510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K163563

B. Purpose for Submission:

To obtain a substantial equivalence determination for Gentamicin for testing of gram negative bacilli on the VITEK® 2 and VITEK® 2 Compact Antimicrobial Susceptibility Test (AST) Systems

C. Measurand:

The VITEK® 2 AST-Gram Negative card contains the following concentrations of Gentamicin: 4, 8, and 32 µg/mL (equivalent standard method concentration by efficacy in µg/mL). The Gentamicin MIC reporting range for the card is ≤ 1 – ≥16 µg/mL.

D. Type of Test:

Automated quantitative or qualitative antimicrobial susceptibility test for Gentamicin

E. Applicant:

bioMérieux, Inc.

F. Proprietary and Established Names:

VITEK® 2 AST- GN Gentamicin (≤ 1 – ≥ 16 µg/mL)

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.1645: Fully Automated Short-Term Incubation Cycle Antimicrobial Susceptibility System

2. Classification:

   Class II

3. Product code(s):

   LON – Fully automated short-term incubation cycle antimicrobial susceptibility system
   LTW – Susceptibility Test Cards, Antimicrobial
   LTT – Panels, Test, Susceptibility, Antimicrobial

4. Panel:

   Microbiology (83)

H. Intended Use/Indications for Use:

1. Intended Use (s):

   The VITEK® 2 Antimicrobial Susceptibility Test (AST) is intended to be used with the VITEK® 2 Systems for the automated quantitative or qualitative susceptibility testing of isolated colonies for the most clinically significant aerobic gram-negative bacilli, *Staphylococcus* spp., *Enterococcus* spp., *Streptococcus* spp. and clinically significant yeast.

2. Indications for Use:

   VITEK® 2 AST-Gram Negative Gentamicin 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 Gentamicin is a quantitative test. Gentamicin has been shown to be active against most strains of the microorganisms listed below, according to the FDA label for this antimicrobial.

   Active *in vitro* and in clinical infections
   Citrobacter species
   Enterobacter species
   Escherichia coli
   Klebsiella species
   Proteus species
   Serratia species
   Pseudomonas aeruginosa

   The VITEK® 2 Antimicrobial Susceptibility Test (AST) is intended to be used with the VITEK® 2 Systems for the automated quantitative or qualitative susceptibility testing of isolated colonies for the most clinically significant aerobic gram-negative bacilli, *Staphylococcus* spp., *Enterococcus* spp., *Streptococcus* spp. and clinically significant yeast.

3. Special conditions for use statement(s):

For Prescription Use Only

Limitations:
"The ability of the VITEK 2 AST card to detect resistance with the following combination(s) is unknown because resistant strains were not available at the time of comparative testing:
Gentamicin: Proteus vulgaris"

"The ability of the VITEK 2 AST card to detect resistance with the following combination(s) is unknown because an insufficient number of resistant strains were available at the time of comparative testing:
Gentamicin: Citrobacter koseri, Enterobacter aerogenes, Enterobacter cloacae, and *Serratia marcescens"*

4. Special instrument requirements:

VITEK 2 and VITEK 2 Compact Systems using VITEK 2 Systems 8.01 software

I. 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 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 (up to 24 hours for *Streptococcus* species). 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 anti-microbial 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-GN Gentamicin has the following concentrations in the card: 4, 8, and 32 µg/mL (equivalent standard method concentration by efficacy in µg/mL). The MIC result range for the VITEK 2 is ≤ 1 – ≥ 16µg/mL.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   VITEK 2 AST-GN Doxycycline

2. Predicate 510(k) number(s):

   K121546

3. Comparison with predicate:

Table 1: Comparison with Predicate Device

Similarities		
Item	**Device: VITEK 2 AST-GN Gentamicin (K163563)**	**Predicate Device: VITEK 2 AST-GN Doxycycline (K121546)**
Intended Use	The VITEK 2 Antimicrobial Susceptibility Test (AST) is intended to be used with the VITEK 2 Systems for the automated quantitative or qualitative susceptibility testing of isolated colonies for the most clinically significant aerobic gram-negative bacilli, *Staphylococcus* spp,, *Enterococcus* spp., *Streptococcus* spp. and clinically significant yeast.	Same
Test Method	Automated quantitative antimicrobial susceptibility test for use with the VITEK 2 and VITEK 2 Compact Systems to determine the *in vitro* susceptibility of Gram negative bacilli	Same
Inoculum	Standardized saline suspension of test organism	Same
Test Card	VITEK 2 Gram Negative Susceptibility Test Card	Same
Instrument	VITEK 2 and VITEK 2 Compact Systems	Same

