510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K222430

B Applicant

bioMérieux SA, Inc.

C Proprietary and Established Names

VITEK 2 AST-Gram Negative Fosfomycin (≤4 - ≥256 µg/mL), VITEK 2 AST-GN Fosfomycin (≤ - ≥256 µg/mL), VITEK 2 AST-GN Fosfomycin

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LON	Class II	21 CFR 866.1645 - Fully Automated Short-Term Incubation Cycle Antimicrobial Susceptibility System	MI - Microbiology
LTW	Class II	21 CFR 866.1640 - Antimicrobial susceptibility test powder	MI - Microbiology
LTT	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 for testing of Gram-negative bacilli on the VITEK 2 and VITEK 2 Compact Antimicrobial Susceptibility Test (AST) Systems

B Measurand:

Fosfomycin (≤4 - ≥256 µg/mL)

C Type of Test:

Automated quantitative or qualitative antimicrobial susceptibility test.

III Intended Use/Indications for Use:

A Intended Use(s):

The VITEK 2 Gram-Negative Susceptibility Card is intended for use with the VITEK 2 Systems in clinical laboratories as an *in vitro* test to determine the susceptibility of clinically significant aerobic Gram-negative bacilli to antimicrobial agents when used as instructed.

B Indication(s) for Use:

VITEK 2 AST-Gram Negative Fosfomycin is designed for antimicrobial susceptibility testing of Gram negative bacilli and is intended for use with the VITEK 2 and VITEK 2 Compact Systems as a laboratory aid in the determination of *in vitro* susceptibility to antimicrobial agents. VITEK 2 AST-Gram Negative Fosfomycin is a quantitative test. Fosfomycin has been shown to be active against most strains of the microorganism listed below, according to the FDA label for this antimicrobial.

Active *in vitro* and in clinical infections:

Escherichia coli

The VITEK 2 Gram-Negative Susceptibility Card is intended for use with the VITEK 2 Systems in clinical laboratories as an *in vitro* test to determine the susceptibility of clinically significant aerobic Gram-negative bacilli to antimicrobial agents when used as instructed.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

VITEK 2 and VITEK 2 Compact Systems using VITEK 2 Systems 9.04 software

IV Device/System Characteristics:

A Device Description:

The VITEK 2 AST card is a miniaturized, abbreviated and automated version of the doubling dilution technique for determining the minimum inhibitory concentration (MIC). Each VITEK 2 AST card contains 64 wells. A control well(s) which contain only nutrient medium is resident on all cards. The remaining wells contain premeasured portions of antimicrobials combined with the nutrient media. The isolate to be tested is diluted to a standardized concentration with 0.45% to 0.50% saline before being used to rehydrate the antimicrobial medium within the card. The VITEK 2 System will automatically (or allow operator to manually) dilute the bacterial suspension to prepare an inoculum for susceptibility cards. Then, the VITEK 2 will fill, seal and place the card into the incubator/reader. The VITEK 2 Compact has a manual filling, sealing, and

loading operation. The VITEK 2 Systems monitor the growth of each well in the card over a defined period of time. The analysis program determines when a well demonstrates growth based on attenuation of light measured by an optical scanner. This data is used to determine the minimum inhibitory concentration or "MIC" values for the antimicrobial agent. At the completion of the incubation cycle, a report is generated that contains the MIC value along with the interpretive category result for each antimicrobial contained on the card.

VITEK 2 AST-Gram Negative Fosfomycin has the following concentrations in the card: 2, 8, 16, and 64 µg/mL (equivalent standard method concentration by efficacy in µg/mL). The fosfomycin MIC result range for the VITEK 2 Gram Negative Fosfomycin is ≤ 4 to ≥ 256 µg/mL.

B Principle of Operation:

The VITEK 2 and VITEK 2 Compact Systems utilize automated growth-based detection using attenuation of light measured by an optical scanner. The optics in the systems use visible light to directly measure organism growth within each of the 64 micro-wells. Transmittance optics is based on an initial light reading of a well before significant growth has begun. Every 15 minutes throughout the incubation cycle (defined period of time based on the VITEK 2 card), light transmittance readings of each well determine organism growth by the amount of light that is prevented from passing through the well. At the completion of the incubation period, the MIC values and their associated interpretive category results for each antimicrobial on the test card are displayed in an automatically generated report.

