Food and Drug Administration
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www.fda.gov

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K192345

B Applicant

Liofilchem s. r. l.

C Proprietary and Established Names

MTS Ampicillin-Sulbactam 0.016/0.008 - 256/128 µg/mL

D Regulatory Information

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

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for Ampicillin-sulbactam at concentrations of 0.016/0.008-256/128 µg/mL for susceptibility testing of non-fastidious Gram-negative organisms.

B Measurand:

MTS Ampicillin-sulbactam in the dilution range of 0.016/0.008-256/128 µg/mL. The concentrations represent the ratio of Ampicillin to Sulbactam in 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:

MTS (MIC Test Strip) Ampicillin-sulbactam 0.016/0.008-256/128 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. MTS Ampicillin-sulbactam at concentration of 0.016/0.008-256/128 µg/mL should be interpreted at 16-20 hours of incubation.

Ampicillin-sulbactam has been shown to be active both clinically and in vitro against these bacterial species according to the FDA drug approved label:

Gram-negative bacteria

Enterobacter asburiae

Enterobacter cloacae

Escherichia coli

Klebsiella aerogenes

Klebsiella oxytoca

Klebsiella pneumoniae

Proteus mirabilis

Acinetobacter baumannii/Acinetobacter calcoaceticus complex

Ampicillin-sulbactam has been shown to be active in vitro only against the non-fastidious bacteria listed below according to the FDA drug approved label:

Gram-negative bacteria

Morganella morganii

Proteus vulgaris

Providencia rettgeri

Providencia stuartii

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

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 ampicillin-sulbactam, across 15 two-fold dilutions similar to dilutions used by conventional MIC methods. One side of the strip is labelled with the ampicillin-sulbactam code (AMS) 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 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 0.125 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 ug/mL

B Predicate 510(k) Number(s):

K153687

C Comparison with Predicate(s):

Table 1. Comparison with the Predicate

Device & Predicate Device(s):	Device K192345	Predicate K153687
Device Trade Name	MTS Ampicillin-sulbactam 0.016/0.008-256/128 µg/mL	Liofilchem MIC Test Strip (MTS)-Vancomycin 0.016 - 256 ug/mL
General Device Characteristic Similarities		
Media	Mueller Hinton agar	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 for even distribution of inoculum.	Same
Reading	Manual; the point where the edge of inhibition ellipse intersects the MIC Test Strip	Same
Results	MIC (µg/mL)	Same
General Device Characteristic Differences		
Intended Use/Indications for Use	Quantitative susceptibility to antimicrobial agents against Gram-negative organisms	Quantitative susceptibility to antimicrobial agents against Gram-positive organisms
Antibiotic	Ampicillin-sulbactam (AMS) combination	Vancomycin (VA)
Drug Concentration Range	0.016/0.008-256/128 µg/mL	0.016-256 µg/mL
Incubation	35ºC ±2ºC for 16-20 hours	35ºC ±2ºC for 24 hours

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 negative organisms. Each isolate was tested in triplicates over three days. The reproducibility panel included one *A. baumannii*, one *E. cloacae*, three *E. coli*, one *K. aerogenes*, one *K. oxytoca*, two *K. pneumoniae*, and one *P. mirabilis* isolates. The mode of MIC value was pre-determined, 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. The testing resulted in overall reproducibility of greater than 95%.

   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 to achieve turbidity equivalent to a 0.5 McFarland standard. 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 1x 10^8 CFU/mL Colony counts was 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 Failure Rate:
   None of the clinical and challenge strains in the study failed to grow with both the broth microdilution panels and the MTS agar plates.

Quality control (QC) Testing:
The QC strains recommended for routine testing by the CLSI for testing the Ampicillin-sulbactam, namely, *E. coli* ATCC 35218, and *K. pneumoniae* ATCC 700603 were tested at three sites for a minimum of 20 times at each testing site. Ampicillin-sulbactam MIC results for these QC strains are summarized in Table 2.

Table 2: QC Results for Ampicillin-Sulbactam with the CLSI Recommended QC Strain

Organism	Concentration (µg/mL)	Reference BMD (All Sites)	MTS (All Sites)
E. coli ATCC 35218	4	0	0
	8	0	2
Expected Results:	16	49	24
	32	12	35
8/4-32/16 µg/mL	64	0	0
K. pneumoniae ATCC 700603	4	0	0
	8	0	0
	16	13	13
Expected Results:	32	48	45
8/4-32/16 µg/mL	64	1[a]	4[a]

[a] One (1) BMD (MIC = 64µg/mL) and 4 MTS (MICs all at 48 µg/mL) outlier QC results for *K. pneumoniae* ATCC 700603 from site 1. There were 1 to 2 additional replicates from this strain from site 1 that were tested on the same day and results were in range for both BMD and MTS. Also, *E. coli* ATCC 35218 results on those days were within QC range. Therefore, MTS and BMD MIC results for clinical isolates tested on those days were acceptable.

