510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I Background Information:

A 510(k) Number

K200308

B Applicant

Liofilchem s. r. l.

C Proprietary and Established Names

MTS Lefamulin 0.016-256 µg/mL

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
JWY	Class II	21 CFR 866.1640 - Antimicrobial Susceptibility Test Powder	MI-Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for Lefamulin at concentrations of 0.016-256 µg/mL for susceptibility testing of non-fastidious and fastidious organisms.

B Measurand:

MTS Lefamulin in the dilution range of µg/mL 0.016-256 µg/mL

C Type of Test:

Quantitative Antimicrobial Susceptibility Test growth-based detection

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The MTS (MIC Test Strip) Lefamulin 0.016-256 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 Lefamulin at concentrations of 0.016-256 µg/mL should be interpreted at 16-20 hours (non-fastidious organisms) and 20-24 hours (fastidious organisms) of incubation.

Lefamulin 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
Streptococcus pneumoniae
Staphylococcus aureus (methicilin-susceptible isolates)

Lefamulin has been shown to be active *in vitro* only against the bacterial species listed below according to the FDA drug approved label:

Gram-negative bacteria
Haemophilus influenzae

C Special Conditions for Use Statement(s):

Rx-Prescription Use Only

Limitations:
The following Limitations are included in the labeling:

Per the FDA-Recognized Susceptibility Test Interpretive Criteria website, the safety and efficacy of antimicrobial drugs for which antimicrobial susceptibility is tested by this AST device, may or may not have been established in adequate and well-controlled clinical trials for treating clinical infections due to microorganisms outside of those found in the indications and usage in the drug label. The clinical significance of susceptibility information in those instances is unknown. The approved labelling for specific antimicrobial drugs provides the uses for which the antimicrobial drug is approved.

The current absence of resistant or intermediate isolates to Lefamulin precludes defining any results other than Susceptible. Isolates yielding MIC results other than Susceptible should be submitted to a reference laboratory for further testing.

Resistance mechanism characterization was not available for all organisms at the time of comparative testing, and therefore the performance of the MTS Lefamulin for non-fastidious

Gram-positive cocci and fastidious Gram-positive and Gram negative species is unknown for the following: S. aureus [vga(B), sal(A)]; S. pneumoniae [vga(A), vga(B), vga(E), lsa(E),sal(A), Cfr methyl tranferase]; H. influenzae [vga(A), vga(B), vga(E), lsa(E), sal(A), Cfr methyl tranferase].

D Special Instrument Requirements:

Manual reading only

IV Device/System Characteristics:

A Device Description:

The MIC Test Strip (MTS) consists of specialized paper impregnated with a predefined concentration gradient of Lefamulin, across 15 two-fold dilutions similar to dilutions used by conventional MIC methods. One side of the strip is labelled with the Lefamulin code (LMU) and the MIC reading scale is µg/mL. MIC values are determined by identifying the drug concentration at which growth of the ellipse ends.

B Principle of Operation:

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 diffuses into the agar for over an hour. After appropriate 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.

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.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Liofilchem MIC Test Strip (MTS)-Vancomycin 0.016 -256 µg/mL

B Predicate 510(k) Number(s):
K153687

C Comparison with Predicate(s):

Table 1. Comparison with the Predicate

Device & Predicate Device(s):	**Device: K200308**	**Predicate: K153687**
Device Trade Name	MTS Lefamulin 0.016-256µg/mL	Liofilchem MTS Vancomycin 0.016-256 µg/mL
General Device Characteristic Similarities		
Intended Use/Indications for Use	Quantitative susceptibility to antimicrobial agents	Same
MTS Strip Material	High quality paper impregnated with a predefined concentration of gradient antimicrobial agent	Same
Inoculation	Isolated colonies from culture in a suspension equivalent to 0.5 McFarland. Inoculum is applied to agar with swab manually or with rotation plate	Same
Result	MIC in µg/mL	Same
Drug Concentration Range	0.016-256 µg/mL	Same
General Device Characteristic Differences		
Indicated Organisms	Fastidious Gram-positive and Gram-negative and Non-fastidious Gram-positive organisms	Non-fastidious Gram-positive organisms
Antimicrobial Agent	Lefamulin (LMU)	Vancomycin (VA)
Plate Media	Mueller Hinton agar + 5% sheep blood (fastidious organisms) Mueller Hinton agar (non-fastidious organisms)	Mueller Hinton agar (non-fastidious organisms)
Incubation	35°C ± 2°C in 5% CO_2 for 20-24 hours (fastidious organisms) 35°C ± 2°C in ambient air for 16-20 hours (non-fastidious organisms)	35°C ± 2°C in ambient air for 16-20 hours (non-fastidious organisms)
Reading	Manual, interpret the MIC as 100% inhibition for fastidious	Manual, interpret the MIC as 80% in inhibition when

	organisms and interpret the MIC as 80% in inhibition when trailing is seen (non-fastidious organisms).	trailing is seen (non-fastidious organisms).

