Fluorescent Light Emitting Diode (LED) Microscopy for the Detection of *Mycobacterium tuberculosis*: a Systematic Review & Meta-analysis

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INTRODUCTION

In most of the world, the diagnosis of tuberculosis (TB) continues to rely primarily on traditional microscopy to detect *Mycobacterium tuberculosis* bacilli in patient specimens. Efforts to maximize the yield and sensitivity of smear microscopy have led to changes in specimen collection, processing, and microscopy techniques (1-4). The development of fluorescent microscopy (FM) using auramine staining has been a significant improvement over Ziehl-Neelsen (ZN) staining for use with light microscopy. FM has been shown to have approximately 10% higher sensitivity compared to routine light microscopy, with no significant compromise in specificity (4). The efficiency of reading slides with FM is also much greater than that of ZN due to the lower magnification needed to screen for the brightly fluorescing bacilli (4, 5). This translates into valuable time savings for many understaffed and overworked laboratories (6, 7). In most of the developed world, FM has now been widely adopted and is used routinely either in addition to or in replacement of ZN staining.

Light emitting diode (LED) microscopy is a novel diagnostic tool developed primarily to provide resource-poor parts of the world access to the benefits of FM (8). Compared to conventional mercury vapour fluorescent microscopes (CFM), 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 they are broken, and are reported to perform equally well without a darkroom (9, 10). These qualities make them feasible for use in low resource settings and have the potential to bring the benefits of FM to areas where their improved sensitivity and efficiency are needed most.

While the first use of LED technology was seen as people began converting existing fluorescent microscopes to LED light sources (9, 11), there are now several commercial LED microscopy products on the market (12). Table 1 compares the major commercial LED units for TB detection. This includes a stand-alone bright light-fluorescent hybrid model developed by Zeiss in collaboration with FIND, the Primostar iLED. This is being sold as a high quality all-in-one solution for laboratories looking to upgrade their microscopy capabilities, and special pricing has been negotiated for high-burden countries. The CyScope (Partec, Germany) and the FieldLab (Cytoscience, Switzerland) are stand-alone LED microscopes which are smaller and built for maximum portability. Two products (Lumin, LW Scientific, USA and ParaLens, QBC Diagnostics, USA) are LED enabled objective lenses which can be swapped for a regular objective on an existing light microscope to confer epi-fluorescent capability. The FluoLED attachment (Fraen, Italy) requires installation onto an existing light microscope and then provides full 2-in-1 light and trans-fluorescent functionality.

OBJECTIVES

We undertook a systematic review of the literature concerning the use of LED microscopy for the detection of *Mycobacterium tuberculosis* and performed meta-analysis of data examining its diagnostic accuracy.

METHODS

To conduct this systematic review and meta-analysis, we used a standard protocol [Appendix 1](13).
Search Strategy

We systematically searched 3 databases for relevant citations: PubMed, EMBASE and BIOSIS (January 1990 – February 2009 inclusive). The search strategy used for PubMed is shown in Appendix 2. All searches were performed with the help of an experienced librarian. Publications in English, French or Spanish were considered. Reference lists from included studies were hand searched. Additionally, experts and manufacturers were contacted to identify additional studies. Unpublished studies were considered eligible if detailed methods and results were provided in manuscript format by August 2009.

Eligibility Criteria

Predetermined eligibility criteria for the primary analysis were: assessment of the diagnostic accuracy or performance characteristics of LED microscopy for the detection of mycobacteria in patient specimens, use of culture as a reference standard, and adequate information to populate a diagnostic 2 by 2 table. Studies using alternate reference standards, such as expert rechecking of smears, were included and evaluated separately. Studies evaluating other characteristics such as time to read slides, cost-effectiveness, user assessments, or implementation issues such as training, staining or smear fading with respect to LED microscopes were also considered and incorporated.

Study Selection

Titles and abstracts were screened for relevance by one reviewer (JM) and obtained for full text review. Full text review was performed by one reviewer (JM). Articles retrieved for full text review along with reasons for exclusion are available from the authors.

Data Extraction

We created and piloted a data extraction form with a subset of eligible studies. Based on experience gained in the pilot study, the extraction form was finalized [Appendix 3]. All studies included in the final review were extracted independently by 2 reviewers (JM and LB) and any disagreements were resolved by consensus.

Outcome Measures

Data were extracted to construct 2 by 2 tables of true positive (TP), false positive (FP), false negative (FN), and true negative (TN) values. True positives were defined as specimens found smear positive by LED microscopy and positive by the reference method. False positives were defined as specimens found smear positive by LED microscopy, but negative by the reference method. False negatives were defined as specimens found smear negative by LED microscopy, but positive by the reference method. True negatives were defined as specimens found smear negative by LED microscopy and negative by the reference method. From these data we calculated the sensitivity and specificity of LED compared to culture and compared to microscopic reference standards.

Studies which provided head to head comparisons between LED and CFM or ZN microscopy had these data extracted and the differences in sensitivity and specificity were calculated and pooled (see Analysis
Methods below). Time to read smears was extracted and summarized; the relative time to read smears using LED compared to ZN was calculated, if available. Studies which performed costing analyses were reviewed and summarized narratively. When described, studies’ approaches to training were compiled and described. Studies which provided head to head evaluations of different LED devices are elaborated on separately. Other outcomes of interest that were reviewed and are presented include mycobacterial fluorochrome staining methods, fluorochrome stain fading, and user reviews. Studies which reported on alternative or novel evaluation methods of LED microscopy are described narratively.

Assessment of Study Quality

Using the QUADAS criteria (14) for assessment of quality of diagnostic studies, we assessed quality characteristics that were considered important for this particular review: (i) blinded interpretation of the test results with reference standard results and vice-versa, (ii) complete verification of test results with the same reference standard, (iii) recruitment of patients/specimens either consecutively or randomly, and (iv) study design (cross-sectional vs. case-control; prospective vs. retrospective). The full QUADAS instrument is reproduced within Appendix 3.

Analysis

Data were analyzed using STATA/IC 11.0. Forest plots visually displaying sensitivity and specificity estimates and their 95% confidence intervals (CIs) from each study were constructed using MetaDiSc software (15). Since these measures tend to be correlated and vary according to thresholds (either explicit or implicit cut-off values determining positive vs. negative results), hierarchical summary receiver operating characteristic (HSROC) curves were analyzed to explore the influence of those thresholds (13, 16).

Accuracy measures were pooled using bivariate random effects regression models (17), using the user-written program “metandi” in STATA (18). Sensitivity and specificity differences between LED and ZN or CFM were pooled using “metan, rd”, a random effects regression model for differences in proportions. Heterogeneity of accuracy estimates was assessed using the I² statistic. If fewer than 4 studies were available, their results were pooled using fixed effects models because bivariate random effects models do not converge.

Results from studies with multiple sites using the same study protocol were pooled directly and considered a single study for the purpose of the meta-analysis (i.e. these studies were treated as multicentric studies). Studies which evaluated more than 1 LED device (e.g. Lumin and Zeiss) were treated as separate study arms and entered as multiple studies for the purpose of the meta-analysis. Studies which reported results separately for significantly different subgroups (concentrated and direct smears; high and low screening magnifications) were not pooled and considered as multiple arms entered individually into the meta-analysis.

Subgroup Analysis
Studies using culture as a reference standard were analyzed and pooled separately from studies using a microscopic reference standard. Subgroup analysis by smear type (direct vs. concentrated) and screening magnification was performed.

