Standard Operating Procedure Utilization for Tuberculosis Microscopy in Mekelle City, North Ethiopia

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ABSTRACT

Objectives: *The aim of this study was to assess the utilization of standard operating procedures for acid-fast bacilli (AFB) smear microscopy.*

Methods: *A facility-based cross-sectional study was conducted in select health institutions in Mekelle City, Ethiopia, from July 1, 2015, through August 30, 2015. Using a simple random sampling technique, 18 health facilities were included in the study. Data were collected using a standard checklist and entered into Epi Info version 3.5.4 (Centers for Disease Control and Prevention, Atlanta, GA) for editing. Analysis was done using SPSS version 20 (SPSS, Chicago, IL).*

Results: *Of the 18 laboratory facilities, only seven (38.9%) had a legible AFB registration book. In three (16.7%) of the laboratories, heat fixation was not applied before adding primary staining reagent. In 12 (66.7%), the staining reagents had precipitates. Two laboratories had microscopes with mechanical stages that could not move freely on both axes. Seven (38.9%) of the laboratories reported samples to be negative before examining all required fields. Most laboratories, 16 (88.9%) and 17 (94.4%), respectively, did not run positive and negative controls after new batch reagent preparation.*

Conclusions: *Tuberculosis microscopy was found to be substandard with clear gaps in documentation, sample collection, and processing.*

In resource-limited settings with a high prevalence of tuberculosis (TB), direct sputum smear microscopy remains the most cost-effective technique for diagnosis and monitoring of treatment.[1] The global TB control target to detect 70% of new cases of smear-positive pulmonary TB by 2000 has still not been met by most World Health Organization member states.[2] The National Tuberculosis Control Program as struggled to surmount the challenges leading to Ethiopia's low detection rate (46%).[3,4] In Ethiopia, although significant improvement in case detection rates for TB smear microscopy has been made in recent years, many cases remain undetected.[5,6]

Laboratories play a vital role in improving TB case detection.[7] In acid-fast bacilli (AFB) smear microscopy, consistent and accurate laboratory procedures save lives and improve public health, whereas errors in diagnosis, reading, and recording of results lead to missing cases and inappropriate treatment that can result in a serious complications.[8,9]

Standard operating procedures (SOPs) are written formal documents that provide detailed step-by-step instructions for carrying out laboratory activities in a safe manner that yield accurate and reliable results. SOPs are essential to good laboratory practices, ensure consistent results, and are required for accreditation.[10-12] Written copies of SOPs should be available (and preferably displayed) in all work areas, and laboratories at each level of care must follow these standard procedures. Use of AFB smear microscopy SOPs for diagnosing pulmonary TB has not been documented in Mekelle City or nationally. Therefore, the aim of this study was to assess utilization of SOPs for AFB smear microscopy in selected health facilities within Mekelle City.

Materials and Methods

Study Area

The study was conducted in 18 randomly selected health facility laboratories in Mekelle City (Tigray region). Mekelle has one university referral hospital (Ayder Referral Hospital), two public hospitals, four private hospitals, eight public health centers, and 21 private clinics. TB laboratory services are provided at each of these facilities.

Study Design and Period

The cross-sectional descriptive study was conducted from July 1, 2015, to August 30, 2015.

Sample Size Determination

Sample size was determined based on a commonly used practice of selecting 30% to 50% of facilities if the number of units/facilities is fewer than 50.[13] Half of the 36 health facilities in Mekelle City were included in the study.

Sampling Technique

A simple random sampling procedure was applied to the 18 selected health facilities.

Data Collection Procedure

Using a standard adopted checklist,[14] data were collected from each laboratory through observation and staff interviews.

Data Quality Control

To avoid errors, data were reviewed daily to ensure that data collection instruments were filled out correctly and completely.

Data Analysis

Data were entered into Epi Info version 3.5.4 (Centers for Disease Control and Prevention, Atlanta, GA) for editing. Analysis was done using SPSS version 20 (SPSS, Chicago, IL). Analyzed data are presented in tables.

