Current Concepts in Tuberculosis Diagnostics

***Dr Aderemi O Kehinde**, MBChB, MPH, FMCPath*
Department of Medical Microbiology, College of Medicine, University College Hospital, Ibadan, Nigeria

Dr A. O. Kehinde is a Senior Lecturer/Consultant
Department of Medical Microbiology,
College of Medicine, University College Hospital,
Ibadan, Nigeria

SUMMARY

The inability of the laboratory to adequately diagnose Tuberculosis (TB) using smear microscopy especially in those with latent infections, TB/HIV co-infections, paediatric and extra-pulmonary infections has led to an upsurge in TB epidemics in the community. While attention is being focused on HIV/AIDS pandemic, little is being heard of TB, especially in the areas of laboratory diagnosis (except of recent) despite the fact that the disease is the commonest cause of death in people living with HIV/AIDS. Efforts should be geared towards diagnostic TB research in developing countries to facilitate early diagnosis of cases and prompt initiation of therapy for TB control programme to have a meaningful impact in the community.

INTRODUCTION

Tuberculosis (TB) is of great public health concern worldwide, more so in the developing countries of Africa and Asia where 95% of cases are seen and 98% of deaths attributable to the disease occur [1]. As a result of the high burden of the disease, the United Nations has fashioned out Millennium Development Goals (MDGs) targets for TB control. These targets are:

(i) By the year 2005, to detect 70% of smear–positive TB cases annually, and to successfully treat 85% of these cases.

(ii) By 2015, to halve the prevalence and death rates associated with TB.

In order to achieve the MDGs for TB control, more efforts should be geared towards TB diagnostics.

All Correspondence to Dr Aderemi O Kehinde
Tuberculosis Research Unit,
Dept. of Medical Microbiology & Parasitology,
University College Hospital,
Ibadan, Nigeria
aokehinde@yahoo.com

A diagnosis of tuberculosis is undisputed when the causative organism is isolated from the clinical specimen. The extent to which this is achieved depends largely on the diagnostic capacity of the mycobacteriology laboratory. Tuberculosis is a preventable and curable disease. Research studies and clinical trials have shown that the treatment of this disease is not only effective but is also among the most cost-effective ways of prolonging healthy living [2,3]. This means that the scourge should have been eradicated by now or reduced to a mere shadow of itself. In fact, there are more cases of TB in the world today than in previous epochs of human history [3]. The problems posed by this disease necessitated the World Health Organization (WHO) to label it a global emergency in 1993. The situation in sub-Saharan Africa is even more worrisome. This is as a result of poor environmental living conditions, ignorance, poverty, HIV/AIDS scourge and more importantly absence of rapid diagnostic tools to facilitate an early diagnosis of the infection leading to a wide spread of the disease.

Situation on Ground

Rapid and accurate diagnosis of symptomatic patients is the cornerstone of global TB control strategies. Remarkable progress has recently been made upgrading the speed and quality of TB diagnostic services in developed countries but for most of the world where TB is a large public health burden, these gains are still unrealized [4]. Thus, the primary laboratory tool supporting case detection in vast majority of cases in disease endemic countries remains microscopic examination of the stained sputum smear. The shortcomings of this method seriously limit the extent and quality of its application, and ultimately, its impact in TB control.

Smear microscopy performs poorly in latent infections and in patients with TB/HIV co-infections. It is not specific as both pathogenic and environmental mycobacteria are indistinguishable. Apart from this, it is grossly inadequate for the diagnosis of paediatric and extra-pulmonary infections. Culture on Lowenstein-Jensen medium requires six to eight weeks incubation to detect TB isolates thus contributing to diagnostic delay associated with TB. This often leads to widespread transmission of the disease.

Good TB control depends on a balanced equation of case detection and treatment delivery. The global expansion of "DOTS" (Directly Observed Treatment Short Course) - a WHO strategy to combat TB) and the advent of Global Drug Facility (GDF) have contributed significantly to good accessibility of TB drugs and improved cure rates. This is good news but the other side of the equation, that is, diagnostics - case detection was left unattended to for many years. For example, WHO recommended diagnostic tool, smear microscopy has been in use since 100 years ago. Reports from twenty-two high TB burden countries show that sixteen reported treatment success rate of over 70% but alarmingly, only four high burden countries have overall smear positive detection rates of over 60%.[5] Majority of cases remained undiagnosed thus perpetuating the epidemic.

How Did We Find Ourselves in this Predicament ?

Two factors are responsible for this. The first is smear microscopy as a case defining diagnostic tool. This tool is insensitive, as it requires a minimum of 10,000 Acid Fast Bacilli (AFB) per high power field to be reported positive, it requires multiple visits by the patients and is technically burdensome, apart from other drawbacks that have been enumerated previously. Clearly, simpler, more sensitive and more patient-friendly diagnostic tools are urgently needed. The Bills and Melinda-Gates funded TB Diagnostic Initiative (TDI) at the United Nations Development Programme/World Back/WHO Special Programme for Research and Training in Tropical Diseases (TDR), along with commercial and academic partners are currently working on new tools that will be affordable and adapted for use in developing countries with high TB burden. The international TB community and general medical community itself, which has always emphasized quality of treatment over quality of diagnosis. In part because of the urgent need to increase cure rate and in part because public health thinking has been dominated by clinicians without much input from microbiologists, hence, drug treatment has always remained higher on global TB agenda than diagnostics.

