Mycobacterial Diseases
ISSN: 2161-1068

Sımsek, Mycobact Diseases 2011, 1:2
DOI: 10.4172/2161-1068.1000101

Review Article Open Access

# Requirement of Quality Assessment for Modern Tuberculosis Laboratory Services

**Hulya Sımsek***

*Refik Saydam National Public Health Agency, National Tuberculosis Reference Laboratory, Ankara, Turkey*

## Abstract

Tuberculosis (TB) which is caused by *M. tuberculosis* complex is still the most important public health problem worldwide. Especially taking into account the recent increase in multidrug-resistant TB (MDR-TB) cases and the emergence of extensively drug-resistant TB (XDR-TB), this disease has to be diagnosed as soon as possible, and started to treatment to prevent TB spread in society. Thus, laboratory diagnosis of TB including drug susceptibility testing for all TB patients should be rapid, accurate and reliable. A dependable laboratory service is an essential component of TB treatment.

The proper standards of TB laboratories are far from being universally applied. All laboratories providing TB diagnostic services must be included in QAS by implicating the standard, internationally recommended, TB management practices, and must provide accurate and reliable service. All TB laboratories serving within the framework of TB Control Program (TCP) not excepting National TB reference laboratory must be taken part in Quality Assessment System (QAS). Quality Assessment (QA) is designed to continuously improve the reliability and efficiency of laboratory services, and composes of internal quality control (IQC), external quality assessment (EQA), and quality improvement. Quality system includes in all of the laboratory's policies, processes, procedures, and resources required achieving quality testing. In conclusion, a good QA program will enhance credibility of laboratory for client.

**Keywords:** Tuberculosis; Quality assessment system; Microscopy; Culture; Molecular tests; Drug susceptibility testing

## Introduction

The laboratory plays an important role in diagnosis of TB and monitoring of its treatment. It is obvious that the control of TB requires the active support of the entire laboratory community and coordination of the appropriate levels of service for smears, cultures, and drug susceptibility testing. The recent increase in MDR-TB cases and the emergence of XDR-TB refers that TB laboratory services should be performed universally according to the proper standards. Delayed both diagnosis of TB and recognition of drug resistance have contributed to the dissemination of MDR- and XDR-TB. The main purpose in tuberculosis laboratory is to generate accurate and reliable test results for clinicians [1-3].

In mycobacteriology, there are three levels of laboratory in terms of the service. Level I laboratory provides the service by testing direct smear from sputum, and sends the same sample to the superior laboratory (level II) to process and culture from sample [4]. Level II laboratory processes the sample, after interpreting acid-fast stain and culture, sends level III laboratory the culture for identification and first and second line antimycobacterial susceptibility tests [5]. Level III laboratory is also called as a reference laboratory, and its number changes according to country's population [6]. Ministry of Health in the country has recognized the reference laboratory as National Reference Laboratory (NRL) [7]. NRL, the reference institution in the country, prepares cultures from samples and undertakes the identification of *M. tuberculosis* strains as well as drug susceptibility testing (DST). As to other tasks of the NRL are to ensure quality control of culture and of DST performed by regional or peripheral laboratories by establishing a regular "on-site" supervision programme for those laboratories, and by providing training in, and the QA systems for the laboratory procedures. NRL should collaborate with Supranational Reference Laboratory (SRL) which is a laboratory belonging to the World Health Organization (WHO) / International Union Against Tuberculosis and Lung Disease (IUATLD) network. SRL guides and advises the national coordinator during the preparation, implementation, and evaluation of the antituberculous drug resistance surveillance system in a country. It also ascertains the accuracy of susceptibility test methods used in the NRL.

Any TB laboratory-based diagnostic procedure should be performed by appropriately trained staff, working to standardized operating procedures in appropriately equipped and safe laboratories, against clear national and international proficiency and quality standards [7].

QAS has 3 main components which are internal quality control (laboratory continuously to control itself including test guides, staff training and supervision), improvement of quality (continuous improvement, error identification and correction) and external quality control (laboratory to control by the external quality control bodies and to compare the performance of inter-laboratories in terms of accuracy and proficiency) [2].

