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QBC ParaLens™ LED fluorescent microscope attachment with QBC *F.A.S.T.*™ AFB staining system*

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ABSTRACT

This study was designed to evaluate the QBC ParaLens™ LED fluorescent microscope attachment and the QBC *F.A.S.T.*™ AFB staining system for the detection of Acid Fast Bacilli in pathological samples in Abidjan, Cote d'Ivoire. A total of 50 patients were tested using direct smear specimens with both Ziehl-Neelsen (ZN) light microscopy and LED fluorescence microscopy with QBC *F.A.S.T.* AFB stain. The samples were also cultured and tested using an immunochromatograpic test for detection of antigen MPT 64 and the results were compared to direct examination. ZN light microscopy detected 20 positive cases and LED fluorescent microscopy with QBC *F.A.S.T.* AFB stain detected 21. The sensitivity and specificity of ZN light microscopy was determined to be 84.2% and 87.1% respectively. The sensitivity and specificity of LED fluorescent microscopy with QBC *F.A.S.T.* AFB stain was determined to be 94.7% and 90.3% respectively. Compared to ZN light microscopy, LED fluorescent microscopy with QBC *F.A.S.T.* AFB stain increased the sensitivity of direct examination without concentration by 10.5%.

Keywords: Tuberculosis; AFB; Ziehl-Neelsen; LED Fluorescence; QBC ParaLens; QBC *F.A.S.T*.

*Conflicts of Interest: B. J. Hnatkovich is employed by QBC Diagnostics, Inc. and assisted in the preparation of this manuscript for publication. Other authors in this study have no conflict of interest or financial relationship with the manufacturer or distributor of the QBC ParaLens, QBC *F.A.S.T.* AFB stain or QBC Diagnostics, Inc.

1. INTRODUCTION

Tuberculosis remains a global scourge throughout the developing world because of its endemoepidemic character and person to person transmission. The TB control strategy is focused on the identification of cases that transmit infection and on implementation of therapeutic regimes. In poorer countries, the diagnostic and therapeutic decisions on patients with suspected TB are often based on direct examination of Ziehl-Neelsen stained slides using light microscopy, a technique whose sensitivity is variable [1]. Detection of AFB in stained smears may provide the initial evidence of the presence of mycobacterium in suspected TB patients, however, smear examination provides only a presumptive diagnosis of TB as the AFB may be acid-fast organisms other than *M. tuberculosis*.

A systematic review conducted by Steingart *et al.* has shown that the sensitivity of direct examination for the detection of Acid-Fast Bacilli (AFB) from pathological samples is better with fluorescent microscopy that with conventional light microscopy [2]. The routine use of fluorescent microscopy for the diagnosis of TB has been limited for various reasons including the short lifetime of the lamp and the overall cost of implementation [3]. Technological advances have allowed for the development of light-emitting diode (LED) fluorescent microscopes. LED fluorescence microscopy is more economic than conventional fluorescent microscopes that utilize halogen or mercury bulbs [2,3].

The QBC ParaLens LED fluorescent microscope attachment transforms a light microscope into a fluorescent microscope. The QBC ParaLens is easier to use than more conventional fluorescent microscopes [3,4] and does not require change to existing infrastructure.

Previous work has shown that there is a good correlation between the results obtained with the QBC ParaLens and those results obtained with classical fluorescent microscopy [5]. In some cases, the performance of LED FM is better than conventional fluorescent microscopy methods [5,6].

This study was designed to evaluate the QBC Para Lens LED fluorescent microscopy attachment and the QBC *F.A.S.T.* AFB staining system for the detection of AFB in pathological specimens of suspected TB patients in Abidjan, Côte d'Ivoire in 2010.

2. METHODS

2.1. Sample Collection

Patient samples were collected at the CHU Services of Cocody, Centre Antituberculeux de Treichville and Adjamé. Samples were collected from 50 patients with symptoms suggestive of tuberculosis. The 50 samples included 31 spontaneous sputum samples, 16 gastric aspirates sample, 1 bronchial aspiration sample, and 2 pleural liquid samples **Table 1**. Samples were then transported at 4˚C to the Tuberculosis Laboratory at the Institute Pasteur of Cote d'Ivoire. The samples were examined for non-conformance per internal quality control procedures and accepted for use in the study.

