510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K180339

B. Purpose for Submission:

To obtain a substantial equivalence determination of the Liofilchem MIC Test Strip (MTS) containing azithromycin at concentrations of 0.016 – 256 µg/mL for susceptibility testing of select gram positive organisms.

C. Measurand:

Azithromycin 0.016 – 256 µg/mL

D. Type of Test:

Quantitative Antimicrobial Susceptibility growth-based detection

E. Applicant:

Liofilchem s.r.l.

F. Proprietary and Established Names:

Liofilchem MIC Test Strip (MTS), Azithromycin 0.016 – 256 µg/mL

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.1640 Antimicrobial Susceptibility Test Powder

2. Classification:

   Class II

3. Product code:

   JWY – Manual Antimicrobial Test Systems

4. Panel:

   Microbiology (83)

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 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 Azithromycin MTS concentrations of 0.016 – 256 µg/mL should be interpreted at 16 – 20 hours of incubation.

   Azithromycin has been shown to be active both clinically and *in vitro* against the non-fastidious bacteria listed below according to the FDA label.

   Staphylococcus aureus (including methicillin-resistant isolates)

2. Indication(s) for use:

   Same as intended use

3. Special conditions for use statement(s):

   For Prescription Use Only

4. Special instrument requirements:

   Manual reading only

I. Device Description:

The azithromycin MIC Test Strip (MTS) consists of specialized paper impregnated with a predefined concentration gradient of azithromycin across 15 two-fold dilutions like those of a conventional MIC method. One side of the strip is labelled with the azithromycin code (AZM); the MIC reading scale is in µg/mL. When the MTS, Azithromycin is applied onto an inoculated agar surface, the preformed exponential gradient of antimicrobial agent is transferred to the agar matrix. After 16 - 20 hours of 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, Azithromycin. The MTS, Azithromycin is single use only. Since the MTS Azithromycin strip generates

MIC values that may fall between two-fold dilutions designations, the MIC value is recorded as 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

Similarities		
Item	**Device Liofilchem MTS, Azithromycin (K180339)**	**Predicate Liofilchem MTS, Vancomycin (K153687)**
Intended Use	Quantitative susceptibility to antimicrobial agents	Same
Media	Mueller Hinton agar	Same
Inoculum	Isolated colonies from culture in suspension equivalent to a 0.5 McFarland turbidity standard. Inoculum is applied to agar with swab manually or with rotation plate.	Same
Reading	Manual: the point where the edge of the inhibition ellipse intersects the MIC Test Strip	Same
Result	MIC (µg/mL)	Same

Differences		
Item	Device	Predicate
Antimicrobial Agent	Azithromycin (AZM)	Vancomycin (VA)
Incubation	35 ± 2°C for 16 – 20 hours	35 ± 2°C for 24 hours

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

FDA Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems; Guidance for Industry and FDA (Issued August 28, 2009)

CLSI M07-10, "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-Sixth 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. When the MIC Test Strip is applied onto an inoculated agar surface, the preformed exponential gradient of antimicrobial agent is transferred to the agar matrix. After 16-20 hours incubation, a symmetrical inhibition ellipse centered along the strip is formed. The minimum inhibitory concentration (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.

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:

   A reproducibility study testing the Liofilchem MIC Test Strip (MTS) containing azithromycin was conducted at three clinical sites using ten isolates of *Staphylococcus aureus* consistent with the intended use. Testing was performed on three separate days and in triplicate for a total of 270 data points among the sites. The isolates tested in the reproducibility study included four isolates of methicillin resistant *Staphylococcus aureus* (MRSA) and six isolates of methicillin susceptible *Staphylococcus aureus* (MSSA).

   The mode MIC was determined and the reproducibility was calculated based on MIC values that fell within ± one doubling dilution from the mode MIC value. Both intra-site and inter-site reproducibility for MTS, Azithromycin was calculated. There were

no off-scale results.

The combined reproducibility results for all three sites were acceptable and demonstrated ≥ 95% reproducibility.

b. *Linearity/assay reportable range:*

Not applicable

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

CLSI recommended QC organisms were tested throughout the comparative study at the three study sites. The organism tested was *S. aureus* ATCC 29213. The recommended QC strain was tested a minimum of 20 times at each site by both the azithromycin MTS and the CLSI broth microdilution reference method.

All results were within the expected range. Results are summarized in Table 2 and were acceptable.

Table 2: Azithromycin MTS QC Results

Organism	Expected Range (µg/mL)	Concentration (µg/mL)	Reference	MTS
S. aureus ATCC 29213	0.5 - 2	0.25		
		0.5	2	3
		1	21	59
		2	39	10
		4		

Inoculum Density Check: The inoculum was prepared to achieve a turbidity equivalent to a 0.5 McFarland standard. Colony counts were performed as part of QC and reproducibility testing as well as during the clinical studies to demonstrate that the inoculum concentrations were within the expected CFU/mL. The colony counts were acceptable.

