510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K182557

B. Purpose for Submission:

To obtain a substantial equivalence determination for Eravacycline (ERV) at concentrations of 0.002-32 µg/mL for susceptibility testing of non-fastidious Gram-negative and non-fastidious Gram-positive organisms

C. Measurand:

Eravacycline 0.002-32 µg/mL

D. Type of Test:

Quantitative Antimicrobial Susceptibility Test growth-based detection

E. Applicant:

Liofilchem s.r.l.

F. Proprietary and Established Names:

MTS Eravacycline 0.002-32 µg/mL

G. Regulatory Information:

1. Regulation section:

   866.1640 Antimicrobial Susceptibility Test Powder

2. Classification:

   II

3. Product code:

   JWY - Manual Antimicrobial Susceptibility Test Systems

4. Panel:

83 – Microbiology

H. Intended Use:

1. Intended use(s):

The Liofilchem MTS (MIC Test Strip) Eravacycline 0.002-32 µg/mL is a quantitative method intended for the *in vitro* determination of antimicrobial susceptibility of bacteria. MTS consists of specialized paper impregnated with a pre-defined concentration gradient of an antimicrobial agent, which is used to determine the minimum inhibitory concentration (MIC) in µg/mL of antimicrobial agents against bacteria as tested on agar media using overnight incubation and manual reading procedures.

The MTS Eravacycline at concentrations of 0.002-32 µg/mL should be interpreted at 16-20 hours of incubation.

MTS Eravacycline can be used to determine the MIC of eravacycline against the following bacteria. Eravacycline has been shown to be active both clinically and *in vitro* against these bacterial species according to the FDA drug approved label:

Gram-positive bacteria:
Enterococcus faecalis
Enterococcus faecium
Staphylococcus aureus

Gram-negative bacteria:
Citrobacter freundii
Enterobacter cloacae
Escherichia coli
Klebsiella oxytoca
Klebsiella pneumoniae

Eravacycline has been shown to be active *in vitro* only against the non-fastidious bacteria below according to the FDA drug approved label:

Citrobacter koseri
Klebsiella (Enterobacter) aerogenes

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

- *For prescription use*

- *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. If critical to patient care, testing should be repeated using an alternative testing/reference method prior to reporting results when the MTS Eravacycline is 0.5 µg/mL for K. pneumoniae and E. cloacae.*

- *Resistance mechanism characterization was not available for all organisms at the time of comparative testing, and therefore the performance of the MTS Eravacycline for non-fastidious gram-negative bacilli and gram-positive cocci is unknown for the following: Enterobacteriaceae [tet(B)]; Enterococcus species [tet(K)].*

- *The ability of the MTS to detect non-susceptible isolates with the following drug/bacterial species combinations is unknown because non-susceptible isolates were either not available or an insufficient number was encountered at the time of comparative testing.*
  Eravacycline: Citrobacter koseri

- *A trend towards lower MIC readings was observed in the overall performance of S. aureus and E. faecium, although no very major errors were reported. However, due to the lack of an intermediate category for eravacycline and the observed trending, there is a concern for the potential of very major errors. If critical to patient care, testing should be repeated using an alternative testing/reference method prior to reporting results for S. aureus and E. faecium, when the MTS Eravacycline is 0.06 µg/mL.*

- *The safety and efficacy of eravacycline in treating clinical infections due to Gram-negative organisms other than C. freundii, E. cloacae, E. coli, K. oxytoca and K. pneumoniae and Gram-positive organisms other than Enterococcus faecalis, E. faecium, and S. aureus may not have been established in adequate and well-controlled clinical trials. The clinical significance of susceptibility information in such instances is unknown.*

4. Special instrument requirements:

   Manual reading only

I. Device Description:

The Eravacycline MIC Test Strip (MTS) consists of specialized paper impregnated with a predefined concentration gradient of eravacycline across 15 two-fold dilutions similar to dilutions used by conventional MIC methods. One side of the strip is labelled with the Eravacycline code (ERV) and the MIC reading scale in µg/mL. When the MIC Test Strip is

applied onto an inoculated agar surface, the preformed exponential gradient of antimicrobial agent is immediately transferred to the agar matrix. After 16-20 hours incubation, a symmetrical inhibition ellipse centered along the strip is formed. The MIC is read directly from the scale in terms of µg/mL at the point where the edge of the inhibition ellipse intersects the MIC Test Strip. Since MTS strip generates MIC values which fall between two-fold dilutions for interpretation, the MIC value read is recorded to the next two-fold dilution value.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Liofilchem MTS, vancomycin

