

**LED FLUORESCENCE MICROSCOPY: LEADS THE FUTURE OF RAPID AND EFFECTIVE PULMONARY TUBERCULOSIS DIAGNOSIS****VISHNU PRASAD SHENOY, SEEMA SHETTY\*, CHAITANYA TELLAPRAGADA AND CHIRANJAY MUKHOPADHYAY***Department of Microbiology, Kasturba Medical College, Manipal University, Manipal.***ABSTRACT**

Early diagnosis and appropriate treatment play an important role in combating the mortality and morbidity caused by Tuberculosis and a rapid, reliable microscopy that can detect acid fast bacilli can be a very useful diagnostic modality. We have evaluated the performance of Light Emitting Diode Fluorescence Microscopy as an alternative to the existing conventional methods in the diagnosis of Pulmonary Tuberculosis. total of 181 sputum samples received at the Microbiology laboratory of Kasturba Hospital, Manipal were examined by three different microbiologists using Ziehl-Neelsen's (ZN), Fluorescence microscopy (FM) and Light Emitting Diode fluorescence microscopy (LED). Results were independently tabulated by all the three examiners for comparison of the efficacies of FM and LED in detecting the tubercle bacilli. Of the 181 samples, 25 (13.8%) were positive by all the three microscopic examinations. We did not observe any better sensitivity of the FM or the LED compared with the conventional ZN method. The measure of agreement between FM and LED was excellent and statistically significant (Kappa= 0.817; P value <0.01). LED is a promising reliable alternative that can replace both conventional ZN and FM. As it shortens the diagnostic process and being cost effective should be considered by all TB diagnostic laboratories for increased case finding and rapid diagnosis of Pulmonary Tuberculosis.

**KEY WORDS:** Sputum; Ziehl Neelsen's; Fluorescence Microscopy; LED Microscopy**SEEMA SHETTY**

Department of Microbiology, Kasturba Medical College, Manipal University, Manipal.

## INTRODUCTION

Tuberculosis, the Captain of the Men of Death, has been and remains a major threat to human health. This antique, lethal disease is a disease of overcrowding, poverty and poor nutrition. Effective and early diagnosis would help in combating this disease. With the advancing technology, a lot number of diagnostic techniques have been devised. But in high incidence and low resource settings, a rapid, accurate, cost effective method is of sole importance<sup>1, 2</sup>. Smear microscopy has been the backbone in diagnosing pulmonary tuberculosis. Since the earlier description of Auramine-O stain for staining AFB, numerous studies have reported the superiority of fluorescence microscopy over conventional ZN staining. A systematic review has shown that fluorescence microscopy is 10% more sensitive than conventional light microscopy and allows more rapid screening of sputum specimens<sup>3</sup>. The main reason for traditional fluorescence microscopy not being used widely was because of the expensive cost of purchasing and maintenance issues. Moreover, the mercury vapor lamps used as a light source had a limited life span, needed extensive power supply and if the bulb broke could release toxic metal into the environment<sup>3</sup>. LED microscopes provide a reliable and cheap light source with a usable life span of about 20,000 hrs and do not release toxic wastes on breakage. These microscopes are less expensive, have lower maintenance costs, require less power and are able to run on batteries<sup>4, 5, 6</sup>. World Health organization has recommended that traditional fluorescence microscopy can be replaced by LED microscopy and that LED microscopy is included in a phased manner in place of Ziehl-Neelsen light microscopy<sup>7</sup>. This study was undertaken to evaluate the applicability of Light Emitting Diode (LED) as an efficient replacement of Fluorescence Microscopy (FM) for the diagnosis of Pulmonary Tuberculosis taking conventional light microscopy as reference standard.

## MATERIALS AND METHODS

A total of 181 sputum specimens received for TB smear microscopy in the Department of

Microbiology, at a tertiary care hospital from June to August 2011 were utilized for the study. Direct smears were stained with Auramine-O for FM [Leica DML B2.inc] and using the Paralens as LED attachment (Power used: 30W, Filter block: Blue). Same slides were used for ZN staining. Performing ZN staining for Auramine-O stained smears should not affect the light microscopy results and is accepted as a standard practice. One set of AFB smears was stained by the hot ZN technique<sup>8</sup>. In house made fluorescence staining reagents were used for fluorescence staining. Smears were flooded with Auramine-O for 15 minutes, then rinsed with water, destained with acid-alcohol for 2 minutes, then rinsed again with water. They were finally counterstained with potassium permanganate for 1 minute, then rinsed with water and allowed to air dry<sup>9</sup>. The ZN stained slides were read by experienced microbiologist as part of routine laboratory work. Each Auramine-stained slide was read by two independent microbiologists, hereafter referred to as FM and LED, using the Paralens LED attachment to an Olympus CH2 light microscope using 60 × magnification. All the microbiologists were blinded to all other smear results. The number of AFB observed was quantified according to RNTCP guidelines<sup>8, 9</sup>. The results were compared considering the ZN staining as the gold standard. With Auramine-O staining, mycobacteria appear as bright yellow fluorescent rods when viewed under an excitatory light source. Auramine-O is excited by blue light (wavelength, 450–480 nm) and emits in the green-yellow range (wavelength, 500–600 nm)<sup>10</sup>. Smears were graded by three different observers who were blinded to the results of each other. Smear grading was done based on the RNTCP guidelines<sup>8, 9</sup>.

