Light-emitting diode technologies for TB diagnosis: what is on the market?

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EXPERT REVIEWS

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Tuberculosis continues to be the world's most important infectious cause of morbidity and mortality among adults. Nearly 9 million people develop TB disease each year, and an estimated 1.6 million die from the disease [1]. Despite this enormous global burden, case detection rates are low, posing an enormous hurdle for TB control. Conventional TB diagnosis approaches continue to rely on century-old tests, such as sputum smear microscopy, solid media culture, tuberculin skin tests and chest radiography. These tests have several limitations and perform quite poorly in populations affected by the HIV epidemic [2]. For example, sputum smear microscopy using standard direct Ziehl–Neelsen (ZN) staining has low sensitivity in HIV-infected individuals and, by definition, it is of no value in smear-negative TB. Smear microscopy is also unhelpful in many cases of extrapulmonary TB and childhood TB.

Although much work is currently being conducted in order to develop new diagnostics, in most resource-limited countries, sputum smear microscopy remains the primary means for the diagnosis of TB. Given the known limitations of smear microscopy, considerable research has been conducted to identify methods that can increase the sensitivity and optimize its yield [3]. A series of recent systematic reviews has demonstrated that microscopy can be optimized using at least three different approaches: chemical and physical processing (e.g., treatment with bleach or centrifugation), fluorescence microscopy (FM) and the examination of two (rather than three) sputum specimens [4–6].

Fluorescence microscopy has several advantages over light microscopy using ZN staining and is widely used in most developed nations. First, the fluorochrome staining procedure used with FM is simpler than that of ZN staining. Second, it has been estimated, using meta-analysis, that FM has approximately 10% greater sensitivity for detecting acid-fast bacilli in patient specimens [5]. Third, and possibly most important, since FM can be examined at a lower magnification than ZN (20–40 vs 100×), slides are read more quickly and efficiently with FM. It has been estimated that using FM may take up to 75% less time than ZN [5]. This advantage would be of tremendous benefit for overburdened laboratory systems in many low-resource settings.

Unfortunately, it is these low-resource settings where FM has failed to be widely implemented. An often cited but simplistic explanation for this is the high capital costs for conventional mercury vapor (MV) fluorescent microscopes. However, cost analyses have demonstrated that, despite a higher initial purchase price, the higher sensitivity and greater time efficiency of FM makes it cost effective, even in low-income settings [7,8]. Rather, the pragmatic issues of using MV microscopes have been the more obstinate road blocks to global implementation of FM [9]. MV fluorescent microscopes require significant maintenance and must be kept away from dusty environments; the bulbs have a limited lifespan, which is further shortened by fluctuating power supply or by turning them on and off repeatedly, and they also pose a toxic hazard if broken and require hazardous materials disposal. User acceptance has also been a problem,

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where concerns regarding the production of UV light, heat production and the requirement to work in a dark room have prevented FM from being used in many TB-endemic areas [10].

There is now considerable interest in developing simpler, field-friendly FM technologies. Nonprofit groups, such as the Foundation for Innovative New Diagnostics (FIND), Special Programme for Research & Training in Tropical Diseases (TDR), WHO, and the Stop TB Partnership's Working Group on New Diagnostics, have all played major roles in the development of new technologies and innovative approaches for TB diagnosis [11–15].

> "Recently, it has been demonstrated that low-cost, ultra-bright light-emitting diodes could be a viable alternative to mercury vapor lamps used in fluorescence microscopy."

Recently, it has been demonstrated that low-cost, ultra-bright light-emitting diodes (LEDs) could be a viable alternative to MV lamps used in FM. LEDs can produce very narrow spectrum light and are able to excite auramine and other commonly used fluorescent stains without the production of UV light. LEDs have an expected bulb life of up to 50,000 h (compared with 200 h for a conventional mercury bulb), produce minimal heat and contain no hazardous materials. Power consumption is much lower, to the point where portable battery operation or solar power is feasible. Finally, it has been reported that image quality remains good outside of a darkroom [16], a significant advantage where space constraints and lack of air conditioning are important barriers to user acceptance.

LED technologies for TB diagnosis in the market

Table 1 shows the major commercial LED products that are currently marketed for direct detection of TB:

- Primo Star iLED™ (Carl Zeiss, Oberkochen, Germany)
- Lumin™ (LW Scientific, Lawrenceville, GA, USA)
- ParaLens™ (QBC™ Diagnostics, Philipsburg, PA, USA)
- FluoLED™ (Fraen Corporation Srl, Settimo Milanese, Italy)
- CyScope® (Partec, Gorlitz, Germany)

The Primo Star iLED microscope from Zeiss was developed through collaboration with FIND. This is a standalone microscope with a switch that easily changes the fluorescent LED function to traditional light microscopy. The Partec CyScope is also a standalone unit, using LED illumination for both fluorescent and white-light illumination. The other products come as attachments that transform a user's existing light microscope into a fluorescent-enabled microscope. The Lumin and the ParaLens involve the removal of one of the objective lenses and replacement with an LED-equipped objective that is connected to an external power supply. The Fraen FluoLED attachment involves the installation of the light source and condenser on the base of the unit and a barrier filter on the head of the microscope.

The FluoLED utilizes transmitted light (or transfluorescence) for excitation of the fluorochrome-stained slide: light comes from beneath the slides, excites the fluorochrome and then passes through the objective lens to the user's eye. The other products are based on reflected (or epifluorescent) illumination, where the excitatory light comes from above the slide and excites the fluorochrome stain, which is then emitted back up into the objective lens to the user's eye. Transfluorescent illumination generally creates a lighter background, whereas epifluorescent light yields a darker field of view.