VI Standards/Guidance Documents Referenced:

- Guidance for Industry and FDA: "Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems, August 28, 2009
- CLSI M07-A11 "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically"; Approved Standard, Eleventh Edition, January 2018
- CLSI M100-29th ed "Performance Standards for Antimicrobial Susceptibility Testing"; Approved Standard, Twenty-Ninth Edition, January 2019

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

   Reproducibility testing was conducted at three sites using ten Gram-positive and Gram-negative fastidious bacteria (5 *H. influenzae* and 5 *S. pneumoniae* isolates) and ten Gram-positive non-fastidious bacteria (10 methicillin-susceptible *S. aureus* isolates). Each isolate was tested in triplicate over three days.

   The mode of the MIC value was pre-determined for each organism, 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. MTS Lefamulin results for both fastidious and non-fastidious organisms were within a doubling dilution of the mode MIC determined by reference broth microdilution results for an overall reproducibility of 100%. The results were acceptable.

2. Linearity:

   Not applicable

3. Analytical Specificity/Interference:

   Not applicable

4. Assay Reportable Range:

   Not applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Inoculum Density Check:
The inoculum was prepared from an overnight agar plate into saline to achieve turbidity equivalent to a 0.5 McFarland standard. The inoculum was applied to agar with swab manually or with a rotation plate.
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 1 x 10^8 CFU/mL.
Colony counts were 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.

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 Rate:
All clinical and challenge isolates grew in both the reference broth microdilution panels and the MTS agar plates.

Quality Control:
The QC strains recommended for routine testing by the CLSI for testing Lefamulin, namely, *S. aureus* ATCC 29213 (for non-fastidious organism), *S. pneumoniae* ATCC 49619 and *H. influenzae* ATCC 49247 (for fastidious organisms) were tested at three sites for a minimum of 20 times at each testing site. Lefamulin MIC results for these QC strains are summarized in Tables 2A (non-fastidious organism) and 2B (fastidious organisms).

Table 2A: QC Results for Lefamulin with the CLSI Recommended QC Strains for Non-Fastidious Organisms

Organism	Concentration (µg/mL)	Reference BMD (All Sites)	MTS (All Sites)
S. aureus ATCC 29213 Expected Results: 0.06-0.25 µg/mL	0.03	0	0
	0.06	4	56
	0.12	56	4
	0.25	0	0
	0.5	0	0

Table 2B: QC Results for Lefamulin with the CLSI Recommended QC Strains for Fastidious Organisms

Organism	Concentration (µg/mL)	Reference BMD (All Sites)	MTS (All Sites)
H. influenzae ATCC 49247 Expected Results: 0.5-2 µg/mL	0.25	0	0
	0.5	4	3
	1	55	48
	2	1	11
	4	0	0
S. pneumoniae ATCC Expected Results: 0.06-0.25 µg/mL	0.03	0	0
	0.06	6	22
	0.12	55	18
	0.25	5	20
	0.5	0	0

6. Detection Limit:

   Not applicable

7. Assay Cut-Off:

   Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Results obtained with Liofilchem MTS with Lefamulin were compared to results obtained from frozen reference MIC panels for susceptibility testing of fastidious and non-fastidious organisms. Reference MIC panels were prepared with Mueller Hinton broth for non-fastidious organisms, cation adjusted Mueller Hinton broth (CAMHB) + 5% lysed horse blood (LHB) for fastidious Gram-positive organisms, and *Haemophilus* test medium (HTM) broth for fastidious Gram-negative organisms and tested as outlined in CLSI recommendation in M07-A11.

   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 plus 5% sheep blood plates in an inverted position at 35°C ±2°C for 20-24 hours in 5% CO_2 (fastidious organisms), in ambient air for 16-24 hours (non-fastidious organisms). At the end of the appropriate incubation, the MIC value where the edge of the inhibition ellipse intersects the strip was compared to MIC results obtained with the CLSI reference broth microdilution method.

   Clinical:
   Clinical testing was performed at three U.S. sites with both MTS Lefamulin and the reference method using a total of 330 non-fastidious Gram-positive clinical isolates of *Staphylococcus aureus* (methicillin-susceptible) and 246 fastidious Gram-positive and Gram-negative clinical

isolates including (150 *S. pneumoniae* and 96 *H. influenzae*). Of 576 fastidious and non-fastidious clinical isolates, 414 clinical isolates (71.8%) were contemporary.

