Abstract—Performing culture and characterization of mycobacteria in low resource settings like Nigeria is a very difficult task to undertake because of the very few and limited laboratories carrying out such an experiment; this is a largely due to stringent and laboratory nature of the tests. Hence, a rapid, simple and accurate test for characterization is needed. The “SD BIOLINE TB Ag MPT 64 Rapid®” is a simple and rapid immunochromatographic test used in differentiating Mycobacteria into Mycobacterium tuberculosis (NTM). The 100 sputa were obtained from patients suspected to be infected with tuberculosis and presented themselves to hospitals for check-up and treatment were involved in the study. The samples were cultured in a class III Biosafety cabinet and level III biosafety practices were followed. Forty isolates were obtained from the cultured sputa, and there were identified as Acid-fast bacilli (AFB) using Ziehl-Neelsen acid-fast stain. All the isolates (AFB positive) were then subjected to the SD BIOLINE Analyses. A total of 31 (77.5%) were characterized as MTBC, while nine (22.5%) were NTM. The total turnaround time for the rapid assay was just 30 minutes as compared to a few days of phenotypic and genotypic method. It was simple, rapid and reliable test to differentiate MTBC from NTM.

Keywords—Culture, mycobacteria, non-tuberculous mycobacteria, SD bioline.

I. INTRODUCTION

NIGERIA has a population of over 130 million people and ranks 4th among the world’s 22 countries with a high TB burden [1]. Among those countries, Nigeria has the highest estimated number of new TB cases with nearly 368,000 new cases and an estimated 30,000 deaths annually [1]. Infectious and parasitic diseases, malnutrition and respiratory diseases are among the causes of tuberculosis and HIV/AIDS case detection is increasing, and thereby constituting serious public health problems [2]. Little is known about the epidemiology of MTB complex species associated with pulmonary TB in Nigeria due to limited facilities for TB culture and molecular assays until the recent introduction of the US President’s Emergency Program for AIDS Relief (PEPFAR) and the Global Fund. A better understanding of the circulating MTB complex species and their resistance to drugs is essential to guide diagnostic and therapeutic measures aimed at controlling this major public health burden in Nigeria, especially with the increase in TB cases due to the prevailing HIV epidemic. Over three million people live with HIV/AIDS in Nigeria with a national prevalence of the disease estimated at 4.1% in 2010, as released by the country’s National Agency for the Control of AIDS (NACA) in its Global AIDS Response Progress Report [13].

Presumptive diagnosis of pulmonary tuberculosis can be made on the basis of patient histories and clinical and radiological findings; the definitive bacteriological diagnosis of tuberculosis continues to depend on the microscopic examination of acid-fast stained sputum smears and then cultural confirmation. Direct microscopy by Ziehl-Neelsen staining to identify AFB is the method, most rapid, but it lacks sufficient sensitivity and specificity.

Tuberculosis is caused by members of Mycobacterium tuberculosis complex (MTBC), which includes M. tuberculosis, M. bovis, M. africanum, M. carnetti and M. microti. Recently, diseases caused by mycobacteria other than tuberculosis (MOTT), also known as non-tuberculous mycobacteria (NTM) are on the rise. This has been attributed to a parallel increase in HIV/AIDS infection and other immune compromised conditions [3].

The clinical presentation of pulmonary disease caused by MTB is similar to that caused by MTBC, as such, NTM as a cause of pulmonary tuberculosis are often misdiagnosed in a low resource constrained settings like Nigeria lacking culture and identification facilities. Therefore, it is imperative to accurately characterize mycobacteria since NTM are usually resistant to conventional anti-tuberculous drugs, require modified treatment regimens and are often misdiagnosed as multi-resistant tuberculosis (MDR) [3].

Characterization of mycobacteria can be done either phenotypically or genotypically. Conventional phenotypic methods for identifying mycobacterial species are based on the results of growth rate, pigmentation of calories and various biochemical tests. These methods are time consuming, also
involves use of hazardous chemicals, some of which are carcinogenic, (Niacin test) and are prone to subjective error in interpretation of results (Global Tuberculosis Programme [1]). While, molecular methods that identify specific nucleic acid sequences are rapid, sensitive and specific, but are expensive and require trained personnel and special laboratory set-up. [4]. Therefore, there is the need for a rapid, accurate and simple test for characterization of mycobacteria.

