International Journal of Community Medicine and Public Health
Faridi M et al. Int J Community Med Public Health. 2024 Aug;11(8):3057-3063
http://www.ijcmph.com
pISSN 2394-6032 | eISSN 2394-6040

DOI: https://dx.doi.org/10.18203/2394-6040.ijcmph20242163

Original Research Article

A comparative study of diagnostic yield of Ziehl-Neelsen staining and fluorescent staining in diagnosis of primary pulmonary tuberculosis in a resource limited country like India

Maryam Faridi[1]*, Nazish Fatima[1], Sumit Varshney[1], Mohammad Shameem[2], Indu Shukla[1]

[1]Department of Microbiology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India
[2]Department of Tuberculosis and Other Respiratory Diseases, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Received: 25 December 2020
Revised: 06 June 2024
Accepted: 09 July 2024

***Correspondence:**
Dr. Maryam Faridi,
E-mail: maryamfaridi0@gmail.com

ABSTRACT

Background: Tuberculosis (TB) is a major public health disease. Early diagnosis and treatment are essential for effective TB control program. In developing countries, diagnosis of TB is mainly by microscopy because of the simplicity of the procedure and cost-effectiveness. In the present study the two methods were compared to derive a conclusion to adopt the better method to provide more effective services for early diagnosis of TB.
Methods: This study was conducted to compare the Ziehl-Neelsen (ZN) staining with Fluorescent staining in the diagnosis of primary pulmonary TB patients. A total of 514 pulmonary tuberculosis suspected patients were included for a period of 2 years. Sputum samples were taken and subjected to both ZN staining and fluorescent staining and results were compared. All samples were subjected to solid culture and taking it as gold standard sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of both the staining methods were defined.
Results: Out of total 514 samples, 265 (51.56%) were ZN positive and 326 (63.43%) samples were positive for FM. Positive samples detected by ZN staining were compared with positive samples by FM. Sensitivity, specificity, PPV, NPV for ZN microscopy were 80.76%, 97.27%, 98.05% and 74.79% respectively. Sensitivity, specificity, PPV, NPV for FM were 98.71%, 95.63%, 97.47% and 97.76% respectively.
Conclusions: In developing country like India where there are large number of cases and gross financial constraints microscopy can be adopted as the main method of diagnosis of pulmonary TB and preferably FM because of higher sensitivity and lesser time taking character of the method.

Keywords: *Mycobacterium tuberculosis*, ZN staining, Fluorescent microscopy, TB

INTRODUCTION

Tuberculosis (TB) is a predominant infectious cause of mortality today.[1,2] According to World Health Organization (WHO), tubercular infections are currently spreading at the rate of one person per second per million people, with three million dying from it.[1,3] An estimated global total of 10.6 million people fell ill with TB in 2022, equivalent to 133 incident cases per 100 000 population. Among all incident TB cases, 6.3% were among people living with human immune-deficiency virus (HIV). Most TB cases in 2022 were in the WHO regions of South-East Asia (46%), Africa (23%) and the Western Pacific (18%), with smaller shares in the Eastern Mediterranean (8.1%), the Americas (3.1%) and Europe (2.2%).[4]

Early diagnosis and case finding has an important role in the early and effective treatment of the patient and

containment of the disease and reducing the disease load. Smear examination is believed to be simple, cheap, quick and effective case finding method for developing countries. Culture results are more reliable but as tuberculosis bacilli are very slow growing organisms, culture results are available after a period of four to six weeks.

In many developing countries, the diagnosis of tuberculosis is mostly based on the Ziehl-Neelsen (ZN) staining technique.[5] The sensitivity of sputum smear microscopy by ZN method, however, is reported to be low and variable, ranging from 20% to 80% leaving a number of positive cases undetected.[5-8]

Another fast and effective method of staining is fluorescent staining by auramine. Under low power objective auramine makes the bacteria fluoresce like white rice against dark background. The sensitivity of conventional Fluorescent microscopy (FM) provides far better yield and better detection of positive smears than the ZN and takes less time to perform.[9-11]

The present study compared the efficacy of both the staining methods in detection of *Mycobacterium tuberculosis* (*M. tb*) in sputum samples of primary pulmonary TB patients.