***Corresponding author:** Dr. Hulya Simsek, Refik Saydam National Public Health Agency, Tuberculosis Reference and Research Laboratory, Saglik Mah. Adnan Saygun Cad. No: 55 Sihhiye Ankara 06100, Turkey, Tel: +90 312 4582211; Fax: +90 312 4582437; E-mail: hsimsek_tr@yahoo.com

**Received** October 29, 2011; **Accepted** November 07, 2011; **Published** November 11, 2011

**Citation:** Sımsek H (2011) Requirement of Quality Assessment for Modern Tuberculosis Laboratory Services. Mycobact Diseases 1:104. doi:10.4172/2161-1068.1000101

All TB laboratories must perform QAS including systematic internal monitoring of working practices, technical procedures, equipment, and materials, including quality of stains, and the responsibility of all laboratory personnel. To meet QA objectives, the Mycobacteriology laboratories should adhere to comprehensive QA and the Clinical Laboratory Improvement Amendments (CLIA) in the U.S. or ISO 15189 implementations that apply to the pre-analytical, analytical, and post-analytical components of the laboratory processing paradigm for all clinical specimens [8,9]. As a result of compliance with QAS consists of a laboratory accredited. In an accredited laboratory, appropriate and sufficient number of samples collecting, accurate record-keeping of samples received, sample processed, laboratory results and samples sent to referral or regional laboratories for culture and susceptibility testing are essential for the proper management of the control programme strategy. Some studies show that implementation of rejection criteria and training on the sample especially how to get increase quality of test results, since quality of sample affects test result [1,10,11].

## Microscopy

Microscopic examination of samples is the most widely used method in clinical practice in diagnosis of TB and in monitoring of its treatment especially in developing countries and countries with high prevalence, and also it represents one of the five pillars in the Directly Observed Treatment Short course (DOTS) strategy for National TCP [12-14]. Acid Fast Smear (AFS) microscopy should be done within a working day from the acceptance of sample to the laboratory. But, sensitivity of microscopy varies depending on staining method, microscopist and various reasons [15,16]. Therefore every step in microscopy should be supervised in terms of QC. Appropriate specimen, appropriate decontamination process of specimen, correct smear preparation, correct staining, correct microscopic evaluation of smear, experienced personnel and a qualified microscope are main factors which increase the quality of test results. The quality of stain, its expiration date, preparation and storage conditions of stain can also affect microscopic evaluation [2,16]. For example, recently Light Emitting Diode (LED) microscopy has been recommended by WHO following standards appropriate for evaluating both the accuracy and patient health impact of TB diagnostics [17]. The commercial QC slides should be used to control every staining after preparing slides for IQC. Whenever the staining is done, one quality control slide on which is found one positive (*M. kansasii*) and one negative (*E. coli*) smear is used. QC slides can be prepared in laboratory; a QC slide prepared in laboratory would be more cost-effective than that of commercial. They show the qualities of both staining procedure and staining solution. After smear examination, it is reported as "negative" for negative control and "positive" for positive control. If one of them has wrong result, all of procedures must be repeated, and an investigation must be done as part of the QA procedures. The solutions such as NaOH- NALC and phosphate buffer, which are used decontamination process of specimen, should be controlled before use. If there is any contamination, they should be prepared again.

EQA for microscopy is the most important implement to provide the reliability and validity of tests. In countries where NTP are performed, this task is fulfilled by the NRL according to the guidelines of WHO. EQA compares participant laboratories to assess their capabilities with the results of other laboratories in TB laboratory network through panel testing and blinded rechecking [18,19].

Random blinded rechecking of a sufficient number of slides provides internal accountability, but often "rechecked" results agree identically with screened slide results, indicating a fault in blinding. The international guidelines on EQA of sputum AFS microscopy have suggested a lot quality assurance sampling (LQAS) method, which is designed to recheck a minimum number of slides examined in another laboratory to identify the faulty centers with an unsatisfactory level of performance [20,21]. However, the efficiency of EQA can be increased by selecting sample size parameters and interpretation criteria that take into account the local working conditions. The greater attention should be paid to the provision of timely feedback and correction of the causes of substandard performance at poorly performing laboratories.

