

Establishment of Quality Control Ranges for Testing the Susceptibility of Target Organisms to Tedizolid by Disk Diffusion

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Abstract

Background: Tedizolid (TR-700), formerly known as torezolid, is an oxazolidinone with potent activity against gram-positive organisms currently undergoing Phase 3 trials for the treatment of acute bacterial skin and skin structure infections (ABSSSI). It is important to have available reliable methods to evaluate in vitro activity against target pathogens and clinical isolates. The feasibility of susceptibility testing TR-700 by disk diffusion has been established based on correlation of disk zones to broth microdilution MICs. This study reports quality control (QC) ranges to be used for disk diffusion susceptibility testing of TR-700 during development and post-approval.

Methods: QC ranges for the disk diffusion testing of TR-700 disks (20 micrograms) were determined per CLSI guidelines (M23-A3). Nine separate laboratories each tested 10 independent replicates of each relevant ATCC QC organism (*S. aureus* ATCC 25923 and *S. pneumoniae* ATCC 49619) across three lots of media from different manufacturers against two disk lots of TR-700 (one from MAST and one from BioRad). One disk lot of linezolid was tested as an in class control. Statistical methods (Gavin statistic and Rangefinder) were used to evaluate the data and generate ranges suitable for their overall performance in the current study are shown in the table below.

Results: QC ranges approved for the disk diffusion testing of TR-700 and their overall performance in the current study are shown in the table below:

Organism	QC range (mm)	# mm	Total N	n (%) in range
<i>S. aureus</i> ATCC 25923*	22-29	8	540	538 (99.6)
<i>S. pneumoniae</i> ATCC 49619	24-30	7	540	533 (98.7)

*read with transmitted light

The above ranges take into account intra-laboratory, intra-media lot, and intra-disk lot variation.

Conclusions: The CLSI approved ranges are suitable for QC of TR-700 disks at the indicated disk mass. The performance of these ranges should be continually monitored during disk testing that takes place as part of the clinical development of TR-700 going forward.

Background

- Tedizolid (TR-700), formerly known as torezolid, is a novel oxazolidinone with potent activity against key gram-positive pathogens and is currently undergoing clinical development by Trius Therapeutics for the treatment of acute bacterial skin and skin structure infections (ABSSSI).
- As part of the tedizolid development plan, and post approval use, it is anticipated that TR-700 will be tested by both broth microdilution methods and by disk diffusion.
- To ensure consistency and accuracy of disk testing quality control ranges for testing TR-700 are required
- This study presents the findings of the CLSI M23 disk diffusion quality control study that was done to establish appropriate zone diameter ranges for the appropriate ATCC strains

Methods

- This study was strictly conducted according to guidelines provided in CLSI M23-A3, *Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters: Approved Guideline 3rd Edition*
- Overall:
 - Nine laboratories participated
 - Each site used three agar lots of each medium type for the test drug TR-700 and three agar lots for the control agent (linezolid)
 - Each site used two TR-700 disk lots and a single linezolid disk lot
 - Overall, testing resulted in 540 disk zone diameter values for TR-700 an 270 for linezolid against each organism tested TR-700
- Organisms tested: *S. aureus* ATCC 25923 and *S. pneumoniae* ATCC 49619

Methods

- Media Lots-Agar
 - Mueller Hinton agar plates used:
 - BD/BBL Lot 1047275
 - Teknova Lot M002524C1101
 - Remel Lot 017484
 - Mueller Hinton agar with lysed sheep's blood (*S. pneumoniae*):
 - BD/BBL Lot 1055217
 - Teknova Lot M12329C1101
 - Remel Lot 017483
- Disk Lots
 - TR-700 (20 mg):
 - BioRad Lot 1C0010
 - MAST Lot 275702
 - Linezolid (30 mg):
 - BD/BBL Lot 1026509

Results

Table 1. CLSI approved QC ranges for the disk diffusion testing of TR-700

Organism	QC range (mm)	# mm	Total N	n (%) in range
<i>S. aureus</i> ATCC 25923*	22-29	8	540	538 (99.6)
<i>S. pneumoniae</i> ATCC 49619	24-30	7	540	533 (98.7)

*read with transmitted light

Table 2. TR-700 disk zones against *S. aureus* ATCC 25923 across laboratories, media lots, and disk lots