METHODS

The present study was a prospective analytical study conducted in culture and DST laboratory (RNTCP certified), Department of Microbiology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, on the sputum samples received of the primary pulmonary tuberculosis suspected patients (according to PMDT guidelines) from the outpatient and inpatient departments of the hospital and from various tuberculosis units in and around Aligarh region (including Hathras, Badaun, Kasganj, Etah, Bijnor, Bulandsheher districts) for a period of 2 years from October 2015 to October 2017.

Patient selection criteria

The study population consisted of suspected cases of primary pulmonary tuberculosis. According to PMDT annexure-1 patients are divided into following 8 categories - failure (all failure of new TB cases, failure by definition is any TB patient who is smear positive at 5 months or more after starting treatment); re-treatment case S+ at 4th month (Smear +ve previously treated cases who remain smear +ve at 4th month onwards); contact of known MDR TB case (all pulmonary TB cases who are contacts of known MDR TB case); S+ at diagnosis, re-treatment case (smear +ve previously treated pulmonary TB cases at diagnosis); any follow up S+ (any smear +ve follow up result in new or previously treated cases); S- at diagnosis, re-treatment case (all smear -ve previously treated pulmonary TB cases at diagnosis); HIV TB case; and others (programme guidelines on programmatic management of drug resistant TB (PMDT) in India; May 2012).

For selection of the study group (primary drug resistant cases) exclusion and inclusion criterion was defined.

Exclusion criteria

Cases excluded from the study were failure, re-treatment case S+ at 4th month, S+ at diagnosis, re-treatment case, and any follow up S+.

Inclusion criteria

Cases included in the study were contact of known MDR TB case, any follow up S+, HIV TB case, and others.

Total 514 patient samples were taken. After proper collection they were subjected to ZN staining and fluorescent staining. All the samples were put to culture on LJ (Lowenstein-Jensen) media.

The patients were advised to collect 4 to 5 ml of early morning sputum in a sterilized 50 ml falcon tube. They were instructed to rinse their mouth with pure water and clean their teeth before collection to avoid contamination with food and other particles. The two consecutive days' sputum samples were collected as per RNTCP criteria: one spot specimen when the patient first attends the hospital, and one next day early morning specimen.

On a new unscratched slide labelled with the Lab serial number a smear was made from yellow purulent potion of the sputum using a sterile stick spread evenly in an area of 2×3 cm in size. All smears were heat fixed and then stained.

ZN staining

After arranging all smears in serial order on staining bridge, they were flooded with filtered 0.1% carbol fuchsin. The smears were intermittently heated for 5 minutes, rinsed with water, and drained. They were decolorized with 3% acid until all free carbol fuschin was washed out, rinsed with water, and drained. They were then counterstained with 0.1% malachite green solution for 30 seconds and rinsed with water. The smear was allowed to air dry and examined microscopically using the oil immersion (100x) objective and grading was done of each smear according to RNTCP criterion.

Fluorescent staining

The smears were flooded with freshly filtered 0.1% auramine-phenol for at least 7-10 minutes. They were then rinsed with water and drained. Smears were decolorized by covering completely with acid-alcohol for 2 minutes, twice, rinsed with water, and drained. They were then flooded with 0.1% potassium permanganate counterstain for 30 seconds and rinsed with water. The smears were

allowed to air dry and examined microscopically using the dry (40X) objective lens of a LED illumination-based fluorescence microscope.

Solid culture on LJ media

Sputum specimens were decontaminated according to the specimen decontamination procedure using N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH). The samples were put to solid culture on Modified Lowenstein-Jensen's (LJ) medium as described by the International Union against tuberculosis (IUAT). Two slopes per specimen were inoculated by 4 mm loop of the centrifuged sediment, distributed over the surface. All cultures were incubated at 35-37°C. Contaminated slopes were also discarded. All cultures were examined 48-72 hours after inoculation to detect any type of contamination. Thereafter cultures were examined Monday to Monday, up to 60 days before a negative report was given. Negative cultures and contaminated vials were discarded during the study and for contaminated samples another specimen of the concerned patient was requested. Typical colonies of *M. tuberculosis* are rough, crumbly, waxy, non-pigmented buff coloured and slow-growers.

