

Original Article

Ziehl Neelsen vs. Auramine staining technique for detection of acid fast bacilli

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Abstract

Background: The culture and molecular test are the best methods for isolation and identification of *Mycobacterium tuberculosis* in developed countries. But, in developing countries like Nepal with a significant number of tuberculosis (TB) cases and limited resources, the diagnosis of TB relies primarily on smear microscopy for Acid fast bacilli (AFB).

Objective: To compare the results of direct sputum examination for AFB stained by Ziehl Neelsen and Auramine technique.

Method: Cross sectional comparative study was conducted in tuberculosis research laboratory, BPKIHS from April to June 2013. A total of 100 sputum samples were collected randomly. Four slides were smeared and labeled for each as neat ZN, neat Auramine, concentrate ZN and concentrate Auramine. Slides were processed as per WHO laboratory guidelines.

Results: The findings of this study revealed that 3% positive with neat Auramine was negative for ZN stain. Similarly, 5% positive cases with Auramine concentrate were negative with ZN concentrate technique. Auramine stain was able to detect all ZN positive as positive but only 83 cases were detected as negative among 88 case of ZN negative. Both concentration techniques showed 12% of positive with significant relationship. With this; Auramine showed 100% sensitivity, 94.6% specificity, positive predictive values and negative predictive values 70.5, 100% respectively.

Conclusion: Auramine stain stands efficient on comparison and can be used as an alternative to ZN stain, with added value of allowing a large number of sputum specimens to be examined in a given time as low power is used for examination.

Key words: Identification, *Mycobacterium tuberculosis*, sputum examination.

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Introduction

Tuberculosis (TB) remains one of the major public health concerns in the WHO South-East Asia Region (SEAR). The Region accounts for 39%

of the global burden of TB in terms of incidence, and India alone accounts for 26% of the world's TB cases.¹ In Nepal, 45% of the total population is infected with TB, out of which 60% are in the productive age group (15- 45). Every year, 40,000 people develop active TB, of whom 20,000 have an infectious pulmonary disease.² It is estimated that nearly one billion people of the world will be infected with TB, 200 million develop the disease and 35 million will die with it during 2000- 2020.³

At present, mostly, the sputum staining is done by two methods, viz. Ziehl-Neelsen (ZN) or Auramine fluorochrome.⁴ Its staining techniques are based on the relatively unique property of Mycobacterium species to retain the primary stain even after exposure to strong acid-alcohol, thus the term, AFB. Although, culture is viewed as to be the gold standard for diagnosis of TB, despite its enhanced sensitivity and specificity, it is of impractical laboratory use, because of associated cost, labour intensive procedure, time factors and specialized safety measures.⁵ Hence, this study was conducted focusing mainly on two most commonly used sputum staining technique (ZN and Auramine stain) to compare and evaluate their sensitivity and specificity in detecting AFB.

Methods

In this cross-sectional comparative study, a total of 100 sputum samples were collected according to the National tuberculosis guidelines⁶ and examined in Tuberculosis research laboratory at Department of Microbiology, B. P. Koirala Institute of Health Science (BPKIHS), Dharan during April to June 2013. BPKIHS TB Laboratory receives approximately 20 samples each day, out of those, five samples were selected using simple random technique on every fourth day for convenience. Samples collected using not standard procedure and less than 2ml of the amount were excluded considering insufficient amount for processing the procedure. The purpose of the study was clearly explained and verbal consent was obtained from each patients.

Processing

Following exclusion criteria, all the samples were collected, recorded into study log book using their allocated laboratory numbers and processed in a bio-safety cabinet. Four slides were labeled for each sample as a neat ZN, neat Auramine, concentrate ZN and concentrate Auramine. Initially, neat smears were prepared and then, remaining sputum samples were processed by modified Petroff's method to prepare smear for concentrate slides.

Smear preparation, staining technique and Microscopy reporting was done according to Laboratory services in Tuberculosis control guidelines.⁷

Analysis

The generated data were compiled in a data entry form and also stored in Microsoft Office Excel programme and later, exposed to SPSS 17.0 version software of windows for analysis. Kappa test of an agreement was calculated from SPSS to establish the relationship between two stains. McNemar's chi-square test was calculated from 'EPI info software 2000' to demonstrate any relationship between discordant results shown by the stains.

Results

The results showed that 3% of the samples that were positive with neat Auramine was negative when ZN techniques were performed. Similarly, the percentage of case that was negative with Auramine but positive with Zn was zero. It shows that Auramine was able to detect all positive cases detected by ZN (total 9) correctly; in addition, it could detect 3 more positive cases which were missed by ZN technique.

However, there was a significant relationship (i.e. very good agreement for κ) for neat techniques in the performance of Auramine when compared to ZN ($p=$

0.05). Details are shown in table1 and their statistical values are given in table 3.

This study also found that 5% positive cases with Auramine concentrates were negative with ZN concentrates. Also same is the case with concentrate technique that no case found where negative with Auramine but positive with ZN i.e. Auramine able to detect all ZN positive as positive but could only detect 83 as negative among 88 cases of ZN negative (true negative). However, both preparations for concentration showed 12% of positive (true positive) with significant relationship (i.e. good agreement for κ) between both techniques ($p= 0.05$). Details are shown in table 2 and their statistical values are given in table 3.

This study also included testing of the discordant variable for establishing any kind of relationship. For this McNemar's χ^2 test was used which showed 'Not significant'. This implies that the discordant result shown by these two stains (shown in table1 and 2) was just due to chance variation which strongly suggests that both Auramine and ZN stains are strongly related. Details are shown in table 3.

