510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K190252

B. Purpose for Submission:

To obtain a substantial equivalence determination for the Liofilchem MIC Test Strip (MTS) containing Gentamicin (CN) at concentrations of 0.016-256 µg/mL for susceptibility testing of non-fastidious Gram-negative organisms

C. Measurand:

Gentamicin 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 Gentamicin 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 (83)

H. Intended Use:

1. Intended use(s):

   The MTS (MIC Test Strip) Gentamicin 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 Gentamicin at concentrations of 0.016-256 µg/mL should be interpreted at 16-20 hours of incubation.

   MTS Gentamicin can be used to determine the MIC of Gentamicin against the following bacteria.

   Gentamicin 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
   Citrobacter freundii
   Citrobacter koseri
   Enterobacter cloacae
   Escherichia coli
   Klebsiella aerogenes
   Klebsiella oxytoca
   Klebsiella pneumoniae
   Proteus mirabilis
   Proteus vulgaris
   Pseudomonas aeruginosa
   Serratia marcescens

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use

The following limitations are included in the labeling:

- 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.

  Gentamicin: *P. vulgaris*

- Characterization of 16S rRNA methyltransferases, aminoglycoside modifying enzymes (AMEs), altered efflux and loss of outer membrane porins was not available for organisms at the time of comparative testing, and therefore the performance of MTS Gentamicin for non-fastidious Gram-negative bacilli with these resistance mechanisms is unknown for the following: *Enterobacteriaceae*, *P. aeruginosa.*

4. Special instrument requirements:

   Manual reading only

I. Device Description:

The MIC Test Strip (MTS) Gentamicin consists of specialized paper impregnated with a predefined concentration gradient of Gentamicin across 15 two-fold dilutions similar to dilutions used by conventional MIC methods. One side of the strip is labeled with the Gentamicin code (CN) and the MIC reading scale in µg/mL. 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. Since MTS 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, **Gentamicin** (**K190252**)	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 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

Differences		
Item	Device Liofilchem MTS, **Gentamicin** (**K190252**)	Predicate Liofilchem MTS, **Vancomycin** (**K153687**)
Intended Use	Quantitative susceptibility to antimicrobial agents against Gram-negative organisms	Quantitative susceptibility to antimicrobial agents against Gram-positive organisms
Antibiotic	Gentamicin code (CN)	Vancomycin code (VA)
Incubation	35 ± 2°C for 16 – 20 hours	35 ± 2°C for 24 hours

K. Standard/Guidance Document Referenced (if applicable):

- 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-Ed29 "Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Ninth Edition, January 2019".

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 0.125µg/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 (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility testing was conducted at three sites using ten Gram-negative 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 one *E. coli,* one *K. pneumoniae,* one *E. cloacae,* one *K. aerogenes,* one *K. oxytoca,* one *P. mirabilis,* one *C. freundii,* one *S. marcescens,* and two *P. aeruginosa* isolates. All MIC results were on scale. The testing resulted in overall reproducibility of greater than 95%.

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 were tested a sufficient number of times (i.e., at least 20/site) at each testing site using both MTS and broth microdilution (BMD) reference methods. The results are summarized in **Table 2** below.

The quality control results are acceptable.

Table 2: MTS Gentamicin QC Results

Organism	Concentration (µg/mL)	BMD Reference	MTS Gentamicin
E. coli ATCC 25922 Expected Result: 0.25 - 1 µg/mL	0.12	0	0
	0.25	3	22
	0.5	46	34
	1	12	5
	2	0	0
P. aeruginosa ATCC 27853 Expected Result: 0.5 - 2 µg/mL	0.25	0	0
	0.5	35	1
	1	23	51
	2	3	9
	4	0	0

Inoculum Density Check:

The inoculum was prepared to achieve turbidity equivalent to a 0.5 McFarland standard. Colony counts were performed for all QC replicates (2 isolates x 20 replicates = 40) as well as one replicate of each reproducibility isolate tested on each of the three days (10 isolates x 3 days = 30), and a minimum of 10% of clinical strains tested. Colony counts were within the recommended range of approximately 1 - 2 x 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:

All isolates tested grew in the broth microdilution panels and the Mueller Hinton agar with MTS Gentamicin.

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 Gentamicin was evaluated at three sites located within the United States. There were 369 Gram-negative clinical isolates and 80 Gram-negative challenge isolates tested for a total of 449 isolates.

Each clinical isolate was tested one time by MTS Gentamicin and the reference method using the same initial standardized suspension. A total of 369 clinical isolates were tested which included 309 *Enterobacteriaceae* (15 *C. freundii*, 15 *C. koseri*, 35 *E. cloacae*, 83 *E. coli*, 15 *K. aerogenes*, 30 *K. oxytoca*, 45 *K. pneumoniae*, 35 *P. mirabilis*, 21 *P. vulgaris*, 15 *S. marcescens*) and 60 *P. aeruginosa* isolates. Isolates were defined as either contemporary (i.e., tested within six months of the organism's original isolation from clinical culture) or stock (i.e., no time limit on time from isolation prior to testing). A total of 65.3% of the clinical isolates were considered contemporary isolates.

Challenge testing was performed at one internal site. A total of 80 challenge isolates were tested which included 70 *Enterobacteriaceae* (2 *C. freundii*, 2 *C. koseri*, 10 *E. cloacae*, 18 *E. coli*, 2 *K. aerogenes*, 10 *K. oxytoca*, 10 *K. pneumoniae*, 10 *P. mirabilis*, 3 *P. vulgaris*, 3 *S. marcescens*) and 10 *P. aeruginosa* isolates.

