



# Quality of Same-Day Sputum Smears Microscopy and Presumptive Tuberculosis Patients Drop-out at Health Facilities of Addis Ababa, Ethiopia

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**Background:** Same-day sputum microcopy is recommended in areas where sputum smear microscopy external quality assessment (EQA) is effectively implemented and sturdy. In Addis Ababa, the status of EQA and drop-out of same-day sputum smear microcopy has not yet been assessed. The objective of this study was to assess the quality of same-day sputum smear microscopy and diagnostic drop-out of presumptive tuberculosis (TB) patients in health facilities (HFs) across Addis Ababa, Ethiopia.

**Methods:** A multi-analysis was conducted from September 2016 to July 2017 to determine the status of external quality assessment and diagnostic drop-out of presumptive TB patients registered for same-day sputum smear microscopy. Data was coded and entered in Microsoft Excel, and subsequently transferred and analyzed using SPSS version 20.0.

**Results:** The drop-out of same-day sputum smear microscopy was 209 (6.2%). More than 33% of the specimens collected for purposes of same-day sputum smears were of poor quality. Among the selected HFs for the study: 13 (46.4%) used filter reagents prior to sputum smear staining while 75% of the selected HFs for the study used smear microscopy services interruption in a year. The sensitivity and specificity of the HFs participating in regional quality assessment scheme for the diagnosis of TB was 97.4% and 99.6%, respectively.

**Conclusion:** The diagnostic drop-out of same-day sputum smear microscopy was high in Addis Ababa. Strengthening EQA, competency-based laboratory professionals training on sputum smear microscopy might reduce the reading errors in sputum smear. Awareness creation of the community on the benefits gained from completion of specimen provision for the same-day approach decreases diagnostic drop-out and enhances TB control program.

**Keywords:** Same-Day; Sputum Smear Microscopy; Drop-out; External Quality Assessment

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**Received:** Apr. 15, 2019

**Revised:** May. 23, 2019

**Accepted:** Jul. 3, 2019

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## Introduction

In developing countries, sputum smears microscopy remains the most widely used and is the means by which universal access to diagnosis and treatment can be achieved<sup>1</sup>. Even though 2-day sputum microscopy is better in sensitivity, people make repeated visits to the health care centers for specimen delivery, results collection, and anti-tuberculosis (TB) treatment initiation. For many patients, the cost of repeated visits to health facilities (HFs) was prohibitive and patients drop-out is a significant problem<sup>2,3</sup>. As a result, the World Health Organization (WHO) has recommended a reduction in the number of specimens from 2-day to same-day

in settings where appropriate sputum smear microscopy is strongly implemented<sup>1</sup>.

The fact that mucociliary clearance of respiratory tract is high overnight and sputum specimens obtained early in the morning is more likely productive than spot. The difference in diagnostic sensitivity of the 2 days—and same-day sputum microscopy approaches can be minimized with appropriate instruction of presumptive TB patients to spout out productive sputum specimen, proper smear preparation, staining, careful microscopic examination of stained smears and performing internal quality control for the reagents<sup>1</sup>.

Presumptive TB patients diagnosed by same-day sputum smear microscopy were more likely submit both specimens (drop-out, 2%) than those screened by 2-day approach (drop-out, 5.8%)<sup>1,3</sup>. In Ethiopia, diagnostic drop-out of presumptive TB patients screened for TB by same-day sputum smear microscopy was not yet studied.

On the bases diagnostic shift from 2-day to same-day sputum smear microscopy approach, morning sputum is missed and same-day sputum specimens are used to diagnose pulmonary tuberculosis (PTB). Spot specimens inevitably contain respiratory secretions from both the healthy airway tract and the diseased; the produced sputum specimens are more saliva which has an impact on smearing quality and poor quality sputum specimens might lead to miss diagnoses<sup>2,4</sup>. Therefore, in this context strengthening external quality assessment (EQA) is the sole parts of sputum smear microscopy.

In Addis Ababa, Ethiopia, the status EQA of same-day sputum microscopy in HFs participating in regional quality assessment scheme (REQAS) was not so far studied and documented. The present study assessed the status of EQA of same-day smear microscopy in HFs participating in REQAS, smear microscopy service quality, and diagnostic drop-out of presumptive TB patients diagnosed by same-day sputum smear microscopy approach.