This study also included the comparison between concentrate ZN with neat ZN

(table 3) and between concentrate Auramine with neat Auramine (table 4).

Table 3 revealed 2 cases which were negative with neat ZN but positive with concentrate ZN but such case increases to 5 which are negative with neat Auramine but positive with concentrates Auramine. However, no difference is recorded in detecting negative (true negative) cases by neat preparation compared with concentrates of both techniques. Data are shown in table 4 and 5.

Taking comparison between concentrate ZN and concentrate Auramine as standard procedure, table 2 is used to calculate sensitivity, specificity, positive predictive value and negative predicting value of Auramine against well-established ZN stain as gold standard. Thus, Auramine shows 100% sensitive, 94.3182 specific and positive predictive values, negative predictive value were 70.5882, 100%.

Table1: Comparison of neat ZN and neat Auramine techniques

		Neat ZN preparation		Total (%)
		Positive (%)	Negative (%)	
Neat Auramine preparation	Positive (%)	9	3	12
	Negative (%)	0	88	88
Total (%)		9	91	100

Table 2: Comparison of concentration ZN and concentration Auramine technique

		Concentration ZN preparation		Total (%)
		Positive (%)	Negative (%)	
Concentration Auramine preparation	Positive (%)	12	5	17
	Negative (%)	0	83	83
Total (%)		12	88	100

Based on above table 2

Sensitivity of Auramine	100(%)
Specificity of Auramine	94.31818(%)
Positive predictive value	70.58824(%)
Negative predictive value	100(%)

Table 3: Performance of different preparation on different tests

Test	For	value	strength of Agreement	95% C.I.*
κ test	Table 1	0.841	very good	0.66-1
	Table 2	0.799	Good	0.63-0.97
Test	For	value	P-Value	Agreement
McNemar χ^2	table 1	1.33	0.25	NS**
	table 2	3.2	0.07	NS**

* Class interval

** Not significant

Table 4: Comparison of neat and concentration ZN preparation

		Concentration ZN preparation		Total (%)
		Positive (%)	Negative (%)	
neat ZN preparation	Positive (%)	9	0	9
	Negative (%)	2	89	91
Total (%)		11	89	100

Table 5: Comparison of neat and concentration Auramine preparation

			Concentration Auramine preparation		Total (%)
			Positive (%)	Negative (%)	
neat Auramine preparation		Positive (%)	12	0	12
		Negative (%)	5	83	88
Total (%)			17	83	100

Discussion:

According to the national guidelines for tuberculosis control, a patient with more than three weeks history of a cough should be screened for PTB with smear microscopy for AFB.^{8,9} Because the clinical signs and symptoms of PTB are not specific, the accurate performance of acid-fast microscopy is vital for the early

recognition of PTB patients for the adequate treatment, respiratory isolation, and contact investigation. Although acid-fast microscopy is more than 100 years old, it still remains the initial and most rapid step in the diagnosis of tuberculosis. Acid-fast microscopy is simple to perform and therefore, could be applied successfully in any laboratory.¹⁰

The added advantage of sputum smear microscopy is that it has very close relation with infectiousness: a patient who is sputum smear positive and culture positive are more likely to be infectious than culture positive but smear negative.¹¹

At the moment in the developing countries like Nepal where tuberculosis is a major health problem, sputum microscopy is carried out widely for microscopic examination of sputum smears stained by ZN method. This study aimed to compare Auramine stain with conventional ZN stain and to prove statistic relationship in between the two.

This study found a significant relationship in the performance of Auramine staining when compared to ZN technique that agrees with the finding of other previous studies which concluded that both ZN and fluorescence staining can be used for the diagnosis of TB.¹²⁻¹⁵

The present study showed 3% and 5 % of false positivity of Auramine in comparison with ZN for neat and concentrate technique respectively which may be due to non-specific fluorescence dye binding. This is usually the disadvantage of the fluorescent microscopy technique which, in turn, caused the decrease in specificity. But, it stood 100% sensitive in detecting the positive cases (along with 100% PPP) against the ZN. The false yielding of Auramine can be prevented by over-

staining the smear by ZN method (a more specific one) for bright-light microscopy. These findings are also in accordance with various studies^{12,13} when they compared the sensitivity of both with culture as a gold standard, the result showed even greater sensitivity of Auramine than ZN. This may be taken as Auramine stains better when talking about detection of positive cases.^{13,15,16}

When the present study compares the data on neat vs. neat and concentrate vs. concentrate for both ZN and Auramine as given in table 4 and 5, the results showed that there were 2% and 5% cases which were negative with neat ZN and neat Auramine respectively but came positive with respective concentrate techniques. This may be taken as the significance of following concentration method (Petroff's method as in this study). This is also in accordance with the previous study.¹⁷

From all the result obtained in this study shown above, there was a good relationship (κ values) between these two stains and even comparing the disagreement data on McNemar's chi-square showed they were not significant, this again added that disagreement results were due to by chance only. This is again in accordance with previous studies.¹²⁻¹⁴

Conclusion

The present study showed reliably a good relationship (κ values) between the two

stains also concluded the discordant result were just due to chance as suggested by McNemar chi square values.

Overall, it can be concluded that Auramine stain stands efficient on comparison and can be used as an alternative to ZN with added advantage of allowing a large number of sputum specimens to be examined in a given time as low power is used for examination. It is better technique in detection of paucibacilli (more sensitive) against a dark background, no use of oil immersion, time effective but yet it is not economical technique in rural areas of developing country because of its associated cost and equipment maintenance.

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