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**FLUORESCENT LIGHT EMITTING DIODE (LED) MICROSCOPY FOR
DIAGNOSIS OF TUBERCULOSIS**

- POLICY STATEMENT -

July 2010

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EXECUTIVE SUMMARY

Conventional light microscopy using Ziehl-Neelsen (ZN) stained smears prepared directly from sputum specimens is the most widely available test for diagnosis of tuberculosis (TB) in resource-limited settings. Specificity of ZN microscopy is high but sensitivity is variable (20-80%) and significantly reduced in extra-pulmonary TB and in HIV-infected TB patients. Conventional fluorescent microscopy has documented higher sensitivity than ZN and takes less time, but uptake has been hampered by high cost due to expensive mercury vapour light sources, the need for regular microscopy maintenance, and the requirement for a dark room.

Light emitting diode (LED) technology has been developed over recent years to allow the benefits of fluorescent microscopy without the associated costs. In 2009, the evidence base for LED microscopy was assessed by the World Health Organization (WHO) following standards appropriate for evaluating both the accuracy and patient/public health impact of new TB diagnostics. Results showed equivalent accuracy of LED microscopy to international reference standards, improved sensitivity over conventional ZN microscopy, and qualitative, operational and cost advantages of LED relative to both conventional fluorescent and ZN microscopy.

Based on these findings, WHO recommends that conventional fluorescent microscopy be replaced by LED microscopy, and that LED microscopy be phased in as an alternative for conventional ZN light microscopy. The switch to LED microscopy should be carried out through carefully phased implementation plans at country level, using LED technologies that meet WHO specifications. Countries implementing LED microscopy should address laboratory staff training, country validation, introduction of appropriate quality assurance, and monitoring of impact on TB case detection and treatment outcome.

POLICY STATEMENT

FLUORESCENT LIGHT EMITTING DIODE (LED) MICROSCOPY FOR DIAGNOSIS OF TUBERCULOSIS

1. Background

Direct sputum smear microscopy is the most widely used test for the diagnosis of pulmonary tuberculosis (TB), available in most primary health care laboratories at health centre level. The majority of laboratories use conventional light microscopy to examine Ziehl-Neelsen stained direct smears, documented to be highly specific in areas with a high prevalence of TB, but with varying sensitivity (20-80%).

Fluorescence microscopy (FM) has been documented to have higher sensitivity (10%) than conventional ZN microscopy, and examination of fluorochrome-stained smears takes less time. Uptake of FM microscopy has, however, been hampered by high cost due to expensive mercury vapour light sources, the need for regular microscope maintenance, and the requirement for a dark room.

Light emitting diode (LED) microscopy is a novel diagnostic tool developed primarily to provide resource-limited settings with access to the benefits of fluorescence microscopy. The first use of LED technology was seen when existing fluorescent microscopes were converted to LED light sources. Considerable research and development have subsequently resulted in inexpensive, robust LED microscopes or LED attachments aimed at routine use in resource-limited settings.

Compared to conventional mercury vapour fluorescent microscopes, LED microscopes are less expensive, require less power and are able to run on batteries, the bulbs have a very long half-life and do not pose the risk of releasing potentially toxic products if broken. LED microscopes are reported to perform equally well without a dark room. These qualities make LED microscopy feasible for use in resource-limited settings, having the potential to bring the benefits of fluorescent microscopy (improved sensitivity and efficiency) where needed most.

2. Evidence base for policy formulation

2.1 *Process for evidence synthesis*

In September 2009, WHO assessed the evidence base for LED microscopy through a systematic, structured process: The first step consisted of a systematic review and meta-analysis of available data (published and unpublished) using standard methods

appropriate for diagnostic accuracy studies. The second step involved the convening of an Expert Group to a) evaluate the strength of the evidence base; b) recommend operational and logistical considerations for implementing LED microscopy in national TB control programmes; and c) identify gaps to be addressed in future research. The third step involved presentation of draft recommendations based on the Expert Group findings 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 GRADE system (<http://www.gradeworkinggroup.org>) was used by the Expert Group to assess the findings of the systematic reviews. The GRADE approach provides a systematic, structured framework for evaluating both the accuracy and the patient/public health impact of new interventions.

The Expert Group findings and the final GRADE evaluation are available at <http://www.who.int/tb/dots/laboratory/policy> and were presented to STAG-TB in November 2009. STAG-TB acknowledged a compelling evidence base and large body of work on LED microscopy and advised WHO to proceed with policy guidance on its use (http://www.who.int/tb/advisory_bodies/stag/en/index.html). STAG-TB also requested WHO to develop an overarching Policy Framework to guide the implementation of new TB diagnostics and methods/approaches at country level (http://www.who.int/tb/advisory_bodies/stag/en/index.html).