Challenge:
Challenge testing was performed at one internal site. A total of 40 non-fastidious Gram-positive challenge isolates of *Staphylococcus aureus* (methicillin-susceptible) and 53 fastidious Gram-positive and Gram-negative (including 33 *S. pneumoniae* and 20 *H. influenzae*) challenge isolates were tested.

Because a "susceptible-only" category is defined, isolates with Lefamulin MIC values higher than this breakpoint were considered as "non-susceptible" due to the lack of an intermediate or resistant interpretive category. Results of MTS Lefamulin with non-fastidious and fastidious clinical and challenge isolates are shown in Tables 3A and 3B.

Table 3A. Overall Performance of MTS Lefamulin with Non-Fastidious Clinical and Challenge Isolates

	Tot	EA N	EA %	Eval Tot	Eval EA N	Eval EA %	CA Tot	CA %	No. NS	No. S	maj	vmj
***S. aureus*-MSSA breakpoints (S only, ≤0.25 µg/mL)**												
Clinical	330	326	98.8	330	326	98.8	330	100	1	329	0	0
Challenge	40	39	97.5	39	38	97.4	40	100	10	30	0	0
Total	370	365	**98.6**	369	364	98.6	370	**100**	10	359	0	0

Table 3B. Overall Performance of MTS Lefamulin with Fastidious Clinical and Challenge Isolates

	Tot	EA N	EA %	Eval Tot	Eval EA N	Eval EA %	CA Tot	CA %	No. NS	No. S	maj	vmj
***Streptococcus pneumoniae* breakpoints (S only, ≤0.5 µg/mL)**												
Clinical	150	146	97.3	146	142	97.3	150	100	1	149	0	0
Challenge	33	33	100	33	33	100	33	100	1	32	0	0
Total	183	179	**97.8**	179	175	97.8	183	**100**	2	181	0	0
***Haemophilus influenzae* breakpoints (S only, ≤2 µg/mL)**												
Clinical	96	95	99.0	95	94	98.9	96	100	0	96	0	0
Challenge	20	20	100	20	20	100	18	90	4	16	2	0
Total	116	115	**99.1**	115	114	99.1	114	**98.3**	4	112	2[1]	0

[1]Adjusted major error rate is 0% (0/112)

EA – Essential Agreement
CA – Category Agreement
EVAL – Evaluable isolates
S – Susceptible
NS – Non-Susceptible

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 *Staphylococcus aureus* (methicillin-susceptible) (Table 3A) is acceptable with 98.6% EA and 100% CA. There were no major or very major discrepancies.

The overall performance of *Streptococcus pneumoniae* (Table 3B) is acceptable with 97.8% EA and 100% CA. There were no major or very major discrepancies.

The overall performance of *Haemophilus influenzae* (Table 3B) is acceptable with 99.1% EA and 98.3% CA. There were two (2/112=1.78%) that were considered as potential major errors. Due to the lack of an intermediate or resistant interpretive category, our analysis takes into consideration MIC results that are within essential agreement with the CLSI reference method and the error rate is adjusted accordingly. Both of those results were within essential agreement. The adjusted major error rate is 0% (0/112). There were no very major discrepancies.

Testing/Reporting MIC for Non-indicated Species:
For this review, the interpretative criteria are applied to the organisms/organism groups according to the FDA STIC website. As required under 511A(2)(2)(B) of the Federal Food, Drug and Cosmetic Act, the following statement is added in the labeling:

> *Per the FDA-Recognized Susceptibility Test Interpretive Criteria website, the safety and efficacy of antimicrobial drugs for which antimicrobial susceptibility is tested by this AST device, may or may not have been established in adequate and well-controlled clinical trials for treating clinical infections due to microorganisms outside of those found in the indications and usage in the drug label. The clinical significance of susceptibility information in those instances is unknown. The approved labelling for specific antimicrobial drugs provides the uses for which the antimicrobial drug is approved.*

Testing of Non-Susceptible Isolates:
Due to the lack of resistant or intermediate categories for Lefamulin, the following limitation was included in the labeling:

> *The current absence of resistant or intermediate isolates to Lefamulin precludes defining any results other than Susceptible. Isolates yielding MIC results other than Susceptible should be submitted to a reference laboratory for further testing.*

Resistance Mechanism Characterization:
Challenge isolates harboring the resistance mechanisms against which Lefamulin has been shown to be active were not tested. 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 Lefamulin for non-fastidious Gram-positive cocci and fastidious Gram-positive and Gram negative species is unknown for the following: S. aureus [vga(B), sal(A)]; S. pneumoniae [vga(A), vga(B), vga(E), lsa(E),sal(A), Cfr methyl tranferase]; H. influenzae [vga(A), vga(B), vga(E), lsa(E), sal(A), Cfr methyl tranferase]*.