QC Strain Integrity Check: Additional QC testing was performed with ampicillin alone at the three sites to confirm the integrity of the quality control strains *K. pneumoniae* ATCC 700603 as recommended by the CLSI (Table 2 A).

Table 2A: Quality Control Summary for Ampicillin Alone

Organism	MIC (µg/mL)	Reference BMD (All Sites)
K. pneumoniae ATCC 700603	64	0
Expected Results: >128 µg/mL	>128	46

Auxiliary QC strains: In addition to testing the QC strains recommended for routine testing by CLSI (Table 2), *E. coli* ATCC 25922 was also tested at each site by both the MTS and the reference BMD method. Even though this auxiliary QC strain generally is not relevant for verification of the activity of the ampicillin-sulbactam combination, it does provide verification of the activity of ampicillin component of the drug (Table 2B).

Table 2B. Quality Control Results for Ampicillin-Sulbactam with Auxiliary QC Strain

Organism[b]	Concentration (µg/mL)	Reference BMD (All Sites)	MTS (All Sites)
E. coli ATCC 25922	1	0	0
	2	1	1
Expected Results:	4	58	46
	8	3	15
2/1-8/4 µg/mL	16	0	0

All QC results were acceptable.

6. Detection Limit:

Not Applicable

7. Assay Cut-Off:

Not Applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

Results obtained with Liofilchem MIC Test Strip (MTS) with ampicillin-sulbactam were compared to results obtained from frozen reference MIC panels. Reference panels were prepared with Muller Hinton broth as outlined in CLSI recommendations in M7-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 plates in an inverted position at 35°C ±2 for 16-20 hours. At the end of incubation, the MIC value where the edge of the inhibition ellipse intersects the strip was compared to MIC results obtained with the reference method.

Clinical:
Clinical testing was performed at three US sites with both MTS Ampicillin-sulbactam and the reference method using a total of 321 *Enterobacteriaceae* (12 *Morganella morganii*, 30 *K. aerogenes*, 31 *E. cloacae*, 8 *E. asburiae*, 90 *E. coli*, 30 *K. oxytoca*, 60 *K. pneumoniae*, 15 *P. mirabilis*, 15 *P. vulgaris*, 15 *P. rettgeri* and 15 *P. stuartii*) and 47 *Acinetobacter baumannii* clinical isolates.

A total of 368 non-fastidious Gram-negative isolates tested of which 206 (56%) were tested within 6 months of isolation (contemporary isolates).

Challenge:
Challenge testing was performed at one internal site using MTS Ampicillin-sulbactam and the reference method. A total of 69 *Enterobacteriaceae* (4 *Morganella morganii*, 11 *K.*

aerogenes, 12 *E. cloacae*, 11 *E. coli*, 7 *K. oxytoca*, 11 *K. pneumoniae*, 7 *P. mirabilis*, 2 *P. vulgaris*, 2 *P. rettgeri*, 2 *P. stuartii*) and 7 *Acinetobacter baumannii* challenge isolates were tested.

The total of 444 non-fastidious Gram-negative organisms (390 *Enterobacteriaceae* and 54 *Acinetobacter baumannii*) clinical and challenge isolates is summarized in Table 3.

Table 3. Performance of MTS Ampicillin-Sulbactam

	Tot	EA N	EA %	Eval Tot	Eval EA N	Eval EA %	CA Tot	CA %	No. R	No. S	min	maj	vmj
Enterobacteriaceae [a]													
Clinical	321	318	99.1	218	215	98.6	289	90	194	87	32	0	0
Challenge	69	68	98.6	13	12	92.3	69	100	63	3	0	0	0
Total	390	386	**99**	231	227	**98.3**	358	**91.8**	257	90	32	0	0
Acinetobacter baumannii[b]													
Clinical	47	47	100	41	41	100	42	89.4	20	20	5	0	0
Challenge	7	7	100	2	2	100	7	100	5	0	0	0	0
Total	54	54	**100**	43	43	**100**	49	**90.7**	25	20	5	0	0
All Gram-negative													
Clinical	368	365	**99.2**	259	256	**98.8**	331	**89.9**	214	107	37	0	0
Challenge	76	75	**98.7**	15	14	**93.3**	76	**100**	68	3	0	0	0
Combined	444	440	**99.1**	274	270	**98.5**	407	**91.7**	282	110	37	0	0

[a] *Enterobacteriaceae* is comprised of *E. asburiae, E. cloacae, E. coli, K. aerogenes, K. oxytoca, K. pneumoniae, P. mirabilis, M.morganii, P. vulgaris, P. stuartii*

b Combined clinical and challenge A. baumannii (54) were tested as representative of the A. baumannii/A. calcoaceticus complex

EA – Essential Agreement
CA – Category Agreement
EVAL – Evaluable isolates
R or NS – Resistant or non-susceptible isolates

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 *Enterobacteriaceae* isolates (Table 3) is acceptable with 99% EA and 91.8% CA. There were no major or very major discrepancies.

