510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K183115

B. Purpose for Submission:

To obtain a substantial equivalence determination for the Liofilchem MIC Test Strip (MTS) containing Levofloxacin (LEV) at concentrations of 0.002-32 µg/mL for susceptibility testing of non-fastidious Gram-negative organisms

C. Measurand:

Levofloxacin 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 Levofloxacin 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 (83)

H. Intended Use:

1. Intended use(s):

   The MTS (MIC Test Strip) Levofloxacin 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 Levofloxacin at concentrations of 0.002-32 µg/mL should be interpreted at 16-20 hours of incubation.

   MTS Levofloxacin can be used to determine the MIC of levofloxacin against the following bacteria.

   Levofloxacin has been shown to be active both clinically and *in vitro* against these bacterial species according to the FDA drug approved label:

   Gram-negative bacteria
   Enterobacter cloacae
   Escherichia coli
   Klebsiella pneumoniae
   Proteus mirabilis
   Pseudomonas aeruginosa
   Serratia marcescens

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

   Citrobacter freundii
   Citrobacter koseri
   Klebsiella aerogenes
   Klebsiella oxytoca
   Morganella morganii
   Proteus vulgaris
   Providencia rettgeri
   Providencia stuartii

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use

   The following limitations are included in the labeling:

   - The ability of the MTS to detect resistant isolates with the following drug/bacterial species combinations is unknown because resistant isolates were either not available or an insufficient number was encountered at the time of comparative testing.
     Levofloxacin: *C. koseri, P. vulgaris, P. rettgeri, S. marcescens*

   - The safety and efficacy of levofloxacin in treating clinical infections due to Gram-negative organisms other than *Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa,* and *Serratia marcescens* may or may not have been established in adequate and well-controlled clinical trials. The clinical significance of susceptibility information in such instances is unknown.

   - Characterization of Topoisomerase IV and DNA gyrase quinolone-resistance determining regions (QRDRs) and altered efflux resistance mechanisms was not available for organisms at the time of comparative testing, and therefore the performance of MTS Levofloxacin for non-fastidious Gram-negative bacilli with these resistance mechanisms is unknown for the following: *Enterobacteriaceae*, *P. aeruginosa.*

4. Special instrument requirements:

   Manual reading only

I. Device Description:

The Levofloxacin MIC Test Strip (MTS) consists of specialized paper impregnated with a predefined concentration gradient of levofloxacin across 15 two-fold dilutions similar to dilutions used by conventional MIC methods. One side of the strip is labeled with the Levofloxacin code (LEV) 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, **Levofloxacin** (**K183115**)	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 to agar with swab manually or with rotation plate.	Same
Reading	Manual; interpret the MIC as 100% inhibition	Same
Result	MIC in µg/mL	Same

Differences		
Item	Device Liofilchem MTS, **Levofloxacin** (**K183115**)	Predicate Liofilchem MTS, **Vancomycin** (**K153687**)
Intended Use	Quantitative susceptibility to antimicrobial agents against Gram-negative organisms	Quantitative susceptibility to antimicrobial agents against Gram-positive organisms
Antibiotic	Levofloxacin code (LEV)	Vancomycin code (VA)
Incubation	35 ± 2°C for 16 – 20 hours	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 0.125µg/mL is considered to be the same as 0.12µg/mL for reporting purposes.

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 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 two *E. coli,* one *K. pneumoniae*, one *E. cloacae*, three *P. aeruginosa,* one *P. mirabilis*, one *C. freundii,* and one *S. marcescens* isolates. All MIC results were on scale. The testing resulted in overall reproducibility of greater than 95%.

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 were tested a sufficient number of times (i.e., at least 20/site) at each testing site using both MTS and broth microdilution (BMD) reference methods. The results are summarized in **Table 2** below.

The quality control results are acceptable.

Table 2: Levofloxacin MTS QC Results

Organism	Concentration (µg/mL)	BMD Reference	MTS Levofloxacin
E. coli ATCC 25922 Expected Result: 0.008 - 0.06 µg/mL	0.004		
	0.008	1	
	0.015	31	26
	0.03	29	35
	0.06		
	0.12		
P. aeruginosa ATCC 27853 Expected Result: 0.5 - 4 µg/mL	0.25		
	0.5	1	7
	1	52	52
	2	8	2
	4		
	8		

Inoculum Density Check:
The inoculum was prepared to achieve turbidity equivalent to a 0.5 McFarland standard. Colony counts were performed for all QC replicates (2 isolates x 20 replicates = 40) as well as one replicate of each reproducibility isolate tested on each of the three days (12 isolates x 3 days = 36), and a minimum of 10% of clinical strains tested. Colony counts were within the recommended range of approximately 1 - 2 x 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:
All isolates tested grew in the broth microdilution panels and the Mueller Hinton agar with MTS, Levofloxacin.

