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Acid Fast Bacilli Smear Microscopy Proficiency of 2012 GC Graduating Batch Students in the Field of Medical Laboratory Science in the Three Earliest Universities of Ethiopia

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ABSTRACT

Objective: To assess performance of graduating batch students in field of Medical Laboratory Science in detecting and quantifying Acid Fast Bacilli using sputum smear microscopy. **Methodology:** A total of 124 Medical laboratory sciences 2012 GC. Graduating batch students were evaluated by proficiency testing (PT) consisting of 5 stained slides with known grade of acid fast bacilli (AFB) from February to June 2012 in three earliest universities of Ethiopia. **Results:** Of 124 medical laboratory Science 2012 graduating batch students evaluated by proficiency test (PT), the mean score was 87.1% and overall PT score ranged from 40-100%.Overall there were 24(19.1%) major errors and 117 (81%) minor error. From panel slide reading, the total numbers of students that report major error are 18(14.5%) and there were a total of 80(64.5%) students that report minor error. **Conclusion:** From this study, the proficiency to read sputum smear by graduating batch students who have taken pre-service training (students from University-002) were better than the rest (University-001 and University-003) which hasn't taken pre-service training yet. Students that report major errors are, 6(12.8%), 1(5.3%), 11(19.0%) for university-001, 002and 003 respectively. Thus, the study has highlights the importance of training in improving the microscopy results.

Keywords: Proficiency Panel Testing, Sputum smear microscopy, blind rechecking, EQA

Introduction

Tuberculosis (TB) is a contagious, airborne disease caused by Mycobacterium tuberculosis. It is a disease of poverty affecting mostly young adults in their most productive ages. The vast majority of TB deaths are in the developing world and thus 1.7 million people died from TB (including 380 000 women) in 2009, including 380 000 people with HIV, equal to 4700 deaths a day [1]. Microscopy remains the mainstay of rapid TB case detection, especially for those patients who are most infectious to others, with the bacterial load involved often reflecting the extent of disease requiring immediate treatment.

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In most countries, especially those with the highest burden of TB, the direct Ziehl–Neelsen (ZN) smear is still the most common test. However, its sensitivity depends on the diligence of the technician and on use of the appropriate technique. The co-epidemics of Human Immunodeficiency Virus (HIV) infection and TB, especially in Africa, and concerns that the ZN smear has lower sensitivity in those with HIV infection, have stimulated interest in practical methods to improve microscopy [2,3].There are three methods that can and should be combined to evaluate laboratory performance: on-site Evaluation, panel testing and blinded rechecking. On case of on-site evaluation, the peripheral laboratories are visited by trained laboratory personnel from the reference or intermediate laboratories. These visits allow for the observation of worker performance under actual conditions, including condition of equipment, laboratory safety, adequacy of supplies, and the process for smearing, staining, reading, recording and reporting. When problems are detected,

solutions can be suggested and potentially implemented immediately [4,5].A panel testing exercise usually involves sending test panels with an identical composition (of negatives and positives) to many laboratories at the same time. So that technicians do not expect the same composition of slides each time, there must be variation in the slide sets (number of positives and negatives) sent with each new panel testing exercise[4]. Panel testing is useful to supplement rechecking programs, provide some preliminary data on peripheral laboratory capabilities prior to implementing a rechecking program, assess current status of performance or to quickly detect problems associated with very poor performance, evaluate proficiency of laboratory technicians following training, monitor performance of individuals when adequate resources are not available to implement a rechecking program (5).Tuberculosis can be controlled successfully only in the context of a National Tuberculosis Program (NTP). The first priority of the NTP is case detection and cure by reliable diagnosis and effective treatment. Since case finding relies heavily on laboratory diagnosis, tuberculosis bacteriology is a fundamental component of a national TB control program, including successful implementation of Directly Observed Treatment Short-course (DOTS).Inconsistent laboratory results, reports which correlate poorly with clinical data and reports which are difficult to interpret, often due to lack of awareness of the reason for their occurrence, lead physicians to rely excessively on radiology for management of TB. Therefore, quality control of sputum smear microscopy must be part of a well functioning TB laboratory network [6].Medical Laboratory Technology students learned in higher educational institute should have a good competency in practical performance specifically on TB detection and grading which is currently a sensitive issue. As NTP recommend, for accurate TB detection and early treatment, immense work must be done on increasing detection ability of Laboratory personnel. Therefore, this study provide clue to schools and particularly to policy makers to formulate programs that can fill the gap of the students.

