Poster # P1896

Reproducibility of mycobacterial QC strains in the Sensititre® rapid growing and slowly growing nontuberculosis mycobacteria broth microdilution susceptibility procedure

Brimecombe, M., P. Stagg, G. Hammerton, S. Killian, C. Knapp and R. Grist
TREK Diagnostics Systems, East Grinstead, UK and Cleveland Ohio, USA

Trek Diagnostic Inc.
982 Keynote Circle, Suite 6
Cleveland, OH 44131
800.871.8909 • www.trekds.com

ABSTRACT

OBJECTIVE
Presently there are no NCCLS QC ranges for *M.avium* and only a few for rapid growing mycobacteria. The purpose of this study was to measure reproducibility with *M.peregrinum* ATCC 700686 , *M.smegmatis* ATCC 19420 and *M.avium* ATCC 700898 on commercial Sensititre dried susceptibility panels. For rapid growers, the effect of incubation time and the addition of saline / 0.2% tween with or without glass beads were also investigated. The antimicrobials evaluated were: Amikacin, Ofloxacin, Ansamycin, Streptomycin, Clofazimine, Kanamycin, Capreomycin, Ofloxacin, Rifampicin, Ethambutol and Isoniazid.
METHOD; rapid growers
A 0.5 McFarland standard was prepared from colonies on a 48hr old TSA / blood plate. Suspensions were vortexed for 5 minutes allowing large clumps to settle. 50 µL were transferred to 10 mL Sensititre Mueller Hinton broth. 100 µL were dosed into each well in the plate. Plates were sealed and incubated at 30°C. Plates were examined for visual growth after 72, 96 and 120 hours. Two sites tested 25 and 50 replicate MIC's for each isolate. An additional 10 results were collected for each of the three suspending media.
METHOD; slow growers
The set up procedure was the same except that 7H9 broth or Sensititre Mueller Hinton broth were supplemented with OADC. Plates were sealed and incubated at 35°C and read at 10 to 14 days depending upon extent of growth. A total of 50 replicates MICs were collected.
RESULTS; rapid growers
All onscale MICs fell within one doubling dilution of the mode. The maximum shift in MIC over time was one dilution. The presence of tween resulted in a one well shift in MIC of Ansmycin and Clofazimine with ATCC 19420 and Clofazimine with ATCC 700686.
RESULTS; slow growers
All MICs fell within 4 dilutions with the exception of Ofloxacin. Weak growth requiring 14 days incubation tended overall to give lower MICs but fell within the 4 well range.
CONCLUSION
MICs were highly reproducible allowing tentative QC ranges to be set for testing Sensititre plates. MICs were not affected by the length of incubation. Saline / tween showed notable affects on the MICs with two of the drugs with rapid growers.

INTRODUCTION

Mycobacterium avium Complex (MAC) causes a wide range of infections including disseminated disease in patients with AIDS and invasive pulmonary disease. The rapidly growing pathogenic mycobacteria cause several forms of disease of varying severity, most commonly skin and soft tissue infections but also skeletal, pulmonary and disseminated disease. Antimicrobial susceptibility testing of clinically significant isolates is recommended. NCCLS (1) has published a recommended procedure but there is currently only a limited number of drug QC ranges for testing rapidly growing pathogenic mycobacteria and MAC. Other antimicrobics have to be quality controlled with non-fastidious organisms listed in Table 3 of NCCLS M100-*Performance Standards for Antimicrobial Susceptibility testing* (2).

The interlaboratory reproducibility of Sensititre dried microdilution panels for susceptibility testing of rapidly growing mycobacteria (3) and *Mycobacterium avium* Complex (4) has been evaluated and shown to be acceptable. The purpose of this study was to measure reproducibility with rapid grower QC strains, *M.peregrinum* ATCC 700686, *M.smegmatis* ATCC 19420 and MAC QC strain *M.avium* ATCC 700898 on Sensititre dried susceptibility panels. For rapid growers, the effect of incubation time and the addition of saline / 0.2% Tween with or without glass beads to suspending media was also investigated. MAC testing compared MICs using Mueller Hinton broth with OADC compared to 7HSF broth.

METHODS

Rapid growing *Mycobacteria*
0.5 McFarland standard prepared from colonies on a 48hr old TSA / blood plate.

Suspensions vortexed in sterile water for 5 minutes allowing large clumps to settle

50 µL transferred to 10 mL Sensititre Mueller Hinton broth To obtain a final organism concentration of 1 X 10^5 CFU/mL to 5 X 10^5 CFU/mL

100 µL dosed into each well of the plate. Plates sealed and incubated at 30°C

Plates examined for visual growth after 72 hours.15 plates had incubation extended to 96 and 120 hours. Two sites collected 25 and 50 replicate results. Testing was performed over 5 days. An additional 10 results were collected from suspensions vortexed in saline containing 0.2% Tween 80 with or without glass beads.