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, Levofloxacin was evaluated at three sites located within the United States. Each clinical isolate was tested one time by MTS, Levofloxacin and the reference method using the same initial standardized suspension. A total of 413 clinical isolates were tested which included 308 *Enterobacteriaceae* (15 *C. freundii,* 13 *C. koseri*, 35 *E. cloacae*, 90 *E. coli*, 15 *K. aerogenes*, 15 *K. oxytoca*, 60 *K. pneumoniae*, 10 *M. morganii,* 15 *P. mirabilis,* 9 *P. rettgeri,* 9 *P. stuartii,* 7 *P. vulgaris,* 15 *S. marcescens*) and 105 *P. aeruginosa* isolates. 63.0% of the clinical isolates were considered contemporary isolates and were collected within 6 months of isolation.

Challenge testing was performed at one internal site. A total of 99 challenge isolates were tested which included 81 *Enterobacteriaceae* (1 *C. freundii,* 2 *C. koseri*, 13 *E. cloacae*, 20 *E. coli*, 5 *K. aerogenes*, 5 *K. oxytoca*, 20 *K. pneumoniae*, 2 *M. morganii,* 5 *P. mirabilis,* 1 *P. rettgeri,* 1 *P. stuartii,* 1 *P. vulgaris,* 5 *S. marcescens*) and 18 *P. aeruginosa* isolates.

Results obtained with the MTS, Levofloxacin were compared to results obtained with the CLSI broth microdilution frozen reference MIC panel. The reference panel contained two-fold serial dilutions of levofloxacin 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 100% 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.

At the time of comparative testing, resistant isolates were not available for several bacterial species. Thus, the following limitation is included in the labeling:

The ability of the MTS to detect resistant isolates with the following drug/bacterial species combinations is unknown because resistant isolates were either not available or an insufficient number was encountered at the time of comparative testing.

Levofloxacin: C. koseri, P. vulgaris, P. rettgeri, S. marcescens

The results obtained from the 512 clinical and challenge isolates are summarized in **Table 3** below.

Table 3: Overall Performance of Clinical and Challenge Isolates (Combined)

Levofloxacin	EA Tot	EA N	EA %	Eval. EA Tot	Eval. EA N	Eval. EA%	CA N	CA %	#R	min	maj	vmj
Enterobacteriaceae Clinical	308	306	99.4	274	272	99.3	305	99.0	45	3	0	0
Challenge	81	81	100	44	44	100	78	96.3	51	3	0	0
Combined	389	387	99.5	318	316	99.4	383	98.5	96	6	0	0
P. aeruginosa Clinical	105	104	99.0	93	92	98.9	101	96.2	24	4	0	0
Challenge	18	18	100	2	2	100	17	94.4	17	1	0	0
Combined	123	122	99.2	95	94	98.9	118	95.9	41	5	0	0
All Organisms	512	509	99.4	413	410	99.3	501	97.9	137	11	0	0

EA – Essential Agreement
CA – Category Agreement
EVAL – Evaluable isolates
R – Resistant isolates
min – minor errors
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 *Enterobacteriaceae* isolates is acceptable with 99.4% EA and 98.5% CA. There were six minor discrepancies and no major or very major errors.

The overall performance of *P. aeruginosa* is acceptable with 98.9% EA and 95.9% CA. There were five minor errors and no major or very major errors.

The overall performance of all organisms combined is acceptable with 99.3% EA and 97.9% CA.

Resistance Mechanisms:

Molecular characterization was not evaluated for all organisms as this information was not available of the time of testing. This was addressed by adding the following footnote in the labeling:

"Characterization of Topoisomerase IV and DNA gyrase quinolone-resistance determining regions (QRDRs) and altered efflux resistance mechanisms was not available for organisms at the time of comparative testing, and therefore the performance of Liofilchem MTS Levofloxacin for non-fastidious Gram-negative bacilli with these resistance mechanisms is unknown for the following: Enterobacteriaceae, P. aeruginosa"

Trending:

Trending was assessed with data from challenge and clinical isolates (**Table 4**) using current trending review practices (i.e., ≥30% difference). Trending was observed for *P. stuartii* and *S. marcescens* which tended to be in exact agreement or at least one doubling dilution lower when compared to the CLSI reference broth microdilution. Trending was also observed for *P. vulgaris* which tended to be in exact agreement or at least one doubling dilution higher when compared to the CLSI reference broth microdilution.