Global sputum microscopy proficiency test perspective

Sputum smear microscopy for AFB is considered to be the most appropriate method for case finding in TB control program. Quality control of their results therefore seems indispensable. Africa home to 11%of the world's population carries 29% of the global burden of tuberculosis cases and 34%of related deaths, and the challenges of controlling the disease in the region have never been greater with the emergence of HIV infection [7].A study conducted by Paresh *et al* indicates that there was a high level of concordance in Z-N smear grading found between microbiologist and district laboratory staff. District tuberculosis Laboratory center (DTLC) readers reported overall consistency level of more than 98% in Z-N grade agreement. The tendency to over-grade the panel slides was much higher (more than 22%) as compared to under-grade (less than 2%) them in "correct slides"[10].In Cebu Provinces, Philippines in 1997, 90% of rural health units participated in the quality control activity. The proportion of good quality smear increased markedly and the FP and FN rates did not change during the period, but most of the FN was observed among the scanty positives of the field reading and no FN were noted, among the heavily positives slides [12].Study conducted in Northern Province of South Africa, at March and July 2000 for the first round and second round respectively in 21 province laboratory showed that, overall performance of first round laboratories was 85.5% and the second round was 95%. The false positive and false negative rate was 20.5% and 9.4% respectively. The sensitivity and specificity was 92.1% and 76.3% respectively. For the second round quality assessment overall agreement of peripheral laboratory and central laboratory was 97.4% and overall false reading rate was 2.63%. Sensitivity and specificity of their performance was 96.5% and 100% respectively [13].The study undergone in Southern Ethiopia by Tadesse *et al* showed that, of the 60 laboratory professionals evaluated by Proficiency test, 10(16.7) scored less than 80 percent, 9(15%) marginal score (60 -70) and 1(1.67%) poor score(<60). The mean score was 87.9%. The overall proficiency test score range score range from 55-100%. The sensitivity, specificity, positive predictive value and negative predictive value of reading smears were 63.9%, 97.1%, 93.8% and 61.9% respectively [14].

Methods and Materials

A cross-sectional study was conducted to assess the performance of graduating batch students on identification and grading of AFB sputum smear. The study was conducted among 124 graduating batch students of medical laboratory science of the three earliest universities of Ethiopia, namely Addis Ababa University, Jimma University and University of Gondar. The ethical committee of Addis Ababa

University, School of Medical Laboratory Science approved the study protocol. The study participants were given unique identification (code) and their post assessment result is kept confidential. Duration of the study was from February 01 to June 25, 2012. Percentage of Major error (HFP and HFN) and minor error (LFP, LFN and QE) are dependent variable and grade of bacteriology course, sex and age of the student are independent variable of the study.

Data Collection

Data Collection Tools

Panel slides are the major tool that was used to collect the information concerned with study participants. 500 panel slides with different grading scales (negative, scarce, 1+, 2+ and 3+) were prepared following WHO standard operating procedure in four batches at National Tuberculosis Reference Laboratory, Ethiopian Health and Nutrition Research Institute by principal investigators under continues supervision of senior technologists. Validation was done for single batch by senior technologists. One batch contains 100 panel slides. Therefore, five times validation was carried out prior to staining. All slides were stained using ZiehlNeelsen staining procedure. Microscopes used for the study were checked for their proper function by laboratory assistants of the respective universities where the research was conducted and by principal investigators as well.

Data Collection Procedures

Five stained panel slides were provided to each study participants for sputum smear microscopy examination. One slide from each grade/ level (negative, scanty, +1, +2 and +3) was provided to the participants and each participant are allowed to read and grade one slide within five minutes. The proficiency results filled on result report format by study participant.

Data Entry and Analysis

After completing data collection, the data was entered using *Epidata version 3.1database* and exported to *SPSS version 16.* Percentage of major error (HFP and HFN) and minor error (LFP, LFN and QE) was calculated.