2. Predicate 510(k) number(s):

   K153687

3. Comparison with predicate:

Table 1: Comparison with the Predicate Device

Similarities		
Item	**Device Liofilchem MTS, Eravacycline (K182557)**	**Predicate Liofilchem MTS, vancomycin (K153687)**
Media	Mueller Hinton agar	Same
Inoculation	Isolated colonies from culture in suspension equivalent to 0.5 McFarland. Inoculum is applied manually using the manual plate inoculation method or plate rotator for even distribution of inoculum	Same
Result	MIC	Same

Differences		
Item	**Device Liofilchem MTS, Eravacycline (K182557)**	**Predicate Liofilchem MTS, vancomycin (K153687)**
Intended Use	Quantitative susceptibility to antimicrobial agents against Gram-negative and Gram-positive organisms	Quantitative susceptibility to antimicrobial agents to Gram-positive organisms
Antibiotic	Eravacycline code (ERV)	Vancomycin code (VA)
Reading	Manual; the point where the edge of inhibition ellipse intersects the MIC Test Strip; interpret the MIC as 80% inhibition when trailing is seen	Manual; the point where the edge of inhibition ellipse intersects the MIC Test Strip; interpret the MIC at 100% inhibition
Incubation	35 ± 2°C for 16 - 20hrs	35 ± 2°C for 24 hours

K. Standard/Guidance Document Referenced:

- Guidance for Industry and FDA - Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems – August 28, 2009.
- CLSI M07-A10 "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard, Tenth Edition January 2015".
- CLSI M100-S26 "Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement, January 2016".

L. Test Principle:

MTS are made of specialized paper impregnated with a predefined concentration gradient of antibiotic, across 15 two-fold dilutions similar to dilutions used by conventional MIC methods. When the MIC Test Strip is applied onto an inoculated agar surface, the preformed exponential gradient of antimicrobial agent is immediately transferred to the agar matrix. After 16-20 hours incubation, a symmetrical inhibition ellipse centered along the strip is

formed. The MIC is read directly from the scale in terms of µg/mL at the point where the edge of the inhibition ellipse intersects the strip MIC Test Strip.

Growth along the entire gradient (i.e., no inhibition ellipse) indicates that the MIC value is greater than or equal to (≥) the highest value on the scale. An inhibition ellipse that intersects below the lower end of the scale is read as less than (<) the lowest value. An MIC of 0.125 µg/mL is considered to be the same as 0.12 µg/mL for reporting purposes. Given that eravacycline is a bacteriostatic drug, the ellipse should be interpreted at 80% inhibition when trailing is observed.

An MTS MIC value which falls between standard two-fold dilutions must be rounded up to the next standard upper two-fold value before categorization.

M. Performance Characteristics:

1. Analytical performance:

   a. Precision/Reproducibility:

   Reproducibility testing was conducted at three sites using ten Gram-negative and ten Gram-positive organisms. Each isolate was tested in triplicate over three days. The mode of MIC values was determined for each isolate and the reproducibility was calculated based on the number of MIC values that fell within ±1 doubling dilution of the mode. The Gram-negative reproducibility panel included three *E. coli,* three *K. pneumoniae*, two *E. cloacae*, one *K. oxytoca*, and one *C. freundii* isolate and the Gram-positive panel included four *S. aureus*, three *E. faecalis*, and three *E. faecium* isolates. All MIC results were on scale. The testing resulted in overall reproducibility of greater than 95% for both Gram-negative and Gram-positive isolates.

   The results were acceptable.

   b. Linearity/assay reportable range:

   Not applicable

   c. Traceability, Stability, Expected values (controls, calibrators, or methods):

   Quality Control (QC) Testing:

   The CLSI recommended QC strains, namely *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 for Gram-negative organisms and *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 for Gram-positive organisms were tested a sufficient number of times (i.e., at least 20/site) at each testing site using both MTS and reference methods. The results are summarized in Table 2 below. The quality control results are acceptable.

