510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K161175

B. Purpose for Submission:

To obtain a substantial equivalence determination for the Liofilchem MIC Test Strip (MTS) containing ceftolozane/tazobactam at concentrations of 0.016/4 -256/4 µg/mL for susceptibility testing of *Enterobacter cloacae, E. coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis,* and *Pseudomonas aeruginosa*

C. Measurand:

Ceftolozane/tazobactam 0.016/4 -256/4 µg/mL

D. Type of Test:

Quantitative AST growth based detection

E. Applicant:

Liofilchem s.r.l.

F. Proprietary and Established Names:

Liofilchem MIC Test Strip (MTS) Ceftolozane/Tazobactam 0.016/4 – 256/4 µg/mL

G. Regulatory Information:

1. Regulation section:

   866.1640 Antimicrobial Susceptibility Test Powder

2. Classification:

   II

3. Product code:

   JWY - Manual Antimicrobial 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 non-fastidious Gram negative and Gram positive aerobic bacteria (for example, *Enterobacteriaceae, Pseudomonas, Enterococcus* and *Staphylococcus* species) and fastidious bacteria (for example, anaerobes, *Haemophilus* and *Streptococcus* species and *N. gonorrhoeae*). 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.

2. Indication(s) for use:

The Liofilchem MIC Test Strip (MTS) is a quantitative method intended for the *in vitro* determination of antimicrobial susceptibility of non-fastidious Gram negative and Gram positive aerobic bacteria (for example, *Enterobacteriaceae, Pseudomonas, Enterococcus* and *Staphylococcus* species) and fastidious bacteria (for example, anaerobes, *Haemophilus* and *Streptococcus* species and *N. gonorrhoeae*). 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 purpose of this 510(k) is specifically for the ceftolozane/tazobactam MTS at concentrations of 0.016/4 – 256/4 µg/mL interpreted after 16-20 hours of incubation.

Ceftolozane/tazobactam has been shown to be active against the following bacteria, both clinically and *in vitro* according to the FDA label:

Enterobacter cloacae
Escherichia coli
Klebsiella oxytoca
Klebsiella pneumoniae
Proteus mirabilis
Pseudomonas aeruginosa

3. Special conditions for use statement(s):

For prescription use

The following limitations are included in the Liofilchem Ceftolozane/Tazobactam MIC Test Strip (MTS) package insert supplement:

1. Enzyme group characterization was not available for organisms at the time of comparative testing, and therefore the performance of Liofilchem MIC Test Strip Ceftolozane/Tazobactam for non-fastidious gram negative bacilli is unknown for the following: Enterobacteriaceae (ESBL, TEM, SHV, CTX-M, and OXA); Pseudomonas aeruginosa (chromosomal AmpC, loss of OprD, up-regulation of MexXY, and MexAB).

2. Ceftolozane/Tazobactam is not active against bacteria that produce serine carbapenemases [K. pneumoniae carbapenemase (KPC)] and metallo-beta lactamases.

4. Special instrument requirements:

   Manual reading only

I. Device Description:

The ceftolozane/tazobactam MIC Test Strip (MTS) consists of specialized paper impregnated with a predefined concentration gradient of ceftolozane/tazobactam across 15 two-fold dilutions like those of a conventional MIC method. One side of the strip is labelled with the ceftolozane/tazobactam code (C/T) 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.

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	Predicate 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	Predicate
Antibiotic	Ceftolozane/tazobactam	Vancomycin
Incubation	35 ± 2°C for 16- 20hrs	35 ± 2°C for 24hrs

K. Standard/Guidance Document Referenced:

"Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems; Guidance for Industry and FDA"

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 like those of a conventional MIC method. 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 (if/when applicable):

a. Precision/Reproducibility:

Reproducibility testing was performed using six *Pseudomonas aeruginosa* isolates and four *Enterobacteriaceae* isolates (two *E. coli*, one *K. pneumoniae*, and one *Enterobacter cloacae*). These ten organisms were tested at three sites in triplicates on three days. The mode of MIC value was determined and the reproducibility was calculated based on the number of MIC values that fell within ±1 doubling dilution of the mode. 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):

The recommended QC isolates were tested a sufficient number of times at all three sites with acceptable results in comparison to the reference method. All results were within the expected range greater than 95% of the time. The results are summarized in Table 2 below.

