510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K234000

B Applicant

bioMérieux, Inc.

C Proprietary and Established Names

VITEK 2 AST-Gram Positive Lefamulin (≤0.03 - ≥4 µg/mL)

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
LTT	Class II	21 CFR 866.1640 - Antimicrobial susceptibility test powder	MI - Microbiology
LTW	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 the VITEK 2 AST-Gram Positive Lefamulin (≤0.03 - ≥4 µg/mL) assay for testing of *Staphylococcus aureus* (methicillin-susceptible isolates) on the VITEK 2 and VITEK 2 Compact Antimicrobial Susceptibility Test (AST) Systems.

B Measurand:

Lefamulin (Imu01n) ≤0.03 - ≥4 µg/mL

C Type of Test:

Automated quantitative or qualitative antimicrobial susceptibility test for Lefamulin

III Intended Use/Indications for Use:

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

B Indication(s) for Use:
VITEK 2 AST-Gram Positive Lefamulin is designed for antimicrobial susceptibility testing of Gram positive microorganisms 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 Positive Lefamulin is a quantitative test. Lefamulin has been shown to be active against most strains of the microorganisms listed below, according to the FDA label for this antimicrobial.

Active both *in vitro* and in clinical infections:

Staphylococcus aureus (methicillin-susceptible isolates)

The VITEK 2 Gram-positive Susceptibility Card is intended for use with the VITEK 2 Systems in clinical laboratories as an *in vitro* test to determine the susceptibility of *Staphylococcus* spp., *Enterococcus* spp., and *S. agalactiae* to antimicrobial agents when used as instructed.

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

The current absence of resistant isolates precludes defining any results other than "Susceptible." Isolates yielding MIC results other than “Susceptible” should be submitted to a reference laboratory for further testing.

D Special Instrument Requirements:

VITEK 2 and VITEK 2 Compact systems using VITEK 2 Systems v9.02 software or newer

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 which only contains microbiological culture media is resident on all cards. The remaining wells contain premeasured portions of antimicrobials combined with 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-GP Lefamulin has the following concentrations in the card: ≤0.125, 0.5, 1, 2 (equivalent standard method concentration by efficacy in µg/mL). The MIC result range indicates that the VITEK 2 system can produce and report the following MIC results: ≤0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, ≥4 µg/mL for the VITEK 2 AST-GP Lefamulin test.

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 Positive Daptomycin (≤0.12 - ≥8 µg/mL)

B Predicate 510(k) Number(s):
K230864

C Comparison with Predicate(s):

Device & Predicate Device(s):	**Device:** K234000	**Predicate:** K230864
Device Trade Name	VITEK 2 AST-GP Lefamulin (≤0.03 - ≥4 µg/mL)	VITEK 2 AST-GP Daptomycin (≤0.12 - ≥8 µg/mL)
General Device Characteristic Similarities		
Intended Use/Indications For Use	The VITEK 2 Gram-positive Susceptibility Card is intended for use with the VITEK 2 Systems in clinical laboratories as an *in vitro* test to determine the susceptibility of *Staphylococcus* spp.,	Same

Device & Predicate Device(s):	Device: K234000	Predicate: K230864
	Enterococcus spp., and *S. agalactiae* 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	Standardized saline suspension of organism	Same
Test Card	Gram-positive (AST-GP) Susceptibility Card	Same
Instrument	VITEK 2 and VITEK 2 Compact systems	Same
Type of test	Quantitative	Same
General Device Characteristic Differences		
Antimicrobial Agent	Lefamulin	Daptomycin
Concentrations of antimicrobial on card	0.125, 0.5, 1, 2	0.5, 1, 2, 4, 16
Reporting Range	≤0.03 - ≥4 µg/mL	≤0.12 - ≥8 µg/mL
Indicated Organisms	*Staphylococcus aureus* (methicillin-susceptible isolates)	*Enterococcus faecalis* (vancomycin-susceptible isolates), *Staphylococcus aureus* (including methicillin-resistant isolates), *Enterococcus faecalis* (vancomycin-resistant isolates)

VI Standards/Guidance Documents Referenced:

- CLSI M100: "2023 Performance Standards for Antimicrobial Susceptibility Testing," 33rd Edition (March 2023)
- CLSI M07: "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically," 11th edition (January 2018)
- FDA Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems; Guidance for Industry and FDA (Issued August 28, 2009)

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Reproducibility testing for the VITEK 2 AST- Gram Positive Lefamulin was conducted at three external sites using a panel of ten methicillin-susceptible *Staphylococcus aureus* strains consistent with the indications for use. Each isolate was tested in triplicate, using separate inocula, over three days for a total of 270 data points. The inocula were prepared using both the auto-dilution and manual dilution methods for testing with the VITEK 2 System. In addition, inocula were prepared by the manual dilution method for testing 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 ± one doubling dilution of the mode MIC value. The majority of data points were on-scale and within ± one doubling dilution agreement as compared to the mode MIC. The data was 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 performance is shown in **Table 1**. The results are acceptable.

