PHAGE AMPLIFICATION TECHNOLOGY IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS: APPLICABILITY IN NIGERIA LABORATORIES.

Olive-Igbezu, Ejio J.

Tuberculosis Unit, Nigerian Institute of Medical Research, 6, Edmund Crescent, P.M.B. 1013, Yaba Lagos, Nigeria.

Though of global importance, the developing world bears the highest burden of tuberculosis (TB) worldwide and Nigeria has been rated amongst 225 countries where TB prevalence is highest worldwide. In Nigeria, diagnosis of TB is by direct smear microscopy using the Ziel-Neelsen method. Studies have shown that the sensitivity of smear microscopy varies between 20 - 30% depending on whether direct or fluorescent slides are used. This is likely this to half or more of TB cases to be missed. In Nigeria are not automated, automated culture and molecular methods exist but the requirements for especially dedicated, very expensive instrumentation and reagents prohibit their use in developing countries including Nigeria. The World Health Organisation recognizes the need for new, affordable, rapid and highly sensitive diagnostic for use in developing countries. Phage amplification technology employs a specific mycobacteriophage which infects a live TB bacteria to replicate in a sample. These replicate and lyse the cells to release progeny phage. The presence of progeny phage is detected visually as plaques on a lawn of a rapid-growing, non-pathogenic Mycobacterium. Phage Amplification Technology has been evaluated and found to detect most cases missed by smear microscopy and to give results with good correlation with culture (which is highly sensitive requiring 8 to 10 weeks incubation to give results), within 24 hours of sample preparation. It is thus faster than culture and cheaper than the new rapid automated methods, as it requires no especially dedicated instrumentation.

INTRODUCTION

Since the emergence of HIV/AIDS in the early 1980s, there has been a dramatic resurgence of tuberculosis (TB) worldwide (1,2). HIV/AIDS and tuberculosis are in a synergistic lethal alliance with one telescoping the onset of the other (3). Besides HIV/AIDS, the resurgence of TB several years after its apparent decline and its high toll on the developing world can be traced to several factors. These include economic crisis, food shortage, homelessness resulting from wars and natural disasters and overcrowding in refugee camps (4).

Though a global problem, the greatest burden of TB, like every other disease related to the standard of living of the people, lies with the world’s poor nations. Though 95% of TB sufferers (5) Between 1980 and 1991, 20-67% levels of increase in the incidence rate of TB were reported for developing countries compared to 3.5% levels of increase for developed countries (6,7). Africa, South of the Sahara faces a double jeopardy. In addition to the 8.3 million of the 36.1 million people affected globally with HIV, a potent facilitator of the reactivation of dormant TB infection to overt TB in TB countries in Africa, more than 10% of adults aged 15-49 are HIV infected. Nigeria bears 10% of the African burden of HIV/AIDS and has recorded a consistent increase in HIV prevalence from 1.8% in 1993 to 3.8 in 1994, 4.5% in 1996 and 5.4% in 199. Thus of her 120 million population, 2.5 million adults are HIV-infected (8,9) HIV/AIDS does not only facilitates the reactivation of dormant TB infection to overt TB but also interferes with correct diagnosis of TB (especially with serological methods and smear microscopy) as it alters immune response to TB (10).