This document provides a pragmatic summary of the evidence and recommendations related to LED microscopy, and should be read in conjunction with the detailed findings from the Expert Group Report (which include the GRADE tables), and the WHO Framework for Implementing TB Diagnostics (<http://www.who.int/tb/dots/laboratory/policy>). The Framework provides the context for implementation of one or more of the currently approved WHO diagnostic tools/methods, within the local context of country infrastructure, resources, TB epidemiology, and TB policy reform.

None of the existing TB diagnostic tools are mutually exclusive and they can be implemented 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 in individual countries, guided by WHO standards (eg. for laboratory biosafety) and procedures, and within the context of overall, integrated, laboratory strengthening activities.

2.2 Management of Declaration of Interest

Expert Group members were asked to submit completed Declaration of Interest (DOI) forms. These were reviewed by the WHO-STB secretariat prior to the Expert Group meeting. No member declared any conflict of interest. DOI statements were summarised by the co-chair (WHO-STB) of the Expert Group meeting at the start of the meeting. No additional declarations were made.

Selected individuals with intellectual and/or research involvement in LED microscopy were invited as observers to provide technical input and answer technical questions on the respective methods. These individuals did not participate in the GRADE evaluation process and were asked to recuse themselves from the Expert Group meeting during the final discussions when recommendations were developed. They were also not involved in the development of the final Expert Group meeting report, nor in preparation of the STAG-TB documentation or preparation of 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 approved in June 2010.

Target date for review: 2015

3. Summary of results

- Accuracy of LED compared to reference standards: LED microscopy showed 84% sensitivity (95CI 76% - 89%) and 98% specificity (95CI 85% - 97%) against culture as reference standard. When a microscopic reference standard was used, overall sensitivity was 93% (95CI 85% - 97%) and overall specificity was 99% (95CI 98% - 99%). A significant increase in sensitivity was reported when direct smears were compared to concentrated smears (89% and 73% respectively);
- Accuracy of LED compared to ZN microscopy: LED microscopy showed a statistically significant increase in sensitivity of 6% (95CI 0.1% - 13%), with no appreciable loss in specificity, when compared to direct ZN microscopy;
- Accuracy of LED compared to conventional fluorescence microscopy: LED microscopy was 5% (95CI 0% - 11%) more sensitive and 1% (95CI -0.7% - 3%) more specific than conventional fluorescent microscopy;
- Many studies evaluated qualitative assessments on user-important characteristics and important outcomes relating to implementation, such as time to reading, cost-effectiveness, training and smear fading. Main findings were:
 - Compared to ZN, timing data showed that LED has similar gains in efficiency as conventional fluorescence microscopy, while requiring around half the time than ZN for smear examination;
 - Cost assessments predict improved cost-effectiveness of LED compared to ZN microscopy, with improved efficiency being a key factor;

- Qualitative assessments of LED microscopy confirmed many anticipated advantages, including the ability to use LED 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 provide a technology platform for other diagnostic services; eg. malaria and trypanosomiasis, reducing costs involved in providing integrated laboratory services;
- Possible barriers to large-scale implementation of LED include training of laboratory staff unfamiliar with fluorescent microscopy and the fading of inherently unstable fluorochrome stains. Evidence from standardised training suggests that full proficiency in LED microscopy can be achieved within a period of one 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 regarding the effect of fading of fluorochrome stains on the reproducibility of smear results over time suggests that current external quality assurance programmes have to be adapted;
- LED introduction may affect cost of other diagnostic modalities, eg. light microscopy for urine/stool/blood examinations which will have to be retained at peripheral health laboratory level;
- No studies evaluated the direct impact of LED microscopy on patient-important outcomes such as cure and treatment completion;
- Further research is required on patient important outcomes of LED microscopy, as well as research into combining LED microscopy with novel approaches for early case detection and/or sputum processing.

4. Policy recommendations

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

- Conventional fluorescence microscopy be replaced by LED microscopy using auramine staining in all settings where fluorescence microscopy is currently used;

- LED microscopy be phased in as an alternative for conventional ZN light microscopy in both high- and low-volume laboratories;
- The switch to LED microscopy be carried out through a carefully phased implementation plan, using LED technologies that meet WHO specifications;
- Countries implementing LED microscopy should address the following issues:
 - Training requirements, especially for laboratory staff unfamiliar with fluorescent microscopy techniques;
 - Country validation, ie. demonstrating equivalent performance of LED with ZN and/or conventional fluorescence microscopy at country level during the introductory phase;
 - Introduction of WHO-endorsed programmes for internal quality control and external quality assurance;
 - Monitoring of trends in TB case detection and treatment outcomes after introduction of LED microscopy;

WHO will assist countries with implementation of LED microscopy by:

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

5. Target audience

This policy statement should be used to guide implementation of LED microscopy for TB diagnosis within national TB control programmes, and is intended to be used 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. Individuals responsible for programme planning, budgeting, resource mobilization, and training activities for TB diagnostic services may also benefit from using this document.

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