Evaluation of the Fluo-RAL Module for Detection of Tuberculous and Nontuberculous Acid-Fast Bacilli by Fluorescence Microscopy


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The World Health Organization has advised that light-emitting diode (LED)-powered fluorescence microscopes replace mercury vapor lamp (MVL)-powered fluorescence microscopes for detection of acid-fast bacilli (AFB) stained with auramine (1). Interested by the advantages of new LED systems, we evaluated the performance of the Fluo-RAL module (RAL Diagnostics Company) in our everyday practice. The Fluo-RAL module is a LED source of light that can be fixed on a microscope and can replace the MVL as the source of light.

The study was conducted at the National Reference Center for Mycobacteria, Pitié-Salpêtrière hospital, between January and April 2010. The Fluo-RAL module was set on a Nikon microscope in replacement of the MVL.

Specimens were prepared according to the Kubica method (2). Smears were spread on a glass slide and fixed with heat (100°C for 15 min) and then with the Fluo-RAL fixator for 15 min. Auramine staining was done as follows: application of auramine for 15 minutes, rinsing with distilled water, application of an RAL bleaching agent for 30 seconds, application of thiazine red for 4 minutes, and then rinsing with water. Microscopic examination of smears was done at >200 magnification, double blinded, with two fluorescence microscopes, one having an MVL and the other the Fluo-RAL module. Specimens were categorized according to the number of suspected AFB forms, as follows: 1 to 9/field, 10 to 99/field, 100/field.

We compared 673 samples, including 562 of respiratory origin. Sixty-two (9%) samples were AFB+, among which 53 were culture positive (46 Mycobacterium tuberculosis and 7 nontuberculous mycobacterium [NTM] samples), 4 were culture negative (sputum was sampled under treatment), and 5 were contaminated. Six hundred eleven samples were AFB−, among which 24 were culture positive (12 M. tuberculosis and 12 nontuberculous mycobacteria samples). Compared to the results obtained using the MVL, there was no major difference observed with the LED: no AFB+ specimen was categorized as negative, and no AFB− specimen was categorized as positive. Among AFB+ specimens, there were some nonsignificant differences (Table 1): 10 specimens were categorized as having >100 AFB/field with the LED versus 9 for the MVL (P = 1), and 20 were categorized as having <1 AFB/field and >10 AFB/slide with the LED versus 17 for the MVL (P = 0.58).

These results confirm the existing literature evaluating other types of LEDs (3–6) and another study conducted with the Fluo-RAL module (7). The Fluo-RAL module, like other LED light sources, can replace an MVL without any negative impact on detection of AFB but with a longer expected life (10,000 versus 300 h) and no chemical hazard risk due to mercury.

Although comparing the rates of detection of tuberculous and nontuberculous bacilli was not the aim of the study, we were struck by the difference. There was a clear (although not significant) trend toward less detection of NTM by fluorescence microscopes regardless of the source of light (Table 2). Underperformance of fluorescence microscopy for detection of NTM has also been reported by Minion et al. (8). Clinicians should be aware of this limit, especially in low-income countries where the incidence of disease caused by NTM can be as high as that of tuberculosis (9).

The Fluo–RAL module appears to be a less dangerous and more durable source of light than MVL for microscopic detection of auramine-stained AFB.
ACKNOWLEDGMENT

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REFERENCES


<table>
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<th>No. of colonies</th>
<th>M. tuberculosis complex</th>
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<td>&lt;10</td>
<td>5/10 (50)</td>
<td>0/5 (0)</td>
<td>0.1</td>
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<td>14/17 (82)</td>
<td>3/8 (38)</td>
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<td>&gt;100</td>
<td>32/32 (100)</td>
<td>6/6 (100)</td>
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*The nontuberculous mycobacteria included 9 Mycobacterium intracellulare strains, 4 M. kansasii strains, 1 M. xenopi strain, 1 M. szulgai strain, 1 M. chelonae strain, 1 M. fortuitum strain, 1 M. mucogenicum strain, and 1 M. peregrinum strain.