ADDIS ABABA UNIVERSITY

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External Quality Assessment Performance and Associated Factors in Selected Governmental Clinical Bacteriology Laboratories in Ethiopia

By: Dawit Assefa

Advisors: Dr. Mistire Woldie (PhD)

Mr. Abay Sisay (MSc.)

A research thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Laboratory Management and quality assurance track)

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This is to certify that the thesis prepared by Dawit Assefa Wendifraw entitled: **External Quality Assessment Performance and Associated Factors in Selected governmental Clinical Bacteriology Laboratories in Ethiopia**

A research thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Laboratory management and quality assurance track) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Advisor: ___________________ Signature ________________ Date ____________

Advisor: ___________________ Signature ________________ Date ____________

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Chairman of the department of graduate program coordinator

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Abbreviations /acronyms

AST	Antimicrobial susceptibility testing
ATCC	American Type Culture Collection
CDC	Centers for Disease Control and Prevention
CLIA	Clinical Laboratory Improvement Amendments
CLSI	Clinical and Laboratory Standards Institute
EQA	External quality assessment
EQAS	External quality assessment schemes
ES	Educational Sample
ESBL	Extended Spectrum Beta-Lactamase
HIV	Human Immunodeficiency Virus
ICARE	INTENSIVE CARE ANTIMICROBIAL RESISTANCE EPIDEMIOLOGY
ID	Microbial identification (bacterial identification)
ISO	International Organization for Standardization
MHA	Mueller-Hinton agar (MHA)
MIC	Minimum inhibitory concentration
MRN	Mutual Recognition Arrangement
MRSA	Methicillin-resistant *Staphylococcus aureus*
NCCLS	National Committee for Clinical Laboratory Standards
NEQAPP	National External quality assurance program Pakistan
PT	Proficiency testing
QA	Quality assurance
SPSS	Statistical Package for the Social Sciences
STGG	Skim milk tryptic soya glucose glycerol
TB	Tuberculosis
TSB	Tryptic soya broth
UK NEQAS	United Kingdom National External Quality Assessment Service
WHO	World Health Organization

Abstract

Background: External quality assessments (EQA) at various level of the health system are very helpful in the identification of possible nonconformance in laboratory. Moreover, it helps develop guidelines for remedial actions to be taken for the improvement of laboratory services with ultimate goal of attaining better health care and patient outcome.

Objective: To assess selected government clinical bacteriology laboratories external quality assessment performance and associated factors in Ethiopia from July 2018 up to May 2019.

Methods: A cross sectional study design using both a prospective and retrospective data approach was employed among 16 bacteriology laboratories from July 2018 to May 2019. From One world accuracy PT sample provider they obtained seven panel tests: bacterial identification from sputum, blood, eye swab, wound and CSF; throat swab; urine; genital; *N. gonorrhoea* identification; gram stain; and AST. In addition, four standard bacterial strains were prepared in house and distributed to participant laboratories. Data were collected using pre-tested structured questionnaire as well as on-site observation. The collected data was cleaned and analyzed using Stata14.2 and exact logistic regression. P <0.05 was considered as statistically significant.

Results: The overall failure rate of bacterial identification during event two from CSF, blood and Abdominal abscess, was 7/13(53.8 %); from throat culture 8/13(61.5%), from urine 5/13(38.46%), and from genital cultures 8/13(61.5%) from gram staining 7/15(46.6%) and for *N. gonorrhea* ID the failure rate was 2/14(14.2%). On the other hand, the failure rate from event three were: from sputum, blood and eye swab 14/16 (87.5 %), from urine 7/16(43.8%), from throat 8/15(53.3%), from genital culture 9/16(56.3%), from gram staining 5/16(31.3%) and for *N.gonorrhoea* ID the failure rate was 7/15(46.7%). The overall AST failure rate of the laboratories during event 2 and 3 were 8/10(80%), and 8/13(62%) respectively. Fifteen laboratories were participated in house prepared PT samples for bacterial ID and AST were the failure rate from pus 1/15(6.66%),sputum 3/15(20%),blood 5/15(33.3%),AST 1/15(6.66%) .The foremost identified contributing factories for unsuccessful PT results includes mis utilization of updated SOPs, no use of McFarland standard, unable to perform internal quality control for Gram staining, culture media, identification reagents, and antibiotic discs (p<0.05).

Conclusion: The overall EQA performance of participant clinical bacteriology laboratories was poor. The main associated factors were poor implementation of quality management system such as poor records and documentation, poor internal quality control practices, no regular competency assessment,

lack of refreshment on basic microbiology training Therefore, continues training and mentorship for laboratories participating in external quality assurance are mandatory.
Key words: *Proficiency testing; Quality assessment;* bacterial identification; antibiotic susceptibility testing

1. Introduction

1.1. Back ground

Proficiency testing (PT) is a valuable tool for assessing laboratory performance and verifying the accuracy and reliability of test results. Participation is required by the Clinical Laboratory Improvement Amendments (CLIA) of 1988 for each of the microbiology subspecialties (bacteriology, mycobacteriology, mycology, Parasitology, and virology), and the regulations include specific PT requirements for each subspecialty(1).

Proficiency Test (PT) for bacteriology laboratories assesses the ability of clinical microbiology laboratories to identify and characterize clinically important bacteria, and conduct antimicrobial susceptibility tests. It is also helping to improve the performances of laboratories and ensure high quality and reliable testing in the field of clinical bacteriology. In other countries, external quality assessment schemes (EQAS) for clinical microbiology had been influencing the improvement of the quality of testing and performance of participating laboratories in different time. Improvement of laboratory performance can be attained when sources of errors that result to poor performance are identified and addressed (2).

External quality assessment (EQA) schemes allow laboratories to assess their performance to identify and type a pathogen, even when different methodologies have been used. The EQA results can also document that laboratory procedures at a certain time point in accordance with internationally agreed procedures(3).

External quality assessment provides objective evidence of laboratory competence for customers, accrediting bodies and regulatory agencies, and serves as a unique source of information that is not obtainable in other ways. For medical laboratories, they have been found useful, in that they initiate a “peer-review” process towards solving technical and methodological problems to improve the quality of service for each individual laboratory as well as to achieve comparability of results among different laboratories. For accrediting bodies and regulatory agencies, they provide objective data on the quality of delivered services as EQA/PT performance has been shown to reflect the quality of patient specimen testing(4).

The pathology community has long held the belief that the quality of the testing result produced by a laboratory is one of the most critical measures for ensuring public safety. Because of this, the laboratory community has embraced proficiency testing at the laboratory level. Laboratories are required to complete PT to document quality as a part of the accreditation process. Proficiency testing is an important educational and quality assessment tool used to assist laboratories in identifying and solve problems as well as evaluation of laboratory personnel skill and improvement of laboratory test results(5).

Diagnostic testing today typically involves multistep processes which are subjected to multiple sources of error. Many laboratory tests in small to medium-sized laboratories, whether it is manual or automated, can exposed inaccuracy associated with either lack of operator competency or failure to adhere to standard test procedures. There are also other sources of error, such as incorrect reagent storage or expiration and instrument inaccuracy. Even the simplest tests have multiple stages at which errors could be introduced. Commonly used tests (e.g. rapid test strips, TB sputum smear microscopy, semi-automated chemistry analysis, and HIV nucleic acid testing) each have up to 45 steps that are susceptible to error (6).

Regular EQA assessment compares laboratory performance against a set of standards or compares between laboratories. Sometimes EQA can also help to define good standards (best practice), although this does depend on the type of EQA test. EQA interprets best practice standards (quality) into a numerical score (quantity). While international bodies or professional organizations set these standards, EQA is able to assess whether these standards are met, with any omissions resulting in a reduction in the total score. Although EQA has an educational role rather than a punitive role, critical errors affecting clinical management will result in laboratory receiving a poor performance categorization (7).

There are many External Quality Control schemes that come in different varieties. There are EQA schemes that can be mandatorily required either by accreditation or law. Others can participate in EQA on voluntary bases to ensure that they are carrying out accurate testing and improve the quality of the laboratories' performance (8).

1.2. Statement of the Problem

Providing health care in resource-limited settings is admittedly a complex problem, and, for clinics or district hospitals with minimal-to-no laboratory support, diagnoses are often made clinically (e.g., by use of clinical algorithms for malaria and tuberculosis). Reliance on clinical diagnosis is attractive in areas with a high prevalence of disease, incurs no extra cost, and requires no special laboratory equipment or supplies; however, diagnoses based on clinical signs and symptoms can be nonspecific, unreliable, and associated with increased mortality (9).

Studies in the United States and Europe have demonstrated that errors occur throughout the testing process, including the pre analytical stage, analytical and post analytical stage . The majority of errors occurs outside of the laboratory in the pre analytical (46%-68%) and post analytical stages (18%-47%). This does not include clinic-based errors that occur in deciding which tests to order and in the interpretation of test results, both areas of high error risk. The frequency of errors during the analytical stage is lower but remains significant, estimated to be between 7% and 12%, despite years of quality management regulation. In the United States, it is estimated that 6% to 12% of laboratory errors put patients at risk of inappropriate care and potentially of adverse events, whereas 26% to 30% of errors have a negative impact on other aspects of patient care. (6).

In June 2018, the Ethiopian government health institute national laboratory capacity building directorate EQA team has analyzed the result of 29 participating laboratories on 6 events of One World accuracy PT program. The time period includes results from the 2015 event 1 up to 2017 event1 nationally for all PT programs. The PT distributed to the participating laboratories include: bacteriologic identification panels and throat culture identification. The result from the test revealed that it was an unacceptable 35 % for bacteriologic identification panels and 33 %for throat culture identification and also among 28 participant laboratories, the result from genital culture (32.5%), *Neisseria gonorrhoeae* (24.2%) and urine culture (27.6%) were categorized as unacceptable (10)

In addition, institutional based retrospective follow-up study was carried. A total of 12 laboratories participated in all six cycles of 20 test parameters panel tests. The overall acceptable analytical performance score was 2643 of 6984 PT challenges (37.85%) EQA fail rates among the 12 laboratories were,88.9% for gram stain, was identified in this study(11).

Generally, there is lack of information on the EQA performance of bacteriology laboratories. In light of this, in Ethiopia, the status of External Quality Assessment (EQA) performance and associated factors in selected governmental clinical bacteriology laboratories was not yet studied and documented. Hence the aim of this study was to assess the external quality assessment performances of selected clinical bacteriology laboratories of Ethiopia and associated factors for their performance.

1.3. Significance of the Study

- This study provides valuable information on whether bacteriology laboratories in Ethiopia are fulfilling defined criteria (accreditation requirement or international standards).
- The study identifies or indicates the possible causes of errors.
- The finding can assist the development of national EQA /PT programs, which in turn minimize hard currency that were paid for PT sample.
- The study provides evidence for establishment of standardized laboratory systems for the delivery of accurate, timely and reliable patient result.

2. Literature Review

Eight EQA test events from 2014 up to 2017 for five samples (KWIK-STIK™), various matrixes (blood, urine, sputum, swab, wound) challenged in each EQA test event ,84% average participation rate across the study arc (participation rate varied based on sample matrixes). The most unsatisfactory performance was shown in bacterial identification from blood matrixes were as 17.6%, 19.79%, 69.69%, 21.3% and 19.35%. The study identified other issues encountered by participating laboratories These, most notably, included: Wrong culture media employed and missed correlation between source material and clinical history, inappropriate enriched medium: and misidentification Gram Positive bacteria identified as Gram negative (12).