RESULTS

The selection of included studies is summarized in Figure 1. We identified 2489 citations from the initial searches, and 2102 unique articles were left after excluding duplicate articles. After screening titles and abstracts, 34 articles were eligible for full-text review. Of these, 2 studies were included in the review. An additional 10 studies were identified by contacting experts, manufacturers and subsequent hand searches.

Characteristics of Included Studies

Studies that met our selection criteria are described in Table 2. We identified 3 published (19-21) and 9 unpublished (22-27) studies. Evaluations performed by the Foundation for Innovative Diagnostics (FIND) were reported in a single document (24), but for the purposes of our review we considered 4 separate studies: Feasibility Study, Evaluation Study, Demonstration Study, and a Comparative sub-study.

Four of the commercial LED products were represented in these evaluations: Lumin (6 studies), Primostar iLED (4 studies), FluoLED (3 studies), ParaLens (1 study). Included in these were 2 head-to-head evaluations: FluoLED vs. Lumin; Primostar iLED vs. FluoLED vs. Lumin. A single study used a conventional fluorescent microscope adapted for use with LED illumination.

There were 8 studies which used mycobacterial culture as a reference standard. The remaining 4 studies used a microscopic reference standard: 2 used a pre-specified expert rechecking mechanism and 2 used CFM. Five studies each evaluated direct smears and concentrated smears; 2 studies reported data on both direct and concentrated smears separately. Different magnifications were used for the screening of smears with 400x being the most common (6 studies) and 200x being the most common alternative magnification (4 studies). A single study used 600x and one study compared 200x with 400x.

Direct comparisons of LED with ZN (7 studies) and LED with CFM (6 studies) were also available for analysis.

Quality of Included Studies

Table 3 provides an overview of the key quality indicators found in the included studies. All of the included studies reported blinding their evaluation of slides using the LED microscopes. Nine studies reported complete verification using their respective reference standards (3 used partial or differential verification). Specimen recruitment was reported as prospective in all but 1 study, and sampling was reported as either consecutive or random in 8 of the 12 studies (the remaining 4 were unclear). Seven of the evaluations used a case-control study design and the remaining 5 used a cross-sectional design.

Accuracy of LED in Comparison to a Reference Standard
Figures 2 and 3 display sensitivity and specificity estimates from individual studies, using culture and microscopic reference standards respectively. Estimates from reading direct smears are shown as closed squares and estimates from reading concentrated or processed smears are shown as open squares. Using a culture reference standard, sensitivity estimates ranged from 67 – 96% and specificity estimates ranged from 89 – 100%. Not surprisingly, studies which compared LED to a microscopic reference standard yielded generally higher estimates of sensitivity (73 – 100%) and specificity (range 98 – 100%).

Pooled estimates of sensitivity and specificity, along with $I^2$ measures of heterogeneity are shown in Table 4. Overall, when culture was used as a reference standard, LED achieved 83.6% sensitivity (95% CI: 76.3, 89.0) and 98.2% specificity (95% CI: 96.6, 99.0). When a microscopic reference standard was used, overall sensitivity was 92.7% (95% CI: 84.9, 96.7) and overall specificity was 98.5% (95% CI: 98.2, 98.8).

**Hierarchical Summary Receiver Operating Curves (HSROC)**

Figures 4 and 5 plot the sensitivity (or TPR) and 1-specificity (or FPR) in an HSROC curve for each of the reference definitions. The curves show greater variation in sensitivity than specificity, with minimal specificity variation when a microscopic reference standard was employed.

**Head-to-head Comparisons of LED with ZN and CFM**

Tables 5 and 6 summarize studies with head-to-head comparisons between LED and ZN or LED and CFM respectively. When compared to ZN microscopy, LED sensitivity ranged from being 9% less sensitive to 24% more sensitive and LED specificity ranged from being 7% less specific to 1% more specific. When compared to CFM, LED sensitivity ranged from being 4% less sensitive to 16% more sensitive and LED specificity ranged from being 1% less specific to 5% more specific.

Pooled differences in sensitivity and specificity using random effects regression estimated LED sensitivity to be 6% (95% CI: 0.1, 13) greater than ZN and 5% (95% CI: 0, 11) greater than CFM. Pooling of specificity differences find LED to be 1% (95% CI: -3, 1) less specific than ZN and 1% (95% CI: -0.7, 3) more specific than CFM.

**Concentrated vs. Direct Smears**

Subgroup analysis was performed depending on whether direct or concentrated smears were used (Table 4). For subgroups with at least 4 studies, bivariate random effects pooling was performed; for subgroups with less than 4 studies available, a fixed effects model was used. Based on non-overlapping confidence intervals, there was a significant increase in sensitivity when direct smears were used (88.9%, 95% CI: 81.1, 93.7) compared to concentrated smears (72.7%, 95% CI: 69.2, 76.0) in the studies using culture as a reference standard. This difference was even more pronounced in those studies using a microscopic reference standard (direct smear sensitivity=93.6%, 95% CI: 99.8, 96.4 vs. concentrated smear sensitivity=78.0%, 95% CI: 69.0, 85.0), although this was based on only 2 studies using concentrated smears and this estimate was derived from a fixed effects model that would result in narrower confidence intervals. One of the studies which included a head to head evaluation of direct and concentrated smears found improved sensitivity and specificity using direct smears. The second
study which compared direct and concentrated smears found no difference in sensitivity or specificity overall, but noted that 2 of their 4 participating sites did find concentrated smears to have a lower sensitivity than direct smears.

**Screening Magnification**

Subgroup analysis was also performed depending on whether the screening magnification used was 200x or higher (400x or 600x were combined) (Table 4). Within the group of studies using culture as a reference standard, those which used a lower screening magnification had a significantly lower pooled specificity compared to studies using a higher screening magnification, based on non-overlapping confidence intervals: 94.4% (95% CI: 91.5, 96.4) with 200x screening vs. 99.0% (95% CI: 98.0, 99.5) with 400x/600x screening. This difference in specificity was not seen in pooled estimates of studies using a microscopic reference standard, but was observed in the single head to head evaluation comparing 200x vs. 400x readings (96.4%, 95% CI: 93.6, 98.0 vs. 100%, 95% CI: 98.6, 100). A difference in sensitivity was also detected between studies using higher vs. lower screening magnifications (+5% sensitivity using 400x/600x), however these pooled estimates were both calculated using fixed effects models which would result in narrower confidence intervals.

**Time to Read Slides**

Six studies provided measures of the time needed for readers to examine smears using LED (Table 7). There were a total of 14 comparisons made to ZN and 7 comparisons made to CFM, with varying proportions of smear +/-, smear type, and screening magnification used. Using simple averages (with equal weighting given to each study arm) the mean time saved compared to ZN was 46%. Considering estimates for only smear + slides, LED was 48% more efficient than ZN, and considering only smear - slides, LED was 59% more efficient than ZN. Compared to CFM, the time to read slides was approximately equal (LED examination taking 4% more time).

The FIND Demonstration study measured the time to read slides 1 month after introduction of the iLED, and again 3 months after its introduction (24). Although after 1 month a 20% reduction in reading time was seen, this increased to 45% after 3 months showing that efficiency continued to increase after the first month of use and full benefits were not realized immediately following introduction.

In another measure of time to read slides, the FIND Evaluation study recorded the sensitivity of reading smears for 30 sec, 1 min, 3 min and 5 min (24). They found that when using either CFM or LED >80% of positive slides were correctly identified within 30 sec and increasing the reading time from 3 min to 5 min did not result in significant addition yield. In contrast, when using ZN <50% of positive slides were correctly identified within 30 sec and the full 5 min was required to maximize yield.