Table 2: Ceftolozane/tazobactam MTS QC results

Organism	Concentration (µg/mL)	Reference	MTS
E. coli ATCC 25922 Expected Results 0.12/4 – 0.5/4µg/mL	0.06/4		
	0.12/4		
	0.25/4	39	11
	0.5/4	21	49
	1/4		
K. pneumoniae ATCC 700603 Expected Results 0.5/4 – 2/4 µg/mL	0.5/4	1	
	1/4	37	10
	2/4	22	49
	4/4		1
P. aeruginosa ATCC 27853 Expected Results 0.25/4 – 1/4 µg/mL	0.12/4		
	0.25/4	1	1
	0.5/4	37	4
	1/4	22	53
	2/4		2

The inoculum density of the quality control organisms was determined each day of testing. A total of 180 inoculum density checks were performed; the average colony counts of each QC strain were within the recommended range of approximately 1x 10^8 CFU/mL.

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:

Clinical testing was conducted at three sites (two U.S. sites and one outside the U.S.). A total of 401 organisms were tested and all organisms grew in the studies. There were 338 (84.3%) isolates that were tested within seven days of collection and 63 (15.7%) isolates that were tested within one year of collection. The study included 296 *Enterobacteriaceae* and 190 *Pseudomonas aeruginosa.* The *Enterobacteriaceae* tested were *Enterobacter cloacae, Esherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae,* and *Proteus mirabilis.* The performance is listed in Table 3 below:

Table 3: Performance of *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates*

Ceftolozane/ Tazobactam	EA Tot	EA N	EA %	Eval. EA Tot	Eval. EA N	Eval. EA%	CA N	CA%	#R	min	maj	vmj
	Enterobacteriaceae* (≤2/4, 4/4, ≥8/4)*											
E. cloacae												
Clinical	30	29	96.7	30	29	96.7	28	93.3	8	2	0	0
Challenge	9	9	100	9	9	100	9	100	8	0	0	0
Combined	39	38	97.4	39	38	97.4	37	94.9	16	2	0	0
E. coli												
Clinical	104	104	100	90	90	100	104	100	16	0	0	0
Challenge	15	14	93.3	15	14	93.3	7	46.7	4	8	0	0
Combined	119	118	99.2	105	104	99.0	111	93.2	20	8	0	0
K. oxytoca												
Clinical	29	28	96.6	29	28	96.6	27	93.1	1	2	0	0
Challenge	3	3	100	3	3	100	3	100	2	0	0	0
Combined	32	31	96.9	32	31	96.9	30	93.8	3	2	0	0

Ceftolozane/ Tazobactam	EA Tot	EA N	EA %	Eval. EA Tot	Eval. EA N	Eval. EA%	CA N	CA%	#R	min	maj	vmj
K. pneumoniae												
Clinical	59	57	96.6	49	47	95.9	58	98.3	10	1	0	0
Challenge	14	13	92.9	12	12	100	13	92.9	11	1	0	0
Combined	73	70	95.9	61	59	96.7	71	97.3	21	2	0	0
P. mirabilis												
Clinical	31	30	96.8	30	29	96.7	31	100	4	0	0	0
Challenge	2	2	100	2	2	100	2	100	1	0	0	0
Combined	33	32	97.0	32	31	96.9	33	100	5	0	0	0
Total *Enterobacteriaceae*	**296**	**289**	**97.6**	**269**	**263**	**97.8**	**282**	**95.3**	**65**	**14**	**0**	**0**
Pseudomonas aeruginosa* (≤4/4, 8/4, ≥16/4)*												
Clinical	148	140	94.6	124	116	93.5	141	95.3	32	7	0	0
Challenge	42	36	85.7	40	34	85.0	33	78.6	27	8	1	0
Combined	190	176	92.6	164	150	91.5	174	91.6	59	15	1	0
All Isolates	**486**	**465**	**95.7**	**423**	**413**	**97.6**	**456**	**93.8**	**124**	**29**	**1**	**0**

* **EA -** Essential Agreement **maj** – major discrepancies
CA - Category Agreement **vmj**- very major discrepancies
R- resistant isolates **min**- minor 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.

** Parenthesis show the ceftolozane/tazobactam MIC values that correspond to the interpretive categories of S/I/R

In the challenge study, low CA was observed for *E. coli* (46.7%, 7/15) and *Pseudomonas aeruginosa* (78.6%, 33/42); low EA (85.7%, 36/42) was also noted for *Pseudomonas aeruginosa.*

E. coli: There were eight minor discrepancies, seven of which were within ± one doubling dilution. The challenge set included six susceptible, five intermediate, and four resistant isolates. The CA was 100% in the clinical study which included 88 susceptible and 16 resistant isolates; there was no intermediate isolates. The resistant isolates were in exact agreement with the reference and the susceptible isolates were all within EA. The combined clinical + challenge *E. coli* CA was 93.2% meeting the acceptance criterion.

