%)	
Government(*N* = 560)	16(2.85)	37(6.60)	3((0.53)	38(6.78)	55(9.82)	149(26.60)
Private (*N* = 90)	2(2.22)	6(6.67)	2(2.22)	6(6.67)	8((8.89)	24(26.66)
Total (*N* = 650)	18((2.77)	43(6.61)	5(0.77)	44(6.77)	63(9.69)	173(26.61)

Where N is number of slide and percentage was calculated from the total slide number given for each health institution

Table 6 Association between demographic characteristics and performance of laboratory professionals in TB smear microscopy at selected peripheral public and private diagnostic laboratories of East Gojjam Zone, North west Ethiopia, 2023

Variables	Categories	Passed ≥ 80% N (%)	Failed < 80% N (%)	Chi-square	Degree of freedom	*P*- value
Sex	Male	22(73.3)	8(26.7)	0.177	1	0.674
	Female	24(68.6)	11(31.4)			
Educational level	Diploma	38(67.9)	18(32.1)	1.658	1	0.198
	Degree	8(88.9)	1(11.1)			
Institution of education	Government	25(86.2)	4(13.8)	6.302	1	0.014
	Private	21(58.3)	15(41.7)			
Working institution	Government	40(71.4)	16(28.6)	0.085	1	0.771
	Private	6(66.7)	3(33.3)			
Work experience	< 2 years	7(31.8)	15(68.2)	24.437	2	0.000
	2–5 years	26(89.7)	3(10.3)			
	> 5 years	13(92.9)	1(7.1)			
TB microscopy training	Yes	26(96.3)	1(3.7)	14.548	2	0.000
	No	20(52.6)	18(47.4)			
Overall performance in TB microscopy		46(70.8)	19(29.2)			

The findings of this study showed that the overall agreement between study participants and reference readers was 83.08%, with a kappa statistic of 0.66, suggesting substantial agreement. However, the agreement level of this study is slightly lower than the study reports (87.1% and 88.2%) conducted in Ethiopia and

Malaysia respectivel [22, 23]. The difference may be due to the difference in the study area settings.

Similarly, in this study, considerable proportion (26.61%) of panel slides were misread by participants, indicating the occurrence of major errors(9.38%) and minor errors (17.23%). Eventhough the total errors reported (26.61%) in this study is slightly lower than the study results(40.5% and 29.5%) conducted at Addis Ababa and Hawassa respectively, it is found to be higher than the study report(13.74%) conducted in the southern Ethiopia [19, 21, 24]. These errors highlight areas for improvement in TB laboratory practice, emphasizing the importance offering TB smear microscopy training to all laboratory professionals to increase the overall performance.

Analysis of the association between demographic characteristics and TB smear microscopy performance revealed significant correlations with institution of education, work experience, and TB microscopy training. Participants who graduated from governmental institutions, those with longer work experience, and those undergoing TB microscopy training demonstrated better performance in TB smear microscopy. One study conducted at three earliest Universities of Ethiopia showed that majority of students did not get adequate training on AFB smear reading and grading [18] In addition, another study conducted at southern region of Ethiopia noted that the most probable reason to under performance in smaer microscopy was lack of refreshment training [25]. As noted by a study conducted at Kinshasa, marked improvemet in smear microscopy can be achived with adequate practical training on AFB smear microscopy [26]. These all findings underscore the importance of educational and training interventions in enhancing the competence of laboratory professionals, particularly in resource-limited settings.

Conclusions and recommendation

The overall TB smear microscopy performance level of laboratory professionals at peripheral diagnostic laboratories in the East Gojjam Zone, Northwest Ethiopia, was satisfactory, indicating a good level of competence. However, notable technical errors related to smear reading and reporting were observed. Previous TB smear microscopy training, work experience, and institution of education had a significant association with the overall performance of laboratory professionals in TB smear microscopy. Therefore, higher education institutions, especially private ones, along with the Zonal Health Department, should implement educational and training interventions to address the identified gaps and ultimately contribute to the national TB control program.

Abbreviations

AFB	Acid Fast Bacilli
EQA	External Quality Assessment
FM	Fluorescent Microscopy
HFN	High False Negative
HFP	High False Positive
LFN	Low False Negative
LFP	Low False Positive
PT	Proficiency Testing
QA	Quality Assurance
QC	Quality Control
QE	Quantification Error
TB	Tuberculosis
ZN	Ziehel-Neelsen

Supplementary Information

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Supplementary Material 1.

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Authors' contributions

B.M contributed for the conception and design of the work M.B and Z.H wrote the main manuscript A.F prepared figures and tables.

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Data availability

Data cannot be shared openly but are available on request from authors.

Declarations

Ethics approval and consent to participate

The study was reviewed and approved by Research and Ethical Review Committee of College of Health Sciences, Debre Markos University (reference number HSC/RCS/192/11/12). The methods used in the study were performed in accordance with the guidelines and regulations. Informed consent was obtained from all study participants.

Consent for publication

Not aplicable.

Competing interests

The authors declare no competing interests.

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