## Materials and Methods

### 1. Study design and setting

Cross-sectional retrospective study was conducted on HFs EQA results archived in EQA centers that performed blinded rechecking of sputum smear microscopy, from September 2016 to July 2017 to determine EQA status of same-day sputum microscopy. All newly presumptive TB patients' data registered for same-day sputum smear microscopy in 13 selected HFs for the study were reviewed to determine diagnostic drop-out rate of the same-day approach. The study was supplemented by checklist based assessment TB laboratory infrastructures related to same-day sputum smear microscopy services quality.

Addis Ababa has a total of 540 km<sup>2</sup> having 10 sub-cities, 116 districts, and an estimated total population of 3,384,569, with annual growth rate of 3.8%<sup>5</sup>. It encompasses more 896 public and private HFs, of which 101 public and 52 private were being providing acid-fast bacilli (AFB) microscopy and Directly Observed Treatment Short Course (DOTS) services<sup>6</sup>.

### 2. Sample size determination and sampling procedure

Blinded–rechecked EQA results of 153 HFs participating in collected according to lot quality assurance techniques<sup>7,8</sup> were reviewed from the archived data in EQA centers and Addis Ababa Public Health Research and Emergency Management (AAPHREM). New presumptive TB patients' data registered in 12 public and one private HFs providing TB diagnostics and DOTS services were purposively selected to determine diagnostic drop-out rate same-day sputum smear microscopy.

### 3. Data collection tools and collection procedures

Ethical clearance was obtained from Addis Ababa Health Bureau Ethical Review Committee (#AAHB/2058/321) and letter of support were obtained from AAPHREM and HFs participated in the study were informed that all data collected would remain anonymous treated statistically and remain confidential. Written informed consents were obtained. Checklists were adapted from the national TB-EQA guidelines to assess TB laboratory infrastructures related to same-day smear microscopy services quality. TB laboratory results records books were reviewed since same-day sputum smear microscopy implementation to determine the diagnostic drop-out rate.

### 4. Data management and analysis

The collected data were checked for completeness, consistency and code number were assigned, entered to micro soft Excel (Microsoft Inc., Redmond, WA, USA). Before analysis, it was cleaned and edited by simple frequencies, cross-tabulation and transferred to SPSS version 20.0 (IBM Corp., Armonk, NY, USA) for analysis. Descriptive statistics, numerical summaries, and tables were used to describe relevant variables.

### 5. Data quality assurances

One-day training was given for data collectors and appropriate supervision was made throughout data collection. Before submission, data collectors were instructed to check the completeness of each data. The principal investigator rechecked the completeness of the data while receiving from data collectors.

## 6. Operational definitions

- Good labeling: Sputum smear slides contain the initials name of HFs from which smear slides were collected for blinded rechecking, date smears prepared, serials numbers of slides on registration book and sequences of smear slides.

- Good smear size: When the sputum smears prepared is 1 by 2 or 2 by 3 cm from the center of microscopic slides covered.

- Cleanness: Sputum smear slides with no artifacts during the microscopic examination and clear background with no remnant staining reagents left on the smear.

- Evenness: Sputum smear with even distribution from the center of microscopic slides to the border with appropriate sizes and good coiling.

- Thickness: Good smearing thickness when smear is not too thick and not too thin with partially translucent that enable to read any of written words on the paper through the smear.

- Staining quality: Sputum smear with no remnants of reagents (well decolorized) on the smears when examined microscopically.

- Diagnostic drop-out: New presumptive TB patients registered for same-day sputum smear microscopy who do not submitted any of the two or one sputum specimen.

- Specimens quality: Sputum smears containing more than 10 white blood cells when examined microscopically.

- External quality assessment: A system of objectively checking the laboratory's performance using an external agencies

or facilities (EQA centers, regional, or central laboratories) through on-site evaluation of the laboratory, panel testing and blinded rechecking.