Another study shows that in Canada, by Clinical Microbiology Proficiency Testing (CMPT), Proficiency testing results obtained from simulated throat swab cultures sent between 1999 and 2013. Eight percent of all pathogen-positive samples were reported as false negatives, and 11 % of the pathogen-negative ones were reported as false positives. The presence of small colony-forming β-hemolytic streptococcus (*Streptococcus anginosus* group) proved to be challenging for many laboratories as 66 % of all unacceptable grades for pathogen-negative throat swabs occurred in these PT samples. Moreover, performance in this group did not improve with subsequent surveys. Results obtained in this study support the added value of clinical relevancy challenges for proficiency testing as laboratories that may not have problems with the analytical aspect of the testing process struggle with the appropriate interpretation of results (13)

In Philippines, the study shows that a total of 468 laboratories participated over the seven-year period for identify three analytes perform antimicrobial susceptibility test (AST) the number of participating laboratories obtaining a passing score of 80% and above had significantly increased from 2009 to 2015. Out of the 144 laboratories consistently enrolled over the seven-year period. Throughout year ,33.3% were good performers(who had passed more than 50% of their annual PT participation); 6.6% were fair performers(who had met the passing rate in 50% of their annual PT participation); and 60.0% were poor performers(who had not met the 80% passing rate for more than 50% of their annual PT participation (2).

In Pakistan, there were 88 laboratories enrolled with NEQAPP in 2011, which increased to 140 laboratories in 2015. Ninety-six percent of the enrolled laboratories participated in the clinical chemistry program followed by hematology (30%), microbiology (28%) and immunoassay (14%). Failure rates of newly enrolled laboratories were higher than those which were participating for the last two years. Laboratories were grouped as defense, government and private laboratories. The percentage of unsatisfactory results of participating laboratories was 19%,15% and10% in the years 2014 and 2015(14)

Another study, showed that performance of microbiology laboratory in the 10^{th} run of the external quality assessment scheme [EQAS] in Tehran and districts. Each laboratory was sent 2 species of bacteria for identification. Of the 487 laboratories that participated, 437 returned their findings. While 77.0% and 69.9% correctly identified *S. saprophyticus* and *C. freundii* respectively, only 29.8% correctly identified *A.baumanii*, 25.3% identified *E. faecalis* and 35.6% identified *Enterobacter agglomerans*. However, 78.7% and 79.5% of the laboratories reported correct -results for susceptibility testing for *S. saprophyticus* and *C. freundii* respectively(15).

A study conducted by Wattal.et.al 2018 New Delhi, India in 2014, 4 analytes per year was sent for 3 exercises, i.e. smear culture and serology. 2018 onwards PT analytes were increased from 4 to 12 and comparative performance of techniques analyzed Out of the 22 smears sent for gram staining, completely correct results ranged between 29.55%-79.9%. Correct results for culture isolate identification & susceptibility testing and serology exercises varied between 70 & 92.4% and 73.1 & 98.59%, respectively. In the year 2018, 470 responses were received for bacterial culture identification & antibiotic susceptibility testing out of which manual and automated systems were used by 54% & 46% and 52.5% & 47.5% participants, respectively.(16)

A study conducted In Thailand,2008 External quality assessment for evaluation of individual competency in isolation and identification of bacteria for clinical microbiological laboratory Lyophilized or freeze- dried bacteria were prepared and a panel of lyophilized bacteria was shipped once a year. The first set consisted of two mixed cultures and one pure culture. 43 laboratories and 38 laboratories participated first and second set respectively. Fifteen (34.9%) laboratories and 21 (55.3%) laboratories were correctly reported for the first and second proficiency sets, respectively. (17)

By the end of 2009, 48 African Member States were involved, with 76 laboratories enrolled in the bacterial enteric diseases and bacterial meningitis components bacterial enteric diseases the Cumulative acceptable performance was 65%. Although 64% of responses involving decisions about antibiotic selection for antimicrobial susceptibility testing were acceptable, 43% involving test reporting were unacceptable. For bacterial meningitis components Cumulative acceptable performance was 52%; Antibiotic choice for antimicrobial susceptibility testing was poor (54% of responses were acceptable) and reporting of corresponding findings was very poor (only 25% of responses were acceptable)(18).

In other study showed that, number of specimens during the evaluation period ranged from 976 to 1,864 specimens per site, and there was a total of 6,115 specimens. Gram stain results were discrepant from culture for 5% of all specimens. 85% of discrepant results were specimens with no organisms reported on Gram stain but significant growth on culture, while 42% of discrepant results had reported organisms on Gram stain that were not recovered in culture. Upon review of available slides, 24% (63/263) of discrepant results were due to reader error, which varied significantly based on site (9% to 45%). The Gram stain error rate also varied between sites, ranging from 0.4% to 2.7%(19).

The number of microbiology laboratories declined from 335 in 1974 to 190 in 1994. Twenty-one percent failed expected performance standards on initial review. Twenty-five laboratories were declared no proficient. Since 1989, 50% of the laboratories have scored at or above 80% for isolation and identification, but 25% have scored at or below 50% on susceptibility testing, and 10% or fewer have scored at or above 80%. Poor susceptibility testing performance is due to inappropriate agent selection, not testing errors(20).

As was the case with chemistry and hematology analytes, PT performance with microbiology analytes improved from 1994 to 2004; however, the failure rates for positive genital/GC cultures, positive urine cultures, and Gram stains remained above 5% throughout the 10-year period. Moreover, the failure rates for the positive cultures greatly exceeded the failure rates for the negative cultures. These results imply that many positive cultures and Gram stains from patient samples may be missed as well(21).

Any deviation from the standard AST procedure can significantly impact succeeding areas of laboratory workflow which in turn could later affect patient diagnosis, treatment, and management. Support systems of the laboratory workflow require strict monitoring, and laboratory personnel should be well trained and competent enough to perform the procedure. For instance, A perfectly carried out inoculation to Muller hiton agar (MHA) plate using “mixed colonies” will turn out unsatisfactory results. Poor standardization of bacterial suspension and a "longer depth of agar" could yield misleading endpoints. Purchasing poor quality MIC panels can lead to "dehydrated wells" or "mixed wells." also inadequacy and lack of supplies needed to perform AST will extend turn-around time and therefore, decrease laboratory productivity and delay patient therapy (22)

Another study showed that, the performances of EXCEL PT program participants on the yearly group C streptococcus challenge. Each year approximately 20% of participants failed to recognize that the β-hemolytic streptococcus isolated was group C and not group A. There was no significant improvement in performance noted over time, and no trend toward improved performance was evident; in fact, the largest percentage of unsatisfactory performance was noted in 2000, the fifth year of monitoring (23).

Clinical microbiology laboratory plays several important roles in the management of bacterial infections. Isolation, identification of pathogenic microorganisms in cultures and subsequent antimicrobial susceptibility testing always assists in selecting appropriate antimicrobial agent and prevention of unnecessary complications. The most important and primary test to perform directly on some special samples such as cerebrospinal fluid and positive cultures is Gram staining which serves as the most rapid and simplest test to characterize microorganisms.(24)

A study conducted on AST performance and reporting from positive blood cultures at hospital microbiology laboratories in Iowa on 116 episodes of bacteremia from 14 participating hospitals were examined. They detected AST or ID errors for 18 (16%) and judged reporting of AST results to be inappropriate for 38 (33%). The most common problem identified was reporting results for drugs that should not be reported for a given organism 28(24%). In 16 (14%), they detected at least one AST error. In four cases (3%), one very major error was detected, including two cases in which an oxacillin (methicillin)-resistant *S. aureus* isolate was reported as susceptible. In 10 (9%), laboratories did not report results for drugs that according to the NCCLS should be routinely provided for the organism(25).

Laboratory services are an essential component of health care delivery in tropical countries and play a vital role in improving the accuracy of clinical diagnosis and the investigation of disease outbreaks. In developing countries, laboratories face numerous constraints to providing quality services, including poor selection of techniques, difficulties in equipment availability and maintenance, and shortages of supplies, staffing and supervision (26).

Clinical and Laboratory Standards Institute document M52-Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems provides recommendations for verification of commercial US Food and Drug Administration cleared antimicrobial susceptibility testing (AST) and microbial identification (ID) systems by clinical laboratory professionals to fulfill regulatory or QA requirements for the use of these systems for diagnostic testing and also applicable to manual methods for ID and AST(27).

Regarding Gram stain microscopy, 560 out of 745 (75.2%) of compared parameters were concordant. As many as 67 out of 84 (79.8%) pairs of culture results were similar, including 16 sterile pairs, 27 having identical identification or description and semi-quantification and 24 only showing variations in semi-quantification with identification of colonies. Seventeen pairs had differing identification. Regarding antibiotic susceptibility testing, a major discrepancy was observed in 5 out of 48 results (10.4%). Conventional microbiology was less robust with major discrepancies reaching 39.5% of the samples for microscopy. Similarly, culture and antibiotic susceptibility testing were prone to discrepancies(28).

In other study showed that, a total of 213 laboratory professionals participated in the study. Seventy five (35.2%) respondents believed that their laboratories did not provide quality laboratory services and the major reported factors affecting provision of quality services were shortage of resources (64.3%), poor management support (57.3%), poor equipment quality (53.4%), high workload (41.1%), lack of equipment calibration (38.3%) and lack of knowledge (23.3%). The logistic regression analysis showed that provision of quality laboratory service was significantly associated with result verification (AOR=9.21, 95% CI=2.26, 37.48), internal quality control (AOR= 6.11, 95% CI=2.11, 17.70(29)

The study conducted by Adugna T. et.al 2017 from all 1140 observations for skill of medical laboratory professionals on gram stain examination and interpretation, found,44 observations (4%) with major errors and 321 observations (28%) with very major errors. of 148 (12.9%) also reported without grading with gram positive bacteria reported as gram negative bacteria while 43 observations (3.7%) reported without grading with gram negative bacteria reported as gram positive bacteria.(30)

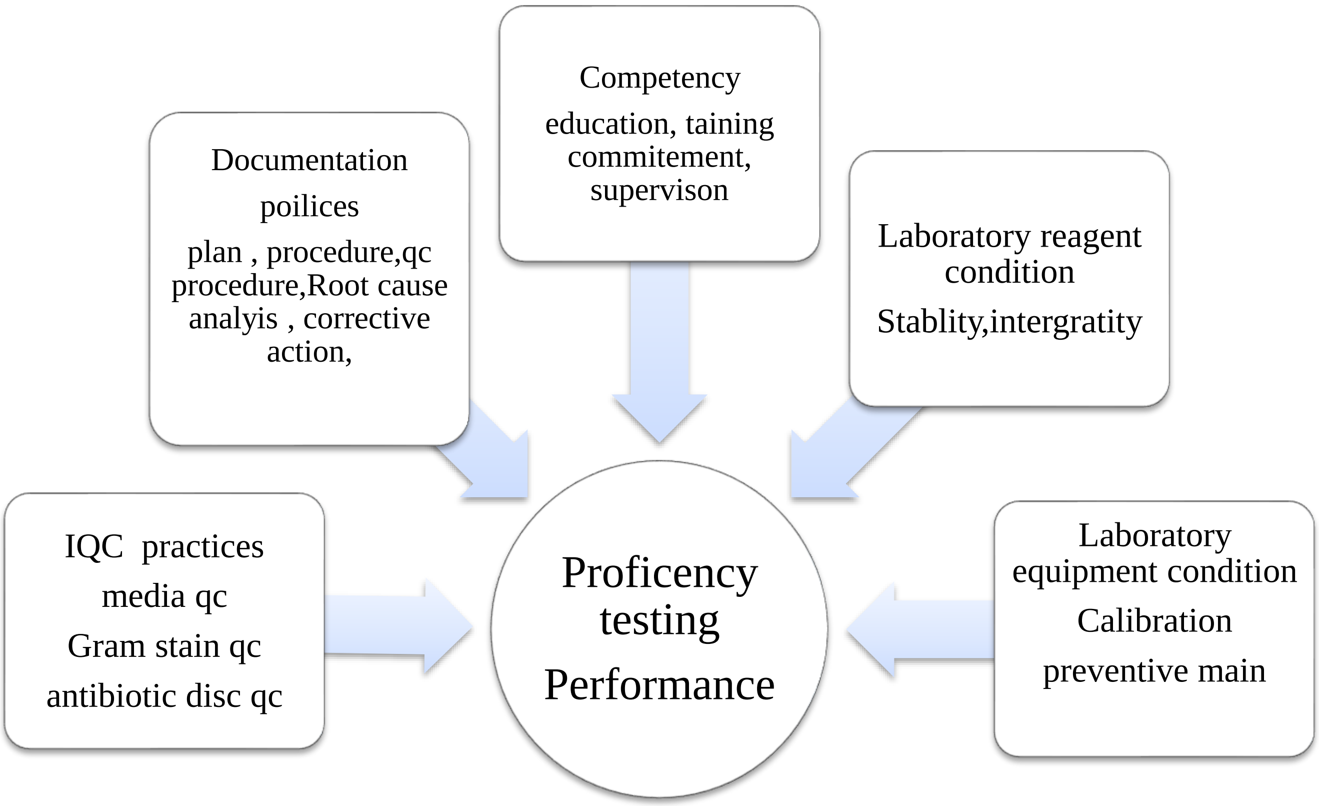

Figure 1:- Conceptual framework for External Quality Assessment Performance and Associated Factors in Selected Governmental Clinical Bacteriology Laboratories in Ethiopia, 2019

