510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K210757

B Applicant

bioMerieux SA

C Proprietary and Established Names

ETEST Fosfomycin (FO) (0.032-512 µg/mL)

D Regulatory Information

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

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for Fosfomycin at concentrations of 0.032-512 µg/mL for susceptibility testing of non-fastidious Gram-negative organisms.

B Measurand:

Fosfomycin 0.032-512 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:

ETEST is a manual, quantitative technique for the determination of antimicrobial susceptibility of non fastidious Gram negative and Gram positive aerobic bacteria and fastidious bacteria. The system comprises a predefined antibiotic gradient which is used to determine the Minimum Inhibitory Concentration (MIC, in µg/mL) of different antimicrobial agents against microorganisms tested on agar media after overnight incubation.

Fosfomycin has been shown to be active against the Gram-positive and Gram-negative aerobic microorganisms listed below according to the FDA label for this antimicrobial agent.

ETEST Fosfomycin can be used to determine the MIC of Fosfomycin against the following microorganisms:

Active both in vitro and in clinical infections:

•*Escherichia coli*

•*Enterococcus faecalis*

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:

ETEST is a thin, inert and non-porous plastic strip carrying the MIC reading scale in µg/mL on one side and a predefined antibiotic gradient on the other side.

The ETEST gradient technology is based on a combination of the concepts of dilution and diffusion principles for susceptibility testing.

The ETEST consists of a thin, inert, nonporous plastic strip that is used to determine the antimicrobial susceptibility of bacteria. One side of the strip carries the minimum inhibitory concentration (MIC) reading scale expressed in µg/mL. The other side of the strip contains a predefined continuous exponential gradient of antibiotic concentrations. ETEST Fosfomycin contains a range of Fosfomycin from 0.032 to 512 µg/mL.

B Principle of Operation:

When the ETEST strip is applied to an inoculated agar surface, the preformed antibiotic gradient immediately transfers into the agar matrix, then forming a stable, continuous and exponential gradient of antibiotic concentrations directly underneath the strip. Bacteria growth becomes visible during incubation, and a symmetrical inhibition ellipse centered along the strip appears. After incubation, the MIC value is read from the scale in terms of µg/mL at complete inhibition of bacterial growth, where the pointed end of the ellipse intersects the strip. Since ETEST generates MIC values which fall between two-fold dilutions for interpretation, the MIC value read must be recorded to the next two-fold dilution.

V Substantial Equivalence Information:

A Predicate Device Name(s):
ETEST Eravacycline (ERV) (0.002 - 32 µg/mL)

B Predicate 510(k) Number(s):
K192050

C Comparison with Predicate(s):

Table 1: Predicate Comparison

Device & Predicate Device(s):	**Device:** K210757	**Predicate:** K192050
Device Trade Name	ETEST Fosfomycin (FO) (0.032-512 µg/mL)	ETEST Eravacycline (ERV) (0.002 - 32 µg/mL)
General Device Characteristic Similarities		
Intended Use	ETEST is a manual, quantitative technique for determination of antimicrobial susceptibility of non-fastidious Gram-negative and Gram-positive aerobic bacteria and fastidious bacteria. The system comprises a predefined antibiotic gradient which is used to determine the Minimum Inhibitory Concentration (MIC, in µg/mL) of different antimicrobial agents	Same

Device & Predicate Device(s):	Device: K210757	Predicate: K192050
Device Trade Name	ETEST Fosfomycin (FO) (0.032-512 µg/mL)	ETEST Eravacycline (ERV) (0.002 - 32 µg/mL)
	against microorganisms tested on agar media after overnight incubation.	
Test Design	Predefined exponential gradient of the dried and stabilized antibiotic covers a continuous concentration range across 15 two-fold dilutions of a conventional MIC method	Same
Inoculum	Isolated colonies from culture	Same
Incubation	35º ± 2º C for 16 – 20 hours	Same
Result	MIC in µg/mL	Same
General Device Characteristic Differences		
Antimicrobial Agent	Fosfomycin	Eravacycline
Drug concentration Range	0.032-512 µg/mL	0.002 – 32 µg/mL
Claimed Organisms	Gram-negative: *Escherichia coli* Gram-Positive: *Enterococcus faecalis*	Gram-negative: *Citrobacter freundii* *Enterobacter cloacae* *Escherichia coli* *Klebsiella oxytoca* *Klebsiella pneumoniae*

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 11th Edition, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically* (09/17/2018)
- CLSI M100 29th ed. *Performance Standards for Antimicrobial Susceptibility Testing* (January 2019)

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

   Reproducibility testing was conducted at three sites using 5 Gram-negative and 5 Gram-positive isolates. Each isolate was tested in triplicate over three days for a total of 270 data points. The mode MIC value 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 consisted of 5 *E. coli* isolates and the Gram-positive panel consisted of 5 *E. faecalis* isolates. All MIC results were on scale. The testing resulted in overall reproducibility of greater than 95%. The results were acceptable.

