Am. J. Trop. Med. Hyg., 83(4), 2010, pp. 906–908
doi:10.4269/ajtmh.2010.10-0153

Short Report: The CyScope® Fluorescence Microscope, a Reliable Tool for Tuberculosis Diagnosis in Resource-Limited Settings

Leopold G. Lehman,* Arlette L. Ngapmen Yamadji, Françoise Ngo Sack, and Charles F. Bilong Bilong

Faculty of Science, University of Douala, Douala, Cameroon; Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Douala, Cameroon; Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon

Abstract. Poor laboratory equipment and few human resources have made it difficult to implement microscopic diagnosis of pulmonary tuberculosis (TB) on a large scale basis worldwide. Three hundred sputum samples from patients in Cameroon were studied by using the CyScope®, a new light-emitting, diode-based, fluorescence microscope, to compare auramine-rhodamine fluorescence with the conventional Ziehl-Neelsen staining method. Five fluorescence protocols were tested to reduce manipulation time. Smear positivity for acid-fast bacilli with the Ziehl-Neelsen staining method was 27.7% (83 of 300) compared with 33.3% (100 of 300) with the fluorescent method. Staining time with the modified fluorescence protocol could be reduced from 21 minutes to 10 minutes. This study confirmed that the fluorescence staining method is more sensitive than the Ziehl-Neelsen staining method. It is suggested that the training of laboratory technicians on fluorescence microscopy should be scaled up for increased disease control.

In the context of human immunodeficiency virus/acquired immunodeficiency syndrome pandemics, tuberculosis (TB) is the most common opportunistic infection. More than 9 million cases in 2007 were found in Africa and Southeast Asia.[1] The absence of accurate diagnostic techniques constitutes a serious hindrance in the strategy of TB control in Africa.[2] The increase of false negative-smear pulmonary TB is highlighted as one of the main reasons for the upsurge of this disease during the past decade in poor countries.[3]

In an effort to improve methods to diagnose pulmonary tuberculosis, the World Health Organization has encouraged the development of simplified and accurate diagnostics, such as fluorescence microscopy for early detection of *Mycobacterium tuberculosis* in sputum.[4] The CyScope® (Partec, Görlitz, Germany) belongs to a new generation of fluorescence microscopes that use light-emitting diodes (LEDs) as a light source.[5] It can be plugged into an ordinary electrical outlet or operated with a built-in rechargeable battery. It is equipped with a high power Royal Blue LED (wavelength = 455 nm) for incidence fluorescence excitation and a white light LED for transmitted light. The LED fluorescence microscope provided similar results as standard mercury vapor lamp fluorescence microscope at a much lower cost.[6] In this study, we used the LED fluorescence microscope CyScope® for TB diagnosis and investigated different staining procedures to shorten the handling time for samples.

During August–November 2009, 300 sputum samples were collected from patients in Cameroon with suspected pulmonary TB or patients receiving treatment. The age range of the patients was 2–74 years. Two slides were prepared from the same specimen for direct sputum smears by using the unconcentrated specimens in the solid or most dense particles of the sputum.[7] The smears were dried in air and heat-fixed. Staining was classically processed as described by the World Health Organization and the International Union Against Tuberculosis and Lung Disease. A solution with 0.3% basic fuchsin (pararosaniline chloride with 88% P1528 dye; Sigma, St. Louis, MO) was heated to the steaming point, decolorized with 25% sulfuric acid, and counterstained with 0.3% methylene blue.[7,8]

The fluorescent method of Degommier[9] was applied. The staining reagent Tb-fluor (Merck, Darmstadt, Germany) was used according to the manufacturer's procedure. Briefly, smears were stained with auramine-rhodamine, rinsed with tap water, decolorized with HCl-isopropanol, and counterstained with $KMnO_4$. Five protocols with variable staining and counterstaining durations were used to establish overall minimal staining time with the fluorescent dye. (Table 1).