3. Objective

3.1. General objective

- To assess the External Quality Assessment performances and associated factors of selected clinical bacteriology laboratories in Ethiopia from July 2018 up to May 2019

3.2. Specific objectives

- To assess the External Quality Assessment Performances of selected clinical bacteriology laboratories from July 2018 up to May 2019
- To assess the associated factors for the External Quality Assessment performances of selected clinical bacteriology laboratories. from July 2018 up to May 2019

4. Hypothesis

HA: External quality assessment performance of clinical bacteriology laboratory in Ethiopian is at chaotic stage.

5. Materials and Methods

5.1 Study Area

The study was conducted in 16hospitals 'clinical bacteriology laboratories of Ethiopia that perform bacteriological tests. In addition to one world accuracy, Proficiency test samples were prepared in Clinical Bacteriology and Mycology Reference Laboratory at the Ethiopian public Health Institute. The Clinical Bacteriology and Mycology National Reference Laboratory (CBM-NRL) is under Bacteriology, Parasitology and Zoonosis directorate of the Ethiopian public Health Institute. It is a biosafety level two laboratory that serves as the National Reference Laboratory for Ethiopia. The laboratory provides bacteriological and mycological test services for health institutes, hospitals, health centers and private health facilities and for the community in general. The laboratory has six sections these are: sterilization and disinfection, media preparation, inoculation, identification, molecular testing and data entry. (31)

The laboratory is accredited in July 2017 by the Ethiopian National Accreditation Office (ENAO), by fulfilling the ISO 15189:2012 standards requirements in conventional or manual methods for identification of aerobic bacteria and antimicrobial susceptible testing. The ENAO is the mutual recognition arrangement (MRN) signatory for the African Accreditation Cooperation (AFRAC) and for the International Laboratory Accreditation Cooperation (ILAC). In addition, the laboratory had completed microbiology mentoring program that was conducted by the American Society for Microbiology (ASM) and graduated on September 2017 with a 5-star score.(32)

The study sites include sixteen Governmental laboratories found in Ethiopia. These laboratories are namely: Ethiopian public Health Institute., Hawassa referral hospital, ALERT hospital, Tikur Anbessa specialized hospital, Yekatit-12 medical college hospital, Zewditu memorial hospital, St Paulos millennium college hospital, Tigray public Health Institute, Ayder compressive referral hospital, Gondar compressive referral hospital, Felegehiwot referral hospital, Amahra public Health Institute Dessie branch, Adama public Health Institute, Hawassa regional laboratory, Jimma referral hospital, and Afar regional laboratory.

5.2. Study design and Period

A cross-sectional study design using both a prospective and retrospective data collection approach was employed from July 2018 up to May 2019 by assessing the performance of selected laboratories on detection and identification of bacteria as well as performance of antimicrobial susceptibility testing from one world accuracy PT sample provider and additional 4 standard bacterial species were distributed to participant laboratories for the study.

5.3. Population

5.3.1. Source population

All Governmental clinical bacteriology laboratories in Ethiopia.

5.3.2. Study population

Selected Government clinical bacteriology laboratories in Ethiopia

5.4. Inclusion and exclusion Criteria

5.4.1. Inclusion Criteria:

- ✓ Laboratories that perform clinical bacteriology services
- ✓ Laboratories that had previous experience on participation in PT testing programs
- ✓ Laboratories which able to identify panel test

5.4.2. Exclusion Criteria:

- ✓ Those laboratories with no sufficient supply to perform the panel and those with high staff turn over.

5.5. Study variables

5.5.1. Dependent variables

- ➢ Proficiency test performance

5.5.2 Independent variables

- ➢ Availability of SOPs or utilization
- ➢ Laboratory reagent condition (storage, viability …)
- ➢ Laboratory equipment condition (calibration, inventory, Preventative Maintenances)
- ➢ Competency (annual competences, every 6 months)
- ➢ Experience of EQA participation

- Periodic monitoring and evaluation root cause analysis and corrective actions
- Internal quality control records and practices (media, stains, antibiotic disc)

5.6. Sample size/ Number of participants

All 16 governmental laboratories that perform clinical bacteriology tests in Ethiopia were our sample size.

5.7. Measurement and Data collection

5.7.1 Data collection method

As part of EQA, the PT of participant bacteriology laboratories were captured by using pre-tested structured questionnaire were tested in Harari regional laboratory, in addition, on site observational study was conducted. The type of PT panel was urine, pus, sputum and blood sample components in the context of the patient history and sample composition.

5.7.2. Study materials from one world accuracy

PT sample with seven test parameters were sent to the participant laboratories by one world accuracy. Which was samples consisting of standard strain inoculated loops or KWIK-STIK™ Ampoule/Swab.

5.7.3. Study materials from in house prepared PT samples

Standard strain was used for preparing PT panels suchas Escherichia *coli* (ATCC25922), *Staphylococcus aureus* (ATCC, 25923), *Pseudomonas aeruginosa* (ATCC27853), *Klebsiella pneumoniae* (ATCC700603). The PT sample were sent by using of STGG medium, which consisted of 3% (wt/vol) tryptone soya broth, 0.5% (wt/vol) glucose, 2% (wt/vol) skim milk powder, and 10% (vol/vol) glycerol in distilled water. The media was sterilized by autoclaving at 15 kpa for 15 min in 121 ^{o}C. Then, 4-ml aliquots were dispensed into 7ml sterile swab transport container (Bijoux), which was used for viability counts.

Quality Control for Transport Media and Packaged Analyte

The sterility of STGG Medium, that was used as transport media was assured for sterility and tested for preservative before sending to the participant laboratories. For confirmation the analytes were subculture and re-identified through conventional methods and commercial identification systems by VITEK® 2 system at EPHI Clinical bacteriology and mycology reference laboratory.

Each Proficiency Testing consists of 4 samples with known pathogen. The PT was sent to each participant laboratories blindly, but labeled with a unique panel number; in a suitable transportation medium and container at ambient temperature that was monitored using thermometer. The panel was distributed in compliance with international packaging and transportation requirements for infectious substances according to the International Air Transportation Association (IATA) Dangerous Goods Regulations UN3373 Category B.

5.8 Operational definitions

Proficiency testing (PT) is an assessment that determines the performance of individual laboratories for specific tests or measurements and is used to monitor laboratories' continuing performance.

External quality assessment (EQA)-refers to the challenge of quality management systems in a medical laboratory. The term external refers to the fact that an organizer outside of the laboratory's organization provides a statement of quality to the laboratory.

Quality assurance (**QA** -is the total process whereby the quality of laboratory reports can be guaranteed.

Failure rate: - The frequency of a laboratory to score less than 80% analytical performance score in each test parameter. (80% performance score is a regulatory requirement of WHO-Afro) (19).

Acceptable performance of participating laboratory: - Defined a minimum achievement of 80% or more for each test run in proficiency testing program (2,12)

Event-Round or single complete sequence of distribution of proficiency test items, and the evaluation and reporting of results to all participants in a proficiency testing scheme (ISO/IEC 17043:2010).

Participant: laboratory, organization or individual that receives proficiency test items and submits results for review by the proficiency testing provider (ISO/IEC 17043:2010).

Acceptable - correct result

Unacceptable -incorrect result

Bacterial identification BACT 5 SAMPLE: PT panel that is composed of 5different sample to pathogenic bacterial identification from different biological sources (bloods, sputum, eye swab and wound)

Urine culture(URIC) 2 SAMPLE: - 2sample for identification of pathogenic bacterial pathogen that disease causing UTI

Throat culture: - 3 sample except to identified *streptococcus pyogen* and candida spp

N. gonorrhea: -2 sample except to identify *N. gonorrhea (present or absent)*

Genital culture: -5 sample from Genital (Vaginal swab, Urethral swab, Endocervical swab and Cervical swabexpect to identified different pathogen (*Gardnella vaginalis* (clue cells present), *Listeria species, S. aureus, candida species, N. gonorrheae, beta hemolytic streptococci Group A and group B).*

Gram stain: -5 unstained sample expect to staining and morphological identification

Antimicrobial susceptibility test: -2 specimen expected perform Antimicrobial susceptibility test

Major Error Type: -Genus correct, species incorrect

Very Major: - Genus and species incorrect

AST error interpretation grading as indicated in (table 1)

Table: 1 Interpretations of AST grading (27)

AST Error Type	PT site result	EXPECT result
Minor	I(intermediate)	S or R
Minor	S or R	I
Major	R	S
Very Major	S(susceptibility)	R

5.9. Quality assurance

SOPs, forms, CLSI documents and quality control strains were used throughout the study. One day training on data collection was given for six data collectors at EPHI.

5.10. Method of data analysis, Interpretation and Presentation

Two consecutive participation events of proficiency testing result were analyzed and computed. Exact logistic regression was used to model binary outcome variables in which the log odd of the outcome is modeled as a linear combination of the predictor variables. This statistical approach applied since the sample size was too small for a regular logistic regression. In addition, the estimates given by exact logistic regression do not depend on asymptotic results, with Stat 14.2 and the P value $\leq$0.05.presentation

5.11. Ethical Consideration

All ethical considerations and obligations were duly addressed, and the study was conducted after the approval of the Departmental Research and Ethics Review committee (DRERC) of the department of Medical Laboratory Sciences, college of health sciences, Addis Ababa University and from EPHI Written informed consent was obtained from the participants before data collection. Each laboratory was given the right to refuse to take part in the study and to withdraw at any time during the study period. All the information obtained from the participating laboratories was coded to maintain confidentially.

5.12. Dissemination of Results

The findings of the study are submitted to the department of medical laboratory science, college of health sciences, Addis Ababa University, Ethiopian public health institute. In addition, the research findings will be presented at different conferences and published in peer reviewed scientific journal.