In Nigeria, there are a quarter of a million registered cases of TB with 25,000 new cases registered annually. In the year 2000 alone, 26,641 cases were reported (12). In an epidemiology study of TB in Lagos, Nigeria, a significant increase from an incidence rate of 21% in 1982 to 42% was reported (13). This was a hospital-based study. Also, a study by Wokoma in 1990 showed a 7.74% increase in the incidence rate of TB in Port Harcourt between 1993 and 1994 (14). It is important to note that disease surveillance is too incomplete to provide exact numbers of new cases and deaths occurring from TB in a year. Also, owing to the stigma attached to TB in the Nigerian society, several cases are unreported. Of the cases reported up to a half or more are never diagnosed and are therefore not treated. This is a dangerous trend, which, amongst other things, would increase morbidity from TB, increase the spread of TB within the community, increase self-medication, especially the use of anti-TB drugs in sub-inhibitory doses since these drugs are available over the shelf (15) and thus increase the incidence of multi-drug resistant TB. In Nigeria, TB control was incorporated into the Primary Health Care programme in 1991 and she has reported to the World health organisation (WHO) under the Directly Observed Treatment, Short Course (DOTS) programme since 1995. Available data shows that as at 1997 cohort study, treatment success was 73% amongst the cases evaluated. Case detection rate was however in the range 9-15%. Meanwhile, Nigeria has been rated among the 22 countries with the highest burden of TB worldwide (16). With increase budgetary allocation to the National TB control programme and Donor support
there is an urgent need to beef up case finding to meet the WHO target of 70% case detention.

CONVENTIONAL DIAGNOSIS OF TUBERCULOSIS IN NIGERIA

Owing to the infectious nature of the disease, there is need for prompt initiation of rational treatment. This demands a diagnosis (17). The need for the diagnosis of TB before treatment is even stronger because Mycobacteria, like several other bacteria, have a remarkable ability to develop resistance to antimicrobial agents even on a single exposure. For a country like Nigeria, a first requirement for rational diagnosis is that the method be cheap and easy to adapt to local laboratories under already available infrastructure while maintaining a high specificity and sensitivity. In addition, any diagnostic method that would be applicable in Nigeria must be one whose performance is not altered by the presence of HIV/AIDS because of its increasing prevalence in TB patients in Nigeria (9,13). There exist today, a multiplicity of test kits and methodologies for the diagnosis of TB. Each has its unique features and advantages. It is however not possible to say categorically that a single method meets all the demands for full diagnosis. There is often the need for a combination of two or more methods depending on the information sought, the availability of test materials and trained personnel as well as the ability of the patient to pay.

TB diagnosis in most developing countries is hinged on acid-fast microscopy (a) because it is relatively cheap and rapid. Acid-fast microscopy is a major component of the WHO's TB control strategy the Directly Observed Treatment, Short Course (DOTS) (18,19). In Nigeria, TB diagnosis under the DOTS programme of the NTLCP is by the Ziehl-Neelsen (ZN) technique (20). Typical, smear microscopy is useful in identifying TB patients that expectorate acid-fast bacilli and therefore pose a public health hazard. It is also employed in identifying acid-fast bacilli from culture. Beside its use in diagnosis, it is also employed in the management of TB as it provides the opportunity to monitor smear-conversion during treatment (23).

Microscopy has several advantages. It is fast and highly specific. It is relatively cheap and easily overcomes the problem of power outages, a common occurrence in Nigeria, as slides can be examined with a light microscope in a well-illuminated laboratory. The WHO has recommended that 3 sputum samples (with at least two submitted on 2 consecutive days) should be examined and that in order to get reliable results, the whole slide must be viewed and examination done for about 15 minutes (21). There are peculiarities on ground that reduce the value of smear microscopy as a diagnosis tool in Nigeria. Generally, there is a shortage of peripheral laboratories located close to TB patients, many of who are poor, rural dwellers. Patients often have to travel several kilometres to submit samples. The possibility of complying with the WHO recommendation for the examination of three sputum samples is very bleak since it goes with the added cost of transportation and inconvenience. With the exception of the newly identified National TB Reference Laboratory in Lagos, several peripheral laboratories suffer form a shortage of adequately trained and experienced personnel. Since the identification of AFBs on a slide requires expertise, there is the risk of human error. In other words, what party A identifies as acid fast bacilli (AFBs) may be identified by party B as artefacts especially when direct smears are examined (22) as is invariably the case in peripheral laboratories in Nigeria (20).

