

Multi-Site Evaluation of Eravacycline MIC Test Strip (MTS) Compared To Broth Microdilution MICs

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Abstract:

Background: MIC Test Strips (MTS, Liofilchem, Roseto degli Abruzzi, Italy) consist of specialized paper impregnated with a pre-defined concentration gradient of an antimicrobial agent, which is used to determine the minimum inhibitory concentration against bacteria as tested on agar media using overnight incubation and manual reading procedures. Eravacycline (ERV) was recently approved in the U.S. for the treatment of patients 18 years of age and older with complicated intra-abdominal infection caused by the following susceptible microorganisms: *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, *Streptococcus anginosus group*, *Clostridium perfringens*, *Bacteroides species*, and *Parabacteroides distasonis*. This study was performed to evaluate the performance of ERV MTS compared to a broth microdilution method (BMD) for FDA 510(k) submission.

Methods: Clinical and challenge isolates were tested by ERV BMD with frozen panels (according to CLSI M7-A11 and M100-S28) and by ERV MTS. Clinical isolates were collected and tested at 3 sites, 10 reproducibility isolates/agent were shared and tested in triplicate on 3 days at each of the 3 sites and challenge isolates were tested at 1 site. Challenge isolates included a majority with MIC results near or above the susceptible breakpoint. The organism species and total number of strains are shown in the results table. QC strains (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212) were tested a minimum of 20 times by each site.

Results: As shown in the table, ERV MTS MIC results for consolidated clinical and challenge organisms were within +/- one doubling dilution (essential agreement) of BMD MIC results for >90% of isolates.

Organism	N	% Essential Agreement	% Category Agreement
Enterobacteriaceae	426	99.5	97.4
<i>S. aureus</i>	240	93.8	100
<i>E. faecalis</i>	134	94.0	99.3
<i>E. faecium</i>	154	90.3	99.4

For reproducibility strains, 100% of ERV MTS results for Enterobacteriaceae and 98.9% of Gram-positive isolates were within a doubling dilution of BMD results. All MTS and BMD QC results were within CLSI ranges.

Conclusion: The ERV MTS performed similar to the reference broth microdilution method against study isolates and QC strains. The ERV MTS received clearance by FDA, Center for Devices and Radiological Health, for testing of relevant Enterobacteriaceae species, *S. aureus*, *E. faecalis* and *E. faecium*.

Introduction

- Liofilchem (Roseto degli Abruzzi, Italy) manufactures MIC Test Strips (MTS) for a variety of antimicrobial agents, including eravacycline. The Liofilchem MIC Test Strip is a quantitative agar-based diffusion assay for determining the minimum inhibitory concentration (MIC).
- This study was performed as part of a 510(k) study (for "in vitro diagnostic use" label in the U.S.)
- This study compared the eravacycline (ERV) MTS MIC to broth microdilution MIC for the clinically indicated organisms and for additional Enterobacteriaceae species included in "in vitro only list" of the ERV label (*Citrobacter koseri* and *Klebsiella (Enterobacter) aerogenes*).

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References:

- Clinical and Laboratory Standards Institute. 2018. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 11th ed. Approved standard, CLSI M7-11, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2018. Performance Standards for Antimicrobial Susceptibility Testing. Approved Standard – 28th Edition. CLSI document M100-28, Wayne, PA
- http://www.liofilchem.net/en/mov/mic_test_strip.php

Methods

Study Strains (Total clinical isolates collected at three sites and challenge isolates)

Organism Group	Clinical/site	Challenge
<i>S. aureus</i> (MSSA)	134	1
<i>S. aureus</i> (MRSA)	61	44
<i>E. faecalis</i> (VSE)	90	14
<i>E. faecalis</i> (VRE)	29	1
<i>E. faecium</i> (VSE)	86	17
<i>E. faecium</i> (VRE)	37	14
<i>C. freundii</i>	30	5
<i>C. koseri</i>		2
<i>E. coli</i>	120	17
<i>E. cloacae</i>	45	16
<i>K. aerogenes</i>	10	10
<i>K. pneumoniae</i>	111	30
<i>K. oxytoca</i>	30	10
TOTAL	773	181

At least 25% of clinical isolates were recently collected (within 6 months).

Among the 181 challenge strains tested, there were 45 Gram-negative and 23 Gram-positive organisms with resistance mechanisms that were molecularly characterized.

