Food and Drug Administration
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510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K190914

B Applicant

Liofilchem s. r. l.

C Proprietary and Established Names

MTS Doxycycline 0.016 - 256 µg/mL

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
JWY	Class II	21 CFR 866.1640 - Antimicrobial Susceptibility Test Powder	IM - Immunology & MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

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

B Measurand:

Doxycycline 0.016 – 256 µg/ml

C Type of Test:

Quantitative Antimicrobial Susceptibility Test growth-based detection

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
MTS (MIC Test Strip) Doxycycline 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.

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

MTS Doxycycline can be used to determine the MIC of doxycycline against the following bacteria.
Doxycycline 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
Escherichia coli
Klebsiella aerogenes
Klebsiella oxytoca
Klebsiella pneumoniae

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:
Manual reading only

IV Device/System Characteristics:

A Device Description:
MTS Doxycycline is made of special high-quality paper impregnated with a predefined concentration of gradient doxycycline across 15 two-fold dilutions like those of a conventional MIC method. One side of the strip is labeled with the Doxycycline code (DXT) and the MIC reading scale in µg/mL.

The MTS is removed from its packaging aseptically and applied to an inoculated agar surface. When the MIC Test Strip is applied onto an inoculated agar surface, the performed exponential gradient of antimicrobial agent diffuses into the agar for over an hour. 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.

B Principle of Operation:

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

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.

V Substantial Equivalence Information:

A Predicate Device Name(s):

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

B Predicate 510(k) Number(s):

K153687

C Comparison with Predicate(s):

Table 1. Comparison with the Predicate Device

Device & Predicate Device(s):	**K190914**	**K153687**
Device Trade Name	**MTS – Doxycycline**	**MTS – Vancomycin**
General Device Characteristic Similarities		
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
Result	MIC in µg/mL	Same
General Device Characteristic Differences		
Intended Use	Quantitative susceptibility to antimicrobial agents	Quantitative susceptibility to antimicrobial agents

	against Gram-negative organisms	against Gram-positive organisms
Reading	Manual; interpret the MIC at 80% inhibition	100% inhibition
Antibiotic	Doxycycline (DXT)	Vancomycin (VA)
Incubation	35 ± 2°C for 16 – 20 hours	35 ± 2°C for 24 hours

VI Standards/Guidance Documents Referenced:

- Guidance for Industry and FDA - Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems – August 28, 2009.
- CLSI M07-A11 "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard, 11th Edition, January 2018".
- CLSI M100-Ed28 "Performance Standards for Antimicrobial Susceptibility Testing; 28th Edition, January 2018".

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Reproducibility testing was conducted at three external sites using eight Gram-negative organisms (3 *E. coli*, 1 *K. aerogenes*, 1 *K. oxytoca*, and 3 *K. pneumoniae*) and one internal site using two Gram-negative organisms (1 *E. coli* and 1 *K. pneumoniae*). 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. All MIC results were on scale. The testing resulted in overall reproducibility of greater than 95%.

The reproducibility results are acceptable.

2. Linearity:

Not applicable

3. Analytical Specificity/Interference:

Not applicable

4. Assay Reportable Range:

Not applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Quality Control (QC) Testing:
The CLSI recommended QC strain *E. coli* ATCC 25922 was 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 with 100% of tests within the expected MIC range.

Table 2: MTS Doxycycline QC Results

Organism	Concentration (µg/mL)	BMD Reference	MTS Doxycycline
E. coli ATCC 25922 Expected Result: 0.5 - 2 µg/mL	0.25	0	0
	0.5	24	11
	1	38	53
	2	0	1
	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 as well as one replicate of each reproducibility isolate tested on each of the three days, and a minimum of 10% of clinical strains tested.

The mean inoculum densities for all isolates (QC, reproducibility, clinical and challenge) tested with the MTS ranged from 6.7×10^7 to 9.05×10^7 CFU/mL. The mean inoculum densities for all isolates tested with the reference broth microdilution method ranged from 3.35×10^5 to 4.36×10^5 CFU/mL (final concentration in each well of panel).

Although the QC inoculum densities were slightly low (ranged from 4.00×10^7 to 1.79×10^8 CFU/mL), the QC results were within the acceptable range.