Purity Checks: Purity checks were performed on all isolates following MTS inoculation. Only results from pure cultures were evaluated.

Growth Rate: All clinical and challenge isolates tested showed growth in the reference MIC panels and on the Mueller Hinton agar with MTS, Azithromycin. The growth rate 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 Azithromycin was evaluated at three sites located within the United States. Each clinical isolate was tested one time by MTS Azithromycin and the reference method using the same initial standardized suspension. A total of 300 of clinical *Staphylococcus aureus* comprising of 121 methicillin susceptible *Staphylococcus aureus* (MSSA) and 179 methicillin resistant *Staphylococcus aureus* (MRSA) were tested. The 300 *S. aureus* isolates included 81 (27.0%) fresh isolates that were tested within seven days of isolation, 108 (36.0%) recent isolates that were tested within one year of isolation and 111 (37.0%) stock isolates that were tested within three years of isolation. All 300 clinical isolates grew on the Mueller Hinton plate with the MTS Azithromycin strip and in the broth microdilution reference plate. The 300 clinical *S. aureus* isolates follows the recommendation for non-fastidious organisms as stated in the AST Special Control guidance.

A total of 57 challenge isolates were tested at a single site and included six MSSA and 51 MRSA.

Results obtained with the Liofilchem MTS were compared to results obtained with the CLSI broth microdilution reference panel. The performance is presented in Table 3 below.

Table 3: Performance of *Staphylococcus aureus* isolates*

Azithromycin	EA Tot	EA N	EA %	Eval. EA Tot	Eval. EA N	Eval. EA %	CA N	CA %	#R	min	maj	vmj
	***Staphylococcus aureus* ≤2 (Susceptible), 4 (Intermediate), ≥8 (Resistant)**											
MSSA												
Clinical	121	119	98.3	68	66	97.1	121	100	57	0	0	0
Challenge	6	6	100	2	2	100	5	83.3	4	1	0	0
Combined	127	125	98.4	70	68	97.1	126	99.2	61	1	0	0
MRSA												
Clinical	179	177	98.9	36	36	100	179	100	144	0	0	0
Challenge	51	49	96.1	15	13	86.7	49	96.1	42	2	0	0
Combined	230	226	98.3	51	49	96.1	228	99.1	186	2	0	0
Total	**357**	**351**	**98.3**	**121**	**117**	**96.7**	**354**	**99.2**	**247**	**3**	**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 (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 *Staphylococcus aureus* was acceptable at 98.3% EA and 99.2% CA. Of the 357 isolates tested, 121 isolates were considered evaluable; the EA of evaluable results was 96.7%. There were 247 resistant isolates tested but the vast majority (i.e. 236 isolates) had MIC values ≥256 µg/mL which were non-evaluable results; there were seven and four results in the range of 8 - 32 µg/mL and 64 - 128 µg/mL respectively. Three minor discrepancies with no major or very major discrepancies were observed.

Azithromycin is bacteriostatic and Liofilchem MTS Azithromycin is read at 80% inhibiton when trailing is seen. Photographic guide is included in the labeling for MTS Azithromycin.

MIC Trends. Using the combined clinical and challenge data for MSSA and MRSA, an analysis of trending was conducted. 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. Though the trending analysis indicated that the Liofilchem MIC is one-dilution lower for MSSA when compared to the reference method, the difference was considered acceptable as most of the results (54.67%) was in exact agreement with the reference method. No further notation was required in labeling. The evaluable data for trend analysis is presented in Table 4.

Table 4: Trending Analysis of Evaluable MSSA and MRSA Results

Azithromycin 0.016- 256 µg/mL	**Total (Trending Evaluable)**	**Difference in MIC as Compared to the CLSI Reference Method**				
		≥2 dil. lower	**1 dil. lower**	**Exact**	**1 dil. higher**	**≥2 dil. higher**
MSSA						
Clinical + Challenge	75	0	26	41	6	2
		26 (34.67%)[a]		(54.67%)	8 (10.67%)[a]	
MRSA						
Clinical + Challenge	59	2	15	22	18	2
		17 (28.81%)[b]		(37.29%)	20 (33.90%)[b]	

[a] Percent difference between the higher and lower dilution trends for MSSA: -24.00% (95% CI; -36.40% to 10.71%)
[b] Percent difference between the higher and lower dilution trends for MRSA: 5.08% (95%CI; -11.46% to 21.26%)
Note: A positive percent difference value indicates higher MIC when compared to the reference method;
A negative percent difference value indicates lower MIC when compared to the reference method**.**

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: Interpretive Criteria for Azithromycin

Organism	**FDA/CLSI Interpretive Criteria (µg/mL)**		
	Susceptible	**Intermediate**	**Resistant**
Staphylococcus aureus	≤2	4	≥8

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.