Table 2: Eravacycline MTS QC Results

Organism	Concentration (µg/mL)	Reference	MTS
E. coli ATCC 25922 Expected Result: 0.03 – 0.12 µg/mL	0.015		
	0.03		1
	0.06	40	57
	0.12	21	3
	0.25		
P. aeruginosa ATCC 27853 Expected Result: 2 - 16 µg/mL	1		
	2	9	6
	4	49	31
	8	3	22
	16		2
	32		
S. aureus ATCC 29213 Expected Result: 0.016 – 0.12 µg/mL	0.008		
	0.016		2
	0.03	4	29
	0.06	50	26
	0.12	6	3
	0.25		
E. faecalis ATCC 29212 Expected Result: 0.016 – 0.06 µg/mL	0.008		
	0.016	1	12
	0.03	36	22
	0.06	23	26
	0.12		

Inoculum Density Check:
The inoculum was prepared to achieve turbidity equivalent to a 0.5 McFarland standard. Colony counts were performed periodically at each site for all QC replicates, from at least one replicate of each reproducibility isolate on each of the three days of testing, and from a minimum of 10% of the clinical and challenge strains tested. Inoculum density checks were performed and the colony counts obtained for each isolate were within the recommended range of approximately 1x 10^8 CFU/mL.

Purity Checks:
Purity checks were performed on all isolates following MTS inoculation. All isolates were pure in both the broth microdilution reference panels and the MTS agar plates.

Growth Failure Rate:
None of the isolates in the study failed to grow with the Eravacycline MTS.

d. Detection limit:

Not Applicable

e. Analytical specificity:

Not Applicable

f. Assay cut-off:

Not Applicable

2. Comparison studies:

a. Method comparison with predicate device:

The MTS, Eravacycline was evaluated at three sites located within the United States. Each clinical isolate was tested one time by MTS, Eravacycline and the reference method using the same initial standardized suspension. A total of 437 clinical non-fastidious Gram-positive isolates of which 76.9% were tested within six months of isolation and 336 non-fastidious Gram-negative isolates were tested of which 67.6% were tested within six months of isolation. The Gram-positive organisms included 134 methicillin-susceptible *S. aureus*, 61 methicillin-resistant *S. aureus*, 90 vancomycin-susceptible *E. faecalis*, 29 vancomycin-resistant *E. faecalis*, 86 vancomycin-susceptible *E. faecium*, and 37 vancomycin-resistant *E. faecium* isolates. The Gram-negative organisms included 30 *C. freundii*, 45 *E. cloacae*, 120 *E. coli*, 30 *K. oxytoca*, and 111 *K. pneumoniae* isolates.

Challenge testing was performed at one internal site. A total of 91 Gram-positive and 90 Gram-negative challenge isolates were tested. The Gram-positive organisms included one methicillin-susceptible *S. aureus*, 44 methicillin-resistant *S. aureus*, 14 vancomycin-susceptible *E. faecalis*, one vancomycin-resistant *E. faecalis*, 17 vancomycin-susceptible *E. faecium*, and 14 vancomycin-resistant *E. faecium* isolates. The Gram-negative organisms included 5 *C. freundii*, 16 *E. cloacae*, 17 *E. coli*, 10 *K. oxytoca*, 30 *K. pneumoniae,* two *C. koseri*, and 10 *K. aerogenes* isolates.

Results obtained with the Liofilchem MIC Test Strip (MTS), Eravacycline were compared to results obtained with the CLSI broth microdilution reference panel. The reference panel contained two-fold serial dilutions of eravacycline with a range of 0.002 – 32 μg/mL. The testing conditions for the reference method were consistent with CLSI guidelines as listed in the CLSI document M07-A10. Isolated colonies from an overnight blood agar plate were suspended in saline to achieve a 0.5 McFarland standard turbidity (approximately 10^8 CFU/mL). Testing conditions consisted of incubation of the inoculated Mueller Hinton agar plates in an inverted position at 35°C ± 2° for 16-20 hours. At the end of incubation, the MIC value determined at 80% inhibition of growth along the strip was compared to MIC results obtained with the reference method. Visual aids were provided in reading guides to each laboratory to assist in interpretation.

The performance for the total (combined clinical and challenge) 528 Gram-positive and 426 Gram-negative isolates is summarized in Table 3 and 4 below.

Table 3: Overall Performance of Gram-positive Clinical and Challenge Isolates

Eravacycline	EA Tot	EA N	EA %	Eval. EA Tot	Eval. EA N	Eval. EA%	CA N	CA%	#NS	maj	vmj
S. aureus (MSSA+MRSA) Clinical	195	181	92.8	195	181	92.8	195	100	8	0	0
Challenge	45	44	97.8	45	44	97.8	45	100	33	0	0
Combined	240	225	93.8	240	225	93.8	240	100	41	0	0
E. faecalis (VSE+VRE) Clinical	119	111	93.3	119	111	93.3	118	99.2	9	1	0
Challenge	15	15	100	15	15	100	15	100	9	0	0
Combined	134	126	94	134	126	94	133	99.3	18	1	0
E. faecium (VSE+VRE) Clinical	123	108	87.8	123	108	87.8	122	99.2	11	1	0
Challenge	31	31	100	31	31	100	31	100	13	0	0
Combined	154	139	90.3	154	139	90.3	153	99.4	24	1	0
Gram-positive (all) Clinical	437	400	91.5	437	400	91.5	435	99.5	28	2	0
Challenge	91	90	98.9	91	90	98.9	91	100	55	0	0
Combined	528	490	92.8	528	490	92.8	526	99.6	83	2	0