Result

Of 124 medical laboratory Science 2012GC graduating batch students evaluated by proficiency test (PT), 84(67.7%) scored greater than 90%, 21(16.9%) scored less than 80%,15(12.1%) marginal score (60-79%) and 6(4.8%) poor score (less than 60%). The mean score was 87.1% and overall PT score ranged from 40-100%.

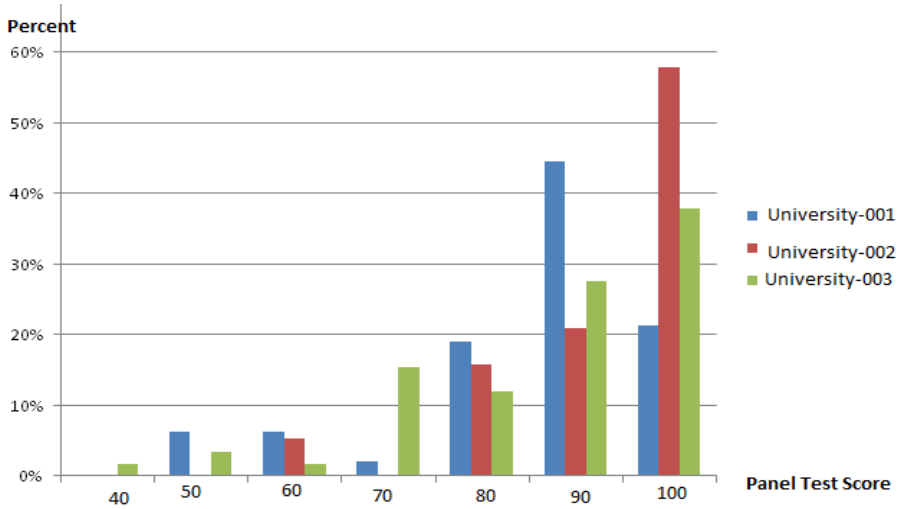

Figure1: Proficiency results of the three university students

From University-001, of 47 students evaluated by PT, 31(66%) scored greater than 90%, 7(14.9%) scored less than 80%, 4(8.51%) marginal score (60-79%), and 3(6.38%) poor score (less than 60%). The mean score was 85.34% and over all PT score ranged from 50-100%.

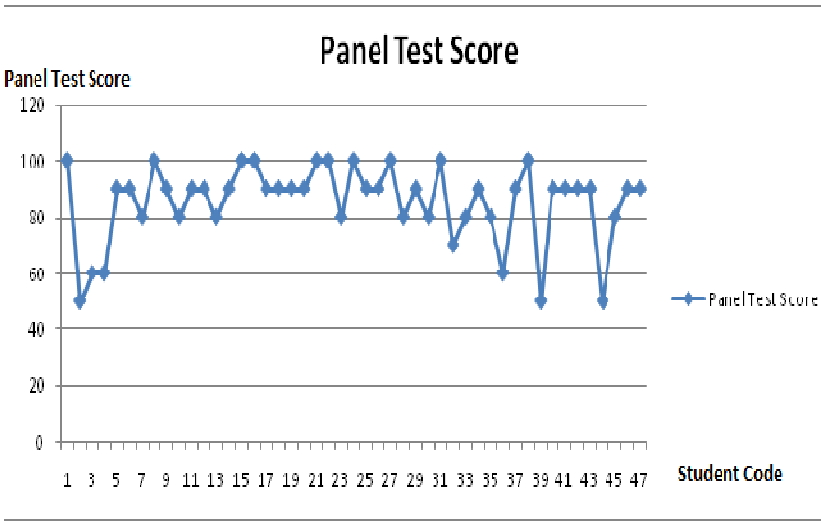

Figure 2: Perform ancerating ofUniversity-001students

From University-002, a total of 19 students participated in the study. Among the participant 15(78.9%) scored greater than 90%, 1(5.26%) scored less than 80%, 1(5.26%) marginal score (60-70%) and no students score less than 60%. The mean score in this university was 92.6% and overall PT result ranged from 60-100%.