## RESULTS

Of the 181 sputum samples, 25 were positive for TB by all the three microscopic examinations. There were no significant differences observed in declaring the presence of TB using all the three methods. We did not observe any better sensitivity of

the FM or the LED as compared with the conventional ZN method. The Cohen's Kappa coefficient for detecting the agreement between FM and LED was performed using the SPSS software which showed a

statistically significant measure of agreement ( $\kappa=0.817$ ,  $p$  value  $< 0.01$ ). The mean time taken to smear examination was approximately 3 mins by ZN method while was only about a min by FM and LED.

**Table 1**  
**Comparison of conventional, fluorescence and led microscopy for grading acid fast bacilli in sputum smear.**

Grade of the Sputum smears*	ZN staining	Fluorescence Microscopy	LED Fluorescence Microscopy
Negative	156	156	156
Scanty	4	0	0
1+	4	7	6
2+	5	6	7
3+	12	12	12
Total	181	181	181

\*Grading of the sputum smears were performed as per the RNTCP guidelines.

## DISCUSSION

This study has not provided any statistical difference, but our findings support previous studies which prove better diagnostic performance of fluorescence microscopy compared to the ZN microscopy<sup>11</sup>. The time taken to examine a smear using FM or LED fluorescence microscopy was short indicating the potential of using LED microscopy widely throughout the country like India, especially in resource limited settings with high burden of tuberculosis. The lower magnification used for fluorescence and LED microscopy compared with light microscopy also has the advantage of screening the smear fast and increased sensitivity results. Simplicity of fluorescence staining compared to Ziehl-Neelsen staining is an added advantage<sup>3</sup>. Our study also confirms that the LED provides an alternate reliable light source for fluorescence microscopy. The availability of a robust technique and LED light source which is cheaper makes fluorescence microscopy cheaper and more feasible option in resource limited settings<sup>12</sup>. The low energy requirements of LED microscopy provides the prospect for the development of battery operated microscopes which can be used in those areas where there is frequent power failures as is the case in remote and rural parts of our country<sup>13</sup>. In LED microscopy our observation was that mycobacteria were visible without a darkroom. But this observation needs to be further evaluated as

the microscopist was comfortable in screening the slide when microscope was in the dark environment. If this evaluation is substantiated by further evidences as reported in previous studies<sup>14</sup>, then the practical feasibility of using LED microscope in remote and rural areas can be extended and this will improve case detection and reduce diagnostic delay<sup>15</sup>. Auramine-O stained smears were observed on the same day. There were no significant differences in grading of the smears for Fluorescence Microscopy and LED Microscopy. But we can conclude that LED microscopy is no less sensitive in detecting mycobacteria in comparison to the Fluorescence Microscopy as observed in the previous studies<sup>16,17</sup>. Our study was limited to a small number of specimens and failed to demonstrate statistically significant differences. However, this study has proven that LED microscopy offers an effective alternative with at least similar diagnostic performance to ZN microscopy. Performance of ZN staining on the same slide which was subjected to Auramine-O staining can affect the sensitivity of the staining as mentioned in previous studies<sup>13</sup> but our study has refuted it as in 4 sputum smears where the fluorescence microscopy was negative compared to scanty reports by ZN microscopy. Recently, however, one study suggested that LED-FM had lower specificity than conventional ZN smear microscopy in patients co-infected with HIV in Uganda, suggesting that the loss of specificity was

more evident in patients who had scanty AFB in sputum<sup>18</sup>. Despite the potential reservation, the decision was to use the very same slide to ensure optimal comparison, as different smears made from the specimens may be highly variable. High cost, requirement of special settings for FM pose to be some of its major drawbacks. Light Emit Diode Microscopy, even though not so expensive once installed in Laboratory settings, will definitely reduce the maintenance cost and can be very economical in the long run in diagnosing pulmonary tuberculosis<sup>12</sup>. In our country RNTCP has started providing LED Microscopes to Designated Microscopy Centers (DMC) in selected Medical Colleges

and once the case finding rates increase from these centers, these microscopes may be supplied to all DMC in a phased manner. LED fluorescence microscopy being rapid, cost effective, energy efficient, are its favorable attributes and can be recommended for usage as an effective diagnostic tool in resource-limited settings. However, this facility should be carefully implemented in newer laboratories. Adequate training to microscopists, following implementation of standard operating procedures and quality control can increase the accuracy of diagnosis of pulmonary tuberculosis using LED Microscopy.

