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# Preliminary results from a new TREK Sensititre® *Mycobacterium tuberculosis* MIC plate (MYCOTB)

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## ABSTRACT

**Background:** With the global rise in *Mycobacterium tuberculosis (Mtb)* resistance, there is a need for a simple, rapid, quantitative susceptibility method for testing *Mtb* strains. The reference method for testing *Mtb* susceptibility is Agar Proportion (APM), which is a one or two -concentration breakpoint test (M24A). The Sensititre MYCOTB plate has been developed for susceptibility testing of *Mtb* to first and second line drugs. MYCOTB plate, a 96-well microtiter dry format, contains full dilution ranges for 12 antibiotics: Amikacin, Cycloserine, Ethambutol, Ethionamide, Isoniazid, Kanamycin, Moxifloxacin, Ofloxacin, Para-aminosalicylic acid, Rifabutin, Rifampin, Streptomycin.
**Methods:** The MYCOTB results were compared to APM using five *Mtb* and two *Mycobacterium* spp. other than tuberculosis (MOTT), all ATCC strains. A total of 40 data points were collected for each drug with five *Mtb* strains testing two lots of broth and plates in duplicate. Reproducibility testing involved four strains tested in triplicate for three days with separate McFarland suspensions per replicate and resulted in 36 data points per drug.
**Results:** Test results demonstrated a 100% categorical correlation between APM and MYCOTB Sensititre MIC results for each test organism. MIC results for four mycobacteria tested in triplicate for three days with separate McFarland suspensions showed good reproducibility (within a 2-fold dilution) from plate-to-plate. All MICs fell within expected in-house ranges for QC test organisms. Optimum growth for MYCOTB plate readings was between 10 and 14 days, whereas the APM read times were 14-21 days.
**Conclusions:** Initial testing of the MYCOTB plate shows good correlation with the APM, and appears to be a more rapid, quantitative susceptibility method for testing *Mtb* strains. Clinical trials are now being conducted with a wide variety of resistant *Mtb* strains to evaluate the performance of the plate.

## INTRODUCTION

WHO estimates that there are 2 billion people world-wide that are infected with *Mycobacterium tuberculosis (Mtb)* and that 2 million people die from the disease each year. There are 8 million new cases of tuberculosis, and in developing countries, MDR and XDR strains can represent up to 40% of all new cases. Therefore, a more rapid and accurate susceptibility test is of primary importance to identify the resistant strains and initiate proper treatment in a timely manner.

Previously, there was only one commercial format that reports minimal inhibitory concentration results (Etest) and no commercial microbroth dilution formats were available. The reference method for *Mtb* susceptibility testing is performed with one or two drug concentrations (break point agar proportion), and thus cannot determine emerging resistance or provide quantitative results that may aid in more appropriate drug treatments.

In November 2009, TREK Diagnostic Systems launched a new Sensititre MIC plate for the susceptibility testing of *Mtb*. The plate contains 12 first and second line drugs and has a minimum of 7 dilutions per drug. The plate is simple to prepare and inoculate and the plate can be read manually using a view box or the Sensititre Vizion® System with the SWIN software platform. Resistant results can be detected in as little as 7-10 days

The purpose of this preliminary study was to compare the MYCOTB plate to the reference agar proportion method (APM) and two lots each of MYCOTB plates and 7H9 broths with five strains of *M. tuberculosis*, and determine reproducibility between the lots using two ATCC isolates of *M. tuberculosis, M. avium* and *M. smegmatis.*

## METHODS AND MATERIALS

**Test strains.** Two different reproducibility experiments were performed. In the first experiment, five isolates of *Mycobacterium tuberculosis* were chosen from ATCC reference strains: *M. tuberculosis* ATCC 27294, 25177, 25618, 35828, and 35833. The second reproducibility experiment was performed with 4 mycobacterium strains: *M. tuberculosis* ATCC 27294 and 25177, *M. avium* ATCC 700898, and *M. smegmatis* ATCC 19420. Both experiments compared the MIC results with APM. With exception of isolate ATCC 35833, all *Mtb* strains were pan-susceptible. ATCC 35833 was resistant to Streptomycin.

