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## Rapid confirmation of drug susceptibility in *Mycobacterium tuberculosis* using MPT 64 Ag based test

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## PEER REVIEW

## ABSTRACT

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**Comments**

This study is commendable in that the adoption and modification of available techniques as shown clearly by this study is a beacon in the right direction in the management and control of tuberculosis which is hinged on rapid and accurate diagnosis.

(Details on Page 209)

**Objective:** To evaluate the possible use of MPT64 based rapid test to detect multi–drug resistant *Mycobacterium tuberculosis* (*M. tuberculosis*) in antibiotic broth dilution cultures. **Methods:** Twenty five isolates of *M. tuberculosis* whose susceptibility pattern had previously been identified by HAIN Genotype MTBDRplus (HainLifeceience, Herhen, Germany) were processed and cultured according to the microscopic–observation drug susceptibility technique. These included 20 susceptible, two multi–drug resistant *M. tuberculosis* and three isoniazid mono–resistant isolates. After 10–day incubation, aspirates from each well were tested with the MPT64 rapid test. **Results:** The rapid test correctly identified all 25 isolates and detected rifampicin and isoniazid resistance in all but one isoniazid mono–resistant isolate. **Conclusions:** MPT64 rapid test could be useful in detecting *M. tuberculosis* and drug resistance from Middlebrook 7H9 antimicrobial broth dilution in resource poor settings without an inverted microscope.

## KEYWORDS

Tuberculosis, MDR–TB, Rapid detection, MPT64 protein, Middlebrook 7H9

### 1. Introduction

In the last three years, tuberculosis has killed more than 4.6 million people<sup>[1,2]</sup>. Yet, case detection of this treatable disease has remained low in most high burden countries due to continued dependence on smear microscopy for diagnosis which misses more than half of incident cases<sup>[3]</sup>. Most of the new techniques of improved detection have remained out of reach of the field where they are most needed<sup>[4]</sup>. Detection of multi–drug resistant tuberculosis (MDR–TB) which has complicated control efforts has also remained elusive to most of these countries. Recent

techniques such as the microscopic–observation drug susceptibility (MODS) assay offer great promise<sup>[3,5,6]</sup>. The liquid culture medium used in this technique is easy to formulate. However, the requirement of an inverted microscope which is still relatively expensive has limited its wide application.

MPT64 based rapid test, has been shown to rapidly detect the presence of *Mycobacterium tuberculosis* (*M. tuberculosis*) in cultures<sup>[7,8]</sup>. Most studies have used the test to discriminate between *M. tuberculosis* complex and non–tuberculous mycobacteria from liquid portions of Lowenstein–Jensen (LJ) slants and BACTEC mycobacterial

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growth indicator tubes cultures[7–9]. These media may either take too long for appreciable growth to occur and/or too expensive. The applicability of this rapid test in detecting MDR-TB to the best of our knowledge has also not been studied.

This study evaluated the possibility of using the MPT64 rapid test to detect *M. tuberculosis* in Middlebrook 7H9 cultures prepared according to the MODS assay protocol thus detecting growth and drug susceptibility in broth without an inverted microscope.