Though considered cheap, the availability of functional microscopes and fresh slides could be a problem in many laboratories. Where slides are wasted and re-cycled as is often the case in Nigeria, there is an increase risk of misdiagnosis since acid-fast materials may be retained in scratches on recycled slides (20,23). Added to this is the fact that when the oil-immersion objective is used to examine slides, the bacilli sometimes float into the oil and are carried to subsequent slides thereby increasing the risk of false-positive results (24). Practically, adherence to the WHO recommendation that each slide be examined for 15 minutes is labour intensive in a busy laboratory considering the fact that three samples will be examined for each patient. Meanwhile, non-compliance with the procedure would reduce its sensitivity.

The major drawback of smear microscopy is the variability of its sensitivity. For direct smears, it could be as low as 30% while for concentrated smears, it varies between 30% and 70% requiring the presence of up to 1x10-1x10 cells/ml of sputum to be
able to detect AFBs. This implies that its maximal value as a diagnostic method can only be harnessed when the sample is processed (concentrated). This requires the availability of a centrifuge. Since most peripheral laboratories in Nigeria do examine direct sputum smears (20) can it be expected that more than of TB patients requiring treatment be not diagnosed and therefore, not treated. \(18\) The requirement for the presence of up to \(1 \times 10^5\) cells/ml of sputum to get a positive result means that smear negatively does not exclude TB. It has been reported that some of the missed cases will become infectious. In fact, active smear negative TB cases do occur and are infectious.\(23\)

In a country like Nigeria where the prevalence of HIV/AIDS in TB patients (HIV/AIDS/TB coinfection) is on the increase (9,13) the value of smear microscopy as a diagnostic tool is further reduced because often, there is reduced number of AFBs in sputum samples (10). Under the DOTS programme in Nigeria, symptomatic patients whose sputum samples are consistently negative are diagnosed by radiographic methods (2). Though rapid X-ray results could be misleading. They lack specificity and have a low positive predictive value. Suspected TB patients may have one or more of a multiplicity of respiratory tract infections capable of causing abnormalities similar to those seen in TB. This is precisely the case with HIV/AIDS patients. Also, correct interpretation of X-ray results depends very much on the experience of the radiographer (27).

Sputum culture improves the diagnosis of smear negative TB tremendously because of its high sensitivity in providing a definite diagnosis of Mycobacterium Tuberculosis. (23) Solid and liquid culture require the use of processed (concentrated/decontaminated) sputum samples because of the high levels of sputum contamination by other bacteria. Thus a centrifuge is also required. Conveniently, the TB reference Laboratory in Lagos, Nigeria uses the egg-based Lowenstein Jensen (LJ) medium. It is relatively cheap to prepare and yields good results. It however requires 6-8 weeks (or more) incubation period (as MTB is a slow grower) before cultures are regarded as negative (3). This is therefore a very slow method. In a busy Laboratory, incubator space could be a problem. Since it involves the propagation of a highly infectious pathogen there is an increased risk of transmitting the bacillus.

Other Methods of TB Diagnosis

Besides the manual culture methods which include the use of LJ, Middlebrook 7H11, 7H10, 7H9 and in some countries, Ogawa medium, there are automated culture methods which can detect growth within one or two weeks but usually require up to 4 weeks incubation. These include the Bactec 460 TB automated Mycobacterial Detection and Susceptibility Testing System (28,29) MB Bact/TB system (Organon Teknika) and the Mycobacterial Growth Indicator Tube (MGIT\(^TM\)) (Becton Dickinson (30). These systems employ liquid media for the detection of MTB. It is therefore not possible to directly study the colony morphology of the organism. In the Bactec 460 system, growth and metabolism. The use of radioactive materials is restricted in many countries. This is a major disadvantage of the Bactec 460 system. Also the culture medium employed has to be purchased specifically from the manufacturer of the machine.

Since it involves the incubation of the specimen into a sealed container by syringe and needle, there is the added risk of needle stick injuries. Generally, these newer (automated) methods have the advantages of high sensitivity and specificity and are comparatively faster than the conventional manual culture methods. They however require specially dedicated and expensive instrumentation. The high cost of these machines, the reagents and servicing of the machines make them unsuitable for use in most Nigerian laboratories. The conventional manual methods will therefore enjoy patronage for a very long time (31).