Pseudomonas aeruginosa: Forty-two isolates were tested in the challenge study. The challenge set included eight susceptible, seven intermediate and 27 resistant isolates. The low CA was caused by minor (19.0%, 8/42) and major (12.5%, 1/8) discrepancies. The major discrepancy rate was high at 12.5% because of the low number of susceptible isolates tested. The EA was also low at 85.7% in the challenge study. However, there were no major or very major discrepancies in the clinical study which resulted in a CA of 95.3% and EA of 94.6%. The overall performance of *P. aeruginosa* was acceptable, with an EA of 92.6%, a CA of 91.6%, and a major discrepancy rate of 0.82%.

The combined clinical and challenge on-scale data of the *Enterobacteriaceae* and *Pseudomonas aeruginosa* were further analyzed for trending as presented in Table 4 below:

Table 4: Trending Summary of on-scale Clinical and Challenge Isolate Results

Ceftolozane/ Tazobactam	Total	Difference in MIC as Compared to the CLSI Reference Method						
		>2 dil. lower	2 dil. lower	1 dil. lower	Exact	1 dil. higher	2 dil. higher	>2 dil. higher
Enterobacteriaceae	296	0.3% (1/296)	0.7% (2/296)	14.9% (44/296)	54.7% (162/296)	28.0% (83/296)	1% (3/296)	0.3% (1/296)
P. aeruginosa	190	0	0	6.8% (13/190)	45.8% (87/190)	40.0% (76/190)	1.6% (3/190)	5.8% (11/190)

The trending analysis indicated that the MTS MIC results tended to be higher than the reference method. For *Pseudomonas aeruginosa*, 40.0% of the MTS MIC results were one doubling dilution higher than the reference method results, while 45.8% of the MTS MIC results matched the reference method exactly. For the *Enterobacteriaceae*, 28.0% of the MTS results were one doubling dilution higher than the reference method results, while 54.7% of the MTS results matched the reference method exactly. Only one major discrepancy was observed from *Pseudomonas aeruginosa*, and no major discrepancies were observed from *Enterobacteriaceae.* The MIC trends were noted as the following footnotes to the Performance Characteristics Table in the ceftolozane/tazobactam MTS package insert supplement:

- *The Liofilchem MIC Test Strip (MST) ceftolozane/tazobactam MIC values tended to be one doubling dilution higher when testing Enterobacteriaceae compared to the reference broth microdilution (out of 296 Enterobacteriaceae isolates tested, 0.3% were >2 dilutions lower, 0.7% were 2 dilutions lower, 14.9% were 1 dilution lower, 54.7% were equivalent, 28.0% were 1 dilution higher, 1.0% were 2 dilutions higher, 0.3% were >2 dilutions higher compared to the CLSI broth microdilution results).*

- *The Liofilchem MIC Test Strip (MST) ceftolozane/tazobactam MIC values tended to be one doubling dilution higher when testing P. aeruginosa compared to the reference broth microdilution (out of 190 P. aeruginosa isolates tested, 6.8% were 1 dilution lower, 45.8% were equivalent, 40.0% were 1 dilution higher, 1.6% were 2 dilutions higher, 5.8% were >2 dilutions higher compared to the CLSI broth microdilution results).*

Enzyme group characterization

The FDA approved pharmaceutical antimicrobial agent package insert provides a detailed description of enzyme groups for organisms tested in the drug study. However, this information was not available at the time when the device comparative study was conducted. The Liofilchem MST ceftolozane/tazobactam study included 220 susceptible, 11 intermediate and 65 resistant isolates for *Enterobacteriaceae;* 122

susceptible, 9 intermediate and 59 resistant *Pseudomonas aeruginosa.* The enzyme group characterization has not been evaluated. This information is noted in the limitation section of ceftolozane/tazobactam package insert supplement:

1. Enzyme group characterization was not available for organisms at the time of comparative testing, and therefore the performance of Liofilchem MIC Test Strip Ceftolozane/Tazobactam for non-fastidious gram negative bacilli is unknown for the following: Enterobacteriaceae (ESBL, TEM, SHV, CTX-M, and OXA); Pseudomonas aeruginosa (chromosomal AmpC, loss of OprD, up-regulation of MexXY, and MexAB).

2. Ceftolozane/Tazobactam is not active against bacteria that produce serine carbapenemases [K. pneumoniae carbapenemase (KPC)] and metallo-beta lactamases.

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: FDA Interpretive Criteria for Ceftolozane/Tazobactam (µg/mL)

Organisms	S	I	R
Enterobacteriaceae	≤2/4	4/4	≥8/4
Pseudomonas aeruginosa	≤4/4	8/4	≥16/4

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.