510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K180330

B. Purpose for Submission:

To obtain a substantial equivalence determination of the Liofilchem MIC Test Strip (MTS) containing linezolid at concentrations of 0.016 – 256 µg/mL for susceptibility testing of select gram positive organisms.

C. Measurand:

Linezolid 0.016 – 256 µg/mL

D. Type of Test:

Quantitative Antimicrobial Susceptibility growth-based detection

E. Applicant:

Liofilchem s.r.l.

F. Proprietary and Established Names:

Liofilchem MIC Test Strip (MTS), Linezolid 0.016 – 256 µg/mL

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.1640 Antimicrobial Susceptibility Test Powder

2. Classification:

   Class II

3. Product code:

   JWY – Manual Antimicrobial Test Systems

4. Panel:

   Microbiology (83)

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 Linezolid MTS concentrations of 0.016 – 256 µg/mL should be interpreted after 16 – 20 hours of incubation.

   Linezolid has been shown to be active both clinically and in vitro against the non-fastidious bacteria listed below, according to the FDA label.

   Staphylococcus aureus (including methicillin-resistant isolates)
   Enterococcus faecium (vancomycin resistant isolates only)

2. Indication(s) for use:

   Same as intended use

3. Special conditions for use statement(s):

   For Prescription Use Only

4. Special instrument requirements:

   Manual reading only

I. Device Description:

The linezolid MIC Test Strip (MTS) consists of specialized paper impregnated with a predefined concentration gradient of linezolid across 15 two-fold dilutions like those of a conventional MIC method. One side of the strip is labelled with the linezolid code (LNZ); the MIC reading scale is in µg/mL. When the MTS, Linezolid 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 of 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 MTS, Linezolid. The MTS,

Linezolid is single use only. Since the MTS Linezolid strip generates MIC values that may fall between two-fold dilutions designations, the MIC value is recorded as 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

Similarities		
Item	**Device Liofilchem MTS, Linezolid (K180330)**	**Predicate Liofilchem MTS, Vancomycin (K153687)**
Intended Use	Quantitative susceptibility to antimicrobial agents	Same
Media	Mueller Hinton agar	Same
Inoculum	Isolated colonies from culture in suspension equivalent to a 0.5 McFarland turbidity standard. Inoculum is applied to agar with swab manually or with rotation plate.	Same
Incubation	35 °C, 16 – 20 hours	Same
Reading	Manual: the point where the edge of the inhibition ellipse intersects the MIC Test Strip	Same
Result	MIC (µg/mL)	Same

Differences		
Item	**Device**	**Predicate**
Antimicrobial Agent	Linezolid	Vancomycin

K. Standard/Guidance Document Referenced (if applicable):

FDA Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems; Guidance for Industry and FDA (Issued August 28, 2009)

CLSI M07-10, "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard – Tenth Edition (January, 2015)

CLSI M100-S27, "Performance Standards for Antimicrobial Susceptibility Testing", Twenty-Seventh Informational Supplement (January 2017)

L. Test Principle:

The Liofilchem MIC Test Strips (MTS) are made of specialized paper impregnated with a predefined concentration gradient of antibiotic. 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 minimum inhibitory concentration (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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   A reproducibility study testing the Liofilchem MIC Test Strip (MTS) containing linezolid was conducted at three clinical sites using ten isolates of gram-positive cocci consistent with the Intended Use. Testing was performed on three separate days and in triplicate for a total of 270 data points among the sites. The isolates tested in the reproducibility study included: *S. aureus* (MRSA) 3 isolates, *S. aureus* (MSSA) 4 isolates and *E. faecium* (vancomycin resistant) 3 isolates.

   The mode MIC was determined and the reproducibility was calculated based on MIC values that fell within ± one doubling dilution from the mode MIC value. Both intra-site and inter-site reproducibility for MTS, Linezolid was calculated. There were no off-scale results.

