LED-based fluoroscopy and the ParaLens system: illuminating the future of TB diagnostics

Tuberculosis is a major cause of morbidity and mortality globally. The situation is often exacerbated by insensitive diagnostic procedures. The recent introduction of LED (Light-emitting diode) fluorescent techniques for the rapid identification of AFB (Acid-Fast Bacilli) provides laboratorians and healthcare workers around the globe with a proven sensitive technique for microscopic observation of AFB.

By D. Armstrong

The global problem of TB

Tuberculosis is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*, a major cause of morbidity and mortality, especially in the developing world. Transmission occurs through airborne particles from infected individuals who harbour bacilli in their lungs. Once the microbes enter a person's lungs, the body mounts an immune response that can wall-off the bacilli, where they remain latent for a period of time. It is estimated that one-third of the world's population is infected with this latent form of tuberculosis.

If a person has a weakened immune system (such as HIV+ individuals), TB is more likely to cause active infections and lead to classic symptoms such as a productive cough, weight loss and night sweats. Left untreated, this disease, once called "consumption", can rapidly lead to serious illness and death. In 2007, 9.27 million new cases of TB were diagnosed, and approximately 1.3 million deaths resulted from tuberculosis infections. This does not include the additional 465,000 deaths attributed to those suffering from both TB and HIV.

Diagnosis of tuberculosis infections

The diagnosis of a tuberculosis infection is not only reliant on clinical findings, but also on laboratory analysis. Since the late 19th century, laboratories have relied on a sputum smear test to diagnose AFB in pulmonary specimens. More recently, solid and liquid media cultures and the rise of molecular techniques have made much progress, providing an accurate diagnosis and reducing the turnaround time between diagnosis and treatment of tuberculosis infections. Most tuberculosis infections, however, occur in areas with limited resources, thus it is not necessarily possible to implement these expensive techniques.

The mainstay of TB laboratory diagnosis has been the sputum smear. Since pulmonary infections with TB cause a chronic cough, sputum is an excellent medium in which to identify AFB. Once smears are made, slides are stained using a fuchsin-based staining system such as the Ziehl-Neelsen (ZN) method, and then observed using a traditional light microscope under 100x oil immersion. However, a simple sputum smear, stained with the ZN technique, only provides about 50% sensitivity in diagnosis. This is reduced to around 20% when examining smears from patients co-infected with HIV. Poor sample quality and weak laboratory infrastructure reduces the sensitivity of the test even further, thus causing potentially infectious cases to remain undiagnosed. This is the situation in many areas in Southeast Asia and Sub-Saharan Africa, where the prevalence of TB continues to rise. With the rise of drug-resistance strains around the globe, it is crucial to diagnose cases quickly and accurately, to commence effective treatment and to halt transmission of this deadly pathogen.

Figure 1. The ParaLens LED attachment on a traditional light microscope.

Basis of fluorescence microscopy

Fluorescence microscopy is not a brand new technique; it was introduced in the early 20th century by scientists eager to use UV-based light sources to create higher resolution images. However, it was not until the 1940s when an antibody-labelling technique was introduced that the practice of fluoroscopy became more widely used in many scientific disciplines. Traditionally, the diagnosis of tuberculosis by fluorescence techniques involves the use of fluorescent stains that tag the bacilli with fluorophores, which are then visualised under UV-light as brightly stained rods, easily identifiable to microscopists. Compared to light-microscopy, these methods reduce eye-strain, use lower magnification and reduce the time needed for slide scanning. Fluorescence microscopy has also been shown to provide an increase in sensitivity of about 10% when examining positive smears, and it provides a cost-effective alternative to the traditional Ziehl-Neelsen process.

Problems arise, though, when these techniques are implemented in resource-limited settings. The high cost of traditional fluorescence microscopes (upwards of 30,000 USD per microscope) and peripherals, as well as the dangers of UV light and mercury vapour-based lamps have prohibited many countries from introducing these techniques.

Need for low cost-diagnostics: the LED system

Much interest has been generated in recent years in the widespread introduction of low-cost diagnostics for TB diagnosis, especially in areas of sputum smear microscopy. With the introduction of LED-based fluorescence, there is the potential to transform the diagnosis of tuberculosis worldwide by increasing sensitivity.

