



Same-Day Diagnosis versus the Conventional ‘Spot-Morning-Spot’ Using Ziehl-Neelsen and Fluorescent Microscopy: A Cross-Sectional Study

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Authors' contributions

This work was carried out in collaboration between all authors. All Authors AM, DM, YG, FA and CU involved in designing the study, writing the protocol and managed the analyses of the study. All authors read and approved the final manuscript

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ABSTRACT

Introduction: Diagnosis of spot-morning-spot (SMS) smear microscopy is inconvenient for patients, who have to make multiple visits to health facilities to submit multiple sputum specimens over two days and may visit also for an extra day to collect the result. Optimization of smear and microscopy will decrease the inconvenience of the patients and possibly increase the detection rate.

Objective: To determine the sensitivity, specificity and predictive values of a proposed “same day” strategy of one-day diagnosis of tuberculosis (TB) and compare it to the conventional method, as culture is reference standard.

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Methods: A cross-sectional study was conducted from June to August, 2013 from University of Gondar Hospital [UoGH] and Debretabor Rural Hospital [DTH], North West Ethiopia. A total of 180 TB suspected patients were enrolled. Patients suspected for TB submitted SMS samples [conventional method]. One additional sample was collected ≥ 1 h after the first sputum (the proposed same-day method) and one sample selected and cultured. Open Epi data & Mc Nemar's tests were used to compare the test.

Result: The sensitivity of the conventional method (27/160) was 81.8%, 95%CI (65.6-91.4) and that of proposed spot method (25/160), was 75.8%, 95%CI (58.9- 87.2) by Ziehl-Neelson (ZN) but the difference was statistically significant; P-value = 0.298. Their specificity was similar 100 % (97.1-100); P-value = 1.00. The light emitted diode (LED-FM) sensitivity was 84.9% (69.1- 93.4) Vs 81.8% (65.6- 91.4) in conventional and proposed method respectively. The difference of sensitivity wasn't significant; P-value=0.568. The specificity was [84.9 % (69.1-93.4) Vs 81.8 % (65.6-91.4)] conventional Vs proposed same day method respectively; P- value=0.155.

Conclusion and Recommendation: Since the sensitivity and specificity was statistically non-difference in conventional and proposed spot-next spot specimen of ZN and LED-FM method, but 6% difference in sensitivity in ZN methods. This difference happens in two cases, this may be due to poor sample preparation (especially first-day next spot smear). But this study shows, it is possible to diagnosis PTB in one day by giving extensive and comprehensive training for laboratory technicians and technologist, and the practicability needs further research.

Keywords: Same day diagnosis; TB; ZN; LED-FM.

ABBREVIATIONS

TB	: Tuberculosis,
PTB	: Pulmonary Tuberculosis,
ITD	: Infectious and tropical disease,
PPV	: Positive predictive value,
NPV	: Negative predictive value,
UoGH	: University of Gondar Hospital,
DTH	: Debretabor Hospital,
LED-FM	: Light emitted diode-Fluorescent Microscopy,
ZN	: ZiehlNeelson.
MTB	: Mycobacterium tuberculosis,
AFB	: Acid-fast bacilli,
SMS	: Spot-morning-spot,
CI	: Confidence interval

1. INTRODUCTION

M. tuberculosis (MTB) is a major public health problem throughout the world. Nearly one-third of the world's population is infected with MTB and hence the risk of developing active disease [1,2]. The greatest burden of morbidity and mortality occurs in the developing world, notably sub-Saharan Africa [2]. Ethiopia ranks seventh among the world's 22 high-burden TB countries [1].

The control of TB in high-incidence countries relies upon passive case finding among individuals self-presenting to health-care facilities and diagnosis by sputum smear microscopy. The requirement to process and examine three

specimens from each TB suspect can create heavy workloads for laboratories, which may impact upon the quality of the service [3]. Patients with suggestive symptoms are asked to make repeated visits to submit serial sputum samples and collect results [4]. The cost of repeated visits is prohibitive and patients need to make repeated visits to the health facility to submit sputum and obtain results [3]. This has an effect on patient's costs and laboratory work load [4]. As a result, there have been several initiatives to optimize smear microscopy including the change in specimen collection, specimen processing and microscope technique [5].

