

Quality Control Parameters for TR-700 Broth Microdilution Susceptibility Tests

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Abstract

Background: TR-701, a promising new oxazolidinone, is a prodrug under development by Trius Therapeutics; the active moiety is TR-700. TR-700 has activity against Gram-positive microorganisms. **Methods:** An eight-laboratory study was conducted to generate data to determine quality control (QC) limits for five standard QC organisms when testing susceptibility to TR-700 by the broth microdilution method. Three different lots of Mueller-Hinton broth were used. The broth was supplemented with 3% lysed horse blood for testing *S. pneumoniae*. All susceptibility tests were performed by methods outlined by the CLSI. Each laboratory performed 30 MIC determinations for each QC strain. **Results:** Colony counts ranged from 1.5 X 10⁴ to 2.0 X 10⁶. Significant lot-to-lot variation was not observed. All tests were very reproducible and the following quality control limits are proposed:

Organism (ATCC)	Proposed QC Limits (µg/ml)	% in Range
<i>S. aureus</i> (29213)	0.25 – 1	99.2%
<i>E. faecalis</i> (29212)	0.25 - 1	100%
<i>S. pneumoniae</i> (49619)	0.12 – 0.5	99.6%

Conclusions: Microbroth dilution quality control ranges are proposed for TR-700 against 3 quality control strains recommended by the CLSI. These ranges have been proposed and accepted by the CLSI Subcommittee on Antimicrobial Susceptibility Testing.

Introduction

TR-700 (Figure 1.) is a new oxazolidinone antimicrobial currently under clinical development. In the course of our in vitro evaluations, a collaborative study was undertaken to define quality control parameters for broth microdilution susceptibility tests. Linezolid was used as the control drug for the MIC study. The participating laboratories were (in alphabetical order):

Director	Institution & City
D. Bade	Microbial Research Inc., Fort Collins, CO
S. Brown	Clinical Microbiol. Inst., Wilsonville, OR
J. Daly	Primary Children's Med. Ctr., Salt Lake City, UT
G. Hall	Cleveland Clinic Foundation, Cleveland, OH
D. Hardy	Univ. of Rochester Med. Ctr., Rochester, NY
J. Hindler	UCLA, Los Angeles, CA
C. Knapp	TREK Diagnostic Systems, Cleveland, OH
R. Rennie	U. of Alberta Hospital, Alberta, Canada

Materials & Methods

- An 8-laboratory broth microdilution susceptibility study was performed exactly as outlined by CLSI documents M7-A¹ and M23-A2².
- Three different lots of cation adjusted Mueller-Hinton broth (CAMHB) were used. The media was made up as plain CAMHB or as CAMHB + 3% lysed horse blood.
- Serial dilutions of the control drug, linezolid, were prepared in one lot of the same media.
- TR-700 (Lot #DP-70-146-5/wt) was obtained from Trius Therapeutics, Inc.
- Linezolid (Lot#1000891018) was obtained from Pfizer Pharmaceuticals, Inc and used as the control drug.
- On separate days of testing, each of the control strains was inoculated into microbroth dilution MIC trays providing 3 TR-700 MICs and 1 control drug MIC.
- MIC quality control ranges were selected as recommended by the Clinical & Laboratory Standards Institute document M23-A2² (2001).
- Proposed ranges were confirmed using the Rangefinder Method of Turnidge and Bordash³.

Results

Figure 1. Structure of TR-700, the active moiety of TR-701.

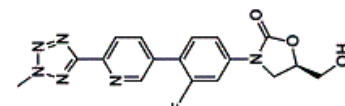


Table 1. Statistical Analysis Using the RangeFinder³ Method.

TR700 MIC QC	<i>Staphylococcus aureus</i> ATCC 29213	<i>Enterococcus faecalis</i> ATCC 29212	<i>Streptococcus pneumoniae</i> ATCC 49619
	Calculated QC Range	Calculated QC Range	Calculated QC Range
	0.25 to 1	0.25 to 1	0.125 to 0.5
Dilution Range	Dilution Range	Dilution Range	
	3	3	3
% Obs. Captured	% Obs. Captured	% Obs. Captured	
	99.2%	100.0%	99.6%