**Cost Estimates**

The equipment costs of the major commercial LED devices, as obtained from their respective companies, are summarized in Table 1 (12).
In conjunction with the demonstration studies performed by FIND, costing data were collected for three participating settings: India, Lesotho, and Peru (24). Taking into account equipment costs, staffing costs, chemicals and reagents, consumables, building and overhead costs, they estimated that the average unit costs (cost per test) would be 10 – 12% lower for iLED compared to ZN. An important factor in this analysis was the time savings of using iLED (estimated to require 55% less reading time), which resulted in significant savings in staff costs. Assuming an overall iLED sensitivity of 96.3% and ZN sensitivity of 90.5%, both with 100% specificity, the cost per new case diagnosed was lower using iLED across all settings and across a wide range of TB prevalence (see FIND report). They concluded that implementation of this technology would not require significant modifications to the budgets of current TB programs, except for the initial capital investment for equipment purchase.

Using retrospective data collected from an urban centre in Malawi, Ramsay et al (28) modeled the effects of implementing LED microscopy along with the adoption of a 2-specimen diagnostic strategy (from 3-specimen) and changes to the threshold for definition of a positive smear and a smear-positive case. Not only would these combined approaches significantly increase case detection (nearly doubling the detection of smear-positive cases), but the workload and time savings would enable a currently overburdened diagnostic laboratory to meet recommended smear examination times using existing human resources and minimal additional equipment.

Previous cost analyses have shown that despite higher upfront equipment costs, CFM can be a cost-effective alternative compared to ZN given the savings in labour and reagents (6, 7). Considering the lower equipment costs for LED devices compared to CFM, their lower maintenance and their lack of a need for a dark room, LED technology can be considered a more cost effective option compared to both ZN and CFM.

**Training**

During the FIND Demonstration study, personnel with experience in ZN microscopy (but no experience with FM) were given between 1 – 5 days of training before entering the first phase of the study (24). Accuracy estimates were calculated separately for three distinct phases: Validation phase (1 month post initial training), Implementation phase (3 months following validation), and Continuation phase (6 months following implementation). Overall accuracy was strong even during the validation phase (sensitivity 94.2%, 95% CI: 92.2, 94.6; specificity 98.2%, 95% CI: 97.9, 98.5), and remained consistent throughout implementation (sensitivity 96.7%, 95% CI: 95.6, 97.2; specificity 98.4%, 95% CI: 97.8, 98.5) and continuation (sensitivity 96.7%, 95% CI: 92.2, 98.6; specificity 97.3%, 95% CI: 95.2, 98.4)(continuation phase estimates based on data collected to date).

Standardized proficiency testing post-training was performed, and repeated at 1 month and 3 months. Target specifications were required to be met at the end of the validation phase, before continuing on to the implementation phase. These targets (>95% accuracy, 100% acceptable staining quality, >80% proficiency) were met by 27/28 of the study sites, with the remaining site achieving the targets 1 month later. Feedback from the microscopists undergoing this training emphasized the importance of practical hands-on training, with the availability of a supervisor to help distinguish AFB from artefacts. Most
microscopists thought that 5 days of training was optimal for those experienced with ZN microscopy, and at least 13 days would be required for those without.

Training issues were not addressed in most of the other reports. However, three studies which did not include such extensive training and standardized proficiency testing noted the possible underperformance of LED upon its introduction due to insufficient training of staff (20, 23, 27).

**Head-to-head LED Device Comparisons**

Two studies included head-to-head evaluations of different LED models. Affolabi et al compared the LW Scientific Lumin and the Fraen FluoLED (22). When using 200X magnification, significantly more positive smears were detected using the Fraen module compared to the Lumin. This difference did not persist when comparing the two using 400X magnification in a secondary analysis. Additionally, the technologists unanimously preferred using the Fraen module, citing easier focusing and better image quality.

In the FIND Comparison study, all three LED devices (Zeiss iLED, Fraen FluoLED, LW Scientific Lumin) resulted in improved sensitivity over ZN and received positive feedback from users (24). Estimates of sensitivity gains compared to ZN resulted in +5.7% for iLED, +7.7% for FluoLED, and +3.8% for Lumin (statistically significant increase for FluoLED). Estimates of specificity gains compared to ZN resulted in +0.8% for iLED, -3.1% for FluoLED, and +0.8% for Lumin (statistically significant decrease for FluoLED). The time to examine slides was significantly less for all models compared to ZN, however, the Lumin examination times were higher than the other 2 models (2.94 min/slide Lumin vs. 2.3 min/slide iLED and 2.38 min/slide FluoLED). User assessments of the 3 models indicated a preference for the iLED citing its high quality optics, operational characteristics and ease if viewing in full light as significant advantages.

**Staining Methods**

Another sub-study reported by FIND compared the performance and suitability of different commercial and in-house stains for use with FM (24). Users reported that all of the fluorochrome staining methods were easier to perform than the ZN stain (likely due to the absence of a heating step). Although experienced users preferred the Auramine O/KMnO$_4$ stain (and the reading time was significantly shorter for both Auramine/KMnO$_4$ and Auramine-Rhodamine/KMnO$_4$), less experienced users found it easier to focus and less tiring to read the stains with coloured backgrounds (Auramine/Methylene Blue and Auramine/Thiazine Red). In a separate evaluation of staining preference done in conjunction with the FIND Feasibility study, users preferred the Auramine/KMnO$_4$ stain over Auramine-Rhodamine/KMnO$_4$ and Auramine/Methylene Blue.

All studies included in this review used auramine O/KMnO$_4$ staining.

**Fading of Fluorochrome-Stained Slides**

Given the current reliance of many External Quality Assurance (EQA) programs on quarterly rechecking of a selection of stored slides, the potential for fading of the fluorescent stained smears was evaluated by 2 studies.
In a sub-study reported by FIND, 6 microscopy centers kept a set of 10 positive smears at room temperature (without air conditioning) and re-read them on a monthly basis for 4 months (24). None of the monthly readings changed from the initial positivity grading at months 1, 2 or 3 and a single center reported misclassifying a single positive slide as negative during the month 4 reading. Qualitative assessments from all sites reported no impairment of reading at month 1 or 2, 1 site reported mild fading at month 3, and 3 sites reported impairment ranging from mild to significant by week 4.

A study performed by Minion et al in Mumbai, India, stored sets of 120 slides in different environments (air conditioned room temperature, 22°C; humidified incubator, 30°C; refrigerator, 4°C – all in slide boxes sealed against light) and re-read them on a monthly basis for up to 5 months (29). A mixture of negative, low positive and high positive smears were included and reading was done in conjunction with a larger LED evaluation study to ensure technologist blinding (i.e. readers did not know they were reading stored smears, but read them as if they were routine). Selected results are presented in Figure 6. Overall, the proportion of positive slides that remained positive decreased to 63% at month 1, 43% at month 2, 26% at month 3, 15% at month 4, and 11% at month 5 for slides stored at air conditioned room temperature. Slides stored in a humidified incubator faded faster than those at room temperature, and surprisingly, slides stored in a refrigerator experienced the fastest fading.

Other Assessments

The study published by Van Deun et al also reports on a field evaluation in 2 high throughput Tanzanian laboratories (20). The field evaluation centres did not have access to culture references, and thus historical comparisons were made to the yield of positive specimens using ZN staining in previous years. In addition to overwhelming user-acceptance and approval of the new LED microscopes, they found a 20% proportional increase in yield of positive smears (from 10 – 12% positives during years of using ZN, to 13 – 16% positives during 2 years of LED use at the two laboratories)(20).

