Fluorescent light-emitting diode (LED) microscopy for diagnosis of tuberculosis

Policy statement

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Contents

Abbreviations

CI	confidence interval
GRADE	grades of recommendation assessment, development and evaluation
LED	light-emitting diode
STAG-TB	Strategic and Technical Advisory Group for Tuberculosis
TB	tuberculosis
WHO	World Health Organization

Executive summary

Conventional light microscopy of Ziehl-Neelsen-stained smears prepared directly from sputum specimens is the most widely available test for diagnosis of tuberculosis (TB) in resource-limited settings. Ziehl-Neelsen microscopy is highly specific, but its sensitivity is variable (20–80%) and is significantly reduced in patients with extrapulmonary TB and in HIV-infected TB patients. Conventional fluorescence microscopy is more sensitive than Ziehl-Neelsen and takes less time, but its use has been limited by the high cost of mercury vapour light sources, the need for regular maintenance and the requirement for a dark room.

Light-emitting diodes (LED) have been developed to offer the benefits of fluorescence microscopy without the associated costs. In 2009, the evidence for the efficacy of LED microscopy was assessed by the World Health Organization (WHO), on the basis of standards appropriate for evaluating both the accuracy and the effect of new TB diagnostics on patients and public health. The results showed that the accuracy of LED microscopy was equivalent to that of international reference standards, it was more sensitive than conventional Ziehl-Neelsen microscopy and it had qualitative, operational and cost advantages over both conventional fluorescence and Ziehl-Neelsen microscopy.

On the basis of these findings, WHO recommends that conventional fluorescence microscopy be replaced by LED microscopy, and that LED microscopy be phased in as an alternative for conventional Ziehl-Neelsen light microscopy. The switch to LED microscopy should be carefully phased in at country level, with LED technology that meets WHO specifications. Countries using LED microscopy should train laboratory staff, validate the technique, introduce appropriate quality assurance and monitor the effect on TB case detection rates and treatment outcomes.

Policy statement

Fluorescent light-emitting diode (LED) microscopy for diagnosis of tuberculosis

1. Background

Direct sputum smear microscopy is the most widely used means for diagnosing pulmonary TB and is available in most primary health-care laboratories at health-centre level. Most laboratories use conventional light microscopy to examine Ziehl-Neelsen-stained direct smears; this has been shown to be highly specific in areas with a high prevalence of TB but with varying sensitivity (20–80%).

Fluorescence microscopy is more sensitive (10%) than conventional Ziehl-Neelsen microscopy, and examination of fluorochrome-stained smears takes less time. Uptake of fluorescence microscopy has, however, been limited by its high cost, due to expensive mercury vapour light sources, the need for regular maintenance and the requirement for a dark room.

LED microscopy was developed mainly to give resource-limited countries access to the benefits of fluorescence microscopy. First, existing fluorescence microscopes were converted to LED light sources. Considerable research and development subsequently resulted in inexpensive, robust LED microscopes or LED attachments for routine use in resource-limited settings.

In comparison with conventional mercury vapour fluorescence microscopes, LED microscopes are less expensive, require less power and can run on batteries; furthermore, the bulbs have a long half-life and do not pose the risk of releasing potentially toxic products if broken, and LED microscopes are reported to perform equally well in a light room. These qualities make LED microscopy feasible for use in resource-limited settings, bringing the benefits of fluorescence microscopy (improved sensitivity and efficiency) where they are needed most.

2. Evidence for policy formulation

2.1 Synthesis of evidence

In September 2009, WHO assessed the evidence for the efficacy of LED microscopy in a systematic, structured way. The first step was a systematic review and meta-analysis of published and unpublished data with standard methods appropriate for studies of diagnostic accuracy. The second step was the convening of an expert group to evaluate the strength of the evidence, recommend operational and logistical considerations for use of LED microscopy in national TB control programmes and identify gaps to be addressed by future research. The third step was presentation of draft recommendations to the WHO Strategic and Technical Advisory Group for Tuberculosis (STAG-TB) for endorsement.

In accordance with current WHO standards for evidence assessment in the formulation of policy recommendations, the grades of recommendation assessment, development and evaluation (GRADE) system (*1*) was used by the Expert Group to assess the findings of the systematic review. This approach provides a systematic, structured framework for evaluating both the accuracy of new interventions and their impact on patients and public health.

The Expert Group's findings and the final GRADE evaluation (*2*) were presented to STAG-TB in November 2009. STAG-TB recognized that the evidence was compelling and that there was a large body of work on LED microscopy and advised WHO to proceed with policy guidance on its use (*3*). STAG-TB also asked WHO to prepare an overarching policy framework to guide the use of new TB diagnostics, methods and approaches at country level (*3*). This document provides a pragmatic summary of the evidence and recommendations for LED microscopy. It should be read

in conjunction with the detailed findings of the Expert Group (which include the GRADE tables) and the WHO framework for using TB diagnostics (*2*). The framework gives the context for use of

one or more of the currently approved WHO diagnostic tools and methods in relation to country infrastructure, resources, TB epidemiology and TB policy reform.

The existing TB diagnostic tools are not mutually exclusive: they can be used in various combinations in country screening and diagnostic algorithms, which are highly setting- and resource-specific. Expert laboratory input is therefore needed to define the most cost-effective and efficient algorithms for individual countries, guided by WHO standards (e.g. for laboratory biosafety) and procedures and in the context of overall, integrated, laboratory strengthening.