Statistical analysis

MedCalc developed by MedCalc software (Acacialaan 22, 8400 Ostend, Belgium) was used to analyze the data. Data were presented as frequency (percentage) and mean (standard deviation). Sensitivity, specificity, negative predictive value, and positive predictive value with 95% confidence intervals were calculated. P<0.05 was taken as statistically significant.

RESULTS

On ZN microscopy of total 514 samples, 265 (51.56%) were found to be positive for AFB and 249 (48.44%) were found to be negative for AFB as shown in Figure 1.

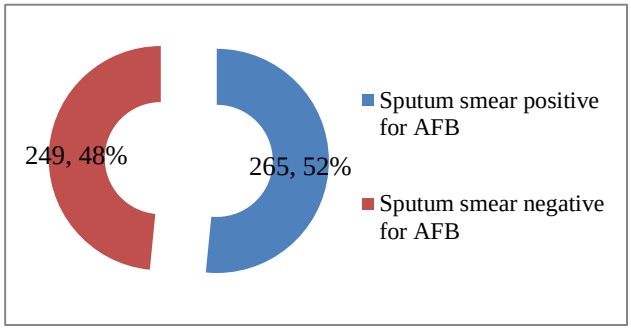

Figure 1: Sputum smear examination of primary pulmonary TB suspected patients by ZN staining for AFB.

On FM of total 514 samples, 326 (63.43%) were found to be positive by FM and 188 (36.57%) were found to be negative by FM as shown in Figure 2.

In Table 1, it is shown that higher number were positive by fluorescent staining 326 (63.43%) than ZN staining 265 (51.56%).

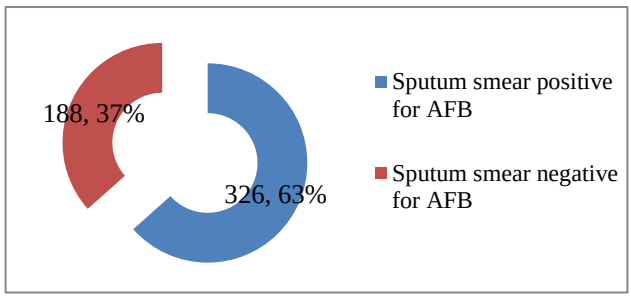

Figure 2: Sputum smear examination of primary pulmonary TB suspected patients by fluorescent microscopy.

Table 1: Comparison of ZN and fluorescent staining: (n=514).

Staining method use	No. of positive smear (%)	No. of negative smear (%)
ZN stain	265 (51.56)	249 (48.44)
Fluorescent stain	326 (63.43)	188 (36.57)

Figure 3 depicts distribution of positive slides by grading using two different staining techniques. Paucibacillary cases (scanty and 1+) detected by ZN microscopy were 178 (67.17%) and 194 (59.51%) by FM. Multibacillary cases detected were 87 (32.83%) on ZN microscopy and 132 (42.31%) on FM.

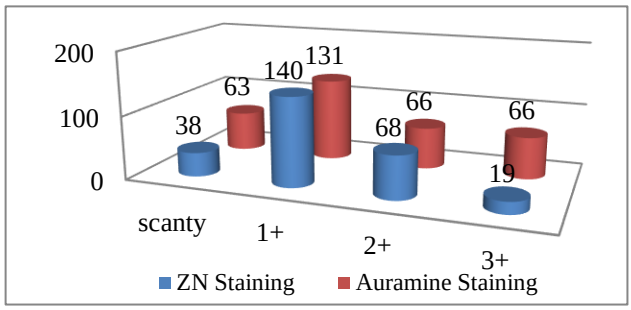

Figure 3: Distribution of positive slides by grading and technique used.