For microscope detection of TB bacilli LED-FM using Auramine staining has been shown to have 10% higher sensitivity compared to ZN staining, without compromising specificity [6]. LED-FM is also more time efficient, it takes only 25-65% of the time required for ZN examination [6].

The morning sputum is likely to contain more bacilli, equal numbers of on the- spot and early morning specimens are reported to be positive in areas with a high prevalence of TB. Operational research is now focused on reducing the burden of smear microscopy without compromising its effectiveness as a case-finding strategy. The diagnostic process could be made much more efficient and convenient for patients if it could be completed in a single day by examining two same-day specimens [4].

Therefore, this study was aimed to determine the sensitivity, specificity and predictive values of a "spot, next spot" samples in a one-day strategy for the diagnosis of TB and compare it to the standard strategy "conventional method-SMS". Furthermore, LED-FM was a new program implemented to scale up PTB diagnosis in Amhara Region-Ethiopia, so it also assesses the effectiveness of LED-FM as compared to ZN microscopy.

2. MATERIALS AND METHODS

2.1 Study Design, Study Area and Period

A cross-sectional laboratory-based study was conducted from June to Sep, 2013. In University of Gondar referral teaching Hospital (UoGH), and Debrtabor Rural Hospital (DTH), this is one of the referral teaching Hospitals in Amhara National Regional State and district (rural) hospital respectively. These facilities provide health service for a population who were living in North-west Amhara Regional State, Ethiopia.

2.2 Source and Study Population

The source population for this study included all those patients suspected of PTB infection from outpatients, inpatients in different wards, TB clinics and ambulatory patients attending the two Hospitals, whose age was ≥ 15 years old.

2.3 Inclusion Criteria

All those patients who had cough for more than two weeks symptomatic for PTB, age ≥ 15 years old were included in the study.

2.4 Exclusion Criteria

All those patients suspected of other kinds of tuberculosis and those patients of sputum conversion at end of the intensive and continuous phase of the drug were excluded from the study.

2.5 Specimen Collection

Patients were requested to submit three sputum samples as "SMS" for diagnosis PTB [the conventional method]. In addition, patients were asked to stay in the Hospital to submit a further sputum sample one or more hour after the first sample [called the 'first-day next spot', with the first on-the-spot plus the next spot constituting the same-day method].

2.6 Specimen Processing

Patients were instructed on the production of good quality sputum samples as per the National TB guideline of Ethiopia. Specimens were assessed macroscopically inspecting the quality and quantity, and a record was made. An appropriate portion of the sputum sample (from the most purulent portion) were collected with an applicator stick and spread on the labelled side of the microscope slide.

The slides were air-dried and heat-fixed. All sputum samples were stained using the standard hot ZN technique and the LED-FM for smear microscopy as recommended for National TB Control Program [7]. Two batches of smears were prepared per specimen. One was stained with the hot ZN method using (Carbol-Fuchsin 85%,3% acid alcohol & 0.3% w/v methylene blue, GCC diagnostics, Netherlands) and the other with the Auramine-O (USA, MSDS available, MW10242) for fluorescence microscopy. Auramine smears were counterstained with potassium permanganate for 60 seconds. Attribution of staining method per smear was randomized.

2.7 Examination

ZN slide from UoGH and DTH were first stained there and then all slides were transported to Bahir-Dar regional laboratory and read by 2 independent laboratory technologists who were blind to the LED-FM smears result when reading the ZN smears from same specimen and vice versa, any discordant result was read by Regional Laboratory Medical Microbiologist and a decision was made. All the results at each testing area were recorded in a separate log book. Table 1 presents the Acid fast Bacilli (AFB) grading reading scales used during the study for the ZN and LED-FM, respectively. No darkroom was used for LED-FM. ZN slides were examined by bright-field microscopy (magnification, X 1,000) on Realux, France, serial number 100129 microscopes. Auramine slides were read by ILED-FM [Labomicroscope, model AFTiLED, Flourscent 3w, Germany) (magnification, X200).

The number of AFB read per standard length of 2 cm long and 1 cm large was reported. A length corresponded to 100 fields under 1000 magnification and was estimated to be equivalent to 30 fields under 200-250x which is equal to 300 HPF magnification Table 1 [8].