Purity Checks:
Purity checks were performed on all isolates following 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 Doxycycline.

6. Detection Limit:

Not applicable

7. Assay Cut-Off:

Not applicable

B Comparison Studies:

1. Method Comparison with the Reference Method:

Performance of MTS Doxycycline with 255 clinical isolates was evaluated at three external sites (2 located within the U.S. and 1 located outside the U.S.). Performance of MTS Doxycycline with 45 clinical isolates and all 75 challenge isolates was evaluated at one internal site (located within the U.S.). A total of 375 isolates were tested.

Each isolate was tested one time by MTS Doxycycline and the reference method using the same initial standardized suspension. A total of 300 clinical isolates were tested which included 134 *E. coli*, 35 *K. aerogenes*, 35 *K. oxytoca* and 96 *K. pneumoniae* isolates. The clinical isolates were comprised of 60% contemporary isolates (i.e., tested within six months of the organism's original isolation from clinical culture) and 40% stock isolates (i.e., no time limit on time from isolation prior to testing). A total of 75 challenge isolates were tested which included 21 *E. coli*, 15 *K. aerogenes*, 15 *K. oxytoca* and 24 *K. pneumoniae.*

Results obtained with the MTS Doxycycline were compared to results obtained with the CLSI broth microdilution reference MIC panel. The reference panel contained two-fold serial dilutions of doxycycline 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-A11. Isolated colonies from overnight blood agar plates were suspended in saline to achieve a 0.5 McFarland standard turbidity (approximately 10^8 CFU/mL). Inoculated Mueller Hinton agar plates were incubated 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 results obtained from the 375 clinical and challenge isolates are summarized in **Table 3**.

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

Doxycycline	EA Tot	EA N	EA %	Eval. EA Tot	Eval. EA N	Eval. EA %	CA N	CA %	#R	min	maj	vmj
Enterobacteriaceae												
Clinical	300	296	98.7	299	295	98.7	287	95.7	71	13	0	0
Challenge	75	74	98.7	74	73	98.6	74	98.7	45	1	0	0
Combined	**375**	**370**	**98.7**	**373**	**368**	**98.7**	**361**	**96.3**	**116**	**14**	**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 MTS result agrees exactly or within one doubling dilution of the reference broth microdilution result. Category Agreement (CA) is when the MTS result interpretation agrees exactly with the reference broth microdilution result interpretation.

The overall performance of all *Enterobacteriaceae* isolates was acceptable with 98.7% EA and 96.3% CA. There were 14 minor errors and no major or very major errors.

To address testing of non-indicated species, the following statement is included 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:

Resistance mechanism characterization was not available for all organisms at the time of comparative testing, and therefore the performance of MTS Doxycycline is unknown for the following: Enterobacteriaceae [tet(B)].

MIC Trends:
A trending analysis was conducted using the combined data (clinical and challenge) for each species. 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**). There were no species in which the difference between the percentage of isolates with higher versus lower MIC values was ≥30%. Thus, there was no evidence of significant trending.

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

Organism	Total Evaluable for Trending	≥1 dil. Lower # (%)	Exact # (%)	≥1 dil. Higher # (%)	Percent Difference	Trending Noted
E. coli	155	14 (9.0)	100 (64.5)	41 (26.5)	17.4	No
K. aerogenes	49	4 (8.2)	35 (71.4)	10 (20.4)	12.2	No
K. oxytoca	50	5 (10.0)	35 (70.0)	10 (20.0)	10.0	No
K. pneumoniae	120	23 (19.2)	77 (64.2)	20 (16.7)	-2.5	No
Enterobacteriaceae (all)	374	46 (12.3)	247 (66.0)	81 (21.7)	9.4	No

2. Matrix Comparison:
Not applicable

C Clinical Studies:

1. Clinical Sensitivity:
   Not applicable

2. Clinical Specificity:
   Not applicable

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
   Not applicable

D Clinical Cut-Off:
Not applicable

E Expected Values/Reference Range:
The FDA susceptibility interpretive criteria for doxycycline are listed in **Table 5**.

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

Organisms	S	I	R
Enterobacteriaceae	≤4	8	≥16

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

VIII Proposed Labeling:

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

IX Conclusion:

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

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 doxycycline 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 doxycycline 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.