In Table 2, it is seen that out of the total 514 samples 312 (60.70%) showed growth of mycobacteria on LJ medium whereas 183 (35.60%) failed to show any growth on LJ medium.

Comparison of ZN staining, fluorescent staining and culture examination on LJ medium was done on sputum samples from primary pulmonary tuberculosis suspected cases as seen in Figure 4. FM showed 326 (65.85%) sample positive for AFB, culture positive on LJ were 312 (63.03%), and 265 (53.53%) samples were positive on ZN microscopy.

Table 2: Mycobacterial culture examination on LJ medium of primary pulmonary tuberculosis suspected patients: (n=514).

Total no. of patients	No. of mycobacterial culture positive (%)	No. of mycobacterial culture negative (%)	Dry/contaminated culture (%)
514	312 (60.70)	183 (35.60)	19 (3.70)

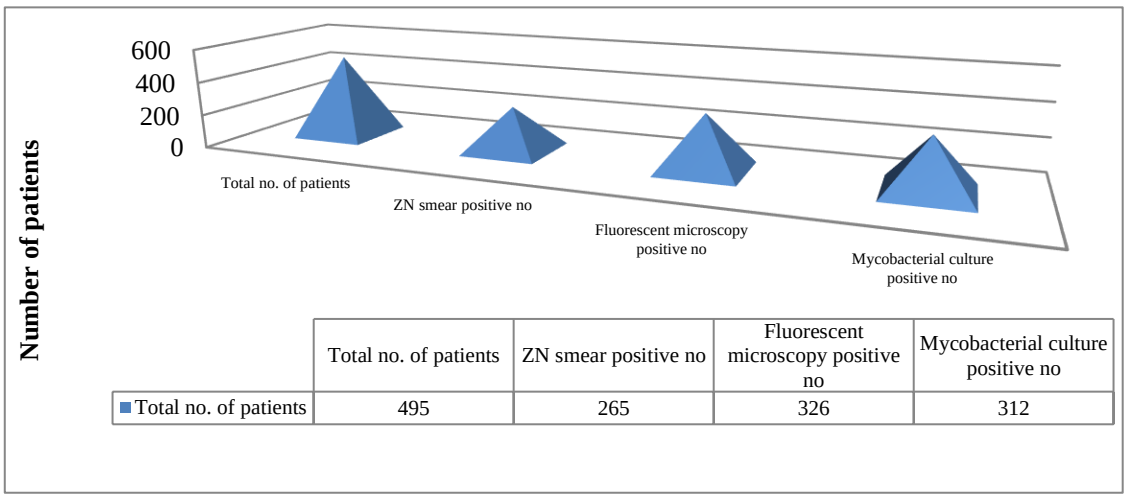

Figure 4: Comparison of ZN smear examination, fluorescent microscopy and mycobacterial culture on LJ medium of primary pulmonary TB suspected patients.

Table 3 shows comparison of results of smear examination by ZN staining, fluorescent staining and mycobacterial culture. It was observed that 252 (50.91%) were both ZN smear and culture positive, 178 (35.96%) were both ZN smear and culture negative. 60 (12.12%) were ZN smear negative and culture positive. On the contrary 5 (1.01%) were ZN smear positive and culture negative. Similarly, 308 (62.22%) samples were both FM and culture positive, 175 (35.35%) samples were both FM and culture negative. 8 (1.62%) were FM positive and culture negative. On the contrary 8 (1.62%) were FM positive and culture negative. There was a total difference of 16 between FM positive and culture positive samples, out of the total 16 samples, 8 samples were negative for growth, 8 samples got contaminated and 2 samples got dried.

Considering *Mycobacterial* culture as gold standard sensitivity, specificity, positive predictive value, and negative predictive value were calculated as follows: true positive-252, true negative-178, false positive-5, false negative-60, sensitivity of smear-80.76%, specificity of smear-97.27%, positive predictive value-98.05%, and negative predictive value-74.79%.

P value is less than 0.0001 which is considered to be extremely statistically significant.

Sensitivity, specificity, positive predictive value and negative predictive value of ZN staining technique was 80.76%, 97.27%, 98.05% and 74.79% respectively.