Table 1. Reproducibility Performance

	VITEK 2		**VITEK 2 Compact**
	Manual Dilution	**Auto-Dilution**	**Manual Dilution**
Best case	98.9%	99.6%	100%
Worst case	98.9%	99.3%	100%

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, *Staphylococcus aureus* ATCC 29213 was tested a sufficient number of times (i.e., at least 20/site) at each testing site using both the VITEK 2 AST-GP Lefamulin and Broth Microdilution (BMD) 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 results are summarized in **Table 2** below. Both the auto dilution and manual dilution methods for VITEK 2 and the manual dilution method for VITEK 2 Compact QC results were within the expected range >95% of the time. Although trending toward lower MIC values was observed, QC was determined to be acceptable.

Table 2: Quality Control Results for Lefamulin: VITEK 2 (Auto Dilution and Manual Dilution Methods) and VITEK 2 Compact (Manual Dilution Method)

Organism	VITEK 2 Result Range (µg/mL)	BMD Result Range (µg/mL)	VITEK 2 Auto Dilution	BMD	VITEK 2 Manual Dilution	BMD	VITEK 2 Compact Manual Dilution	BMD
Staphylococcus aureus ATCC 29213 Expected Result: 0.06-0.25 µg/mL		≤0.015						
	≤0.03	0.0.3						
	0.06	0.06	92	1	61		20	
	0.125	0.125		82		56		20
	0.25	0.25		9		5		
	0.5	0.5						
	1	1						
	2	2						
	≥4	4						
		8						
		16						
		≥32						

BMD: reference broth microdilution

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. Any purity checks found not to be pure were retested in accordance with the testing protocol. Only those cultures that were pure were evaluated in the study.

Device Failures:
During the performance of the comparative study, there were instrument processing errors that resulted in loss of AST cards during 5 device failures with the VITEK 2 System and 2 device failures with the VITEK 2 Compact. All isolates affected by these errors were retested in accordance with the testing protocol.

Growth Failure Rate:
A total of 405 clinical and challenge isolates were tested by VITEK 2 AST-GP Lefamulin. One growth failure was recorded for a clinical isolate of MSSA. Results for 404 isolates on the VITEK AST were available.

6. Detection Limit:

   Not applicable.

7. Assay Cut-Off:

   Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Testing of Lefamulin on the VITEK 2 AST-Gram Positive card was performed at three external sites. There were 329 clinical isolates and 75 challenge isolates tested for a total of 404 isolates tested. Results obtained with VITEK 2 AST-Gram Positive Lefamulin were compared to results obtained with the CLSI broth microdilution (BMD) reference method. The MIC range for the VITEK 2 AST-Gram Positive Lefamulin is ≤ 0.03 - ≥ 4 µg/mL for all species. The reference method testing consisted of two-fold serial dilutions of Lefamulin with a range of ≤0.015 to ≥ 32 µg/mL. The testing conditions for the reference method consisted of the following:

- Medium: Mueller Hinton broth with the appropriate dilutions of antimicrobial solution added
- Inoculum: Direct colony suspension
- Incubation: 35 ± 2 °C; 16-20 hours

The VITEK 2 AST cards were inoculated with test organisms using the auto-dilution method (VITEK 2) and using the 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 DensiCheck Plus instrument.

During the Comparison Studies, it was noted that when tested with methicillin-susceptible *Staphylococcus aureus* (MSSA), Lefamulin required prolonged incubation times to generate MIC values. The following footnote is included in the device labeling to address the extended incubation time:

> *The incubation time (Time of Call) for AST GP cards containing Lefamulin may exceed 16 hours. This extended incubation time is required to generate MIC values.*

A total of 329 clinical isolates of MSSA were evaluated using the auto-dilution and VITEK 2. Of these isolates, 100% were recent isolates (isolated within the last 6 months). No clinical stock isolates were tested.

A total of 75 challenge isolates of MSSA were evaluated. This 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.

Clinical and Challenge Data-VITEK 2 Auto-Dilution
The results obtained using the auto-dilution method of the VITEK 2 from the 404 total isolates (329 clinical isolates and 75 challenge isolates) are summarized in **Table 3**.

Table 3. Performance of All Clinical and Challenge Isolates for Lefamulin: VITEK 2 Auto-Dilution

	Tot	No. EA	EA %	Eval EA Tot	No. Eval EA	Eval EA %	No. CA	CA %	No. NS	No. S	Min	Maj	VMJ
Methicillin Susceptible *Staphylococcus aureus*, S: ≤0.25, NS: ≥0.5													
Clinical	329	297	90.3	322	290	90.1	328	99.7	2	327	0	1	0
Challenge	75	70	93.3	71	66	93.0	75	100	1	74	0	0	0
Combined	404	367	90.8	393	356	90.6	403	99.8	3	401	0	1	0

EA – Essential Agreement
CA – Category Agreement
EVAL – Evaluable Isolates
NS – Non-susceptible Isolates
min – minor errors
maj – major errors
vmj – very major errors
S – Susceptible Isolates

Essential Agreement (EA) occurs when there is agreement between the MIC result of the reference method and that of the VITEK 2 test card within plus or minus one serial two-fold dilution of the antibiotic. Evaluable results are those that are on scale for both the VITEK 2 test card and the reference method or those 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 of the VITEK 2 test card.

