510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K211672

B Applicant

Liofilchem s.r.l.

C Proprietary and Established Names

MTS Piperacillin-tazobactam 0.016/4 - 256/4µg/mL

D Regulatory Information

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

II Submission/Device Overview:

A Purpose for Submission:
To obtain a substantial equivalence determination of the Liofilchem MIC Test Strip (MTS) containing piperacillin-tazobactam at concentrations of 0.016/4 – 256/4 µg/mL for susceptibility testing of select gram negative organisms.

B Measurand:
Piperacillin-tazobactam in the dilution range of 0.016/4 - 256/4 µg/mL

C Type of Test:
Quantitative AST 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) Piperacillin-tazobactam 0.016/4-256/4 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 Piperacillin-tazobactam at concentrations of 0.016/4-256/4 µg/mL should be interpreted at 16-20 hours of incubation.

MTS TZP can be used to determine the MIC of piperacillin-tazobactam against the following microorganisms for which piperacillin-tazobactam has been shown to be active clinically and/or in vitro according to the FDA drug approved label:

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

Escherichia coli
Klebsiella pneumoniae
Pseudomonas aeruginosa
Acinetobacter baumannii

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

Citrobacter koseri
Proteus mirabilis
Serratia marcescens

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

The ability of the MTS to detect non-susceptible isolates with the following drug/bacterial species combinations is unknown because resistant isolates were either not available or an insufficient number was encountered at the time of comparative testing.

Piperacillin-tazobactam: *Citrobacter koseri, Proteus mirabilis, Serratia marcescens*

D Special Instrument Requirements:

Manual readings only

IV Device/System Characteristics:

A Device Description:

MTS Piperacillin-tazobactam 0.016/4-256/4 µg/mL is made of special high quality paper impregnated with a predefined concentration of gradient piperacillin-tazobactam, across 15 two-fold dilutions like those of a conventional MIC method. One side of the strip is labeled with the piperacillin tazobactam code (TZP) and the MIC reading scale in µg/mL. The MIC Test Strip (MTS) is single use only.

B Principle of Operation:

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 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 MTS.

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: Predicate Comparison

Device & Predicate Device(s):	**Device:** K211672	**Predicate:** K153687
Device Trade Name	MTS Piperacillin-tazobactam 0.016/4 - 256/4 µg/mL	Liofilchem MIC Test Strip (MTS)-Vancomycin 0.016 -256 µg/mL
General Device Characteristic Similarities		
Plate 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.	Same
Reading	Manual; Interpret the MIC as 100% inhibition	Same
Result	MIC in µg/mL	Same
General Device Characteristic Differences		
Intended Use/Indications For Use	Quantitative susceptibility to antimicrobial agents against specified Gram-negative organisms.	Quantitative susceptibility to antimicrobial agents against specified Gram-positive organisms

Antimicrobial Agent	Piperacillin-tazobactam (TZP)	Vancomycin (VA)
Incubation	35°C ± 2°C for 16-20 hours	35°C ± 2°C for 24 hours

VI Standards/Guidance Documents Referenced:

Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems; Guidance for Industry and FDA, 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-S31 "*Performance Standards for Antimicrobial Susceptibility Testing"* January 2021

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

   Reproducibility testing of the Liofilchem Piperacillin-tazobactam MIC test strip (MTS) was performed using 10 isolates (1 *A. baumannii*, 1 *P. aeruginosa*, 3 *K. pneumoniae*, and 5 *E. coli*). Testing was performed at three sites in triplicate on three separate days (270 data points) to determine site to site and overall reproducibility. The mode of the MIC values was determined for each organism and the reproducibility was calculated based on the number of MIC values that fell within ± one doubling dilution of the mode. Results were within one +/- doubling dilution agreement as compared to the mode MIC value of Piperacillin/tazobactam for all organisms at all sites and there were no off-scale results. The results were acceptable and demonstrated 100% reproducibility.