Table 3: Profile of ZN smear, fluorescent microscopy, and mycobacterial culture examination of primary pulmonary tuberculosis suspected cases (n=495).

S. no.	Description	Total	%
1	Total ZN smear positive	265	53.53
2	Total fluorescent positive	326	65.85
3	Total culture positive	312	63.03
4	Both ZN smear and culture positive	252	50.91
5	Both fluorescent microscopy and culture positive	308	62.22
6	ZN smear negative and culture positive	60	12.12
7	ZN smear positive and culture negative	5	1.01
8	Both ZN smear and culture negative	178 (32+146)	35.96
9	Fluorescent microscopy negative and culture positive	4	0.81
10	Fluorescent microscopy positive and culture negative	8	1.62
11	Both fluorescent microscopy and culture negative	175 (31+144)	35.35

Table 4: Sensitivity and specificity of ZN microscopy method.

Variables	Mycobacterial culture positive	Mycobacterial culture negative
ZN smear positive	252	5
ZN smear negative	60	178

Table 5: Sensitivity and specificity of fluorescent microscopy.

Variables	Mycobacterial culture positive	Mycobacterial culture negative
Fluorescent microscopy positive	308	8
Fluorescent microscopy negative	4	175

Considering *Mycobacterial* culture as gold standard sensitivity, specificity, positive predictive value, and negative predictive value were calculated as follows: true positive-308, true negative-175, false positive-8, false negative-4, sensitivity of smear-98.71%, specificity of smear-95.63%, positive predictive value-97.47%, and negative predictive value-97.76%.

P value is less than 0.0001 which is considered to be extremely statistically significant.

Sensitivity, specificity, positive predictive value and negative predictive value of fluorescent staining technique was 98.71%, 95.63%, 97.47% and 97.76% respectively.

DISCUSSION

Worldwide, TB is the second leading infectious killer after COVID-19 (above HIV and AIDS). A total of 1.3 million people died from TB in 2022 (including 167 000 people with HIV).[4] In March 2017 the Government of India (GoI) announced that the new aim with regard to TB in India was the elimination of TB by 2025. Elimination as defined by the World Health Organisation (WHO) means that there should be less than 1 case of TB for a population of a million people. Prompt detection is essential for controlling the development and spread of TB as it facilitates the appropriate and timely delivery of antitubercular therapy reducing overall cost of treatment and transmission of cases. Direct microscopic examination of appropriately stained sputum specimens for acid-fast bacilli is an important tool in the diagnosis of tuberculosis. Though culture is more sensitive than microscopy, in developing countries, diagnosis is primarily based on AFB microscopy owing to its simplicity, less cost and rapidity.

In the present study a total number of 514 primary pulmonary tuberculosis suspected patients were included. In our study out of the total 514 samples, 265 (51.56%) were AFB positive and 249 (48.44%) were smear negative. In a similar study Tripathy et al reported 47.65% cases were smear positive for AFB which is comparable to our study.[12] Mukherjee et al in their study found 38.64% samples positive by ZN staining.[13] Sahoo et al reported smear positivity in 74.5% of cases.[14] Myneedu et al reported 76.4% smear positivity in their study.[15]

The present study showed that out of total 514 samples, 326 (63.43%) samples were positive for FM and 188 (36.57%) were negative.

In this study the number of paucibacillary cases that were detected by ZN staining was 178 whereas, the number of paucibacillary cases detected by fluorescent staining was 194 and 3.13% paucibacillary cases were missed on ZN microscopy. Thapa et al reported that 1.4% cases were detected by FM which were missed on ZN microscopy.[16] In a study by Hooja et al the cases that were missed on ZN microscopy was 9.29% which is high as compared to our study but it clearly shows that FM was better in detecting cases which is shown in our study too.[17]

In the present study, out of 514 samples 312 (60.70%) samples were positive for culture on LJ media and 183 (35.60%) samples were negative however, 19 (3.70%) samples were dry/contaminated. Kelamane et al in their study also faced contamination rate of 3%.[18] Singh et al reported 4.9% contamination in their study.[19] In a study by Myneedu et al 2.0% cultures got contaminated.[15]

Culture examination is very efficient, most reliable and gold standard technique, which is the prerequisite for determining strength of bacteria to antibiotics and differentiating from other non-pathogenic mycobacterium by growth rate and biochemical test. But the requirement of longer time duration (6-8 weeks), high cost, well trained manpower and chances of contamination are posing difficulties in it in developing countries.