Table 1. IUATLD/WHO semi-quantitative scales of AFB smear grading

IUATLD/WHO Scale (1000 X fields = HPF) Results	Microscopic method used		
	Bright field (1000X magnification; 1 length=2CM=100 HPF)	Fluorescence (200-250X magnification; 1 length= 30 fields= 300HPF)	Fluorescence (400X magnification; 1 length=40 fields= 200 HPF)
Negative	Zero AFB/1 length	Zero AFB/1 length	Zero AFB/1 length
Scanty(Actual Count)	1-9 AFB/ 1 length or 100 HPF	1-29 AFB/ 1 Length	1-19 AFB/ 1 Length
1+	10-99 AFB/ 1 length or 100 HPF ()	30-299 AFB/ 1 Length	20-199 AFB/ 1 Length
2+			
3+			

2.8 Reading Time

Randomly selected smears were selected in pair wise fashion; the time afforded was used for measurement of examination time using each method. An average examination time was calculated per result [negative, very low positive [scanty], low positive [1+] and high positive [2+ and 3+] for each method. At the end pooled positive smear time was also measured in both methods using Wilcoxon signed rank test. The examination time included the time taken to record results.

2.9 Data Collection

A structured questionnaire was developed to collect information on the patients' socio-demographic and clinical data. The study was agreed by patients in terms of oral and written information and consent, after due explanations of the study purpose. All patients' results were registered in a single log book to monitor the finding of patients enrolled in the study.

2.10 Data Quality Management

Smears were graded according to IUATLD 2000 with the exception that smears graded as scanty ZN (1–9 AFB per 100 HPF) were considered as positive for the purpose of the analysis. For the LED-FM microscopy, 1-29 AFB per length read were also counted as scanty positive for the purpose of analysis. All smears were read by two independent persons and any discordant result was read by Medical Microbiologist who was blind for the first and second result. The qualities of the AFB reagents for the two methods were controlled by positive and negative sample smear. The LED-FM slides were first read in the hospital by the hospital laboratory technician and technologist who were trained for LED-FM microscopy by Amhara Regional Research Laboratory Center for 7 days and worked for the patients. The result was recorded and at the end of the study, all Auramine slides were transported to regional-lab for re-staining and re-read by Regional Laboratory Medical Microbiologist staffs. The LED-FM reading scheme was first screening of the slide under 200 magnification and confirmation under 400 magnification. Positive LED-FM slides were not confirmed by re-staining and reading with ZN [9].

2.11 TB Culture

Patients were asked to produce additional specimen i.e one additional sample was collected ≥ 1 h after the first sputum. Randomly selected one from a set of 4 sputum sample per patient were stored in the fridge and sent to Bahir-Dar Regional Research Laboratory Center for MTB culture every Friday. Pulmonary TB suspected patients were tested for HIV at the first visit of the each Hospital. Specimens, once a week in Bahir-Dar Regional Health Research Laboratory Center where the MTB cultured on 2 solid Lowenstein Jensen (LJ) medium was performed. Specimens were decontaminated using N-acetyl-L-cysteine/sodium hydroxide (NALC/NaOH) followed by neutralization with phosphate buffer, centrifuged and the deposits inoculated on LJ media and incubated at 37°C up to 8 weeks. The cultures were inspected weekly and reported in accordance with the world health organization (WHO) culture grading scale. All positive cultures were confirmed for the presence of acid-fast bacilli by ZN microscopy.

2.12 Data Processing and Analyses

The case detection rates were calculated using current case definition of WHO in both methods, PTB⁺ if ≥ 2 smear and culture as the gold standard. Rates were adjusted by the expected sensitivity of the gold standard [7,10]. OpenEpi, the Version 2 open source calculator-Diagnostic Test, was used to compare the sensitivity, specificity and predictive values of the same day with standard approaches. McNemar's test used to compare smear positivity rate, P-value's 0.05 or lower is used to declare that the association is statistically significant. Results were displayed using tables and figures. The intra-reader reliability was assessed between the Hospital laboratory technicians and technologists with the regional laboratory staffs by kappa value calculation. A kappa >0.75 was considered a very good agreement.

