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Comparative cost and performance of light-emitting diode microscopy in HIV–tuberculosis-co-infected patients

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ABSTRACT: Light-emitting diode (LED) microscopy has recently been endorsed by the World Health Organization (WHO). However, it is unclear whether LED is as accurate and cost-effective as Ziehl–Neelsen (ZN) microscopy or mercury vapour fluorescence microscopy (MVFM) in tuberculosis (TB)–HIV-co-infected subjects.

Direct and concentrated sputum smears from TB suspects were evaluated using combinations of LED microscopy, ZN microscopy and MVFM. Median reading time per slide was recorded and a cost analysis performed. Mycobacterial culture served as the reference standard.

647 sputum samples were obtained from 354 patients (88 (29.8%) were HIV-infected and 161 (26%) were culture-positive for *Mycobacterium tuberculosis*). Although overall sensitivity of LED compared with ZN microscopy or MVFM was similar, sensitivity of all three modalities was lower in HIV-infected patients. In the HIV-infected group, the sensitivity of LED microscopy was higher than ZN microscopy using samples that were not concentrated (46 *versus* 39%; p=0.25), and better than MVFM using concentrated samples (56 *versus* 44; p=0.5). A similar trend was seen in the CD4 count <200 cells·mL^{-1} subgroup. Median (interquartile range) reading time was quicker with LED compared with ZN microscopy (1.8 (1.7–1.9) *versus* 2.5 (2.2–2.7) min; p⩽0.001). Average cost per slide read was less for LED microscopy (US$1.63) compared with ZN microscopy (US$2.10).

Among HIV–TB-co-infected patients, LED microscopy was cheaper and performed as well as ZN microscopy or MVFM independent of the staining (ZN or auramine O) or processing methods used.

KEYWORDS: HIV, light-emitting diode microscopy, smear microscopy, tuberculosis

Despite numerous advances, microscopy remains the cornerstone of tuberculosis (TB) diagnosis, particularly in developing countries [1]. Fluorescent stains increase sensitivity by ⩽10% over carbol-fuchsin-based stains and reduce the time required to read smears [2]. However, fluorescent microscopes using mercury vapour lamps (MVLs) are relatively expensive, have a short life span (the bulb lasts ~250 h), require a reliable electricity supply and replacement bulbs may be difficult to obtain [3]. These factors have delayed the wider implementation of fluorescent microscopy and have led to an interest in fluorescent microscopy using light-emitting diodes (LEDs). LEDs have a lifespan of up to 50,000 h, may be battery operated and do not require a dedicated darkroom [3]. These advantages, together with a potential cost benefit, make LED technology particularly appealing for high-burden resource-limited settings [4].

In 2010, the World Health Organization (WHO) issued as policy statement, recommending that conventional fluorescence microscopy be replaced by LED microscopy using auramine staining in all settings where fluorescence microscopy is currently used, and that LED microscopy be phased in as an alternative for conventional Ziehl–Neelsen (ZN) light microscopy in both high- and low-volume laboratories [5]. A meta-analysis commissioned by WHO, of published and unpublished data, found that LED microscopy was significantly more sensitive (~6%) and without appreciable loss in specificity when compared with direct ZN microscopy [5]. Other studies have also shown good concordance between the performance of

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LED and conventional fluorescent microscopy [6–8]. However, these studies had a low proportion of HIV-infected participants.

Given the lower concentration of bacilli in the sputa of HIV–TB-co-infected patients and the relevance to large parts of Africa where ZN microscopy is the norm, it remains unclear whether LED microscopy performs as well as other microscopy methods in samples obtained from HIV-infected patients. The aim of our study was to assess the performance and cost of LED fluorescence microscopy compared with conventional light microscopy and MVL fluorescent microscopy in HIV–TB-co-infected patients.

METHODS

Patients

Consecutive ambulant patients with suspected TB (aged ⩾18 yrs) were recruited from two primary care clinics in Cape Town, South Africa, during 2009. Informed consent was obtained from all participants and the study was approved by the University of Cape Town Human Research Ethics Committee. HIV and CD4 count testing (if HIV-infected) was performed in all consenting study participants. Two expectorated sputum samples were collected from each patient where possible.

