510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K183140

B. Purpose for Submission:

To obtain a substantial equivalence determination for Tetracycline at concentrations of 0.016-256 µg/mL for susceptibility testing of non-fastidious Gram-negative organisms and *Staphylococcus aureus*

C. Measurand:

Tetracycline 0.016-256 µg/mL

D. Type of Test:

Quantitative Antimicrobial Susceptibility Test growth-based detection

E. Applicant:

Liofilchem s.r.l.

F. Proprietary and Established Names:

MTS Tetracycline 0.016-256 µg/mL

G. Regulatory Information:

1. Regulation section:

   866.1640 Antimicrobial Susceptibility Test Powder

2. Classification:

   II

3. Product code:

   JWY - Manual Antimicrobial Susceptibility Test Systems

4. Panel:

83 – Microbiology

H. Intended Use:

1. Intended use(s):

   The Liofilchem MTS (MIC Test Strip) Tetracycline 0.016-256 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.

   The MTS Tetracycline at concentrations of 0.016-256 µg/mL should be interpreted at 16-20 hours of incubation.

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

   Staphylococcus aureus
   Acinetobacter baumannii
   Escherichia coli
   Klebsiella aerogenes
   Klebsiella oxytoca
   Klebsiella pneumonia

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   - *For prescription use*
   - *Liofilchem MIC Test Strip (MTS) Tetracycline MIC values tended to be in exact agreement or at least one doubling dilution higher when testing A. baumannii, and Enterobacteriaceae (specifically when testing K. oxytoca and K. pneumoniae) compared to the CLSI reference broth microdilution.*
   - *The safety and efficacy of tetracycline in treating clinical infections due to species of Enterobacteriaceae other than K. aerogenes, K. pneumoniae, K. oxytoca, and E. coli, and Staphylococcus species other than S. aureus may or may not have been established in adequate and well-controlled clinical trials. The clinical significance of susceptibility information in such instances is unknown.*

4. Special instrument requirements:

Manual reading only

I. Device Description:

The Tetracycline MIC Test Strip (MTS) consists of specialized paper impregnated with a predefined concentration gradient of Tetracycline across 15 two-fold dilutions similar to dilutions used by conventional MIC methods. One side of the strip is labelled with the Tetracycline code (TE) 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. 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:

Table 1: Comparison with the Predicate Device

Similarities		
Item	**Device Liofilchem MTS, Tetracycline (K183140)**	**Predicate Liofilchem MTS, Vancomycin (K153687)**
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
Result	MIC	Same

Differences		
Item	**Device Liofilchem MTS, Tetracycline (K183140)**	**Predicate Liofilchem MTS, Vancomycin (K153687)**
Intended Use	Quantitative susceptibility to antimicrobial agents against Gram-negative and *S. aureus*	Quantitative susceptibility to antimicrobial agents to Gram-positive organisms
Antibiotic	Tetracycline code (TE)	Vancomycin code (VA)
Reading	Manual; the point where the edge of inhibition ellipse intersects the MIC Test Strip; interpret the MIC as 80% inhibition when trailing is seen	Manual; the point where the edge of inhibition ellipse intersects the MIC Test Strip; interpret the MIC at 100% inhibition
Incubation	35 ± 2°C for 16 – 20 hours	35 ± 2°C for 24 hours

K. Standard/Guidance Document Referenced:

- Guidance for Industry and FDA - Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems – August 28, 2009.
- 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 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 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 0.125 µg/mL is considered to be the same as 0.12 µg/mL for reporting purposes. Given that tetracycline is a bacteriostatic drug, the ellipse should be interpreted at 80% inhibition when trailing is observed.

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:

1. Analytical performance:

   a. Precision/Reproducibility:

   Reproducibility testing was conducted at three sites using ten Gram-negative and ten Gram-positive organisms. Each isolate was tested in triplicate over three days for a total of 270 data points. The mode of MIC values was determined for each isolate and the reproducibility was calculated based on the number of MIC values that fell within ±1 doubling dilution of the mode. The Gram-negative reproducibility panel included three *E. coli,* three *K. pneumoniae*, one *K. oxytoca*, one *K. aerogenes*, and two *A. baumannii* isolates and the Gram-positive panel included five methicillin-resistant *S. aureus* five methicillin-susceptible *S. aureus* isolates. All MIC results were on scale. The testing resulted in overall reproducibility of greater than 95% for both Gram-negative and Gram-positive isolates.

