Paper # **C-020**

Mycobacterium peregrinum ATCC 700686 Susceptibility Testing: A Multi Site Evaluation to Establish Microbroth Dilution Quality Control (QC) Ranges for 5 Antimicrobial Agents

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ABSTRACT

Background: The purpose of this study was to determine the QC ranges for 5 antimicrobial agents used in the treatment of rapidly growing mycobacteria infections against the QC organism, *M.peregrinum* ATCC 700686.The frozen reference broth microdilution (BMD) method, with plates produced by TREK Diagnostic Systems, Cleveland, OH was used. The criteria utilized were based on the NCCLS M23 document.
Methods: The NCCLS M24 method was used as the BMD method in this study; using cation adjusted Mueller Hinton Broth (CAMHB) and incubating for 72 hrs. in ambient air at 30ºC. Eight laboratories performed the BMD method on 10 different replicates of the QC strain over several days. The isolates were tested against cefoxitin, linezolid, meropenem, ertapenem and tobramycin on 3 different lots of CAMHB.
Results: The BMD QC ranges were established and included greater than 95% of the observed values. The proposed BMD QC ranges are as follows: cefoxitin, 4-16µg/ml, linezolid, 1-8µg/ml, meropenem, 2-8µg/ml, ertapenem, 8-32µg/ml, and tobramycin, 2-8µg/ml.
Conclusions: Of the 5 proposed BMD QC ranges, 3 drugs (cefoxitin, linezolid and tobramycin) were re-tested, following the NCCLS M23, to extend their earlier published QC ranges and meropenem will be added to table 9 in the NCCLS M24 standard.

OBJECTIVE

In reviewing the CLSI (NCCLS) standard M24-A, it was observed that cefoxitin, tobramycin and linezolid only had 2 well quality control ranges for testing ATCC 700686 *Mycobacterium peregrinum*. Quality control limits for meropenem also needed to be established.

PURPOSE OF THE STUDY

The purpose of this study was to conduct a multi laboratory trial to evaluate quality control ranges for *Mycobacterium peregrinum* ATCC 700686 with 5 antimicrobial agents for the microbroth dilution method using the CLSI M23 document.

MATERIALS

Testing was performed at 8 different sites:

- ARUP Research Institute
- TREK Diagnostic Systems
- Cleveland Clinic Foundation
- Wisconsin State Lab
- Lab Corp
- Mayo Clinic
- Winnipeg-Canadian Science Center for Human and Animal Health
- University of Texas Health Center

Quality Control Strains

Mycobacterium peregrinum ATCC 700686
Staphylococcus aureus ATCC 29213

- **The frozen reference microdilution plates were prepared by TREK Diagnostic Systems, Cleveland, OH, as per the CLSI M7 standard.**

3 Different Lots of Mueller Hinton Broth

Supplied by Becton Dickinson (Sparks, MD)

Lot #1- 3126205 DIFCO
Lot #2- 3311419 DIFCO
Lot #3- 2218968 BBL

5 Antimicrobial Agents

Antimicrobials	Range Tested (µg/ml)	Supplied By
Cefoxitin	0.5-64	**Sigma-Aldrich** St. Louis, Missouri
Linezolid	0.5-8	**Pfizer Pharmaceuticals** New York, New York
Meropenem	0.015-32	**Astra Zeneca** Wilmington, Delaware
Tobramycin	0.06-16	**Sigma-Aldrich** St. Louis, Missouri
Ertapenem	0.03-64	**Merck & Co., Inc.** North Wales, PA

METHODS

- The study design followed the CLSI M23 guidelines for establishing quality control ranges. These studies evaluate the reproducibility of inter to intra laboratory testing and between reagent lots.[2]
- The MIC plates were inoculated, incubated, and read according to the CLSI M24-A section 5 and M7-A6 standards.[1,3]
- Colony counts were performed for each ATCC Quality Control strain per day of testing as per the protocol.
- Data analysis for establishing the expected ranges was based on 95% of the results included in the proposed range

RESULTS

- For MIC QC ranges, a 3 dilution range is preferred. However, in order to obtain 95% of the values included, a four dilution range may be needed.
  - Based on the results from the study, the proposed ranges for ATCC 700686 *M. peregrinum* were 4-32 µg/ml cefoxitin, 1-8 µg/ml linezolid, 2-16 µg/ml meropenem, 8-32 µg/ml ertapenem, and 2-8 µg/ml tobramycin. The distribution of results can be observed in Tables 2-6.
  - The MIC values for tobramycin, linezolid, and cefoxitin were all expanded from their original 2 well range published in CLSI document M24-A Table 9.
- The 8 laboratory study also tabulates the inter and intra laboratory performances for the five different antimicrobials along with variations in the 3 different reagent lots of Mueller Hinton Broth. This can be observed in Table 1.