2.13 Ethical Clearance

Ethical clearance was obtained from the Office of Amhara Regional Health Bureau ethical committee and Bahir-Dar Regional Health Research Laboratory center, Bahir-Dar. The official letter from the Research and Technology Process owner office were written to UoGH and DTH. Patients with at least one positive smear result (>1 AFB/length), regardless of the

microscopy method, were started on treatment. Smear-negative patients were referred to the clinician for further investigation. Patients who were later found to be culture positive were traced and started on treatment.

3. RESULT

3.1 Socio-Demographic Data PTB Suspected Patients

A total of 180 PTB suspected patients were involved in this study. The median (IQR) age of all respondents was 40(b/n 27 and 52.7) within a minimum and maximum age of 17 – 77 years. Among these study subjects, 101 (56.1%) were male and 79 (43.9%) were females (Table 2).

Table 2. Socio-demographic characteristics of PTB suspected patients (June-Sept 2013, N=180)

Characteristics	Number (N=180)	Percentage
Age of the respondents		
15-19	11	6.1
20-29	47	26.1
30-39	26	14.4
40-49	39	21.7
50- 59	23	12.8
>60	34	18.9
Sex		
Female	101	56.1
Male	79	43.9
Marital status		
Married	96	53.3
Unmarried	84	46.7
Educational status		
Illiterate	98	54.4
Literate	82	45.6
Residency		
Urban	53	29.4
Rural	127	70.6
Occupation		
Non farmer	96	53.3
Farmer	84	46.7

Of the 180 subjects screened for PTB, 15.0 % (27/180) were positive by ZN as compared to 15.0% (28/180) smear positive by LED-FM microscopy in the conventional method; P-value<0.001. Among those screened patients [101] were male patients, from these patients PTB were positive 15.8% (16/101) by LED-FM and 14.9% (15/101) by ZN microscopy while of the female patients [79], 14.9% (12/79) were positive by ZN as compared to 15.2% (13/79) by

LED-FM. No statistical difference between male and female; P-value>0.05.

Regarding age distribution, the highest smear positivity rates were seen in the age group of 20-29 years (11.1%) (P-value<0.05), and the lowest smear positive were \geq 50 years.

The clinical presentation was assessed for the study subjects, all 180 patients had cough for \geq 2 weeks, Fig. 1. Out of 720 sample [(540 specimens by conventional method, SMS, and 180 specimens by the first day next spot specimens (in one day method)], 523 (72.6%) were macroscopically purulent or mucopurulent, 151 (21.0%) mucoid, 6(0.8%) blood-stained and 40 (5.6%) salivary. The morning specimen (B) was of significantly better quality (purulent or Mucopurulent) (162/180) than the first day next spot (115/180) specimens; P value<0.001.

All study patients submitted a set of four sputum samples requested, and culture was done for 166 patients using a randomly selected one sample. After exclusion of contaminated and invalid cultures and 4 insufficient samples, 20.6% (33/160) of the patients had a confirmed infection with MTB by culture. The 4 single invalid cultures resulted from reagent preparation.

Seventy eight (16.3%) of the 480 spot (first day spot(A=27), first day next spot(E=25) and second day spot(C=25)) specimens and 27 (16.9%) of the 160 morning(B) specimens were smear positive by ZN as compared to 83(17.3%) of the 480 spot samples (first spot (A=28), first day next spot(E=27) and second day spot(C=28)) and morning(B=28) sample by LED-FM; P-value< 0.05. There was a difference in the number of positive smears between the three spot samples. In both methods second-day spot (C) samples did not give additional case detection. In general the smear positivity & negative rate were [104(16.3%) and 536(83.8%)] Vs [111(17.3%) and 529(82.7%)] for ZN and LED-FM methods respectively.

Using 1 AFB/length cut-off to define a positive smear, there were 104 positive smears (16.3%, 95%CI [13.5-19.3]) with ZN compared to 111 (17.3%, 95%CI [14.6–20.4]) with LED-FM, P-value = 1.00. Among all 31 positive (scanty) smears by LED-FM, culture was negative in 2(6.5%)cases. Eight specimens LED-FM were scanty positive and ZN were negative. Thirty-seven scanty positive smears were read by ZN and culture were positive (100%).