## 2. Materials and methods

### 2.1. Specimens processing

The study was carried out at the Microbiological Reference Laboratory, Lawrence Henshaw Memorial Hospital, Calabar, Cross River State, Nigeria. Ethical approval was previously obtained from the Cross River State Ministry of Health Ethical review Committee. Twenty five isolates of *M. tuberculosis* obtained from LJ culture of clinical and external quality assessment isolates were used. Susceptibility of the isolates to isoniazid and rifampicin was previously determined with HAIN GenoType MTBDRplus assay (HAIN Lifescience Herhen, Germany). The isolates included 20 susceptible strains, three isoniazid resistant strains and two isoniazid and rifampicin resistant strains. The *M. tuberculosis* colonies from LJ cultures were harvested into a sterile tube containing 100  $\mu$ L water-tween-80 solution and six glass beads. The tubes were vortexed for 2 min and allowed to stand for 5 min. Three millilitres of water-tween-80 solution was added and vortexed again for 20 seconds and allowed to stand for 30 min. The supernatant was transferred to another tube and the turbidity adjusted to 0.5 McFarland standard. Five microlitre of the 0.5 McFarland suspensions was added to 5 mL of Middlebrook 7H9 medium supplemented with casitone, glycerol and mycobacterial growth indicator tube supplement made up of oleic acid, albumin dextrose and catalase. The preparation was cultured in 24-wells tissue culture plates as described for the MODS technique[10]. Each sample was cultured in one drug free well, and one well each containing final volumes of 500  $\mu$ g/mL p-Nitrobenzoic acid (inhibitor of *M. tuberculosis*), 0.4  $\mu$ g/mL isoniazid and 1  $\mu$ g/mL rifampicin. A non-tuberculous mycobacteria identified from the external quality assessment samples was also processed and cultured for use as MPT64 rapid test control. One row out of the six rows of 24-wells plates contained only media as negative control to evaluate the possibility of cross contamination as recommended in the MODS technique.

The culture plates were secured in a zip-lock bag and incubated at 37 °C. The culture plates were examined

for mycobacterial growth from Day 5 using an inverted light microscope. *M. tuberculosis* was identified by its characteristic cordlike growth and inhibition of growth in the presence of p-Nitrobenzoic acid. The plates were examined daily till Day 10 to allow for sufficient growth and probably maximal production of MPT64 antigen.

At Day 10, all the plates were removed from the zip-lock bag in a class 2A bio-safety cabinet and renumbered to blind the negative control and NTM containing wells. Aspirates of 100  $\mu$ L of culture from the drug free, isoniazid and rifampicin containing wells were tested with the SD Bioline TB Ag MPT64 rapid test (Standard Diagnostics Inc., Kyonggi-do, South Korea) according to manufacturer's instructions (Figure 1). All SD Bioline MPT64 rapid tests were read blind to the results of MODS culture. Isolates were considered resistant to a drug if the SD Bioline test of the aspirate from the drug containing well was positive.



**Figure 1.** SD Bioline TB Ag MPT64 test result of an INH and RIF susceptible *M. tuberculosis* isolate.

### 2.2. Data analysis

All data were entered into and analysed using SPSS statistical software version 17.0 (SPSS Inc, Chicago, USA). Kappa statistic was used to evaluate reliability. The kappa value, a measure of reliability of a test was interpreted as follows: 0–0.2, poor; 0.21–0.4, fair; 0.41–0.6, moderate; 0.61–0.8, good; >0.8, good.

## 3. Results

### 3.1. *M. tuberculosis* detection

MPT 64 rapid test correctly detected *M. tuberculosis* growth in all 25 drug free MODS culture wells (Table 1). All negative

**Table 1**

Comparison of the concordance of MPT64 test with MODS cultures.

Tests	detection			INH 0.4 µg		RIF 1.0 µg	
	M.Tb (n=25)	NTM (n=1)	Neg control (n=5)	Susceptible (n=20)	Resistant (n=5)	Susceptible (n=23)	Resistant (n=2)
MODS assay (%)	25 (100.0)	1 (100.0)	5 (100.0)	20 (100.0)	5 (100.0)	23 (100.0)	2 (100.0)
MPT64 test (%)	25 (100.0)	1 (100.0)	5 (100.0)	20 (100.0)	4 (80.0)	23 (100.0)	2 (100.0)
	Kappa value=1.0 $P<0.01$			Kappa value=0.87 $P<0.01$		Kappa value=1.0 $P<0.01$	

M.tb–*M. tuberculosis*; NTM–non tuberculous mycobacteria; neg control–negative control; INH–Isoniazid; RIF–Rifampicin.

controls (NTM and media only wells) were also correctly identified as negative.

