

Critical Appraisal Of Fluorescent And Ordinary Light Microscopies In Mycobacterium Tuberculosis Diagnosis At The National Hospital Abuja, Nigeria

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Citation

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Abstract

Despite the discovery of tubercle bacillus more than a hundred years ago, and all the advances made in our knowledge of the disease since then, tuberculosis still remains one of the major health problems facing mankind, particularly in developing countries. From a global perspective, many laboratories use the same methods today that were in use nearly half a century ago: conventional stains such as Ziehl-Neelsen or Kinyoun for staining sputum smears, given rise to low case detection rate. This necessitated this study-- to compare the iLED fluorescence with the Zeihl- Neelsen Techniques. We randomly collected Seven hundred and sixty eight (768) sputum samples from three hundred and eighty four TB suspect patients aged 15-65years, their smears were prepared, fixed and stained according to laid down procedures; results showed that two hundred and seven were positive for acid fast bacilli (AFB) with a percentage positivity rate of 26.95% , from smears stained with Auramine-O and examined with the iLED Fluorescence microscope ; while seventy five were positive with percentage positivity rate of 9.77 % with smears stained with the Ziehl-Neelsen staining technique. The yield achieved with fluorescence microscopy exceeded the yield achieved with light microscopy "26.95% compared with 9.77%", but the difference was not statistically significant ($t=1.76$; $P>0.05$). The case detection rate was 17.18%. This clearly shows the superiority of fluorescence microscopy over the conventional light microscopy for the diagnosis of tuberculosis.

BACKGROUND OF STUDY

Currently, sputum smear microscopy by Ziehl-Neelsen (ZN) staining method is the simplest and most rapid test available to detect acid fast bacilli (AFB) in clinical specimens.

Although it is a robust technique and quite inexpensive with a specificity of almost 100%, it suffers from a low sensitivity ranging from 22 to 78% and requires at least 5×10^3 bacilli per ml of sputum

Conventional fluorescence microscopy is more sensitive than Ziehl-Neelsen and takes less time, but its use has been limited by the high cost of mercury vapour light sources, the need for regular maintenance and the requirement for a dark room. Light-emitting diodes (LED) have been developed to offer the benefits of fluorescence microscopy without the associated costs. In 2009, the evidence for the efficacy of LED microscopy was assessed by the World Health Organization (WHO), on the basis of standards appropriate for evaluating both the accuracy and the effect of new TB diagnostics on patients and public health. The results showed that the accuracy of LED microscopy was equivalent to that of international reference standards, it was more sensitive

than conventional Ziehl-Neelsen microscopy and it had qualitative, operational and cost advantages over both conventional fluorescence and Ziehl- Neelsen microscopy. On the basis of these findings, WHO recommends that conventional fluorescence microscopy be replaced by LED microscopy, and that LED microscopy be phased in as an alternative for conventional Ziehl-Neelsen light microscopy. The switch to LED microscopy should be carefully phased in at country level, with LED technology that meets WHO specifications. Countries using LED microscopy should train laboratory staff, validate the technique, introduce appropriate quality assurance and monitor the effect on TB case detection rates and treatment outcomes . 2

METHODS

SOURCES AND COLLECTION OF SPECIMENS

Randomized routine sputum samples from TB suspect patients from Microbiology laboratory, National Hospital, Abuja were used. Beginning from May, 2011, duplicate smears were made for each sputum specimen received at the

laboratory; one smear was stained with the Aura mine-O and the other by the hot Ziehl-Neelsen staining technique. Each slide was examined independently.

SAMPLE SIZE

Within the period under study, two sputum samples were collected from 384 TB suspect patients given rise to 768 smears. Sample was determined using the formula $n = \frac{Z^2 \cdot a \cdot 2P(1-P)}{d^2}$ where P= Anticipated population proportion (50%), d= relative precision (10 percentage points), and P(1-P)%=confidence level (95%), . 3

SMEAR PREPARATION

Smears were selected from fine, pale -white moist (cheesy), yellowish purulent or blood tinged particles of the sputum specimen and spread over a 2x1 cm area in a clean, grease-free slide with a wooden applicator stick. These were allowed to air-dry and heat-fixed with Bunsen burner flame. For each sample, the smears were made in duplicate. Positive and negative control smears were also prepared.

Staining Procedure (ZN)

The smears were arranged in serial order on staining bridge, with smear side up and were flooded with filtered 0.1% Carbol Fuchsin. The smears were steamed and allowed to stain for 10 minutes after which they were rinsed with water and drained. Decolorization was followed with 3% acid alcohol for 3 minutes. The smears were rinsed with water, drained and counter stained with 0.1% Methylene blue solution for 1 minute, they were rinsed with water, drained and were allowed to air-dry. The smears were examined microscopically using the oil immersion (100x) objective.