Results obtained with the MTS Gentamicin were compared to results obtained with the CLSI broth microdilution frozen reference MIC panel. The reference panel contained two-fold serial dilutions of Gentamicin 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 100% 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.

At the time of comparative testing, resistant isolates were not available for *P. vulgaris*. Thus, the following limitation is included in the labeling:

> *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.*
>
> *Gentamicin: P. vulgaris*

The results obtained from the 449 clinical and challenge isolates are summarized in **Table 3** below.

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

Gentamicin	EA Tot	EA N	EA %	Eval. EA Tot	Eval. EA N	Eval. EA%	CA N	CA %	#R	min	maj	vmj
Enterobacteriaceae Clinical	309	303	98.0	303	297	98.0	308	99.7	25	1	0	0
Challenge	70	70	100	54	54	100	68	97.1	46	2	0	0
Combined	379	373	98.4	357	351	98.3	376	99.2	71	3	0	0
P. aeruginosa Clinical	60	60	100	51	51	100	58	96.7	12	2	0	0
Challenge	10	10	100	1	1	100	10	100	9	0	0	0
Combined	70	70	100	52	52	100	68	97.1	21	2	0	0
All Organisms	449	443	98.7	409	403	98.5	444	98.9	92	5	0	0

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 (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 all *Enterobacteriaceae* isolates is acceptable with 98.4% EA and 99.2% CA. There were three minor errors and no major or very major errors.

The overall performance of *P. aeruginosa* is acceptable with 100% EA and 97.1% CA. There were two minor errors and no major or very major errors.

The overall performance of all organisms combined is acceptable with 98.7% EA and 98.9% CA.

To address testing of 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 labeling for specific antimicrobial drugs provides the uses for which the antimicrobial drug is approved.*

Resistance Mechanisms:

Molecular characterization was not evaluated for all organisms as this information was not available at the time of testing. This was addressed by adding the following footnote in the labeling:

> *Characterization of 16S rRNA methyltransferases, aminoglycoside modifying enzymes (AMEs), altered efflux and loss of outer membrane porins was not available for organisms at the time of comparative testing, and therefore the performance of MTS Gentamicin for non-fastidious Gram-negative bacilli with these resistance mechanisms is unknown for the following: Enterobacteriaceae, P. aeruginosa.*

Trending:

A trending analysis was conducted using the combined data (clinical and challenge) for each organism species and group. This trending calculation analyzes device MIC values that are determined to be one or more doubling dilutions lower or higher than the reference method. MIC values that are off-scale for both the reference and device are not considered in the trending analysis.

Trending results were stratified by species to determine if species-related trends were observed (**Table 4**). Species for which the difference between the percentage of isolates with higher versus lower MIC values was ≥30% and for which the confidence interval was determined to be statistically significant were considered to have evidence of significant trending and is addressed in labeling.

A trend toward lower MIC values was observed for *P. vulgaris* and *S. marcescens* while a trend toward higher MIC values was observed for *P. aeruginosa*. The sponsor included the following footnotes to the performance table to address the trending observed for MTS Gentamicin.

> *Liofilchem MIC Test Strip (MTS) Gentamicin MIC values tended to be in exact agreement or at least one doubling dilution lower when testing P. vulgaris and S. marcescens compared to the CLSI reference broth microdilution.*

> *Liofilchem MIC Test Strip (MTS) Gentamicin MIC values tended to be in exact agreement or at least one doubling dilution higher when testing P. aeruginosa compared to the CLSI reference broth microdilution.*

Table 4. Trending by Species (clinical + challenge isolates)

Organism	Total Evaluable for Trending	≥1 dil. Lower # (%)	Exact # (%)	≥1 dil. Higher # (%)	Percent Difference (95% CI)	Trending Noted
C. freundii	16	4 (25.0)	10 (62.5)	2 (12.5)	-12.5%	No
C. koseri	17	2 (11.8)	12 (70.6)	3 (17.6)	5.9%	No
E. cloacae	40	5 (12.5)	27 (67.5)	8 (20.0)	7.5%	No
E. coli	95	34 (35.8)	52 (54.7)	9 (9.5)	-26.3%	No
K. aerogenes	17	6 (35.3)	10 (58.8)	1 (5.9)	-29.4%	No
K. oxytoca	40	6 (15.0)	28 (70.0)	6 (15.0)	0.0%	No
K. pneumoniae	51	6 (11.8)	33 (64.7)	12 (23.5)	11.8%	No
P. mirabilis	44	11 (25.0)	29 (65.9)	4 (9.1)	-15.9%	No
P. vulgaris	24	8 (33.3)	16 (66.7)	0	-33.3% (-53.3 to -12.7)	Yes
S. marcescens	18	6 (33.3)	12 (66.7)	0	-33.3% (-56.3 to -8.8)	Yes
Enterobacteriaceae (all)	362	88 (24.3)	229 (63.3)	45 (12.4)	-11.9%	No
P. aeruginosa	53	4 (7.5)	29 (54.7)	20 (37.7)	30.2% (14.5 to 44.4)	Yes

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 Gentamicin are as listed in **Table 5**.

Table 5: FDA Recognized Interpretive Criteria for Gentamicin (µg/mL)[a]

Organisms	S	I	R
Enterobacteriaceae	≤4	8	≥16
Pseudomonas aeruginosa	≤4	8	≥16

[a]According to CLSI M100-Ed28 and FDA STIC Website
https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm410971.htm

N. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device when evaluated with the current FDA-recognized gentamicin breakpoint.

O. Conclusion:

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

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 gentamicin 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 gentamicin 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.