   The results were acceptable.

   b. Linearity/assay reportable range:

   Not applicable

   c. Traceability, Stability, Expected values (controls, calibrators, or methods):

   Quality Control (QC) Testing:

   The CLSI recommended QC strains, namely *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 for Gram-negative organisms and *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 for Gram-positive organisms were tested a sufficient number of times (i.e., at least 20/site) at each testing site using both MTS and reference methods. The results are summarized in Table 2 below. The quality control results are acceptable.

Table 2: Tetracycline MTS QC Results

Organism	Concentration (µg/mL)	Reference	MTS
E. coli ATCC 25922 Expected Result: 0.5 - 2 µg/mL	0.25		
	0.5	27	12
	1	34	43
	2		6
	4		
P. aeruginosa ATCC 27853 Expected Result: 8 - 32 µg/mL	4		
	8	42	12
	16	19	42
	32		7
	64		
S. aureus ATCC 29213 Expected Result: 0.12 - 1 µg/mL	0.06		
	0.12	3	4
	0.25	47	41
	0.5	10	15
	1		
	2		
E. faecalis ATCC 29212 Expected Result: 8 - 32 µg/mL	4		
	8	20	13
	16	35	43
	32	5	4
	64		

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, from at least one replicate of each reproducibility isolate on each of the three days of testing, and from a minimum of 10% of the clinical and challenge strains tested. Inoculum density checks were performed and the colony counts obtained for each isolate were within the recommended range of approximately 1x 10^8 CFU/mL.

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 isolates in the study failed to grow with the Tetracycline MTS.

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:

The MTS, Tetracycline was evaluated at three sites located within the United States. Each clinical isolate was tested one time by MTS Tetracycline and the reference method using the same initial standardized suspension. A total of 315 clinical *S. aureus* isolates of which 79.7% were tested within six months of isolation (contemporary isolates) and 315 non-fastidious Gram-negative isolates were tested of which 65.7% were tested within six months of isolation. The Gram-positive organisms included 156 methicillin-susceptible *S. aureus* and 159 methicillin-resistant *S. aureus*. The Gram-negative organisms included 120 *E. coli*, 30 *K. aerogenes*, 30 *K. oxytoca*, 75 *K. pneumoniae,* and 60 *A. baumannii* isolates.

Challenge testing was performed at one internal site. A total of 75 Gram-positive and 78 Gram-negative challenge isolates were tested. The Gram-positive organisms included one methicillin-susceptible *S. aureus* and 74 methicillin-resistant *S. aureus*. The Gram-negative organisms included 19 *E. coli*, 15 *K. aerogenes*, 15 *K. oxytoca*, 19 *K. pneumoniae,* and 10 *A. baumannii* isolates.

Results obtained with the MTS Tetracycline were compared to results obtained with the CLSI broth microdilution reference panel. The reference panel contained two-fold serial dilutions of Tetracycline with a range of 0.016 – 256 µg/mL. The testing conditions for the reference method were consistent with CLSI guidelines as listed in the CLSI document M07-A10. 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 determined at 80% inhibition of growth along the strip was compared to MIC results obtained with the reference method. Visual aids were provided in reading guides to each laboratory to assist in interpretation.

The performance for the total (combined clinical and challenge) 390 *S. aureus*, 323 *Enterobacteriaceae*, and 70 *A. baumannii* isolates is summarized in Table 3 and 4 below.

Table 3: Overall Performance of *S. aureus* Clinical and Challenge Isolates

Tetracycline	EA Tot	EA N	EA %	Eval. EA Tot	Eval. EA N	Eval. EA%	CA N	CA%	#R	min	maj	vmj
S. aureus (MSSA+MRSA) Clinical	315	315	100	315	315	100	313	99.4	15	2	0	0
Challenge	75	75	100	73	73	100	74	98.7	25	1	0	0
Combined	390	390	100	388	388	100	387	99.2	40	3	0	0

Table 4: Overall Performance of Gram-negative Clinical and Challenge Isolates

Tetracycline	EA Tot	EA N	EA %	Eval. EA Tot	Eval. EA N	Eval. EA%	CA N	CA%	#R	min	maj	vmj
Enterobacteriaceae (all) Clinical	255	255	100	227	227	100	249	97.6	55	6	0	0
Challenge	68	68	100	43	43	100	67	98.5	41	1	0	0
Combined	323	323	100	270	270	100	316	97.8	96	7	0	0
A. baumannii Clinical	60	57	95.0	34	31	91.2	57	95.0	32	3	0	0
Challenge	10	10	100	3	3	100	10	100	10	0	0	0
Combined	70	67	95.7	37	34	91.9	67	95.7	42	3	0	0

EA – Essential agreement
CA – Category agreement
EVAL – Evaluable isolates
R – Resistant isolates
maj – Major errors
vmj – Very major errors
min – Minor errors

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.