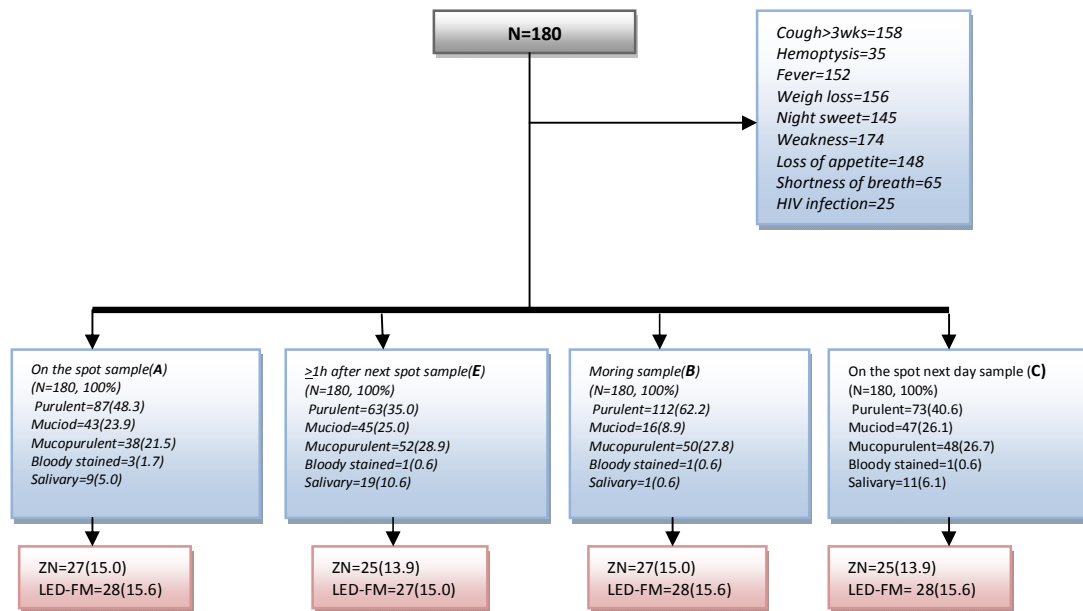


Fig. 1. Clinical data of PTB patients

Eight LED-FM smears were read by controllers had the scanty positive result but negative by the first reader (lab technicians and technologists), culture was negative in all cases. Eight smears were reported ZN negative by the first and second reader. Microbiologist reported positive also culture confirms positive. Among 13 discordant results by ZN (positive by first reader and negative by second reader and vice versa) microbiologist reported positive, also culture confirms positive.

One smear ZN positive (1+) by the second reader, negative by the first reader, microbiologist reported negative and culture confirmed negative. One smear ZN positive (1+) by the second reader, negative by the first reader, microbiologist reported positive (1+) and culture confirmed positive. Table 3, presents the smear microscopy results per specimens based on culture results.

Table 3. Smear microscopy grading result

ZN	LED-FM				
	Negative	Scanty	1+	2+	3+
Negative	529	15	2	0	0
Scanty	1	12	19	5	0
1+	0	2	14	8	8
2+	0	1	1	2	2
3+	0	1	3	4	7

Based on the current WHO case definition criteria, PTB was positive having 2 or more smears positive, the SMS (conventional method) and the proposed spot-next spot (proposed same-day method) would identify 27 and 25 of the 33 culture-positive cases by ZN. As culture was the reference standard & using 2 smears as a case definition, the accuracy of conventional method & of the proposed method is shown in Table 4. The sensitivity of the ZN conventional method was 81.8%, 95%CI [965.6-91.4] & that of proposed spot method 75.8%, 95%CI [58.9-87.2], but the differences weren't statistically significant; P-value=0.298. The specificity of the two methods was similar; P-value=1.0, Table 4.

Using the above case definition and results by culture were the reference standard, the conventional SMS and the proposed same day method were identified in 28 and 27 cases of 33 culture positive cases by LED-FM respectively (Table 5). The sensitivity were 84.9% (69.1-93.4) Vs 81.8% (65.6- 91.4) in conventional and proposed methods respectively. The difference in Sensitivity wasn't significant; P-value=0.568. Both methods also have similar high specificity and there is no statically difference P-value=1. The NPV and PPV of the conventional method were 96.2%, 95%CI (91.3-98.4) and 93.3%, 95%CI (78.7-98.2) respectively, and that of the proposed same day diagnosis were 95.4%, 95%CI (90.4-97.9) and 93.1%, 95%CI (78.0- 98.1) respectively, by LED-FM.