## Results

### 1. Blinded-rechecked results of same-day sputum smear microscopy

Since same-day sputum smear microscopy implementation in Addis Ababa over 1 year, a total of 7,551 Ziehl-Neelsen (ZN)-stained sputum smear slides were collected for blinded rechecking from HFs participating in REQAS. Sputum smear reading agreement between peripheral laboratories and EQA centers that performed blinded rechecking were 99.6% for negative and 98.8% for positive with overall concordant rates 99.4%. From the total discordant: 44 (0.6%), of which 18 (0.2%) were false-negative and 26 (0.4%) were false-positive. Among false-negatives, 14 (77.8%) were high false-negative and among false-positives, 22 (84.6%) were low false-positive, as the detailed illustrated in Table 1.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of peripheral laboratories in diagnosing TB against the results of EQA centers as reference standard were 97.4% (95% confidence interval [CI], 95.9–98.5), 99.6% (95% CI, 99.4–99.7), 96.3% (95% CI, 94.6–97.4), and 99.7% (95% CI, 99.6–99.8), respectively.

Specimens, smearing, and staining qualities of same-day

**Table 1.** Sputum smear reading agreement between peripheral HFs laboratories and EQA center lead hospitals or regional laboratory, Addis Ababa, Ethiopia, September 2016 to July 2017

Peripheral laboratory	EQA center			Error			
	Positive	Negative	Total	HFP	HFN	LFP	LFN
Positive	677 (8.9)	26 (0.3)	703 (9.3)	4 (15.4)	14 (77.8)	22 (84.6)	4 (22.2)
Negative	18 (0.2)	6,830 (90.5)	6,848 (90.7)				
Total	695 (9.2)	6,856 (90.8)	7,551 (100)				

Values are presented as number (%).

HF: health facility; EQA: external quality assessment; HFP: high false-positive; HFN: high false-negative; LFP: low false-positive; LFN: low false-negative.

**Table 2.** Quality of same-day sputum smears rechecked in Addis Ababa, Ethiopia

Specimens quality	No. (%)	Smearing quality					Staining quality
		Labeling	Size	Thickness	Cleanness	Evenness	
Good	5,001 (66.5)	6,521 (86.7)	5,321 (70.7)	4,956 (65.9)	5,368 (81.3)	5,017 (66.7)	5,692 (75.6)
Poor	2,523 (33.5)	1,003 (13.3)	2,203 (29.3)	2,568 (34.1)	1,233 (18.7)	2,507 (33.3)	1,832 (24.5)
Total	7,524 (100)	7,524 (100)	7,524 (100)	7,524 (100)	6,601 (100)	7,524 (100)	7,524 (100)

Values are presented as number (%).

sputum smears of HF's participated in REQAS were reviewed. From the total of 7,524 ZN-stained sputum smear results evaluated and documented 2,523 (33.5%) had poor specimen quality as the detailed depicted in Table 2.

## 2. Smear positivity rate and bacillary loads of same-day sputum smears examined in HF's of Addis Ababa

Sputum smear positivity rate and the bacillary loads of smears positive registered in selected HF's for the study were calculated to evaluate the impacts same-day sputum smear microscopy implementation on the bacillary of smears positives. From the total of 6,397 new sputum smear slides examined in HF's included in the study, 249 (3.9%) were positive (129 first spot and 120 second spot). Of 129 first spot sputum smear positive, seven did not submit second spot; two were negative for the second spot smear. Of 3,361 new presumptive TB patients registered in laboratories of selected HF's, 120 (3.8%) were positive for both first and second spot sputum smears. The bacillary loads of sputum smear microscopy were determined using international sputum smear microscopy grading scale<sup>6</sup>. The majorities of smear positive 106 (42.6%) were 1+ as the detailed illustrated in Table 3.

## 3. Laboratory infrastructures and service quality related to same-day sputum smear microscopy

The infrastructures of HF's laboratories performing sputum smear microscopy was assessed using checklists adapted from national sputum smear microscopy guidelines as variable indicating quality of same-day sputum smear microscopy. Nearly 46.4% of HF's performing sputum smear microscopy had no separate area for TB smear microscopy as the detailed showed in Table 4.

## 4. Diagnostic drop-out of same-day sputum smear microscopy in Addis Ababa, Ethiopia

From the total of 3,361 new presumptive TB patients registered in selected HF's after implementations of same-day spu-

tum smear microscopy, 93 (2.7%) had submitted the first spot but did not submit second spot sputum specimen. One hundred sixteen (3.4%) did not submit any of the two spot sputum specimens. The overall diagnostic drop-out rate of same-day sputum smear microscopy among the newly diagnosed presumptive TB patients was 209 (6.2%).