Most peripheral laboratories in Nigeria do not culture sputum. This is a problem, which is bound to hamper effective TB control because so far there has not been a good liaison between peripheral laboratories and the reference laboratory.

Molecular biology has been adapted in several ways to improve the diagnosis of TB. Several tests employ nucleic acid probes and gene amplification. These include the Gen-probe Amplified Mycobacterium Tuberculosis Direct test (AMTD), Strand Displacement Assay (SDA), Polymerase Chain reaction (PCR), and Lipase Chain Reaction (LCR) amongst others. These offer a rapid approach to
diagnose because of their abilities to detect a few cells a specimen within a few hours as opposed to the 6-8 weeks required for culture (32). They are however, difficult to adapt for routine use in an average laboratory (33). Under currently available infrastructure in Nigerian laboratories, it is not feasible to use these methods because just like the automated culture methods, they require especially dedicated and very expensive instrumentation. Also, there is the need to perform every step of the procedure in a separate room to avoid cross contamination of the genetic material. Just like smear microscopy, molecular tests do not distinguish between live and dead bacilli. Therefore, treated TB can be confused with active TB. Besides, many laboratories in Nigeria lack skilled personnel capable of performing these tests.

Chromatography has emerged as a useful diagnostic tool for TB in recent times. It is based on finding that each Mycobacterium species synthesizes a unique set of mycolic acids and that a species-specific pattern can be produced. High performance liquid chromatography (HPLC) has been reported to produce good identification results within 4 hours after culture is available. This is a rapid test that can replace an entire battery of biochemical tests. Results can be as good as 100% specificity and sensitivity for MTB complex (34). However, besides the requirement for culture, there is the added requirement for special, very expensive instrumentation and expertise for sample preparation and assay. It therefore has very limited application in Nigerian Laboratories.

**Phage-based Test in the Diagnosis of Tuberculosis**

The use mycobacteriophage in the diagnosis of TB is fast gaining ground today. Research into the use of phages in diagnosis dates back to 1947, when Gardner and Weiser identified phages that are specific for the Mycobacteria (35). In 1960, Redmond and Mcdermott isolated phages that specifically infect MTB and M. bovis (35). The fact that phages are specific in their infection of hosts has been employed in developing two diagnostic methods today. These are the Luciferase Reporter phage (LRP) technology and phage amplification technology (5,37). In both methods, a specific phage that infects members of the MTB complex is used to target viable MTB in a specimen. The presence of viable cells is then determined in one of two ways. One is by the incorporation of the bacterial luciferase enzyme (Lux: AB) or the firefly enzyme (Flux) into the phage genome that will be expressed when the phage infects viable Mycobacteria. This is the case with the Luciferase Reporter phage technology where the presence of viable cells is detected by the emission of light (38). The other detects the presence of viable MTB when progeny phages are released from the infected bacilli (5). This is method is simpler than the Luciferase phage Reporter system because results are read visually as opposed the LRP in which a luminometer or photographic film is required to detect positive results.

