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

A. 510(k) Number:

K173817

B. Purpose for Submission:

To obtain a substantial equivalence determination of the Liofilchem MIC Test Strip (MTS) containing Ceftazidime/avibactam at concentrations of 0.016/4 – 256/4 µg/mL for susceptibility testing of select Gram-negative bacilli

C. Measurand:

Ceftazidime/avibactam 0.016/4 – 256/4 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), Ceftazidime-avibactam 0.016/4 – 256/4 µg/mL

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.1640 Antimicrobial Susceptibility Test Powder

2. Classification:

   Class II

3. Product code(s):

   JWY – Manual Antimicrobial Test Systems

4. Panel:

   Microbiology (83)

H. Intended Use/Indications for 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 Ceftazidime-avibactam MTS at concentrations of 0.016/4-256/4 µg/mL should be interpreted at 16-20 hours of incubation.

   Ceftazidime-avibactam has been shown to be active both clinically and *in vitro* against the non-fastidious bacteria listed below:

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

   The following *in vitro* data are available but their clinical significance is unknown:

   Citrobacter koseri
   Enterobacter aerogenes
   Providencia stuartii

2. Indications for Use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For Prescription Use Only

   Limitations:

   - *Enzyme group characterization was not available for organisms at the time of comparative testing, and therefore the performance of Liofilchem MIC Test Strip*

Ceftazidime/avibactam MTS for non-fastidious gram negative bacilli is unknown for the following: Enterobacteriaceae (TEM, SHV, CTX-M, KPC, AmpC, and OXA); Pseudomonas aeruginosa (chromosomal AmpC, loss of OprD).

- *Due to the lack of an intermediate interpretive category for ceftazidime/avibactam, testing of P. aeruginosa with this drug has resulted in major discrepancies for isolates that are otherwise within essential agreement of the reference method. Testing should be confirmed using an alternative testing/reference method prior to reporting results for P. aeruginosa when the MTS MIC is 16 µg/mL.*

4. Special instrument requirements:

   Manual reading only

I. Device Description:

The Ceftazidime/avibactam MIC Test Strip (MTS) consists of specialized paper impregnated with a predefined concentration gradient of Ceftazidime/avibactam across 15 two-fold dilutions like those dilutions of a conventional MIC method. One side of the strip is labelled with the Ceftazidime/avibactam code (CZA) and the MIC reading scale is 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. The MIC Test Strip is single use only. 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:

Similarities		
Item	**Device: Liofilchem MTS, Ceftazidime/avibactam (K173817)**	**Predicate Device: 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 0.5 McFarland. Inoculum is applied to agar with swab manually or with rotation plate	Same
Reading	Manual; the point where the edge of inhibition ellipse intersects the MIC Test Strip	Same
Result	MIC (µg/mL)	Same
Differences		
Item	**Device: Liofilchem MTS, Ceftazidime/avibactam (K173817)**	**Predicate Device: Liofilchem MTS, vancomycin (K153687)**
Antimicrobial Agent	Ceftazidime/avibactam	Vancomycin
Incubation	35°C ± 2°C for 16-20 hours	35°C ± 2°C for 24 hours

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:

MIC Test Strips (MTS) are made of special high quality paper impregnated with a predefined concentration gradient of antibiotic, across 15 two-fold dilutions like those of a conventional MIC method. When the MTS 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 MTS.

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:

Reproducibility testing was conducted at three clinical sites using ten isolates of Gram-negative bacilli 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 *E. coli* (two isolates), *K. pneumoniae* (one isolate), *E. cloacae* (one isolate), and *P. aeruginosa* (six isolates). The mode MIC value 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 Ceftazidime/avibactam MTS was calculated. There were no off-scale MIC 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):

Quality Control (QC) Testing

FDA/CLSI recommended QC organisms were tested throughout the comparative testing at three study sites. The organisms tested were *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 700603, and *E. coli* ATCC 25922. These recommended QC organisms were tested a minimum of 20 times/site by both the Liofilchem Ceftazidime/avibactam MTS and the CLSI broth microdilution reference method.