6. Result

6.1. Characteristics of participated laboratories institutions/facilities

A total of 16 clinical bacteriology laboratories participated in proficiency testing program from one world accuracy. From these, 7 (43.75%) were owned by federal ministry of health, 5 (31.25%) were under regional health bureaus, 3 (18.75%) were regional hospitals, and1 (6.25%) was national reference laboratory. Among 352 samples sent to 16 participating laboratories, on event two 298 (84.7%) and on event three 347(98.2 %) responded in different bacterial identification panels. On the other hand, from those laboratories participated in an antimicrobial susceptibly testing, in event two and even three, 10(62.5%) and e 13(81.3 %) were responded (table 2), respectively

Table 2: PT participation frequency for bacteria identification and antimicrobial susceptibly testing among 16 hospital laboratories from July – October 2018.

Variables	**EVENT -2** **Frequency (Percent)**	**EVENT -3** **Frequency (Percent)**
Bacteria identification:		
Success full participation	298(84.7)	347(98.2)
Failure to participate	29(8.2)	0
Reagent unavailable	5(1.4)	2(0.57)
Testing suspended during test event	20(5.7)	3(0.85)
Test not performed	0	0
Total **test**	352	352
Antimicrobial susceptibly testing:	**Frequency (Percent)**	**Frequency (Percent)**
Success full AST participation	10(62.5)	13(81.3)
Failure to participate	3(18.8)	1(6.3)
Test not performed	3(18.8)	2(12.5)
Total **sites**	16	16

The main reasons for unsuccessful participations in event two and three include, failure to participant, testing suspended during test event, delay of PT distribution at the site, antibiotic stock out and reagent unavailability. Similarly, reasons for unsuccessful participation on AST PT includes failure to participant and test not performed, as shown in table 2.

From 10 to 16 laboratories responded on the submitted PT samples during the two PT events. Accordingly during event two, the failure rate for submitted PT samples; from CSF, blood ,and abdominal abscess were 7/13 (53.8 %); from throat culture was 8/13(61.5%), from urine cultures identification were 5/13(38.46%), from genital cultures were 8/13(61.5%), from *N. gonorrhea* identifications were 2/14(14.2%) and from gram staining 7/15(46.6%). On the other hand, the failure rate from even three were; from sputum , blood and eye swab were 14/16 (87.5 %), from urine culture identification were 7/16(43.8%), from throat culture were 8/15(53.3%), from *N.gonorrhoea* identification were 7/15(46.7%), from genital culture were 9/16(56.3%) and from gram staining 5/16(31.3%). The overall AST performance during event 2 and 3 were 8/10(80%), and 8/13(62%) respectively as indicate in (figure 2).

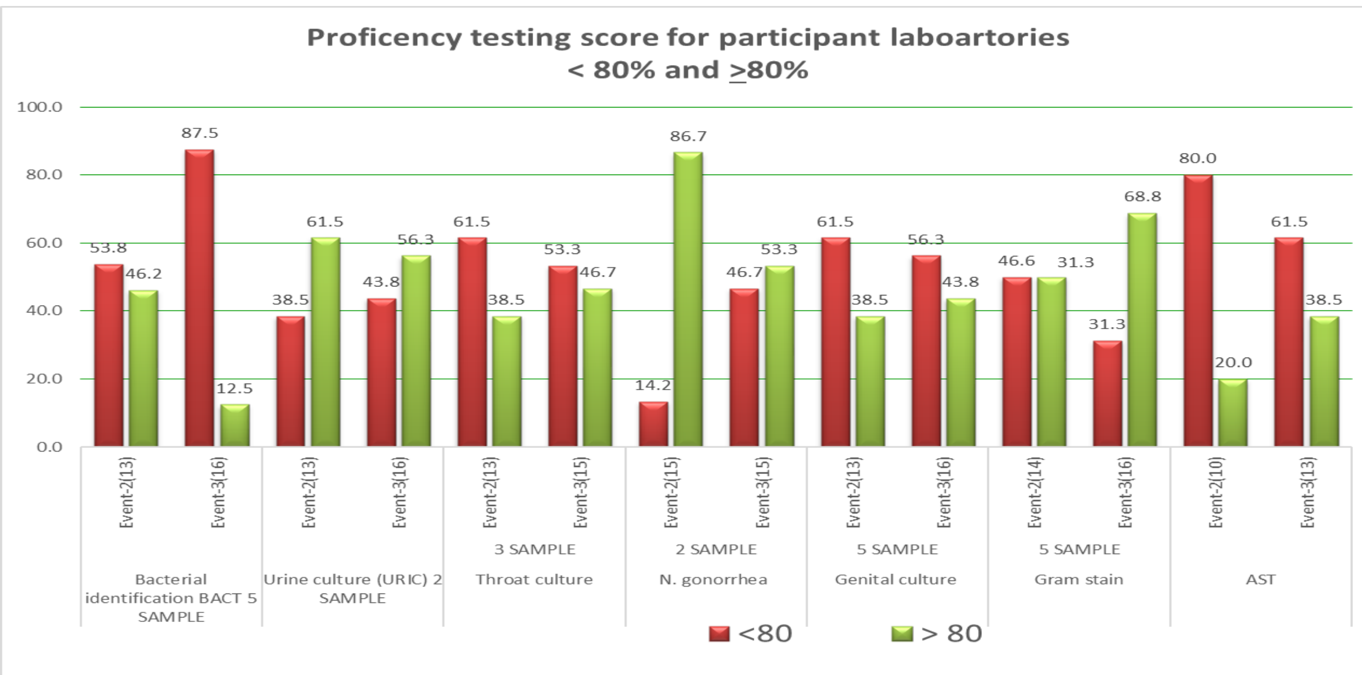

Figure 2: PT performance results of participant laboratories below 80 % and >80 %

The performances of all laboratories were less than 80% in all events except N. gonorrhea identification in event two. The AST performance of the participating laboratories was also less than 80%(table3)

Table 3: Distribution of laboratory performances in 2 cycles of proficiency testing program carried out from July- Oct, 2018.

Code of sites	Bacterial identification BACT 5 SAMPLE		Urine culture (URIC)2 SAMPLE		Throat culture 3 SAMPLE		N. gonorrhea 2 SAMPLE		Genital culture 5SAMPLE		Gram stain 5SAMPLE		AST		Individual lab. Performance	
	Event-2	Event-3	Event-2	Event-3	Event-2	Event-3	Event-2	Event-3	Event-2	Event-3	Event-2	Event-3	Event-2	Event-3	Event 2	Event 3
8	80	40	100	100	100	100	100	100	100	80	60	40	65	100	86	80
3	100	60	100	100	100	100	100	50	60	80	100	100	25	50	84	77
1	80	40	100	100	67	67	100	100	80	60	100	80	90	100	88	78
10	100	80	100	100	67	100	100	100	80	80	100	80	80	50	90	84
5	fail pa	20	fail pa	0	fail p	33	fail p.	50	fail	40	80	80	fail pa.	25	20	35
6	fail pa	20	fail p	50	fail p	67	fail p	50	fail	80	60	60	fail pa	83	60	59
2	80	20	50	50	33	67	100	50	60	40	40	100	75	100	63	61
11	80	40	100	100	67	100	100	100	100	80	80	80	not done	not done	75	71
7	60	100	50	100	100	100	100	100	60	60	100	100	not done	100	67	94
13	Suspended test	0	Suspended test	0	Suspended test	67	100	50	Suspended test	80	80	80	72.9	72	84	50
16	50	40	100	0	0	100	50	100	75	100	60	55	not participate	not participate	56	79
12	60	40	50	100	100	67	100	50	0	20	60	100	test not perform	test not perform	62	63
9	60	0	50	50	67	100	100	100	33	60	No reagent	72.5	73	50	63.8	64
15	0	40	50	100	33	33	0	50	80	50	40	40	25	50	33	52
4	40	20	100	0	100	test suspended	100	Reagent not available	60	50	80	100	64	64	78	47
17	20	40	100	100	67	33.3	100	100	20	20	60	80	55	77	60	64
Average	62.3	37.5	80.77	70	69.3	71	89	72	62	61	73.3	78	62	71		
<80 %	7(53.8)	14(87.5)	5(38.5)	7(43.8)	8(61.5)	8(53.3)	2(14.2)	7(46.7)	8(61.5)	9(56.3)	7(46.6)	5(31.3)	8(80.0)	8(61.5)	11/16(68)	13/16(81.25)
No of Participant	13	16	13	16	13	15	14	15	13	16	15	16	10	13	16	16

Evaluation of each study participant laboratory PT performances at two cycles has been done. Accordingly, on average point 5/16 and 3/16 laboratories perform successfully (average PT >80) on event two and event three respectively, as shown in (table 3)

The individual laboratory most of the laboratory have below 80%   as shown in (figure3)

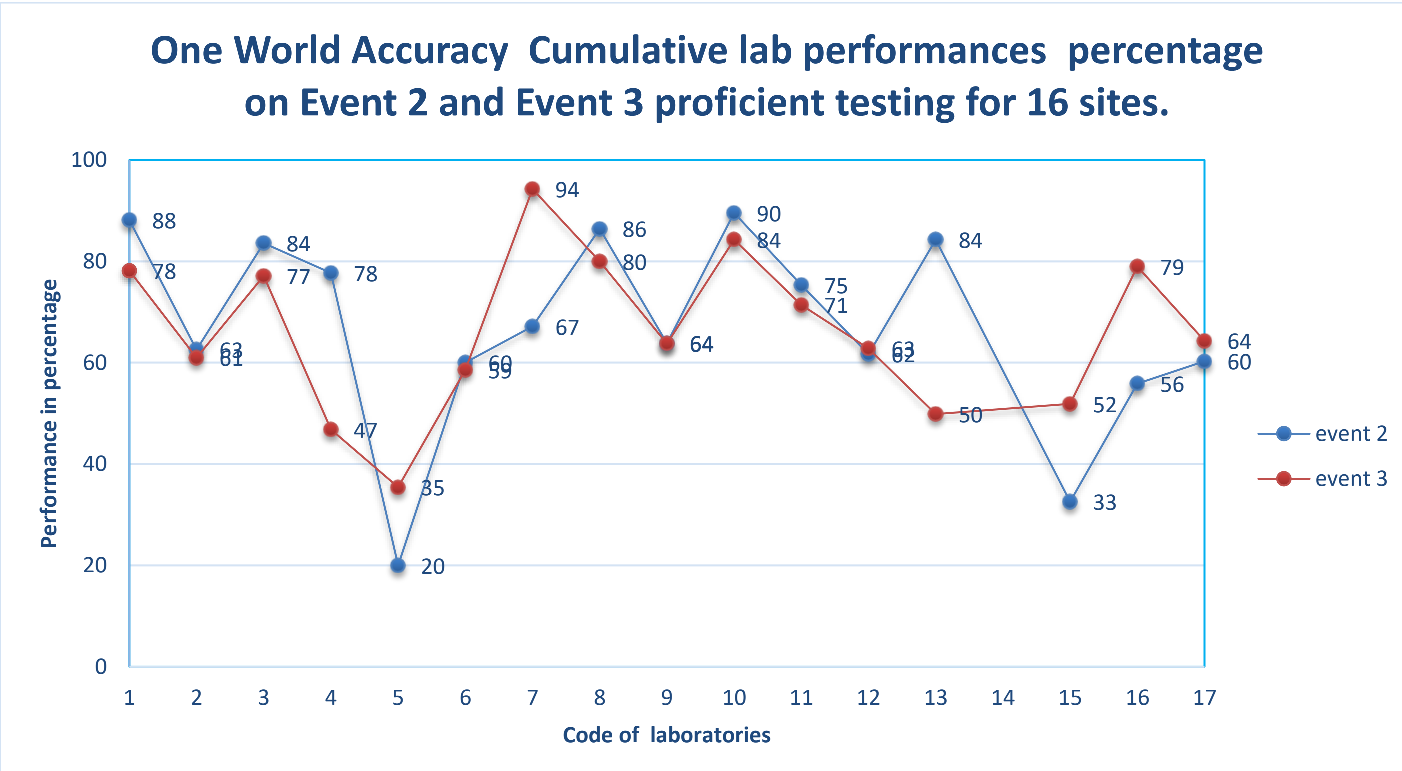

Figure 3: One World Accuracy Cumulative lab performances percentage on Event 2 and Event 3 proficient testing for 16 sites.