**FASTPLAQUE TB (FPTB) ASSAY**

FASTplaque TB is a commercially available diagnostic kit that detects the presence of the TB bacilli in sputum as well as other samples from TB patients. For the diagnosis of pulmonary TB, the sputum sample is processed (decontaminated and concentrated) using the NaOH-NALC (N-acetyl-L-cysteine) method which has been reported to concentrated more viable cells in sputum than the modified Petroff's method commonly used in Nigeria (32). The retention of as many viable cells as possible in the specimen is important because the FASTplaque assay does not detect the presence of killed bacilli as phage replication can only occur in live cells. Processing is thus a very important step in the procedure. This is followed by the addition of the mycobacteriophage (Adphiophage) to the sample in a reagent bottle. The mixture is incubated at 370C for 1 hour to allow the phage sufficient time to infect viable MTB if present in the sample. A potent virucide (virucide) is then added. This destroys any phage that has not infected MTB cells but does not affect phage within a cell. This is incubated for 5 minutes on the bench after which the virucide is neutralized. A non-pathogenic, rapid-growing suspension (sensor cells), which is susceptible to the phage, is added. This is then plated in an agar medium, using the pour plate method and incubated overnight at 370C. These sensor cells grow overnight to form a lawn. Plaques are seen on a positive plate (5,41,42). The underlying principle is that of phage replication. When MTB cells in the sputum sample become infected by the phage, replication occur within the
bacilli. After maturation, infected cells lyse to release the progeny phages. These become available to infect the sensor cells. Thus plaques are a confirmation that infected sensor cells are undergoing lysis. This is not possible if there were no viable MTB cells within which the phages were initially propagated. A negative plate will thus have a confluent growth with plaques.

FASTPLAQUETB VERSUS CONVENTIONAL DIAGNOSTIC METHODS

FASTplaque assay has several advantages. Assay results are available within 24 hours of sample preparation, it does not involve the propagation of the pathogen, and there is reduced risk, if any, of misdiagnosis because it does not detect dead bacilli as is the case with smear microscopy as it has the ability to detect as few as 100-300 cells/ml of sputum (43).

In one study in Cape Town, the performance of FASTplaque TB assay in the correct diagnosis of auramine smear negative sputum sample was compared with that of chest X-ray. FPTB detected 55% of cases, negative by auramine smear, which is even more sensitive than ZN smears. Over 70% of the chest x-rays that were suggestive of TB were from patients who did not have TB. This demonstrates the fact that chest x-ray have a negative predictive value. These cases were confirmed by culture and/or clinical diagnosis (44).

In another study at the Aga Khan Hospital in Nairobi, Kenya, the FASTplaque TB was compared with AFB smear microscopy and the BACTEC 460 TB culture using samples from patients, 70% of whom were co-infected with HIV. There was 100% sensitivity and 98.2% specificity. Also FASTplaque TB detected all cases missed by AFB smear microscopy (45). This demonstrates that the performance of FPTB is not affected by the presence of HIV/AIDS. In yet another trial at the South African Institute of Medical Research (SAIMR), Cape Town, results from FASTplaque assay were compared with those obtained from concentrated auramine smear microscopy, solid culture (LJ) and clinical symptoms. The sensitivity of FPTB as compared with culture was 73.1% with 99.0% specificity. Also, FPTB was able to detect the presence of the TB bacilli in 55.3% of the samples that were negative by concentrated auramine smear microscopy. Overall, FPTB had positive and negative predictive values of 99.0% respectively. There is therefore little or no risk of false positive and false negative results.

Similar results were obtained in an evaluation conducted at the Sindh Institute of Urology and Transplantation (SIUT), Karachi, Pakistan. In results from FPTB assay was compared with those from concentrated auramine smear using the Ziehl-Neelsen method and LJ culture. Positive results were confirmed by PNB testing and a PCR assay specific for MTB complex. Of mail samples tested, FPTB showed 68% and 98% sensitivity and specificity in the diagnosis of smear negative TB (25). The fact that the FASTplaque assay requires no specially dedicated centrifuge and an incubator. It is an in vitro laboratory, which intends to use smear microscopy for diagnosing TB, would need a centrifuge. The technique can thus be applied either as a complement to smear microscopy with better results (increased sensitivity, specificity, and positive predictive value (46, 25). The assay is easy to run as it only involves simple pipetting, mixing of reagents and the plate culture method. It is convenient as the reagents, virucide, sensor cell, agar, growth supplement and reagent bottles all come in the same pack. It does not require extra-ordinary skills or instruments to read the results. Plaques are visible to the naked eyes. FAST plaque TB assay cannot replace culture that is more sensitive. It can however reduce the need for culture due to its ability to detect most cases that are missed by smear microscopy. It completes favourably with culture, greatly reducing the time required to obtain results from 6-8 weeks or more to 24 hours after sample preparation. While culture requires further confirmation and differentiation, FASTplaque results are conclusive of the presence of MTB complex. The WHO does not emphasize the use of culture in diagnosis except in retreatment cases. Culture requires a lot of incubator space in a busy laboratory.