Table 4: Overall Performance of Gram-negative Clinical and Challenge Isolates

Eravacycline	EA Tot	EA N	EA %	Eval. EA Tot	Eval. EA N	Eval. EA%	CA N	CA%	#NS	maj	vmj
Enterobacteriaceae (all) Clinical	336	334	99.4	336	334	99.4	326	97	21	4	6
Challenge	90	90	100	90	90	100	89	98.9	54	0	1
Combined	426	424	99.5	426	424	99.5	415	97.4	75	4	7

EA – Essential agreement
CA – Category agreement
EVAL – Evaluable isolates
NS – Non-susceptible isolates
maj – Major errors
vmj – Very major errors

Essential Agreement (EA) is when the Liofilchem MIC Test Strip (MST) results agree exactly or within one doubling dilution of the reference broth microdilution results. Category Agreement (CA) is when the Liofilchem MIC Test Strip (MST) result interpretation agrees exactly with the reference broth microdilution result interpretation.

The overall performance of all Gram-positive isolates is acceptable with 92.8% EA and 99.6% CA. There were two major errors (0.4%) reported, however, both results were within essential agreement of the reference method result. In addition, there were no very major errors.

The overall performance of all *Enterobacteriaceae* isolates is acceptable with 99.5% EA and 97.4% CA. There were four major errors (1.4%) reported, however, all four results were within essential agreement of the reference method result. In addition, there were seven very major errors (9.3%) reported, however, six of those seven very major errors, which consisted of five *K. pneumoniae* and one *E. cloacae* isolate, were within essential agreement of the reference method result. Given that the errors were within essential agreement of the reference method result, the adjusted major error rate is 0% and adjusted very major error rate is 1.3% (1/75) and therefore, acceptable. Furthermore, given that there is no intermediate breakpoint for eravacycline and high very major error rate for *Enterobacteriaceae,* the following limitation was included in the package labeling to instruct the end-user to retest isolates that have a result of 0.5 µg/mL.

- *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. If critical to patient care, testing should be repeated using an alternative testing/reference method prior to reporting results when the MTS Eravacycline is 0.5 µg/mL for K. pneumoniae and E. cloacae.*

Resistance Mechanisms:

Molecular characterization of challenge isolates was provided with respect to tetracycline-specific resistance mechanisms to include efflux mediated *tet*(a), *tet*(B), and *tet*(K) and ribosomal protection encoded by *tet*(M) and *tet*(Q). Specifically, 11 *K. pneumoniae* and 2 *E. cloacae* encoding *tet*(A) were evaluated in which all isolates were found to be non-susceptible to eravacycline by both the MTS strip and reference method except for one *K. pneumoniae* isolate. In addition, one *S. aureus* isolate encoding *tet*(k) was evaluated and was found to be non-susceptible. Furthermore, three *S. aureus* and one *E. faecalis* isolate encoding *tet*(M) were evaluated and all were found to be non-susceptible to eravacycline. Given that some claimed organisms with their associated resistance mechanisms were not available during the time of testing, the following limitation was included in the labeling:

- *Resistance mechanism characterization was not available for all organisms at the time of comparative testing, and therefore the performance of the MTS Eravacycline for non-fastidious gram-negative bacilli and gram-positive cocci is unknown for the following: Enterobacteriaceae [tet(B)]; Enterococcus species [tet(K)].*

Trending:

Trending was assessed separately for Gram-positive and Gram-negative groups using data for challenge and clinical isolates (Tables 5 and 6). No trending was observed for Gram-negative isolates; however, trending was observed for *S. aureus* and *E. faecium*

which tended to be in exact agreement or lower when compared to the reference method indicating a potential for the occurrence of very major errors due to the absence of an intermediate breakpoint. The difference between higher and lower dilutions for these organisms was ≥30%. Given the lack of intermediate category and observed trending, the following limitation was included in the labeling to instruct users should they receive a result of 0.06 µg/mL (susceptible breakpoint):