Ethical Clearance

Ethical clearance was obtained from Mekelle University, College of Health Sciences Research and Community Services Council prior to data collection. Verbal consent was also obtained from employees who were interviewed and administrators of each health institution.

Operational Definitions

SOP: Approved manuals that describe how to perform various activities in the laboratory.

SOP utilization: Step-by-step instruction followed by laboratory staff to produce the right result at the right time on the right specimen from the right patient with result interpretation based on correct reference data using consistent smear and safe laboratory practices.

Results

The 18 laboratories providing TB diagnostic services were evaluated using the following parameters for TB microscopy:

Recordkeeping

During spot observations, only seven (38.9%) of the 18 laboratories maintained legible AFB registration books. In eight (44.4%) of the laboratory facilities, frosted slides were not available for permanent labeling of smear samples. Five (27.8%) laboratory facilities did not have a separate laboratory request format and identification codes for labeling. Half of the facilities did not store smear slides for at least 3 months or were storing them inappropriately **Table 1**.

Sputum Collection, Smearing, and Staining Procedures

In three (16.7%) laboratories, patients were required to provide satisfactory and adequate sputum specimens. There were inadequate supplies of clean sputum containers, new slides, and clean applicator sticks in eight (44.4%), 11 (61%), and four (22.2%) laboratories, respectively. From each laboratory, 10 smeared slides were arbitrarily selected and evaluated against the standard. Four (22.2%), three (16.7%), and three (16.7%) laboratories respectively fulfilled required criteria in terms of size, evenness, and thickness. In two (11.1%) laboratories, reagents did not have labels indicating date of preparation and expiry. One (5.6%) laboratory that did use proper labels was using reagents after their expiry date. In eight (44.4%) laboratories, staining reagents were not properly stored, and in 12 (66.7%), reagents were contaminated with precipitants. With regard to staining procedures, three (16.7%) laboratories did not apply heat fixative before adding primary staining reagent, and two (11.1%) laboratories used inappropriate staining time intervals between the successive reagents. From each of the laboratories, five smear-positive slides were arbitrarily selected and evaluated. Slides were found to be well stained in only four (22%) of these **Table 2**.

Microscopy and Reading

Two (11.1%) laboratories were using microscopes with mechanical stages that could not move freely on both axes. In seven (38.9%) laboratories, slides were reported as negative before the required number of fields. Sixteen (88.9%) laboratories did not run positive controls, and 17 (94.4%) did not run negative controls to test newly prepared batches of reagent or at least every week. In 14 (77.8%) laboratories, positive smear slides were not cross-

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Table 1
Recordkeeping and Documentation Trend of the Selected Health Facilities (n = 18) for Acid-Fast Bacilli Microscopy in Mekelle City, Ethiopia (July-August 2015)[a]

SN	Criteria	Score	No. (%)
1	Laboratory log/register is legible	0	1 (5.6)
		1	1 (5.6)
		2	1 (5.6)
		3	8 (44.4)
		4	7 (38.9)
2	Slides labeled permanently	0	8 (44.4)
		3	10 (55.6)
3	Laboratory uses one result form per patient and labels form with identification number or name	0	5 (27.8)
		1	5 (27.8)
		2	8 (44.4)
4	Laboratory keeps smears for at least 3 months and stores them appropriately	0	2 (11.1)
		1	7 (38.9)
		2	9 (50.0)

SN, serial number.
[a]Maximum score of 4 for criterion 1, 3 for criterion 2, and 2 for criteria 3 and 4.

checked with second readings. In seven (38.9%), the oil immersion in use was not clean enough, and in three (16.7%), the microscope area had inadequate light and seating space **Table 3**.