Under the NTLCP in Nigeria, direct smear microscopy, which is far less sensitive than concentrated smear microscopy is the method used. In contrast with smear microscopy, chest x-rays and serological methods, the presence of HIV/AIDS does not alter the results of the test (45). It is thus suitable for Nigeria where over 2.6 million adults were said to be living with HIV/AIDS as at 1999.
Coast as a Determinant of the Applicability of New Diagnosis in Nigeria.

It should be noted that despite the availability of sophisticated rapid methods for the diagnosis of TB over the years, none of these are being employed in developing countries. Therefore one of the important factors that would determine the applicability of the FASTplaque TBM in the Nigerian TB Control Programme is its cost as compared with those of conventional methods. TB is a disease symptomatic of poverty and so ability to pay is a major determinant of what tests are likely to be recommended for individual patients. The cost of AFB x 3 varies between six hundred and eight hundred naira (N600 - N8000) (about $4 - $5) in non-profit set ups. If 3 or 2 sputum samples are negative by smear microscopy, chest x-ray is conducted. This gives an additional cost of between N400- N8000 and more depending on where it is done. Culture goes for about N6000. These rates are highly variable. This could be higher in private clinics which are profit-oriented and may be lower it is subsidized. The FASTplaqueTBM cost $200 per kit of 60 tests plus freight if one buys in small quantities from Biotec Laboratories, Ipswich, UK (47). It is difficult to guess what the actual cost per test will come to in Nigeria after freight is added, if bulk purchase attracts a discount or if the kits are bought from a local distributor. It is unlikely that the final cost for end users will be more than the WHO stipulated $5.00 for diagnostic. It has been speculated that it may be possible to reduce the cost of phage amplification technology by propagating the phages and sensor cells (M. Smegmatism, a non Mycobacterium) in a local laboratory. It has however been reported that a gene has been discovered in M. smegmatis which, when over-expressed, was found to induce resistance to infection by certain phages (40). Coupled with the problem of standardization, it may be better for now to use the commercially available kit produced by Biotec laboratories, Ipswich, UK which is tested to ensure high performance standards before plunged into the market.

Cost is unlikely to prohibit the use of FPTB in Nigeria especially if one recalls that active cases of smear negative TB do occur and are infectious. Having to wait for 6 - 8 weeks or more to get results of culture from the reference laboratory in Lagos before treatment is commenced increases the morbidity of TB on the patient and gives him/her adequate time to infect his/her contacts. This would in turn increase the number of people that would be requiring tests as well as treatment. Since FPTB detects most smear negatives, treatment is commenced promptly. Besides, the use of one AFB and one FPTB increases case detection tenaciously. (46) Saving from the second and third AFBs, transportation to and from clinics to submit 3 samples and future testing and treatment of contacts could be used to pay for the diagnostic. With increased budgetary allocation to the NTLCP and donor support, the cost of diagnosis and treatment on individual patients could be significantly low.

LIMITATIONS OF THE FASTPLAQUE TBM
The use of FASTplaque TB assay does have limitations. One of these is the fact that a positive result does not distinguish between all four members of the Mycobacterium tuberculosis (MTB) complex, M. tuberculosis, M. bovis, and M. africanum. This is not a problem if FPTB is used for diagnosis since all four members are etiologic agents of TB invariably requires treatment. For purposes of research, there may be need to differentiate between members of the Mycobacterium tuberculosis complex by other means. In a sputum sample containing more than 10% of blood, the performance of the test is reduced. Also, treatment with anti-TB drugs reduces the sensitivity of the test hence results are interpreted in relation to clinical information as well as other laboratory test. Most importantly, the efficacy of the test depends on the quality and quantity of the sputum, its storage and the number of viable organisms present. To ensure a good number of live organisms, it has been recommended that the NALC/NaOH (39) method be used to process the specimen as opposed to the modified Petroff's method currently in use at the TB reference laboratory in Lagos.