- *A trend towards lower MIC readings was observed in the overall performance of S. aureus and E. faecium, although no very major errors were reported. However, due to the lack of an intermediate category for eravacycline and the observed trending, there is a concern for the potential of very major errors. If critical to patient care, testing should be repeated using an alternative testing/reference method prior to reporting results for S. aureus and E. faecium, when the MTS Eravacycline is 0.06 µg/mL.*

Table 5. Trending for Gram-positive Organisms by Species

Total	≥2 dil. lower	1 dil. lower	Exact	1 dil. higher	≥2 dil. higher
***S. aureus* (MSSA+MRSA)[a]**					
240	15	141	83	1	0
	(65.0%)		(34.58%)	(0.42%)	
***E. faecalis* (VSE+VRE)[b]**					
134	8	38	79	9	0
	(34.33%)		(58.96%)	(6.72%)	
***E. faecium* (VSE+VRE)[c]**					
154	15	64	72	3	0
	(51.3%)		(46.75%)	(1.95%)	
All Gram-positive[d]					
528	38	243	281	13	0
	(53.22%)		(53.22%)	(2.46%)	

[a]Difference between the higher and lower dilutions for *S. aureus* is: -64.58%; 95% C.I. (-70.35% to -58.07%)

[b]Difference between the higher and lower dilutions for *E. faecalis* is: -27.61%; 95% C.I. (-36.56% to -18.28%)

[c]Difference between the higher and lower dilutions for *E. faecium* is: -49.35%; 95% C.I. (-57.22% to -40.72%)

[d]Difference between the higher and lower dilutions for all Gram-positive organisms is: -50.76%; 95% C.I. (-55.10% to -46.17%)

Table 6. Trending for *Enterobacteriaceae* by Species

Total	≥2 dil. lower	1 dil. lower	Exact	1 dil. higher	≥2 dil. higher
***C. freundii*[a]**					
35	0	6	26	3	0
	(17.14%)		(74.29%)	(8.57%)	
***C. koseri*[b]**					
2	0	0	2	0	0
	(0%)		(100%)	(0%)	

***K. aerogenes*[c]**					
10	0	0	7	3	0
	(0%)		(70.0%)	(30.0%)	
***E. cloacae*[d]**					
61	0	14	47	0	0
	(22.95%)		(77.05%)	(0%)	
***E. coli*[e]**					
137	0	31	103	3	0
	(22.63%)		(75.18%)	(2.19%)	
***K. oxytoca*[f]**					
40	2	9	26	3	0
	(27.5%)		(65.0%)	(7.5%)	
***K. pneumoniae*[g]**					
141	0	18	102	21	0
	(12.77%)		(72.34%)	(14.89%)	
All *Enterobacteriaceae*[h]					
426	2	78	313	33	0
	(18.78%)		(73.47%)	(7.75%)	

[a]Difference between the higher and lower dilutions for *C. freundii* is: -8.57%; 95% C.I. (-25.09% to 7.93%)
[b]Difference between the higher and lower dilutions for *C. koseri* is: 0%; 95% C.I. (-65.76% to 65.76%)
[c]Difference between the higher and lower dilutions for *K. aerogenes* is: 30.0%; 95% C.I. (-3.76% to 60.32%)
[d]Difference between the higher and lower dilutions for *E. cloacae* is: -22.95%; 95% C.I. (-34.91% to -12.38%)
[e]Difference between the higher and lower dilutions for *E. coli* is: -20.44%; 95% C.I. (-28.27% to -13.03%)
[f]Difference between the higher and lower dilutions for *K. oxytoca* is: -20.0%; 95% C.I. (-36.10% to -3.19%)
[g]Difference between the higher and lower dilutions for *K. pneumoniae* is: 2.13%; 95% C.I. (-6.05% to 10.30%)
[h]Difference between the higher and lower dilutions for all *Enterobacteriaceae* is: -11.03%; 95% C.I. (-15.57% to -6.52%)

b. Matrix comparison:

Not Applicable

3. Clinical studies:

a. Clinical Sensitivity:

Not Applicable

b. Clinical specificity:

Not Applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not Applicable

4. Clinical cut-off:

Not Applicable

5. Expected values/Reference range:

The FDA susceptibility interpretive criteria for Eravacycline are as listed in **Table 7**.

Table 7: FDA Interpretive Criteria for Eravacycline (µg/mL)

Organisms	S	I	R
Enterobacteriaceae	≤0.5	-	-
S. aureus	≤0.06	-	-
E. faecalis and *E. faecium*	≤0.06	-	-

N. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.