Safety Measures

None of the laboratories had a biological safety cabinet. Fourteen (77.8%) did not have a separate area with good ventilation for smear processing, and 17 (94.4%) had no well-ventilated airflow from less-contaminated areas. Eleven (61.1%) laboratories did not restrict access, and 16 (88.9%) did not have a separate room/office for administrative works. Thirteen (72.2%) laboratories did not decontaminate materials prior to disposal, and in 10 (55.6%), staff did not clean work benches before and after preparation of smears or immediately after spills.

Overall Status of the Laboratories

The health facilities assessed in this study were rated "excellent," "good," or "poor" based on overall SOP utilization for TB microscopy. No health facility was rated "excellent." Health facilities rated "good" were as follows: Semhal Specialty Clinic (83.5%), Ayder Referral Hospital (82.0%), Dr. Mekonen Specialty Clinic (78.7%), SeAme Specialty Clinic (76.5%), Mekelle Hospital (72.4%), Rohobot Specialty Clinic (72.4%), and Universal Diagnostic Laboratory (70.2%). Health facilities rated "poor" included the following: Markos Hospital (66.5%), Quiha Health Center (65.7%), Ben Meskerem Hospital (65.0%), Semen Health Center (60.2%), Ethio-Medium Clinic (59.1%), Hiwot Specialty Clinic (58.7%), Adishimdihun Health Center (55.7%), Kidus Specialty Clinic (52.0%), Mekelle Health Center (52.0%), Marry Medium Clinic (51.3%), and Amanuel Medium Clinic (50.2%).

Discussion

In the present study, there was significant failure in SOP utilization in each of the assessed laboratories, even with the presence of SOPs for TB microscopy. Clear gaps in documentation, patient orientation, sample collection, preparation, and processing were found. Thus, the existing gaps may lead to mismanagement and inaccurate diagnosis and treatment by increasing the risk of false-positive and false-negative results. Due to risk of contamination, the gaps may also expose laboratory professionals and patients to infection.

Many laboratory facilities did not have legible AFB registration books and did not store smeared slides as required for external quality assessment and rechecking. In a significant proportion of laboratories, patients did not receive clear instruction on how to provide satisfactory and representative sputum specimens. Safety precautions during sample collection, smear preparation, and processing were inadequate in almost all of the laboratories. Precipitate was present in the staining reagents in most facilities. Smears were not prepared appropriately in terms of size, thickness, and evenness in most laboratories. In a significant proportion of laboratories, slides were reported as negative before the required number of fields and minimum time spent per slide. In most laboratories, there was no trend to run positive and negative control smears regularly. The possible reasons for failure in SOP utilization could be due to knowledge gaps, heavy workload, negligence, lack of facility and supplies, and lack of regular assessment and feedback.

In general, TB microscopy was found to be substandard in the assessed laboratories, which is similar to

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Table 2
Sample Collection, Smearing, and Staining Procedures of the Selected Health Facilities (n = 18) for AFB Microscopy in Mekelle City, Ethiopia (July-August 2015)[a]