To get a good picture of the finding, PT test result analysis and interpretation of data has been done Accordingly, on event two and three, participant laboratories done minimum and maximum number of major errors in association with throat culture (5.10% and 0%) and gram staining procedure (18.7% and 18.4%) respectively. On the other hand on Identification very major errors, minimum number of mistakes were done on event two and three on *N.gonorrhea* (3.60%) and gram stain procedures 3.8%s respectively; whereas maximum number of mistakes done in event two and three were on bacterial identification which was 25.40% and 46.90% respectively. (table 4)

Table 4: The bacterial identification and AST with the magnitude of error among the 16 bacteriology laboratories in Event-2 and Event-3 PT participation, July –October 2018.

PT Events		Bacterial ID	Urine culture	Throat culture	N. gonorrheae	Genital culture	Gram stain	AST
Event -2								
Error	**Minor error**	NA	NA	NA	NA	NA	NA	24.20%
	Major error	12.30%	11.50%	5.10%	7.10%	13.30%	18.70%	12.20%
	V. Major error	25.40%	7.70%	25.70%	3.60%	24.10%	8%	1.40%
Total error (n(%)		9(37.70%)	5(19.20%)	8(30.80%)	2(10.70%)	11(37.4%)	11(26.7%)	37.8%
Event -3								
Error	**Minor error**	NA	NA	NA	NA	NA	NA	26%
	Major error	14%	13.3%	0	6.70%	7.50%	18.40%	1.70%
	V. Major error	46.90%	23.30%	24.40%	16.70%	31.30%	3.80%	1.20%
Total error (n(%)		14(61%)	7(36.6%)	8(24.4%)	6(23%)	15(39%)	11(22%)	28.9%

Table 5: - Cumulative analytical performance rate for 16 sites from one world accuracy

The overall cumulative unsatisfactory rate were in both events were in bacterial identification 75/145 (51.72 %) , from urine identification in two events 16/58 (27.59 %) , in throat culture 23/84 (27.39%), the *Neisseria gonnnoria* 10/58 (17.24) in genital culture 54/140 (38.57 %) , in gram staining 154 (24.68 %) and the over all in 6 parameters in both events were 216/639(33.80)%. as indicated in (table 5).

Cumulative Analytic Performance score per cycle for each test apartment						Total in Two Rounds		
		EVENT 2		EVENT 3				
analytic/ panels	Grade offered for PT	#	%	#	%	# Total	%	Total
BACT	ACC	40	60.6	30	38.0	70	48.28	
	UNACC	26	39.4	49	62.0	75	51.72	145
URIC	ACC	21	80.8	21	65.6	42	72.41	
	UNACC	5	19.2	11	34.4	16	27.59	58
THROAT	ACC	27	69.2	34	75.6	61	72.62	
	UNACC	12	30.8	11	24.4	23	27.38	84
NEGO	ACC	25	89.3	23	76.7	48	82.76	
	UNACC	3	10.7	7	23.3	10	17.24	58
GENITAL	ACC	38	61.3	48	61.5	86	61.43	
	UNACC	24	38.7	30	38.5	54	38.57	140
GRAM STAIN	ACC	55	73.3	61	77.2	116	75.32	
	UNACC	20	26.7	18	22.8	38	24.68	154
TOTAL	ACC	206	69.6	217	63.3	423	66.20	
	UNACC	90	30.4	126	36.7	216	33.80	639

PT Performance Using In-House Prepared Panel

Fifteen laboratories were participated in house prepared PT samples on bacterial identification from Urine samples 100 % correct , whereas 1/15 on Pus ,sputum and blood PT samples 5/15 and 3/15 were unsuccessful respectively, regarding on AST result from sample 'A' except one laboratory all are score ≥ 80%

The PT performance of participant laboratories on in house prepared samples were much better than the imported "one world accuracy" PT panel results the average of 4/15(26.6%) Vs 7/13(53.8 and 14/16(87.5) Of 4/15(26.6%) of the participant laboratories scored below 80% and the sample D from blood source 5/15(33.3%) score<80 %. (Table 6)

The difference between one world accuracy and in-house PT sample performances by participant laboratories could be rapid and also the cost was very cheap.

Table 6: - Distribution of laboratory performances in-house prepared panel and laboratories feedback result 1 cycles of proficiency testing program carried during the year's 2019.

CODE	Sample A/Pus	Sample B Urine	Sample C Sputum	Sample D Blood	AST For Sample A PUS	Individual sites result in %
1	100	100	100	100	100	100
2	100	100	100	0	87.5	77.5
3	100	100	100	100	80	96
4	100	100	0	100	100	80
5	100	100	0	0	100	60
6	100	100	100	100	100	100
7	100	100	100	0	100	80
8	100	100	100	100	100	100
9	100	100	100	100	100	100
10	100	100	100	100	100	100
11	100	100	100	0	100	80
12	100	100	0	0	100	60
13	100	100	100	100	100	100
15	100	100	100	100	100	100
16	**Failed to participate**					
17	0	100	100	100	0	60
Commutative in %	**93.3**	**100**	**80**	**66.7**	**91.1**	**86**
< 80%	1(6.66%)	0%	3(20%)	5(33.3%)	1(6.66%)	4(26%)

EQA performance associated factors in bacteriology test at selected government clinical bacteriology laboratories in Ethiopia

Factors that affect the quality assurance practice of bacteriology laboratories was assessed and analyzed using exact logistic regression analysis. The key contributing factors of proficiency testing is summarized in table 7.The major factors that affect the quality assurance practice at bacteriology laboratories were: using updated sops, lack of knowledge on how to do corrective action and calibration of major equipment and IQC practice for media, gram stain, AST, purity plates, use of calibrated PH meter, Training delivered on basic microbiology , use of densitometer or McFarland standard and updated CLSI M100 for selection and interpretation of antibiotics based on the organism.

The study also bare that statistically significant association with PH meter calibration of equipment, Internal quality control for Antibiotics disc in weekly and in new lot shipment get 13 times good PT result than those who are not using calibrated PH meter & not practices internal quality control for Antibiotics disc while performing prepared Müller-Hinton agar for disc diffusion test those who are not using of calibrated PH meter and internal quality for antibiotics disc the P value 0.035 this statistically significant association with PH meter calibration of equipment with AST performance.

The study also revealed that statistically significant association with internal quality control for media and antibiotic disc, PH meter calibration of equipment, updated SOPs and evidence of root cause analysis and corrective action and use of Standardized 0.5 McFarland standard A perfectly carried out inoculation to MHA plate using “mixed colonies” will turn out unsatisfactory results. The analysis also showed ,those who are using updated CLSI M100 antibiotics selection 14 times better result on proficiency testing result than those who are not using the update CLSI-M100used (OR =14.0, P =0.0714). The analysis also showed that 26 times positive association evidence of PT success with laboratories performing a regular competency assessment than those did not. (OR=26.14), p value =0.0256). (table 7)

Table 7: Exact regression output indicated that factor associated with laboratory proficiency testing performances in selected governmental Clinical Bacteriology Laboratories in Ethiopia, 2019

Factors		Number of Sites	PT ≥80%	OR	P-value
Utilization of Standard operating procedures	Yes	5	4(80.00%)	26.14505	0.0256*
	No	11	1(9.90%)	1.	
Regular Competency assessment	Yes	5	4(80.00%)	26.14505	0.0256*
	No	11	1(9.90%)	1	
Internal QC. For Media Performed	Yes	7	5(71.43%)	18.49614	0.0096*
	No	9	0(0.00%)	1.	
Internal QC for Antibiotics	Yes	5	4(80.00%)	26.14505	0.0256*
	No	11	1(9.90%)	1.	
Internal QC for gram stain	Yes	5	4(80.00%)	26.14505	0.0256*
	No	11	1(9.90%)	1	
Evidence of trouble shoot for unacceptable QC or PT failure	Yes	3	2(66.67%)	5.764119	0.4286
	No	13	3(23.08%)	1	
Using 0.5 McFarland standard	Yes	5	4(80.00%)	26.14505	0.0256*
	No	11	1(9.90%)	1	
Use of updated CLSI –M100 for antibiotic selection	Yes	6	4(66.6)	14.00421	0.0714
	No	10	1(10)	1.	
Use PH meter for using preparing media for Müller-Hinton agar	Yes	3	3(100.00%)	13.18126	0.0357*
	No	13	2(15.38%)	1	
Training delivered on basic microbiology	Yes	6	4(66.67%)	18	0.034*
	No	10	1(10.00%)	1.	

PT = proficiency testing OR = un adjusted odds ratio; 95% CI confidence interval,1-reference point,

** statically significant*

7. Discussion

Findings of the present study indicated that identification of organisms and antibiotic susceptibility test was difficult for majority of selected participant laboratories. Bacteriology laboratories in the Ethiopia used conventional methods and manual commercial kits. From ten up to sixteen laboratories responded to the submitted PT samples for the two PT events. During event two, the failure rate from submitted PT samples were as follows: from CSF, blood and Abdominal abscess, 7/13 (53.8 %); from throat culture 8/13(61.5%), from urine, 5/13(38.46%), from genital cultures 8/13(61.5%), and for *N. gonorrhea* identifications the failure rate was 2/14(14.2%). also, among ten laboratories AST performance was below the requirement 8(80%). The current study finding was comparable with a study shown from Ontario medical laboratory Toronto, Canada, even though the time and place were varies on survey conducted from 1974 to 1994 which involves 25 laboratories majority declared non proficient with 50% score for isolation and 80% for identification. In the same study, 25% have score at or below 50% for susceptibility test while 10% or few have scored at or above 80% (20). The reason might be due to no regular competency assessment, use of standard operating procedure as well as lack of experience and shortage of training for laboratory personnel about the current situation.

In the present study, on the other hand, the failure rate from event three were; from sputum, blood and eye swab14/16(87.5%),from urine7/16(43.8%), from throat 8/15(53.3%), from genital culture 9/16(56.3%), from gram staining 5/16(31.3%) and for *N.gonorrhoea* the identification the failure rate was 7/15(46.7%).these study was by far higher than those study conducted *Ashebir* on the analysis 6 events of one world accuracy PT program, form the 2015 event 1 up to 2017 event 1 for among that the bacteriologic identification panels and throat culture identification participate 29 laboratories, the unacceptable result was 35 % and 33.% respectively and also among 28 participant laboratories genital culture, *Neisseria gonorrhoeae* and urine culture 32.5%,24.2% and 27.6% unacceptable PT result had been found respectively(10) and also another study shows that lower accuracy in bacterial identification from blood matrixes the unsatisfactory performance in eight event which is 17.6%,19.79%, 69.69% ,21.3% and 19.35% respectively . This result might be due to no improvement plan and follow up with corrective action had taken.(12)

The results of event 2 among 13 laboratories were; on urine culture identification 5(38.5.8%), genital culture 8(61.5%), while the results of event 3 among 16 laboratories, genital culture 9 (56.3%), urine culture identification 7(43.8%) scored below 80 %. This finding was similar to the study conducted by Edson, et al in 1994, in which the failure rates for positive microbiology cultures was reported to range from 14.9% to 35.0%. A continuation of the same study in 1995, reported that failure rates for positive genital/GC cultures, positive urine cultures and Gram stains increased to 26.5% ,45.5% and 18.5%, respectively(21). This also may be due to lack of quality control of culture media; regular competency testing and adherence of laboratory standard operating procedure as well as internal quality control gram stain reagents.