CONCLUSION AND RECOMMENDATION
It is pertinent to note that for now, no diagnostic can stand on its own as the singular method for the diagnosis of TB. There may be need for a combination of laboratory methods and clinical analysis for rational diagnosis to be made. Due to the
debilitating nature of the disease, its ability to spread fast and the inherent ability of the MTB bacilli to rapidly develop resistance to anti-TB drugs, there is need for prompt diagnosis to ensure prompt treatment. Molecular, chromatographic and other automated methods require special skills to perform and specially dedicated and expensive instrumentation. It is therefore for feasible to apply these in the Nigerian TB control. In contrast, phage amplification technology is a manual method that does not require special instrumentation. It is conclusive that smear microscopy performs best with processed sputum. So also does culture. Phage amplification technology places very little demand on the scarce resources of Nigerian Laboratories because it relies on the basic instruments that ought to be available in an average laboratory. There are a centrifuge, an autoclave and an incubator.

In the diagnostic algorithm in Nigeria, FASTPlaqueTB assay could be used either in the place of smear microscopy where it can afforded or in combination with one smear microscopy as opposed to AFB x 3. This combination has been reported to improve case-detection to about 91%. Most cases missed by smear microscopy will be picked up by the FASTPlaqueTB assay. This would drastically reduce the number of samples to be cultured.

There is an urgent need to establish a strong a liaison peripheral laboratories and the National Reference Laboratory in Lagos so that when necessary, sputum from symptomatic cases that are smear and FASTPlaque negative as well as those from re-treatment cases can be submitted to the reference laboratory for culturing. This will involve the provision of more incubator space at the Reference Laboratory and the provision of vehicles for the transportation of samples. Before FASTPlaque TB™ assay can be introduced into the diagnostic algorithm in Nigeria, there is need for the evaluation of this kit at the National Reference Laboratory to ascertain excellent performance under peculiar circumstances in Nigeria. There will be need for the National TB Control Programme to embark on laboratories inspection and identification of suitable centers with a view to providing basic equipment functional microscopes, centrifuges incubator, glass and plastic wares and reagents.

There is the need for staff training and sanitization on the advantages and disadvantages of available tests as well as the new kits and how and when to apply what method or a combination of methods. Prior to introduction of this kit into the Nigerian TB control, there is the need to set performance standards as well as set up monitoring groups who will over see work in peripheral laboratory from time to time. The kits can be used in a specified region for a trial period of six months. An impact assessment must be done after this period to ascertain whether their use has significantly improved TB Control in Nigeria or not. This should precede its full incorporation into the National TB Control Programme.

There is a dire need to patronize TB control especially as it has been reported to complicate HIV/AIDS as well as predispose to infection. Beyond the expression of the political will to do so, all tiers of government must allocate adequate towards the control of TB in Nigeria, as it is difficult for only the Federal government assisted by foreign donors to shoulder the responsibility for this task. This would de-emphasize cost as a major determinant of the use of diagnostics and create an opportunity to use only the best.

ACKNOWLEDGEMENTS:
I am most grateful to the management and staff of Biotec Laboratories, Ipswich, UK especially Messrs Rowland King, Andre Trolip, and Dr. Heidi Albert for giving me my first hands-on training in phage Amplification Technology. Special thanks to the Director general of the Nigerian Institute of Medical research for providing the opportunity to attend the training in Cape Town and to Andre for his useful suggestions during the preparation of this paper.

REFERENCES
29. Hieflies, L.: Qualitative and quantitative: drug


42. FASTPlaqueTM Pack Insert. Biotec laboratories Limited, Ipswich, UK.


47. Personal Communication with Rowland King, Biotec Laboratories, Ipswich, UK.