## Culture

The definitive diagnosis of TB is done by demonstrating bacillus. The culture of bacterium in media is regarded as the gold standard and it is recommended that at least two media, the one of which is liquid, should be used for culturing. The purpose of a QA programme is to improve the efficiency and reliability of culture services. QC of culture is a process of effective and systematic internal and external monitoring of the performance of bench work in the culture laboratory. QC ensures that the information generated by the laboratory is accurate, reliable and reproducible. This is accomplished by assessing - against acceptable established limits - the quality of specimens, the performance of homogenization-decontamination-digestion and culture procedures, the quality of reagents, media and equipment, by reviewing culture results and by documenting the validity of culture methods [22]. IQC shows variation according to the medium used for culture. 10 media are selected randomly for IQC of the prepared solid media and they are checked in terms of color, air bubbles and smooth surface. They are incubated for sterility control at 36 ± 1°C for 2 days. *Mycobacterium tuberculosis* or *Mycobacterium fortuitum* is cultured for testing the media to ability of growth and speed. The cultured media are controlled firstly on third day in terms of contamination, and then they are checked on seventh day and every week in terms of growth [23]. For QC of commercial solid Media (LJ) the manufacturer must indicate that the QA protocol conforms to NCCLS (now CLSI) standards. The manufacturer must supply to the user test organisms used, the pH, the performance criteria evaluated, behavior of test organisms and assessment of contamination (microbial load). As a user, the items such as cracked bottles, unequal filling, cracked medium, color, excessive number of bubbles or pits, contamination and moisture must be inspected for in each lot of media used. Any deficiencies of these types should be noted and checked by this laboratory. Corrective action, up to deficient during use, this observation should be documented and the company notified. Corrective action taken should be recorded. QC for liquid media used in MGIT 960 is only suggested for new shipment or lot number of BBL MGIT 7 ml tubes. American Type Culture Collection (ATCC) control organisms (*M. tuberculosis* ATCC 27294, *M. kansasii* ATCC 12478, *M. fortuitum* ATCC 6841) prepared in a suspension in Middlebrook 7H9 Broth are used for QC. A suspension in Middlebrook 7H9 Broth is prepared from solid media cultures less than 15 days old. The suspension is allowed to sit for 20 minutes. The supernatant is transferred to an empty sterile tube and allowed sitting for an additional 15 minutes. Transfer the supernatant to another empty, sterile tube. The turbidity is adjusted to a 0.5 McFarland standard. Control organisms (*M. tuberculosis* ATCC 27294 = 1:500, *M.*

*kansasii* ATCC 12478 = 1:50,000, *M. fortuitum* ATCC 6841 = 1:5,000) are diluted and inoculated the BBL MGIT 7ml tubes. *M. tuberculosis* ATCC 27294 grows 6-10 days, *M. kansasii* ATCC 12478 grows 7-11days, *M. fortuitum* ATCC 6841 grows 1-3 days. Two BBL MGIT 7 ml tubes are used for QC of PANTA. 900 ul PANTA are added to one tube, and the other tube is without PANTA. The 2 tubes are incubated at the same condition for 42 days. Then they are evaluated. The result must be negative [24,25].

The Center for Disease Control and Prevention (CDC) announces that isolation and identification of *M. tuberculosis* should be completed within 21 days [26]. Therefore MGIT 960 is a very important machine for isolation of mycobacteria; all indicators are controlled on the MGIT 960 machine with 2 or 4 weeks intervals. Then results are printed and kept.

EQC for culture is prepared from an artificial set of sputum samples with known results. TB laboratory can participate in either any international or national EQA programme to compare its capability with the results of other TB laboratories. It has been attained opportunity to review all steps from decontamination to identification by these systems.

## Molecular testing

Today, to hasten the decline of the prevalence of TB, new technologies that provide rapid detection, identification and drug susceptibility testing of *M. tuberculosis*, have been used by many countries, especially developed laboratories [27].

Some molecular tests detecting very fast, simultaneously both active TB disease and multidrug-resistant (MDR) TB have been endorsed by WHO, although the conventional techniques are the cornerstone in TB diagnosis and its drug susceptibility testing [28]. Especially, nucleic acid amplification techniques for diagnosis of TB have drawn considerable attention for shortening the time required to detect and identify *M. tuberculosis* in respiratory specimens [29]. There are various methods used to detect mycobacteria DNA. But, the requirement of sophisticated infrastructure limits the utility of these tests [30]. The reliabilities of molecular tests for TB are related to experience and accuracy of the personnel conducting the tests.