V Substantial Equivalence Information:

A Predicate Device Name(s):
VITEK 2 AST-Gram Negative Eravacycline (<=0.12 - >=4µg/mL)

B Predicate 510(k) Number(s):
K191766

C Comparison with Predicate(s):

Device & Predicate Device(s):	**Device K222430**	**Predicate K191766**
Device Trade Name	VITEK 2 AST-Gram Negative Fosfomycin (≤ 4 - ≥ 256 µg/ml)	VITEK 2 AST-GN Eravacycline (≤0.12 - ≥4 µg/mL)
General Device Characteristic Similarities		
Intended Use/Indications for Use	The VITEK 2 Gram-Negative Susceptibility Card is intended for use with the VITEK 2 Systems in clinical laboratories as an *in vitro* test to determine the susceptibility of clinically	Same

Device & Predicate Device(s):	Device K222430	Predicate K191766
	significant aerobic Gram-negative bacilli to antimicrobial agents when used as instructed.	
Test Methodology	Automated quantitative antimicrobial susceptibility test for use with the VITEK 2 and VITEK 2 Compact Systems to determine the *in vitro* susceptibility of microorganisms.	Same
Inoculum	Saline suspension of organism	Same
Test Card	Gram Negative (AST-GN) Susceptibility Card	Same
Instrument	VITEK 2 and VITEK 2 Compact Systems	Same
Analysis Algorithm	Growth Pattern Analysis	Same
Type of Test	Quantitative	Same
General Device Characteristic Differences		
Antimicrobial Agent	Fosfomycin	Eravacycline
Concentrations of antimicrobial on card	2, 8, 16, 64 µg/ml	0.25, 1, 2, 4 µg/ml
Indications for Use	VITEK 2 AST-Gram Negative Fosfomycin is designed for antimicrobial susceptibility testing of Gram-negative bacilli and is intended for use with the VITEK 2 and VITEK 2 Compact Systems as a laboratory aid in the determination of *in vitro* susceptibility to antimicrobial agents. VITEK 2 AST-Gram Negative Fosfomycin is a quantitative test. Fosfomycin has been shown to be active against most strains of the microorganism listed	VITEK 2 AST-Gram Negative Eravacycline is designed for antimicrobial susceptibility testing of Gram-negative bacilli and is intended for use with the VITEK 2 and VITEK 2 Compact Systems as a laboratory aid in the determination of *in vitro* susceptibility to antimicrobial agents. VITEK 2 AST-Gram Negative Eravacycline is a quantitative test. Eravacycline has been shown to be active against most strains of the microorganisms listed below, according to the

Device & Predicate Device(s):	Device K222430	Predicate K191766
	below, according to the FDA label for this antimicrobial. Active *in vitro* and in clinical infections: *Escherichia coli*	FDA label for this antimicrobial. Active *in vitro* and in clinical infections: *Citrobacter freundii* *Enterobacter cloacae* *Escherichia coli* *K. oxytoca* *Klebsiella pneumoniae* *In vitro* data are available but clinical significance is unknown: *Citrobacter koseri* *Klebsiella (Enterobacter) aerogenes*

VI Standards/Guidance Documents Referenced:

- FDA Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems; Guidance for Industry and FDA (Issued August 28, 2009)
- 7-279: M07, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Eleventh Edition", (January 2018)
- 7-294: M100, "Performance Standards for Antimicrobial Susceptibility Testing; 32nd Edition (January 2022)

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Reproducibility testing for the VITEK 2 AST-Gram Negative Fosfomycin was conducted at three external clinical sites using a panel of 10 *E. coli* isolates. Each isolate was tested in triplicate, using separate inocula, over three days for a total of 270 data points. Inocula were prepared using both the auto-dilution and manual dilution methods for testing in the VITEK 2 System. In addition, inocula were prepared by the manual dilution method for use with the VITEK 2 Compact. 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 data were analyzed taking into consideration best-case and worst-case scenarios as described in the Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems.

The reproducibility data for both of the VITEK 2 System card inoculation options and the manual inoculum for VITEK 2 Compact are summarized in Table 1 below. The results are acceptable.

Table 1. Reproducibility Data for Each Inoculum Method

VITEK 2 System Card Inoculum Method	Best Case	Worst Case
Automatic Dilution	**100%**	**100%**
Manual Dilution	**99.6%**	**99.3%**

VITEK 2 Compact Card Inoculum Method	Best Case	Worst Case
Manual Dilution	**99.3%**	**99.3%**

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 recommends four QC strains for testing fosfomycin, namely *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213.

