LED fluorescence microscopy for diagnosis of tuberculosis in countries with high disease burden

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Sputum light microscopy is still the cornerstone of tuberculosis (TB) diagnosis in developing countries. The advent of fluorescence microscope (FM) was a major step forward in the direction of improved diagnosis of TB. But the high cost of the mercury lamp and its maintenance cost gave way to a cheaper alternative, i.e. light-emitting diode (LED). Thus, increased sensitivity, low cost and more rapid screening of smear specimens make LED FM operationally desirable in a poor resource setting with high disease burden. Supported by enough evidence, scaling-up LED FM introduction for improved case detection and reduced diagnostic delays in rural and remote areas of countries with high TB/HIV burden is the need of the hour. Before going all out, various operational, quality and management issues should be identified and sorted out.

Care of patients with tuberculosis (TB) starts with a quality assured diagnosis. Successful DOTS implementation and management of drug-resistant and HIV-associated TB require a robust network of TB laboratories with adequate biosafety, modern methods for diagnosis, standard operating procedures and appropriate quality assurance. However, arguably the weakest component of health systems, the laboratory services have historically been grossly neglected and underfunded. Among various reasons, slow policy change and technology transfer, especially in middle and low income countries, has been cited as one of the causes of poor diagnostic capacity.

The development of newer diagnostic tools is an important component of the global plan to stop TB[1]. The world spends an estimated US$ 1 billion per year on diagnostics for TB[2]. It is important to ensure that such expenditure on research is translated into policy and practice, especially in low-middle income countries, where the disease burden is high.

Despite numerous advances, microscopy remains the cornerstone of TB diagnosis, particularly in developing countries. In high-incidence countries, TB control relies on passive case finding among individuals presenting to healthcare facilities followed by clinical or laboratory diagnosis using sputum smear microscopy. The sensitivity of sputum smear microscopy has been reported to vary (20–80%)[3] and is significantly reduced in children, patients with extrapulmonary TB and in HIV-infected TB patients[4].

Considering these limitations of light microscopy, the advent of fluorescence microscope (FM) was a major step forward in the direction of improved diagnosis for TB. The FM illuminates the smear with a high-pressure mercury vapour lamp allowing a much larger area of the smear to be seen, resulting in more rapid examination of the specimen. Because the specimens are stained with auramine O/rhodamine, the bacilli shine fluorescent yellow against the blue background, which also makes them much easier to see and count. Additional advantages of FM include the simplicity of the fluorochrome staining method, compared with ZN methods[5,6].

Due to the above-mentioned reasons, FM smears can be examined in a fraction (about 25%) of the time needed for ZN smears[7]. In another study, conventional FMs took 1–1.4 min/slide, whereas it took 3.6–4 min/slide with a light microscope[7–9].

A systematic review by Steingart *et al.*[10] revealed that the sensitivity of FM was on an average 10% (95% CI = 5–15%) more than conventional microscopy with similar specificities. Similar results have been demonstrated in various other settings, including in developing economies with high TB burden[9,11–13].

Thus, increased sensitivity and more rapid screening of smear specimens make FMs operationally desirable in a resource-poor setting with high disease burden. However, conventional FM using a mercury vapour lamp is expensive with high maintenance cost and limited lifespan (typically 200–300 h). Repeated on-and-off switching, as may occur with unreliable local power supply in low-resource settings, shortens the lifespan even further. In addition, they may release toxic mercury into the environment[14,15]. Therefore FMs never got the desired attention.

Light-emitting diode (LED) is a novel illumination system to allow the resource-poor settings reap the benefits of FM without the associated high costs. LED technology provides a cheap and reliable light source with a usable lifespan of > 50,000 h; repeated on-and-off switching does not reduce its usable lifespan, and it does not pose a potential toxicity risk[14]. Compared to mercury vapour FMs, LED microscopes are less expensive and have lower maintenance requirements[16].

In a comparative study of cost and performance of LED microscopy with ZN microscopy among HIV-TB co-infected patients, LED microscopy showed similar overall sensitivity, but median reading time was much quicker with LED[8]. Average cost per slide read was cheaper for LED microscopy (US$ 1.63) compared to ZN microscopy (US$ 2.10)[8]. In another study conducted in South India, the sensitivity of FM was found to be 16% higher than that of conventional microscopy among HIV-infected patients[17]. But there are studies which show that LED FM has low specificity than conventional microscopy, although careful interpretation of the findings is needed in some cases because of different types of specimens used[18,19]. In another study in Indonesia, a country with high TB burden, lower specificity was reported in LED FM over ZN microscopy[20] (although both greater than 90%). Some studies reported similar specificities in certain settings[10,16]. Khatun *et al.*[21] have shown LED FM to be a more useful test than conventional FM and light microscope (ZN method) to distinguish smear-

negative cases. Marais *et al.*[7] also found high inter-reader kappa value for LED FM over conventional FM and light microscope[7]. Also, various cost-assessment studies have shown higher cost-effectiveness of LED over ZN microscopy[22]. The fact that LED FM does not require a darkened environment[14] greatly enhances the practical operability of using it to provide decentralized diagnostic services.

Thus, higher sensitivity, faster reading time, better acceptability and ease of staining support the introduction of LED FM at the peripheral laboratory level in countries with high TB and HIV burden.

Experts still argue that there is need for more reliable data on specificity for effective implementation of this technique in laboratories[20]. Considering the evidence supporting improved case detection and ease of operation, The International Union Against Tuberculosis and Lung Disease has introduced LED FM at 200 medical colleges across India in collaboration with the Revised National Tuberculosis Control Programme (RNTCP). It resulted in the detection of 5495 more new sputum smear-positive TB cases in 2012, more than double that in 2011. Another measure of success of this project has been the cost-effectiveness of the diagnostic tool, which was less than half (US$ 175 per additional positive case detection) as proposed[23]. Thus it is high time we consider scaling up introduction of LED FM up to Designated Microscopy Centre (DMC) level for improved case detection and reduced diagnostic delays in rural and remote areas of countries with high TB burden like India.

Now with the recent development of the real-time PCR-based instrument called GeneXpert, rapid detection of TB and rifampicin resistance is possible within 2 h (ref. 24). However, cost is a major constraint. Moreover, it can process only two samples per hour; so it may not be suitable for laboratories receiving large number of samples each day[25]. However, GeneXpert should be used as the initial diagnostic test in the case of MDR-TB or HIV-TB, specially in countries with rising disease burden.

The successful and widespread implementation of LED FM in countries with high disease burden might be reasonably expected to improve TB case-finding through increase in direct smear sensitivity and decrease in time spent on smear examination. To reap these benefits, meticulous planning and foresight is needed while introducing LED FM into laboratories unfamiliar with the technique. Adequate training of the laboratory staff at technical and management levels, enhancement of results-reporting mechanisms, reliable mechanisms for instrument maintenance, detailed standard operating procedures and efficient quality assurance programmes will help maximize accuracy and hence benefits. Work should be pursued in the form of implementation studies or operational research to identify newer challenges and deal with them in a timely and effective manner.

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