The performance of both events in our study is similar to a study conducted in Philippine where 468 participating laboratories were involved throughout 2009 to 2015 and 33.3% were good performers; 6.6% were fair performers; and 60.0% were poor performers(2). And also, the study conducted in Thailand a by Wijit Wonglumsom et.al.2008 forty-three laboratories and thirty-eight laboratories participated first and second set respectively. 15 (34.9%) laboratories and 21 (55.3%) laboratories were correctly reported for the first and second proficiency sets, respectively. (17)This result has a good comparative or our study even if the size of the laboratory that shown as a good comparative result.

In the present study, during event 2 and 3 cycles, among 13 and 15 laboratories participated on the program, those laboratories that scored< 80% on throat culture were 8/13(61.5%), and 8/15(53.3%) respectively. Our findings were comparable to performance of Canadian clinical laboratories processing throat culture proficiency testing surveys, where 8% of all pathogen-positive samples were reported as false negatives, and 11 % of the pathogen-negative ones were reported as false positives.66 % of all unacceptable grades for pathogen-negative throat swabs occurred in these PT samples(13).Additionally, in throat culture proficiency samples, the failure rate in our study was higher than those study conducted by Novak RW.et.al. 2014 in which 20% of participants failed to recognize that the b-hemolytic streptococcus isolated was group C and not group A (23).

In our study Gram staining result of participating laboratories, both staining and morphological characteristics, from the two consecutives events, scored below 80%. This finding was similar to the study conducted by Samuel et al2016, where 58% of discrepant results were specimens with no organisms reported on Gram stain but significant growth on culture, while 42% of discrepant results had reported organisms on Gram stain that were not recovered in culture(19) This poor findings might be due to poor quality of staining material, lack of internal quality control practices, poor skill on Gram stain preparations and identification of gram positive and gram-negative bacteria.

Furthermore, our study result on the gram stain showed a failure rate of 24.68%wihich was similar to the study conducted by *scherz V et.al 2017* on Gram stain microscopy (75.2%) of compared parameters were concordant ,that mean 24.8% were dis concordant(28) but lower than the study by conducted by *Ashebiret.al*.2012 to 2013 G.C, where they reported with EQA fail rates of 88.9% among the laboratories participating for gram stain PT(11).This might be due the number of sample and also the hospital laboratories may be different in terms of their scope.

Reporting Gram stain results may guide the selection of the appropriate initial antibiotic before the results of culture identification and susceptibility testing become available especially for infections like sepsis and meningitis where a delay in the institution of appropriate initial antibiotic results in high mortality however ,our result that shown that the magnitude of error in gram staining from event two major and very major errors 18.70%and 8%, in event three 18.40% and 3.8% respectively this result comparable with the study conduct by *Adugna T, et.al 2017* ,from all 1140 observations for skill of medical laboratory professionals on gram stain examination and interpretation, found,44 observations (4%) with major errors and 321 observations (28%) with very major errors.(30) this might be due to quality control of reagents and competency assessment

In the current study, antimicrobial susceptibility testing result from 10 laboratories were classified as Minor error (24.2%), major error (12.20%) and very major error (1.40%) interpretation in event 2. Our finding is similar to the study conducted by Diekema,2004 in which 116 episodes of bacteremia from 14 participating hospitals were examined detected common problem identified was reporting results for drugs that should not be reported for a given organism 28 /116(24%) of cases, in 16/116 cases (14%), they detected at least one AST error. In 4/116 cases (3%), they detected one very major error, In 10 cases (9%), laboratories did not report results for drugs that according to the NCCLS should be routinely provided for the organism(25).That implies associated to selection of drug based on organism and also reading error while major error and very major error associated with poor quality of antibiotic discs and no weekly and new /lot shipment of antibiotic quality control and no follow up of updated CLSI.

Use of Standard operating procedures, regular competency assessment, Internal QC for (culture media, gram stain, antibiotics), use of 0.5 McFarland standard and Use PH meter for using preparing media for Müller-Hinton agar are among the factors that significantly affected the performance rate of participating laboratories in the present study. findings which was similar the study Carter JY et al,2002 to In developing countries, laboratories face numerous constraints to providing quality services, including poor selection of techniques, difficulties in equipment availability and maintenance, and shortages of supplies (26)

In current study, factors that contributes for poor performance similar to study by Mondoy.et al,2013 assessment of poor performing government laboratories for bacteriology in which 31 government-owned laboratories that performed poorly in the 2009–2012 PTs were assessed. Only 12.9% (4/31) of the laboratories had personnel with updated training; 45.2% (14/31) used ATCC biological standards for quality control; and only 29.0% (9/31) implemented SOPs and quality control of culture media and antibiotics 9.7% (3/31) had complete essential culture media and antibiotics for AST(2).This result is also similarity to the study by Eyob et al, 2013-2014 ,with Seventy five (35.2%) respondents believed that their laboratories did not provide quality laboratory services and the major reported factors affecting provision of quality services were shortage of resources (64.3%), poor management support (57.3%), poor equipment quality (53.4%), high workload (41.1%), lack of equipment calibration (38.3%) and lack of knowledge (23.3%)(29) .

Limitation of the study

Shortage of adequate literature pertinent to Ethiopia for making adequate comparison and also few numbers of laboratories participating in the current EQA program.

Conclusion

The overall EQA performance of participant clinical bacteriology laboratories was poor. The main associated factors for poor performance was poor implementation of quality management system such as poor records and documentation, unable to perform internal quality control, lack or no regular competency assessment, lack of refreshment on basic microbiology training.

Recommendation

Based on the finding of our study the following points are recommended

- The EPHI and regional laboratories together with partners should work hard to provide continuous training on the basic microbiology and quality management system for laboratory personnel
- Supportive supervision and onsite training and mentorship on how to do root cause analysis and corrective action for proficiency error with timely feedback should be given to laboratories
- Developing innovative training sessions and guidelines to help supervisory staff effectively use PT to assure regulatory compliance and improve quality in their laboratories;
- Developing, distributing and analyzing a larger nationwide PT survey should be considered
- Standardized monitoring of Gram stains is an essential quality control tool for laboratories and is necessary for the establishment of a quality benchmark across laboratories.

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Annex I: Informed Consent form

Topic: - Informed Consent form

Purpose: To assess External Quality Assessment Performances and Its Associated Factors in Selected government Clinical bacteriology Laboratories in Ethiopia

Procedure: On site observation and questionnaire, information on the procedure of routine clinical bacteriology and four tubes of STGG inoculated with the four standard control bacteria sent to each participant laboratory.

Benefits: This study will help your laboratory errors have been identified that involve either the clerical, technical, analytical or interpretive processes of a laboratory.

Participant Agreement: The proposal has been explained for me. I have been given a chance to ask questions. I feel that all my questions have been answered. Being in this study is my choice. I may change my mind and leave the study any time during the interview.

Participant Signature_____________

Date_________________________

Name of persons obtaining consent_____________________

Signature of persons obtaining consent_____________________

Date____________________________

Supervisor Name ____________________

Supervisor signature_________________

Date________________

Annex II: Checklist

GENERAL INFORMATION							
Date of Assessment				Name(s) and Affiliation(s) of Assessor(s)_______________			
Laboratory Name				Laboratory Address			
Name of Laboratory Head				Telephone			
Name of Laboratory Section Head/Supervisor				Telephone			
Laboratory Level (check those that apply)				Laboratory Affiliation (check those that apply)			
	National		Regional/Provincial		government		Academic
	Zonal		District		Private		NGO/Religious Institution
Specimen receiving schedule							
Types of Specimens Processed							

Bacterial Cultures (Number per month)			
Blood: Referred:	CSF: Referred:	Stool: Referred:	Genital: Referred:
Pus/Aspirate/Tissue: Referred:	Sputum: Referred:	Urine: Referred:	Throat: Referred:

Part 1: BASIC LABORATORY CAPABILITY AND INFRASTRUCTURE

1. Is the bacteriology lab currently accredited to either ISO 15189 or CAP standards for any of the following? (Check all that apply)

☐ Blood cultures❒ Stool cultures ❒ Urine cultures ❒ Gonorrhoeae cultures Other ❒No

2. If accredited, when was accreditation awarded and who performed the accreditation inspection (which accrediting body)? (Review certificate to ensure accreditation is current)
   a. N/AAccredited in (most recent year) ___________ by:

Laboratory staff

3.Indicate the highest level of microbiology training achieved by the technical staff reading bacteriology cultures and performing antibiotic susceptibility testing, and the number of staff that fall into each training level category.

Training level	Number of staff
Advance degree level (e.g., PhD, MD)	
Graduate degree level (e.g., MS)	
Undergraduate degree in Medical Microbiology (e.g. BS, BA)	
Undergraduate degree other than Med Micro (e.g. BS, BA in food microbiology)	

Medical Microbiology lab training program	
Non-degree certificate/diploma course	
In-laboratory training only	
No specific laboratory training	
Other (specify)	

4. Does the lab have bacteriology -specific training policies and procedures for orienting new employees?

❒Yes ❒ No

5. Does the lab have up-to-date documentation showing which benches/tests each staff member has been trained on and approved to work independently? (Review such records)

❒Yes ❒ No

6. Do lab staff receive regular competency assessments for each of the areas in which they perform testing? (Review competency records)

- ☐ Yearly or more frequently
- ☐ Other frequency, please Specify: _______________________________
- ☐ No

Laboratory facility

7. Is deionized water (DI) or distilled water available at the laboratory?

❒Yes ❒ No

8. Is there a functioning generator to provide backup power to the lab in the case of power failure?

❒Yes ❒ No

9. Is there a contingency plan in place for continued testing in the event of prolonged electricity disruption (e.g. power outage lasting several days)?

❒Yes ❒ No

10. Has the laboratory had a safety audit within the last year (e.g., via WHO Lab assessment tool or ASM checklist)?

- ☐ Yes, date: _____________________________
- ☐ No (if checked, please fill out Safety Appendix)

Laboratory instruments and equipment

11. In the last 6 months, has prolonged equipment failure caused a disruption in routine bacteriology services?

❒Yes ❒ No

Inventory and storage

12. Are all media, reagents and test kits in use and in stock currently within the manufacturer-assigned expiry dates or within stability from date of reconstitution? (Verify by random sampling)

❒Yes ❒ No

13. In the last 6 months, have media/reagent/test kit stock outs caused a disruption in routine bacteriology services?

❒Yes ❒ No

Data management and reporting

14. Where does the lab record the bench testing results (e.g., colony morphology, hemolysis, reagent +/- results, zone sizes/MICs)? Check all that apply.
    - ☐ Handwritten on a paper work card or logbook
    - ☐ Recorded in a commercial Lab Information System (List vendor: ______________________
    - ☐ Recorded in some other electronic database/computer program. Specify: ________________
    - ☐ Other, please describe: ________________________________
15. Does the lab retain these bench testing result records for a defined length of time?
    ❒Yes ❒ No
16. How does the lab report the final organism ID and AST results back to the physician?
    - ☐ Handwritten paper form
    - ☐ Printout from Lab Information System or another electronic database/computer program (Name of LIS/computer program: ____________________________
    - ☐ Electronically via an HIS or EMR interface to the LIS.
17. Does the lab retain a copy of the final organism ID and AST report for a defined length of time?
    ❒Yes ❒ No
18. If final organism ID and AST results are stored electronically, how often is the data backed up? (e.g., on USB, CD, facility server, or external hard drive)