   One organism, *E. coli* ATCC 25922, was tested a sufficient number of times (i.e., at least 20/site) at each testing site using both the VITEK 2 card and agar dilution (AD) reference method. Both the automatic dilution and manual dilution methods were used for the VITEK 2 and the manual dilution method was used for the VITEK 2 Compact. The QC organism was tested by the automatic dilution method at the four clinical sites and at the sponsor's laboratory while the manual VITEK 2 and COMPACT testing was performed at only the four clinical sites. This QC organism yielded off-scale VITEK 2 fosfomycin MICs of ≤ 4 µg/mL (Table 2). A VITEK result of ≤ 4 µg/mL is considered acceptable for this organism.

Table 2. Fosfomycin VITEK 2 Quality Control Data

QC Organism	VITEK Result Range (µg/mL)	BMD Result Range (µg/mL)	VITEK 2 Auto Dilution	Reference Result	VITEK 2 Manual Dilution	Reference Result	VITEK 2 COMPACT Manual Dilution	Reference Result
E. coli ATCC 25922 Expected Result 0.5-2 (µg/mL)		≤ 0.25						
		0.5		84		35		37
		1		86		50		52
		2		16		11		11
	≤4*	4	186	0	96	0	100	0

* ≤ 4 µg/mL (i.e. lowest reporting value for VITEK 2 and VITEK 2 COMPACT Gram-Negative (AST-GN) Susceptibility Card

The off-scale results necessitated identifying an additional Gram-negative QC organism. *K. pneumoniae* ATCC BAA-2814 for use specifically for quality control testing of Fosfomycin with the VITEK 2 System. This isolate was evaluated retrospectively at three laboratories and yielded an expected QC range of 16-64 µg/mL, as noted in the device labeling.

The two Gram-positive QC organisms, *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213, were used as ancillary QC organisms for only the agar dilution reference method. This was done to perform further quality control of the agar dilution plates. QC results for the agar dilution method were within the expected result range 100% of the time for both organisms (Table 3).

Table 3. Ancillary Quality Control Data for Fosfomycin

ATCC QC Strain	MIC (µg/mL)	Reference Result
S. aureus ATCC 29213 Expected Result 0.5-4 (µg/mL)	≤ 0.25	
	0.5	2
	1	26
	2	89
	4	72
Total		**189**
E. faecalis ATCC 29212 Expected Result 32-128 (µg/mL)	≤ 0.16	
	32	76
	64	113
	128	
Total		**189**

Inoculum Density Control

The DensiCHEK Plus was used to standardize the inoculum to a 0.5 McFarland standard. The instrument was standardized daily with all results recorded at each site. Calibration values were within the expected range.

Purity Check

A purity check of all organisms was performed on the dilution tube used to prepare the VITEK 2 card inoculum. Only those cultures that were pure were evaluated in the study.

Device Failure
Device failures occurred at each of the test sites on 12 different days. These involved failure of an instrument to fill cards properly, pipettor errors, failure of an instrument to connect to the computer, failure of an instrument to recognize a card, an instrument rejecting cards, a reader error, and a card being dropped on the floor before testing. All failures were resolved by one of the following: repeat testing with the original inoculum; card replacement; straightening of kinked tubing; technician intervention, or BioMérieux intervention. There was no impact on testing or results.

Growth Failure Rate
A total of 380 clinical and challenge isolates were tested by VITEK 2 Fosfomycin. No growth failure was recorded and all 380 isolates have VITEK 2 AST results available.

6. Detection Limit:

   Not applicable

7. Assay Cut-Off:

   Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Testing of fosfomycin on the VITEK 2 AST-Gram Negative card was performed at four clinical sites. There were 300 clinical isolates and 80 challenge isolates tested for a total of 380 isolates. Results obtained with the VITEK 2 AST-Gram Negative card with fosfomycin were compared to results obtained with the CLSI recommended agar dilution testing conditions. The MIC result range for the VITEK 2 AST-Gram Negative Fosfomycin is ≤4 to ≥256 µg/mL for *Escherichia coli*. The reference method testing consisted of two-fold serial dilutions of fosfomycin with a range of ≤0.25 to ≥1024 µg/mL. The testing conditions for the reference method consisted of the following:

   - Medium – Mueller Hinton agar with addition of 25 µg/mL of glucose-6-phosphate and appropriate dilutions of antimicrobial solution added
   - Inoculum – Direct colony suspension
   - Incubation – 35°C; 16-24 hours

   The VITEK 2 AST cards were inoculated with test organisms using the auto-dilution method (VITEK 2) and manual dilution method (VITEK 2 and VITEK 2 Compact). All test inocula used for the VITEK 2 AST cards and the reference method were standardized using the DensiCHEK Plus instrument.

   A total of 300 clinical isolates were evaluated at four sites: the majority were recently isolated from clinical specimens and the remainder were stock isolates. Complete test results

are available for all 300 clinical isolates. All clinical isolates were tested with the auto-dilution option of the VITEK 2.