The overall performance of *Acinetobacter baumannii* isolates (Table 3) is acceptable with 100% EA and 90.7% CA. There were no major or very major discrepancies.

Results of comparative testing with clinical and challenge isolates combined demonstrated an EA of 99.1% and CA of 91.7 for all non-fastidious Gram-negative organism which was acceptable (Table 3).

To address testing and reporting of MIC results for non-indicated species the sponsor included the following statement in the Precautions section of the device 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.

Resistance Mechanism Characterization

Challenge isolates of *Enterobacteriaceae* and *A. baumannii* harboring various molecular mechanisms of resistance were evaluated with MTS Ampicillin-sulbactam. Isolates harboring the following resistance mechanisms were evaluated: NDM, OXA, *KPC, TEM, SHV, CTX-M, CMY, ACT, IMP, AmpC, ESBL, VIM, DHA, tet(A)*

MIC Trending Analysis

Using the combined clinical and challenge data, an analysis of trending was conducted for both *Enterobacteriaceae* and *A. baumannii*. 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.

No significant trending was observed for *Enterobacteriaceae* overall, however, a trend toward higher MIC reading was observed for *E. cloacae, E. coli, K. aerogenes, M. morganii, P. mirabilis, P. stuartii* and *A. baumannii* with MTS Ampicillin-sulbactam when compared to the reference method (Table 4).

Table 4. Trending for MTS Ampicillin-Sulbactam with Gram-Negative Organisms

Organism	Total Evaluable for Trending	≥ 1 Dilution lower No. (%)	Exact No. (%)	≥ 1 Dilution Higher No. (%)	Percent Difference (CI)*	Trending Noted
E. asburiae	7	0 (0.00)	4 (57.14)	3 (42.86)	42.9	No
E. cloacae	22	1 (4.55)	10 (45.45)	11 (50.00)	45.5	Yes
E. coli	73	7 (9.59)	36 (49.32)	30 (41.10)	31.5	Yes
K. aerogenes	26	1 (3.85)	11 (42.31)	14 (53.85)	50.0	Yes
K. oxytoca	24	3 (12.50)	19 (79.17)	2 (8.33)	-4.2	No
K. pneumoniae	35	3 (8.57)	22 (62.86)	10 (28.57)	20	No
M. morganii	13	1 (7.69)	4 (30.77)	8 (61.54)	53.8	Yes
P. mirabilis	20	1 (5.00)	10 (50.00)	9 (45.00)	40.0	Yes
P. vulgaris	17	6 (35.29)	7 (41.18)	4 (23.53)	-11.8	No
P. rettgeri	15	5 (33.33)	6 (40.00)	4 (26.67)	-6.7	No
P. stuartii	17	0 (0.00)	10 (58.82)	7 (41.18)	41.2	Yes
Enterobacteriaceae	269	28 (10.41)	138 (51.30)	103 (38.29)	27.9	No
A.baumannii	48	3 (6.25)	24 (50.00)	21 (43.75)	37.5	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.

To address the observed high trend, the following footnote has been added to the labeling in the performance characteristics section:

MTS Ampicillin-sulbactam MIC values tended to be in exact agreement or at least one doubling dilution higher compared to the CLSI broth microdilution reference method when testing E. cloacae, E. coli, K. aeruginosa, M. morganii, P. mirabilis, P. stuartii and A. baumannii.

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 recognized susceptibility interpretive criteria for Ampicillin-sulbactam are listed in Table 5.

Table 5. FDA Identified Interpretive Criteria for Ampicillin-Sulbactam

Organism	Interpretive Criteria for Ampicillin-Sulbactam MIC (µg/mL) [a]		
	Susceptible (S)	Intermediate (I)	Resistant (R)
Enterobacteriaceae [b]	**≤8/4**	**16/8**	**≥32/16**
Acinetobacter spp	**≤8/4**	**16/8**	**≥32/16**

[a] FDA STIC Webpage
https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm410971.htm
[b] *E. asburiae, E. cloacae, E. coli, K. aerogenes, K. oxytoca, K. pneumoniae, P. mirabilis, M. morganii, P. vulgaris, P. stuartii*

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 Ampicillin-sulbactam 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 Ampicillin-sulbactam 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.