2.2. Direct Examination

Direct examination without concentration was performed on each of the samples. Two smears were prepared according to internal protocols. The first smear was made on a common use microscope slide. The second smear was made on a QBC *F.A.S.T.* SureFocus slide. Both smears were air-dried and fixed using flame. The first smear was stained according to accepted procedures with a 0.5% Carbol Fuschsin solution prepared in the laboratory. The second smear was stained using the QBC *F.A.S.T.* AFB Stain kit according to the manufacturer's instructions for use.

Direct examination was conducted using an Olympus CX21 microscope. To facilitate fluorescent examination, the QBC ParaLens fluorescent microscope attachment with 60× objective was mounted to the nosepiece of the same microscope.

Ziehl-Neelsen (ZN) stained smears were reviewed using a 100× objective under oil. QBC *F.A.S.T.* AFB fluorescent stained smears were reviewed using the QBC ParaLens with 60× objective under oil. Two technicians conducted blinded microscope examination according to accepted procedures for both the ZN and QBC *F.A.S.T.* AFB examination techniques. Discordant results were reviewed by a third, more experienced technician. Direct examination results were quantified using the WHO scale [7].

2.3. Culture

Each of the samples was also cultured according to accepted procedures. Samples were decontaminated using a 2% N-Acetyl-L-Cysteine (NALC) solution and incubating for 15 minutes. After incubation, the samples were centrifuged at 3000 × g for 20 minutes. The supernatant was removed and the pellet re-suspended in a phosphate suspension buffer (pH 7). The solution was homogenized by aspiration. Each sample was used to inoculate three cultures of Lowenstein-Jensen (LJ) media. The inoculants were incubated at 37˚C for eight weeks. After incubation, all positive LJ cultures were tested using an Immunochromatographic (SD BioLine) test for detection of antigen MPT 64. Data was collected and analyzed using Epi-Info 6.04 (CDC, Atlanta, GA, USA).

3. RESULTS

Direct examination of ZN stained specimens resulted in 40%; 95% C.I. [0.26 - 0.54]% (20 of 50 samples) positive incidence rate. When LED fluorescent microscopy was used to examine the specimens stained with the QBC *F.A.S.T.* AFB stain, a 42%; 95% C.I. [0.28 - 0.56]% (21 of 50 samples) positive incident rate was seen (**Table 2**).

Of the samples examined, positive results were observed using ZN stain in 16 spontaneous sputum samples, 3 gastric aspirates and 1 liquid bronchial aspiration. LED fluorescent microscopy with QBC *F.A.S.T.* AFB stain resulted in positive samples being observed in 17 spontaneous sputum samples, 3 gastric aspirates and 1 liquid bronchial aspiration. The MPT 64 antigen detection method identified 18 positive cases and 1 strongly suggestive suspected case.

Compared to the MPT 64 antigen, the sensitivity, specificity, positive and negative predictive value of ZN were determined to be 84.2%; C.I. 95% [0.74 - 0.91]%, 87.1%; C.I. 95% [0.78 - 0.96]%, 80% and 90% respectively (**Table 3**).

Compared to the MPT 64 antigen, the sensitivity, specificity, positive and negative predictive value of LED fluorescent microscopy with QBC *F.A.S.T.* AFB stain were determined to be 94.7%; C.I. 95% [0.88 - 1.00]%, 90.3%; C.I. 95% [0.82 - 0.99]%, 85.7% and 90.6% respectively (**Table 4**).

ZN resulted in 5 discordant results; 3 false negatives and 2 false positives compared to LED fluorescent microscopy with QBC *F.A.S.T.* AFB stain. Of the 3 false negative findings, 2 were found to be positive for MPT 64 and 1 was found to be negative. The results of the smear examination of the 2 false positives were recorded as "scanty" and in both cases the sample was negative for MPT 64.

LED fluorescent microscopy with QBC *F.A.S.T.* AFB

Table 1. Distribution of samples according to origin.

Origin	Sputum	Gastric Aspiration	Pleural Fluid	Bronchial Aspiration	Total
CHU Cocody	20	16	1	2	39
CAT Adjamé	3	0	0	0	3
CAT Treichville	8	0	0	0	8
Total	31	16	1	2	50

Table 2. Distribution of the results according to the method used.