Table 4. Comparison of the conventional method (SMS) and proposed one day (spot- next spot) approaches against culture by ZN smear microscopy

Approach	Culture		Sensitivity (95%CI)	Specificity (95%CI)	PPV (95% CI)	NPV (95% CI)
	Positive	Negative				
Two day diagnosis , SMS(conventional)						
Positive	27	0	81.8% (65.6- 91.4)	100%(97.1-100)	100%(87.5-100)	95.5%(90.5-97.9)
Negative	6	127				
Proposed first day diagnosis(spot-next spot)						
Positive	25	0	75.8%(58.9- 87.2)	100%(97.1- 100)	100%(86.7-100)	
Negative	8	127				
Total	33	127				94.1%(88.7-96.9)

NPV: Negative predictive value, PPV: Positive predictive value, CI: confidence interval

Table 5. Comparison of the conventional method (SMS) and proposed same-day (spot- next spot) approaches against culture by LED- FM microscopy

Approach	Culture		Sensitivity (95%CI)	Specificity (95%CI)
	Positive	Negative		
SMS[conventional FM]				
Positive	28	2		
Negative	5	125	84.9% (69.1- 93.4)	98.4%(94.4, 99.6)
Proposed first day diagnosis				
Positive	27	2		
Negative	6	125	81.8%(65.6- 91.4)	98.4%(94.4- 99.6)
Total	33	127		

Table 6, presents the comparison of LED-FM and ZN performance when using SMS smear case definition. Sensitivity of both microscopy methods was equal to 81.8%, 95%CI (65.6-91.2) for ZN and 84.9%, 95% CI (69.1-93.4) for LED-FM microscopy, LED-FM were 3.1% more sensitive than ZN. Even though LED-FM was more sensitive than ZN, but statistically no difference between two methods P-value=0.706 was found on the basis of the SMS smear-positive case definition. When we compared the specificity of both methods, the conventional method LED-FM achieved 98.4%, 95%CI (94.4-99.6) and ZN also achieves 100%, 95%CI (97.1-100), but the difference wasn't statistically significant; P-value=0.155 as seen in the Table. Lastly, 2 cases, 8 slides, were positive by LED-FM microscopy but negative by culture, and also the specificity slightly lower than ZN. All false positive results were very low scanty positive results by the LED-FM method. There was no difference in sensitivity and specificity between LED-FM and ZN microscopy.

One hundred seventy-three (96.1%) patients were screened for HIV and 25 (13.9%) were HIV positive, 7(3.9%) were of unknown sero-status. Due to the small size of their number, it is not necessary to go or analyze further.

3.2 Reading Time

Random selected cases of 10 pair of scanty, 4 pair of low positive (1+), 4 pairs and 10 pairs of high positive (2+,3+) respectively and in addition to these, 10 pairs of negative slides were assessed for the time that took during examination by both methods and computed in different parametric test (Table 7). The mean (standard deviation, SD) reading time of ZN scanty had 8.5(3.6) minute as compared to 2.4(0.6) minute with LED-FM scanty smear. There was a high statistical difference; P-value<0.001.

The median (range) reading time (in minutes) with pooled positive ZN result was higher than the median (range) pooled positive result of LED-FM microscopy in comparison [4.05(13.0) Vs 2.01(3.72)]. There was a statistically significant difference time for reading in the two methods', P=value<0.001. When we compared the negative result between the two methods, the mean (SD) of ZN microscopy was higher than the mean (SD) of LED-FM microscopy in minutes [13.4(3.9) Vs 5.1(1.4)].