Acid-fast–stained smears were colored by a variety of protocols and observed on the CyScope®. This microscope uses white light and fluorescence. When the fluorescence function was turned on, the acid-fast bacilli were visualized at magnifications of 200× and 400×. Their number in the expectoration fluid was related to the sample contagiousness, and the result was expressed quantitatively.[1,7]

Solid Löwenstein-Jensen medium containing pyruvate was considered as the gold standard[4,10,11] for samples with discrepant microscopic results. Each sputum sample was liquefied and decontaminated with 3% sodium laurylsulfate and 1% NaOH and neutralized with H_2SO_4-bromocresol purple solution.[12] Two slants of Löwenstein-Jensen medium were simultaneously inoculated with each specimen and incubated at 37°C for 8 weeks. Identification of TB strains was based on growth rate and colony morphology.[13]

Data were analyzed by using the database/statistical package SPSS version 10.0 (SPSS Inc., Chicago, IL). The chi-square test and proportion test for a binomial distribution were used to compare differences between the fluorescent method and the Ziehl-Neelsen method. Statistical analysis was conducted with a 5% significance level.

A total of 300 sputum specimens were included in the study. Of these specimens, 17 (6%) of Ziehl-Neelsen–negative samples were positive under by the fluorescent method and confirmed by Löwenstein-Jensen culture (Table 2). Smear positivity for acid-fast bacilli with the fluorescent method (33.3%, 100 of 300) was higher than with the Ziehl-Neelsen method (27.7%, 83 of 300), but the difference between the two methods showed borderline significance ($P = 0.06$). The relationship of smear-positive results between the two methods showed that a scanty result was more common with the fluorescent method (Table 3). The modified protocols of Degommier made

*Address correspondence to Léopold G. Lehman, Faculty of Science, University of Douala, PO Box 2326, Douala, Cameroon. E-mail: lblehman@yahoo.fr

TABLE 1
Protocols for auramine-rhodamine fluorescence staining

Protocols	Steps (minutes)			Duration (minutes)
	Auramine-rhodamine	Hydrochloric acid and isopropanol	Potassium permanganate	
I	15	1	5	21
II	10	1	5	16
III	10	1	1	12
IV	8	1	1	10
V	4	1	1	6

it possible to obtain a qualitative assessment of the samples (Table 4). Our results confirmed that the fluorescence staining method is more sensitive than the Ziehl-Neelsen method, particularly for cases of paucibacillary TB.[11,14–17]

Although this study did not evaluate the influence of concurrent HIV infection on the diagnostic capabilities of LED fluorescence microscopy, we presume that the sensitivity in a person with a co-infection would be improved because smears are often reported as negative because of a low bacillary content.[11,16,18–20] Mycobacterial culture with Löwenstein-Jensen medium is not used for routine diagnosis of TB but it is still considered a gold standard even though this method is time-consuming.[10,18,21] Culture with Löwenstein-Jensen medium was only used to determine discrepancies. The fluorescence method is already known for its better sensitivity compared with the Ziehl-Neelsen method. In previous comparisons between the fluorescent method and the Ziehl-Neelsen method, specimens with positive smear results and negative culture results were considered false-positive results likely because of dead mycobacteria or fluorescent artifacts.[11] Thus, microscopy has an important role in the diagnosis of pulmonary TB.

The high sensitivity of fluorescence microscopy is caused by increased contrast between the stained bacilli and the background, which makes the bacilli more visible.[11,14] Acid-fast bacilli appear as bright yellow rods against a dark background under the CyScope® microscope. Slides can be examined at a lower magnification, enabling observation of a much larger area per unit of time. Fluorescence microscopy requires only two minutes to examine an area; 10 minutes are needed with bright-field microscopy.[10,11,13] Although using lower magnification to screen for bacilli may occasionally lead to artifacts being identified as positive, we did not encounter this problem. Staining of artifacts can be reduced by using either phenol or $KMNO_4$ as a counterstain. However, the use of low magnification may increase the proportion of artifacts in the fluorescence method, but this risk can be reduced by using higher magnification and immersion oil. It has been suggested that the addition of phenol satisfactorily reduces fluorescent artifacts.[22] The use of $KMNO_4$ as a counterstain has also been reported to greatly reduce staining artifacts.[23]