The overall performance of *S. aureus* (both MRSA and MSSA) isolates is acceptable with 100% EA and 99.2% CA. There were no major or very major errors.

The overall performance of all *Enterobacteriaceae* isolates is acceptable with 100% EA and 97.8% CA. In addition, the performance for each Gram-negative organism was evaluated separately and results for each demonstrated >90% EA and >90% CA with no major or very major errors and was, therefore, deemed acceptable. In addition, the overall performance of *A. baumannii* isolates was acceptable with 95.7% EA and 95.7% CA.

Trending:

Trending was assessed separately for *S. aureus* and Gram-negative organisms using data for challenge and clinical isolates (Tables 5 and 6). Trending was assessed using current

trending review practices (i.e., ≥30% difference between higher and lower dilution readings). No significant trending was observed for *S. aureus*; however, trending was observed for *A. baumannii, K. oxytoca,* and *K. pneumoniae* which tended to be in exact agreement or higher when compared to the reference method. The difference between higher and lower dilutions for this organism was >30%. Given the observed trending, the following was included in the labeling:

- *Liofilchem MIC Test Strip (MTS) Tetracycline MIC values tended to be in exact agreement or at least one doubling dilution higher when testing A. baumannii, and Enterobacteriaceae (specifically when testing K. oxytoca and K. pneumoniae) compared to the CLSI reference broth microdilution.*

Table 5. Trending Analysis for *S. aureus*

Total	≥1 dil. lower	Exact	≥1 dil. higher
***S. aureus* (MSSA+MRSA)[a]**			
390	81	277	32
	(20.77%)	(71.03%)	(8.21%)

[a]Difference between the higher and lower dilutions for *S. aureus* is: -12.56%; 95% C.I. (-17.46% to -7.68%)

Table 6: Tetracycline Trending Analysis for Gram-negative organisms

Total	≥1 dil. lower	Exact	≥1 dil. higher
***A. baumannii*[a]**			
38	1	12	25
	(2.63%)	(31.58%)	(65.79%)
***E. coli*[b]**			
115	13	67	35
	(11.30%)	(58.26%)	(30.43%)
***K. aerogenes*[c]**			
42	1	31	10
	(2.38%)	(73.81%)	(23.81%)
***K. oxytoca*[d]**			
45	2	23	20
	(4.44%)	(51.11%)	(44.44%)
***K. pneumoniae*[e]**			
82	2	40	40
	(2.44%)	(48.78%)	(48.78%)
All *Enterobacteriaceae*[f]			
284	18	161	105
	(6.34%)	(56.69%)	(36.97%)

[a]Difference between the higher and lower dilutions for *A. baumannii* is: 63.16%; 95% C.I. (43.90% to 76.34%)
[b]Difference between the higher and lower dilutions for *E. coli* is: 19.13%; 95% C.I. (8.70% to 29.16%)
[c]Difference between the higher and lower dilutions for *K. aerogenes* is: 21.43%; 95% C.I. (7.09% to 36.28%)
[d]Difference between the higher and lower dilutions for *K. oxytoca* is: 40.00%; 95% C.I. (22.96% to 54.74%)
[e]Difference between the higher and lower dilutions for *K. pneumoniae* is: 46.34%; 95% C.I. (34.22% to 57.12%)

[f]Difference between the higher and lower dilutions for *Enterobacteriaceae* is: 30.63%; 95% C.I. (24.22% to 36.83%)

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 FDA susceptibility interpretive criteria for Tetracycline are as listed in **Table 7**.

Table 7: FDA Recognized Interpretive Criteria for Tetracycline (µg/mL)

Organisms	S	I	R
Enterobacteriaceae	≤4	8	≥16
Acinetobacter spp.	≤4	8	≥16
Staphylococcus spp.	≤4	8	≥16

N. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.