Zone Diameter (mm)	Laboratory (incidence):										Media Lot Code (incidence):		Disk Lot Code (incidence):		Total
	A	B	C	D	E	F	G	H	I	Lot A	Lot B	Lot A	Lot B		
19															
20															
21															
22															
23															
24															
25															
26															
27															
28															
29															
30															
31															
32															
33															
Total	60	60	60	60	60	60	60	60	60	180	180	180	270	270	540
Median	26	24	26	24	26	25	24.5	24	27	25	26	25	25	25	25
Mode	26	25	26	24	26	25	24	24	28	25	26	25	25	26	25
Mean	26.5	24.2	26.2	24.2	26.2	25.4	24.9	24.0	27.1	24.9	25.9	25.5	25.4	25.4	25.4
Range	6	5	6	5	6	6	6	5	7	7	9	8	9	9	9

Gavin statistic: All Lab median = 25
 Median of Ranges (MR) = 6
 1/2 MIR rounded up (R) = 3
 All Lab Median +/- R = 22 - 28
 Rangefinder: 22 - 29

Table 3. TR-700 disk zones against *S. pneumoniae* ATCC 25923 across laboratories, media lots, and disk lots

Zone Diameter (mm)	Laboratory (incidence):										Media Lot Code (incidence):		Disk Lot Code (incidence):		Total
	A	B	C	D	E	F	G	H	I	Lot A	Lot B	Lot A	Lot B		
21															
22															
23															
24															
25															
26															
27															
28															
29															
30															
31															
32															
33															
Total	60	60	60	60	60	60	60	60	60	180	180	180	270	270	540
Median	26	24	26	24	26	25	24.5	24	27	25	26	25	25	25	25
Mode	26	25	26	24	26	25	24	24	28	25	26	25	25	26	25
Mean	26.5	24.2	26.2	24.2	26.2	25.4	24.9	24.0	27.1	24.9	25.9	25.5	25.4	25.4	25.4
Range	6	5	6	5	6	6	6	5	7	7	9	8	9	9	9

Gavin statistic: All Lab median = 27
 Median of Ranges (MR) = 6
 1/2 MIR rounded up (R) = 3
 All Lab Median +/- R = 24 - 30
 Rangefinder: 24 - 31

FIGURE 1. Distribution of disk zone results overall, by media lot, and by disk lot against *S. aureus* ATCC 25923. Dashed lines indicate CLSI approved QC range.

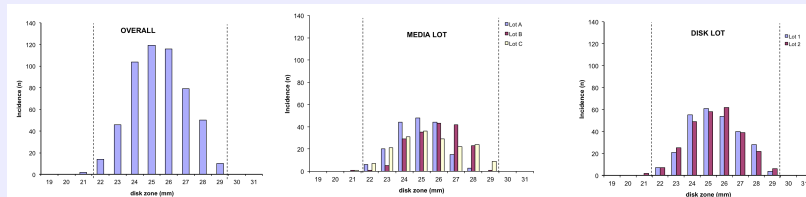
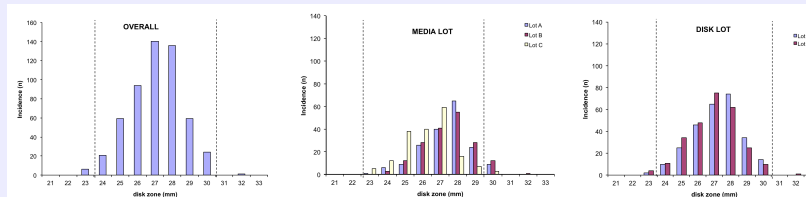


FIGURE 2. Distribution of TR-700 disk zone results overall, by media lot, and by disk lot against *S. pneumoniae* ATCC 49619. Dashed lines indicate CLSI approved QC range.



Conclusions

- The results of this study, which followed strict CLSI M23 Guidelines for QC range development, established reproducible quality control ranges for disk diffusion testing of TR-700 against target gram-positive pathogens.
- These ranges will be used to monitor disk test results throughout continued development of TR-700, and the results obtained throughout all phases of development will be analyzed to determine if the currently established ranges need any alteration.
- Until that time these TR-700 QC ranges should be used to monitor the disk diffusion testing performance in any laboratory testing this compound.

Acknowledgement

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