510(k) SUBSTANTIAL EQUIVALENCE DETERMINATIONS
DECISION SUMMARY

A. 510(k) Number:

K181889

B. Purpose for Submission:

To obtain a substantial equivalence determination for Penicillin (P) at concentrations of 0.002-32 µg/mL for susceptibility testing of fastidious Gram-positive organisms

C. Measurand:

Penicillin 0.002-32 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 Penicillin 0.002-32 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) Penicillin 0.002-32 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™ Penicillin at concentrations of 0.002 - 32 µg/mL should be interpreted at 20-24 hours of incubation for *Streptococcus* spp.

   MTS™ Penicillin can be used to determine the MIC of penicillin against the following bacteria. Penicillin has been shown to be active both clinically and *in vitro* against these bacterial species according to th e FDA drug approved label:

   Streptococcus pneumoniae
   Streptococcus pyogenes (Group A)
   Streptococcus dysgalactiae (Group C & G)
   Streptococcus anginosus (Group C & G)
   Streptococcus constellatus (Group C & G)
   Streptococcus intermedius (Group C & G)

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   - For prescription use
   - The ability of the MTS to detect resistant 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.

     Penicillin:
     Streptococcus pyogenes (Group A)
     Streptococcus dysgalactiae (Groups C & G)
     Streptococcus anginosus (Groups C & G)
     Streptococcus constellatus (Groups C & G)
     Streptococcus intermedius (Groups C & G)

4. Special instrument requirements:

   Manual reading only

I. Device Description:

The Penicillin MIC Test Strip (MTS) consists of specialized paper impregnated with a predefined concentration gradient of Penicillin across 15 two-fold dilutions similar to dilutions used by conventional MIC methods. One side of the strip is labelled with the Penicillin code (P) 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 20-24 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, Penicillin (K181889)	Predicate Liofilchem MTS, vancomycin (K153687)
Intended Use	Quantitative susceptibility to antimicrobial agents	Same
Inoculum	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 (µg/mL)	Same

Differences		
Item	Device Liofilchem MTS, Penicillin (K181889)	Predicate Liofilchem MTS, vancomycin (K153687)
Media	Mueller Hinton Agar with 5% sheep blood	Mueller Hinton Agar
Antibiotic	Penicillin code (P)	Vancomycin code (VA)
Incubation	35 ± 2°C for 20 – 24 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:

The Liofilchem MIC Test Strips (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 20-24 hours incubation (time specific to Liofilchem MTS, Penicillin), 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:

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility testing was conducted at three sites using ten Gram-positive organisms. Each isolate was tested in triplicate over three days. The reproducibility panel included four *S. pyogenes,* one *S. anginosus*, one *S. constellatus*, one *S. intermedius*, and three *S. pneumoniae* isolates. The mode MIC values were pre-determined and the reproducibility was calculated based on the number of MIC values that fell within ±1 doubling dilution of the mode MIC values. All MIC results were on scale. The testing resulted in overall reproducibility of 100%.

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 strain, *S. pneumoniae* ATCC 49619, was 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: Quality Control Results Summary for Penicillin MTS

Organism	Concentration (µg/mL)	Reference	MTS
S. pneumoniae ATCC 49619 Expected Result: 0.25-1 µg/mL	0.12	0	0
	0.25	14	16
	0.5	61	64
	1	1	1
	2	0	0

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 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. Only results from pure cultures were evaluated.

Growth Rate:
The growth rate for the Liofilchem MIC Test Strip (MTS) with Penicillin was 100%.

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, Penicillin was evaluated at three sites located within the United States. Each clinical isolate was tested one time by MTS, Penicillin and the reference method using the same initial standardized suspension. A total of 239 clinical fastidious Gram-positive isolates were tested which included 100 β-hemolytic *Streptococcus* spp. (60 *S. pyogenes* and 40 *S. dysgalactiae*), 48 Viridans *Streptococcus* spp. (23 *S.*

anginosus, 4 *S. intermedius*, and 21 *S. constellatus*), and 91 *S. pneumoniae* isolates. Of the clinical isolates, 54% were contemporary isolates tested within 6 months of isolation.

All clinical isolates grew on the Mueller Hinton with 5% blood plate with the Penicillin MTS strip.

Challenge testing was performed at one internal site. A total of 50 challenge fastidious Gram-positive isolates were tested which included 10 *S. pyogenes*, 15 Viridans *Streptococcus* spp. (7 *S. anginosus*, 2 *S. constellatus*, 6 *S. intermedius*), and 25 *S. pneumoniae* isolates.

Results obtained with the Liofilchem MIC Test Strip (MTS), Penicillin were compared to results obtained with the CLSI broth microdilution reference panel. The same clinical and challenge isolates for *S. pneumoniae* were evaluated using both sets of interpretive criteria for meningitis and non-meningitis. The reference panel contained two-fold serial dilutions of penicillin with a range of 0.002 – 32 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 with 5% sheep blood in an inverted position at 35°C ± 2° for 20-24 hours. At the end of incubation, the MIC value at which the edge of the inhibition ellipse intersected the strip was compared to MIC results obtained with the reference method.

The performance for the total 289 clinical and challenge isolates is summarized in Table 3 below.

