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Can LED fluorescence microscopy replace Ziehl-Neelsen microscopy in tuberculosis detection?

Ziehl-Neelsen (ZN) acid-fast bacilli (AFB) microscopy, which detects only about 50% of pulmonary tuberculosis patients in the community, is the cornerstone worldwide for the diagnosis of tuberculosis, as, apart from other constraints, it is influenced by the laboratory workload, stain quality and light microscope conditions in the field. In comparison to ZN microscopy, conventional (mercury vapour lamp) fluorescence microscopy (CFM), based on auramine phenol staining, can detect approximately 5–10% more AFB positive smears.[1] However, CFM is used only in specialised laboratories and not in peripheral health institutions due to the requirement for expensive mercury vapour lamps and darkroom facilities. The light emitting-diode (LED) microscope, with its inexpensive, long-life LED lamps, is a boon to mycobacteriologists.

The LED microscope can be housed and slides examined in an ordinary, well-lit room. Its major advantage is the ease of examination with 40×/20× objectives. Laboratory technicians (LTs) feel less fatigue on examining the slides, thus increasing the chances of AFB detection in paucibacillary samples.

The World Health Organization recently recommended both the use of LED FM and replacing ZN microscopy in a phased manner. However, this gives rise to the following issues: 1) less experienced LTs are likely to commit false-positive errors,[2] as impurities in auramine stains and artefacts such as blood[3] in the sputum will fluoresce like AFB; 2) detection of at least three AFB in a smear is likely to be highly confirmative of culture-positive TB;[4] 3) AFB damaged by anti-tuberculosis drugs during treatment stain better with auramine than carbol fuchsin;[5] 4) positive smears are not checked before reporting the results in the field; and 5) there is a paucity of knowledge about the adequacy of training on LED FM and on the quality of FM reporting in programmatic conditions: doubtful smears encountered with LED FM should be restained by ZN and verified until the technicians are fully familiar with LED FM microscopy.

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In reply

Drs. Das and Selvakumar warn against rolling out LED fluorescence microscopy (FM) without a validation period during which scanty and doubtful results are confirmed by Ziehl-Neelsen microscopy (ZN). We disagree. Confirmation or validation of FM by ZN microscopy may reduce the gains in sensitivity and laboratory efficiency anticipated upon switching to LED FM. As the less sensitive method, ZN will often miss rare acid-fast bacilli (AFB) detected in FM, which may also be washed off during restaining,[1] so that freshly trained technicians start to mistrust and no longer report scanty results. In our experience, alternative approaches can ensure quality without adversely affecting sensitivity or workload.

Initial training must be provided by staff experienced in using LED FM in everyday practice. Building a cadre of such trainers is a necessary prerequisite for widespread implementation of LED FM. Training should include sufficient supervision and practice time to allow the trainees to become confident in differentiating artefacts from AFB, a skill that makes proficiency in LED FM harder to acquire than in ZN microscopy. Where there is uncertainty, trainees should be taught to switch from a 20× to a 40× or 50× objective lens for confirmation.[2] On-site supervision and rechecking of a sufficiently large sample should be performed rapidly after LED FM implementation, with re-training, additional supervision and rechecking as needed to ensure proficiency and to reduce not only false positives, but also false negatives.

Concerning objective lenses, some manuals have recommended scanning with a 40× rather than with the preferred 20× lens. The field of view of the 20× objective is 3–4 times larger than that of the 40× objective and 25 times larger than that of the 100× objective used for ZN microscopy (older texts mistakenly state that the field of view is 10–12 times larger).[3] Under time pressure, technicians may tend

to review fewer fields than recommended before declaring a sputum smear negative, but viewing just four fields through the 20× objective will already correspond to the 100 fields required by ZN. Examining one full length of a smear corresponds to 500 ZN fields, and gives the greatest chance for LED FM to fulfil its promise of increased sensitivity.

As with any technology, poor implementation of LED FM may lead to underperformance. Fortunately, each of these pitfalls—hasty and poor training, overreading of scanty smears with ZN, and the use of 40× or even 100× objectives for screening—can be overcome by using better approaches for training and quality assurance. These may vary from setting to setting and with the experience of the microscopist. To date, we have not seen any published studies measuring LED FM proficiency after different lengths of training. Such studies are critical to ensure that scale-up of LED FM achieves the goals of reducing laboratory workload and increasing case detection, without increasing false-positive diagnoses.

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Change to patient-centred terminology in tuberculosis: an important step, but what about the treatment strategies?

We read with great interest the article of Zachariah et al. and welcome the reflection and recommendation towards more patient-centred terminology in tuberculosis (TB) programmes. The article laudably mentions that the lack of a strong, patient-centred approach to TB services is probably the most important reason for poor treatment completion rates. But will a change in terminology and/or definitions suddenly enhance treatment outcomes?

We are concerned that TB programmes continue to focus mainly on directly observed therapy (DOT) as their recommended method of supervision. DOT has not been shown to improve treatment outcomes compared to self-administered therapy (SAT).[1] While there is increasing emphasis on approaches that allow DOT to become more patient-focused, it still views patients as passive subjects of their treatment programmes. In many settings with a high prevalence of human immunodeficiency virus (HIV) infection and TB that are often challenged with inadequate human resources for health, DOT is neither feasible nor practised. Rather, weeks of TB medication are dispensed to patients with little health education or treatment support. In recognition of this reality, the endorsement of the SAT strategy is critical.

After more than a decade of prescribing antiretroviral therapy (ART), the HIV world has learned much about patient empowerment and its ability to enhance treatment outcomes. Recent interesting strategies include the use of community ART groups (CAGs) in Mozambique,[2] and adherence clubs in Cape Town,[3] where patients are not only educated and prepared, they are also considered co-responsible for the treatment of their illness.

HIV treatment programmes have learned that treatment strategies need not only to be adapted to the reality of patients' daily lives, they should also be owned by them to achieve optimal outcomes.[4] A recent cluster randomised trial in Uganda found that home-based ART delivery was equivalent to facility-based ART delivery in terms of survival and virological suppression.[5] In Eastern Africa, various community models involving people living with HIV support drug distribution and patient follow-up, leading to reduced loss to follow-up.[6]

While the World Health Organization recognises the need to establish mechanisms for delivering integrated TB and HIV services to co-infected patients,[7] only South Africa has created a manual to guide integration.[8] In most settings two separate services continue to exist, with different and sometimes conflicting treatment approaches.

When will we dare to integrate TB and HIV services, especially in settings with high co-infection, and create patient-centred strategies that encourage the participation of patients as responsible, educated partners in their own treatment?

We urge the TB world to move beyond lip-service to a patient-centred approach. Words are important, but evidence-based strategies to accompany them exist and must be implemented.