For all organisms evaluated using the auto-dilution method of the VITEK 2, EA was acceptable at 90.8% **(Table 3)**. The CA was acceptable with 99.8% with only one major error and no minor errors or very major errors.

Challenge Data: VITEK 2 and VITEK 2 Compact Manual Dilution

The 75 challenge isolates were also tested at one site with the manual dilution option for the VITEK 2 and the manual dilution of the VITEK 2 Compact. The results are summarized in **Table 4**.

Table 4. Performance of Challenge Isolates for Lefamulin: VITEK 2 and VITEK 2 Compact Manual Dilution

	Tot	No. EA	EA %	Eval Tot	No. Eval EA	Eval EA %	CA Tot	CA %	No. NS	No. S	min	maj	vmj
Methicillin Susceptible *Staphylococcus aureus*, S: ≤0.25, NS: ≥0.5													
VITEK 2	75	71	94.7	70	66	94.3	75	100	1	74	0	0	0
VITEK 2 Compact	75	72	96.0	70	67	95.7	75	100	1	74	0	0	0

EA – Essential Agreement
CA – Category Agreement
EVAL – Evaluable Isolates
NS – Non-susceptible Isolates
min – minor errors
maj – major errors
vmj – very major errors
S – Susceptible Isolates

Essential Agreement (EA) occurs when there is agreement between the MIC result of the reference method and that of the VITEK 2 test card within plus or minus one serial two-fold dilution of the antibiotic. Evaluable results are those that are on scale for both the VITEK 2 test card and the reference method or those 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 of the VITEK 2 test card.

The overall performance for the VITEK 2 AST-Gram Positive Lefamulin when testing MSSA with the manual dilution option of the VITEK 2 system is acceptable with an EA of 94.7%, a CA of 100%, and no category errors (0%).

The overall performance for the VITEK 2 AST-Gram Positive Lefamulin when testing MSSA with the VITEK 2 Compact system is acceptable with an EA of 96.0%, a CA of 100%, and no category errors (0%).

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.*

Trending

A trending analysis was performed using both the clinical and challenge data. 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. Organisms in which the difference between the percentage of isolates with higher vs. lower MIC values was ≥ 30% and for which the confidence interval was determined to be statistically significant were considered to show evidence of trending. Trending that shows higher or lower MIC values compared to the reference is addressed in the labeling.

The VITEK 2 AST-Gram Positive Lefamulin MIC values for MSSA tended to be equal to or at least one doubling dilution lower than the reference broth microdilution method, as seen in **Table 5**. The following footnote was added to the performance table in the device labeling to address trending:

> *VITEK 2 Lefamulin MIC values tended to be in exact agreement or at least one doubling dilution lower when testing methicillin-susceptible S. aureus compared to the CLSI reference broth microdilution method.*

Table 5. Trending Analysis for methicillin-susceptible Staphylococcus aureus (clinical and challenge isolates) with Lefamulin and the VITEK 2 system

Inoculation/Read Method	Organism Group	Total On Scale for Trending	≥ 1 Dilution Lower No. (%)	Exact No. (%)	≥ 1 Dilution Higher No. (%)	Percent Different (95% CI)	Trending Noted
Auto/VITEK 2	*Staphylococcus aureus*	400	222, (55.5)	147	31, (7.75)	-48% (42-53)	Yes

Inoculation/Read Method	Organism Group	Total On Scale for Trending	≥ 1 Dilution Lower No. (%)	Exact No. (%)	≥ 1 Dilution Higher No. (%)	Percent Different (95% CI)	Trending Noted
Manual/VITEK 2	*Staphylococcus aureus*	73	43, (58.9)	27	3, (4.11)	-55% (41-66)	Yes
Manual/VITEK 2 Compact	*Staphylococcus aureus*	71	44, (61.67)	26	1, (1.41)	-61% (47-71)	Yes

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 recognized susceptibility interpretive criteria for Lefamulin are listed in **Table 6**.

.

Table 6. FDA Recognized Interpretive Criteria for Lefamulin[a]

Organism	Minimum Inhibitory Concentration (µg/mL)		
	S	I	R
Staphylococcus aureus (methicillin-susceptible)	≤0.25	-	-

S = Susceptible; I = Intermediate; R = Resistant
[a]FDA STIC Website

The current absence of resistant isolates precludes defining any results other than "Susceptible". Isolates yielding MIC results other than “Susceptible” should be submitted to a reference laboratory for further testing.

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 incorporated by reference a breakpoint change protocol that was reviewed and accepted by the FDA in submission K230864 cleared on July 5, 2023. The referenced 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/development-resources/fda-recognized-antimicrobial-susceptibility-test-interpretive-criteria). The referenced protocol outlined the specific procedures and acceptance criteria that bioMérieux intends to use to evaluate the VITEK 2 AST-Gram Positive Lefamulin when revised breakpoints for Lefamulin 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 VITEK 2 AST-Gram Positive Lefamulin 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.