SN	Criteria	Score	No. (%)
1	Adequate supply of clean sputum containers with wide mouth and screw top (enough for 3 months of patients)	0	8 (44.4)
		4	10 (55.6)
2	Tell patients how to give satisfactory sputum specimen	0	3 (16.7)
		4	15 (83.3)
3	Obtain at least one morning sputum	0	0 (0)
		4	18 (100)
4	Use new, clean slides and have an adequate supply	0	11 (61.1)
		2	7 (38.9)
5	Use clean applicator (stick, pipette, or wire loop) for smearing	0	4 (22.2)
		2	14 (77.8)
6	Use appropriate smear size (1-2 cm × 2-3 cm)	0	2 (11.1)
		3	10 (55.6)
		4	2 (11.1)
		5	4 (22.2)
7	Use appropriate evenness (even throughout)	0	2 (11.1)
		2	1 (5.6)
		3	9 (50.0)
		4	3 (16.7)
		5	3 (16.7)
8	Use appropriate thickness (once dry, able to read print through thick film at 4-5 cm)	0	1 (5.6)
		2	2 (11.1)
		3	8 (44.4)
		4	4 (22.2)
		5	3 (16.7)
9	Stain the slides so AFB are not faded, even after 3 months (AFB stain red)	0	3 (16.7)
		2	10 (55.6)
		3	1 (5.6)
		4	4 (22.2)
10	Use stains without precipitate. Filtering stain before use can reduce precipitate	0	12 (66.7)
		2	6 (33.3)
11	Use reagent grade stains (commercial or prepared on site)	0	2 (11.1)
		4	16 (88.9)
12	Store stains at room temperature and away from bright light or heat source	0	1 (5.6)
		0.5	7 (38.9)
		1	10 (55.6)
13	Use commercial stains within expiration date	0	1 (5.6)
		9	17 (94.4)
14	Use stains within 12 months of opening/preparing (preferably within 6 months)	0	7 (38.9)
		2	11 (61.1)
15	Air-dry slide	2	18 (100)
16	Heat fix slide (with flame or slide warmer to 65-75°C)	0	3 (16.7)
		1	1 (5.6)
		2	14 (77.8)
17	Use approved times	0	2 (11.1)
		3	16 (88.9)
18	Stain individual slides to prevent cross-contamination	0	8 (44.4)
		1	10 (55.6)
19	Change solution in bottles used to stain slides every 2 weeks and record change	0	5 (27.8)
		0.5	11 (61.1)
		1	2 (11.1)

AFB, acid-fast bacilli; SN, serial number.
[a]Maximum score of 1 for criteria 12, 18, and 19; 2 for criteria 4, 5, 10, 14, 15, and 16; 3 for criterion 17; 4 for criteria 1, 2, 3, 9, and 11; 5 for criteria 6, 7, and 8; and 9 for criterion 13.

previous findings from some parts of the nation and other settings on the African continent.[15-18] Therefore, to improve SOP utilization and thereby enhance the quality of TB microscopy, there is a need to provide on-the-job training, supportive supervision, and timely feedback for service providers/professionals. In addition, since the Ethiopian government has been given due attention to strengthen diagnostic health services, there is also a need

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Table 3
Microscopy and Reading Trend of the Selected Health Facilities (n = 18) for Acid-Fast Bacilli Microscopy in Mekelle City, Ethiopia (July-August 2015)[a]

SN	Criteria	Score	No. (%)
1	Have ×100 magnification (plus ×10 eyepiece)	2	18 (100)
2	Use clean oil for slide and remove oil from slide before storing with absorbent paper	0	2 (11.1)
		0.5	5 (27.8)
		1	11 (61.1)
3	Read each slide for 5 minutes or 100-150 fields	2	7 (38.9)
		4	11 (61.1)
4	Use microscope in good working order	0	2 (11.1)
		2	16 (88.9)
5	Have microscope area with appropriate lighting and sufficient seating space	0	1 (5.6)
		0.5	2 (11.1)
		1	15 (83.3)
6	Use positive control smear at least every week and after new reagent	0	16 (88.9)
		2	1 (5.6)
		3	1 (5.6)
7	Use negative control smear at least every week and after new reagent	0	17 (94.4)
		1	1 (5.6)
8	Perform external proficiency testing and results observed by evaluator	0	17 (94.4)
		4	1 (5.6)
9	Process >15 specimens each week (about 65 each month)	0	12 (66.7)
		1	6 (33.3)
10	Use internationally accepted grading system for reporting results	2	18 (100)
11	Perform second reading on all positive slides	0	14 (77.8)
		1	4 (22.2)
12	Report results within 24 hours from specimen receipt	1	18 (100)

SN, serial number.
[a]Maximum score of 2 for criteria 1, 4, and 10; 1 for criteria 2, 5, 7, 9, 11, and 12; 4 for criteria 3 and 8; 3 for criterion 6.

to create awareness to all stakeholders to fully equip laboratories with the required facilities, materials, supplies, and manpower.

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