Laboratory processing

Two direct smears were prepared from each sample prior to *N*-acetyl-L-cysteine/NaOH decontamination [9]. One of these was ZN stained, whereas the other was stained with auramine O and read at 200× magnification using the Lumin™ (LW Scientific, Lawrenceville, GA, USA) LED attachment fitted to a light microscope. Thereafter, the specimens were decontaminated and centrifuged and 0.5 mL of the deposit inoculated into a Mycobacterial Growth Indicator Tube (MGIT; Becton Dickinson Diagnostics, Franklin Lakes, NJ, USA). Two further smears were prepared from the deposit, both were auramine O stained, and one read with the LED attachment and the other with a conventional MVL microscope (Zeiss Axioskop; Carl Zeiss, Oberkochen, Germany). Batches (maximum 20 slides) with varying proportions of smear-positive and -negative slides were read by a qualified medical technologist blinded to other microscopy and culture results. The total time taken to read each batch was recorded and the average time to read each slide calculated. Positive slides were graded according to WHO guidelines and the grading of the auramine-stained smears was converted to account for the difference in magnification between fluorescent and light microscopy [10].

Cultures positive for acid-fast bacilli were identified as *Mycobacterium tuberculosis* complex using either an in-house PCR method [11], or the Hain MTBDRplus® assay (Hain LifeSciences, Nuhren, Germany) if susceptibility testing had been requested.

Analysis

The reference standard was at least one positive MGIT culture for *M. tuberculosis*. Test accuracy results were computed as sensitivity and specificity, along with 95% confidence intervals. Categorical variables were compared

TABLE 1 Sensitivities, specificities, and positive and negative predictive values for each of the four different smear detection methods stratified by HIV status and CD4 cell count

Study group	Sputum samples n	Sensitivity				Specificity			
		Unconcentrated sputum sample		Concentrated sputum pellet		Unconcentrated sputum sample		Concentrated sputum pellet	
		ZN	LED	MVFM	LED	ZN	LED	MVFM	LED
All	616	49[#,¶] (42–57)	52[#,+] (44–59)	66 (58–73)	66[¶,+] (58–73)	99 (98–100)	100 (98–100)	99 (98–100)	99 (98–100)
HIV-negative	377	55[§] (45–65)	57[ƒ] (46–66)	74[##] (64–82)	71[¶¶] (61–79)	99 (97–100)	99 (97–100)	99 (97–100)	99 (97–100)
HIV-positive	150	39[§,++,§§] (26–54)	46[ƒ,++] (32–61)	46[#,ƒƒ] (32–61)	54[¶,¶,§§,ƒƒ] (39–68)	100 (97–100)	100 (97–100)	100 (97–100)	99 (95–100)
CD4 count <200 cells·mL^{-1}	69	39 (20–61)	50 (29–71)	44[###] (25–66)	56[###] (34–75)	100 (93–100)	100 (93–100)	100 (93–100)	100 (93–100)

Culture positivity to *Mycobacterium tuberculosis* served as the reference standard. Data are presented as median (95% CI), unless otherwise stated. ZN: Ziehl–Neelsen staining light microscopy; LED: auramine O staining light-emitting diode fluorescent microscopy; MVFM: auramine O staining conventional mercury vapour fluorescent microscopy. [#]: p=0.33; [¶]: p=0.001; [+]: p=0.005; [§]: p=0.08; [ƒ]: p=0.28; [##]: p=0.002; [¶¶]: p=0.06; [++]: p=0.25; [§§]: p=0.092; [ƒƒ]: p=0.5; [###]: p=0.5.

TABLE 2 Average unit cost per acid-fast bacilli (AFB) smear on sputum sample/slide for light-emitting diode (LED) with auramine O *versus* Ziehl–Neelsen (ZN) light microscopy

Type of microscopy	Cost per AFB smear US$						
	Overhead[#]	Building space[¶]	Equipment[+]	Staff[§]	Reagents and chemicals[ƒ]	Consumables[##]	Total
LED	0.81	0.01	0.08	0.47	0.04	0.22	1.63
ZN	1.06	0.01	0.08	0.69	0.04	0.22	2.10

[#]: maintenance, running, management and supervision costs; [¶]: cost relating to the use of specific physical (laboratory space) for procedures relevant for microscopy; [+]: costs based on annualised cost of laboratory equipment, inclusive of procurement costs; [§]: complete staff hands-on time from the receipt of specimen to dispatch and filing of the result forms; [ƒ]: based on costs of ready-made staining reagents procured by National Health Laboratory Services and ~3 mL of use per slide for each staining reagent; [##]: cost associated with the use of general consumables such as sputum collection cups, gloves, glass slides, *etc.*

using the Chi-squared or Fisher's exact tests. Concordance between tests was measured using the κ coefficient.

