

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

K153687

**B. Purpose for Submission:**

To obtain a substantial equivalence of Liofilchem's MIC Test Strip (MTS) containing vancomycin at concentrations of 0.016-256µg/mL for susceptibility testing of non-fastidious species (*Staphylococcus aureus*, *Staphylococcus epidermidis* (including methicillin resistant strains), *Enterococcus faecalis* and *Enterococcus faecium*).

**C. Measurand:**

Vancomycin 0.016-256µg/mL

**D. Type of Test:**

Quantitative AST growth based detection

**E. Applicant:**

Liofilchem s.r.l

**F. Proprietary and Established Names:**

Liofilchem MIC Test Strip (MTS)-Vancomycin 0.016-256µg/mL

**G. Regulatory Information:**

1. Regulation section:

866.1640 Antimicrobial Susceptibility Test Powder

2. Classification:

II

3. Product code:

JWY - Manual Antimicrobial Test Systems

4. Panel:

83 - Microbiology

**H. Intended Use:**

1. Intended use(s):

The Liofilchem MIC Test Strip (MTS) is a quantitative method intended for the in vitro determination of antimicrobial susceptibility of non-fastidious Gram negative and Gram positive aerobic bacteria (for example, *Enterobacteriaceae*, *Pseudomonas*, *Enterococcus* and *Staphylococcus* species) and fastidious bacteria (for example, anaerobes, *Haemophilus* and *Streptococcus* species and *N. gonorrhoeae*).

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  $\mu\text{g/mL}$  of antimicrobial agents against bacteria as tested on agar media using overnight incubation and manual reading procedures.

2. Indication(s) for use:

The Liofilchem MIC Test Strip (MTS) is a quantitative method intended for the in vitro determination of antimicrobial susceptibility of non-fastidious Gram negative and Gram positive aerobic bacteria (for example, *Enterobacteriaceae*, *Pseudomonas*, *Enterococcus* and *Staphylococcus* species) and fastidious bacteria (for example, anaerobes, *Haemophilus* and *Streptococcus* species and *N. gonorrhoeae*).

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  $\mu\text{g/mL}$  of antimicrobial agents against bacteria as tested on agar media using overnight incubation and manual reading procedures.

The purpose of this 510(k) is specifically for the vancomycin MTS at concentrations of 0.016-256  $\mu\text{g/mL}$  interpreted after 24 hours incubation.

The non-fastidious bacteria that have been shown to be active both clinically and in vitro against vancomycin according to the FDA label are:

*Staphylococcus aureus* and *Staphylococcus epidermidis* (including methicillin resistant strains)

*Enterococcus faecalis*

*Enterococcus faecium*

3. Special conditions for use statement(s):

For prescription use

The following limitations are included in the device labeling:

*“The ability of the Liofilchem MIC Test Strip (MTS) to detect vancomycin resistant *S. aureus* strains is unknown due to the limited number of resistant strains available at the time of comparative testing”.*

*“The ability of the Liofilchem MIC Test Strip (MTS) to detect vancomycin intermediate or resistant *S. epidermidis* is unknown because intermediate or resistant strains were not encountered at the time of testing. Any *S. epidermidis* isolate for which the vancomycin MIC is  $\geq 8\mu\text{g/mL}$ , should be submitted to a reference laboratory for further testing”.*

4. Special instrument requirements:

Manual readings only

**I. Device Description:**

The vancomycin MIC Test Strip (MTS) is made of special high quality paper impregnated with a predefined concentration of gradient vancomycin, across 15 two-fold dilutions like those of a conventional MIC method. One side of the strip is labelled with the vancomycin code (VA) and the MIC reading scale in  $\mu\text{g/mL}$ . 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 24 hours incubation, a symmetrical inhibition ellipse centered along the strip is formed. The MIC is read directly from the scale in terms of  $\mu\text{g/mL}$  at the point where the edge of the inhibition ellipse intersects the MIC Test Strip.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

Etest® Ceftriaxone

2. Predicate 510(k) number(s):

K121002

3. Comparison with predicate:

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate K121002</b>
Intended Use	Quantitative susceptibility to antimicrobial agents	Same
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	Same
Result	MIC	Same
Reading	Manual; the point where the edge of inhibition ellipse intersects the MIC Test Strip	Same

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Strip Material	Special High Quality Paper	Plastic
Antibiotic	Vancomycin	Ceftaroline
Incubation	35°C ±2 for 24h	16-20h

**K. Standard/Guidance Document Referenced:**

“Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems; Guidance for Industry and FDA”

CLSI M07-A10 “Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard, Tenth Edition January 2015”

CLSI M100-S25 “Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement, January 2015”

**L. Test Principle:**

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 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. 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:*

Testing using Liofilchem MIC Test Strip (MTS) was performed using 10 isolates (five staphylococci and five enterococci). Testing was performed at three sites in triplicate on three separate days to determine site to site reproducibility. Results were within +/- one doubling dilution agreement as compared to the mode MIC value of vancomycin for all organisms at all sites and there were no off-scale results. The results were acceptable and demonstrated  $\geq 95\%$  reproducibility.

b. *Linearity/assay reportable range:*

Not applicable

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

The recommended QC isolates were tested a sufficient number of times with acceptable results with the reference method. The MTS results demonstrate that the system can produce QC results in the recommended range (Tables 1).