In recent years, a variety of antigens have recently emerged for the immunodiagnosis of TB. The Mycobacterium tuberculosis protein 64 (MPT-64) antigens is Mycobacterium tuberculosis complex (MTC) specific antigen secreted during bacterial growth. It is also known as protein RV 1980C, which is a 24 kDa secretory protein secreted by the MTBC, except some strains of Mycobacterium bovis BCG [5]. This antigen is encoded by RD2 region which is specific for MBTC and can be detected in culture isolates and biopsy samples [6]. MPT 64 induces a strong delayed type hypersensitivity reaction similar to that induced by purified protein derivatives in sensitized guinea pigs [7].

The Standard Diagnostic (SD, Korea) developed a simple and rapid assay called “SD BIOLINE TB Ag MPT Rapid ®” (Commercial assay) to discriminate between MTBC and NTM by immunochromatography (ICT). The SD Bioline TB Ag MPT64 Ag MPT64 Rapid test (SDMPT64 Rapid test), a simple immunochromatographic test (ICT) for the M. tuberculosis complex, has been developed and uses monoclonal antibodies to detect the MPT64 protein [13], [15]. MPT64 is one of the major culture filtrate proteins (24 kDa), encoded by the RD2 region genes and has been shown to be a specific antigen that differentiates the M. tuberculosis complex from the mycobacteria other than tuberculosis (MOTT) species, otherwise known as environmental mycobacteria[15]. The SD MPT64 Rapid Test is used widely to confirm the identity of M. tuberculosis complex from humans. Furthermore, the test is highly sensitive and specific. The SD MPT64 Rapid test is potentially useful for identifying members of the M. tuberculosis complex, because the MPT64 antigen is expressed by all members of the M. tuberculosis complex [10].

This present study was undertaken to evaluate the commercial assay, SD TB Ag MPT 64 Rapid ® for characterization of mycobacteria already isolated on Lowenstein Jensen (LJ) media. LJ media containing glycerol which favours the growth of M. tuberculosis and LJ media containing pyruvate which favours the growth of M. bovis were used for each Sputum sample. These were then incubated for eight weeks in an upright position. Colonies suspected of being mycobacteria were examined for the presence of AFB by ZN staining technique.

II. MATERIAL AND METHODS

The study was carried out in Gombe State, North-Eastern Nigeria. Four General Hospitals (Gombe, Kaltungo, Zambuk and Bajoga) were selected for the study. A total of 100 sputa were collected in sterile universal tubes from suspected TB patients. The sputum samples were processed (decontaminated and neutralized) for mycobacterial culturing according to the standard operating procedure described by [1].

A. Ethical Clearance

Ethical clearance letter was obtained from Gombe state hospital management board for permission to collect samples from the designated hospitals.

B. Collection of Samples

Three consecutive sputum samples were obtained from all patients with cough of more than two weeks and reported to the respective hospitals for investigation. Patients sputum that were found to be positive for tuberculosis after smearing, staining and viewing through the microscope were stored at 4°C for up to three days and transported to the Zankali Medical Centre TB Laboratory, Abuja, for culture.

C. Staining of the Sputum Sample

Ziehl-Nelson (ZN) staining method was used perform and light-emitting diode (LED) microscope was used to view the smear.

D. Preparation and Decontamination of Specimen

All direct sputum specimens from patients that were positive for AFB were subjected to a treatment called N-Acetyl Cysteine and Sodium Hydroxide (NALC and NAOH) method of decontamination, digested and concentrated under the biosafety cabinet according to Centre for Disease Control (CDC) guidelines for public health mycobacteriology, a guideline for the level II laboratory [12]. Decontamination of sputum was done by adding 1 ml of 4% Sodium hydroxide (NaOH) to 1 ml of sputum to make 2 ml of solution, the caps of the McCartney bottles were tightened and mixed well using well using a Vortex machine inside the biosafety cabinet and allowed to stay for 15 minutes. The 2 ml of sterile water was added to 2 ml of the solution, to make up to 4 ml. These (sputum + NaOH + sterile water) were centrifuged at 300 rpm for 15 minutes. The McCartney bottle was gently removed from the centrifuge and the supernatant were discarded.

E. Sputum Culture for Mycobacteria

The sediments obtained were cultured onto Lowenstein-Jensen (LJ) media. LJ media containing glycerol which favours the growth of M. tuberculosis and LJ media containing pyruvate which favours the growth of M. bovis were used for each Sputum sample. These were then incubated for eight weeks in an upright position. Colonies suspected of being mycobacteria were examined for the presence of AFB by ZN staining technique.