A total of 80 *E. coli* challenge isolates were evaluated at one site. The challenge set was tested with the auto-dilution and manual dilution options of the VITEK 2 and with the manual dilution method on the VITEK 2 Compact.

At the time of comparative testing, 14 resistant isolates and 1 intermediate isolate were available for *E. coli*. The 14 resistant isolates comprise 3.6% of 380 total isolates. However, since the resistant breakpoint is at the upper limit of the reporting range, an upward shift in MIC values may not be easily detected. Since resistance rate to Fosfomycin is relatively low, the occurrence of such shifts may be rare. However, the following footnote is included in the device labeling to inform users of this issue:

An MIC shift in the resistance range (≥256 µg/mL) will be unknown since the resistant breakpoint and the upper limit of the reporting range is the same.

Clinical and Challenge Data –VITEK 2 Auto-Dilution

VITEK 2 AST-GP Fosfomycin performance was determined with 380 isolates (300 clinical isolates and 80 challenge isolates). The 80 challenge isolates were also tested at one site with the manual dilution option for the VITEK 2 Compact system. The data are summarized in Table 4. The overall performance of using the VITEK 2 auto-dilution is acceptable with an EA of 99.2% and a CA of 99.7%. There were no major or very major errors. The overall performance of the manual dilution option of the VITEK 2 and VITEK 2 Compact systems is acceptable with an EA of 100% and a CA of 98.8%% with the VITEK 2 Compact system. There were no major or very major errors.

Table 4. Essential and Category Agreement for VITEK 2 Combined Challenge and Clinical *E. coli* Isolates

Organism Group	Total Tested	# EA	% EA	Total Eval	# EA of Eval	% EA of Eval	# CA	% CA	# R	# S	# min	# maj	# vmaj
Inoculation Method Auto - Read Method VITEK 2													
Clinical	300	297	99	7	4	57.1	300	100	0	300	0	0	0
Challenge	80	80	100	11	11	100	79	98.8	14	65	1	0	0
Combined	380	377	99.2	18	15	83.3	379	99.7	14	365	1	0	0
Inoculation Method Manual – Read Method VITEK 2													
Challenge	80	79	98.8	11	10	90.9	79	98.8	14	65	1	0	0
Inoculation Method Manual – Read Method VITEK 2 Compact													
Challenge	80	80	100	11	11	100	79	98.8	14	65	1	0	0

EA – Essential Agreement
CA – Category Agreement
Eval - Evaluable isolates
R – Resistant isolates
S – Susceptible isolates
min – minor errors
maj – major errors
vmaj – very major errors

Essential agreement (EA) occurs when the result of the reference method and that of the VITEK 2 AST-Gram Negative Fosfomycin are within plus or minus one serial two-fold dilution of the antibiotic. Evaluable results are those that are on-scale for both the reference method and the VITEK 2 AST-Gram Negative Fosfomycin or results in which an off-scale result is at least two doubling dilutions from the on scale result. Category agreement (CA) occurs when the interpretation of the result of the reference method agrees exactly with the interpretation provided by the VITEK 2 AST-Gram Negative Fosfomycin.

Resistance Mechanism Characterization

The FDA drug label for Fosfomycin does not indicate any specific mechanisms of resistance.

As required under 511A(b)(2)(C)(ii)(I) of the Federal Food, Drug and Cosmetic Act, the following statement is included in the Precautions section of the device labeling to address testing and reporting of non-indicated species:

> *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.*

MIC Trends

A trending analysis was conducted using the combined data (clinical and challenge) obtained for each inoculation method (Table 5). 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. A difference between the percentage of isolates with higher or lower MIC values ≥30% with a statistically significant confidence interval are considered to have evidence of trending. No trending was detected for 22 on-scale results with VITEK 2 auto dilution.

Table 5. Trending (clinical and challenge isolates)

Inoculation/Read Method	Organism	Total On Scale for Trending	≥ 1 Dilution Lower # (%)	Exact #(%)	≥ 1 Dilution Higher # (%)	Percent Difference (95% Cl)	Trending Noted
Auto\VITEK 2	*E. coli*	22	6, (27.3)	8	8, (36.4)	9% , (-18%, 34%)	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-identified recognized interpretive criteria for fosfomycin are listed in Table 6

Table 6. FDA-Recognized Interpretive Criteria for Fosfomycin (µg/mL)[a]

Organisms	S	I	R
Escherichia coli	≤64	128	≥256

[a]According to FDA STIC Website.

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 VITEK 2 AST-GN Fosfomycin 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, bioMérieux 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.