510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K171906

B. Purpose for Submission:

To obtain a substantial equivalence for the addition of Delafloxacin at concentrations of 0.002-32 µg/mL for susceptibility testing of non-fastidious Gram positive organisms

C. Measurand:

Delafloxacin 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:

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

   The non-fastidious bacteria that have been shown to be active both clinically and *in vitro* against Delafloxacin according to the FDA label are:

   Staphylococcus aureus (including methicillin-resistant and methicillin-susceptible isolates)
   Staphylococcus haemolyticus
   Staphylococcus lugdunensis
   Enterococcus faecalis

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use

4. Special instrument requirements:

   Manual reading only

I. Device Description:

The Delafloxacin MIC Test Strip (MTS) consists of specialized paper impregnated with a predefined concentration gradient of Delafloxacin across 15 two-fold dilutions similar to dilutions used by conventional MIC methods . One side of the strip is labelled with the Delafloxacin code (DLX) 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, Delafloxacin (K171906)	Predicate Liofilchem MTS, vancomycin (K153687)
Intended Use	Quantitative susceptibility to antimicrobial agents	Same
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
Reading	Manual; the point where the edge of inhibition ellipse intersects the MIC Test Strip	Same
Result	MIC	Same

Differences		
Item	Device Liofilchem MTS, Delafloxacin (K171906)	Predicate Liofilchem MTS, vancomycin (K153687)
Antibiotic	Delafloxacin code (DLX)	Vancomycin code (VA)
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 0.125µg/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 positive organisms. Each isolate was tested in triplicates over three days. The reproducibility panel included seven *S. aureus* (three MRSA and four MSSA), and three *E. faecalis* isolates. The mode of MIC value was pre-determined and the reproducibility was calculated based on the number of MIC values that fell within ±1 doubling dilution of the mode. 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 QC isolates recommended by both FDA and CLSI, namely *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were tested a sufficient number of times (i.e., at least 20/site) at each testing site. The results are summarized in Table 2 below.

The expected range for *S.aureus* ATCC with Delafloxacin is 0.001-0.008 µg/mL. However, the Delafloxacin concentrations included in the Liofilchem MIC test Strip (MTS) is 0.002-32 µg/mL. Therefore, most results for the QC strain *S. aureus* ATCC 29213 were off scale as the Liofilchem MTS report the lowest end of the scale as ≤0.002 µg/mL. The concentration range on the reference test panel was also 0.002 to 32µg/mL and also did not include the low end of the acceptable range for this QC strain (0.001 µg/mL). The footnote as shown under Table 2 was included in the device labeling.

However, *E. faecalis* ATCC 29212 was also tested to verify the performance of the device and all results were on-scale since the reporting range of the Liofilchem Delafloxacin MTS and the reference panel covers the expected range for this organism (Table 2).

The quality control results are acceptable.

Table 2: Delafloxacin MTS QC Results

Organism	Concentration (µg/mL)	Reference	MTS
S. aureus[a] ATCC 29213 Expected Result: 0.001-0.008µg/mL	**≤0.002**	48	2
	0.004	11	58
	0.008	2	8
	0.016		
E. faecalis ATCC 29212 Expected Result: 0.015-0.12 µg/mL	0.008		
	0.015		
	0.03	46	12
	0.06	16	54
	0.12		
	0.25		

[a]Note: *"The Liofilchem MIC Test Strip (MTS), Delafloxacin does not include the full CLSI/FDA-recommended dilution range for QC testing with S. aureus ATCC 29213".*

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. Inoculum density checks were performed and the colony counts obtained for each QC strain were within the recommended range of approximately 1x 10^8 CFU/mL. Colony counts was also determined from one replicate of each reproducibility isolate on each of the three days of testing and from a minimum of 10% of the clinical strains tested.

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:

Results obtained with Liofilchem MIC Test Strip (MTS) with Delafloxacin were compared to results obtained from frozen reference MIC panels. Reference panels were prepared and interpreted as outlined in CLSI recommendations in M7-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 and inverted position at 35°C ±2 for 16-20 hours. At the end of incubation, the MIC value where the edge of the inhibition ellipse intersects the strip was compared to the reference method.

Growth Rate:
The growth rate for the Liofilchem MIC Test Strip (MTS) with Delafloxacin was 100%

Clinical:
Clinical testing was performed at three US sites. A total of 299 clinical isolates were tested which include 199 *Staphylococcus* spp (75 MSSA, 94 MRSA, 15 *S. haemolyticus* and 15 *S. lugdunensis)* and 100 *E. faecalis isolates* (two vancomycin resistant *E. faecalis* and 98 vancomycin susceptible*).* There were 105 (35.1%) fresh isolates that were tested within seven days of isolation, 122 (40.8%) recent isolates that were tested within one year of isolation and 72 (24.1%) of recent isolates that were tested within three years of isolation.

Challenge:
Challenge testing was performed at one internal site. A total of 89 challenge isolates were tested which included 84 *Staphylococcus spp* (7 MSSA, 48 MRSA, 21 *S. haemolyticus* and 8 *S. lugdunensis*)*,* and five *E. faecalis* isolates (two vancomycin resistant and three vancomycin susceptible)

The total of 388 clinical and challenge isolates is summarized in Table 3 below.