The combined reproducibility results for all three sites were acceptable and demonstrated ≥ 95% reproducibility.

b. *Linearity/assay reportable range:*

Not applicable

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

CLSI recommended QC organisms were tested throughout the comparative study at the three study sites. The organisms tested were *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212. These recommended QC strains were tested a minimum of 20 times at each site by both the MTS, Linezolid and the CLSI broth microdilution reference method.

All results were within the expected range >95% of the time. Results are summarized in Table 2; all QC results were acceptable.

Table 2. Quality Control Results Summary for Linezolid MTS

Organism	Expected Range (µg/mL)	Concentration (µg/mL)	Reference	MTS Linezolid
S. aureus ATCC 29213	1 - 4	0.25	1	0
		0.5	0	1
		1	3	11
		2	52	57
		4	5	5
		8	0	0
E. faecalis ATCC 29212	1 - 4	0.25	0	0
		0.5	0	0
		1	20	0
		2	41	41
		4	0	30
		8	0	0

Inoculum Density Check: The inoculum was prepared to achieve a turbidity equivalent to a 0.5 McFarland standard. Colony counts were performed as part of QC and reproducibility testing as well as during the clinical studies to demonstrate that the inoculum concentrations were within the expected CFU/mL. The colony counts were acceptable.

Purity Checks. Purity checks were performed on all isolates following MTS inoculation. Only results from pure cultures were evaluated.

Growth Failure. All isolates tested showed growth on the Mueller Hinton agar with

MTS, Linezolid. One *E. faecium* isolate did not grow in the reference method test; this isolate was excluded from the study.

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, Linezolid was evaluated at three sites located within the United States. Each clinical isolate was tested one time by MTS, Linezolid and the reference method using the same initial standardized suspension. A total of 299 clinical isolates comprised of the following species were tested: *S. aureus* (MSSA,75 isolates), *S. aureus* (MRSA,105 isolates) and *E. faecium* (vancomycin resistant, 119 isolates). The 299 clinical gram-positive isolates tested included 77 (25.8%) fresh isolates that were tested within seven days of isolation, 104 (34.8%) recent isolates that were tested within one year of isolation and 118 (39.5%) stock isolates that were tested within three years of isolation. A total of 300 isolates grew on the Mueller Hinton plate with the Linezolid MTS strip; one isolate of *E. faecalis* did not grow in the broth microdilution reference plate and was removed from the study.

A total of 77 challenge isolates were tested at a single site and included *S. aureus* (MSSA, one isolate), *S. aureus* (MRSA, 29 isolates), *E. faecium* (vancomycin resistant, 32 isolates) and coagulase negative staphylococci (15 isolates). Coagulase negative staphylococci are not included in the list of intended organisms and were not included in the determination of device performance.

Results obtained with the Liofilchem MIC Test Strip (MTS), Linezolid were compared to results obtained with the CLSI broth microdilution reference panel. The reference panel contained two-fold serial dilutions of linezolid with a range of 0.016 – 256 µg/mL. The testing conditions for the reference method were consistent with CLSI guidelines as listed in the CLSI document M07-A10. Clinical sites were instructed to record if trailing/hazy end-points were observed and to read linezolid MICs at the point of 90% inhibition when such trailing was noted, as recommended in the Liofilchem Technical Sheet "*Cidal-Static*" found on the Liofilchem website.

For *S. aureus* (MSSA and MRSA) the results from clinical and challenge testing demonstrated a combined EA of 99.0% and CA of 99.5% with one major error and no very major errors. All results were evaluable; the EA of evaluable results was 99.0%. For *E. faecalis* the results from clinical and challenge testing demonstrated a combined EA of 98.7% and CA of 94.0%. There were no major or very major errors. (See Table 3). Review of the data showed that hazy endpoints/trailing growth occurred at a frequency of 98% and 97% for *S. aureus* and *E. faecium*, respectively.