Currently, many molecular diagnostic kits for TB are commercially available, but rate of their false positivity could be high or their sensitivity is often unclear, due to lack of international standards or reference reagents for molecular testing. Therefore, the tests approved by CDC or WHO should be prefer and worked by highly skilled technicians in well equipped laboratory, and EQC and IQC for molecular tests should be used, taken into consideration sensitivity problem. For example, for IQC, both positive and negative internal controls are used for every laboratory work for molecular tests of TB. Automatic pipettes used for tests should be calibrated. Reagents and test kits should be stored at the proper conditions.

GeneXpert System technology which was developed recently to rapidly detect *M. tuberculosis* is also the cornerstone of unique approach to molecular diagnostics product suite. This system allows accurate test results with unprecedented speed, cost effective and ease-of-use [31].

If TB laboratory using molecular tests participates in EQA programme, it will be attained opportunity to compare its capability with the results of other TB laboratories in terms of reproducibility, sensitivity and specificity. There are some EQA organizations such as QCMD, NEQAS, INSTAND, and EQUALIS implying proficiency testing for molecular diagnostics. The purpose of proficiency panels in molecular tests is to help laboratories to determine their own performance.

## Drug susceptibility testing

The CDC declared that susceptibility testing against first-line antituberculous drugs of every *M. tuberculosis* strain isolated from patients must be performed [32]. There are various laboratory methods of susceptibility testing for mycobacteria. But, the laboratory methods for anti-TB drug susceptibility testing (DST) should be selected from among those that are recommended by Clinical and Laboratory Standards Institute (CLSI). Four DST methods have been standardized and are widely used throughout the world [33], DST methods are proportion method and its economic and standard variants, resistance ratio method, absolute concentration method and BACTEC 460® radiometric method.

In 1994, WHO and IUATLD launched the Global Project on Antituberculosis Drug Resistance Surveillance. In this project, purpose is to measure the prevalence and monitor the trend of anti-TB drug resistance worldwide using a standardized methodology, and to study the correlation between the level of drug resistance and treatment policies in the different countries [34,35]. Inter-laboratory QC of DST was implemented within the global network (22 SRL) together with this project in the same year. Sensitivity, specificity and reproducibility of susceptibility testing were calculated for each SRL and for each of four drugs used in treatment of TB [34].

It is very important that QCS for DST has been established within the National TCP performed in a country in terms of the confidential results and the true resistance's profile. For IQC of DST, first of all, standardized procedures should be followed whether proportion method, BACTEC radiometric method, resistance ratio method, or absolute concentration method is used for susceptibility testing and for formulation of media. The quality of the medium should be controlled for each batch. The standard H37Rv strain or known drug-resistant strains in each new batch of LJ medium and for each drug are used for IQC. Drugs added to the medium must be pure drugs obtained from a known company and the percentage of potency must be clearly indicated. Drug dilutions and their addition to the medium should be performed according to the CLSI.

NRL should participate in an EQA programme for DST every year, and EQA programmes, organized by the SRL, validate the results of susceptibility tests done by the NRL. NRL should ensure EQC of DST performed by all laboratories in the country. If laboratory results are discordant, it should arrange on-site supervision programme for that laboratory, or provide training for the technicians. The regular proficiency testing can significantly improve the quality of DST, even in the most sophisticated TB laboratories [36]. The application of EQC is useful to measure the test standardization and efficiency of the laboratories performing DST. Therefore, it is necessary that all the laboratories performing DST are enforced to join the EQC programmes by law or by performance applications [37].

The panel tests containing 20 strains have been sent our NRL by WHO SRL since 2002, to monitor the quality of DST in our country [38]. NRL results have been assessed and validated in terms of susceptibility, specificity, efficiency, and reproducibility for streptomycin, isoniazid, rifampicin, and ethambutol. Eventually, to join EQC has improved the quality of DST in NRL, and expansion of EQC programme by NRL to all the laboratories performing DST contributes to better diagnosis of drug-resistant TB, and leads to more effective control of TB in our country.