Plates inoculated with suspensions prepared in Saline/Tween with or without beads were incubated for 72 hours

M.avium
The set up procedure same as for rapid growers except for use of 7H9 broth supplemented with 5% OADC (7HSF) or Sensititre Mueller Hinton broth pH 7.3 supplemented with 5% OADC (MHB-OADC)

Plates inoculated with MHB-OADC broth were sealed and incubated at 35°C and read at 10 or 14 days depending upon extent of growth. A total of 50 replicates from one site MICs were collected.

Ten plates dosed with 7HSF broth were read at 6 to 21 days.

RESULTS

Table 1. Effect of incubation time and suspension medium on reproducibility of *M. peregrinum* ATCC 700686 MICs

Antibiotic/Experimental variable	Number of results at MIC (µg/mL)				
Amikacin	0.25	0.5	1	2	4
72h		66	9		
96h		5	10		
120h		1	14		
Saline/Tween		10			
Saline/Tween beads		9	1		
Ansamycin	0.5	1	2	4	8
72h		1	46	28	
96h			4	11	
120h				15	
Saline/Tween			6	4	
Saline/Tween beads			6	4	
Capreomycin	1.25	2.5	5	10	20
72h	13	53	9		
96h		8	7		
120h			15		
Saline/Tween		5	5		
Saline/Tween beads		4	6		
Clofazamine	0.12	0.25	0.5	1	2
72h		45	25	5	
96h		7	3		
120h		1	12	2	
Saline/Tween	10				
Saline/Tween beads	8	2			
Ethambutol	2	4	8	16	32
72h	2	16	54	3	
96h			10	5	
120h			4	11	
Saline/Tween			10		
Saline/Tween beads			10		
Ethionamide	>20				
72h	75				
96h	15				
120h	15				
Saline/Tween	10				
Saline/Tween beads 10					
Isoniazid	>4				
72h	75				
96h	15				
120h	15				
Saline/Tween	10				
Saline/Tween beads 10					
Kanamycin	1.25	2.55	10	20	
72h	7	46	22		
96h		1	14		
120h			14	1	
Saline/Tween			10		
Saline/Tween beads			10		
Ofloxacin	≤0.25	0.5	1	2	4
72h	65	10			
96h	14	1			
120h	14	1			
Saline/Tween	10				
Saline/Tween beads	10				
Rifampicin	>16				
72h	75				
96h	15				
120h	15				
Saline/Tween	10				
Saline/Tween beads 10					
Streptomycin	16	32	>32		
72h	1	40	34		
96h		1	14		
120h			15		
Saline/Tween		4	6		
Saline/Tween beads		2	8		

Table 2. Effect of incubation time and suspension medium on reproducibility of *M. smegmatis* ATCC 19420 MICs

Antibiotic/Experimental variable	Number of results at MIC (µg/mL)				
Amikacin	0.12	0.25	0.5	1	2
72h		72	3		
96h		15			
120h		15			
Saline/Tween		10			
Saline/Tween beads		10			
Ansamycin	0.5	1	2	4	8
72h		2	72	1	
96h			14	1	
120h			13	2	
Saline/Tween	3	2	5		
Saline/Tween beads	1	5	4		
Capreomycin	0.06	1.25	2.5	5	10
72h		15	60		
96h		1	14		
120h			15		
Saline/Tween		4	6		
Saline/Tween beads		3	7		
Clofazamine	0.06	0.12	0.25	0.5	1
72h		22	47	6	
96h		2	12	1	
120h		1	12	2	
Saline/Tween	2	8			
Saline/Tween beads		10			
Ethambutol	≤ 0.5				
72h	75				
96h	15				
120h	15				
Saline/Tween	10				
Saline/Tween beads	10				
Ethionamide	20	>20	40	80	160
72h	16	59	11*	44*	
96h				43*	1*
120h				23*	21*
Saline/Tween	2	7			
Saline/Tween beads	5	5			
Note Different panel with extended concentration range to bring MICs onscale					
Isoniazid	0.5	1	2	4	>4
72h	1	34	24	16	
96h		10	5		
120h			14	1	
Saline/Tween	2	3			
Saline/Tween beads		4	1		
Kanamycin	≤ 0.06	1.25	2.5	1	2
72h	75				
96h	15				
120h	15				
Saline/Tween	10				
Saline/Tween beads	10				
Ofloxacin	0.25	0.5	1	2	4
72h		71	4		
96h		15			
120h		15			
Saline/Tween		10			
Saline/Tween beads		10			
Rifampicin	>16				
72h	75				
96h	15				
120h	15				
Saline/Tween	10				
Saline/Tween beads	10				
Streptomycin	0.25	0.5	1	2	4
72h		29	46		
96h		6	9		
120h		4	11		
Saline/Tween		9	1		
Saline/Tween beads			10		

