

EVALUATION OF DTECH AND HEXAGON TB KITS USED FOR DIAGNOSIS OF TUBERCULOSIS IN NIGERIA

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ABSTRACT

A Total of 345 Sputum Samples were collected from patients who presented with complaints reminiscent of tuberculosis. Fast staining with Ziehl Neelsen (ZN) revealed that a total of 297 (86.09%) of the sample were positive for acid fast bacilli (AFB). Blood samples from one hundred of the smear – positive patients were used for the Dtech and Hexagon TB (Human GmbH-D-65205, Wiesbaden, Germany) diagnosis to test for the presence of tuberculosis antibodies in the blood samples. Blood samples were also collected from ten healthy donors as control. Dtech showed sensitivity for 81 of the 100 smear-positive patients while Hexagon TB showed sensitivity for 76 of the total 100 test samples. None of the two test kits detected tuberculosis (TB) antibodies in the blood samples from the ten healthy donors neither did the ZN AFB staining technique. At acceptance region of 95% ($P < 0.05$), 76 of the 100 smear positive patients as well as 81 of the same population showed no significant difference statistically from each other but were both statistically different from the 100 smear positive patients ($P < 0.05$) which served as the control.

Key words: Dtech, Hexagon TB, Tuberculosis, Ziehl Neelsen, and Acid fast bacilli.

INTRODUCTION

Historically, the first immunodiagnostic test was the tuberculin skin test (Runyon, 1959; Lunn, 1983). Although some of the problems of tuberculin skin test with respect to diagnosis are sited as the inability to distinguish active tuberculosis from passed sensitization by tubercle bacilli, BCG or

environmental *Mycobacteria* (Ten Dam and Hitzen, 1980; ACCP/ATS, 1995) an appropriate criterion for defining a positive skin test reaction is regarded as ones dependent on the likelihood of TB infection and the risk of TB if infection has occurred (ATS-CDC, 1990) or as a dependent on the population being tested (ATS-CDC, 1994; Bass, 1993). For persons with HIV infection, close contacts of an infectious patient with tuberculosis (ACCP/ATS, 1995) and those with fibrotic lesions on chest radiograph, a reaction of $\geq 5\text{mm}$ is considered positive. For others at risk, including infants and children younger than 4 years of age, a reaction of $\geq 10\text{mm}$ is positive. Persons who are not likely to be infected with *M. tuberculosis* should generally not be skin tested because the predicted value of a positive skin test in low risk populations is poor. If a skin test is performed on a person who is not in a high risk category or who is not exposed to a high risk environment, a cut off point of $\geq 15\text{mm}$ is positive (ATS-CDC, 1994). Although tuberculin testing is the standard method for screening asymptomatic populations for TB and tuberculosis infection, chest radiography or sputum smear examinations and culture (Cook, 1992; Esteban *et al*, 1998) are the initial screening method of choice when the objective is to rapidly identify persons with clinically active disease (ATS – CDC, 1992). At present however, radiometric or colorimetric detection techniques provide the earliest identification of growth. Serodiagnosis of TB as a colorimetric diagnostic method has recently had attention focused on it (Reggiardo and Vazquez, 1981; Daniel and Debanne 1987; Caplin *et al*, 1989; Grange, 1989). These methods (serodiagnosis) are aimed at competing with sputum microscopy and radiometrics with respect to sensitivity, specificity, ease of organization/diagnosis and quality control, acceptability to patients, technical simplicity and cost (Grange, 1989).

Of recent, due to the fact that antibodies to this antigen (*Mycobacterium tuberculosis*; causative agent of tuberculosis) appears in the blood stream before appearing in the sputum, from where smears are made on slides for acid-fast staining, serological tests are the other of the day. These serological tests that could involve either radiometric or colorimetric detection techniques provide the earliest identification of growth (ATS-CDC, 1994). It is with respect to the speed and ease of diagnosis using these methods, which with respect to our environment embraces the colorimetric technique that this work is hinged on.

It is with respect to this that we decided to investigate into the various TB diagnostic test kits available in recent times in Nigeria. These test kits employ serological techniques and are meant to be spot tests. These techniques are therefore attractive and there seems to be a rush for them. The threat of the neglect of order methods of TB diagnosis and subsequent misdiagnosis call for the verification of the sensitivity and specificity of these new techniques.

MATERIALS AND METHODS

SAMPLE COLLECTION

A total of 345 patients from the metropolis of Abuja suspected to be suffering from Tuberculosis and are attending the Medical Diagnostic Unit of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja investigated. Early morning sputum samples were collected from the patients as well as that of the 10 control patients (who also are from the metropolis of Abuja) and all the samples immediately subjected to staining using the Ziehl-Neelsen Acid – fast staining technique.