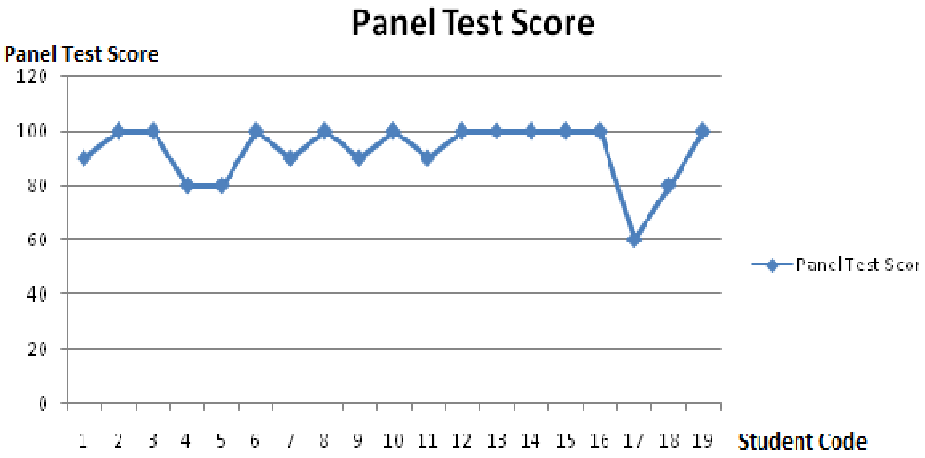

Figure 3: Performance rating of University-002 students

University-003, of 58 students evaluated by PT, 38(66.5%) scored greater than 90%, 13(22.4%) scored less than 80%, 10(17.2%) marginal score (60-79%) and 4(6.9%) poor score (less than 60%). In this university the mean score was 86.7% and overall PT score ranged from 40-100%.

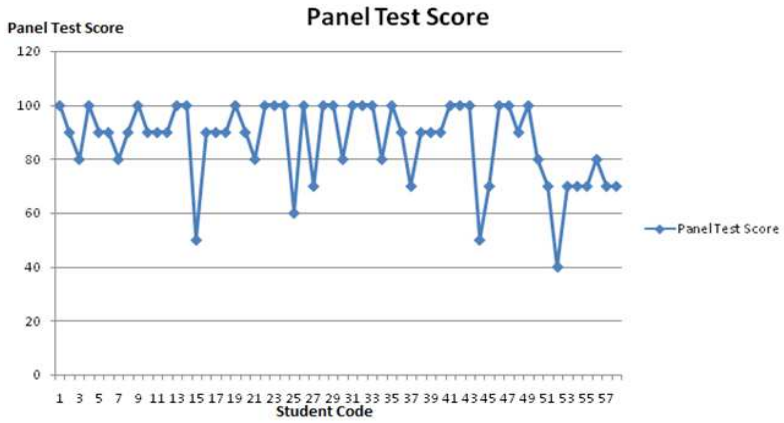

Figure 4: Perform ancerating of University-003students

From panel slide reading, major errors (HFP and HFN errors) were observed, with HFN results being much frequent than HFP results. Minor errors (i.e., LFP, LFN and QEs) were observed in the majority of PT results LFN being more frequently observed than LFP and QEs.In university-001, there were 8 (6.4%) major errors (6(4.8%) HFN and 2 (1.6%) HFP) and 46 (36.6%) minor errors 3 (2.4%) LFP, 24(19.0%) LFN and 19 (15.1%) QE). In university-002, there was 1 (0.8%) major error, 1 (0.8%) HFN) and 11 (8.8%) minor error (2(1.6%) LFP, (6(4.8%) LFN and 3 (2.4%) QE). No HFP observed among study participants from university-002. Major errors account 15 (12%) i.e10 (8%) HFN and 5 (4%) HFP for study participants from university-003.35(35.7%) mirror errors (29(23%) LFN and 16 (12.7%) QE). No LFP observed among study participants from university-003.