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

Delafloxacin	EA Tot	EA N	EA %	Eval. EA Tot	Eval. EA N	Eval. EA%	CA N	CA %	#R	min	maj	vmj
Staphylococcus aureus (MRSA and MSSA)	224	216	96.4	168	165	98.2	210	93.8	49	14	0	0
S. haemolyticus	36	36	100	31	31	100	24	66.7	16	12	0	0
S. lugdunensis	23	23	100	23	23	100	**NA**	**NA***	**NA**	**NA**	**NA**	**NA**
E. faecalis	105	105	100	105	105	100	100	95.2	17	5	0	0
All Organisms*	388	380	97.9	327	324	99.1	334	95.1*	82	31	0	0

*Note: "*Category Agreement is not calculated because Delafloxacin breakpoints for S. lugdunensis have not been established by the FDA; All organisms Category Agreement is based on a total of 365 (excludes 23 S. lugdenensis").*

EA – Essential Agreement
CA – Category Agreement
EVAL – Evaluable isolates
R or NS – Resistant or non-susceptible isolates

min – minor discrepancies
maj – major discrepancies
vmj – very major discrepancies

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 organisms is acceptable with 97.9% EA and 91.5% CA.

The overall performance of *Staphylococcus aureus* (MSSA and MRSA) was acceptable with 96.4% EA and 93.8% CA. There were 14 minor discrepancies and no major or very major discrepancies.

The overall performance of *S. lugdunensis* is acceptable with 100% EA. However, due to lack of interpretive criteria, this organism was excluded from the overall CA. The footnote as shown under Table 3 was included in the labeling.

The overall performance of *S. haemolyticus* is acceptable with 100% EA. The overall CA is lower than 90%; however, based on the AST Guidance, this was considered acceptable because all the discrepancies were minor and the data shows good EA of evaluable. There were no major or very major errors.

The overall performance of *E. faecalis* is acceptable with 100% EA, and 95.2% CA. There were five minor discrepancies with no major or very major discrepancies.

Resistance Mechanism:
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:

"*Resistance mechanism characterization was not provided for all organisms at the time of comparative testing, and therefore the performance of the Liofilchem MIC Test (MTS) Delafloxacin for non-fastidious gram positive organisms in unknown for isolates with the following resistance mechanisms: topoisomerase IV and DNA gyrase Quinolone-Resistant Determining Regions (QRDRs) or altered efflux*".

Trending

Using the combined clinical and challenge data for *Staphylococcus aureus* an analysis of trending was conducted. This trending calculation takes into account MIC values that are determined to be one or more doubling dilution lower or higher compared to the reference method irrespective whether the device MIC values are on-scale or not. The trending analysis is shown in Table 4 below:

Table 4. Tending in Combined Clinical and Challenge-*S.aureus*

Organism	Difference in MIC as Compared to the CLSI Reference Method			
	# Eval Isolates for Trending	**≤1 dil lower**	**Exact**	**≥1 dil. higher**
***S.aureus* (MRSA and MSSA Combined)**	209	25 (11.96%)*	91 (43.54%)	**93** (44.50%)*

*Difference between the higher and lower dilutions for *S. aureus* is: 32.54% 95% CI: (24.21% to 40.27%)
Note: A positive percent difference value indicates higher MIC when compared to the reference method.

A higher MIC reading trend was observed in the overall performance of *Staphylococcus aureus* (MRSA and MSSA combined) compared to the CLSI broth microdilution reference method, which raises concerns for potential major discrepancy. This trending and the potential for occurrence of major discrepancies were addressed by adding the following footnote in the Performance Characteristics section of the labeling, "Drug Specific Supplement for Delafoxacin MIC Test Strip (MTS)":

"Liofilchem MIC Test Strip (MTS) Delafloxacin MIC values tended to be in exact agreement or at least one doubling dilution higher when testing Staphylococcus aureus (including methicillin resistant and methicillin susceptible isolates) compared to the reference broth micro-dilution

An analysis of the overall clinical and challenge data of *S. lugdunensis* was also performed and revealed a lower trending in the MIC of the Liofilchem MIC Test Strip (MTS), Delafloxacin compared to the CLSI reference method as summarized in Table 5 below.

Table 5. Trending in Combined Clinical and Challenge-*S. lugdunensis*

Organism	Difference in MIC as Compared to the CLSI Reference Method			
	# Eval Isolates for Trending	≤1 dil lower	Exact	≥1 dil. higher
S. lugdunensis	23	14 (60.87%)[c]	8 (34.78%)	1(4.35%)[c]

[c] Difference between the higher and lower dilutions is: -56.52% 95% CI: (-73.87% to -30.44%)
Note: A negative percent difference value indicates lower MIC when compared to the reference method.

A lower MIC reading trend was observed in the overall performance of *S. lugdenensis* compared to the CLSI broth microdilution reference method, which raises concerns for potential very major discrepancy. This trending and the potential for occurrence of major discrepancies were addressed by adding the following footnote in the Performance Characteristics section of the labeling, "Drug Specific Supplement for Delafoxacin MIC Test Strip (MTS)":

"*Liofilchem MIC Test Strip (MTS) Delafloxacin MIC values tended to be in exact agreement or at least one doubling dilution lower when testing S. lugdenensis compared to the reference broth micro-dilution*".

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 Delafloxacin are as listed in Table 5.

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

Organisms	S	I	R
S. aureus(MRSA and MSSA)	≤0.25	0.5	≥1
Staphylococcus haemolyticus	≤0.25	0.5	≥1
Enterococcus faecalis	≤0.12	0.25	≥0.5

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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