ZIEHL NEELSEN ACID FAST STAINING

Smears were made from the sputum samples on new clean and grease free microscopic slides with a sterile wire loop. A loopful of the sputum was spread on each of the slide over an area of about 1

x 2cm. The loopful was evenly spread over an area not exceeding the above size. The smears were allowed to air dry and then heat fixed.

Ziehl Neelsen acid-fast staining was performed on all the slides. The Ziehl Neelsen stain consisted of a mixture of basic fuchsin and phenol crystal as the primary stain while the counter stain was 0.1% methylene blue. Acid alcohol was used as the decolourizing agent (Kent and Kubica, 1985). All the stained slides were examined under the immersion oil at a magnification of x 100.

SEROLOGICAL PROCEDURE:

Two types of kits were purchased from Nigerian market. These were Diagno Tech rapid TB and Hexagon TB (Human GmbH-0-6520. Wiesbaden, Germany) test kits. Thirteen packets each of these kits were used for the sampling. Blood samples (5.0mls) were collected from 100 sputum smear positive patients and 10 healthy individuals in plain non-heparanized blood sample containers. The blood was immediately centrifuged at a speed of 10,000g for 5 minutes so as to separate the blood into two distinct layers of serum (the top layer) and precipitated red blood cells. The serum was pipetted out while the precipitates discarded. The serum were then diluted 1:10 with normal saline. Turbid samples were filtered using 0.45 – micron filter papers. The tests were performed immediately after specimen collection according to manufacturer's instructions, using each of the kit for eight samples.

RESULTS

The presence of *Mycobacterium tuberculosis* was detected in 297 sputum samples. This represents 86.09% of the patients that were investigated. The smears of the sputum samples of the ten healthy patients were negative for the presence of *M. tuberculosis*. Dtech diagnostic kit showed a positive test in 81 of the 100 smear – positive patients, while Hexagon TB gave a positive test in 76 or 76% patients. The two diagnostic test kit showed negative results for all the ten healthy patients that were tested. Statistically, at $\alpha = 5\%$, $Z_{\alpha/2} = Z_{0.025}$, 81 and 76 were statistically indifferent at the sample number of 100, while 81 and 76 were significantly different from total respectively at the above mentioned statistical boundary. The results are as tabulated below (Table 1).

Table 1: Detection of *M. tuberculosis*. tuberculosis antibodies using two diagnostic test kits.

Patient Type	Sample (N)	Dtech Positive	Hexagon TB Positive
Healthy Individuals	10	0	0
100 Smear Positive Patients	100	81	76

DISCUSSION

The diagnosis of TB had previously depended solely on the laboratory confirmation for the presence of the acid fast bacilli (AFB) of the tubercle, *M Tuberculosis*, in the sputum via the Ziehl - Neelsen acid- fast staining method. This method though rapid, involves the collection of early morning sputa on at least three consecutive days and involves essentially, the making and fixing of

sputum as smears on slides followed by the procedures of staining with various dyes after an acid – alcohol decolorization step.

However, this method in comparison to modern techniques of TB diagnosis is not only obsolete and time consuming, but can only diagnose TB bacilli long after antibody formation and circulation in the blood stream, thereby exposing susceptible victims to the bacilli before diagnosis and subsequent treatment of patients can even commence. Amongst various methods of TB diagnosis are the radiometric and colorimetric methods, which involves diagnosis using chest radiographic films and colour developments via serology techniques respectively. The later, however is of an advantage because of its portability, accessibility to rural areas, its rapid and easy nature of obtaining results as well as the fact that supposedly, it detects antibodies which always appears earlier than the tubercle bacilli would do in sputum (Grange, 1989).

Dtech and Hexagon TB are two serological test kits commonly found in Nigerian market and by virtue of their antibody detecting capability of diagnosing TB, should tilt the diagnosis of TB totally on their side via the visible advantages of serological testing to the conventional Ziehl – Neelsen method commonly used in Nigeria.

However, this is not the case. While out of a 100 smear positive TB patients, Diagno Tech and Hexagon TB showed that they had a sensitivity of 81% and 76% respectively even though they were negative for the ten healthy individuals that were tested, 81 and 76 were statistically insignificant at $\alpha = 0.05\%$ but significantly different statistically from 100 respectively. In other words, their main aims of being embraced at the Nigerian markets have been defeated, viz: the earlier and more accurate diagnosis of TB to the Ziehl – Neelsen method. This implies that the serological methods can not be used in total independence of the ZN method as a diagnostic test since ZN would still be needed as a confirmatory test for any diagnosis done using them and so makes their use invalid and inconsequential.

The authors are of the opinion that in the face of the impediments facing the available serological test kits, the ZN remains the more easily accessible TB diagnostic technique ahead of the chest radiograph and its consequential exposure to heavy doses of radiation and attendant health consequences, in Nigeria as of today.

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