2. Linearity:

   N/A

3. Analytical Specificity/Interference:

   N/A

4. Assay Reportable Range:

   N/A

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

   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 at least 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. One protocol deviation was noted during the reproducibility phase of the trial. The purity result was entered at a later date but the inoculum density check information was not recovered or recorded. This missing value was accepted.
   The inoculum densities were acceptable.

   Purity Check. Verification of isolate purity was conducted on all clinical, challenge and reproducibility organism suspensions for each ETEST and from each growth control well of the agar dilution reference panel. Only results from pure suspensions were evaluated.

   Growth or Device Failure. No device failures occurred in the ETEST Fosfomycin (FO) (0.032-512 µg/mL) clinical trial.

   Quality Control Testing. The CLSI recommended QC strains (*E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 29213) were tested at least 20 times per site at three sites using both ETEST and agar dilution reference methods. The results are summarized in Table 2.

The Quality Control results were within the recommended range > 95% of the time which is acceptable.

Table 2: Quality Control Test Results for ETEST Fosfomycin

QC Organism	Expected Range (Fosfomycin, µg/mL)	Concentration (µg/mL)	Reference Agar Dilution Frequency (All Sites)	ETEST Frequency (All Sites)
E. coli ATCC 25922	0.5-2	<0.5		
		0.5	**31**	**64**
		1	**37**	**5**
		2	**1**	
		>2		
E. faecalis ATCC 29212	32-128	<32	**2**	**1**
		32	**48**	**57**
		64	**19**	**11**
		128		
		>128		
P. aeruginosa ATCC 27853	2-8	<2		
		2	**5**	
		4	**57**	
		8	**6**	
		>8	**1**	
S. aureus ATCC 29213	0.5-4	<0.5		
		0.5		**15**
		1		**6**
		2	**13**	**12**
		4	**53**	**34**
		>4	**2**	**1**

In addition to testing the QC isolates recommended for Fosfomycin, supplemental quality control testing was performed with *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213 to support testing to determine *E. faecalis* Vancomycin resistance status (Table 3).

Table 3. Supplemental Quality Control to Support Testing *E. faecalis* for Vancomycin Resistance.

QC Organism	Expected Range (Vancomycin, µg/mL)	Concentration (µg/mL)	ETEST Frequency (All Sites)
E. faecalis ATCC 29212	1-4	<1	
		1	
		2	**6**
		4	**34**
		>4	
S. aureus ATCC 29213	0.5-2	<0.5	
		0.5	**1**
		1	**21**
		2	**18**
		>2	

6. Detection Limit:

   N/A

7. Assay Cut-Off:

   N/A

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Results obtained with ETEST Fosfomycin were compared to results obtained with the CLSI agar dilution reference panel. The reference panel, prepared and interpreted according to recommendations outlined in the CLSI document M07 11th ed., contained two-fold serial dilutions of Fosfomycin with a concentration range of 0.064 – 512 µg/mL. At the end of incubation, the MIC value obtained from the ETEST (where the complete inhibition of growth intersects the strip) was compared to MIC results obtained with the reference method.

   The testing conditions for ETEST consisted of the following:
   - Inoculum: Direct colony suspension to achieve a suspension equivalent to a 0.5 McFarland standard suspension
   - Medium: Cation-adjusted Mueller Hinton agar
   - Incubation: 35° C ± 2 for 16-20 hours

   Clinical testing was performed at three external sites with both ETEST Fosfomycin and the reference method. A total of 353 clinical isolates were evaluated including 201 *E. coli,* and 152 *E. faecalis*. The clinical testing included 57.2% fresh (isolated no longer than 6 months prior to testing) and 42.8% stock strains (isolated over 6 months prior to testing) clinical isolates. A total of 76 challenge isolates were also evaluated at a single site including 37 *E. coli,* and 39 *E. faecalis*.

A comparison was provided to the reference method with the following agreement (Table 4). The combined clinical and challenge isolate performance for *E. coli* was acceptable with an EA of 90.8% and CA of 99.2% with no major or very major errors. The combined clinical and challenge isolate performance for *E. faecalis* was acceptable with an EA of 97.9% and CA of 93.7% with 1 major error (1/189 = 0.5%) and no very major errors.