2. Linearity:
   Not applicable

3. Analytical Specificity/Interference:
   Not appliable

4. Assay Reportable Range:
   Not applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   The QC strains recommended by the CLSI for routine QC testing the combination of piperacillin/tazobactam (i.e., *E. coli* ATCC 35218 and *K. pneumoniae* ATCC 700603) were tested at three sites for a minimum of 20 times at each site by both the MTS and the reference method. For *E. coli* ATCC 35218 reference method a single outlier result was obtained. Upon repeat testing was performed the following day and the result was within the established range at piperacillin/tazobactam concentration of 2/4 µg/mL. The results demonstrate that the

piperacillin/tazobactam MTS can produce quality control results in the recommended range >95% of the time (Table 2).

Table 2. Quality Control Test results for Piperacillin/Tazobactam

QC Organism	Expected Range (Piperacillin/ Tazobactam, µg/mL)	Concentration (µg/mL)*	Reference BMD Frequency (All Sites)	MTS Frequency (All Sites)
E. coli ATCC 35218	0.5/4-2/4	0.25		
		0.5		
		1	**25**	**11**
		2	**34**	**49**
		4	**1**	
K. pneumoniae ATCC 700603	8/4-32/4	4		
		8	**18**	**11**
		16	**39**	**46**
		32	**3**	**3**
		64		

* Tazobactam concentration was fixed at 4 µg/mL

In addition to testing *E. coli* ATCC 35218 and *K. pneumoniae* ATCC 700603, "Auxiliary" QC strains, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, were tested at each site by both the MTS and the reference. Even though these auxiliary QC strains generally are not relevant for verification of the activity of the tazobactam component of piperacillin/tazobactam combination, they do provide verification of the activity of the piperacillin component of the drug (Table 3).

Table 3. Quality Control Summary for Piperacillin/Tazobactam with Auxiliary QC Strains

QC Organism	Expected Range (Piperacillin/ Tazobactam, µg/mL)	Concentration (µg/mL)*	Reference BMD Frequency (All Sites)	MTS Frequency (All Sites)
E. coli ATCC 25922	1/4-4/4	0.5		
		1	**4**	**3**
		2	**40**	**15**
		4	**16**	**42**
		8		
P aeruginosa ATCC 27853	1/4-8/4	0.5		
		1		
		2		**1**
		4	**48**	**26**
		8	**12**	**33**
		16		

* Tazobactam concentration was fixed at 4 µg/mL

The quality control results are acceptable.

Inoculum Density Check: The inocula were prepared to approximate a 0.5 McFarland standard turbidity. Colony counts were performed periodically at each site to demonstrate that the inoculum procedure results were in the expected CFU/mL (approximately 1x 10^8 CFU/mL).

Purity Check: Purity checks were performed by subculture from the prepared inoculum for all isolates following MTS inoculation. All isolates were pure in both the broth microdilution reference panels and the MTS agar plates.

Growth Failures: There were no growth failures, all isolates grew in both the broth microdilution reference panels and on the MTS agar plates.

6. Detection Limit:
   Not applicable

7. Assay Cut-Off:
   Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Clinical testing was performed at three external sites with both MTS Piperacillin/Tazobactam and the reference method. A total of 483 clinical isolates were evaluated including: 9 *C. koseri,* 12 *P. mirabilis,* 12 *S. marcescens,* 60 *A. baumannii,* 90 *P. aeruginosa,* 150 *E. coli,* and 150 *K. pneumoniae*.

   The clinical testing included 83.1% fresh (isolated no longer than 6 months prior to testing) and 16.9% stock strains (isolated over 6 months prior to testing) clinical isolates.

   A total of 97 challenge isolates were also evaluated at a single site including 20 *A. baumannii,* 20 *K. pneumoniae,* 27 *P. aeruginosa,* and 30 *E. coli*.

   Results obtained with Liofilchem MIC Test Strip (MTS) with Piperacillin/Tazobactam were compared to results obtained from frozen reference MIC panels. Reference panels were prepared, tested and interpreted as outlined in the CLSI document M07-A10.