### 3.2. Drug resistance detection

All 23 (100.0%) rifampicin susceptible isolates were correctly identified as well as resistance in the two MDR isolates. There was a 100% concordance in detection isoniazid susceptible isolates. SD bioline TBAGMPT 64 rapid test<sup>®</sup> correctly detected isoniazid resistance in the MDR isolates and two of the three mono-resistant isolates (kappa value 0.87,  $P<0.01$ ).

## 4. Discussion

The challenge of tuberculosis and MDR–TB detection in resource poor settings urgently calls for evaluation of safe, rapid, specific and sensitive methods of detection. The specificity of the SD Bioline MPT64 assay in detecting *M. tuberculosis* observed in this study is similar to previous studies[8,9]. The SD Bioline test false negative isoniazid resistant isolate was observed in MODS to have few colonies (about 10 colonies). MPT64 protein is a secreted product and thus appears to be affected by the colony count and size as was the observation elsewhere[9]. More studies using larger sample size are therefore necessary to evaluate the sensitivity of indirect and possibly direct susceptibility testing of *M. tuberculosis*.

A limitation of this study was the low number of resistant strains available. Studies with a larger number will therefore be necessary to fully evaluate sensitivities and specificities of MDR–TB detection. MPT64 rapid testing was done 5 d after MODS cultures were observed to be positive. This was to allow for adequate concentration of the MPT64 antigens to be formed. Therefore, there is a need to evaluate the appropriate time for positivity following liquid cultures. The effect of bacterial or fungal contamination which sometimes occurs in liquid cultures also needs evaluation.

In conclusion, the MPT64 rapid test could be useful in detecting positive cultures and drug susceptibility testing of *M. tuberculosis* in easily formulated Middlebrook 7H9 antimicrobial broth dilution in resource poor settings that

do not own an inverted microscope.

### Conflict of interest statement

We declare that we have no conflict of interest what so ever with the manufacturers of the test kit. OSM receives allowances from Family health international 360 (FHI360) which established and manages the tuberculosis laboratory.

### Acknowledgment

The inverted microscope and secretarial support for this study was provided by the Save Lives Initiative Calabar, Nigeria. We also acknowledge the Family Health International 360 (FHI360) which established and manages the tuberculosis laboratory were the study was conducted.

### Comments

#### Background

The high economic burden of tuberculosis diagnosis still remains a public health issue in regions with high burden for the disease. This is compounded by the low detection rate that relies solely on smear microscopy with little recourse to culture and drug sensitivity testing because of prohibitive cost of newer diagnostic techniques.

#### Research frontiers

Study assessed the applicability of MPT64 antigen based rapid test in detection of *M. tuberculosis* complex and MDR–TB from Middlebrook 7H9 liquid culture with the aim of lowering cost as well as increasing rate of detection of the pathogen and its resistance pattern.

#### Related reports

The study used validated protocols in a clinical setting taking into cognizance biosafety issues and quality control.

#### Innovations & breakthroughs

The study has shown that the cost of detection of *M.*

*tuberculosis* complex and MDR–TB could be reduced with adaptation and modification of already validated protocols and techniques.

### Applications

The protocol used in the study using safe, rapid and sensitive detection method is applicable to clinical settings in resource poor countries with high disease burdens.

### Peer review

This study is commendable in that the adoption and modification of available techniques as shown clearly by this study is a beacon in the right direction in the management and control of tuberculosis which is hinged on rapid and accurate diagnosis.

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### Asian Pacific Journal of Tropical Disease

Asian Pacific Journal of Tropical Disease (*APJTD*, ISSN 2222–1808), which was started in February 2011, is a bimonthly publication in English. As an international journal, it mainly publishes articles on tropical biomedicine in the Asian–Pacific region. *APJTD* has been entered eight international indexes, including ZR, Scopus, EM, CABI, Global Health, IC, CA, and Ulrich PD.

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