The following footnotes were included in the labeling to address the trending:

- *Liofilchem MIC Test Strip (MTS) Levofloxacin MIC values tended to be in exact agreement or at least one doubling dilution lower when testing P. rettgeri, P. stuartii and S. marcescens compared to the CLSI reference broth microdilution.*
- *Liofilchem MIC Test Strip (MTS) Levofloxacin MIC values tended to be in exact agreement or at least one doubling dilution higher when testing P. vulgaris compared to the CLSI reference broth microdilution.*

Table 4. Trending by Species (clinical + challenge isolates)

Total	≥2 dil. lower	1 dil. lower	Exact	1 dil. higher	≥2 dil. higher
C. freundii [a]					
16	0	3	12	1	0
	(18.8%)		(75%)	(6.3%)	
C. koseri [b]					
15	0	2	12	1	0
	(13.3%)		(80.0%)	(6.7%)	
E. cloacae [c]					
47	0	9	36	2	0
	(19.1%)		(76.6%)	(4.3%)	
E. coli [d]					
77	1	11	57	8	0
	(15.6%)		(74.0%)	(10.4%)	
K. aerogenes [e]					
19	0	2	16	1	0
	(10.5%)		(84.2%)	(5.3%)	
K. oxytoca [f]					
19	0	4	14	1	0
	(21.1%)		(73.7%)	(5.3%)	
K. pneumoniae [g]					
64	0	12	41	11	0
	(18.8%)		(64.1%)	(17.2%)	
M. morganii [h]					
9	0	1	8	0	0
	(11.1%)		(88.9%)	(0.0%)	

Total	≥2 dil. lower	1 dil. lower	Exact	1 dil. higher	≥2 dil. higher
P. mirabilis [i]					
17	0	1	14	1	1
	(5.9%)		(82.47%)	(11.8%)	
P. rettgeri [j]					
10	0	3	7	0	0
	(30.0%)		(70.0%)	(0.0%)	
P. stuartii [k]					
10	0	4	6	0	0
	(40.0%)		(60.0%)	(0.0%)	
P. vulgaris [l]					
8	0	0	5	3	0
	(0.0%)		(62.5%)	(37.5%)	
S. marcescens [m]					
20	0	8	12	0	0
	(40.0%)		(60.0%)	(0.0%)	
All *Enterobacteriaceae* [n]					
331	1	60	240	29	1
	(18.4%)		(72.5%)	(9.1%)	
P. aeruginosa [o]					
97	1	16	70	10	0
	(17.5%)		(72.2%)	(10.3%)	

[a] Difference between the higher and lower dilutions for *C. freundii* is: -12.5%; 95% C.I. (-37.30% to 12.71%)
[b] Difference between the higher and lower dilutions for *C. koseri* is: -6.6%; 95% C.I. (-31.82% to 18.39%)
[c] Difference between the higher and lower dilutions for *E. cloacae* is: -14.9%; 95% C.I. (-28.64% to -1.62%)
[d] Difference between the higher and lower dilutions for *E. coli* is: -5.2%; 95% C.I. (-16.13% to 5.71%)
[e] Difference between the higher and lower dilutions for *K. aerogenes* is: -5.2%; 95% C.I. (-26.58% to 15.55%)
[f] Difference between the higher and lower dilutions for *K. oxytoca* is: -15.8%; 95% C.I. (-38.49% to -7.29%)
[g] Difference between the higher and lower dilutions for *K. pneumoniae* is: -1.6%; 95% C.I. (-15.16% to 12.03%)
[h] Difference between the higher and lower dilutions for *M. morganii* is: -11.1%; 95% C.I. (-43.50% to 20.16%)
[i] Difference between the higher and lower dilutions for *P. mirabilis* is: 5.88%; 95% C.I. (-16.86% to 28.97%)
[j] Difference between the higher and lower dilutions for *P. rettgeri* is: -30.0%; 95% C.I. (-60.32% to 3.76%)
[k] Difference between the higher and lower dilutions for *P. stuartii* is: -40.0%; 95% C.I. (-68.73% to -3.84%)
[l] Difference between the higher and lower dilutions for *P. vulgaris* is: 37.5%; 95% C.I. (-2.74% to 69.43%)
[m] Difference between the higher and lower dilutions for *S. marcescens* is: -40.0%; 95% C.I. (-61.34% to -15.75%)
[n] Difference between the higher and lower dilutions for all *Enterobacteriaceae* is: -9.4%; 95% C.I. (-14.61% to -4.14%)
[o] Difference between the higher and lower dilutions for *P. aeruginosa* is: -7.2%; 95% C.I. (-17.12% to 2.67%)

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 Levofloxacin are as listed in **Table 5**.

Table 5: FDA Interpretive Criteria for Levofloxacin (µg/mL)

Organisms	S	I	R
Enterobacteriaceae	≤2	4	≥8
Pseudomonas aeruginosa	≤2	4	≥8

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.