Both the reference and test methods were within the expected range >95% of the time. In instances where any organism was out of range for the reference method, all testing data from that day was invalid and repeated. The Ceftazidime/avibactam MIC QC results are summarized in Table 1. All QC results were acceptable.

Table 1. Quality Control Results Summary for Ceftazidime/avibactam MTS

Organism	Concentration (µg/mL)	Reference	MTS
P. aeruginosa ATCC 27853 Expected Range 0.5/4 – 4/4 µg/mL	0.25/4		
	0.5/4	1	
	1/4	14	29
	2/4	43	30
	4/4	3	1
	8/4		
K. pneumoniae ATCC 700603 Expected Range 0.25/4 – 2/4 µg/mL	0.12/4		
	0.25/4		
	0.5/4	32	12
	1/4	29	47
	2/4	1	1
	4/4		
E. coli ATCC 25922 Expected Range 0.06/4 – 0.5/4 µg/mL	0.03/4		
	0.06/4	2	
	0.12/4	28	10
	0.25/4	30	46
	0.5/4	2	4
	1/4		

In addition, it is recommended in both the FDA Pharmaceutical label and CLSI M100/S28 document that *K. pneumoniae* ATCC 700603 be tested against ceftazidime alone to ensure the presence of the plasmid encoding beta-lactamase when testing this beta-lactam/beta-lactamase inhibitor combination. The acceptable MIC range for ceftazidime alone is 16 – 64 µg/mL. The sponsor did not perform this ancillary testing during their studies; however, the following recommendation was included in the Quality Control Section of the Ceftazidime/avibactam Liofilchem MIC Test Strip (MTS) labeling to instruct users regarding this additional QC testing.

> *K. pneumoniae ATCC 700603 should be tested against ceftazidime/avibactam and ceftazidime alone to confirm the activity of avibactam in the combination and to ensure that the plasmid encoding the beat-lactamase has not been lost in this strain. The acceptable range for ceftazidime alone is 16 – 64 µg/mL.*

Inoculum Density Check

The inoculum was prepared to achieve a 0.5 McFarland standard turbidity. Colony counts were performed periodically at each site as part of QC and reproducibility procedures as well as during clinical studies to demonstrate that the inoculum procedure results were in the expected CFU/mL (approximately $1x10^8$ 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:

Results obtained with the Liofilchem MIC Test Strip (MTS), Ceftazidime/avibactam were compared to results obtained with the CLSI broth microdilution reference panel. The reference panel contained two-fold serial dilutions with a range of ≤ 0.016/4 to ≥ 256/4 µg/mL. The testing conditions for the reference method were consistent with CLSI guideline, M07-A10.

The Liofilchem MIC Test Strip (MTS), Ceftazidime/avibactam was evaluated by three sites; two located in the United States and one in the United Kingdom. Every clinical isolate was tested one time by Ceftazidime/avibactam MTS and the reference method using the same initial standardized suspension.

Of the 297 clinical *Enterobacteriaceae* isolates, there were 243 (81.8%) fresh isolates that were tested within seven days of isolation and 54 (18.2%) recent isolates that were tested within one year of isolation. In addition, of the 148 clinical *P. aeruginosa* isolates, there were 120 (81.1%) fresh and 28 (18.9%) recent isolates. All clinical strains grew in both the MTS agar plates and the broth microdilution panels.

A total of 85 challenge isolates (43 *Enterobacteriaceae* + 42 *P. aeruginosa*) were also evaluated at one site.

The comparative study (both clinical and challenge organisms) included 340 *Enterobacteriaceae* which consisted of *C. freundii, C. koseri, E. aerogenes, E. cloacae, E. coli, K. oxytoca, K. pneumoniae, Proteus species* (*P. vulgaris*, *P. mirabilis, P. stuartii*), and 191 *P. aeruginosa* isolates.

The performance of clinical and challenge isolates is summarized in Tables 2 and 3 for *Enterobacteriaceae* and *P. aeruginosa* respectively.