**Table 1. Quality Control Test Results for Vancomycin**

Organism	Concentration	Reference	MTS
<i>S. aureus</i> ATCC 29213 Expected Results 0.5-2 $\mu$ g/mL	<0.5		
	0.5		1
	1	57	71
	2	3	4
	>2		
<i>E. faecalis</i> ATCC 29212 Expected Results 1-4 $\mu$ g/mL	<1		
	1		
	2	15	11
	4	43	63
	>4		

The quality control results are acceptable.

The inoculum was prepared to achieve 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  $1 \times 10^8$  CFU/mL).

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:*

Clinical testing was performed at three external and one internal site. There were 812 clinical isolates [312 *S. aureus* (156 MRSA, 156 MSSA); 60 *S. epidermidis* and 378 *E. faecalis* and 62 *E. faecium*].

The clinical testing included 57.1% fresh (isolated no longer than seven days prior to testing) and 42.9% recent (isolated no longer than one year prior to testing) clinical isolates.

A total of 76 challenge isolates were also evaluated [41 *S. aureus* (2 MSSA, 39 MRSA), 5 *S. epidermidis*, 11 *E. faecalis* and 19 *E. faecium*].

Results obtained with Liofilchem MIC Test Strip (MTS) with vancomycin were compared to results obtained from frozen reference MIC panels. Reference panels were prepared and interpreted as outlined in CLSI recommendations in M7-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  $35^{\circ}\text{C} \pm 2$  for 24h. 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.

A comparison was provided to the reference method with the following agreement (Tables 3).

**Table 3. Overall Performance of Clinical and Challenge Isolates  
Liofilchem MTS for Vancomycin**

	EA TOT	EA N	EA %	Eval EA Tot	Eval EAN	Eval EA%	CA N	CA %	# R	min	maj	vmj
Clinical	812	811	99.9	746	745	99.9	810	99.8	68	2	0	0
Challenge	76	76	100	66	66	100	69	90.8	17	7	0	0
Combined	888	887	99.9	812	811	99.9	879	99.0	85	9	0	0

EA – Essential Agreement

CA – Category Agreement

R – resistant isolates

maj – major discrepancies

vmj – very major discrepancies

min – minor discrepancies

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

For clinical and challenge isolates tested with the Liofilchem MTS for vancomycin, the overall combined %EA and %CA met the acceptance criteria of greater than or equal to 90% (Table 3). However, there were a limited number of resistant *S. aureus* and *S. epidermidis* isolates available during the time of comparative testing. Since the prevalence of resistant strains of *S. aureus* and *S. epidermidis* for vancomycin is low this was addressed by adding the following limitations in the labeling:

*“The ability of the Liofilchem MIC Test Strip (MTS) to detect vancomycin resistant S. aureus strains is unknown due to the limited number of resistant strains available at the time of comparative testing”.*

*“The ability of the Liofilchem MIC Test Strip (MTS) to detect vancomycin intermediate or resistant S. epidermidis is unknown because intermediate or resistant strains were not encountered at the time of testing. Any S. epidermidis isolate for which the vancomycin MIC is  $\geq 8\mu\text{g/mL}$ , should be submitted to a reference laboratory for further testing”.*

In this study, 25 challenge and one clinical vancomycin intermediate *S. aureus* (VISA) isolates were tested. However, five VISA isolates were mis-categorized as minor categorical errors (intermediate by the reference method but susceptible by Liofilchem device). This was mitigated by adding the following footnote in the labeling:

*“The ability of the Liofilchem MIC Test Strip (MTS) to detect vancomycin intermediate S. aureus is unknown due to the limited number of vancomycin intermediate strains tested. Any Staphylococcus isolate for which the vancomycin MIC is 4 or  $8\mu\text{g/mL}$  should be submitted to a reference laboratory for further testing”.*

**MIC Trends:**

Using the data provided by the sponsor in the diagonal table format recommended in the AST Guidance, an analysis was conducted to check for trending in MIC values.

A lower trend was observed in the overall performance of MRSA compared to the CLSI broth micro-dilution reference method, as summarized in Table 4.

**Table 4. Lower Trending of Results Obtained with MTS Vancomycin in Combined Clinical and Challenge Study with MRSA**

Organism	Difference in MIC as Compared to the CLSI Reference Method							Total
	≥ -3	-2	-1	0	+1	+2	≥ +3	
MRSA clinical and challenge	0% (0/195)	0.5% (1/195)	21.0% (41/195)	73.3% (143/195)	5.1% (10/195)	0% (0/195)	0% (0/195)	195

The trending towards lower MIC values and the potential for occurrence of very major error(s) for vancomycin when testing MRSA was addressed in the labeling by adding the following footnote:

*“The Liofilchem MIC Test Strip (MST) vancomycin MIC values tended to be one doubling dilution lower when testing MRSA compared to the reference broth microdilution (out of 195 MRSA isolates tested, 0.5% were two doubling dilutions lower, 21.0% were one doubling dilution lower, 73.3% were equivalent and 5.1% were one doubling dilution higher compared to the CLSI broth microdilution results)”*

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:

**Table 5. FDA Interpretive Criteria for Vancomycin (µg/mL)**

<b>Organisms</b>	<b>S</b>	<b>I</b>	<b>R</b>
<i>Staphylococcus aureus</i>	≤2	4-8	≥16
<i>Staphylococcus epidermidis</i>	≤4	8-16	≥32
<i>Enterococcus spp</i>	≤4	8-16	≥32

**N. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

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