Kuhn et al undertook a field evaluation of the Lumin attachment to assess portability, durability and ease of use (25). All of these characteristics were assessed favourably. It was noted that although viewing was best achieved in completely dark conditions, use was adequate even in bright lighting. This ability to use LED FM without a special dark room was confirmed by several of the other studies (19, 21, 24, 27).

Omar et al also conducted standardized laboratory experiments to compare the Lumin LED with CFM (26). Using smears prepared from known concentrations of MTB suspension, slides were read using standardized protocols in order to quantify their bacillary burden. Concordance between the two microscopes was very high and the technologists preferred the contrast achieved with the LED enabled microscope.

Criticisms that have been noted include reports by technologists working with Omar et al that using the lower magnification of FM resulted in difficulty differentiating artefacts from bacilli (26). Additionally, when using the attachments it is not possible to easily switch to other lenses (requires removing the adapter).
User assessments were also collected during all of the FIND studies (24). Participants in the Feasibility study rated the iLED 3/3 on contrast, resolution, depths of focus, signal-to-noise ratio, and homogeneity of illumination. Evaluation and Demonstration studies asked users to judge the iLED on a number of characteristics including ease of installation, training required, overall handling and features, use of switching between ZN and FM, light intensity/background/contrast, resolution/depth of focus, need for a darkroom, magnification objectives, preferred bulbs for light microscopy, gain in speed, and recommendations for implementation. Overall, reviews were very positive and 94 – 100% of respondents would recommend implementing the iLED system over ZN (24).

DISCUSSION

Principle findings

In this meta-analysis, we performed an extensive literature search and identified 12 studies that evaluated the performance of LED microscopy, 9 of which are currently unpublished. A single evaluation was done using a non-commercial LED adapted microscope, 6 study arms used LED objective lens attachments (5 Lumin, LW Scientific; 1 ParaLens, QBC Diagnostics), 3 study arms used the transfluorescent module FluoLED (Fraen) and 4 study arms using the Primostar iLED (Zeiss). Eight evaluations used culture as a reference standard and 4 used a microscopic reference standard. Pooled estimates of accuracy found LED to have 83.6% sensitivity and 98.2% specificity when compared to culture, and 92.7% sensitivity and 98.5% specificity when compared to a microscopic reference. Direct comparisons estimated LED to have 6% greater sensitivity than ZN and 5% greater sensitivity than CFM, with no appreciable difference in specificity.

Timing data show that compared to ZN, LED microscopy has similar gains in efficiency to CFM, requiring approximately 46% less time than ZN for smear examination. Cost assessments predict improved cost-effectiveness compared to ZN microscopy, with the improved efficiency being a key quality of both LED and CFM. Qualitative assessments of LED microscopes confirmed many touted advantages, including the ability to use without a darkroom, durability and (in the case of the attachment models) portability. User acceptance in all field trials was reported as excellent.

Barriers that remain with respect to implementing wide spread use of LED FM include training of laboratory staff unfamiliar with FM and a proposed mechanism of QC for the inherently impermanent auramine stain used with FM. Insufficient training was cited as a potential limiting factor in several studies, however, with the use of standardized training and assessment, it appears that LED performance can be maximized within a period 1 month. Evidence regarding the effect of fluorochrome fading on the reproducibility of slide reading over time is inconclusive, but suggests that current QC programs may not be able to reliably use stored slides to evaluate a laboratory’s performance.

Strengths and Limitations of the SR

Our SR had several strengths. First, we used a standard protocol for doing the systematic review, including a comprehensive search strategy to retrieve both published and unpublished relevant studies. By contacting several experts and manufacturers, we were able to identify and include several
unpublished studies. In addition to assessing accuracy, multiple other important outcomes were reviewed and summarized including time to read slides, cost analysis, stain fading, training and user assessments. Lastly, we used rigorous methods for data analysis, including bivariate random effects models, HSROC analyses, and tests for statistical heterogeneity. Subgroup analysis using type of smear and magnification used was performed in an attempt to explain some of the observed heterogeneity in estimates of accuracy.

Our SR was limited by the lack of a common reference standard (some studies using culture and others using a microscopic reference), and lack of well-accepted and consistently applied microscopy methods such as smear processing and screening magnification. Considerable heterogeneity was found in many of the pooled estimates, which was not unexpected given the different products, diverse settings and study designs used. The decision to leave subgroup comparisons of smear type and screening magnification unpooled, and thus essentially counted as separate studies, may have overweighted the results from those studies (FIND Feasibility, FIND Evaluation (24), and Shenai et al (27)). However, given the differences between these subgroups it was felt that using the random effects model during meta-analysis would be more appropriate than simple pooling of these data. The other alternative would have been to exclude one of these subgroups from the analysis, however, since neither smear type nor screening magnification could be considered ‘standard’ this was also felt to be inappropriate. The majority of studies included in this review are unpublished and thus are not peer reviewed. Nevertheless, being a new technology we felt it was important to summarize the most up to date evidence available regardless of publication status.

All studies reported on sensitivity and specificity, many studies included qualitative assessments on user-important characteristics and several included comparisons with CFM or ZN. Additionally, 2 head to head comparisons of commercial products were available and evidence was identified on important outcomes relating to implementation such as time to cost-effectiveness, training, and smear fading. However, no studies looked at whether implementation of LED microscopy would have a clinical impact on patient-important outcomes.

Conclusions

LED microscopy has comparable diagnostic accuracy to CFM while using a more durable, safer, and less expensive technology than mercury vapour fluorescent microscopes. The benefits associated with using CFM have been previously established, and current LED evaluations are consistent with the improved sensitivity, simplicity and efficiency of FM compared to ZN light microscopy. The barriers to widespread implementation of FM in many low-income settings have been largely practical and several may be overcome with the introduction of LED fluorescent microscopy. Remaining issues are likely to involve the implementation of an effective quality control system for use with the impermanent fluorescence of the auramine stain, and training for technologists new to using fluorescent microscopy.

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Figure 1. Study Selection

2489 potentially relevant citations identified from electronic databases (PubMed 989, Embase 893, Biosis 607)

387 duplicate citations excluded

2102 titles and abstracts screened

2068 citations excluded based on relevance

34 full-text articles reviewed

articles excluded:
6 - technical notes, letters
2 - reviews
24 - not LED or not TB

12 studies included in review

10 studies added from hand search, contact with experts and industry

database search performed Feb 8, 2009
Figure 2. Forest Plots of LED Studies using Culture as Reference Standard (n=13)

Published studies have year of publication in brackets; remaining studies are unpublished. Point estimates of sensitivity and specificity from each study are shown as solid squares (if direct smears were used) or open squares (if processed/concentrated smears were used). Size of the square is proportionate to the size of the study. Solid lines represent 95% confidence intervals.