F. SD Bioline

Five to seven (5-7) colonies were emulsified in about 200µl of sterile buffered saline, then 100µl of the suspension added into the sample wells and allowed to stay for 15 minutes before being read. Positive result is indicated by the presence of only two color band (one control band and one test band). The presence of only one control band within the result window indicated a negative result. Faint color band was recorded as a weak positive and the sample retested (Fig. 1).
The control band was detected in all the 40 isolates strains tested indicating the validity of the test. The commercial assay was therefore considered satisfactory.

From all 40 MTBC strains tested, 28 showed the presence of MPT64 antigen, while three of the isolates gave a faint band which were retested and found to give a weak positive result both times, and were therefore considered as MTBC. All the 9 NTM strains gave a negative result [Table I].

### TABLE I

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Total No. of Sputum collected</th>
<th>Culture positive</th>
<th>SD Bioline Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gombe (FMC)</td>
<td>20</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Kaltungo</td>
<td>50</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Bajoga</td>
<td>20</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Zambuk</td>
<td>50</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
<td><strong>40</strong></td>
<td><strong>31</strong></td>
</tr>
</tbody>
</table>

FMC: Federal Medical Centre

### IV. DISCUSSION

There is an urgent need in low resource settings to have a simple, rapid yet accurate test for differentiation of mycobacteria. It was therefore, decided to assess the usefulness of “SD BIOLINE TB Ag MPT64 ® assay marketed as a rapid test. The major advantage of using this test was its claim to characterize mycobacterial isolates accurately in 15 minutes. The assay was evaluated using 40 isolates obtained from culturing sputa of patients suspected to have pulmonary TB, and to find out whether it can replace the conventional phenotypic methods. In this study, it was observed that the assay had 100% specificity which concurred with other studies that demonstrated specificity of 100% and sensitivity of 96.5% to 100% [8], [9]. The test is based on the detection of MPT64, an antigen considered highly specific protein for MTBC which has been confirmed by cloning and sequencing of the MPT64 gene of the H37 RV Cultire filtrate [10]. It has been proved that the MPT64 antigen is found only in viable and actively dividing cells of MTBC (5). It is secreted in significant amounts during the early period of culture and decreased with longer cultivation. This antigen is found in the culture fluid of MTBC isolation media. The MPT64 antigen is absent in BCG strain of M. bovis, M. laprae and non-tuberculous mycobacteria [11].

The cultures used to run the assay in this study have stayed for more than three months, yet the assay was able to detect all the positives without giving false positives. This has been confirmed by a previous study that MPT64 once secreted into the culture medium, is stable for at least one whole year [4]. Therefore, instability of the antigen could not be the reason for the false negativity, an advantage that can be exploited in poor or low resource settings [14].

The only limitation of this assay is the requirement of biosafety procedures and equipment while manipulating the culture. This is probably not a true limitation since biosafety is a mandatory requirement for any mycobacteriology laboratory performing cultures.

The advantages of this commercial assay are its rapidity, simplicity, ease of use, economy and non-requirements of technical skills, equipment, hazardous chemicals and low temperature storage. With 99.19% sensitivity and 100% specificity, the test appears to be accurate and has the potential to replace the phenotypic methods of characterization.

### V. CONCLUSION

SD BIOLINE TB Ag MPT64 Rapid ® assay is a simple, rapid, economical and reliable test for characterization of clinical mycobacterial isolates that can be easily incorporated by mycobacteriology laboratories. Rapid characterization of isolates is expected to facilitate treatment decisions in tuberculosis control program. The results of this study suggest that the SD MPT64 Rapid test might be a useful method for quick identification of members of M. tuberculosis complex from human sputum samples. The test could possibly replace conventional confirmation assays. The greater simplicity and lower cost of the SD MPT64 Rapid test compared to other methods make this technique a good choice for confirming members of the M. tuberculosis complex. Using this test for identifying the members of the M. tuberculosis complex from humans might contribute to the establishment of an eradication strategy for tuberculosis in humans.

There is also the need to carry out further research on the evaluation of the assay to identify the MPT64 antigens directly in smear positive clinical samples.

### ACKNOWLEDGMENT

This research was supported by Agri. Research Council, TETFUND and Federal Livestock Department Abuja. The material and financial support given by Management of Zankali Medical Centre and the staff of the Zankali TB Research laboratory are highly appreciated.

### REFERENCES