   For MTS Piperacillin/Tazobactam, isolated colonies from an overnight agar plate were suspended in saline to achieve a 0.5 McFarland standard turbidity (approximately10^8 CFU/mL). Post inoculum application on Mueller-Hinton agar plates, testing conditions consisted of incubation of the inoculated plates in an inverted position at 35°C ±2 for 16-20h and reading of the results by manual methods. During the clinical study, one site utilized a rotator plate inoculator (Retro C80) for the plate inoculation procedure. There was no difference in correlation to the reference broth microdilution for this site as compared to the other two sites. To address the use of the rotator plate inoculator in the comparative study, the sponsor included the following as a footnote to the performance table:

For the plate inoculation procedure, one testing site utilized a plate rotator (Retro C80) to assist even distribution of inoculum. There was no difference in performance for the site using the plate rotator as compared to sites using the manual plate inoculation method.

A comparison was provided to the reference method with the following agreement (Table 4). The combined clinical and challenge isolate performance for members of the *Enterobacterales* was acceptable with an EA of 98.2% and CA of 95.0% with no major or very major errors. The combined clinical and challenge isolate performance for *P. aeruginosa* was acceptable with an EA of 100% and CA of 94.9% with no major or very major errors. The overall performance for *A. baumannii* was acceptable with an EA of 100% and CA of 96.3% with no major or very major errors.

Table 4. Overall Performance of Clinical and Challenge Isolates Liofilchem MTS for Piperacillin/Tazobactam

	Tot	EA N	EA %	Eval Tot	Eval EA N	Eval EA %	CA Tot	CA %	No. R	No. S	min	maj	vmj
Enterobacterales[a] **(Breakpoints (µg/mL): S ≤16/4, I 32/4-64/4, R ≥128/4)**													
Clinical	333	327	98.2%	311	306	98.4%	320	96.1%	23	310	13	0	0
Challenge	50	49	98.0%	29	28	96.6%	44	88.0%	23	27	6	0	0
Total	383	376	98.2%	340	334	98.2%	364	95.0%	46	337	19	0	0
P. aeruginosa **(Breakpoints (µg/mL): S ≤16/4, I 32/4-64/4, R ≥128/4)**													
Clinical	90	90	100%	84	84	100%	88	97.8%	8	82	2	0	0
Challenge	27	27	100%	14	14	100%	23	85.2%	13	14	4	0	0
Total	117	117	100%	98	98	100%	111	94.9%	21	96	6	0	0
A. baumannii **(Breakpoints (µg/mL): S ≤16/4, I 32/4-64/4, R ≥128/4)**													
Clinical	60	60	100%	32	32	100%	58	96.7%	27	33	2	0	0
Challenge	20	20	100%	2	2	100%	19	95%	18	2	0	0	0
Total	80	80	100%	34	34	100%	77	96.3%	45	35	2	0	0

[a] Includes *C. koseri, E. coli, K. pneumoniae, P. mirabilis* and *S. marcescens*

EA – Essential Agreement
CA – Category Agreement
EVAL – Evaluable MIC results
S – Susceptible
min – minor discrepancies
maj – manor discrepancies
vmj – very major discrepancies
R - resistant

Essential agreement (EA) is when the Liofilchem MTS results agree exactly or within one doubling dilution of the reference method. Category agreement (CA) is when the Liofilchem MTS result interpretation agrees exactly with the reference panel result interpretation.

For clinical and challenge isolates tested with the Liofilchem MTS for piperacillin/tazobactam, the overall combined %EA and %CA met the acceptance criteria of greater than or equal to 90% (Table 4).

Testing/Reporting MICs for Non-Indicated Species

For this review, the interpretative criteria are applied to the organisms/organism groups according to the FDA STIC website. As required under 511A(2)(2)(B) of the Federal Food, Drug and Cosmetic Act, the following statements are added in the package insert:

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 labeling for specific antimicrobial drugs provides the uses for which the antimicrobial drug is approved.