Method	Negative	Scanty	1+	2+	3+	Total
ZN	30	4	2	5	9	50
LED	29	4	4	8	5	50

*WHO/IUATLD Scale.

Table 3. Performance of ZN light microscopy compared to antigen MPT 64 test on cultured samples.

Direct Examination	MPT 64 (+)	MPT 64 (−)	Total
ZN (+)	16	4	20
ZN (−)	3	27	30
Total	19	31	50

Table 4. Performance of LED Fluorescent Microscopy with QBC *F.A.S.T.* AFB Stain compared to antigen MPT 64 Test on cultured samples.

Direct Examination	MPT 64 (+)	MPT 64 (−)	Total
LED (+)	18	3	21
LED (−)	1	28	29
Total	19	31	50

stain also resulted in 5 discordant results compared to ZN; 2 false negative and 3 false positives. Of the 2 false negative findings, both were determined to be negative for MPT 64. Of the results of the smear examination of the 3 false positives, 2 were recorded as "scanty" and 1 was recorded as "1+". When cultured and tested, 1 "scanty" sample and 1 "1+" sample were positive for MPT 64

The sensitivity of direct examination, without concentration, of AFB increased from 84.2% with ZN to 94.7% with LED fluorescent microscopy with QBC *F.A.S.T.* AFB stain.

4. DISCUSSION

Despite its low sensitivity compared to culture, the diagnostic decisions and treatment of tuberculosis in low-income countries are based on the direct examination of ZN stained pathological specimens. The current strategy in the fight against TB is centered on the detection of infected individuals and requires detection methods with a higher sensitivity and specificity compared to conventional ZN light microscopy to identify a larger number of positive patients that present with symptoms of TB. With a sensitivity of 94.7%, LED fluorescent microscopy with the QBC ParaLens and QBC *F.A.S.T.* AFB stain is a credible alternative to ZN light microscopy for the detection of TB.

Previous work comparing conventional ZN light microscopy to LED fluorescent microscopy concluded that the sensitivity of the LED method was superior to ZN light microscopy [2,3,6]. In the study presented here, 50 patients from TB centers were used to compare ZN light microscopy to LED fluorescent microscopy. As a diagnostic tool, LED fluorescent microscopy with QBC *F.A.S.T.* AFB stain identified 2 false negative samples compared to ZN light microscopy. When cultured and tested, these samples were determined to be positive MPT 64 antigen.

While showing superior sensitivity to ZN light microscopy, the specificity of the two methods were relatively close; ZN light microscopy resulted in a specificity of 87.1% and LED fluorescent microscopy with QBC *F.A.S.T.* AFB stain resulted in a specificity of 90.3%. **Table 5**.

Compared to previous studies that have compared LED fluorescent microscopy to ZN light microscopy, a sample size of 50 specimens is high [3,6]. The sample size, to a certain extent, contributed to the performance of LED fluorescent microscopy presented herein. Marsh, *et al.*, with 221 sputum samples, found LED fluorescent microscopy to have a sensitivity of 84.7% and a specificity of 98.9% for the routine detection of AFB [6]. It can also be mentioned that 8% of the samples with results classified as "scanty" by direct examination are more likely to be false positives [8] both with ZN light microscopy and LED fluorescent microscopy. In some studies, cultures from samples identified as "scanty" by

Table 5. Comparison of the direct examination methods.

Performance	ZN	LED FM with QBC *F.A.S.T.* AFB Stain	Difference
Sensitivity (95% C.I.)	84.2% (0.74 - 0.94)	94.7% (0.88 - 1.01)	+10.5%
Specificity (95% C.I.)	87.1% (0.78 - 0.96)	90.3% (0.82 - 0.99)	+3.2%
Positive Predictive Value (PPV)	80.0%	85.7%	+5.7%
Negative Predictive Value (NPV)	90.0%	90.6%	+0.6%

direct examination were found to be negative 95% of the time [9].

In conclusion, we believe that LED fluorescent microscopy with QBC *F.A.S.T.* AFB stain to be a credible alternative to ZN light microscopy for routine TB screening in countries endemic for pulmonary tuberculosis.

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