The diagnostic drop-out rate was highest in private HF's. From the total of 691 new presumptive TB patients registered in private HF's 50 (7.2%) were dropped-out from diagnostic paths in contrast to 159 (5.9%) in public HF's. Among the total 209, diagnostic defaulter male and female accounted 44.9% and were 47.4% respectively as the detailed depicted in Table 5.

## Discussion

In this study, the diagnostic drop-out rate of the same-day sputum smear microscopy was 5.9% in public and 7.4% in private HF's with overall drop-out rate of 6.2%. It was much lower than study conducted in Pakistan with overall default rate of 12.9%<sup>9</sup>. The difference might be due to the difference in diagnostic approach. In the present study, the drop-out of same-day smear microscopy were reviewed in the study of Pakistan, the 2-day sputum smear microscopy approach was reviewed.

The finding of the present study was higher than the drop-out (2%) of the WHO same-day policy implementation<sup>1</sup>. The difference might be due to methodologies: in the present study, secondary data were reviewed from records in contrast to WHO randomized control trial study. The overall sputum smear microscopy reading agreement between peripheral laboratories and EQA centers 99.4% was concordant with the study conducted in west Amhara, Ethiopia, and Argentina with smear reading agreement 99.4% and 98%, respectively<sup>10-12</sup>. However, it was higher than the study conducted in Eastern Ethiopia with smear reading agreement 94.5%<sup>13</sup>.

The agreement between peripheral laboratories and EQA centers for negative smear reading in the present study was 99.6%, was higher compared to, 42.9% of the study conducted in Tanzania and 95% of the previous study conducted in Ad-

**Table 3.** Bacillary loads of sputum smear positive examined in HF's of Addis Ababa, Ethiopia, September 2016 to July 2017

Bacillary loads of smear	First spot	Second spot	Total positives
Scanty	11 (8.5)	12 (10)	23 (9.2)
1+	60 (46.5)	46 (38.3)	106 (42.6)
2+	28 (21.7)	35 (29.2)	63 (25.3)
3+	26 (20.2)	23 (19.2)	49 (19.7)
No grading but positive	4 (3.1)	4 (3.3)	8 (3.2)
Total	129 (100)	120 (100)	249 (100)

Values are presented as number (%).  
HF: health facility.

**Table 4.** Laboratory services quality same-day sputum smear microscopy, Addis Ababa, Ethiopia, September 2016 to July 2017

Variable	Yes	No
Separate area for TB smear microscopy laboratory	15 (53.6)	13 (46.4)
Working area looks clean and well organized	16 (57.1)	12 (42.9)
Laboratory have adequate ventilation	19 (67.9)	9 (32.1)
Regular water supply/water tank in place	24 (85.7)	4 (14.3)
Availability regular electricity supply or back-up power	23 (82.1)	5 (17.9)
Availability of national guideline and SOPs in laboratory	6 (21.4)	22 (77.6)
Laboratory professionals trained on AFB microscopy or EQA within in the last 1 year	58 (15.5)	317 (84.5)
Availability of laboratory reagents and service interruption	3 (25.0)	9 (75.0)
Sputum container approved by NTP	15 (53.6)	13 (46.4)
Having filter paper to filter carbol fuchsin and methylene blue	14 (50.0)	14 (50.0)
Check the quality of sputum	12 (42.9)	16 (56.1)
Filter carbol fuchsin and methylene blue prior to stain smears	13 (46.4)	15 (53.6)
Prepare positive and negative smears to check reagents quality	13 (46.4)	15 (53.6)
Record when slides sent for EQA	14 (50.0)	14 (50.0)
Register EQA results and plan for improvement	13 (64.4)	18 (53.6)
Timely EQA feedback for HFs participating in REQAS	101 (66.7)	51 (33.3)
HFs participating in REQAS in Addis Ababa	152 (15.0)	743 (85.0)

Values are presented as number (%).

TB: tuberculosis; SOP: standard operating procedures; AFB: acid-fast bacilli; EQA: external quality assessment; NTP: National Tuberculosis Program; HFs: health facilities; REQAS: regional external quality assessment scheme.