QC strains:

S. aureus ATCC 29213
E. faecalis ATCC 29212
E. coli ATCC 25922
P. aeruginosa ATCC 27853

Reproducibility isolates:

20 isolates (species as shown in Figure 2) were tested by ERV MTS at each of the 3 testing sites in triplicate on three separate days for a total of 27 results/reproducibility isolate

Testing sites:

- Laboratory Specialists, Inc. (LSI), Westlake, OH (Challenge strains only)
- University of Rochester Medical Center, Rochester, NY
- Wake Forest Baptist Medical Center, Winston-Salem, NC
- Santa Maria Hospital, Reggio Emilia, Italy

MIC methods:

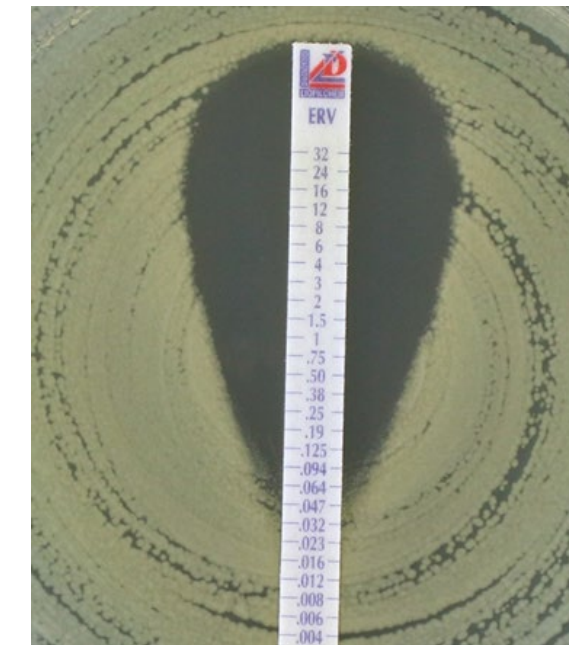
- Each isolate was tested once by broth microdilution according to CLSI method (1) with frozen panels containing eravacycline concentrations of 0.002-32 µg/mL and by eravacycline MTS (ERV) (3) containing concentrations of 0.002-32 µg/mL (Liofilchem, Roseto degli Abruzzi, Italy) on Mueller Hinton Agar II plates (MHA from Becton Dickinson [Sparks, MD]). Quality control strains were tested each day of testing and a total of 20 replicates/site were tested.
- MTS results were rounded up to next doubling dilution for analysis. MIC results were interpreted according to FDA breakpoints. Essential agreement (EA; MTS results +/- 1 dilution of reference MIC) and category agreement (MTS and BMD susceptible/resistant result agreement) were determined.

Results

- Quality Control** (Table 1): All ERV BMD and MTS MIC results for all QC strains were within the CLSI expected ranges.
- Reproducibility** (Table 2): 100% and 98.9% of Gram-negative and Gram-positive consolidated ERV MTS results were within +/-1 doubling dilution of modal MIC.
- Clinical & Challenge**
 - Enterobacteriaceae:** MTS MIC results were within +/- 1 doubling dilution for 424/426 isolates, which included 2/2 *C. koseri*, 35/35 *C. freundii*, 61/61 *E. cloacae*, 137/137 *E. coli*, 10/10 *K. aerogenes*, 38/40 *K. oxytoca* and 141/141 *K. pneumoniae*. Category agreement was 97.4%, major error rate was 1.3% and very major error rate was 9.3%. Due to the lack of an intermediate category for eravacycline, testing of *K. pneumoniae* and *E. cloacae* has resulted in 6 very major errors that are otherwise within essential agreement of the reference method. Given this, the very major error rate of 9.3% (7/75) is adjusted to 1.3% (1/75) if calculated to exclude the errors that are within essential agreement.
 - S. aureus*:** MTS MIC results were within +/- 1 doubling dilution for 225/240 isolates, which included 123/135 MSSA and 102/105 MRSA. Category agreement was 100%. All 14 results that were outside of EA were from one laboratory. Replicate data from the initial test site and a second site (Table 3) show that repeat results were within EA for 8 isolates tested by the initial site and for 12 isolates tested by a second site.
 - Enterococcus spp.*:** MTS MIC results were within +/- 1 doubling dilution for 265/288 isolates, which included 126/134 *E. faecalis* and 139/154 *E. faecium*. Category agreement was 99.3% and major error rate was 0.8%. All 15 results for *E. faecium* that were outside of EA were from one laboratory. Replicate data from the initial test site and a second site (Table 3) show that repeat results were within EA for 13 isolates tested by the initial site and for all 15 isolates tested by a second site.

Conclusions

- The essential and category agreement rates for ERV MTS against all Gram-negative and Gram-positive species was above the 90% acceptance criteria.
- There was also a tendency for one dilution lower ERV MTS results compared to BMD MIC results, particularly for *S. aureus* and *Enterococcus spp.* Due to the tendency of ERV MTS to give MICs one dilution lower than BMD and additionally because of the lack of an intermediate category, there is a potential for very major errors.
- Site specific issues with lower MTS results for *S. aureus* and *Enterococcus spp.* were improved after retesting and taking care in the plate inoculation step to assure more confluent growth.
- The ERV MTS was cleared for in vitro diagnostic use by the FDA, with limitations regarding very major error rate for *K. pneumoniae* and *E. cloacae* and trend for lower MICs for *S. aureus* and *E. faecium*.