The Primo Star iLED was designed to be a true two-in-one microscope. The Zeiss design focuses on simplicity and durability, and aims to provide an affordable, yet high-quality, all-purpose microscope. The CyScope is a more compact and portable standalone available with either monocular or binocular viewing. However, some national TB programs will want to take advantage of the new LED technology without investing in entirely new microscopes. In this case, they may find it easier to retrofit existing microscopes with one of the attachments instead.

Both the Lumin and ParaLens attachments are designed with maximum portability in mind. They come in small, lightweight, hard-shelled carrying cases that can be transported easily. Compatibility requires only standard Royal Microscopical Society threading of the objective lens onto an existing light microscope. In order to switch back to light microscopy, a different objective may be used or the FM objective is replaced with the original. The FluoLED has been designed to fit onto several common light microscope models and requires a somewhat more involved installation process that results in essentially a two-in-one microscope where all objective lenses can be used for both light and fluorescent illumination (alternatively, it can be purchased as a complete, ready-to-use microscope).

Current literature

There have been few published diagnostic evaluations of this new technology applied to direct detection of *Mycobacterium tuberculosis*, and no head-to-head comparisons of the commercial products are available to date.

The first description of new-generation LEDs being used as excitatory light sources for diagnostic fluorescence stains was published by Martin *et al.*, where it was demonstrated that a LED could replace a mercury arc lamp and produce light of sufficient intensity for use with FM [17]. Anthony *et al.* described how to reversibly adapt an epifluorescent MV microscope for use with a high-power LED and reported good results using it with auramine O-stained patient smears [18]. In a letter published in *Lancet Infectious Disease*, Van Hung *et al.* described using a similarly adapted microscope for examination of auramine O-stained patient specimens in parallel with blinded examination using a conventional MV microscope [16]. They reported good concordance between these two readings (98%; κ: 0.93) and confirmed user-favorable qualities, such as the lack of heat produced by the light source and the ability to perform readings without a completely darkened room [16].

Table 1. Comparison of commercial light-emitting diode products currently available for TB diagnostics.

Device	Manufacturer	Standalone microscope	Attachment	Light transmission	Battery powered	Weight (kg)	Cost (US$)	Ref.
Primo Star iLED™ 	Carl Zeiss, Oberkochen, Germany	Yes	NA	Epifluorescent	Yes	9.5	4825*	[101]
Lumin™ 	LW Scientific, Lawrenceville, GA, USA	No	Objective lens replacement (20, 40, 60 and 100× oil)	Epifluorescent	Yes	0.448	700–2000‡	[102]
ParaLens™ 	QBC™ Diagnostics, Philipsburg, PA, USA	No	Objective lens replacement (40, 60 and 100× oil)	Epifluorescent	Yes	1.27	995§	[103]
FluoLED™ 	Fraen Corporation Srl, Settimo Milanese, Italy	No	Adaptor attached to base and filter installed on head of microscope	Transfluorescent	Yes	5	1977–3530¶	[104]
CyScope® 	Partec, Gorlitz, Germany	Yes	NA	Epifluorescent	Yes	2.7	2372–3699#	[105]

Quotes in currencies other than US dollars were converted using rates published 11 June 2009.
*Special pricing available for high-burden countries: €1250.
‡Depending on options.
§When purchased in quantity.
¶Depending on model and quantity of order.
#Special pricing available for high-burden countries: US$1398.
NA: Not applicable.
Images have been reproduced with the permission of the respective companies.

Subsequently, Marais *et al.* and Van Deun *et al.* published diagnostic evaluations using an LED-equipped microscope and the FluoLED attachment, respectively [10,19]. Marais *et al.* brought LED microscopy to the forefront of people's attention, comparing LED microscopy with conventional MV microscopy and using a diagnostic specimen culture as a reference standard. The sensitivity and specificity of the novel LED technology (84.7 and 98.9%, respectively) compared favorably with conventional MV-fluorescent microscopy (73.9 and 99.8%, respectively) and light microscopy using ZN staining (61.1 and 98.9%, respectively) [19]. Inter-reader variation for the LED examinations was 0.87 compared with 0.79 for conventional MV and 0.77 for light microscopy. Van Deun also found good concordance with conventional MV microscopy (99.1%) in a reference setting; however, field evaluations were, ironically, hindered by the enthusiastic acceptance of the FluoLED and refusal to adhere to a planned return to ZN microscopy for comparison [10].

> "The application of high-powered light-emitting diodes for use in diagnostic fluorescent microscopy is an excellent example of using existing technology to fill a practical need..."

Other diagnostic evaluations are ongoing. The WHO has followed the evolution of this technology closely, and a policy regarding its use for TB diagnosis in low-resource settings is also awaited. Evidence-based policy will ultimately need not only studies on the diagnostic characteristics of sensitivity and specificity but also on practical implementation issues, such as quality-control programs and the effects on patient-important and program-level outcomes.

In conclusion, despite the generally archaic system of diagnosis for TB globally, progress is indeed being made. The application of high-powered LEDs for use in diagnostic fluorescent microscopy is an excellent example of using existing technology to fill a practical need, and one that may prove to have an important impact on global TB-diagnostic programs.

Information resources

- Foundation for Innovative New Diagnostics (FIND) www.finddiagnostics.org
- Stop TB Partnership www.stoptb.org
- TDR, a Special Programme for Training and Research in Tropical Diseases http://apps.who.int/tdr

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