510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K163298

B. Purpose for Submission:

To obtain a substantial equivalence determination for the Liofilchem MIC Test Strip (MTS) containing ceftazidime at concentrations of 0.016 -256.0 µg/mL for susceptibility testing of *Citrobacter* spp., *Enterobacter* species*, E. coli, Klebsiella* spp.*, Proteus mirabilis, Proteus vulgaris,* and *Pseudomonas aeruginosa*

C. Measurand:

Ceftazidime 0.016-256.0 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) Ceftazidime 0.016-256.0 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 indications for use of this 510(k) is specifically for the Ceftazidime MTS at concentrations of 0.016-256.0 µg/mL interpreted after 16-20 hours of incubation.

   Ceftazidime has been shown to be active against the following bacteria, both clinically and *in vitro* according to the FDA label:
   Citrobacter spp.
   Enterobacter spp.
   Escherichia coli
   Klebsiella spp.
   Proteus mirabilis
   Proteus vulgaris
   Pseudomonas aeruginosa

3. Special conditions for use statement(s):

   For prescription use

4. Special instrument requirements:

   Manual reading only

I. Device Description:

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

2. Predicate 510(k) number(s):

   K161175

3. Comparison with predicate:

Table 1: Comparison with the Predicate Device

Similarities		
Item	Device	Predicate Liofilchem MTS, ceftolozane/tazobactam K161175
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
Incubation	35 ± 2°C for 16- 20hrs	Same

Differences		
Item	Device	Predicate
Antibiotic	Ceftazidime	Ceftolozane/ tazobactam

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: Ceftazidime MTS QC results

Organism [a]	Concentration (µg/mL)	Reference	MTS
E. coli ATCC 25922 Expected Result 0.06 – 0.5µg/mL	0.03		
	0.06		
	0.12	4	2
	0.25	43	48
	0.5	13	10
	1		
P. aeruginosa ATCC 27853 Expected Result 1 – 4 µg/mL	0.5		
	1	4	18
	2	48	41
	4	8	1
	8		
K. pneumoniae ATCC 700603 Expected Result 16 - 64 µg/mL	8		
	16	5	6
	32	32	28
	64	23	26
	128		

[a] *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 recommended by FDA and CLSI; *K. pneumoniae* ATCC 700603 recommended by CLSI only.

The inoculum was prepared to achieve a 0.5 McFarland standard turbidity. Colony counts were performed periodically at each site. Inoculum density checks were performed and 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 509 organisms were tested and all organisms grew in the studies. There

were 358 (82.5%) isolates that were tested within seven days of collection and 76 (17.5%) isolates that were tested within one year of collection. The study included 327 *Enterobacteriaceae* and 182 *Pseudomonas aeruginosa.* The *Enterobacteriaceae* tested were *Citrobacter* spp., *Enterobacter* species*, E. coli, Klebsiella* spp.*, Proteus mirabilis,* and *Proteus vulgaris.* All organisms grew in the clinical study. The performance is listed in Table 3 below:

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

Ceftazidime	EA Tot	EA N	EA %	Eval. EA Tot	Eval. EA N	Eval. EA %	CA N	CA %	#R	min	maj	vmj
	***Enterobacteriaceae* ≤4 (Susceptible), 8 (Intermediate), ≥16 (Resistant)**											
E. coli												
Clinical	94	88	93.6	81	75	92.6	92	97.9	24	2	0	0
Challenge	13	13	100	8	8	100	12	92.3	11	1	0	0
Combined	107	101	94.4	89	83	93.3	104	97.2	35	3	0	0
K. pneumoniae												
Clinical	59	58	98.3	49	48	98.0	57	96.6	15	2	0	0
Challenge	14	14	100	6	6	100	14	100	11	0	0	0
Combined	73	72	98.6	55	54	98.2	71	97.3	26	2	0	0
K. oxytoca												
Clinical	28	26	92.9	27	25	92.6	27	96.4	3	1	0	0
Challenge	3	3	100	3	3	100	3	100	3	0	0	0
Combined	31	29	93.5	30	28	93.3	30	96.8	6	1	0	0
Proteus mirabilis												
Clinical	31	29	93.5	28	26	92.9	31	100	4	0	0	0
Challenge	2	2	100	2	2	100	0	0	0	2	0	0
Combined	33	31	93.9	30	28	93.3	31	93.9	4	2	0	0
Ent. cloacae												
Clinical	30	26	86.7	22	18	81.8	30	100	15	0	0	0
Challenge	9	9	100	1	1	100	9	100	9	0	0	0
Combined	39	35	89.7	23	19	82.6	39	100	24	0	0	0
C. freundii												
Clinical	12	11	91.7	10	9	90.0	12	100	8	0	0	0
C. koseri												
Clinical	10	10	100	9	9	100	10	100	2	0	0	0
Ent. aerogenes												
Clinical	12	10	83.3	8	6	75.0	12	100	6	0	0	0
Proteus vulgaris												
Clinical	10	10	100	10	10	100	10	100	0	0	0	0
***Enterobacteriaceae* Total**	**327**	**309**	**94.5**	**264**	**246**	**93.2**	**319**	**97.6**	**111**	**8**	**0**	**0**
***Pseudomonas aeruginosa* ≤8 (Susceptible), --, ≥16 (Resistant)**												
Clinical	148	135	91.2	136	123	90.4	146	98.6	57	0	2	0
Challenge	34	34	100	14	14	100	34	100	30	0	0	0
Combined	**182**	**169**	**92.9**	**150**	**137**	**91.3**	**180**	**98.9**	**87**	**0**	**2**	**0**
All Isolates	**509**	**478**	**93.9**	**414**	**383**	**92.5**	**499**	**98.0**	**198**	**8**	**2**	**0**