Cost analysis

Unit costs for both microscopic methods (LED and ZN) were estimated based on a routine diagnostic algorithm implemented at the study site with ~20 specimens processed per batch. All economic costs associated with each respective system was analysed in health services perspective, where we concentrated on laboratory-only costs [12, 13]. Unit costs were calculated using the ''ingredients'' approach, and multiplying the quantity of inputs used by price [14]. All capital costs (laboratory space and equipment) were annualised based on their estimated expected life-yrs. Overhead costs were calculated by fractionating staff costs and time, and space and infrastructure utilised to each test [14]. All pricing and costs are expressed in 2009–2010 US dollars based on the currency exchange rates at the time writing. Overhead costs used in this analysis were provided by the National Health Laboratory Services (NHLS).

RESULTS

A total of 647 sputum samples were collected from 345 patients. 295 patients consented to HIV testing. 88 (29.8%) patients were HIV-infected with a median (interquartile range) CD4 count of 178 (124–320) cells·mL^{-1}. 50 patients either refused HIV testing or had unavailable results and were exclude from analysis. The mean±SD age of patients was 36±7 yrs; the majority were male and black African, and 34.5% had a history of previous TB. Of the 647 samples cultured, 25 were contaminated and nontuberculous mycobacteria were isolated from five, leaving 617 evaluable cultures. Of these, 161 (26%) were positive for *M. tuberculosis*.

Table 1 shows the performance characteristics of LED microscopy compared with ZN light microscopy using unprocessed sputum and conventional mercury vapour fluorescence microscopy (MVFM) using concentrated samples, and stratified by HIV infection and CD4 count. The overall sensitivity of LED and ZN microscopy in direct smears was similar (~50%) with an agreement of 97% (κ=0.871), while in concentrated samples, LED microscopy and MVFM were almost identical (66%) with an agreement of 97% (κ=0.896). The sensitivity of LED and MVFM was better in concentrated *versus* unconcentrated samples (66% *versus* 52%, respectively; p=0.005).

In HIV-infected patients, the sensitivity of all four microscopy modalities decreased compared with non-HIV-infected patients, and the performance of MVFM on concentrated samples was significantly better in non-HIV-infected compared with HIV-infected patients (46 (32–61)% *versus* 74 (64–82)%; p=0.002). However, in both unconcentrated and concentrated sputum samples, the performance of LED fluorescence microscopy, although decreased in the HIV-infected subgroup, did not differ significantly between HIV-infected and non-HIV-infected groups (unconcentrated samples 57 *versus* 46%, p=0.28; concentrated samples 71 *versus* 54%, p=0.06). Amongst HIV-infected patients, the sensitivity of LED microscopy was better than MVFM on concentrated samples, although it did not reach significance (54 *versus* 46%, respectively; p=0.5) (table 1). In HIV-infected patients with CD4 counts <200 cells·mL^{-1}, the sensitivity of LED microscopy was better than MVFM, but did not reach significance (56 *versus* 44%, respectively; p=0.5).

The median (interquartile range) time for reading unconcentrated smears was significantly quicker with LED fluorescence microscopy compared with standard ZN light microscopy (1.8 (1.7–1.9) *versus* 2.5 (2.2.–2.7) min; p⩽0.001). The mean time saved by using LED compared with ZN microscopy was 25%. Reading concentrated smears took 35% less time than unconcentrated smears using either LED microscopy or MVFM.