**Agar Proportion Method.** APM plates were constructed using 24-well tissue culture plates for the drug testing and quadrant plates for the control agar testing. Figures 1 and 2 show the control and drug agar plates. Because of difficulty in counting the control wells, the controls were tested in quadrant plates, which have more surface area. Isolates were suspended to a turbidity equivalent to #0.5 McFarland in a saline tween solution containing glass beads. The final dilutions on the control plates were $10^{-2}$ and $10^{-4}$ and the drug testing was performed at $10^{-4}$ dilution. Plates were incubated at 34-36ºC and colonies were counted at 7, 10, 14 and 21 days. The percent resistant was determined by comparing the number of colonies on the control and drug wells. A strain was susceptible if the percent resistance was <=1%. A resistant strain had a calculated percent resistance of >1%. Figure 3 shows an INH resistant isolate.

## METHODS AND MATERIALS cont.

**Sensititre MYCOTB plates.** Isolates were inoculated using a manual pipettor to a turbidity equivalent to 0.5 McFarland standard in a saline Tween solution containing glass beads. From the saline Tween suspension, 100µl of the suspension was inoculated to a Middlebrook 7H9 (7H9) broth containing OADC. 100µl of the 7H9 broth was inoculated to all the plate wells. The plate was sealed with a plastic seal, disinfected (phenol-based compounds are appropriate, such as Amphyl) and placed in the incubator at 34-36ºC. Depending on the organism, plates were read for growth at 3, 7, 10, 14 or 21 days using the Vizion System. Culture purity was determined by inoculating 50 µl of the 7H9 broth to a Middlebrook 7H10 and blood agar plate and incubated for 3weeks and 48 h, respectively. Figure 4 shows a typical Vizion image for an isolate resistant to OFL, MFX, RIF, STR, RFB and INH.

**Reproducibility experiment 1.** Five strains of *Mtb* were chosen to test the reproducibility of the MYCOTB plate using two lots of MYCOTB plates and two different lots of 7H9 broth. The isolates were tested in duplicate on four separate days. Plates were read on 7, 10, 14 and 21 days and using the Vizion System. Vizion images were saved for later review. APM plates were tested in duplicate and the results were compared to the MYCOTB plate.

**Reproducibility experiment 2**. Four mycobacteria strains were chosen for this evaluation. Two strains were *Mtb* and two were mycobacteria other than Mtb (MOTT). Each strain was tested in triplicate, using a separate McFarland suspension, and tested on three separate days, resulting in 9 values per drug per organism. For the *Mtb* strains results were read on days 10 and 14. The plates were read on day 3 for *M. smegmatis* and day 7 and 10 for *M. avium*.

**Comparison of the APM and MYCOTB methods.** Table 1 shows the drug ranges for all drugs in the MYCOTB plate and the break point concentrations used for the APM method. The determination of S or R was performed by comparing the percent resistance from APM to the MIC value on the MYCOTB panel. A susceptible strain was one that had an MIC result of < = the breakpoint concentration, and resistant if the MIC was > than the breakpoint concentration. The methods were considered to be in agreement if the determination was either susceptible or resistant for both methods. Discrepant results occurred when one method was susceptible or resistant and the other method differed.

## RESULTS

**Reproducibility Experiment 1.** There was 100% agreement between the APM and MIC plate method. Additionally, the MIC spread was no more than +/- one well from the mid MIC value. Table 2 gives the mode and range.

**Reproducibility Experiment 2.** The results for the four test organisms are shown in table 3. Streptomycin and Cycloserine MIC points changed only one well higher with the *M. avium* reading at 10 days. Only Ethionamide had an increased range of 2.5- 40 µg/ml reading at day 10, whereas the range was 2.5-5 µg/ml at day 7. There was a 100% correlation between APM plates and the MIC results.