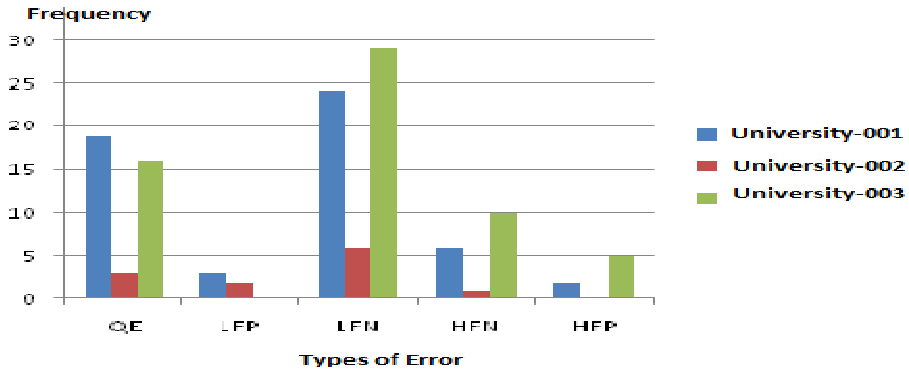

Figure 5: Types and frequency of errors reported in the three university students

From panel slide reading, the total number of students that report major error are 18(14.5%) i.e. (14(11.3%) HFN, 2(1.6%) HFP and 2(1.6%) HFN and HFP). From university-001, 6(12.8%) students report major error (4(8.5%) HFNand 2(4.3%) both HFP and HFN. 1(5.3%) students from university-002, report major (1(5.6%) HFN).From university-003, 11(19.0%) of students report major error (2(3.4%) HFP and 9(15.5%) HFN).In this study, there were a total of 80(64.5%) students that report minor error (10(8.1%) LFP, 31(25.0%) LFN, 14(11.3%) QE and others report multi error (19(15.5%) LFP and QE, 4(3.2%) LFN and QE and 2(1.6%) LFP and LFP).A total of 36(76.6%) of university-001 students report minor errors (3(6.1%) LFP, 14(29.8%) LFN, 9(19.1%) QE and 10(21.3%) LFP and QE). From university-002, 8(42.1%) students report minor errors (4(21%) LFN, 1(5.3%) QE, 1(5.3%) LFP and QE, 1(5.3%) LFN and QE, and 1(5.3%) LFP and LFN). Of 58 students from university-003, 35(60.3%)

students minor errors (7(12.1%) LFP, 13(22.4%) LFN, 4(6.9%) QE, 8(13.8%) LFP and QE, 3(5.2%) LFN and QE).

Table 1: Percentages and types of errors for students evaluated by proficiency test

	Types of Error								
	MajorError			MinorError					
	HFP	HFN	HF+HFN	LFP	LFN	QE	LFP+LFN	LFP+QE	LFN+QE
Number of student	-	4(8.5%)	2(4.2)	3(6.1%)	14(29.8%	9(19%)	-	10(21.3%)	-
Number of students inUniversit	-	1(5.3%)	-	-	4(21%)	1(5.3%)	1(5.3%)	1(5.3%)	1(5.3%
Number of students inUniversit	2(3.4%)	9(15.5%)	-	7(12.1%)	13(22.4%)	4(6.9%)	-	8(13.8%)	3(5.2%

From total participants, 70(56%) had had practical attachment during their vacation period. Most participants 102(82%) had a good knowledge on the proper timing for sputum sample collection that recommended by Ethiopian TB control programme. Seventy one (57%) of them knows the alternative stain for AFB staining if they run out of carbolfuchsin. Furthermore, 93(75%) knows the infectiousness of sputum. Majority of the study participant 67(54%) felt that as they didn't get adequate training on AFB smear reading and grading during their stay in the school. Around two-third 79(64%) of study participants agreed that the number of microscopes available in their school is not enough for practical session during their stay in the school. More participants from university-003 (46 (37%)) than any other reported in adequacy of microscope (25(20%) from university-001 and 9(7%) from university-002. There was a significant statistical association ($p<0.05$, $p=0.04$, CI=95%) between acid fast bacilli microscopy proficiency score and grade (score) of introduction to bacteriology and diagnostic bacteriology courses. Approximately 91(73%) of study participants who scored B and above in diagnostic bacteriology course scored PT scores above 80%. Furthermore, 86(69%) of participants who had scored B and above in introduction to bacteriology course scored PT score above 80%.