Table 3: Performance of Clinical and Challenge Isolates (Combined)

Penicillin	EA Tot	EA N	EA %	Eval. EA Tot	Eval. EA N	Eval. EA%	CA N	CA%	#R	min	maj	vmj
β-hemolytic *Streptococcus* spp.[1]												
Clinical	100	100	100	100	100	100	100	100	0	0	0	0
Challenge	10	10	100	10	10	100	10	100	0	0	0	0
Combined	110	110	100	110	110	100	110	100	0	0	0	0
Viridans *Streptococcus* spp.[2]												
Clinical	48	45	93.8	46	44	95.7	48	100	0	0	0	0
Challenge	15	15	100	15	15	100	15	100	1	0	0	0
Combined	63	60	95.2	61	58	95.1	63	100	1	0	0	0
S. pneumoniae (non-meningitis breakpoints)												
Clinical	91	91	100	91	91	100	89	97.8	4	2	0	0
Challenge	25	25	100	24	24	100	21	84.0	5	4	0	0
Combined	116	116	100	115	115	100	110	94.8	9	6	0	0
S. pneumoniae (meningitis breakpoints)												
Clinical	91	91	100	91	91	100	89	97.8	43	N/A[3]	1	1
Challenge	25	25	100	24	24	100	25	100	24	N/A[3]	0	0
Combined	116	116	100	115	115	100	114	98.3	67	N/A[3]	1	1

[1]Includes *S. pyogenes* and *S. dysgalactiae.*
[2]Includes *S. anginosus, S. intermedius,* and *S. constellatus.*
[3]Not applicable due to lack of intermediate breakpoint.

EA – Essential Agreement
CA – Category Agreement
EVAL – Evaluable isolates
R – Resistant isolates
min – minor errors
maj – major errors
vmj – very major errors

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 for β-hemolytic *Streptococcus* spp. was acceptable with 100% EA and 100% CA. The overall performance for Viridans *Streptococcus* spp. was acceptable with 95.2% EA and 100% CA.

When incorporating *S. pneumoniae* breakpoints for non-meningitis isolates, the overall performance was acceptable with 100% EA and 94.8% CA. There were six minor discrepancies, and no major or very major errors.

When incorporating *S. pneumoniae* breakpoints for meningitis isolates, the overall performance was acceptable with 100% EA and 98.3% CA. There was one major error (0.5%), and one very major errors (1.5%). Both major and very major errors were acceptable.

Trending:

Trending of ≥30% difference between higher and lower dilutions was observed (Table 4) for Penicillin MIC values against *S. dysgalactiae* and *S. constellatus*. For these organisms, MTS Penicillin MIC values tended to be in exact agreement or higher when compared to the reference method. The following footnote was included in the labeling to indicate this trending:

"The MTS Penicillin MIC values tended to be in exact agreement or at least one doubling dilution higher when testing Streptococcus dysgalactiae and Streptococcus constellatus compared to the CLSI reference broth microdilution."

Table 4. Trending of Clinical and Challenge Results for *Streptococcus* spp.

Total	≥2 dil. lower	1 dil. lower	Exact	1 dil. higher	≥2 dil. higher
***S. pyogenes*[a]**					
70	0	0	50	20	0
	(0%)		(71.43%)	(28.57%)	
***S. dysgalactiae*[b]**					
40	0	0	21	19	0
	(0%)		(52.50%)	(47.50%)	
***S. anginosus*[c]**					
30	0	3	20	7	0
	(10.00%)		(66.67%)	(23.33%)	
***S. intermedius*[d]**					
132	12	59	61	0	0
	(53.79%)		(46.21%)	(0%)	
***S. constellatus*[e]**					
23	0	0	10	10	3
	(0%)		(43.48%)	(56.52%)	
***S. pneumoniae (meningitis)*[f]**					
116	0	18	83	15	0
	(15.52%)		(71.55%)	(12.93%)	
***S. pneumoniae (non-meningitis)*[g]**					
116	0	18	83	15	0
	(15.52%)		(71.55%)	(12.93%)	

[a]Difference between the higher and lower dilutions for *S. pyogenes* is: 28.57%; 95% C.I. (17.96% to 40.05%)

[b]Difference between the higher and lower dilutions for *S. dysgalactiae* is: 47.50%; 95% C.I. (30.50% to 62.50%)

[c]Difference between the higher and lower dilutions for *S. anginosus* is: 13.33%; 95% C.I. (-6.09% to 32.1%)

[d]Difference between the higher and lower dilutions for *S. intermedius* is: -22.22%; 95% C.I. (-54.77% to 16.53%)

[e]Difference between the higher and lower dilutions for *S. constellatus* is: 56.52%; 95% C.I. (32.16% to 74.37%)

[f]Difference between the higher and lower dilutions for *S. pneumoniae* (meningitis) is: -2.59%; 95% C.I. (-11.72% to 6.55%)

[g]Difference between the higher and lower dilutions for *S. pneumoniae* (non-meningitis) is: -2.59%; 95% C.I. (-11.72% to 6.55%)

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 susceptibility interpretive criteria for Penicillin are as listed in Table 5.

Table 5: Interpretive Criteria for Penicillin (µg/mL)

Organism	S	I	R
β-hemolytic *Streptococcus* spp.	≤0.12	-	-
Viridans Group *Streptococcus* spp.	≤0.12	0.25-2	≥4
S. pneumoniae meningitis	≤0.06	-	≥0.12
S. pneumoniae (non-meningitis)	≤2	4	≥8

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.