LED-based systems such as the QBC ParaLens utilise epi-fluorescent technology and high-powered ultra-bright light. The operational life of LED light bulbs is long (over 50,000 hours compared to 200-500 for traditional fluorescent bulbs) and hazardous UV light is not emitted.

The commercially available ParaLens system uses a portable attachment that fits on to most light microscopes, using RMT (Royal Microscope Threading). The attachment on to the objective lens transforms a standard light microscope into a fluorescent microscope, without the need of additional lamps or extensive peripherals [Figure 1]. In addition, the ParaLens attachment functions easily using AC power 12V electrical sources. When using this LED-based microscope to observe smears stained with standard fluorescent stains (Auramine-O, Auramine-Rhodamine, etc), brightly fluorescing organisms can be seen against a darkened background. [Figure 2].

This attachment was tested against a traditional fluorescent microscope, comparing functionality, durability and usefulness both in field settings and controlled laboratory settings. Indications were that the ParaLens was comparable to traditional microscopes in brightness and accuracy, and was easy to use and transport in field laboratory settings [unpublished data].

Implementation of LED-based fluorescence: is it feasible?

Recently, fluorescence technology for the diagnosis of AFB has become a top priority according to the WHO Global Health priority list. There is an overwhelming need for improved TB diagnostics, especially in developing countries where the burden of the disease is high. However, implementation is not necessarily straightforward. While portable LED-based fluorescence technology can be inexpensive and does not require a large infrastructure, there is an initial steep learning curve, especially for those who have no experience in dark-field microscopy. Those who do have experience in microscopic examination of Ziehl-Neelsen stained slides may expect to see the typical corded red-coloured AFB, thus will have to train themselves to identify the size, shape and colour of the mycobacteria, in order to avoid false positives. Training courses that highlight not only the importance of fluorescence but also provide hands-on experience in staining and reading slides with fluorescence would be a help to educate those involved in this process; this especially includes TB microbiologists that are unfamiliar with fluorescence techniques.

Fluorescence microscopy, while providing a more sensitive approach to TB microscopy, does have its limitations. A reliable source of electricity (AC power, 12V battery, etc) is needed for power, so in many isolated areas implementation of this technique would be difficult, at best. Initial costs are higher than for traditional ZN techniques, but costs would decrease with prolonged use. Fluorescence techniques do have an initial learning curve, as do all new methods, so there may be resistance to change on the part of microbiology staff who have been performing traditional Ziehl-Neelsen techniques for many years. In addition, fluorescence microscopy is not used to speciate mycobacteria, therefore in areas with high levels of atypical mycobacterial infections (non-TB), culture techniques should still be used to identify smear positive specimens accurately. Overall, these techniques do need a clean and dust-free environment, not only during use of equipment but also for storage of materials. Clear standard operating procedures (SOPs) should be implemented as well, with which all laboratory personnel should be familiar.

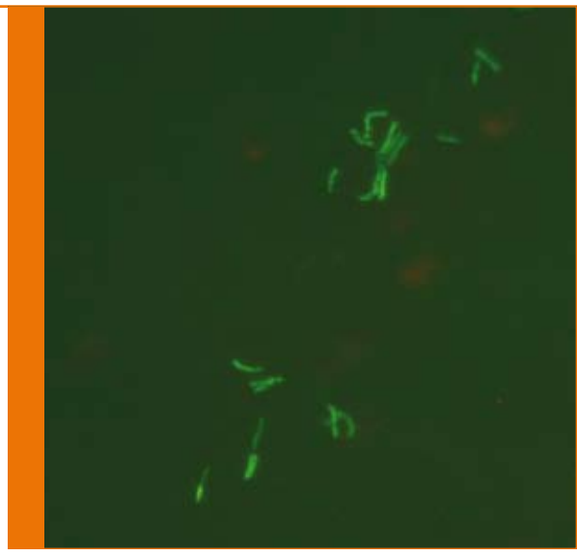

Figure 2. M. tuberculosis seen under fluorescent light using the ParaLens system.

Final thoughts

Ultimately, there is a crucial need for robust and accurate techniques that improve the sensitivity of identifying AFB in smears. A LED-based system such as the ParaLens is a welcomed addition to providing reliable and portable fluorescence microscopy for the identification of AFB. These techniques will provide increased sensitivity for the diagnosis of TB and other mycobacteria, and should therefore be rapidly implemented, especially in areas burdened by high rates of tuberculosis.

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