Discussion

It is very essential that every laboratory technologist should be trained in sputum AFB microscopy. It is also desirable to know the proficiency of smear reading by the laboratory technologists before they are assigned with the responsibility of sputum AFB microscopy. As we will be relying on TB smear microscopy for the foreseeable future, quality assurance of smear microscopy is of utmost importance to National Tuberculosis Control programmes. Both, false positive and negative results have serious implications. Since students graduating (junior technologists) from higher institutions going to serve the community, retention of proficiency in sputum smear microscopy is of utmost importance. All errors were defined as a quantification error (QE), a low-false-negative (LFN) result, a high-false-negative (HFN) result, a low-false-positive (LFP) result, or a high false-positive (HFP) result according to the international EQA classification. EQA results were interpreted by using the most stringent criteria listed in the guidelines, suggesting that any major error (an HFP or HFN result) is unacceptable performance, as well as the least-stringent criteria, suggesting that any HFP result, more than three LFN results, and one or two HFN results define unacceptable performance. Overall, 103(83.1%) students' score above 80% and 21(16.9%) students score below 80%. According to the international EQA guideline, PT scores below 80% are considered as unacceptable performance. In this study, there were 18(14.5%) students which report major errors. The most frequent major error was HFN which account 17(13.5%) of the total errors reported by the students. The main problem associated with this type of error (high false positive and negative)

reporting is due to lack of knowledge on AFB bacilli morphology by laboratory technologists or use of nonfunctional microscope. The later one is not a reason for results that are reported in this research because functional microscopes are selected by the aid of laboratory assistants in each study sites the research has been done and by principal investigators as well .From the total of 124 study participants, 80(64.5%) students report minor error. For reporting the low false positive and negative errors the same above problems could be cited. In addition, following irregular screening technique or screening insufficient microscopic fields are other possible problems. The quantification error problem is purely due to lack of knowledge in grading system based on number of fields examined. It has been reported that the smear reading capability of the Istanbul medical graduate students was less satisfactory with 40% of false-negatives and 26% of false-positives in reading smears (9). It should be pointed out that in the field conditions in Mexico, the agreement achieved before and after a course of refresher training, ranged only from 65% to 67% and from 75% to 80%, respectively in a panel-testing programme (11). Similar results were shown for a few intermediate laboratories in a panel-testing programme in India (8).

Conclusion

In the absence of newer technology that is accessible to resource poor settings, the Ziehl Neelsen test will remain the cornerstone of TB diagnosis. Therefore quality assurance is vital to ensure high quality TB microscopy results. There were 18(14.5%) major errors which was reported by students participated in proficiency test. From this study, the proficiency to read sputum smear by graduating batch students who have taken pre-service training (students from University-002) were better than the rest (University-001 and University-003) which hasn't taken pre-service training yet. Students that report major errors are, 6(12.8%), 1(5.3%), 11(19.0%) for university-001, 002 and 003 respectively. Thus, the study has highlights the importance of training in improving the microscopy results.

Recommendation

The first step to improve the effectiveness of AFB microscopy networks is increasing the ability of laboratory technologists that read sputum smear microscopy to diagnose tuberculosis. Since our findings indicate that students who have taken pre-service training had a good competency in reading and grading AFB relative to the one that hasn't taken pre-service training we recommend that students should take pre-service training before they go to serve the community. In addition, intensive practical session during studentship must be given a great concern.

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Abbreviations

AFB, Acid-Fast Bacilli; DOTS , Directly Observed Treatment Short-course; DTLC, District Tuberculosis Laboratory Center; EQA, External Quality Assessment; FN,False Negative; FP,False Positive; HFN,High False Negative ; HFP ,High False Positive; HIV,Human Immunodeficiency Virus; LFN,Low False Negative; LFP,Low False Positive ; NTP,National Tuberculosis Program; NTRL,National Tuberculosis Reference Laboratory ; QA,Quality Assurance ; QC,Quality Control ; QE,Quantification Error; RL,Reference Laboratory; TB,Tuberculosis; WHO,WorldHealthOrganization;Z-N,Ziehl-Neelsen.

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