## REFERENCES

1. P.M. Small, M. Pai, Tuberculosis diagnosis--time for a game change. *N. Engl. J. Med*, 363: 1070–71 (2010).
2. R.S. Wallis, M. Pai, D. Menzies, T.M. Doherty, G. Walzl, et al., Biomarkers and diagnostics for tuberculosis: progress, needs, and translation into practice. *Lancet*, 375: 1920–1937,(2010).
3. K.R. Steingart, M. Henry, V. Ng., P.C. Hopewell, A. Ramsay, et al., Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. *Lancet Inf. Dis*, 6: 570–581, (2006).
4. A. Van Deun, T.M. Chonde, M. Gumusboga, S. Rienthong, Performance and acceptability of the FluoLED Easy module for tuberculosis fluorescence microscopy. *Int. J. Tuberc. Lung Dis*, 12: 1009–1014, (2008).
5. D. Affolabi, G. Torrea, M. Odoun, et al, Comparison of two LED fluorescence microscopy build-on modules for acid-fast smear microscopy. *Int. J. Tuberc. Lung Dis*, 14: 160–164, (2010).
6. R. M. Anthony, A. H. Kolk, S. Kuijper, P. R. Klatser, Light emitting diodes for auramine O fluorescence microscopic screening of Mycobacterium tuberculosis. *Int. J. Tuberc. Lung Dis*, 10: 1060–1062, (2006).
7. World Health Organization (2010) Fluorescent light emitting diode (LED) microscopy for diagnosis of tuberculosis: policy statement. [http://www.who.int/tb/dots/laboratory/who\\_policy\\_led\\_microscopy\\_july10pdf](http://www.who.int/tb/dots/laboratory/who_policy_led_microscopy_july10pdf).
8. Revised National Tuberculosis Control Program (RNTCP), Module for Laboratory Technicians, Central TB Division, Directorate General of Health Services, Ministry of Health and Family welfare, New Delhi,1999.
9. Manual for sputum smear fluorescence microscopy – TBC India, 2007.
10. S.H. Siddiqi, MGIT procedure manual prepared for the FIND MGIT demonstration project, Sparks, MD: BD Diagnostic Systems, 2005.
11. K. Prasanthi, A.R. Kumari, Efficacy of fluorochrome stain in the diagnosis of pulmonary tuberculosis co-infected with HIV. *Indian J. Med. Microbiol*, 43: 179–185(2005).
12. J. Minion, H. Sohn, M. Pai, Light-emitting diode technologies for TB diagnosis: what is on the market? *Expert Rev. Med. Devices*, 6: 341–345, (2009).
13. B.J. Marais, W. Brittle, K. Painczyk, A.C. Hesselring, N. Beyers, E. Wasserman, D. van Soolingen, R.M. Warren, Use of light-emitting diode fluorescence microscopy to detect acid-fast bacilli in sputum. *Clin. Infect. Dis*. 47: 203-207, (2008).
14. H. Albert, Y. Manabe, G. Lukyamuzi, P. Ademun, S. Mukkada, et al, Performance of three LED-based fluorescence

- microscopy systems for detection of tuberculosis in Uganda. PLoS ONE, 5: e15206, (2010).
15. A.I. El-Sony, S.A. Mustafa, A.H. Khamis, et al, The effect of decentralization on tuberculosis services in three states of Sudan, *Int. J. Tuberc. Lung Dis.* 7: 445–450, (2003).
  16. R.M. Anthony, A.H. Kolk, S. Kuijper, P.R. Klatser, Light emitting diodes for Auramine-O fluorescence microscopic screening Mycobacterium tuberculosis, *Int. J. Tuberc. Lung Dis.* 10: 1060–1062,(2006).
  17. N.V. Hung, D.H. Sy, R.M. Anthony, F.G.J. Cobelens, D. van Soolingen, Fluorescence microscopy for tuberculosis diagnosis. *Lancet Infect. Dis*, 7: 238–239,(2007).
  18. A. Cattamanchi, J.L. Davis, W. Worodria, S. den Boon, S. Yoo, et al, Sensitivity and specificity of fluorescence microscopy for diagnosing pulmonary tuberculosis in a high HIV prevalence setting. *Int. J. Tuberc. Lung Dis*, 13: 1130–1136, (2009).