Table 1. Distribution of Quality Control (QC) MICs Tested with 3 Different Reagent Lots of Mueller Hinton Broth

M. peregrinum ATCC 700686

Method/ Result MIC (µg/ml)	Cefoxitin Lot # 1	2	3	Linezolid Lot # 1	2	3	Meropenem Lot # 1	2	3	Tobramycin Lot # 1	2	3	Ertapenem Lot # 1	2	3
0.015															
0.03															
0.06															
0.12															
0.25															
0.5															
1					1										
2				40	27	39			2	9	19	5			
4				38	44	29	52	50	36	66	60	66			
8	60	42	43	2	8	12	28	30	42	5	1	9	17	17	20
16	20	38	37										62	62	59
32													1	1	1
64															
128															

Table 2. Inter and Intra Laboratory comparisons of the Cefoxitin* MIC results versus *M. peregrinum* ATCC 700686 for eight test sites

Laboratory code (occurrences)

MIC (µg/ml)	A	B	C	D	E	F	G	H	Total
0.5									
1									
2									
4									
8	19	22	20	24	20	12	22	5	144
16	11	8	10	6	10	18	8	25	96
32									
64									
128									
Total	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**240**
MODE	**8**	**8**	**8**	**8**	**8**	**16**	**8**	**16**	**8**
Range	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**

*100% of results within proposed QC Range (4-32 µg/ml)

RESULTS cont.

Table 3. Inter and Intra Laboratory comparisons of the Linezolid* MIC results versus *M. peregrinum* ATCC 700686 for eight test sites

Laboratory code (occurrences)

MIC (µg/ml)	A	B	C	D	E	F	G	H	Total
0.5									
1		3		1					4
2	11	20	26	26	24	2		7	116
4	14	7	4	2	6	24	28	22	107
8	8					4	2	1	15
Total	**33**	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**242**
MODE	**4**	**2**	**2**	**2**	**2**	**4**	**4**	**4**	**2**
Range	**3**	**3**	**3**	**3**	**2**	**3**	**2**	**3**	**4**

*100% of results within proposed QC Range (1-8 µg/ml)

Table 4. Inter and Intra Laboratory comparisons of the Meropenem* MIC results versus *M. peregrinum* ATCC 700686 for eight test sites

Laboratory code (occurrences)

MIC (µg/ml)	A	B	C	D	E	F	G	H	Total
0.5									
1									
2		2							2
4	23	20	21	27	18	2	26	7	144
8	7	8	9	3	12	28	4	23	94
16									
32									
Total	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**240**
MODE	**4**	**4**	**4**	**4**	**4**	**8**	**4**	**8**	**4**
Range	**2**	**3**	**2**	**2**	**2**	**2**	**2**	**2**	**3**

*100% of results within proposed QC Range (2-16 µg/ml)

Table 5. Inter and Intra Laboratory comparisons of the Tobramycin* MIC results versus *M. peregrinum* ATCC 700686 for eight test sites

Laboratory code (occurrences)

MIC (µg/ml)	A	B	C	D	E	F	G	H	Total
0.06									
0.12									
0.25									
0.5									
1									
2		16		3	7	5	2		33
4	30	14	30	25	22	24	28	19	192
8				2	1	1		11	15
16									
Total	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**240**
MODE	**4**	**2**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Range	**1**	**2**	**1**	**3**	**3**	**3x**	**2**	**2**	**3**

*100% of results within proposed QC Range (2-8 µg/ml)

RESULTS cont.

Table 6. Inter and Intra Laboratory comparisons of the Ertapenem* MIC results versus *M. peregrinum* ATCC 700686 for eight test sites

Laboratory code (occurrences)

MIC (µg/ml)	A	B	C	D	E	F	G	H	Total
0.03									
0.06									
0.12									
0.25									
0.5									
1									
2									
4									
8	14	18		15		1	2	4	54
16	16	12	30	15	29	29	28	24	183
32					1			2	3
64									
Total	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**240**
MODE	**16**	**8**	**16**	**16**	**16**	**16**	**16**	**16**	**16**
Range	**2**	**2**	**1**	**2**	**2**	**2**	**2**	**3**	**3**

*100% of results within proposed QC Range (8-32 µg/ml)

CONCLUSIONS

- The results from this 8 laboratory study were adequate to provide acceptable ranges for 4 of the 5 antimicrobials tested. These approved ranges are listed below:

Quality Control Ranges of Minimal Inhibitory Concentrations (MICs) (µg/ml) for *M. peregrinum* ATCC 700686 and *S. aureus* ATCC 29213 when Testing Rapidly Growing Mycobacteria

Antimicrobial Agent	MIC Range (µg/ml) for *M. peregrinum* ATCC 700686	MIC Range (µg/ml) for *S. aureus* ATCC 29213
Cefoxitin	4-32	1-4
Linezolid	1-8	1-4
Meropenem	2-16	0.03-0.12
Tobramycin	2-8	0.12-1
*Ertapenem	8-32	N/A

*Not included in Table 9 in the M24 supplement: It was decided by the subcommittee not to include ertapenem (8-32 µg/ml) QC ranges in the approved table 9 supplement for M24 since ertapenem is not normally utilized for treatment of patients.

- Data analysis for establishing the expected ranges is based on 95% of the values being included in the proposed ranges.
- All of the generated MIC values were incorporated within the recently approved ranges. There were no occurances noted between labs or reagent lots.

REFERENCES

1. National Committee for Clinical Laboratory Standards. 2003. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-sixth edition.* Approved document M7-A6. Wayne, PA: NCCLS
2. National Committee for Clinical Laboratory Standards. 2001. *Development of In Vitro susceptibility testing criteria and quality control parameters; approved guideline second edition.* Approved document M23. Wayne, PA: NCCLS
3. National Committee for Clinical Laboratory Standards. 2003. *Susceptibility testing of Mycobacteria, Nocardiae, and other aerobic actinomycetes; approved standard.* Approved document M24-A. Wayne, PA: NCCLS