In this study positivity rate of ZN microscopy, fluorescent microscopy and mycobacterial culture positive were 265 (53.53%), 326 (65.85%) and 312 (63.03%) respectively. In a similar study by Laifangbam et al 44.1%, 71.6% and 70% cases were found positive by ZN, FM and culture respectively, which is comparable to the data achieved in our study.[20] In another study by Laifangbam et al positivity rates for ZN, FM, and culture were 36.1%, 74.1%, and 72.2%, respectively which is again close to the data of our study however positivity rate of ZN microscopy in our study is quite high in our study.[21] The performance of ZN microscopy and fluorescent microscopy were assessed on the basis of sensitivity and specificity by taking culture as the gold standard.

The sensitivity and specificity of ZN microscopy in the present study was 80.76% and 97.27% respectively. In a

similar study by Bhalla et al showed sensitivity of ZN microscopy as 81.6% and specificity as 83.5%.[22] Mean time to read the smear by ZN staining according to Bhalla et al was 5 min which was approximately 2.5 times to FM which was 2 min.[22] A similar study by Gupta et al the sensitivity and specificity of ZN staining method was found out to be 93.75% and 100.00%, respectively. The positive predictive value was 100.00% and the negative predictive value was 99.59%.[23]

In a similar study by Bansal et al sensitivity of ZN staining came out to be 80% and specificity was 96%.[24] In a similar study by Timalsina et al sensitivity and specificity of direct microscopic examination were found to be 60.03% and 98.51% for ZN method.[25]

In the present study, for FM sensitivity was 98.71% and specificity was 95.63%. In a study by Bhalla et al, sensitivity of FM was 83.1% and specificity was 82.4%.[22] Bansal et al reported sensitivity for fluorescent staining 85% and specificity 96% which was same as that of ZN staining in their study.[24] Sensitivity and specificity was found to be 83.56% and 94.53% for FM respectively in a study by Timalsina et al.[25]

In our study FM came out to be 11.87% more effective than ZN staining in case detection. Suhasini et al reported in their study that FM was 7.8% more effective than ZN microscopy.[26] Kelamane et al reported FM 4.4% more effective than ZN microscopy.[18] Goyal et al also reported in their study FM 7.22% more effective that ZN microscopy.[27] Golia et al also found FM 6.15% more effective than ZN microscopy.[28] Although our study led to a clear and strong conclusion but we could not deny there were some limitations of the study as observations were subjective and needed very trained personnels. The solid culture method was taken as the gold standard which itself is a very time taking procedure. It was a task to keep the sample safe and track the growth for weeks.

CONCLUSION

The study clearly indicated that the case detection rate (efficacy) of FM is remarkably higher than that of ZN microscopy. There are aided advantages of less eye strain, easy visualization, less time consuming and even detection of low number of bacteria (paucibacillary cases) in comparison to ZN method. As the screening was done under lower power magnification (400x) i.e. larger area per field than ZN method (1000x), less time is consumed for examination of same area by FM. Taking culture as gold standard, there is high agreement between fluorescent staining and culture than that of ZN staining and culture. The use of fluorescent staining alone could be reliable microscopic method as there were no cases of ZN positive where fluorescent microscopy was negative. But ZN staining alone missed many of the positive pulmonary cases with respect to fluorescent staining and culture. In case of fluorescent microscopy, though the capital cost is higher for expensive instrumentation, the overall cost with large number of sample processing by limited manpower makes no difference in cost. Thus, it has been recommended as very effective method of choice in high risk areas, where a large number of sputum samples are to be examined.

