Rapid and Inexpensive Detection of MDR Mycobacterium tuberculosis using a Resazurin Microtitreplate Assay in a High Burden Tuberculosis Reference Laboratory

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INTRODUCTION

- The emergence of multidrug-resistant *Mycobacterium tuberculosis (MTB)* and the close association of MTB with HIV accentuates the need for simple, rapid and affordable methods of anti-TB susceptibility testing, especially in resource constrained settings

- The resazurin microtitreplate assay (REMA) is an innovative colorimetric assay using redox indicator salts to detect MTB cell growth and viability in a week.

AIMS/OBJECTIVES

- To assess the performance of the REMA assay against the gold standard DST (drug susceptibility testing) method in a high TB burden reference laboratory.

RESULTS

Preliminary data:

- The sensitivity and specificity of the REMA was found to be 89% (24/27) and 88% (8/9) respectively for the diagnosis of MDR-TB

- Hundred percent of the fully susceptible MTB isolates were correctly detected when compared to the gold standard DST

- Time to detection was within a week of plate inoculation.

METHODS

- Forty well characterised MTB isolates comprising of **27 MDR-TB, 10 susceptible and 3 mono-resistant** were evaluated against 1st and 2nd line antituberculous drugs by the colorimetric method using the Resazurin Microtitre plate Assay (REMA)

- Isolates were characterised using the indirect susceptibility testing method on Middelbrook 7H10 (M7H10) agar [rifampicin (1ug/ml), isoniazid (0.2ug/ml), kanamycin (2ug/ml), moxifloxacin (2ug/ml)]

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- REMA testing was performed in a 96 well microplate containing Middelbrook 7H9 broth (M7H9) and antituberculous drugs [isoniazid (8.0-0.125ug/ml), rifampicin (8.0-0.25ug/ml), kanamycin (20-2.5ug/ml) and moxifloxacin (0.06-8.0ug/ml)]

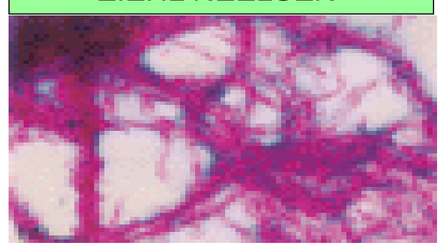
- Plates were incubated at 37°C for 8 days

METHODS

LABORATORY PROCEDURES

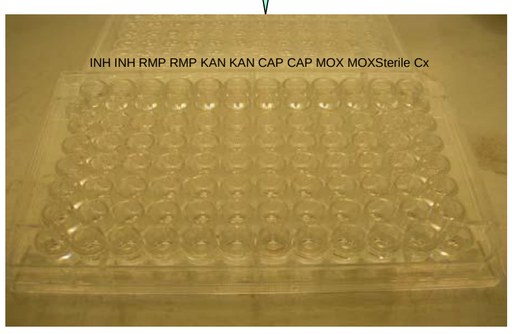
POSITIVE MGIT

ZIEHL NEELSEN

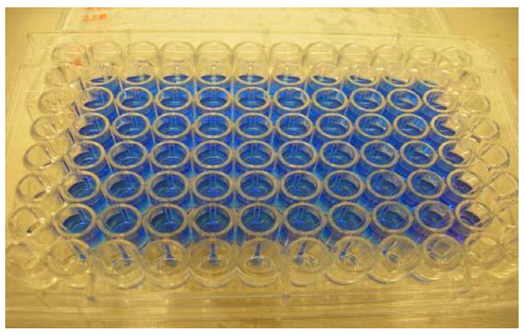


SUBCULTURE MIDDLEBROOK 7H11 AGAR

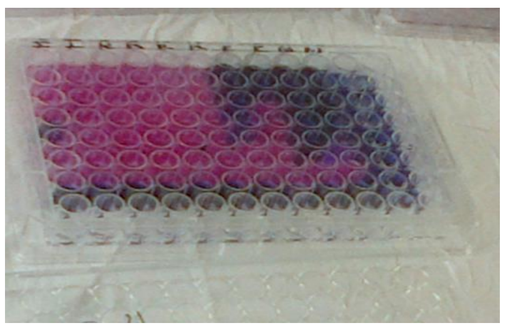
INDIRECT SUSCEPTIBILITY TESTING MIDDLEBROOK 7H10 AGAR



REMA plate containing M7H9 broth, inoculum and antibiotics



Addition of resazurin after 7 days of incubation



A colour change of blue to red was interpreted as a positive result (drug resistant) after addition of the resazurin

CONCLUSION

- The REMA study has shown to have good correlation and improved turnaround times in comparison to the conventional DST

- In a high throughput tuberculosis reference laboratory, the REMA has shown to be useful for the diagnosis of MDR-TB