Intra-reader reliability of LED-FM was measured between Hospital Laboratories and the Regional Laboratory professionals for 640 smears. Therefore the two professionals

Table 6. Diagnostic performance of smear-microscopy using ZN and LED-FM microscopy using conventional (SMS) smear-positive case definitions from PTB suspected patients

	ZN		LED-FM		P-value
	Positive	Negative	Positive	Negative	
Conventional method[SMS]					
Positive culture	27	6	28	5	
Negative culture	0	127	2	125	
Sensitivity %(95CI)	81.8(65.6-91.2)		84.9(69.1-93.4)		0.567
specificity%(95CI)	100(97.1-100)		98.4(94.4-99.6)		0.155
PPV%(95CI)	100(87.5-100)		93.3(78.7-98.2)		
NPV %(95CI)	95.5(90.5-97.9)		96.2(91.3-98.4)		
Diagnostic accuracy	96.3(92.1-98.3)		95.6(91.3-97.7)		

Table 7. Reading time of ZN and LED FM methods, related to smear grading

Smear result[grading]	Reading time mean[SD]		P-value
	ZN	FM	
Negative	13.4[3.8]	5.1[1.35]	P-value<0.001*
Very low pos[scanty]	8.5[3.6]	2.4[0.6]	P-value<0.001*
High pos[3+]	2.9[0.6]	0.6[0.4]	P-value<0.001*
	Reading time median [range]		
Low pos[1+]	4.05[0.33]	2.43[2.0]	P-value=0.021*
High pos[2+]	4.05[1.88]	3.25[2.01]	P-value=0.059*

* Independent T test, † independent Kruskal Wallis test

are in almost a perfect agreement with kappa value of 0.96.

The training of technician for LED-FM had taken 7 days in the Bahir-Dar Regional Health Research Laboratory center and they said that the Auramine staining was as easy as the ZN staining method and reading were easier with LED-FM, using 20X magnification and no oil immersion, and easier to handle all the procedures. As they said they accepted very well.

4. DISCUSSION

The objective of this study was to give a direct comparison of the conventional spot-morning-spot method with one day stop diagnosis of TB in both LED-FM and ZN microscopy. Furthermore, the study also allowed comparison of LED-FM performance as compared to ZN microscopy, because of the LED-FM were a new program implemented in Amhara Region, Ethiopia by March, 2013.

The internationally recommended approach to sputum smear microscopy requires the examination of specimens collected on the spot, when the patient presented to the health-care facility, early morning at home and a second on the spot when the patient returns to the facility to submit the morning sputum [4,7]. Moreover, at least one further visit to the health facility is usually required for the patient to collect the smear result [11]. This may lead to high patient's costs and either to preventable diagnostic delay or to failure to treat positive TB cases in the cases of geographically isolated residences [12].

Many poor people in low and middle-income country, who need to be investigated for tuberculosis, are financially unable to afford repeated visits to health facilities for smear diagnosis and frequently default during the diagnosis process [13].

Some diagnostic benefit might be gained from performing three serial smears [14]. It is likely that, in practice, many of these three specimens are not examined well [15] due to the high work load of technician [10]. Thus, the quality and yield of smear microscopy could be affected by factors such as workload, training and motivation of laboratory staff [16]. Therefore, smear microscopy optimization is likely to solve these challenges.

Several studies on the yield of examining 2 specimen sets, however, have demonstrated that examining the first and second specimens identifies about 96% of patients ultimately found to have positive smears [17]. The first sputum specimen detects 85.8 % [18], 80 % [19], and 85.5% [20] of PTB cases. In this study the first specimen detected almost all PTB patients detected by both methods, (ZN first (A=27)) cases and LED-FM first sample (A=28) cases. Although the morning sputum had a more bacilli load, its load of bacilli did not result in a significant contribution of smear being positive compared with the spot smear [17]. This study also showed that the morning sputum smear did not detect additional smear-positive cases than compared with the first spot samples. Of course, the smear grading of morning sputum was higher than the 3 spot (A, E and C) samples in both methods, but many studies show that scanty samples (<10AFB/100HPF) were true positive by ZN method [21, 12, 22, 23]. This study also showed that scanty ZN bacilli are true bacilli confirmed by culture; on the contrary to LED-FM scanty samples, which are not necessarily true bacilli, because in this study we lost scanty LED-FM bacilli by culture.

Even though the diagnosis of PTB was based on 2 or more smears as case definition of WHO, this study lost 2 ZN smears as negative by first day next spot [E] smear; this may be due to improper sample preparation. But if the diagnosis of PTB ≥ 1 AFB/slide it is possible to diagnose TB in one day, because the first spot (A) sample detected an equal number of cases when compared with morning(B) specimen. But the examination and interpretation of the result need extensive training to health facility technicians and technologists. This result also agrees with the study done in Besholo health center, Ethiopia [4] and Abuja, Nigeria [17] who proposed the diagnosis of PTB in one day by collecting only 2 spot sample.