- **FIND – Feasibility_A** used concentrated smears; **FIND – Feasibility_B** used direct smears
- **FIND – Evaluation_A** used 400x screening magnification; **FIND – Evaluation_B** used 200x screening magnification
- **FIND – Comparative_A** used iLED; **FIND – Comparative_B** used FluoLED; **FIND – Comparative_C** used Lumin
- **Shenai_A** used concentrated smears; **Shenai_B** used direct smears

**Sensitivity (95% CI)**

- Marais (2008) 0.86 (0.71 - 0.95)
- Trusov (2009) 0.76 (0.69 - 0.82)
- Cuevas 0.69 (0.66 - 0.71)
- FIND – Feasibility_A 0.73 (0.67 - 0.78)
- FIND – Feasibility_B 0.73 (0.67 - 0.78)
- FIND – Evaluation_A 0.96 (0.95 - 0.98)
- FIND – Evaluation_B 0.96 (0.94 - 0.97)
- FIND – Comparative_A 0.90 (0.85 - 0.94)
- FIND – Comparative_B 0.88 (0.83 - 0.92)
- FIND – Comparative_C 0.85 (0.79 - 0.89)
- Omar 0.67 (0.56 - 0.76)
- Shenai_A 0.74 (0.70 - 0.77)
- Shenai_B 0.76 (0.73 - 0.79)

Chi-square = 456.42; df = 12 (p = 0.0000)  
Inconsistency (I-square) = 97.4 %

**Specificity (95% CI)**

- Marais (2008) 0.99 (0.96 - 1.00)
- Trusov (2009) 1.00 (0.99 - 1.00)
- Cuevas 0.95 (0.94 - 0.96)
- FIND – Feasibility_A 0.98 (0.96 - 0.99)
- FIND – Feasibility_B 0.96 (0.94 - 0.98)
- FIND – Evaluation_A 1.00 (0.99 - 1.00)
- FIND – Evaluation_B 0.96 (0.94 - 0.98)
- FIND – Comparative_A 0.99 (0.97 - 1.00)
- FIND – Comparative_B 0.97 (0.95 - 0.99)
- FIND – Comparative_C 0.99 (0.97 - 1.00)
- Omar 0.98 (0.97 - 0.99)
- Shenai_A 0.89 (0.85 - 0.93)
- Shenai_B 0.94 (0.91 - 0.97)

Chi-square = 134.47; df = 12 (p = 0.0000)  
Inconsistency (I-square) = 91.1 %
Published studies have year of publication in brackets; remaining studies are unpublished. Point estimates of sensitivity and specificity from each study are shown as solid squares (if direct smears were used) or open squares (if processed/concentrated smears were used). Size of the square is proportionate to the size of the study. Solid lines represent 95% confidence intervals.
Affolabi_A used FluoLED; Affolabi_B used Lumin
Figure 4. HSROC Plot for LED Studies Using Culture as Reference Standard (n=13)

Individual studies are shown as open squares whose size is proportionate to the size of the study. Summary point is shown as a closed circle, representing sensitivity and specificity estimates pooled using bivariate random effects model. HSROC curve is truncated outside of the area for which data exist.
Figure 5. HSROC Plot for LED Studies Using Microscopy as Reference Standard (n=6)

Individual studies are shown as open squares whose size is proportionate to the size of the study. Summary point is shown as a closed circle, representing sensitivity and specificity estimates pooled using bivariate random effects model. HSROC curve is truncated outside of the area for which data exist.
Figure 6a. Fading of Auramine O/KMnO₄ Slides: Proportion of Positive Slides Re-read as Positive (n=120 at each storage condition)*

*RT_month refers to storage at air conditioned room temperature (22°C), re-read monthly
INC_month refers to storage in a humidified incubator (30°C), re-read monthly
FRG_month refers to storage in a refrigerator (4°C), re-read monthly

Figure 6b. Fading of Auramine O/KMnO₄ Slides: Positivity Grading Over Time for Slides Stored at Room Temperature, 22°C (n=120)
Table 1. Comparison of commercial light-emitting diode products currently available for TB diagnostics

<table>
<thead>
<tr>
<th>Device</th>
<th>Manufacturer</th>
<th>Standalone microscope</th>
<th>Attachment</th>
<th>Light transmission</th>
<th>Battery powered</th>
<th>Weight (kg)</th>
<th>Cost (US$)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primo Star iLED™</td>
<td>Carl Zeiss, Oberkochen, Germany</td>
<td>Yes</td>
<td>NA</td>
<td>Epifluorescent</td>
<td>Yes</td>
<td>9.5</td>
<td>4825*</td>
<td>[104]</td>
</tr>
<tr>
<td>Lumin™</td>
<td>LUV Scientific, Lawrenceville, GA, USA</td>
<td>No</td>
<td>Objective lens replacement (20, 40, 60 and 100x oil)</td>
<td>Epifluorescent</td>
<td>Yes</td>
<td>0.448</td>
<td>700–2000*</td>
<td>[102]</td>
</tr>
<tr>
<td>ParaLens™</td>
<td>QBC™ Diagnostics, Philadelphia, PA, USA</td>
<td>No</td>
<td>Objective lens replacement (40, 60 and 100x oil)</td>
<td>Epifluorescent</td>
<td>Yes</td>
<td>1.27</td>
<td>995*</td>
<td>[103]</td>
</tr>
<tr>
<td>FluoLED™</td>
<td>Fraen Corporation Srl, Santarino, Milan, Italy</td>
<td>No</td>
<td>Adaptor attached to base and filter installed on head of microscope</td>
<td>Transfluorescent</td>
<td>Yes</td>
<td>5</td>
<td>1977–3530*</td>
<td>[104]</td>
</tr>
<tr>
<td>CyScope®</td>
<td>Partec, Gorlitz, Germany</td>
<td>Yes</td>
<td>NA</td>
<td>Epifluorescent</td>
<td>Yes</td>
<td>2.7</td>
<td>2372–3699*</td>
<td>[105]</td>
</tr>
</tbody>
</table>

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.

### Table 2. Study Characteristics (n=12)

<table>
<thead>
<tr>
<th>Ref</th>
<th>Author</th>
<th>Year</th>
<th>Total N (Ref+/Ref-)</th>
<th>Country</th>
<th>LED Device</th>
<th>Reference</th>
<th>Comparison</th>
<th>Smear</th>
<th>Screening Magnification</th>
</tr>
</thead>
<tbody>
<tr>
<td>(22)</td>
<td>Affolabi</td>
<td>unpublished</td>
<td>941/996</td>
<td>Benin</td>
<td>Lumin, FluoLED</td>
<td>rechecking</td>
<td>-</td>
<td>direct</td>
<td>200x</td>
</tr>
<tr>
<td>(23)</td>
<td>Cuevas</td>
<td>unpublished</td>
<td>1513/4999</td>
<td>Ethiopia, Nepal, Nigeria, Yemen*</td>
<td>Lumin</td>
<td>LJ</td>
<td>ZN</td>
<td>conc</td>
<td>200x</td>
</tr>
<tr>
<td>(24)</td>
<td>FIND – Feasibility</td>
<td>unpublished</td>
<td>263/282</td>
<td>Thailand, Germany, Peru, Gambia*</td>
<td>iLED</td>
<td>LJ</td>
<td>CFM</td>
<td>direct, conc</td>
<td>400x</td>
</tr>
<tr>
<td>(24)</td>
<td>FIND – Evaluation</td>
<td>unpublished</td>
<td>600/280</td>
<td>Thailand, Vietnam, India, Germany, Peru*</td>
<td>iLED</td>
<td>LJ</td>
<td>ZN, CFM</td>
<td>direct</td>
<td>200x, 400x</td>
</tr>
<tr>
<td>(24)</td>
<td>FIND – Demonstration†</td>
<td>unpublished</td>
<td>1317/8229</td>
<td>multiple‡*</td>
<td>iLED</td>
<td>rechecking</td>
<td>ZN</td>
<td>direct</td>
<td>400x</td>
</tr>
<tr>
<td>(24)</td>
<td>FIND – Comparative</td>
<td>unpublished</td>
<td>205/277</td>
<td>Zambia, Uganda*</td>
<td>iLED, FluoLED, Lumin</td>
<td>LJ</td>
<td>ZN, CFM</td>
<td>direct</td>
<td>400x</td>
</tr>
<tr>
<td>(25)</td>
<td>Kuhn</td>
<td>unpublished</td>
<td>20/5</td>
<td>USA/Bangladesh</td>
<td>ParaLens</td>
<td>CFM</td>
<td>-</td>
<td>conc</td>
<td>600x</td>
</tr>
<tr>
<td>(19)</td>
<td>Marais</td>
<td>2008</td>
<td>36/185</td>
<td>South Africa</td>
<td>Adapted CFM</td>
<td>MGIT, LJ</td>
<td>ZN, CFM</td>
<td>conc</td>
<td>400x</td>
</tr>
<tr>
<td>(26)</td>
<td>Omar</td>
<td>unpublished</td>
<td>93/616</td>
<td>South Africa</td>
<td>Lumin</td>
<td>MGIT</td>
<td>CFM</td>
<td>conc</td>
<td>400x</td>
</tr>
<tr>
<td>(27)</td>
<td>Shenai</td>
<td>unpublished</td>
<td>635/267</td>
<td>India</td>
<td>Lumin</td>
<td>MGIT, LJ</td>
<td>ZN</td>
<td>direct, conc</td>
<td>200x</td>
</tr>
<tr>
<td>(21)</td>
<td>Trusov</td>
<td>2009</td>
<td>199/508</td>
<td>Russia, Macedonia*</td>
<td>Lumin</td>
<td>LJ</td>
<td>ZN, CFM</td>
<td>conc</td>
<td>400x</td>
</tr>
<tr>
<td>(20)</td>
<td>Van Deun</td>
<td>2008</td>
<td>100/361</td>
<td>Tanzania/Thailand</td>
<td>FluoLED</td>
<td>CFM</td>
<td>-</td>
<td>direct</td>
<td>200x</td>
</tr>
</tbody>
</table>