E. coli
 Eravacycline
 MIC = 0.047 µg/mL,
 reported as 0.06 µg/mL

Table 1. Eravacycline BMD and MTS Quality Control Results by Testing Site

QC Organism	MIC µg/mL	Reference BMD				MTS			
		Site 1	Site 2	Site 3	All Sites	Site 1	Site 2	Site 3	All Sites
<i>E. coli</i> ATCC 25922	0.016								
	0.03					1			1
	0.06	2	21	17	40	18	21	18	57
	0.12	18		3	21	1		2	3
	0.25								
<i>P. aeruginosa</i> ATCC 27853	1								
	2	8	1		9	6			6
	4	11	19	19	49	12	3	16	31
	8	1	1	1	3	2	16	4	22
	16						2		2
<i>S. aureus</i> ATCC 29213	0.008								
	0.016					1	1		2
	0.03	1	3		4	17	12		29
	0.06	18	15	17	50	2	7	17	26
	0.12	1	2	3	6			3	3
<i>E. faecalis</i> ATCC 29212	0.008								
	0.016		1		1	12			12
	0.03	18	13	5	36	8	8	6	22
	0.06	2	6	15	23			14	26
	0.12								

Shaded area represents expected QC range

Table 2. Eravacycline MTS MIC reproducibility results

Reproducibility Strain No., Species	Difference in the number of doubling dilutions between test result and test mode						Test Mode
	Off-Scale	-2	-1	0	1	2	
Gram Negative Organisms							
R1, <i>E. coli</i>				23	4		0.06
R2, <i>E. coli</i>				26	1		0.06
R3, <i>E. coli</i>				24	3		0.12
R5, <i>K. pneumoniae</i>			2	24	1		0.25
R6, <i>K. pneumoniae</i>			6	19	2		0.5
R7, <i>K. pneumoniae</i>				15	12		0.5
R8, <i>E. cloacae</i>			1	23	3		0.25
R9, <i>E. cloacae</i>			10	16	1		0.25
R11, <i>K. oxytoca</i>			4	23			1
R15, <i>C. freundii</i>			3	24			0.5
Total	0	0	26	217	27	0	0
Between-site Reproducibility				270/270 = 100%			
Gram Positive Organisms							
R1, <i>S. aureus</i>				10	11	6	0.03
R2, <i>S. aureus</i>				10	17		0.03
R3, <i>S. aureus</i>				8	19		0.03
R4, <i>S. aureus</i>				7	20		0.03
R13, <i>E. faecalis</i>				7	18	2	0.06
R14, <i>E. faecalis</i>					15	12	0.016
R15, <i>E. faecalis</i>				7	14	6	1
R18, <i>E. faecium</i>			3	11	13		0.03
R19, <i>E. faecium</i>			1	16	10		0.016
R20, <i>E. faecium</i>			3	21	3		0.03
Total	0	3	64	164	39	0	0
Between-site Reproducibility				267/270 = 98.9%			

Table 3. Eravacycline MTS and BMD Dilution Difference for Select Isolates, Initial & Repeat Results

Organism	Site, Replicate	Dilution Difference (MTS-BMD MIC)			
		-2	-1	0	1
<i>E. faecium</i>	Site 1, Initial	15			
	Site 1, Repeat	2	12	1	
	Site 2, Repeat		8	7	
<i>S. aureus</i>	Site 1, Initial	14			
	Site 1, Repeat	6	8		
	Site 2, Repeat	2	8	4	

Note: Only initial results were included in the 510(k)

Figure 1. Eravacycline MTS MIC compared to BMD MIC for 426 Enterobacteriaceae (number of results at each MIC)

MTS Results	Reference Results														
	≤0.002	0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	≥32
≤0.002															
0.004															
0.008															
0.016															
0.03					1	1									
0.06						23	13	1							
0.12						2	93	30		1					
0.25							7	104	22						
0.5								15	35	6					
1									4	17	2				
2										3	18	1			
4											1	16	3		
8												1	6		
16															
≥32															
Overall EA				424 / 426		99.5%									
EA (evaluable results)				424 / 426		99.5%									
Category Agreement				415 / 426		97.4%									
Category Major				4 / 351		1.1%									
Errors Very Major				7 / 75		9.3%									

Figure 2. Eravacycline MTS MIC compared to BMD MIC for 240 S. aureus (number of results at each MIC)

MTS Results	Reference Results														
	≤0.002	0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	≥32
≤0.002															
0.004															
0.008					1	3									
0.016						55	11								
0.03							12	54							