Table 3. Performance of Clinical and Challenge Isolates, *S. aureus* (MSSA and MRSA) and *E. faecium*

	Tot	EA N	EA %	Eval Tot	Eval EA N	Eval EA %	CA Tot	CA %	No. R	No. S	min	maj	vmj
***S. aureus*, MSSA**													
Clinical	75	75	100	75	75	100	75	100	0	75	NA*	0	0
Challenge	1	1	100	1	1	100	1	100	1	0	NA*	0	0
Total	76	76	100	76	76	100	76	100	1	75	NA*	0	0
***S. aureus* MRSA**													
Clinical	105	104	99.0	105	104	99.0	105	100	0	105	NA*	0	0
Challenge	29	28	96.6	29	28	96.6	28	96.6	25	4	NA*	1	0
Total	134	132	98.5	134	132	98.5	133	99.3	25	109	NA*	1	0
S. aureus, MSSA and MRSA													
Clinical	180	179	99.4	180	179	99.4	180	100	0	180	NA*	0	0
Challenge	30	29	96.7	30	29	96.7	29	96.7	26	4	NA*	1	0
Total	210	208	99.0	210	208	99.0	209	99.5	26	184	NA*	1	0
E. faecium													
Clinical	119	117	98.3	119	117	98.3	112	94.1	6	110	7	0	0
Challenge	32	32	100	32	32	100	30	93.8	24	1	2	0	0
Total	151	149	98.7	151	149	98.7	142	94.0	30	111	9	0	0

*NA – not applicable due to a lack of an intermediate breakpoint for this drug

EA – Essential Agreement (+/- 1 dilution)
CA – Category Agreement
EVAL – Evaluable isolates
R – Resistant isolates
min – minor discrepancies
maj – major discrepancies
vmj – very major discrepancies
S – Susceptible isolates

Essential agreement (EA) occurs when the result of the reference method and that of the MTS, Linezolid are within plus or minus one serial two-fold dilution of the antibiotic. Evaluable results are those that are on scale for both the reference method and the MTS, Linezolid. Category agreement (CA) occurs when the interpretation of the result of the reference method agrees exactly with the interpretation of the MTS, Linezolid.

MIC Trends. Using the combined clinical and challenge data for *S. aureus* and *E. faecium*, an analysis of trending was conducted. 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. The trending analysis indicated that the MTS, Linezolid gives a one-dilution higher MIC when compared to the reference method; however, the difference was considered to be acceptable and no further notation was required in labeling. The evaluable data for trend analysis is presented in Table 4.

Table 4. Trending Analysis of Evaluable Clinical and Challenge Results

Organism	Total No. Isolates[a]	Difference in MIC as Compared to the Reference Method				
		≥ 2 Dilutions lower	1 Dilution Lower	Exact	1 Dilution Higher	≥ 2 Dilutions Higher
S. aureus	210	0	17	136	55	2
		17 (8.10%)[b] 95% CI (5.12 – 12.58)		64.76%	57 (27.14%)[b] 95% CI (21.58 – 33.53)	
E. faecium	151	0	13	94	42	2
		13 (8.61%)[c] 95% CI (5.10 – 14.17)		62.25%	29.14%[c] 95% CI (22.48 – 36.83)	

[a] Total number of evaluable results for trending analysis
[b] Difference: 19.05%, 95% CI (11.90 - 26.09)
[c] Difference: 20.53%, 95% CI (11.85 – 28.99)

Resistance Mechanisms

The FDA approved pharmaceutical antimicrobial agent package insert indicates that point mutations in the 23S rRNA are associated with linezolid resistance. A total of 18 challenge MRSA isolates with the noted mutation were evaluated in this study. All isolates provided the expected resistant result.

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:

Table 5. Interpretive Criteria for Linezolid

Organism	Interpretive Criteria for Linezolid (FDA and CLSI)		
	S	I	R
Staphylococcus spp.	≤ 4	-	≥ 8
Enterococcus spp.	≤ 2	4	≥ 8

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.