At present, TB diagnostic services receive little attention. Laboratories are marginalized by TB control programmes and too often are staffed with overworked and unmotivated laboratory scientists who are forced to make do with sub-standard reagents and inadequate or broken down equipment.

The poor state of the laboratories leads to poor performance, perpetuating a vicious cycle of laboratory mediocrity reinforcing clinical irrelevance. This is dramatically illustrated in the HIV- prevalent regions of sub-Saharan Africa. Here the percentage of symptomatic pulmonary TB suspects who are sputum smear positive is so low that there is an inevitable drift towards syndromic management.

Countdown to New Diagnostic Tools

If one may ask! What is new in TB diagnostics in Nigeria? The answer is "Not much" This scenario is disturbing because Nigeria rank 4th out of the 22 TB high burden countries globally and it has the highest number of new TB cases in sub-Saharan Africa [5].

New tools that have been developed to be adapted for use in developing countries are:

(i) Radiometric liquid culture systems eg Bactec 460^{TB} are commonly used in level III mycobacteriological laboratories in developed countries but the difficulty working with radioactive materials, necessity of expensive equipment for detection of radioactive gas and cost of materials seriously limit its use in poor resource countries.

(ii) Alternative new solid culture media was developed by TB Diagnostic Initiative (TDI) based at WHO headquarters in Geneva. The activities of TDI later gave birth to FIND (Foundation For Innovative Diagnostics for Infectious Diseases). FIND was formed with the intention of developing

tools for diagnosis of infectious diseases that will be affordable and adapted for use in poor countries with high burden of diseases. Tuberculosis was taken as a prototype of such infectious diseases. In July 2004, FIND in collaboration with an American Pharmaceutical Company (SALUBRICS INC.) [6] started piloting the use of TK medium as an alternative to the Lowestein-Jeesen medium. This new medium shortens TB detection time to half and its speed and sensitivity is comparable to automated systems.

•TK medium indicates growth of Mycobacterium by changing colour thereby avoiding the need to wait for colonies to become apparent on culture media. Speed is enhanced by the medium's ability to detect the metabolic activity of the bacteria, which changes the colour from red to yellow for a positive isolate while a contaminant will change from red to green. "The speed of the test results, simplicity and its' discriminating capacity improves the relevance of culture and make it ideal for use in developing countries" says Mark Perkins. Mark Perkins was formerly Medical Officer in charge of TB Diagnostic Initiative and now the Scientific Director of FIND.

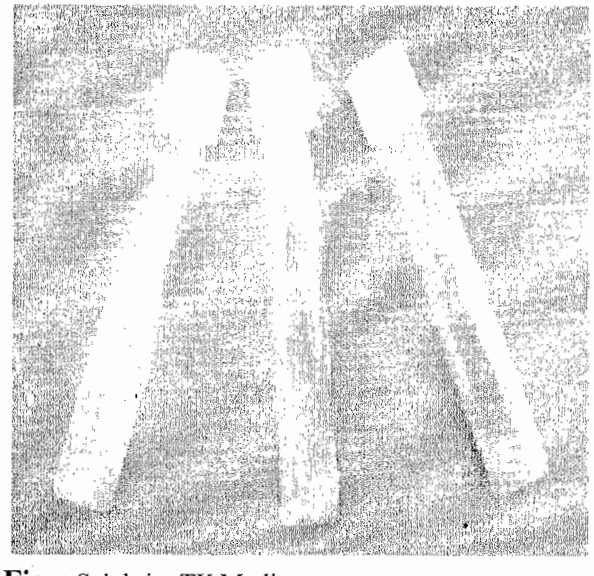

Fig. : Salubrics TK Medium

Summary of Salubrics' Product

•Rapid culture medium

•Original red color turns to yellow by mycobacterial growth before the colonies become visible.

•It differentiates contamination by turning to green when many other species of bacteria or fungi grow

•It is not radioactive. It does not create a radioactive waste problem

•Protective cap makes inoculations and subcultures easy and safe

•TK SLC is the selective type containing five different antimicrobials to inhibit the growth of other bacterial species and fungi

•TK PNB allows rapid differentiation of tuberculosis and non-tuberculosis mycobacteria

•TK Anti-Tb Kit allows susceptibility test easy and fast.