Comparing the times to results, the data for the MYCOTB plate for the *Mtb* strains could be read between 7 and 14 days, whereas the time was 14 to 21 days for the APM.

**Figure 1. Agar proportion control plate at $10^{-2}$ and $10^{-4}$ dilutions**

## RESULTS cont.

**Figure 2. Agar proportion antibiotic wells at $10^{-4}$ dilution.**

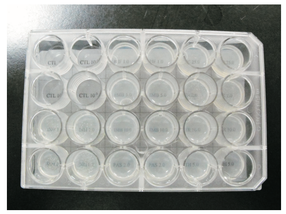


**Figure 3. Agar proportion plate with an INH resistant organism.**

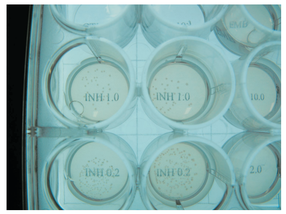



**Table 1. Drug Concentration comparisons for Sensititre *Mtb* plate and APM.**

| | | |
|---|---|---|
| Isoniazid (INH) | 0.03-4 | 0.2, 1.0 |
| Rifampin[1] (RIF) | 0.12-16 | 1.0 |
| Ethambutol (EMB) | 0.5-32 | 5.0, 10.0 |
| Ethionamide (ETH) | 0.3-40 | 5.0 |
| Kanamycin[2] (KAN) | 0.6-40 | 5.0 |
| Ofloxacin[3] (OFL) | 0.25-32 | 2.0 |
| p-Aminosalicylic acid (PAS) | 0.5-64 | 2.0 |
| Rifabutin[4] (RFB) | 0.12-16 | 0.5 |
| Streptomycin (STR) | 0.25-32 | 2.0, 10.0 |
| Cycloserine[5] (CYC) | 2.0-256 | 25.0 |
| Amikacin (AMI) | 0.12-16 | 5.0 |
| Moxifloxacin (MXF) | 0.06-8.0 | 2.0 |

1 - Rifampin class representative for Rifapentene
2 - Kanamycin is class representative for Amikacin
3 - Ofloxacin is the class representative for fluoroquinolones
4 - Some investigators include higher concentrations (1.0-2.0) but clinical relevance is unknown.
5 - Cycloserine break point was recommended by FDA

**Table 2. MIC results from the reproducibility testing with five *Mtb* strains (Results read at day 10).**

| Antibiotic | *M. tuberculosis* strains | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 25177 | | 27294 | | 25618 | | 35828 | | 35833 | |
| | Mode | Range | Mode | Range | Mode | Range | Mode | Range | Mode | Range |
| OFL | 0.5 | 0.5 | 0.5 | 0.5 | 1 | 0.5-1 | 0.5 | 0.5 | ≤0.25 | ≤0.25-0.5 |
| MFX | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06-0.12 | 0.12 | 0.12-0.25 | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06-0.12 |
| RIF | ≤0.12 | ≤0.12 | ≤0.12 | ≤0.12 | ≤0.12 | ≤0.12 | ≤0.12 | ≤0.12 | ≤0.12 | ≤0.12 |
| AMI | 0.5 | 0.5 | 0.5 | 0.5-1 | 0.5 | 0.5 | 0.5 | 0.25-0.5 | 0.25 | 0.25-0.5 |
| STR | 1 | 0.5-1 | 0.5 | 0.5-1 | 1 | 1 | 1 | 0.5-1 | >32 | >32 |
| RFB | ≤0.12 | ≤0.12 | ≤0.12 | ≤0.12 | ≤0.12 | ≤0.12 | ≤0.12 | ≤0.12 | ≤0.12 | ≤0.12 |
| PAS | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5 |
| ETH | 1.2-2.5 | 1.2-2.5 | 2.5 | 1.2-5 | 2.5 | 1.2-2.5 | 5 | 2.5-10 | 5-10 | 5-10 |
| CYC | 4 | 4-8 | 4 | 4-8 | 4 | 4 | ≤2 | ≤2 | 4 | 4 |
| INH | ≤0.03 | ≤0.03 | ≤0.03 | ≤0.03-0.06 | 0.06 | ≤0.03-0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| KAN | 2.5 | 2.5-5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 1.2-2.5 | 1.5 | 1.2 |
| EMB | 1 | ≤0.5-1 | 1 | 1-2 | 1 | 1 | 1 | 1 | 1 | ≤0.5-1 |