Table 4. Performance of ETEST Fosfomycin with *E. coli* and *E. faecalis*

	Tot	EA N	EA %	Eval Tot	Eval EA N	Eval EA %	CA Tot	CA %	No. R	No. S	Min	Maj	Vmj
E. coli													
Clinical	201	183	91.0%	188	170	90.4%	199	99.0%	14	187	2	0	0
Challenge	37	33	89.2%	33	29	87.9%	37	100%	4	33	0	0	0
Total	238	216	90.8%	221	199	90.0%	236	99.2%	18	220	2	0	0
E. faecalis													
Clinical	152	149	98.0%	150	147	98.0%	142	93.4%	2	150	10	0	0
Challenge	39	38	97.4%	39	38	97.4%	37	94.9%	0	39	1	1	0
Total	191	187	97.9%	189	185	97.9%	179	93.7%	2	189	11	1	0

EA – Essential Agreement
CA – Category Agreement
EVAL – Evaluable MIC results
S – Susceptible

min – minor discrepancies
maj – manor discrepancies
vmj – very major discrepancies
R - resistant

Essential agreement (EA) is when the ETEST Fosfomycin results agree exactly or within one doubling dilution of the reference method. Category agreement (CA) is when the ETEST Fosfomycin result interpretation agrees exactly with the reference panel result interpretation.

For clinical and challenge isolates tested with the ETEST Fosfomycin, the combined clinical and challenge %EA and %CA met the acceptance criteria of greater than or equal to 90% (Table 4) for each organism group.

Testing/Reporting MICs for Non-Indicated Species

For this review, the interpretative criteria are applied to the organisms/organism groups according to the FDA STIC website. As required under 511A(2)(2)(B) of the Federal Food, Drug and Cosmetic Act, the following statements are added in the package insert:

> *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.*

Inoculator and ETEST Strip Applicator Options:

Culture media plates for ETEST can be inoculated and streaked by swabs manually or with the RETRO C80 inoculator. ETEST strips can be applied onto inoculated media using forceps or the NEMA C88 vacuum pen.

The ETEST studies for Fosfomycin used manual inoculation with swabs and applied ETEST strips with forceps and the Vacuum Pen NEMA C88 at all test sites. The following statement is included as a footnote to the performance table in the device labeling:

The optional Inoculator RETRO C80 and Applicator SIMPLEX C76 can be used to inoculate plates and apply ETEST strips to agar media. In the ETEST Fosfomycin clinical studies, swabs were used for plate inoculation/streaking, and forceps and the Vacuum Pen NEMA C88 were used for ETEST strip application.

MIC Trends:
An analysis of trending was conducted using the combined clinical and challenge data. 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. Results that are not clearly at least one dilution lower, at least one dilution higher or in exact agreement with the CLSI reference method are not considered in the trending analysis.

Species for which the difference between the percentage of isolates with higher vs. lower MIC reading was ≥ 30% and for which the confidence interval was determined to be statistically significant were considered to show evidence of trending. Trending that provides higher or lower MIC values compared to the reference is addressed in labeling.

A trend toward lower MIC readings was observed for *E. coli* when compared to the CLSI agar dilution reference method, as summarized in Table 5. The following statement is included as a footnote to the performance table in the device labeling to address the observed trending:

"*ETEST Fosfomycin MIC values tended to be in exact agreement or at least one doubling dilution lower when testing Escherichia coli compared to the reference agar dilution method."*

Table 5. Trending Observed with ETEST Fosfomycin

Organism	Total Evaluable for Trending	≥ 1 Dilution lower No. (%)	Exact No. (%)	≥ 1 Dilution Higher No. (%)	Percent Difference (CI)	Trending Noted
E. coli, clinical & challenge	227	121 (53.3%)	87 (38.3%)	19 (8.4%)	-44.9% (-52.0%, -37.1%)	Yes
E. faecalis, clinical & challenge	191	44 (23%)	126 (66%)	21 (11%)	-12.0% (-16.5%, -4.5%)	No

Resistance Mechanisms: Isolates with the following resistance mechanisms were evaluated:
VRE, ESBL, carbapenemase MBL, carbapenemase KPC, carbapenemase OXA-48, acquired penicillinase, acquired cephalosporinase.

2. Matrix Comparison:

N/A

C Clinical Studies:

1. Clinical Sensitivity:

   N/A

2. Clinical Specificity:

   N/A

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

   N/A

D Clinical Cut-Off:

N/A

E Expected Values/Reference Range:

Table 6. FDA-Recognized Interpretive Criteria for Fosfomycin

	Minimum Inhibitory Concentration (µg/mL)[a]		
Organisms	**S**	**I**	**R**
Enterobacteriaceae	≤64	128	≥256
E. faecalis	≤64	128	≥256

[a] FDA STIC webpage

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 bioMérieux intends to use to evaluate the BioMerieux ETEST Fosfomycin (FO) (0.032-512 µg/mL) when revised breakpoints for Fosfomycin are published on the FDA STIC webpage. The breakpoint change protocol included with the submission indicated that if specific criteria are met, BioMerieux will update the Fosfomycin 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.