## Conclusion

The fact that XDR-TB cases as well as MDR TB have recently emerged indicates that proper standards of TB laboratories are far from being universally applied. Thus, TB laboratories should ensure adequate expert input on laboratory design, specimen and process flow, biosafety, standard operating procedures, maintenance of equipment and EQA.

All laboratories providing TB diagnostic services should be included in QAS by implicating the standards, internationally recommended, TB management practices, and should provide accurate and reliable service.

The laboratories should be organized efficiently and the procedures should be carefully selected, taking account programme efficiency, and performed only by well-trained personnel under a systematic, effective, and sustainable quality assurance programme. Eventually, a good QA program will enhance credibility of laboratory for client.

### References

1. Pai M, Daley P, Hopewell PC (2007) International standards for tuberculosis care: Relevance and implications for laboratory professionals. *Indian J Med Microbiol* 25: 89-92.
2. Ozkutuk A (2009) Quality Assurance In Tuberculosis Laboratories. *Mikrobiyol Bul* 43: 699-707.
3. Zignol M, Gemert W, Falzon D, Jaramillo E, Blanc L, Raviglione M (2011) Modernizing Surveillance of Antituberculosis Drug Resistance: From Special Surveys to Routine Testing. *Clin Infect Dis* 52: 901-906.
4. Bass JB Jr, Farer LS, Hopewell PC, Jacobs RF, Snider DE Jr (1990) Diagnostic standards and classification of tuberculosis. *Am. Rev. Respir. Dis* 142: 725-735.
5. Centers for Disease Control. Isolation and identification of Mycobacterium tuberculosis: a guide for the level II laboratory. 1981.
6. Centers for Disease Control. Public health mycobacteriology: a guide for the level III laboratory. U.S. Department of Health and Human Services, 1985.
7. Drobniewski FA, Hoffner S, Rusch-Gerdes S, Skenders G, Thomsen V, the WHO European Laboratory Strengthening Task Force (2006) Recommended standards for modern tuberculosis laboratory services in Europe. *Eur Respir J* 28: 903–909.
8. Federal Register. Final guidelines for clinical laboratory improvement amendment of 1988, 1992; 57: 40 and 148.
9. Berwouts S, Morris MA, Dequeker E (2010) Approaches to quality management and accreditation in a genetic testing laboratory. *Eur J Hum Genetics* 18: 1–19.
10. Mundy CJ, Harries AD, Banerjee A, Salaniponi FM, Gilks CF, et al. (2002) Quality assessment of sputum transportation, smear preparation and AFB microscopy in a rural district in Malawi. *Int J Tuberc Lung Dis* 6: 47-54.
11. Addo KK, Dan-Dzide M, Yeboah-Manu D, Owusu-Darko K, Caulley P, et al. (2006) Improving the laboratory diagnosis of TB in Ghana: the impact of a quality assurance system. *Int J Tuberc Lung Dis* 10: 812-817.
12. Washington JA (1994) Microbiologic diagnosis of Lower Respiratory tract infections. In: Murray JF, Nadel JA, editors. Text book of Respiratory Medicine. 2nd ed. Philadelphia: WH Saunders; 585–600.
13. Enarson DA, Rieder HL, Arnadottir T (2000) Management of tuberculosis: A guide for law income countries. 5th ed. Paris: IUATLD.
14. World Health Organization. Guidelines for national programmes. Geneva: WHO; 1997(document WHO/TB/97.220).
15. Ba F, Rieder HL (1999) A comparison of fluorescence microscopy with the Ziehl-Neelsen technique in the examination of sputum for acid-fast bacilli. Int J Tuberc Lung Dis 3: 1101-1105.
16. Van Deun A, Hossain MA, Gumusboga M, Rieder HL (2008) Ziehl-Neelsen staining: theory and practice. Int J Tuberc Lung Dis 12: 108-10.
17. WHO. Fluorescent Light Emitting Diode (Led) Microscopy for Diagnosis of Tuberculosis- March 2010.
18. Kam KM (2002) Recommended Tools for Quality Assurance in TB Laboratories WHO, Western Pacific Region National TB Program and Laboratory Managers Meeting Cebu, Philippines, 3-6 December.