The average unit cost, expressed as cost per slide read, was cheaper for LED-based methods (US$1.63) as compared with conventional light microscopic method using ZN staining (US$2.10; table 2). Most of the cost savings were as a result of a reduced amount of time required for reading slides and simpler staining process. LED and ZN micrscopy would cost US$1,568 and US$2,049, respectively, to screen 1,000 TB suspects on their first sputum sample using concentration methods.

DISCUSSION

This is, to our knowledge, the first study that comprehensively examines the usefulness and accuracy of LED microscopy in HIV-infected subjects. The major finding of this study is that LED microscopy, despite being cheaper, performs as well as ZN

microscopy or MVFM in HIV-infected subjects using both concentrated and unconcentrated sputum samples. In fact, LED microscopy performed better than ZN staining when using uncentrifuged samples, and better than MVFM when using centrifuged samples, although the difference failed to reach significance. The density of mycobacteria is lower in the sputa of HIV–TB-co-infected patients who have pauci-bacillary disease and thus it is important to confirm that LED microscopy performs as well as other methodologies in this subgroup of patients.

Published studies have already shown that LED microscopy performs as well as conventional microscopy and MVFM in unselected patients with TB in both research and operational settings [6–8, 15]. MARAIS *et al.* [6] showed a slightly better, although not statistically significant, detection rate using LED as compared with MVFM (5 *versus* 12%, n=221), while VAN HUNG *et al.* [16] reported slightly lower sensitivity of LED microscopy, which they attributed to photo-bleaching, as the smears were read on a MVL microscope before the LED microscope. However, there are hardly any data for HIV–TB-co-infected persons and the published WHO guideline does not address performance in this subgroup of patients, although WHO recommendations are meant apply to both HIV-infected and non-HIV-infected TB suspects. Confirming efficacy in HIV–TB-co-infected patients is important to the roll-out of LED microscopy by National TB Programmes (NTPs) in African countries where this technology is most needed, where $\leqslant$80% of patients have HIV–TB-co-infection, and where the electricity supply is erratic and dark-room facilities limited. Thus, these data may enhance and facilitate the widespread uptake of LED microscopy in Africa.

The second major finding is that in HIV–TB-co-infected patients with a CD4 count $<$200 cells·mL^{-1}, LED microscopy performs as well as ZN microscopy or MVFM using both unconcentrated and concentrated sputum samples. A similar pattern of the superiority of LED microscopy over other microscopy modalities was seen in this subgroup. This finding is significant, given that the majority of HIV–TB-co-infected patients presenting to services in Africa have a CD4 count of $<$200 cells·mL^{-1} [17].

Thirdly, there are no published cost analysis data of LED microscopy, although studies have shown that fluorescence microscopy is a cost-effective alternative to ZN in resource-limited settings [18]. We show that LED microscopy using auramine O staining is cheaper than conventional microscopy using ZN staining. This information will be crucial to enhance uptake of this newer technology by policymakers, especially since the WHO recently endorsed LED microscopy for widespread use.

A limitation of our study is the lack of sufficient numbers of HIV-infected patients to be able to demonstrate superiority of LED microscopy over conventional microscopy or MVFM. However, at the very least, there is no evidence of reduced sensitivity or specificity when using LED microscopy, when reading smears from HIV-infected individuals. We did not perform MVFM on unconcentrated samples because of workload considerations and because, in most settings, MVFM is usually carried out on concentrated specimens only. To avoid the effect of photo-bleaching, which biases against LED microscopy, we elected to use separately prepared slides for each form of microscopy.

In 2010, the WHO recommended that conventional fluorescence microscopy be replaced by LED microscopy using auramine O staining [5]. However, there are very few data about its applicability in HIV-infected persons and our data help to fill this gap in knowledge. Our findings, given the superior performance of LED microscopy, and its user-, field- and cost-friendly format, suggest that African NTPs should now initiate and accelerate the roll-out of LED microscopy. This will enhance the availability of fluorescent microscopy in resource-poor settings and thus impact on case detection rates and lowering of disease burden. Studies are also required to evaluate the combination of LED microscopy with other microscopy-enhancing methodologies, such as the field-friendly concentration technique, TB-Beads (Microsens Medtech Ltd., London, UK) [19], which obviates the use of a centrifuge, so that ease of use in resource-poor settings is further improved.

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STATEMENT OF INTEREST

None declared.

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