In both methods (ZN & LED-FM microscopy) next day spot sample(C) did not detect or identified extra PTB positive smears. According to this study, it is possible to spare the 3rd sample which is costly for the patients and increases laboratory workload.

In 2013, the Federal Ministry of Health, Ethiopia, recommended to replace ZN by LED-FM in PTB high burden Health centers and Hospitals, and that FM microscopy be at the stage of development for conventional ZN microscopy. In this study the statistical analysis shows that LED-

FM had the same sensitivity and specificity as that of ZN microscopy; however, this is the case for a small sample size. But in the real world, where LED-FM detecting an additional case compared with ZN, shows that LED-FM microscopy was more sensitive. When we compared the specificity of LED-FM to ZN [98.4%, 95%CI (94.4-99.6)] Vs [100%, 95%CI (97.1-100)] respectively, LED-FM had 1.6% less specificity than ZN but statistically not a difference; this higher sensitivity is true that for scanty bacilli measured by ZN. Also the ZN was more specific than LED-FM in our study, however, also showing that of 8 scanty bacilli, 2 cases were positive but negative by culture. This finding was in agreement with the study done in Uganda which showed loss of specificity in scanty LED-FM smear in HIV co-infected patients [23]. Both of the studies suggest that the loss of specificity is more evident for patients who had scanty bacilli in their sputum. This study does not allow to arrive at a conclusion on the basis of in single day diagnosis by LED-FM microscopy, but needs further research.

Smear reading time was twice lower with LED-FM (median time = 2 min) than by ZN microscopy (median time = 4 min) in a pool of positive cases. While in the negative sample the mean time for LED-FM was 5 min as compared to 13.4 min with ZN. This is consistent with the 25 to 66% time saving when using FM compared to ZN microscopy reported in Kenya [6, 21]. Therefore, in programmatic conditions, the introduction of LED-FM would significantly reduce laboratory workloads and possibly allow better field examination of the smear as compared to ZN microscopy.

The intra-reader agreement reproducibility of LED-FM was excellent (K=0.96). This may be explained by the fact that the sample size was small, and small number of LED-FM smears were positive.

Regarding feasibility aspects, this study confirmed the very good acceptability of the LED-FM by technicians, the simple staining procedure, examination of many fields within a short time, no necessity to use oil immersion, and simple handling. This all contributes to a good acceptance by the technicians and technologists.

However, the study has some limitations: Culture as a reference standard was not processed on all specimens. The apparently lower sensitivity of

FM might be explained by the higher experience of technicians with the ZN microscopy than with the FM microscopy, acquired only during 3 months starting with the beginning of the program. Because of small number of HIV positive patients, an analysis could not be applied to the HIV status.

5. CONCLUSION AND RECOMMENDATION

From the 3 spot smear, first-day smear (A=27) an equal number PTB cases was detected for the morning (B=27) cases, and lower number of PTB cases was identified by next day spot smear (C=25) with ZN microscopy. While with LED-FM, first spot smears (A=28) equal numbers of cases were detected from morning (B=28) and next day smears (C=28). This shows that next day spot smear did not detect additional cases, although at variance with a significant laboratory workload and patients cost. Therefore this study suggested that 3rd sample or smear C should be dispensable, so as to increase laboratory good performance and minimize patient cost. While the sensitivity and specificity was statistically non-different in the conventional (spot-morning-spot) and the proposed cases of spot-spot specimen of ZN method, a 6% difference in sensitivity was found for the two methods. This difference happens in two cases, that could be caused by poor performance of the laboratory work (especially smear E). But this study shows, it is possible to diagnose PTB in one day by giving extensive and comprehensive training for laboratory technicians and technologist and then the feasibility needs further research. The in one day diagnosis of PTB by LED-FM, due to scanty bacilli, could be doubtful due to small number of samples or culture missing the scanty bacilli and needs further research.

This study also shows that LED-FM might not always increase sensitivity as compared to ZN microscopy and its specificity was less comparable to ZN for in cases with scanty bacilli, the specificity of ZN being by far better than LED-FM microscopy. The faster reading of LED-FM smear, however, combined with easy staining and easy labor handling make it more acceptable by the laboratory personnel.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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