†available data from all phases pooled (validation, implementation, continuation)
‡study sites: India, Vietnam, Cambodia, Thailand, Peru, Russia, Lesotho, Ethiopia, South Africa
*study sites pooled
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Frequency (n = 12 studies)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specimen Recruitment</strong></td>
<td></td>
</tr>
<tr>
<td>- Prospective</td>
<td>11</td>
</tr>
<tr>
<td>- Unclear</td>
<td>1</td>
</tr>
<tr>
<td><strong>Study Design</strong></td>
<td></td>
</tr>
<tr>
<td>- Cross-Sectional</td>
<td>5</td>
</tr>
<tr>
<td>- Case-Control</td>
<td>7</td>
</tr>
<tr>
<td><strong>Sampling</strong></td>
<td></td>
</tr>
<tr>
<td>- Consecutive or Random</td>
<td>8</td>
</tr>
<tr>
<td>- Unclear</td>
<td>4</td>
</tr>
<tr>
<td><strong>Verification</strong></td>
<td></td>
</tr>
<tr>
<td>- Complete</td>
<td>9</td>
</tr>
<tr>
<td>- Partial</td>
<td>3</td>
</tr>
<tr>
<td><strong>Blinded Interpretation</strong></td>
<td></td>
</tr>
<tr>
<td>- Yes</td>
<td>12</td>
</tr>
</tbody>
</table>
Table 4. Pooled Estimates of LED Accuracy (against a reference standard) Using Bivariate Random Effects Models

<table>
<thead>
<tr>
<th>Test (# arms)</th>
<th>Pooled Sensitivity (95% CI)</th>
<th>$I^2$ (p-value)</th>
<th>Pooled Specificity (95% CI)</th>
<th>$I^2$ (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Culture Reference</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct Smears only (n=7)</td>
<td>88.9%* (81.1, 93.7)</td>
<td>96.5%</td>
<td>98.3% (96.2, 99.3)</td>
<td>82.6%</td>
</tr>
<tr>
<td>Concentrated Smears only (n=6)</td>
<td>72.7%* (69.2, 76.0)</td>
<td>69.3%</td>
<td>97.9% (94.8, 99.2)</td>
<td>93.9%</td>
</tr>
<tr>
<td>400x/600x Magnification (n=9)</td>
<td>84.1% (76.0, 89.8)</td>
<td>95.3%</td>
<td>99.0%* (98.0, 99.5)</td>
<td>67.4%</td>
</tr>
<tr>
<td><strong>Microscopy Reference</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct Smears only (n=4)</td>
<td>93.6%* (88.8, 96.4)</td>
<td>97.5%</td>
<td>98.5% (98.1, 98.9)</td>
<td>42.4%</td>
</tr>
<tr>
<td>Concentrated Smears only (n=2)†</td>
<td>78.0%* (69.0, 85.0)</td>
<td>91.2%</td>
<td>99.0% (98.0, 99.0)</td>
<td>0%</td>
</tr>
<tr>
<td>400x/600x Magnification (n=3)†</td>
<td>95.0%* (95.0, 96.0)</td>
<td>96.6%</td>
<td>98.0% (98.0, 99.0)</td>
<td>0.0%</td>
</tr>
<tr>
<td>200x Magnification (n=3)†</td>
<td>90.0%* (89.0, 91.0)</td>
<td>95.3%</td>
<td>99.0% (98.0, 99.0)</td>
<td>16.4%</td>
</tr>
</tbody>
</table>

*non-overlapping confidence intervals by subgroup
†too few studies to perform bivariate random effects pooling; fixed effects pooling performed
Table 5. Head to Head Comparisons of LED with ZN (n=8)

<table>
<thead>
<tr>
<th>Ref</th>
<th>Author</th>
<th>Smear Reference</th>
<th>Sample Size</th>
<th>LED</th>
<th>ZN</th>
<th>LED – ZN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>Sensitivity</td>
</tr>
<tr>
<td>(23)</td>
<td>Cuevas</td>
<td>conc culture</td>
<td>6512</td>
<td>0.69</td>
<td>0.95</td>
<td>0.60</td>
</tr>
<tr>
<td>(24)</td>
<td>FIND – Evaluation†</td>
<td>direct culture</td>
<td>880</td>
<td>0.96</td>
<td>1.00</td>
<td>0.91</td>
</tr>
<tr>
<td>(24)</td>
<td>FIND – Demonstration‡</td>
<td>direct microscopy</td>
<td>9546</td>
<td>0.93</td>
<td>0.99</td>
<td>0.78</td>
</tr>
<tr>
<td>(24)</td>
<td>FIND – Comparison§</td>
<td>direct culture</td>
<td>482</td>
<td>0.90</td>
<td>0.99</td>
<td>0.79</td>
</tr>
<tr>
<td>(19)</td>
<td>Marais</td>
<td>conc culture</td>
<td>221</td>
<td>0.85</td>
<td>0.99</td>
<td>0.61</td>
</tr>
<tr>
<td>(27)</td>
<td>Shenai</td>
<td>conc culture</td>
<td>903</td>
<td>0.74</td>
<td>0.94</td>
<td>0.85</td>
</tr>
<tr>
<td>(21)</td>
<td>Trusov</td>
<td>conc culture</td>
<td>707</td>
<td>0.76</td>
<td>1.00</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>Pooled Differences Using Random Effects Models</td>
<td>84.7%</td>
<td>98.8%</td>
<td>77.2%</td>
<td>98.8%</td>
<td>+6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(73.6, 90.4)</td>
<td>(94.7, 99.7)</td>
<td>(67.6, 84.6)</td>
<td>(97.0, 99.5)</td>
<td>(+0.1, +13)</td>
</tr>
</tbody>
</table>