❒Weekly or more frequently❒Monthly❒Other frequency, specify: _________❒Never

19. Does the LIS/electronic database permit the laboratory to produce a cumulative annual antibiogram?
    ❒Yes ❒ No

PART 2. QUALITY ASSURANCE

Specimen quality

20. Does lab policy require that all test requests are accompanied by a laboratory-approved test requisition form?
    ❒Yes ❒ No
21. Are received specimens evaluated according to written acceptance/rejection criteria?
    ❒Yes ❒ No
22. Is there evidence that specimen rejection criteria are enforced (review rejection log)?
    ❒Yes ❒ No
23. Does the lab provide specimen collection guidelines to patient sample collection areas?
    ❒Yes ❒ No

24. Review each specimen- collection guideline. Are guidelines present? ❒Yes ❒ No

Analytical

Temperature & Atmosphere monitoring

25. equipment	Acceptable rangesclearly defined onrecord sheet?			Temperatures arerecorded and documented daily?			Comments
	YES	*NO*	*N/A*	*YES*	*NO*	*N/A*	
Room temperature							
Freezers							

Refrigerators							
Incubators							
Water baths							

26. Is there documentation of action taken in response to out of range temperatures?
    - ☐ Yes
    - ☐ No, records indicate that temperatures are never out of range
    - ☐ No, records indicate occasional out of range temperatures, but no action is documented.
    - ☐ N/A, temperatures are not recorded
27. Are CO2 incubators checked and documented daily (or each day of use if not used daily) for adequate CO2 levels?
    - ☐ Always
    - ☐ Never
    - ☐ N/A

Reference Strains

28. What kind of quality control organisms does the laboratory use? (check all that apply)
    - ☐ Certified Reference Microorganisms

Specify which type(s): ATCC, NCTC, other ___________________________

- ☐ Organisms retained from prior patient specimens and/or EQA challenges
- ☐ Other, specify source: ____
- ☐ No quality control organisms are used

29. Are reference strains stored appropriately?	Yes	No
Reference culture stored in original container and frozen at -80C		
Reference stock culture frozen broth preparation derived from reference culture and stored at -20C or lower		
Working stock culture maintained on a refrigerated slant no longer than one week		
Subculture on a fresh sub plate each day of use		

30. Is media stored at the correct temperature?
❐Yes ❐ No
31. Are ATCC or ATCC-derivative strains used to QC media?
❐Yes ❐ No
32. Do records demonstrate that QC is performed on each newly reconstituted batch or newly received lot number/shipment of media?
❐Yes ❐ No
33. Indicate in the table below whether the lab has at least one functional piece of the equipment listed. If the lab has a functional piece of equipment indicate if it is calibrated routinely and if a user manual is present.

Equipment	At least 1 functional	Calibrated routinely	User manual present
pH meter	Yes / No	Yes / No	Yes / No
Weighing balance	Yes / No	Yes / No	Yes / No

34. Review records for each type of media reconstituted in-house. Is each batch checked for?

a)	Proper pH (using a pH meter)?	All/Some/None
b)	Sterility?	All/Some/None
c)	Proper volume dispensed?	All/Some/None

QC of Reagents and Phenotypic Identification Methods

35. Is QC performed and results recorded on each new preparation or lot number of gram stain reagents?
❒Yes ❒ No
36. Are positive and negative controls run and results recorded weekly with all gram stains?
❒Yes ❒ No
37. Do QC records demonstrate that both positive and negative controls are used and that QC is done on each new batch made or lot number/shipment received of the following differential tests?

Reagent	Not in use?	Positive Control	Negative Control
Catalase		Y / N	Y / N
Coagulase plasma		Y / N	Y / N
Staph latex		Y / N	Y / N
PYR		Y / N	Y / N
Optochin		Y / N	Y / N
Bile solubility		Y / N	Y / N
Oxidase		Y / N	Y / N
Indole reagents		Y / N	Y / N
Citrate		Y / N	Y / N
TSI or KIA		Y / N	Y / N
Urease		Y / N	Y / N
Motility		Y / N	Y / N
Lysine decarboxylase		Y / N	Y / N
Other			

38. Are ATCC or ATCC-derivative strains used to QC the tubed media and reagents referenced above?
❒Yes ❒ No
39. If commercial test kits are used for organism identification (e.g., API, Liofilchem, RapID), do QC records confirm that QC is performed on every new lot numbers/shipment before kits are placed into use? (Review QC records to confirm)

❒Yes ❒ No

40. If automated instruments are used for ID, (e.g., Vitek, Phoenix, Microscan) is QC performed on every new lot number/shipment of ID cards/cartridge before they are placed into use? (Review QC records to confirm)
    - ☐ Yes, for all cards/cartridges
    - ☐ Yes, but only for some cards/cartridges
    - ☐ No, QC not done on these automated instruments ()
    - ☐ N/A – the lab does not use these automated instruments ()

41. For any identification method, are patient results reported if QC of the identification method used was not performed?

❒Yes ❒ No

42. For any identification method, are patient results reported if QC of the identification method used failed to produce acceptable results?

❒Yes ❒ No

43. Is there evidence that the lab troubleshoots unacceptable QC results for tubed media, reagents, kits or automated ID systems?

❒Yes ❒ No

QC of AST Methods

44. Are antibiotics (whether in disk form, gradient strips, trays, cards, liquid, or powder) stored at the correct temperatures?

    ❒Yes ❒ No

45. Is QC of newly received or prepared batches/lot numbers/shipments of each antibiotic disk performed before being placed into use? (Review QC records to confirm)

    ❒Yes ❒ No

46. Is QC of newly received or prepared batches/lot numbers/shipments of each antibiotic disk performed using all of the recommended ATCC or ATCC-derivative reference strains? (Review QC records to confirm)

    ❒Yes ❒ No

47. Has the lab completed a 20-30-day QC conversion plan for each antibiotic in use with disk diffusion?

    ❒Yes ❒ No

48. How often do you perform QC on the antibiotic disks for which the QC conversion plan has been completed? (Confirm by reviewing QC records).
    - Daily when AST is performed
    - Weekly
    - With each new lot number/shipment
    - Other ________________________________❒ Never

49. Does your lab utilize any automated instrument for AST testing? (check all that apply)

    ❒Vitek❒ Phoenix ❒ Microscan

    ❒Other (please list) _____________________________❒_ No

50. Regardless of the AST method used, if QC failed to produce acceptable results are patient AST results ever reported?

    ❒Yes ❒ No

51. Is there evidence that the lab troubleshoots unacceptable QC results?

❒Yes ❒ No

Post-analytical

QC Review

52. Is there an SOP defining the post-analytical QC practices in use at this lab?

❒Yes ❒ No

53. Are QC results recorded and archived after testing is performed?

    ❒Yes ❒ No

54. Does a supervisor (or QC officer designated by the supervisor) review all QC results (including AST)?
    - ☐ Weekly or more frequently
    - ☐ Monthly or less frequently
    - ☐ Not routinely
    - ☐ No
55. Is there evidence that supervisory level QC review is performed at the stated frequency?
    - ☐ Yes, for all QC result
    - ☐ No
    - ☐ N/A

Results review

56. Does a supervisor review positive culture result every day?

❒Yes ❒ No

57. Are there written guidelines stating who is permitted to modify erroneous lab results after they have been reported?

    ❒Yes ❒ No

58. Who is permitted to modify erroneous lab results?
    - ☐ Supervisors and/or persons with supervisory permission
    - ☐ All microbiologists
    - ☐ No one
59. When corrections to patient results are made, what is done with the erroneous result?
    - ☐ Erroneous results remain in place but are amended to reflect that they are erroneous
    - ☐ Erroneous results are deleted from the record
    - ☐ Other ____

External Quality Assurance

60. Answer the following questions about lab participation in an External Quality Assessment (EQA) or Proficiency Testing (PT) program.

Tests	Participate in PT/EQA	PT/EQA supplier	Challenges
Gram stain	❒Yes ❒ No	☐ UK NEQAS ☐ Digital PT/One World Accuracy ☐ Other	
Aerobic Bacteriology: culture, organism identification, and antibiotic susceptibility testing	❒Yes ❒ No	☐ UK NEQAS ☐ Digital PT/One World Accuracy ☐ Other	

61. Are PT/EQA specimens handled and tested the same way as patient specimens? (No special treatment or additional testing above and beyond what would be done for a patient; not sent to other labs for confirmation before submitting results.)

    ❒Yes ❒ No

62. Are PT/EQA specimens tested by the same staff performing patient testing? (Not just by supervisors or senior staff; look for evidence that all staff participate in the challenges)
❒Yes ❒ No
63. On average, how long does it take for EQA performance results to come back to the lab?
- Less than 2 months
- 2 – 6 months
- More than 6 months
64. Is a root cause analysis performed when unacceptable PT/EQA results are obtained? (Request to see a recent example)
- Yes
- No
- N/A – no unacceptable results have been obtained
65. Is corrective action based on the findings of the root cause analysis documented?
- Always
- Never
- N/A – no unacceptable results have been obtained
66. Is laboratory leadership notified of all unacceptable EQA results as soon as they are received?
- Yes
- No
- N/A – no unacceptable results have been obtained

Part 3: SPECIMEN PROCESSING AND ORGANISM ISOLATION

Urine Cultures

67. Does the lab perform urine cultures?
❒Yes ❒ No
68. Does the laboratory have an SOP for how to process urine for bacterial culture? (request to see)
❒Yes ❒ No
69. Which media are used for primary culture of urine? (check all that apply)
- Blood agar
- MacConkey/EMB or another selective gram-negative agar
- Other, describe____
70. Are quantitative cultures (colony counts) performed?
❒Yes ❒ No
71. Are urines plated using a calibrated 1uL loop?
- Yes
- No, they are plated using a calibrated 10uL loop
- Calibrated loops are not used to plate urines

Part 5: ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST)

72. Does the lab have SOPs in place for each of the different AST methods used?
❒Yes ❒ No
73. When performing AST, are fresh isolates (<24 hours old) used?
- Always
- Never

74. When performing AST, are well-isolated, pure colonies (as evidenced by gram stain, colony morphology, etc.) used?
    - Always
    - Never
75. When preparing a bacterial inoculum for AST, is a 0.5 McFarland suspension used?
    - Always
    - Never
76. After inoculation, are purity plates always made from the remaining suspension? (Ask to see one)
    ❒Yes ❒ No
77. Do the AST SOPs outline what actions to take when unusual or unexpected AST results are documented from patient samples (e.g., reconfirm organism ID, reconfirm relevant QC, repeat testing, notify supervisor)?
    ❒Yes ❒ No
78. Is there evidence of such actions being taken?
    ❒Yes ❒ No
79. Is the microbiology lead or supervisor informed when unusual AST results are identified?
    ❒Yes ❒ No
80. Which AST interpretation standard does your lab use (check all that apply)?
    - CLSI
    - EUCAST
    - Other ______________________
81. Which edition of the standard referenced above does your lab possess?
    - Current year
    - 2014 or later
    - 2013 or earlier
    - N/A – no copy available in lab

Manual/Conventional Methods

82. If the lab uses disk diffusion for AST, what kind of disks are used?
    - Commercially manufactured* (list manufacturers) _________________
    - Disks prepared in-house after antibiotic reconstitution
    - Other (please explain) ____
    - N/A, disk diffusion is not used ()
83. Does the lab have an adequate disk diffusion SOP (as defined below)?
    - Yes,
    - No

Safety appendix

<u>To be completed if no record of other safety audit in the past 12 months (e.g. as part of WHO LAT or accreditation visit).</u>

1. Is standard safety equipment available and in use in the laboratory?

Equipment:	Tick for each item			Comments
	Yes	NO	N/A	

Biosafety cabinets (Class IIA				
Covers on centrifuge buckets				
Covers on centrifuge rotors				
Hand-washing station				
Eyewash station/bottle				
Sharps containers				
Flame cabinet				

2. Does lab policy prohibit eating, drinking, and smoking in the laboratory?
❐Yes ❐ No
3. Are hazardous chemicals stored appropriately (acids separate from alkaline, flammables in a flame cabinet)?
❐Yes ❐ No
4. Has each biosafety cabinet been recertified within a year of today's date?
❐Yes ❐ No
5. Is work area (bench and hood) disinfection documented daily? (Review documentation for completeness)
❐Yes ❐ No
6. Is all necessary personal protective equipment (PPE) available? For BSL2: gloves, gowns, and face protection (goggles, mask, face shield, or another splatter guard)
❐Yes ❐ No
7. Does lab policy require microbiology staff to wear close-toed shoes?
❐Yes ❐ No
8. Is PPE utilized appropriately and consistently by laboratory staff? (Observe)
❐Yes ❐ No

Handling control sample; -

1. All control specimens will be accompanied with unequivocal documentation regarding the analysis to be done and other information's needed.