**Table 5.** Drop-out rate among sex and age category of the study participants, Addis Ababa, Ethiopia, September 2016 to July 2017

Age category (yr)	Male	Female	Total
1–10	4 (1.9)	2 (0.9)	6 (2.8)
11–20	5 (2.4)	16 (7.6)	21 (10.0)
21–30	21 (10.0)	39 (18.7)	60 (28.7)
31–40	18 (8.6)	14 (6.7)	32 (15.3)
41–50	10 (4.8)	9 (4.3)	19 (9.1)
51–60	5 (2.4)	4 (1.9)	9 (4.3)
>60	10 (4.8)	2 (0.9)	12 (5.7)
Age not registered	21 (10.1)	13 (6.2)	28 (13.4)
Both age and sex not registered	-	-	16 (7.6)
Total	94 (44.9)	99 (47.4)	209 (100)

Values are presented as number (%).

dis Ababa<sup>14,15</sup>. The difference could be attributed to training of laboratory professionals on AFB microscopy. Prior to implementation of same-day sputum microscopy, many training sections have been conducted to laboratory professionals by the region that might increase the performances. In Ethiopia, laboratory quality management was implemented after the

previous study which might be contributed to the difference. However, the positive smear reading agreement 98.8% in the present study was in line with 100% of study in Tanzania and 99% of study in Addis Ababa<sup>14,15</sup>.

The discrepancy might result from sample size, training laboratory professionals on microscopy. In the present study,

a total of 7,551 sputum smear slides were collected for blinded rechecking whilst 578 ZN-stained sputum smear slides were rechecked in conducted study Eastern Ethiopia.

In the present study, the sensitivity, specificity, PPV and NPV of same-day sputum smear microscopy 97.4%, 99.6%, 96.3%, and 99.7%, respectively. It was higher than the study conducted in Eastern Ethiopia with sensitivity, specificity, PPV, and NPV of 83.5%, 97.8%, 91.7%, and 95.7%, respectively<sup>15</sup>. The difference could be due to laboratory professionals training on sputum smear microscopy which might vary from region to region, the work loads of smear microscopy and hired laboratory professionals might differ from region to region. Besides proper collection of sputum samples from suspected PTB patients, the preparation of good, uniform, thin smears, and staining of smears with high-quality staining reagents is imperative in precise reporting on microscopy<sup>16</sup>.

In this study, 7,524 of smears slides collected for EQA blinded rechecking, 2,523 (33.5%) had poor specimens quality which might result from specimen collected at any time of presumptive TB patient arrival (spot-spot) to HFs. Mucociliary clearance is high overnight hence spot-spot is less concentrated than morning specimens which might lead to poor specimens quality.

Timely feedback on supportive supervision and EQA coverage of the present study were 66.6% and 15%, respectively. It was comparable to the finding of the study conducted in west Amhara and Mekelle, Ethiopia indicating supportive supervision and timely feedback, internal and external quality assurance practices, equity in training and resource distribution were issues given less attention in these areas<sup>17,18</sup>.

In this study lack of training on same-day approach, stock-outs of reagents and supplies and lack of sufficient human power as per workloads of laboratory professionals, motivation, risk allowance, inadequate space infrastructures were some of the challenges identified. Similar findings were reported from study conducted in Uganda on identified key health system barriers hindering TB service quality: stock-outs of drugs/supplies, inadequate space and infrastructure, lack of training, high workload, low staff motivation, and poor coordination of health center services<sup>19,20</sup>.

In conclusion, in Addis Ababa, the diagnostic drop-out rate of same-day sputum smear microscopy was high. Strengthening EQA, sputum smear microscopy services quality in HFs, health education and promotion for presumptive TB patients on sputum provision, training of laboratory professionals on sputum smear microscopy reduces errors in sputum smear reading, increases case detection and plays a great role in the fight against TB.

## Authors' Contributions

Conceptualization: Taddese BD, Misganaw AS. Methodol-

ogy: Taddese BD, Misganaw AS. Formal analysis: Taddese BD, Misganaw AS. Data curation: Taddese BD, Misganaw AS. Software: Taddese BD, Misganaw AS. Validation: Taddese BD, Misganaw AS. Investigation: Taddese BD, Misganaw AS. Writing - original draft preparation: Taddese BD, Misganaw AS. Writing - review and editing: Taddese BD, Misganaw AS. Approval of final manuscript: all authors.

## Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

## Acknowledgments

We would like to thank Addis Ababa City Administration Health Bureau and AAPHREM Core Process for giving us this opportunity. Our thanks also extend to those all who cooperated with us in doing this work.

## Funding

No funding to declare.

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