MIC Trending Analysis:
Using the combined clinical and challenge data, an analysis of trending was conducted for all claimed organisms and for organism groups. Results are stratified by species to determine if species-related trends were observed (Table 4). This trending calculation considers MIC values that are determined to be one or more doubling dilutions lower or higher compared to the reference method irrespective of whether the device MIC values are on-scale or not. Results that are not clearly at least one dilution lower, at least one dilution higher or in exact agreement with the CLSI reference method are not considered in the trending analysis.

Species for which the difference between the percentage of isolates with higher vs. lower readings was ≥ 30% and for which the confidence interval was determined to be statistically significant were considered to show evidence of trending. Trending that provides higher or lower MIC values compared to the reference is addressed in labeling.

A trend toward lower MIC reading was observed for *S. aureus* (MSSA) with MTS Lefamulin when compared to the CLSI reference method (Table 4A). This was addressed by adding the following footnote to the labeling in the performance characteristics section:

> *Liofilchem MIC Test Strip (MTS)* Lefamulin *MIC values tended to be in exact agreement or at least one doubling dilution lower when testing S. aureus (MSSA) compared to the CLSI reference broth microdilution*.

Table 4A. Trending for MTS Lefamulin with Non-Fastidious Organism

Organism(s)	Total Evaluable for Trending	≥ 1 Dilution lower No. (%)	Exact No. (%)	≥ 1 Dilution Higher No. (%)	Percent Difference (CI)*	Trending Noted
S. aureus (MSSA)	369	192	172	5	-50.7 [(-55.8)-(-45.3)]	Yes

*A percent difference ≥30% is considered significant trending; a positive percentage difference value in trending analysis indicates higher MIC observed with the device and could cause potential major discrepancies. A negative percentage difference value in trending analysis indicates lower MIC observed with the device and could cause potential very major discrepancies.

No significant trending was observed for fastidious organisms (*H. influenzae and S. pneumoniae*) as shown in Table 4B below.

Table 4B. Trending for MTS Lefamulin with Fastidious Organisms

Organism(s)	Total Evaluable for Trending	≥ 1 Dilution lower No. (%)	Exact No. (%)	≥ 1 Dilution Higher No. (%)	Percent Difference (CI)*	Trending Noted
H. influenzae	115	20	79	16	-3.5 [(-13.0)-(6.0)]	No
S. pneumoniae	183	47	95	41	-3.3 [(-12.0)-(5.5)]	No

*A percent difference ≥30% is considered significant trending; a positive percentage difference value in trending analysis indicates higher MIC observed with the device and could cause potential major discrepancies. A negative percentage difference value in trending analysis indicates lower MIC observed with the device and could cause potential very major discrepancies.

2. Matrix Comparison:

Not applicable

C Clinical Studies:

1. Clinical Sensitivity:

Not Applicable

2. Clinical Specificity:

Not Applicable

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not Applicable

D Clinical Cut-Off:

Not Applicable

E Expected Values/Reference Range:

The FDA identified susceptibility interpretive criteria for Lefamulin are listed in Table 5.

Table 5. FDA Identified Interpretive Criteria for Lefamulin[1]

Organism(s)	Minimum Inhibitory Concentration, MIC (µg/mL)		
	Susceptible	Intermediate	Resistant
Staphylococcus aureus (methicillin-susceptible isolates)	≤0.25	-	-
Streptococcus pneumoniae	≤0.5	-	-
Haemophilus influenzae	≤2	-	-

[1]FDA STIC Webpage https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm410971.htm

VIII Proposed Labeling:

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

IX Conclusion:

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

To support the implementation of changes to FDA-recognized susceptibility test interpretive criteria (i.e., breakpoints), this submission included a breakpoint change protocol that was reviewed and accepted by the FDA. This protocol addresses future revisions to device labeling in response to breakpoint changes that are recognized on the FDA STIC webpage (https://www.fda.gov/drugs/development-resources/antibacterial-susceptibility-test-interpretive-criteria). The protocol outlined the specific procedures and acceptance criteria that Liofilchem intends to use to evaluate the Liofilchem MIC test strip (MTS) when revised breakpoints for Lefamulin are published on the FDA STIC webpage. The breakpoint change protocol included with the submission indicated that if specific criteria are met, Liofilchem will update the Lefamulin device label to include (1) the new breakpoint, (2) an updated performance section after re-evaluation of data in this premarket notification with the new breakpoints, and (3) any new limitations as determined by their evaluation.