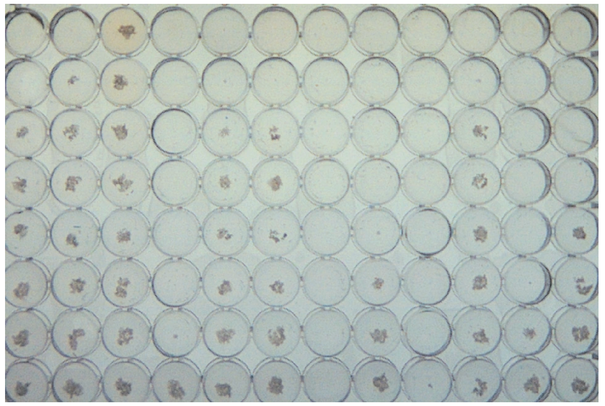
## RESULTS cont.

**Table 3. MIC results from the reproducibility testing with two *Mtb* strains and two MOTT strains.**

| Antibiotic | Mtb 25177[1] | | Mtb 27294[1] | | M. avium[2] 700898 | | M. smegmatis[3] 19420 | |
|---|---|---|---|---|---|---|---|---|
| | Mode | Range | Mode | Range | Mode | Range | Mode | Range |
| OFL | 0.5 | 0.5 | 0.5 | 0.5 | 16 | 16 | 0.5 | 0.5-1 |
| MFX | ≤0.06 | ≤0.06 | ≤0.06 | ≤0.06-0.12 | 1 | 1-2 | 0.12 | 0.12 |
| RIF | ≤0.12 | ≤0.12 | ≤0.12 | ≤0.12 | 2 | 1-2 | >16 | >16 |
| AMI | 0.5 | ≤0.12-0.5 | 0.5 | 0.5 | 4 | 4-8 | 0.5 | 0.5 |
| STR | 1 | 0.5-1 | 0.5 | 0.5 | 8 | 8 | 0.5-1 | 0.5-1 |
| RFB | ≤0.12 | ≤0.12 | ≤0.12 | ≤0.12 | 0.5 | 0.5 | 4 | 4 |
| PAS | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.5 | >64 | >64 | >64 | >64 |
| ETH | 2.5 | 1.2-2.5 | 2.5 | 1.2-5 | 2.5 | 2.5-5 | 5 | 5 |
| CYC | 4 | 4 | 4 | 4-8 | 32 | 32 | 32 | 32 |
| INH | ≤0.03 | ≤0.03-0.06 | ≤0.03 | ≤0.03 | >4 | 2->4 | 2 | 2-4 |
| KAN | 2.5 | 2.5-5 | 2.5 | 2.5 | 5 | 5-10 | 2.5 | 2.5 |
| EMB | ≤0.5 | ≤0.5-2 | 1 | 1 | 4 | 4 | 1 | 1 |

1 - Results are from a 10 day read
2 - Results are from a 7 day read
3 - Results are from a 3 day read

**Figure 4. Vizion image of a resistant XDR strain.**



OFL MXF RIF AMI STR RFB PAS ETH CYC INH KAN EMB

## CONCLUSIONS

- The Sensititre MYCOTB plate is a rapid, reproducible, and simple method to obtain quantitative susceptibility data for *M. tuberculosis* isolates .
- There was a 100% correlation between APM and MYCOTB plate results.
- Turn around time for the pan-susceptible strains of *Mtb* was 7-10 days for the MYCOTB plate and 14-21 days for APM. The one streptomycin resistant data point could be observed at 7 days.
- Clinical trials are currently ongoing to evaluate the MYCOTB plate comparing the reference APM method and more resistant *Mtb* strains.