Table 3 . Effect of incubation time and broth type on reproducibility of *M.avium* ATCC 700898 MICs

Broth	Incubation time (days)	Number of Amikacin MICs (µg/mL)				
		1	2	4	8	>8
MHB OADC	10			6	14	
MHB OADC	14	3	21	5	1	
7HSF	6			4	6	
7HSF	7				5	
7HSF	10				4	1
7HSF	14				4	1
7HSF	21				1	4
Broth	**Incubation time (days)**	**Number of Ansamycin MICs (µg/mL)**				
		0.06	0.12	0.25	0.5	1
MHB OADC	10			12	8	
MHB OADC	14			4	18	6
7HSF	6			10		
7HSF	7			4		
7HSF	10			1	4	
7HSF	14			1	3	1
7HSF	21				2	3
Broth	**Incubation time (days)**	**Number of Capreomycin MICs (µg/mL)**				
		5	10	20	>20	
MHB OADC	10			19	1	
MHB OADC	14	5	15	9		
7HSF	6		1	9		
7HSF	7		1	4		
7HSF	10			5		
7HSF	14			3	2	
7HSF	21				5	
Broth	**Incubation time (days)**	**Number of Clofazimine MICs (µg/mL)**				
		≤ 0.06	0.12	0.25	0.5	1
MHB OADC	10	9	11			
MHB OADC	14	30				
7HSF	6			1	9	
7HSF	7				5	
7HSF	10					5
7HSF	14					2
7HSF	21					
1@>4						
Broth	**Incubation time (days)**	**Number of Ethambutol MICs (µg/mL)**				
		4	8	16	32	64
MHB OADC	10	11	9			
MHB OADC	14	26	4			
7HSF	6				6	
7HSF	7		4	1		
7HSF	10			4		
7HSF	14			2		
7HSF	21			3		
Broth	**Incubation time (days)**	**Number of Ethionamide MICs (µg/mL)**				
		1.25	2.5	5	10	20
MHB OADC	10	1	12	6	1	
MHB OADC	14	2	12	15	1	
7HSF	6			1	1	2
7HSF	7	5				
7HSF	10			2		2
7HSF	14					2
7HSF	21					2
Broth	**Incubation time (days)**	**Number of Isoniazid MICs (µg/mL)**				
		1	2	4	>4	
MHB OADC	10				20	
MHB OADC	14				30	
7HSF	6				10	
7HSF	7				5	
7HSF	10				5	
7HSF	14				5	
7HSF	21				5	
Broth	**Incubation time (days)**	**Number of Kanamycin MICs (µg/mL)**				
		1.25	2.5	5	10	20
MHB OADC	10		1	19		
MHB OADC	14	3	6	19	2	
7HSF	6			7	3	
7HSF	7			3	2	
7HSF	10				5	
7HSF	14				5	
7HSF	21				4	1
Broth	**Incubation time (days)**	**Number of Ofloxacin MICs (µg/mL)**				
		2	4	8	16	>16
MHB OADC	10			1	4	16
MHB OADC	14	8	18	4		
7HSF	6		1	9		
7HSF	7		2	3		
7HSF	10			5		
7HSF	14			4	1	
7HSF	21			3	2	
Broth	**Incubation time (days)**	**Number of Rifampicin MICs (µg/mL)**				
		1	2	4	8	16
MHB OADC	10			12	8	
MHB OADC	14			4	18	8
7HSF	6	2	8			
7HSF	7	4	1			
7HSF	10		1	3	1	
7HSF	14			3	2	
7HSF	21			2	2	1
Broth	**Incubation time (days)**	**Number of Streptomycin MICs (µg/mL)**				
		2	4	8	16	32
MHB OADC	10			11	9	
MHB OADC	14	4	8	12	6	
7HSF	6		7	3		
7HSF	7		1	4		
7HSF	10			4	1	
7HSF	14			1	4	
7HSF	21				4	1

CONCLUSIONS

Rapid growers
Tables 1 and 2 show excellent reproducibility between sites

Onscale MICs fell within one doubling dilution of the mode with exception of Ethambutol with *M.peregrinum* ATCC 700686 (1.9% outliers) and Isoniazid with *M.smegmatis* ATCC 19420 (0.9% outliers)

Use of Saline and Tween to prepare inoculum resulted in a one well shift in the MIC for Anamycin and Clofazimine with *M.smegmatis* ATCC 19420 and Clofazimine with *M. peregrinum* ATCC 700686 on panels read after 72 hours incubation

Extending incubation to from 72 hours to 120 hours increased the modal MIC for all drugs giving onscale MICs by one dilution with *M.peregrinum* ATCC 700686. No shift was observed with *M.smegmatis* ATCC 19420 apart from Isoniazid.