Staining Procedure (FM)

The smears were arranged in serial order on staining bridge, with smear side up and were flooded with filtered 0.1% Aura mine-O and were allowed to stain for 20 minutes after which they were rinsed with water and drained. Decolorization was followed with 0.5% acid alcohol for 3 minutes. The smears were rinsed with water, drained and counter stained with 0.5% Potassium permanganate solution for 1 minute, they were rinsed with water, drained and were allowed to air-dry. The smears were examined microscopically using the dry (40x) objective.

Table A

Examination of smears

IULTD/WHO SCALE	Bright Field (1000x)	Fluorescence (400x) magnification
(100x field=HPF)	Magnification: 1Length	: 1Length=40fields=
Result	=2cm=100HPF	200HPF
Negative	Zero AFB/1 Length	Zero AFB/1 Length
Scanty (actual count)	1-9 AFB/100fields	1-19 AFB/1 Length
1+	10-99 AFB/100fields	20-199 AFB/1 Length
2+	1-10 AFB/Field	5-50 AFB/ 1 field on average
3+	≥10 AFB/Field on average	>50 AFB/1 field on average

(Adopted from IHVN Training Manual, 2010) ⁴

LIMITATION OF STUDY

The samples were not cultured for Mycobacteria due to lack of facilities to control parameters.

RESULTS

Of the seven hundred and sixty eight (768) sputum samples collected from three hundred and eighty four TB suspect patients, two hundred and seven were positive for acid fast bacilli (AFB) with a percentage positivity rate of 26.95 % (table B), from smears stained with Aura mine-O and examined with the iLED Fluorescence microscope while seventy five were positive with percentage positivity rate of 9.77% with smears stained with the Ziehl-Neelsen staining technique.

The yield achieved with fluorescence microscopy exceeded the yield achieved with light microscopy 26.95% compared with 9.77%, but the difference was not statistically significant (Table C). This gave rise to 17.18% improved case detection rate.

Figures 1 and 2 reveal a sharp contrast between two smears made from the same sputum sample stained with Aura mine-O and examined with the iLED fluorescence microscope (fig.2) and the Ziehl-Neelsen (hot method) and examined with the bright field mode of the same microscope. This reveals in more classical terms the superiority of the Aura mine-O iLED fluorescence AFB smear microscopy and the conventional Ziehl-Neelsen AFB smear microscopy.

Table B

The study reveals an increase in case detection rates for the period under study

MONTH	SMEARS	+(FM)	+(ZN)	TOTAL (%)
May	148	18	15	18(12.16)
June	098	17	13	17(17.35)
July	088	11	04	11(12.50)
August	176	93	18	93(52.84)
September	126	37	12	37(29.37)
October	132	31	13	31(23.48)
TOTAL	768	207	075	207(26.95)

The Monthly Percentage Positivity Rates

Table C

The yield achieved with fluorescence microscopy exceeded the yield achieved with light microscopy 26.95% compared with 9.77%, but the difference was not statistically significant

MONTH	TOTAL SMEARS	% POSITIVE (FM)	% POSITIVE (ZN)
May	148	12.16(18/148)	10.14(15/148)
June	98	17.35(17/98)	13.27(13/98)
July	88	12.50(11/88)	4.55(04/88)
August	176	52.84(93/176)	10.23(18/176)
September	126	29.37(37/126)	9.52(12/126)
October	132	23.48(31/132)	9.59(13/132)
TOTAL	768	26.95(207/768)	9.77(075/768)

t=1.76 df=10 p>0.05

The Student 't' Value

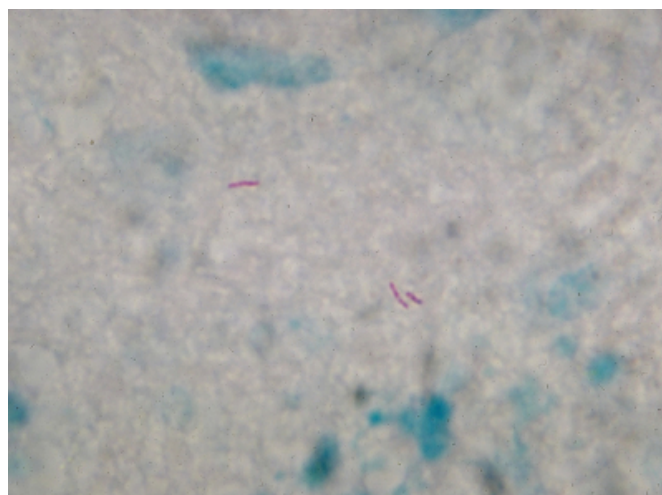
Figure 1

Figures 1 and 2 reveal a sharp contrast between two smears made from the same sputum sample stained with Aura mine-O and examined with the iLED fluorescence microscope (fig.2) and the Ziehl-Neelsen (hot method) and examined with the bright field mode of the same microscope