ACKNOWLEDGEMENTS

Authors would like to thank the Dean and Principal, JNMCH, Aligarh for allowing to carry out this project in the Institution. They are also grateful to the Professor and Heads of Microbiology as well as Respiratory Diseases Departments of JNMCH, Aligarh, for providing all the facilities available in the Departments while carrying out this work successfully. They would also like to extend their thanks to the technical members of Intermediate Reference Laboratory, Department of microbiology, Jawaharlal Nehru medical college, Aligarh Muslim University, Aligarh, Uttar Pradesh for their technical support during research work.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Ba F, Rieder HL. A comparison of fluorescence microscopy with the Ziehl-Neelsen technique in the examination of sputum for acid-fast bacilli. Int J Tuberc Lung Dis. 1999;3(12):1101-5.
2. Deshmukh SR, Mantri SB, Kendre PB, Nagoba BS. A comparison of sputum examination for acid fast bacilli by modified Schaeffer & Fulton stain, Ziehl-Neelsen stain & cold stain. Indian J Med Res. 1996;103:294-5.
3. Goyal R, Kumar A. A comparison of Ziehl-Neelsen staining and fluorescent microscopy for diagnosis of pulmonary tuberculosis. IOSR J Dent Med Sci. 2013;8(5):5-8.
4. World Health Organization. Global tuberculosis report 2023. Available at: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2023#:~:text=The%20WHO%20Global%20Tuberculosis%20Report,TB%20commitments%2C%20strategies%20and%20targets. Accessed on 05 May 2024.
5. Ryu YJ. Diagnosis of pulmonary tuberculosis: recent advances and diagnostic algorithms. Tuberc Respir Dis (Seoul). 2015;78(2):64-71.
6. Steingart KR, Ramsay A, Pai M. Optimizing sputum smear microscopy for the diagnosis of pulmonary tuberculosis. Expert Rev Anti Infect Ther. 2007;5(3):327-31.
7. Cohen RA, Muzaffar S, Schwartz D, Bashir S, Luke S, McGartland LP, et al. Diagnosis of pulmonary tuberculosis using PCR assays on sputum collected within 24 hours of hospital admission. Am J Respir Crit Care Med. 1998;157(1):156-61.