2.2 Management of declarations of interest

Expert Group members were asked to submit completed declaration of interest forms, which were reviewed by the WHO secretariat before the Expert Group meeting. None of the members declared any conflict of interest. The declaration of interest statements were summarized by the co-chair of the Expert Group meeting at the start of the meeting. No additional declarations were made.

Selected individuals with intellectual or research involvement in LED microscopy were invited as observers to provide technical input and answer technical questions on the methods. These individuals did not participate in the GRADE evaluation and were asked to leave the meeting during the final discussions, when the recommendations were developed. They were also not involved in writing the final meeting report, nor in preparation of the STAG-TB documentation or the final WHO policy statements.

The process for evidence synthesis and policy development was reviewed by the WHO Guidelines Review Committee, and the policy recommendations were approved in June 2010.

The target date for review is 2015.

3. Summary of results

- *Accuracy of LED in comparison with reference standards*: LED microscopy showed 84% sensitivity (95% confidence interval [CI], 76–89%) and 98% specificity (95% CI, 85–97%) against culture as the reference standard. When a microscopic reference standard was used, the overall sensitivity was 93% (95% CI, 85–97%), and the overall specificity was 99% (95% CI, 98–99%). A significant increase in sensitivity was reported when direct smears were used rather than concentrated smears (89% and 73%, respectively).

- *Accuracy of LED in comparison with Ziehl-Neelsen microscopy*: LED microscopy was statistically significantly more sensitive by 6% (95% CI, 0.1–13%), with no appreciable loss in specificity, when compared with direct Ziehl-Neelsen microscopy.

- *Accuracy of LED in comparison with conventional fluorescence microscopy*: LED microscopy was 5% (95% CI, 0–11%) more sensitive and 1% (95% CI, -0.7% - 3%) more specific than conventional fluorescence microscopy.

In qualitative assessments of user characteristics and outcomes in relation to implementation, such as time to reading, cost-effectiveness, training and smear fading, the main findings were:

  - In comparison with Ziehl-Neelsen, LED showed similar gains in time for reading as conventional fluorescence microscopy, with about half the time for smear examination.

  - Cost assessments predict better cost-effectiveness with LED than with Ziehl-Neelsen microscopy, with improved efficiency.

- Qualitative assessments of LED microscopy confirmed many anticipated advantages, including use of the devices without a dark room, durability and portability (in the case of attachment devices); user acceptability in all field studies was reported as excellent.

- LED may be useful for diagnosing other diseases, e.g. malaria and trypanosomiasis, reducing the costs involved in providing integrated laboratory services.

- Possible barriers to widescale use of LED include training of laboratory staff unfamiliar with fluorescence microscopy and the fading of inherently unstable fluorochrome stains. Evidence from standardized training suggests that full proficiency in LED microscopy can be achieved within 1 month.

- Adequate evidence is available to recommend the use of auramine stains for LED microscopy. Other commercial and in-house fluorochrome stains are not recommended.

- Evidence on the effect of fading of fluorochrome stains on the reproducibility of smear results over time suggests that current external quality assurance programmes should be adapted.

- The introduction of LED might affect the cost of other diagnostic modalities, e.g. light microscopy for examining urine, stools and blood, which should be retained at peripheral health laboratory level.

- No studies were found on the direct effect of LED microscopy on outcomes important to patients, such as cure and treatment completion.

- Further research is required on the outcomes of LED microscopy that are important to patients and on combinations of LED microscopy with novel approaches for early case detection or sputum processing.

4. Policy recommendations

The GRADE process confirmed that there is sufficient generalizable evidence to recommend strongly the use of LED microscopy. WHO therefore recommends that:

- conventional fluorescence microscopy be replaced by LED microscopy with auramine staining in all settings where fluorescence microscopy is currently used;
- LED microscopy be phased in as an alternative to conventional Ziehl-Neelsen light microscopy in both high- and low-volume laboratories;
- the switch to LED microscopy be made according to a carefully phased implementation plan, with LED technology that meets WHO specifications;
- countries using LED microscopy address the following issues:
  - training requirements, especially for laboratory staff unfamiliar with fluorescence microscopy techniques;
  - country validation, i.e. demonstrating equivalent performance of LED with Ziehl-Neelsen or conventional fluorescence microscopy at country level during the introductory phase;
  - introduction of WHO-endorsed programmes for internal quality control and external quality assurance; and
  - monitoring of trends in TB case detection and treatment outcomes after introduction of LED microscopy.

WHO will assist countries in the use of LED microscopy by:

- preparing and disseminating technical specifications for LED devices to guide countries, technical and funding agencies in the purchase of high-quality equipment;
- preparing and disseminating standard operating procedures for LED microscopy;
- preparing and disseminating programmes for internal quality control and external quality assurance for LED microscopy; and
- facilitating, with partners and technical agencies, a coordinated approach to standardized training on LED microscopy at country level.

5. Intended audience

This policy statement should be used to guide the use of LED microscopy for TB diagnosis in national TB control programmes. It is intended for use by national TB control programme managers and laboratory directors, in coordination with external laboratory consultants, donor agencies, technical advisors, laboratory technicians, laboratory equipment procurement officers, warehouse managers, other service providers, other relevant government officials and implementing partners involved in country-level TB laboratory strengthening. People responsible for programme planning, budgeting, resource mobilization and training for TB diagnostic services may also benefit from reading this document.

6. References

1. http://www.gradeworkinggroup.org
2. http://www.who.int/tb/laboratory/policy_statements/en/index.html
3. http://www.who.int/tb/advisory_bodies/stag/en/index.html

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