Figure 2. *S. aureus* ATCC 29213

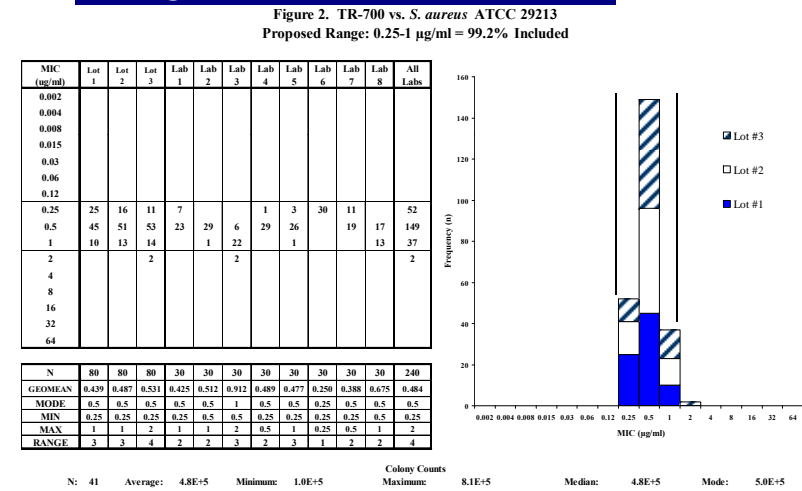


Figure 3. *E. faecalis* ATCC 29212

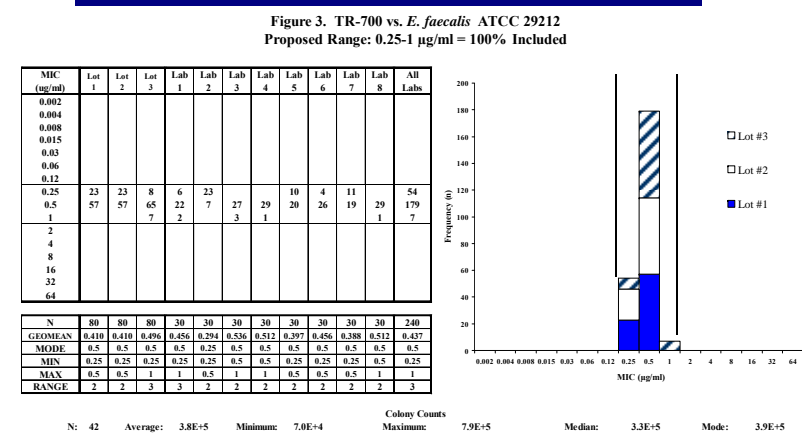
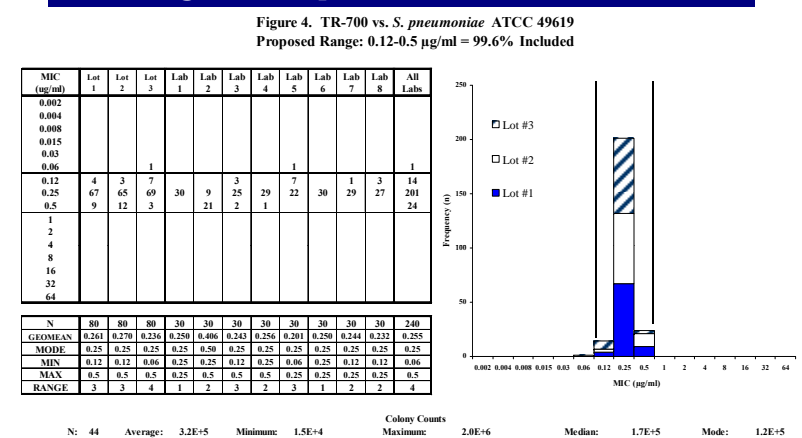


Figure 4. *S. pneumoniae* ATCC 49619



Results

- Figures 2-4 show the breakdown of the individual test values.
- No significant lot-to-lot variability in Mueller-Hinton base media was observed with any of the broth media. The mode for each lot was essentially the same.
- There were no instances in which the values observed for the control drug exceeded the CLSI recommended quality control ranges.
- The proposed MIC quality control ranges are presented in Table 2.

Conclusions

Table 2. Proposed Broth Microdilution Quality Control Ranges for TR-700

Control Strain	Proposed QC Limits (µg/ml) (% Included)
<i>S. aureus</i> ATCC 29213	0.25 to 1 (99.2%)
<i>E. faecalis</i> ATCC 29212	0.25 to 1 (100%)
<i>S. pneumoniae</i> ATCC 49619	0.12 to 0.5 (99.6%)

- Under CLSI standard test conditions following the M23-A2 approved guidelines, TR-700 yielded reproducible susceptibility results for all three aerobic quality control strains.
- Data from this study were presented to the CLSI AST Subcommittee and all ranges approved fell within the required ≥95% of data for all 8 laboratories.
- The approved QC ranges will be published when TR-700 receives a generic name.

References

- Clinical and Laboratory Standards Institute. *Methods for dilution antimicrobial susceptibility tests for Bacteria that Grow Aerobically; Approved Standard-7th Edition.* CLSI Document M7-A7. 2006.
- Clinical and Laboratory Standards Institute. *In Vitro Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline--2nd Edition.* CLSI Document M23-A2. 2001.
- Turnidge, J and G Bordash. 2007. *Statistical Methods for Establishing Quality Control Ranges for Antibacterial Agents in Clinical and Laboratory Standards Institute Susceptibility Testing.* Antimicrob. Agents. Chemother. 51(7):2483-2488.

Acknowledgement

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