8. Mathew P, Kuo YH, Vazirani B, Eng RH, Weinstein MP. Are three sputum acid-fast bacillus smears necessary for discontinuing tuberculosis isolation? J Clin Microbiol. 2002;40(9):3482-4.
9. Hänscheid T. The future looks bright: low-cost fluorescent microscopes for detection of Mycobacterium tuberculosis and Coccidiae. Trans R Soc Trop Med Hyg. 2008;102(6):520-1.
10. Kivihya-Ndugga LE, Van Cleeff MR, Githui WA, Nganga LW, Kibuga DK, Odhiambo JA, Klatser PR. A comprehensive comparison of Ziehl-Neelsen and fluorescence microscopy for the diagnosis of tuberculosis in a resource-poor urban setting. Int J Tuberc Lung Dis. 2003;7(12):1163-71.
11. Bell WJ, Brown PP. Fluorescence microscopy in the laboratory diagnosis and assessment of pulmonary tuberculosis. Central Afr J Med. 1962;8(1):4-9.
12. Tripathy S, Kumar R, Singh SD. Prevalence of multidrug resistant pulmonary tuberculosis in North Bihar. J Clin Diagnost Res. 2015;9(11):LC09.
13. Mukherjee A, Saha I, Sarkar A, Chowdhury R. Gender differences in notification rates, clinical forms and treatment outcome of tuberculosis patients under the RNTCP. Lung India. 2012;29(2):120.
14. Sahoo UC, Singh SK, Basa S, Mohan U. A study of the epidemiological pattern of pulmonary tuberculosis patients undergoing treatment at tuberculosis units of Lucknow. Asian J Biomed Pharm Sci. 2015;20.
15. Myneedu VP, Singhal R, Khayyam KU, Sharma PP, Bhalla M, Behera D, et al. First and second line drug resistance among treatment naïve pulmonary tuberculosis patients in a district under Revised National Tuberculosis Control Programme (RNTCP) in New Delhi. J Epidemiol Global Health. 2015;5(4):365-73.
16. Thapa B, Reza LW, Kumar AM, Pandey A, Satyanarayana S, Chadha S. Light Emitting Diode Fluorescence Microscopy increased the detection of smear-positives during follow-up of Tuberculosis patients in India: program implications. BMC Res Notes. 2015;8:1-4.
17. Hooja S, Pal N, Malhotra B, Goyal S, Kumar V, Vyas L. Comparison of Ziehl Neelsen & Auramine O staining methods on direct and concentrated smears in clinical specimens. Indian J Tuberculosis. 2011;58(2):72-6.
18. Kelamane S, Mispah P. Sensitivity and Specificity of Conventional Fluorescent Microscopy to diagnose Pulmonary Tuberculosis among Pulmonary tuberculosis symptomatics in Karimnagar. Sch J App Med Sci. 2017;5:391-5.
19. Singh BK, Sharma SK, Sharma R, Sreenivas V, Myneedu VP, Kohli M, et al. Diagnostic utility of a line probe assay for multidrug resistant-TB in smear-negative pulmonary tuberculosis. PLoS One. 2017;12(8):e0182988.
20. Laifangbam S, Singh HL, Singh NB, Devi KM, Singh NT. A comparative study of fluorescent microscopy with Ziehl-Neelsen staining and culture for the diagnosis of pulmonary tuberculosis. Kathmandu University Med J. 2009;7(27):226-30.
21. Laifangbam S, Huidrom LS, Khumanthem SD. Detection of Mycobacterium tuberculosis by three methods and their correlation to chest X-ray findings and CD4 T-lymphocyte counts in human immunodeficiency virus-pulmonary tuberculosis coinfection. J Med Soc. 2013;27(3):203-7.
22. Bhalla M, Sidiq Z, Sharma PP, Singhal R, Myneedu VP, Sarin R. Performance of light-emitting diode fluorescence microscope for diagnosis of tuberculosis. Int J Mycobacteriol. 2013;2(3):174-8.
23. Gupta S, Shenoy VP, Bairy I, Muralidharan S. Diagnostic efficacy of Ziehl-Neelsen method against fluorescent microscopy in detection of acid fast bacilli. Asian Pacific J Trop Med. 2010;3(4):328-9.
24. Bansal R, Sharma PK, Jaryal SC, Gupta PK, Kumar D. Comparison of sensitivity and specificity of ZN and fluorescent stain microscopy with culture as gold standard. J Tuberc Res. 2017;5(02):118.
25. Timalsina B, Kutu B, Pradhan R, Maharjan B. A Comparative Study on Ziehl-Neelsen Staining (Light Microscopy), Auramine O Staining (Iled-Fluorescent Microscopy) and Culture on LJ Media of Sputum Samples for the Diagnosis of Pulmonary Tuberculosis. Int J Med Biomed Sci. 2015;1(1):4-14.
26. Suhasini S, Mamilla R. Sensitivity of FM staining versus ZN staining in diagnosing sputum smear positive PTB. IOSR-JDMS. 2015;14(3):29-33.
27. Goyal R, Kumar A. A comparison of Ziehl-Neelsen staining and fluorescent microscopy for diagnosis of pulmonary tuberculosis. IOSR J Dent Med Sci. 2013;8(5):5-8.
28. Golia S, Hittinahalli V, Nirmala AR, Sangeetha KT, Asha SK. A comparative study of auramine staining using led fluorescent microscopy with Ziehl-Neelsen staining in the diagnosis of pulmonary tuberculosis. J Evol Med Dent Sci. 2013;2(20):3450-7.

Cite this article as: Faridi M, Fatima N, Varshney S, Shameem M, Shukla I. A comparative study of diagnostic yield of Ziehl-Neelsen staining and fluorescent staining in diagnosis of primary pulmonary tuberculosis in a resource limited country like India. Int J Community Med Public Health 2024;11:3057-63.