Table 2. Performance[‡] of *Enterobacteriaceae* Isolates

Organism Group	EA Total	EA N	EA %	Eval EA Total	Eval EA N	Eval EA %	CA N	CA %	#R	Min	Maj	Vmj
***Enterobacteriaceae* ≤8/4 (Susceptible), ≥16/4 (Resistant)**												
Clinical	297	291	98.0	271	265	97.8	297	100	35	N/A	0	0
Challenge	43	43	100	40	40	100	43	100	7	N/A	0	0
Combined	340	334	98.2	311	305	98.1	340	100	42	N/A	0	0

[‡]**EA** – Essential Agreement (+/- 1doubling dilution)
CA – Category Agreement
EVAL – Evaluable isolates
R – Resistant isolates
Min – Minor discrepancies
Maj – Major discrepancies
Vmj – Very major discrepancies

Essential Agreement (EA) is when the Liofilchem MIC Test Strip (MTS) results agree exactly or within one doubling dilution of the reference broth microdilution results. Category Agreement (CA) is when the Liofilchem MIC Test Strip (MTS) result interpretation agrees exactly with the reference broth microdilution result interpretation. Evaluable results were defined as when both the reference method results and the Liofilchem MTS results were on-scale. Evaluable results were also defined as when the reference method results were on-scale and off-scale Liofilchem MTS results clearly did not agree within the accepted +/- one doubling dilution.

Table 3. Performance of *P. aeruginosa* Isolates

Organism Group	EA Total	EA N	EA %	Eval EA Total	Eval EA N	Eval EA %	CA N	CA %	#R	Min	Maj	Vmj
***P. aeruginosa* ≤8/4 (Susceptible), ≥16/4 (Resistant)**												
Clinical	148	133	89.9	139	124	89.2	143	96.6	39	N/A	4	1
Challenge	43	42	97.7	27	26	96.3	39	90.7	30	N/A	4	0
Combined	191	175	91.6	166	150	90.4	182	95.3	69	N/A	8	1

Overall Performance:

The overall performance of the Ceftazidime/avibactam MIC Test Strip for *Enterobacteriaceae* is acceptable with an EA of 98.2% and CA of 100%. In addition, there were no very major (0%, 0/42 resistant isolates) or major errors.

The overall performance for *P. aeruginosa* was acceptable with an EA of 91.6% and CA of 95.3%. In addition, there were eight major errors (6.6%, 8/122 susceptible isolates) and one very major error (1.4%, 1/69 resistant isolates). Given that there is no intermediate breakpoint, the MIC data for these isolates was analyzed to determine if the results are within essential agreement of the reference MIC values. Seven of the eight major errors were considered acceptable because they were within essential agreement. Taking this into consideration, the adjusted major error rate would be 0.8% (1/122 susceptible isolates) but the very major error rate remained at 1.4%. However, because of the observed high major error rate with this organism, the following limitation was added to the device labeling to address the possibility of major errors when testing *P. aeruginosa*:

Due to the lack of an intermediate category for ceftazidime/avibactam, testing of P. aeruginosa with this drug has resulted in major discrepancies for isolates that are otherwise within essential agreement of the reference method. Testing should be repeated using an alternative testing/reference method prior to reporting results for P. aeruginosa when the MTS MIC is 16 µg/mL.

Resistant Organisms and Enzyme Group Characterization:
A total of 111 resistant isolates were identified in the combined clinical (n=74) and challenge (n=37) study of the Ceftazidime/avibactam MTS out of 531 organisms (20.9%). However, characterization of resistant isolates was not provided in the submission. Therefore, the following was included as a limitation in the labeling:

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

In addition, given that Ceftazidime/avibactam is not active against bacteria that produce metallo-beta lactamases and may not have activity against Gram-negative bacteria that overexpress efflux pumps or have porin mutations, the following was included in the labeling:

Ceftazidime/avibactam is not active against bacteria that produce metallo-beta lactamases and may not have activity against gram-negative bacteria that overexpress efflux pumps or have porin mutations.

MIC Trends:
Using the combined clinical and challenge data for *Enterobacteriaceae* and *P. aeruginosa*, an analysis of trending was conducted. This trending calculation considers 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 evaluable data for trend analysis is presented in Table 4 for all organisms.