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

Enterobacteriaceae: The overall performance of *Enterobacteriaceae* was acceptable with EA 94.5% EA and 97.6% CA. There were no major or very major discrepancies. When stratified, the EA for *E. aerogenes* was low at 83.3% due to two resistant strains (both gave reference MIC values of 128 µg/mL and device MIC values of >256 µg/mL). The low EA did not affect the CA, which was 100%. The EA for *Enterobacter* species (*E. cloacae* and *E. aerogenes* combined) was 88.2% with 100% CA.

Pseudomonas aeruginosa: The performance of *P. aeruginosa* was 91.3% EA, 98.9% CA and major discrepancy rate 2.1% (2/95) with no vary major discrepancies. The performance met the acceptance criteria.

Trending

The claimed *Enterobacteriaceae* organisms were also evaluated for trending. This trending calculation takes into account MIC values that are determined to be ≤1 and ≥1 doubling dilutions compared to the reference method irrespective whether the device MIC values are on-scale or not. The analysis showed that within EA, trending was observed for *E. coli, K. oxytoca, P. mirabilis* and *E. cloacae.* The trending analysis was shown in Tables 4.1 and 4.2:

Table 4.1: Trending Analysis of Evaluable Clinical and Challenge Results for *E. coli* and *K. pneumoniae*

Ceftazidime 0.016- 256 µg/mL	Total [a]	Difference in MIC as Compared to the CLSI Reference Method				
		≤2 dil. lower	1 dil. lower	Exact	1 dil. higher	≥2 dil. higher
E. coli	91	2	43	35 (38.5%)	7	4
		45 (49.5%) [b] 95% CI (39.41% to 59.54%)			11 (12.09%) [b] 95% CI (6.88% to 20.36%)	
K. oxytoca	31	0	15	12 (38.71%)	3	1
		15 (48.39%) [c] 95% CI (31.97% to 65.16%)			4 (12.90%) [c] 95% CI (5.13% to 28.85%)	

[a] Total number of evaluable results for trending analysis
[b] Difference: 37.36%; 95% CI (24.35% to 48.71%)
[c] Difference: 35.48%; 95% CI (12.60% to 53.97%)

A lower MIC reading trend was observed in the overall performance of *E. coli* and *Klebsiella oxytoca* compared to the CLSI broth microdilution reference method, which raises concerns for potential very major discrepancies. This trending and the potential for occurrence of very major discrepancies for ceftazidime when testing

clinical and challenge isolate results with the Ceftazidime MTS was addressed by adding the following footnote in the Performance Characteristics section of the labeling, "Drug Specific Supplement for Ceftazidime MIC Test Strip (MTS)":

"The Liofilchem MIC Test Strip (MTS) ceftazidime values tended to be in exact agreement or at least one doubling dilution lower when testing E. coli and K. oxytoca compared to the CLSI reference broth microdilution."

Table 4.2: Trending Analysis of Evaluable Clinical and Challenge Results for *E. cloacae* and *P. mirabilis*

Ceftazidime 0.016- 256 µg/mL	**Total [a]**	**Difference in MIC as Compared to the CLSI Reference Method**				
		≤2 dil. lower	**1 dil. lower**	**Exact**	**1 dil. higher**	**≥2 dil. higher**
E. cloacae	35	0	5	13 (37.14%)	13	4
		5 (14.29%) [b] 95% CI (6.26% to 39.37%)			17 (48.57%) [b] 955CI (32.99% to 64.43%)	
P. mirabilis	30	0	1	16 (53.33%)	11	2
		1 (3.33%) [c] 95% CI (0.59% to 16.67%)			13 (43.33%) [c] 95% CI 27.38% to 60.80%)	

[a] Total number of evaluable results for trending analysis
[b] Difference: -34%; 95% CI (-52.06% to -12.60%)
[c] Difference: -40%; 95% CI (-57.68% to -19.20%)

A higher MIC reading trend was observed in the overall performance of *Enterobacter cloacae* and *Proteus mirabilis* 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 for ceftazidime when testing clinical and challenge isolate results with the Ceftazidime MTS was addressed by adding the following footnote in the Performance Characteristics section of the labeling, "Drug Specific Supplement for Ceftazidime MIC Test Strip (MTS)":

"The Liofilchem MIC Test Strip (MTS) ceftazidime values tended to be in exact agreement or at least one doubling dilution higher when testing E. cloacae and P. mirabilis compared to the CLSI reference broth microdilution."

The analysis of the *Pseudomonas aeruginosa* MIC data demonstrated no notable trending.

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 Ceftazidime (µg/mL)

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

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.