Resistant strains

Due to the lack of resistant isolates of *C. koseri, P. mirabilis*, and *S. marcescens* the following limitation is included in labeling:

The ability of the MTS to detect non-susceptible isolates with the following drug/bacterial species combinations is unknown because resistant isolates were either not available or an insufficient number was encountered at the time of comparative testing.

Piperacillin-tazobactam: *Citrobacter koseri, Proteus mirabilis, Serratia marcescens*

Resistance Mechanisms: Isolates with the following resistance mechanisms were evaluated:
ESBL, NDM-1, OXA-72, KPC-3, TEM-1, CMYII, CTX-M9, aac(3)-IId, aac(6')Ib-cr aadA5, catA1, catB3, CTX-M-15, dfrA17, OXA-1, strA, strB, sul2, TEM-1B, tet(B), KPC, IMP, OXA-181, VIM, NDM, OXA-1_OXA-30, SHV-7, SHV-1, OXA-10, VEB-1a, VIM-4, SPM, IMP-14, VIM-2, IMP-1

MIC Trends:
An analysis of trending was conducted using the combined clinical and challenge data (except for *P. mirabilis* and *S. marcescens* for which only clinical data was provided). 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 MIC reading 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.

A trend toward higher MIC readings was observed for *Enterobacterales (including E. coli, K. pneumoniae, P. mirabilis* and *S. marcescens), P. aeruginosa,* and *A. baumannii* when compared to the CLSI broth micro-dilution reference method, as summarized in Table 5.

Table 5. Observed Trending of Results Obtained with MTS Piperacillin/Tazobactam

Organism	Total Evaluable for Trending	≥ 1 Dilution lower No. (%)	Exact No. (%)	≥ 1 Dilution Higher No. (%)	Percent Difference (CI)	Trending Noted
E. coli, clinical & challenge	173	15 (8.7%)	83 (48.0%)	75 (43.4%)	34.7% (25.9%-42.8%)	Yes
K. pneumoniae, clincal & challenge	137	6 (4.4%)	55 (40.2%)	76 (55.5%)	51.1% (41.4%-59.5%)	Yes
P. mirabilis, clinical	12	1 (8.3%)	2 (16.7%)	9 (75.0%)	66.7% (27.6%-84.2%)	Yes
S. marcescens, clinical	12	1 (8.3%)	4 (33.3%)	7 (58.3%)	50.0% (12.2%-73.4%)	Yes
Enterobacterales, clinical & challenge	343	23 (6.7%)	146 (42.6%)	174 (50.7%)	44.4% (37.9%-49.7%)	Yes
P. aeruginosa, clinical & challenge	104	4 (3.9%)	55 (52.9)	45 (43.3%)	39.4% (21.0%-43.3%)	Yes
A. baumannii, clinical & challenge	34	1 (2.9%)	17 (50.0%)	16 (47.1%)	44.1% (24.4%-60.5%)	Yes

The trending towards higher MIC values for Piperacillin/Tazobactam when testing was addressed in the labeling by adding the following footnote:

"*Liofilchem MIC Test Strip (MTS) Piperacillin/tazobactam MIC values tended to be in exact agreement or at least one doubling dilution higher when testing C. koseri, E. coli, K. pneumoniae, S. marcescens, P. mirabilis, P. aeruginosa 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):

D Clinical Cut-Off:
Not applicable

E Expected Values/Reference Range:

Table 6. FDA-Recognized Interpretive Criteria for Piperacillin-Tazobactam

	Minimum Inhibitory Concentration (µg/mL)[a]		
Organisms	**S**	**I**	**R**
Enterobacterales	≤16/4	32/4-64/4	≥128/4
Pseudomonas aeruginosa	≤16/4	32/4-64/4	≥128/4
Acinetobacter baumannii	≤16/4	32/4-64/4	≥128/4

[a] FDA STIC webpage

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 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/DevelopmentApprovalProcess/DevelopmentResources/ucm410971.htm). 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 piperacillin/tazobactam 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 piperacillin/tazobactam device label to include (1) the new breakpoints, (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.