In December 2004, FIND entered into a collaboration with Becton Dickson BD & Co. aimed at improving the diagnosis of pulmonary TB in HIV infected patients in developing countries using a more sensitive and rapid diagnostic kit. TB is particularly difficult to diagnose in HIV/AIDS patients because they produce few TB bacteria in their sputum. Thus while smear microscopy is insensitive in these patients, culture on Lowestein-Jensen medium is notoriously slow. FIND in conjunction with BD & Co.[7] has developed an improved culture method called BD MGIT^{TM} (Mycobacteria Growth Indicator Tube) system, which provides results within 10-14 days. FIND plans to conduct demonstration projects on this product in conjunction with WHO, StopTB Partnership and Consortium To Respond Effectively To AIDS /TB Epidemics (CREATE) based at John Hopkins Center For TB Research, United States of America with the aim of promoting the use of the product in poor resource countries with high burden of TB.

(iii) Serology: Existing commercialized serologic tests make use of well described immunodominant antigens to detect immunoglobulinG or other immunoglobulin classes in dipstick or Elisa format. Promising research developments in serology include:

(a) Availability of highly purified and recombinant antigens

(b) Improved understanding of the heterotypic nature of humoral response to TB and the development of multi-antigen tests that maintain high specificity.

(iv) Phage systems: Phage replication systems (Luciferase Reporter Mycobacteriophages and FastPlaque kits by Biotec Laboratories) detect live mycobacteria in clinical samples or in young liquid cultures using phages that infect and replicate in mycobacterial cells as indicators.

Nigerian Institute of Medical Research, Lagos in collaboration with Biotec laboratories carried out a demonstration project on FastPlaque kits in 2002. Main problem then was the cost of the kits which made it unaffordable for use for generality of people.

(v) Molecular Techniques: This determines the presence of *Mycobacterium tb* in clinical specimens by detecting specific nucleic acid sequences after being amplified. Nucleic acid amplification assays (NAA) have been found to be more sensitive than smear microscopy but less sensitive than culture.[2] Even though commercially available NAA Kits are simple and reliable to use, cost, degree of technical support and quality control requirements limit their use in poor countries.

(vi). New in-vitro assays are in the pipeline to replace Tuberculin skin test, which is the only test currently used to detect latent infection. This is particularly important in high HIV prevalent areas. Tuberculin (PPD) test is not specific for TB because it shares a large number of antigens with BCG and environmental mycobacteria. Also it is plagued by errors due to readers' interpretation and the need for return patient visits.

The Way Forward (Diagnostic Needs Of High Prevalence Countries Like Nigeria)

•*Impediments to TB diagnostics in Nigeria include:*

(i) Widespread use of BCG vaccinations which may affect the specificity of immunologic tests.

(ii) High prevalence of HIV/AIDS infection

.•*Ideal new tools should address the followings:*

(a) Replacement for Microscopy:

(i) New test (s) should yield conclusive results in less than 2hrs while the patient is still in the clinic;

(ii) Be simple enough for use by unskilled workers with less than 3 hrs of training;

(iii) Be specific enough to allow initiation of therapy;

(iv) Function well in HIV patients;

(v) require little or no interpretation.

(b) Replacement for culture:

(i) To augment microscopy for evaluation of complex patients;

(ii) Should be sensitive enough to detect the majority of smear negative culture positive TB patients including those co-infected with HIV;

(iii) It must be fast, simple to perform and inexpensive to be implemented at the peripheral level.

(c) Screening tests: to rapidly screen symptomatic cases thus reducing laboratory workload and also to detect drug resistance within 2-4 weeks of sputum microscopy in areas with high multi-drug resistant (MDR-TB) load.

•Screening tests for latent infection must be specific for *M.tb*, must not require a return visit and must function well in HIV patients.

•Efforts are in top gear at global level to find a suitable vaccine that could prevent *M.tb* from overwhelming its victims. Many scientists have potential vaccines under investigation, and lots of laboratory experiments have suggested that they could work. But there has been no pharmaceutical interest in pushing any of these vaccines to a level required for use in humans. The barriers of financial risks, market forces and the high cost of vaccine development blocked the path between hope and cure. Drug companies could not afford to take the chance that a drug proven to be safe in mice would work in people, and research laboratories could not afford human trials.

•Here, efforts of Sequella Foundation (SF) should be applauded. SF is an international NGO founded by an Immunologist, Dr Carol Nacy, and being funded by Bill and Melinda Gates Foundations. By funding the critical intermediary steps needed to launch vaccine testing in humans, Sequella Foundation is turning imagination to reality. Thus, Sequella can be the matchmaker between academic laboratory scientists and the pharmaceutical industry [8].

How Can We Improve Case Detection to Maximize the Impacts of Dots in Nigeria ?

(i) First step is to give TB laboratories the support they need, allowing them to offer high quality services through provision of equipment and reagents, training and support of laboratory staff, insistence on quality control and proficiency testing and sponsorship of communication links between clinical and laboratory services.

(ii) To coordinate international assistance for capacity building in TB laboratories in Nigeria. This is needed to support not only the performance of standard methodologies but also to support operational research. Strong collaboration between academic researchers and national TB control staff should be encouraged.

(iii) The third step is the development of new diagnostic tools that respond to our needs. Accurate case detection is the Achilles' heel of the DOTS strategy. The success of current concerted efforts to stop TB depends on our ability to detect cases early enough, to institute curative therapy and interrupt the cycle of transmission.

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