2. All control specimens will be kept inside appropriate container (e.g. Leak proof, Ice box)

LONG - TERM STORAGE

I. **Principle**

Strains of *bacteria* and related species are sensitive to environmental factors and must be sub cultured every 24-48h. Unless they are stored frozen at factors and ≤-70°c in a suitable medium. Pure cultures of isolates are suspended densely (≥10^9 colony forming units/ml) in a freezing medium composed of Skim milk tryptic soy glucose glycerol (STGG) OR tryptic soy broth containing 20% glycerol. when stored at low temperatures, approximately 99% of organisms will not survive the freeze/thaw process. Cells are physically destroyed by the formation of crystals in the freezing the suspension in an alcohol/dry ice or acetone/ dry ice bath, although this is not necessary for routine storage. Strains may not be stored frozen at –20°c for more than 1-2weeks and may not survive under these conditions if the freezer door is opened frequently.

II. **Specimen Requirements**

- *Optimum specimen:*

Pure cultures of isolates from non-selective medium incubated at optimum condittion

- *Unacceptable specimen:*

Cultures of isolates on from non-selective medium incubated at optimum condition for ≥24h.

-Contaminated cultures.

Stability of specimens:

- Some bacterial species may lyse after 18-24h of growth. Frozen specimens should be prepared after no longer than 24h incubation.

III. **Reagents and Equipment's**

Preparation of STGG , STGG medium consisted of 3% (wt/ vol) tryptone soya broth (_________), 0.5% (wt/vol) glucose (______), 2% (wt/vol) skim milk powder (___), and 10% (vol/vol) glycerol in distilled water .. One-milliliter aliquots were dispensed into 1.8-ml cryotubes (Nunc) for storage of at _70°C, or 4-ml aliquots were dispensed into 7-ml glass sample containers (Bijoux) for viability counts. We were careful to mix each medium at regular intervals. The media were sterilized by autoclaving at 15 lb/in 15 min in 121 oc Medium: Trypticase Soy Broth plus 20% glycerol CDC Media Formulary #: 9282 Trypticase soy broth (BD)------- 30.0g, ETF (endotoxin-free) H_2O--------- 800.0 ml, Glycerol (Baker) ----------- 200.0 ml

1. Add water to dehydrated trypicase soy broth base; heat to dissolved the medium completely.
2. Add glycerol and dispense in 1.0ml aliquots in 12mm x 75 mm sterile, sealed tubes. .
   Sterilize by autoclaving for 15min at 121^{o}c.
3. Store at 4^{o}c or -20^{o}c (long term) or at room temp until used.
   Storage conditions: Refrigerated, 4-10^{o}c (long-term) or room temp

   Shelf life: 1 year

QC strains are stored at –70^{o}c in a solution of tryptic soy broth containing 20% glycerol for 2 years.

IV. Quality Control Procedure:

1. “Pre incubate” tubes of freezing medium at room temp at least 1 day before use.
2. Examine the tubes for turbidity, which may indicate that the medium is contaminated. If in doubt, inoculate a sample of the medium onto a nonselective medium, incubate it in required atmosphere, and examine the late for growth. The presence of growth after incubation indicates that the medium is contaminated; the absence of growth after the incubation indicates that the medium is not contaminated.
3. Prepare frozen stocks as described in the V. Procedure
4. Re culture QC strains after storage for 24-48h and confirm growth of control strains and properties of organisms.
5. Read result and record in the QC log.

Quality Control Schedule:

- A QC test is performed with each new batch of medium.

- QC results are recorded in the QC log.

  Tolerance limits and Corrective Action:

- *Problem*: QC strains fail to grow when recultured. This may indicate that the medium does not contain glycerol.

Corrective Action: Obtain new medium and repeat procedure.

V. Procedure:

1. Subculture the organism onto non selective medium at 35-36.5^{o}c for 18-24h

4. Verify the purity of the culture by Gram stain or biochemical reaction.
5. With a cotton swab, harvest growth from isolated colonies or the edge of confluent growth.

   Note: A swab with a wooden handle is preferred to one with a plastic handle. Plastic handles flex when the excess fluid is expressed and may spray fluid access the work area if the swab slips out of the tube.

6. Make a dense suspension, as dense as possible, of the culture in approximately 1.0ml of the freezing medium.
7. With a sterile Pasteur pipette, transfer an aliquot (2-3 drops) of the suspension into a microfuge tube; the suspension should occupy no more than one-half the volume of the tube because it will expand when frozen. Cap the microfuge tube firmly.
8. Label the tube with the strain #
9. Store the labeled tube immediately in the appropriate storage container in a –70^{o}c freezer.

HAZARD NOTE: Aerosols may be produced when opening vials. Wear Protective eyewear and open with caution only in Biohazard hood

VII. Limitations of test

- Properly prepared and quality controlled, there should be no limitations to storing isolates in this freezing medium for many years. QC strains could probably be stored much longer than 2 years under these conditions.

Stability and homogeneity testing

Event No: ______________________

Organism used: ___________________

BA/Choc (QC) - batch no__________ expiry: _________

Preparation Date: __________________ Initial: _______________

Swabs batch number and expiry date: ____________________________

1. Sterility check				
STGG broth prior to addition of organism	24 hrs	48 hrs	Date	initial

<u>Stability and Viability QC (performed during shipping and closure of survey</u>
Allmedia incubated for duration and atmosphere optimal for growth of the organism

Sample	Date of sub culture	Date read	Initial	Blood agar	Vitak result
<u>1</u>					
<u>2</u>					
<u>3</u>					
<u>4</u>					

Form 1… final result report format
laboratory code: __________ Date received in lab: ______ Date results returned: ________please indicate with □ if procedure is carried out

FINAL ORGANISM IDENTIFICATON	
SAMPLE A	
FINAL ORGANISM IDENTIFICATON	
SAMPLE B	
FINAL ORGANISM IDENTIFICATON	
SAMPLE C	
FINAL ORGANISM IDENTIFICATON	
SAMPLE D	

Laboratory Code: ____Specimen No: Sample A date Received in Lab: ______Date Results Returned: _____

Form 2… final result report format
PLEASE LIST THE ANTIMICROBIALS YOU TESTSED

ANTIMICROBIAL SUSCEPTIBILITY TESTING: Antimicrobial testing not performed on this isolate□

Disk Diffusion Method □	MIC Method □		
Kirby Bauer □	Agar Dilution□	Etest □	Micro dilution in house □
Other commercial methods □	if yes specify		
	Automated Systems	Vitek 2 □	MicroScan□
GUIDELINES Followed	CLSI □	ECAST □	

No	Antibiotic list	Disc zone diameter (mm)	S	I	R	MIC Symbol			MIC Range			S	I	R
1			□	□	□							□	□	□
2			□	□	□							□	□	□
3			□	□	□							□	□	□
4			□	□	□							□	□	□

INSTRUCTION FOR PROFICIENCY TESTING

Thank you for participating in the Bacteriology Proficiency Testing). We hope that your participation will be beneficial to your laboratory

The participating in the Bacteriology Proficiency Testing aims to determine the laboratory procedures on handling specimens including complete identification, antimicrobial susceptibility testing and recommendations to clinicians. **Samples should be treated in the same way as routine patient specimens.**

Contact Details:

Dawit Assefa

Contact telephone numbers: **+251910156377**

E-mail: dawitassefa8@gmail.com

Selection of samples: Isolates which are included in the proficiency testing for bacteriology are carefully characterized to ensure that typical features are present to facilitate identification.

Please note the following:

We accept no responsibility for results faxed to wrong fax numbers or posted to wrong addresses. It is your responsibility to ensure that we have received your results before the closing date. Please phone or email us to check that your results have been received.

PT SAMPLE AND RESUT SUMMITION deadlines:

EVENT /Round	Mailing	Result deadline
EVENT /ROUNED 1	01 FEB 19	10FEB 19

Result returns deadline:10 working days after receipt in your laboratory; to reach us no later than the dates stipulated above

INSTRUCTION FOR PROFICIENCY TESTING

The package contains either:

- 4 samples for the bacteriology sites

Special handling:

If the package contains broken samples, sterilize (autoclave) the container and contents immediately. Dispose of according to normal procedures in your laboratory. Guard against the production of aerosols. Please follow universal safety precautions at all times.

Instructions for completing the survey questionnaire

Read the clinical details and processing instructions for each sample on next page

Process the samples using standard operating procedures.

Participating laboratories are free to perform routine procedures according to their standard operating procedures. Please observe and adhere to the format in which we request antimicrobial susceptibility results be reported and whether or not you would report the susceptibility test result on a particular antibiotic to the clinician.

Please record your LAB CODE AND SPECIMEN NO in the space provided on the result form.

Evaluation of responses

Major Error Type: -Genus correct, species incorrect

Very Major: - Genus and species incorrect

Table: 1 Interpretations of AST grading (27)

AST Error Type	PT site result	EXPECT result
Minor	I	S or R
Minor	S or R	I
Major	R	S
Very Major	S	R

All bacteriology results (regardless of challenge type) will be evaluated using a two-tiered marking

Grading category	Mark Allocation
Culture and ID – Final Organism Identification	100
Choice of antibiotics	100
Susceptibility result	100
TOTAL (Maximum score)	100
PERCENTAGE	100

Table 2

CLINICAL DETAILS AND PROCESSING INSTRUCTIONS

The PT samples must be processed as soon as they are received in the laboratory.

Please record your LABORATORY CODE, SAMPLE NO, date of receipt in your laboratory and date of returning of results to the EPHI in the space provided on the result form

SPECIMEN	CLINICAL DETAILS	INSTRUCTIONS FOR PROCESSING
SAMPLE A Sample from a wound infection	A wound swab from a patient post-surgery. No further details provided.	Perform identification, and AST
SAMPLE B Sample from urine	78 old females in medical clinic having Dysuria and frequency for the past 2 days	Perform identification
SAMPLE C Sample from sputum	70yearold male with having chronic bronchitis 2-day productive cough difficulty in breathing and fever	Perform identification
SAMPLE D Sample from blood	A 45-year-old male with fever and nausea.	Perform identification

Declaration

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Dawit Assefa Wendifraw entitled: External Quality Assessment Performance and Associated Factors in Selected governmental Clinical Bacteriology Laboratories in Ethiopia to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Laboratory management and quality assurance track) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

M.Sc. Candidate: Dawit Assefa (B.Sc.) Signature ____________________Date ____________

This thesis has been summited with our approval as advisors,

Advisor: Mistire Woldie (PhD) Signature _____________________ Date ____________

Advisor: Mr. Abay Sisay (MSc.) Signature _______________________Date ____________