19. Central TB Division. RNTCP Laboratory Network Guidelines for Quality Assurance of Smear Microscopy for Diagnosing Tuberculosis. New Delhi: Directorate General of Health Services, Ministry of Health and Family Welfare: New Delhi, India, 2005.
20. Simsek H, Ceyhan I, Tarhan G, Guner U (2010) Quality Assessment of Microscopic Examination in Tuberculosis Diagnostic Laboratories: A Preliminary Study. Mikrobiyol Bul 44: 561-569 (Turkish).
21. Association of Public Health Laboratories (APHL). External quality assessment or AFB smear microscopy. APHL and Centers for Disease Control and Prevention, Washington, D.C. 2002.
22. Laboratory Services In Tuberculosis Control. Culture, Part III, IN. De Kantor, SJ. Kim, T. Friden, A. Laszlo, F. Luelmo, P-Yves Norval, H. Rieder, P. Valenzuela, K. Weyer, For the Global Tuberculosis Programme, World Health Organization, 1998 Geneva, Switzerland. (document WHO/TB/98.258).
23. Kim SJ (2008) Challenge of quality assurance for routine cultures. Int J Tuberc Lung Dis 12: 359-60.
24. National Committee for Clinical Laboratory Standards. Quality assurance standards for commercially prepared microbiological culture media; approved standard. NSSLC publication M22-A2. Villanova, Pennsylvania, NCCLS; 1996.
25. Isenberg HD (1994) Clinical Microbiology Procedures Handbook, Vol. 2. American Society for Microbiology, Washington, DC.
26. Ridderhof JC, Deun A, Kam KM, Narayanan PR, Aziz MA (2007) Roles of laboratories and laboratory systems in effective tuberculosis programmes. Bull World Health Organ 85: 354-9.
27. Drobniewski FA, Caws M, Gibson A, Young D (2003) Modern Laboratory diagnosis of tuberculosis. Lancet Infect Dis 3: 141-7.
28. World Health Organization. Policy statement. Molecular line probe assay for rapid screening of patients at risk of multidrug-resistant (MDR) TB. Geneva, 2008.
29. Rattan A (2000) PCR For Diagnosis Of Tuberculosis: Where Are We Now? Ind. J Tub 47: 79 -82.
30. Kar A, Modi-Parekh K, Chakroborty AK. Advances in Tuberculosis Diagnostics. Health Administrator Vol : XV, Numbers : 1-2, pg.118-123
31. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, Kop J, Owens MR, Rodgers R,Banada P et al. (2010) Rapid Detection of Mycobacterium tuberculosis and Rifampin Resistance by Use of On-Demand, Near-Patient Technology. J Clin Microbiol. 48: 229-237.
32. Centers for Disease Control and Prevention. Initial therapy for tuberculosis in the era of multidrug resistance. Recommendations of the advisory council for the elimination of tuberculosis. MMWR 1993; 42: 1-8.
33. Guidelines on the management of drug-resistant tuberculosis. Geneva, World Health Organization, 1996 (document WHO/TB/96.210).

34. Anti-tuberculosis drug resistance in the world: the WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance. Geneva, World Health Organization, 1997 (document WHO/TB/97.229).

35. Anti-tuberculosis drug resistance in the world: the WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance. Report 2: Prevalence and trends. Geneva, World Health Organization, 2000 (document WHO/CDS/TB/2000.278).

36. Laszlo A, Rahman M Espinal M, Raviglione M (2002) WHO/IUATLD Network of Supranational Reference Laboratories Quality assurance programme for drug susceptibility testing of Mycobacterium tuberculosis in the WHO/IUATLD Supranational Reference Laboratory Network: five rounds of proficiency testing, 1994-1998. Int J Tuberc Lung Dis 6:748-56.

37. Ceyhan I, Simsek H, Tarhan G, Guner U (2010) Implementation of External Quality Control for First Line Antituberculous Drug Susceptibility Testing: A Preliminary Study. Klimik Dergisi 23: 110-115.

38. Fattorini L, Iona E, Cirillo D, Migliori GB, Orefici G, et al. (2008) External quality control of Mycobacterium tuberculosis drug susceptibility testing: results of two rounds in endemic countries. Int J Tuberc Lung Dis 12: 214-7.