Figure 2

Figures 1 and 2 reveal a sharp contrast between two smears made from the same sputum sample stained with Aura mine-O and examined with the iLED fluorescence microscope (fig.2) and the Ziehl-Neelsen (hot method) and examined with the bright field mode of the same microscope



DISCUSSION

No statistically significant differences were noted, but our findings support previous studies that demonstrated the superior diagnostic performance of fluorescence microscopy, compared with conventional light microscopy 5 6 7 . From public health point of view a single active case missed is a public health threat. The Stop TB Partnership targets of

halving TB prevalence and death rates by 2015, compared with 1990 levels, thus paving the way for the elimination of TB (defined as less than one case of TB disease per one million population per year) by 2050² will be a mere dream without enhanced case detection rate. The study reveals an increase in case detection rates for the period under study (Table B). Early diagnosis of tuberculosis (TB) and initiating optimal treatment would not only enable cure of an individual patient but will also curb the transmission of infection and disease to others in the community. Of the several distinct components of TB control programme, case finding remains the corner stone for effective control of the disease.⁸ The diagnostic technology recommended in current control strategies is sputum microscopy, which was developed in the 1880s and has remained essentially unchanged since then. Smear microscopy is an attractive technology for public-health programmes, as it requires simple equipment only, can be used for more than one purpose, and provides visual evidence not only of tuberculosis, but of bacterial burden, which in most instances is specific enough that no confirmatory testing is needed (Mark et al, 2006).⁹ In many countries it is based only on the examination results of Ziehl-Neelsen (ZN) stained smears. However, only tiny amounts of material are examined - as little as 0.2 micro Liter (μ l), and hence bacteria must be present in high concentrations to be visible; typically over 10,000 acid fast bacilli per ml. Since the first description of the Auramine O fluorescence microscopy technique by Hageman (Hageman et al, 1937), numerous reports have confirmed the superior diagnostic performance of fluorescence microscopy, compared with Ziehl-Neelsen (ZN) staining and light microscopy^{5 6 10}. In a systematic review of 18 studies, Steingart et al.⁷ reported that fluorescence microscopy of Auramine-stained smears provides similar specificity and increased sensitivity (mean improvement of 10%), compared with light microscopy of ZN-stained smears. In addition to increased sensitivity, fluorescence microscopy also allows more-rapid screening of sputum smear specimens. From an operational perspective, this is highly advantageous, particularly when high numbers of samples are screened per day, because the majority of laboratory time is spent confirming negative smear results.

According to the International Union Against Tuberculosis and Lung Disease technical guidelines for sputum microscopy, at least 5 minutes of screening time is required to correctly identify a negative smear result when

conventional light microscopy is used.¹¹ However, under routine field conditions, the time spent per slide is often far less than the minimum required. An operational study from Cameroon demonstrated a median sputum microscopy examination time of only 2 min.¹² Almost 50% of the cases detected after a thorough 10-min evaluation were missed during routine investigation¹² which demonstrates the negative impact that conventional light microscopy may have on early case detection and diagnostic delay.

Furthermore, the diagnosis of tuberculosis in children is especially difficult as they cannot easily produce sputum and clinical features are nonspecific and chest radiographs are often difficult to interpret. Though the demonstration of mycobacteria in various clinical specimens by culture remains a gold standard, this is not always possible in children due to paucity bacillary nature of illness. The same is true in extra pulmonary manifestations and quite often also in patients suffering from HIV and tuberculosis. To meet these special situations, new techniques are very much needed¹ and among them is the use of iLED fluorescence microscope which has been shown to be promising alternative in developing countries.

TB is closely connected with HIV. People living with HIV, representing over 10% of annual TB cases, are up to 37 times more likely to develop TB than people who are HIV-negative. This situation is worsened by the HIV pandemic, since the risk of death in HIV-infected patients with TB is twice that of HIV-infected patients without TB¹³

Of great concern for the control of the disease is the emergence of drug resistance (DR) since there is no cure for some multidrug-resistant (MDR) strains of *M. tuberculosis*, and there is concern that they may spread rapidly around the world¹⁴. Recognizing the scale of the problem, global targets for reductions in the TB burden have been set within the context of the Millennium Development Goals (MDGs) and by the Stop TB Partnership, a global movement made up of more than 1200 organizations working together to eliminate TB as a public health problem and, ultimately, to secure a world free of TB. The target set within the MDGs is to halt and reverse the incidence of TB by 2015. MDG indicators form measuring progress in TB control are the incidence rate, the prevalence rate, the death rate and the proportion of cases that are detected and cured in DOTS programmes.

CONCLUSION

Although no statistically significant differences were noted between the conventional light microscopy and the fluorescent microscopic techniques in Mycobacterium tuberculosis diagnosis, but our findings support previous studies that demonstrated the superior diagnostic performance of fluorescence microscopy, compared with conventional light microscopy in simple practical terms.

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