TABLE 2
Comparison of the Ziehl-Neelsen method with the fluorescent method for the detection of acid-fast bacilli

Outcome	Ziehl-Neelsen method, no. (%)	Fluorescent method, no (%)	*P*
Positive	83 (27.7)	100 (33.3)	0.06
Negative	217 (72.3)	200 (66.7)	
False-negative	17 (6)	0	< 0.001

TABLE 3
Comparison of type of positive results for Ziehl-Neelsen method and fluorescent method*

Result	Ziehl-Neelsen method, no. (%)	Fluorescent method, no. (%)	*P*
Scanty (1–19 AFB/length)	16 (19.3)	31 (31)	0.10
1+ (20–199 AFB/length)	38 (45.8)	36 (36)	0.23
2+ (5–50 AFB/field)	7 (8.4)	8 (8)	1
3+ (> 50 AFB/field)	22 (26.5)	25 (25)	0.95
Total	83 (100)	100 (100)	

*AFB = acid-fast bacilli.

High costs and difficulties in maintenance were the main factors against use of fluorescence microscopy in developing countries.[11,16,24] Nevertheless, new microscopes equipped with LEDs appeared more durable and less expensive. The LED source is known to last several thousand hours, making the fluorescent technique more accessible[5,6,24] The CyScope® also has useful features for laboratory and field work in developing countries because it has a rechargeable batteries that provide six hours of use without the need for electricity. Moreover, it does not require a darkroom because of the high LED power.

The modified protocols of Degommier enabled reduction of staining time to approximately 10 minutes. Successful reduction of the staining time by approximately 50% in our protocol can be useful to laboratory technicians when the workload is high. Although fluorescent acid-fast bacilli were observed, these protocols showed the most satisfactory results (brightness of fluorescent tubercle bacilli and image contrast). The shortest modified protocol of six minutes showed poor contrast because of weak fluorescence of the acid-fast bacilli.

This study confirmed that the auramine-rhodamine fluorescent staining method is more sensitive than the conventional Ziehl-Neelsen method. It also showed that the staining time can be reduced approximately 50% without losing quality staining. Moreover, the compact CyScope® fluorescenct microscope is a reliable tool for TB diagnosis and does not require a darkroom. The longevity of auramine-rhodamine–stained slides is short but the images can be obtained by using a universal serial bus cable. Because the CyScope® has a rechargeable battery, it is useful for field work in remote areas. Our results and the recent development of other LED fluorescence microscopes imply the necessity to rapidly enhance the training of laboratory technicians on these novel tools for better disease control.

Received March 9, 2010. Accepted for publication July 19, 2010.

Acknowledgments: We thank Alyom Gaspard and Keugni Liliane (Hôpital Protestant de Bonabéri, Douala, Cameroon) for support and Dr. J. Noeske and Professor Wolfgang Göhde for critical comments on the manuscript.

TABLE 4
Qualitative data of modified protocols for the fluorescent method

Modified protocol	Background	Fluorescent bacilli	Contrast
I	Black	Bright yellow	Good
II	Black	Bright yellow	Good
III	Black	Bright green-yellow	Good
IV	Black	Bright green-yellow	Good
V	Black-brown	Pale yellow	Poor

Financial support: This study was supported by the International Society for Health Research and Training, Douala, Cameroon.

Authors' addresses: Leopold G. Lehman, Faculty of Science, University of Douala, Douala, Cameroon, E-mail: lblehman@yahoo.fr. Arlette L. Ngapmen Yamadji and Charles F. Bilong Bilong, Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon, E-mails: arlette2007linda@yahoo.fr and bilong_bilong@yahoo.com. Françoise Ngo Sack, Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Douala, Cameroon, E-mail: nsackmakak@yahoo.fr.

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