I² (p-value) 97.7% 94.1% 98.7% 89.2% 90.8% 96.0%
(p<0.0001) (p<0.0001) (p<0.0001) (p<0.0001) (p<0.001) (p<0.001)

†iLED used for LED data
‡subset of sites with head-to-head ZN comparison, not pooled with culture reference studies
§400x magnification used for LED data
Table 6. Head to Head Comparisons of LED with CFM (n=7)

<table>
<thead>
<tr>
<th>Ref</th>
<th>Author</th>
<th>Smear Reference</th>
<th>Sample Size</th>
<th>LED</th>
<th>CFM</th>
<th>LED – CFM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>Sensitivity</td>
</tr>
<tr>
<td>(24)</td>
<td>FIND - Feasibility</td>
<td>conc culture</td>
<td>545</td>
<td>0.73</td>
<td>0.98</td>
<td>0.68</td>
</tr>
<tr>
<td>(24)</td>
<td>FIND – Feasibility</td>
<td>direct culture</td>
<td>545</td>
<td>0.73</td>
<td>0.96</td>
<td>0.68</td>
</tr>
<tr>
<td>(24)</td>
<td>FIND – Evaluation†</td>
<td>direct culture</td>
<td>880</td>
<td>0.96</td>
<td>1.00</td>
<td>0.96</td>
</tr>
<tr>
<td>(24)</td>
<td>FIND – Comparison‡</td>
<td>direct culture</td>
<td>482</td>
<td>0.90</td>
<td>0.99</td>
<td>0.84</td>
</tr>
<tr>
<td>(19)</td>
<td>Marais</td>
<td>conc culture</td>
<td>221</td>
<td>0.85</td>
<td>0.99</td>
<td>0.72</td>
</tr>
<tr>
<td>(21)</td>
<td>Trusov</td>
<td>conc culture</td>
<td>707</td>
<td>0.67</td>
<td>0.98</td>
<td>0.71</td>
</tr>
</tbody>
</table>

Pooled Estimates Using Random Effects Models

<table>
<thead>
<tr>
<th></th>
<th>LED</th>
<th>CFM</th>
<th>LED – CFM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>Sensitivity</td>
</tr>
<tr>
<td></td>
<td>83.2%</td>
<td>99.2%</td>
<td>77.5%</td>
</tr>
<tr>
<td></td>
<td>(72.3, 90.3)</td>
<td>(97.9, 99.7)</td>
<td>(64.2, 86.9)</td>
</tr>
</tbody>
</table>

I² (p-value)

<table>
<thead>
<tr>
<th></th>
<th>LED</th>
<th>CFM</th>
<th>LED – CFM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>Sensitivity</td>
</tr>
<tr>
<td></td>
<td>96.4%</td>
<td>71.7%</td>
<td>97.3%</td>
</tr>
<tr>
<td></td>
<td>(p&lt;0.0001)</td>
<td>(p=0.002)</td>
<td>(p&lt;0.0001)</td>
</tr>
</tbody>
</table>

†LED used for LED data
‡400x magnification used for LED data
Table 7. Time to Read Slides (n=14)

<table>
<thead>
<tr>
<th>Ref</th>
<th>Author</th>
<th>% Smear</th>
<th>Smear Magnification</th>
<th>Screening Magnification</th>
<th>LED</th>
<th>Time/Slide (LED)</th>
<th>Comparison</th>
<th>Time/Slide (Comparison)</th>
<th>Relative Time (LED/Comparison)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(24)</td>
<td>FIND – Feasibility</td>
<td>100%</td>
<td>direct</td>
<td>400x</td>
<td>iLED</td>
<td>0.6 min</td>
<td>ZN</td>
<td>1.1 min</td>
<td>0.55</td>
</tr>
<tr>
<td>(24)</td>
<td>FIND – Feasibility</td>
<td>0%</td>
<td>direct</td>
<td>400x</td>
<td>iLED</td>
<td>1.4 min</td>
<td>ZN</td>
<td>3.2 min</td>
<td>0.44</td>
</tr>
<tr>
<td>(24)</td>
<td>FIND – Evaluation</td>
<td>100%</td>
<td>direct</td>
<td>400x</td>
<td>iLED</td>
<td>0.6 min</td>
<td>ZN</td>
<td>1.1 min</td>
<td>0.55</td>
</tr>
<tr>
<td>(24)</td>
<td>FIND – Evaluation</td>
<td>0%</td>
<td>direct</td>
<td>400x</td>
<td>iLED</td>
<td>1.4 min</td>
<td>CFM</td>
<td>3.3 min</td>
<td>0.42</td>
</tr>
<tr>
<td>(24)</td>
<td>FIND – Evaluation</td>
<td>100%</td>
<td>direct</td>
<td>200x</td>
<td>iLED</td>
<td>0.5 min</td>
<td>ZN</td>
<td>1.1 min</td>
<td>0.45</td>
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<tr>
<td>(24)</td>
<td>FIND – Evaluation</td>
<td>0%</td>
<td>direct</td>
<td>200x</td>
<td>iLED</td>
<td>1.3 min</td>
<td>CFM</td>
<td>3.3 min</td>
<td>0.39</td>
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<tr>
<td>(24)</td>
<td>FIND – Demonstration†</td>
<td>50%</td>
<td>direct</td>
<td>400x</td>
<td>iLED</td>
<td>1.62 min</td>
<td>ZN</td>
<td>2.02 min</td>
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<td>50%</td>
<td>direct</td>
<td>400x</td>
<td>iLED</td>
<td>1.18 min</td>
<td>ZN</td>
<td>2.13 min</td>
<td>0.55</td>
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<tr>
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<td>direct</td>
<td>400x</td>
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<td>ZN</td>
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<td>0.61</td>
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<tr>
<td>(24)</td>
<td>FIND – Comparison</td>
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<td>direct</td>
<td>400x</td>
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<td>2.59 min</td>
<td>ZN</td>
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<td>0.62</td>
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<tr>
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<td>direct</td>
<td>400x</td>
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<td>ZN</td>
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<td>0%</td>
<td>conc</td>
<td>400x</td>
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<td>ZN</td>
<td>3.6 min</td>
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<td>(27)</td>
<td>Shenai</td>
<td>58%</td>
<td>direct</td>
<td>200x</td>
<td>Lumin</td>
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<td>ZN</td>
<td>2.5 min</td>
<td>0.56</td>
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<td>(27)</td>
<td>Shenai</td>
<td>71%</td>
<td>conc</td>
<td>200x</td>
<td>Lumin</td>
<td>0.6 min</td>
<td>ZN</td>
<td>1.1 min</td>
<td>0.55</td>
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</tbody>
</table>

*mean of LED and CFM  
†after 1 month experience with LED  
‡after 3 months experience with LED
REFERENCES


APPENDIX 1: PROTOCOL FOR A DIAGNOSTIC META-ANALYSIS

A "road map" for systematic reviews of diagnostic test evaluations

1. Define a focused diagnostic review question (Patient/Disease, Index test, Reference standard, and Outcomes)

2. Review guidelines on diagnostic reviews, and guidelines on primary diagnostic studies and prepare a protocol

3. Identify appropriate databases and sources of diagnostic studies

4. Run searches on all relevant databases and sources

5. Save all citations (titles/abstracts) in a reference manager. Document search strategies that were employed. These citations are ready for first screen (N1)