Table 4. Trending Analysis of Evaluable Clinical and Challenge Results for *Enterobacteriaceae*

Ceftazidime/ avibactam 0.016/8 – 256/8 µg/mL	Total Isolates	Difference in MIC as Compared to the CLSI Reference Method				
		≥ 2 dilution lower	1 dilution lower	Exact	1 dilution higher	≥ 2 dilution higher
C. freundii[a]	12	0	1	5 (41.67%)	6	0
		1 (8.33%)			6 (50.0%)	
C. koseri[b]	9	0	2	6 (66.67%)	1	0
		2 (22.22%)			1 (11.11%)	
E. aerogenes[c]	8	0	2	3 (37.5%)	2	1
		2 (25.0%)			3 (37.5%)	
E. cloacae[d]	39	0	8	24 (61.54%)	7	0
		8 (20.51%)			(17.95%)	
E. coli[e]	96	2	14	65 (67.71%)	14	1
		16 (16.67%)			62 (15.63%)	
K. oxytoca[f]	32	1	5	21 (65.63%)	5	0
		6 (18.75_			5 (15.63%)	

K. pneumoniae[g]	64	0	7	47 (73.44%)	9	1
		7 (10.94%)			10 (15.63%)	
Proteus spp.[h]	42	0	6	12 (28.57%)	24	0
		6 (14.29%)			24 (57.14%)	
P. stuartii[i]	10	0	1	5 (50.0%)	4	0
		1 (10.0%)			4 (40.0%)	
All *Enterobacteriaceae*[j]	312	3	46	188 (60.26%)	72	3
		49 (15.73%)			75 (24.04)	
P. aeruginosa.[k]	175	1	24	91 (52.0%)	44	15
		25 (14.29%)			59 (33.71%)	

[a] Difference: 41.67%: 95% CI (5.08% to 67.22%)
[b] Difference: -11.11%: 95% CI (-44.89% to 24.97%)
[c] Difference: 12.5%: 95% CI (-29.07% to 49.08%)
[d] Difference: -2.56%: 95% CI (-20.06% to 15.08%)
[e] Difference: -1.04%: 95% CI (-11.57% to 9.5%)
[f] Difference: -3.13%: 95% CI (-21.86% to 15.78%)
[g] Difference: 4.69%: 95% CI (-7.44% to 16.83%)
[h] Difference: 42.86%: 95% CI (22.69% to 58.54%)
[I] Difference: 30.0%; 95% CI (-8.24% to 59.88%)
[j] Difference: 8.33%; 95% CI (2.07% to 14.54%)
[k] Difference: 19.43%; 95% CI (10.55% to 27.95%)

Note: A positive percent difference value indicates higher MIC when compared to the reference method.
A negative percent difference value indicates lower MIC when compared to the reference method.

Despite the small number of available isolates with evaluable data, a higher MIC reading trend was observed in the overall performance of *C. freundii*, *Proteus* spp., and *P. stuartii* compared to CLSI broth microdilution. This observation raises concerns for potential major errors with these groups of organisms.

This trending appears to be consistent with the observed high major error rate. To address the potential occurrence of major error(s) when using the Ceftazidime/avibactam MTS, the following statement was added as a footnote in the Performance Characteristics section of the labeling, "Drug Specific Supplement for MIC Test Strip (MTS), Ceftazidime-avibactam (CZA)":

> *Liofilchem MIC Test Strip (MTS) Ceftazidime-avibactam MIC values tended to be in exact agreement or at least one doubling dilution higher when testing C. freundii, Proteus species, and P. stuartii compared to the CLSI reference broth microdilution.*

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 interpretive criteria for Ceftazidime/avibactam are shown in Table 5.

Table 5. Interpretive Criteria for Ceftazidime/avibactam (FDA Drug Label)

Organism	FDA Interpretive Criteria for Ceftazidime/avibactam (µg/mL)		
	S	I	R
Enterobacteriaceae and *P. aeruginosa*	≤8/4	NA	≥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.