Table 4 shows tentative QC ranges based upon combining data from Tables 1 and 2 with other published information.

Slow Growers
Onscale MICs fell within 4 dilutions with the exception of Ofloxacin with MHB-OADC broth when incubated for 10 to 14 days

Weak growth requiring 14 days incubation gave lower MICs but fell with the 4 well range for Amikacin, Capreomycin, Clofazimine, Ethambutol, Ofloxacin and Streptomycin

M.avium ATCC 700898 with 7HSF broth gave poorly reproducible results with Ethambutol, Clofazimine and Ethionamide

Table 4 shows tentative QC ranges based upon combining MHB-OADC data from Tables 3 with other published information.

Table 4 Tentative Quality Control Ranges for mycobacteria QC strains[1]

Antimicrobial agent	*M. peregrinum* ATCC 700686	*M. smegmatis* ATCC 19420	*M. avium* ATCC 700898
Amikacin	0.5 – 4	0.12 - 0.5	1 – 8
Ansamycin	1-8	1 – 4	–
Capreomycin	1.25 – 10	1.25 – 5	5 – 40
Cefoxitin	16 – 32*		
Ciprofloxacin	≤ 0.12 – 0.5*	0.25-1 **	
Clarithromycin	≤ 0.06 – 0.5*		
Clofazimine	0.25 – 1	0.12 - 0.5	–
Doxycycline	0.12 – 0.5*		
Ethambutol	2-16		1 – 8
Ethionamide	> 160	20-80****	1.25 – 10
Gatifloxacin	≤0.12 (3)	≤ 0.12 (3)	
Imipenem	2 – 16*		
Isoniazid		1–4	> 4
Kanamycin	1.25 – 10	≤ 0.06	1.25 – 10
Levofloxacin	0.12 – 0.5***	0.12 – 0.5***	
Linezolid	≤ 2 – 4*		
Ofloxacin	≤ 0.25 – 0.5	0.25 – 1	
Rifampicin	8-64	> 16	4 – 32
Streptomycin	16 – 64	0.5 – 2	2 – 16
Sulphamethoxazole	≤ 1 – 4*		
Tetracycline			
Tobramycin	4 – 8*		

Notes 1 Ranges based on Sensititre in-house testing
* NCCLS M24-A QC range (1).
** Range based on data from Reference 5
*** Range based on data from Reference 6
**** Incubation time dependent. Range based on 72 hours incubation only.

REFERENCES

1. National Committee for Clinical Laboratory Standards. 2003. Susceptibility Testing of Mycobacteria, Nocardiae and other Aerobic Actinomycetes; Approved Standard. M23-A. National Committee for Clinical Laboratory Standards, Wayne. Pa
2. National Committee for Clinical Laboratory Standards. 2004. Performance Standards for Antimicrobial Susceptibility Testing; Fourteenth Informational Supplement. M100-S14. National Committee for Clinical Laboratory Standards, Wayne. Pa
3. Woods,G.A. *etal.* 1999. Multisite Reproducibility of results Obtained by the Broth Microdilution Method for Susceptibility Testing of *Mycobacterium abscessus*, *Mycobacterium chelonae* and *Mycobacterium fortuitum.* J. Clin. Microbiol 37:1676-1682
4. Woods,G.A. *etal.* 2003. Multisite Reproducibility of results Obtained by Two Broth Dilution Methods for Susceptibility Testing of *Mycobacterium avium* Complex. J. Clin. Microbiol 41: 627-631
5. Brown-Elliott, B; Wallace, R; Crist, C; Mann, L; and Wilson, R. (2002). Comparison of *In Vitro* Activities of Galtifloxacin and Ciprofloxacin against Four Taxa of Rapidly Growing Mycobacteria. *Antimicrobial Agents and Chemotherapy* 46: 3283-3285
6. Wallace. R; Brown-Elliott, B; Crist, C; Mann, L; and Wilson, R. (2002). Comparison of *In Vitro* Activities of Gatifloxacin and Ciprofloxacin against Four Taxa of Rapidly Growing Mycobacteria. Interscience Conference on Antimicrobial Agents and Chemotherapy Abstract E-541