6. Reviewer 1 screens all titles/abstracts and makes selections for second screen

7. Reviewer 2 screens all titles/abstracts and makes selections for second screen

8. Software suggestions: EndNote, Reference Manager, ProCite

9. Excluded after second screen

10. Keep a log of excluded studies with reasons for exclusion

11. Paper data extraction forms (after pilot test)

12. Get full tests of all articles identified for second screen (N)

13. Articles considered eligible after full-text review by 2 reviewers is the final set of studies for inclusion (N2)

14. Studies included in the final analysis (Np) Each article gets a unique ID number

15. Reviewer 1 extracts data (including quality assessment) from the final selected articles

16. Reviewer 2 extracts data (including quality assessment) from the final selected articles

17. Software suggestions: Access, Excel

18. Collect outcomes as TP, FP, FN and TN; or raw ROC data

19. Contact authors for missing data (e-mail may be more effective than letters)

20. Software suggestions: QUOROM, MOOSE as general guides for report writing (acknowledging that they are not meant for diagnostic reviews)

21. Import data and analyze using software

22. Tabulate study characteristics

23. Forest and ROC plots of SE and SP

24. Look for correlation between TPR and FPR

25. Search for threshold effect

26. Perform SIROC analyses

27. Pool measures like LR and DOR only if appropriate

28. Search for heterogeneity, and reasons for heterogeneity

29. Consider subgroup and sensitivity analyses

30. Interpret, discuss results, and write the report

31. Discuss applicability of results, and limitations of the review

32. Make recommendations for practice or policy, and research

APPENDIX 2: SEARCH STRING USED IN PUBMED


Search #3: #1 AND #2

Restricted to papers published from Jan 2000 to Feb 2009
### APPENDIX 3: DATA EXTRACTION FORM USED IN THE META-ANALYSIS

<table>
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<tr>
<th>Study #: __________</th>
<th>Author: __________</th>
<th>Year: __________</th>
<th>Reviewer: __________</th>
<th>Language: __________</th>
</tr>
</thead>
<tbody>
<tr>
<td>Published: Y / N</td>
<td>Country: __________</td>
<td></td>
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</table>

**LED model:**
- [ ] stand-alone
- [ ] attachment
  - used with: ________

**Bulb/Spectra:**

**Magnification used**

**Screening:**

**Confirmation:**
- [ ] not specified

**Smear:**
- [ ] direct
- [ ] concentrated
- [ ] other processing

**Stain/Counterstain:**

**Criteria for +:**

**Criteria for –:**

**Comparison 1:**
- [ ] different slide used
- [ ] same slide restained
- [ ] same slide

**Time b/w readings**

**Order read:** 1st 2nd 3rd
- [ ] not specified

**Dark Room:**
- [ ] Y / N / ?

**Comparison 2:**
- [ ] different slide used
- [ ] same slide restained
- [ ] same slide

**Time b/w readings**

**Order read:** 1st 2nd 3rd
- [ ] not specified

**Dark Room:**
- [ ] Y / N / ?

**Reference:**
- [ ] culture
- [ ] method: __________
- [ ] duration: __________

**Specimens:**
- [ ] unit of analysis
- [ ] pulmonary only
- [ ] extrapulmonary only
- [ ] mixed
- [ ] not specified

**Patients:**
- [ ] unit of analysis
- [ ] adults only
- [ ] peds only
- [ ] mixed
- [ ] not specified

**Time per slide**

**LED:**
- [ ] not given

**Comp1:**
- [ ] not given

**Comp2:**
- [ ] not given

**Turnaround**

**LED:**
- [ ] not given

**Comp1:**
- [ ] not given

**Comp2:**
- [ ] not given

**Cost Info**

**LED unit:**

**Labour:**

**Reagents:**

**Other:**

**User Identified Pros:**

**User Identified Cons:**

**Implementation Issues:**

**Training Described:**

**QC discussed:**
- [ ] Y / N / ?

**QC described / ref’d:**
- [ ] Y / N

**Prospective**

**Retrospective**

**not specified**

**HIV included**
- [ ] % __________

**HIV not included**
- [ ]

**not specified**

**Specimens:**
- [ ] unit of analysis
- [ ] case control selection
- [ ] consecutive selection
- [ ] random selection
- [ ] not specified

**Patients:**
- [ ] unit of analysis
- [ ] inpatients only
- [ ] outpatients only
- [ ] mixed
- [ ] not specified

**Time per slide**

**LED:**
- [ ] not given

**Comp1:**
- [ ] not given

**Comp2:**
- [ ] not given

**Turnaround**

**LED:**
- [ ] not given

**Comp1:**
- [ ] not given

**Comp2:**
- [ ] not given

**Cost Info**

**LED unit:**

**Labour:**

**Reagents:**

**Other:**

**User Identified Pros:**

**User Identified Cons:**

**Implementation Issues:**

**Training Described:**

**QC discussed:**
- [ ] Y / N / ?

**QC described / ref’d:**
- [ ] Y / N

**Prospective**

**Retrospective**

**not specified**

**HIV included**
- [ ] % __________

**HIV not included**
- [ ]

**not specified**

**Specimens:**
- [ ] unit of analysis
- [ ] case control selection
- [ ] consecutive selection
- [ ] random selection
- [ ] not specified

**Patients:**
- [ ] unit of analysis
- [ ] inpatients only
- [ ] outpatients only
- [ ] mixed
- [ ] not specified

**Time per slide**

**LED:**
- [ ] not given

**Comp1:**
- [ ] not given

**Comp2:**
- [ ] not given

**Turnaround**

**LED:**
- [ ] not given

**Comp1:**
- [ ] not given

**Comp2:**
- [ ] not given

**Cost Info**

**LED unit:**

**Labour:**

**Reagents:**

**Other:**

**User Identified Pros:**

**User Identified Cons:**

**Implementation Issues:**

**Training Described:**

**QC discussed:**
- [ ] Y / N / ?

**QC described / ref’d:**
- [ ] Y / N

**Prospective**

**Retrospective**

**not specified**

**HIV included**
- [ ] % __________

**HIV not included**
- [ ]

**not specified**
### QUADAS CHECKLIST

1. Was the spectrum of patients representative of the patients who will receive the test in practice?  
   - YES  
   - NO  
   - UNCLEAR

2. Were selection criteria clearly described?  
   - YES  
   - NO  
   - UNCLEAR

3. Is the reference standard likely to correctly classify the target condition?  
   - YES  
   - NO  
   - UNCLEAR

4. Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?  
   - YES  
   - NO  
   - UNCLEAR

5. Did the whole sample or a random selection of the sample receive verification using a reference standard of diagnosis?  
   - YES  
   - NO  
   - UNCLEAR

6. Did patients receive the same reference standard regardless of the index test result?  
   - YES  
   - NO  
   - UNCLEAR

7. Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?  
   - YES  
   - NO  
   - UNCLEAR

8. Was the execution of the index test described in sufficient detail to permit replication of the test?  
   - YES  
   - NO  
   - UNCLEAR

9. Was the execution of the reference standard described in sufficient detail to permit its replication?  
   - YES  
   - NO  
   - UNCLEAR

10. Were the index test results interpreted without knowledge of the results of the reference standard?  
    - YES  
    - NO  
    - UNCLEAR

11. Were the reference standard results interpreted without knowledge of the results of the index test?  
    - YES  
    - NO  
    - UNCLEAR

12. Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?  
    - YES  
    - NO  
    - UNCLEAR

13. Were uninterpretable/intermediate test results reported?  
    - YES  
    - NO  
    - UNCLEAR

14. Were withdrawals from the study explained?  
    - YES  
    - NO  
    - UNCLEAR

### RESULTS:

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<th>Specimen</th>
<th>Patient</th>
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<th>FP</th>
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**RESULTS:**