Differences		
Item	**Device: VITEK 2 AST-GN Gentamicin (K163563)**	**Predicate Device: VITEK 2 AST-GN Doxycycline (K121546)**
Antimicrobial Agent	Gentamicin	Doxycycline
Antimicrobial Concentrations	4, 8, 32 µg/mL	1, 4, 16 µg/mL
Reporting Range	≤ 1 - ≥ 16 µg/mL	≤ 0.5 - ≥ 16 µg/mL
Analysis Algorithm	Unique for gentamicin (Growth pattern analysis)	Unique for doxycycline (Discriminate analysis)

K. Standard/Guidance Documents 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-A9, "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard-Ninth Edition" Vol. 32 No. 2 (January 2012)

- CLSI M100-S24, "Performance Standards for Antimicrobial Susceptibility Testing"; Twenty-fourth Informational Supplement, Vol. 33 No. 1 (January 2014)

L. Test Principle:

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 uses 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 measures 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   A reproducibility study for the VITEK 2 AST-GN card with Gentamicin was conducted at three clinical sites using ten isolates of gram-negative bacilli consistent with the Intended Use. Testing was performed on three separate days and in triplicate for a total of 270 data points. The isolates tested in the reproducibility study included *K. pneumoniae pneumoniae* (five isolates), *Citrobacter braakii* (two isolates), *Citrobacter freundii* (one isolate), and *Pseudomonas aeruginosa* (two isolates).

Inocula were prepared using both the auto-dilution and manual dilution methods for testing in the VITEK 2 System. Inocula were prepared by the manual dilution method only for use with the VITEK 2 Compact. The mode MIC value was determined and the reproducibility was calculated based on MIC values that fell within +/- one doubling dilution from the mode MIC value.

For VITEK 2 auto-dilution and manual dilution methods, overall reproducibility was 100% for best- and worst-case scenarios.

For the VITEK 2 Compact manual dilution method, overall reproducibility was 100% for best- and worst-case scenarios.

The combined reproducibility results for all three sites were acceptable.

b. Linearity/assay reportable range:

Not applicable

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

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.

Growth Failure Rate
There was one isolate that failed to grow in the VITEK 2 card in the clinical study. Complete test results were available for 872 isolates in a total of 873 clinical isolates. The growth failure rate was 0.1% and was acceptable.

All 75 challenge organisms grew in the VITEK 2 GN card with Gentamicin using both the auto-dilution and manual dilution methods for the VITEK 2 and manual inoculation for the VITEK 2 Compact System.

A total of 947 VITEK 2 AST results were available.

Quality Control (QC) Testing
The FDA/CLSI recommended QC organisms (*E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) were tested using both the VITEK 2 card and the reference method at each site. 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.

Both the auto-dilution and the manual dilution methods for VITEK 2 and the manual dilution for VITEK 2 Compact were within the expected range >95% of the time.

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

Gentamicin		**VITEK 2 Auto-Dilution**		**VITEK 2 Manual Dilution**		**VITEK 2 Compact Manual Dilution**	
Organism	**Conc. (µg/mL)**	**Reference**	**Test**	**Reference**	**Test**	**Reference**	**Test**
E. coli ATCC 25922 Expected Range 0.25 – 1 µg/mL (VITEK 2: ≤1µg/mL)	≤0.0625						
	0.125						
	0.25						
	0.5	214		105		105	
	(≤)1*	11	225	7	112	7	112
	2						
	4						
	8						
	16						
	32						
	≥64						
P. aeruginosa ATCC 27853 Expected Range 0.5 – 2 µg/mL (VITEK 2: ≤1- 2µg/mL)	≤0.0625						
	0.125						
	0.25						
	0.5	35		17		17	
	≤1*	180	216	85	103	85	102
	2	5		5		5	1
	4						
	8		4		4		4
	16						
	32						
	≥64						

*The lowest dilution of the VITEK 2 Gentamicin MIC range is ≤1 µg/mL. Obtaining this value was considered an indicator that the quality control test results were acceptable.

The Gentamicin expected ranges for *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 are 0.25 – 1 and 0.5 - 2µg/mL respectively. However, the VITEK 2 MIC reporting range is ≤1 – ≥16 µg/mL (MIC results: ≤1, 2, 4, 8, ≥16 µg/mL). The VITEK 2 systems do not provide results lower than 1 µg/mL. Therefore, all results for *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were off scale.

A MIC value of ≤1 µg/mL indicated that the quality control test results were acceptable.

bioMérieux included the following footnote to the QC table in the device labeling:

"The VITEK 2 Gram Negative Gentamicin does not include the full CLSI/FDA-recommended dilution ranges for QC testing with E. coli ATCC 25922 and P. aeruginosa ATCC 27853".

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:

Results obtained with the bioMérieux VITEK 2 AST-Gram Negative card with Gentamicin were compared to results obtained with the CLSI broth microdilution reference panel. The following concentrations of Gentamicin are contained in the VITEK 2 AST-GN test card: 4, 8, and 32 µg/mL (equivalent standard method concentration by efficacy in µg/mL) and the reporting range is ≤ 1 – ≥ 16 µg/mL (i.e., ≤1, 2, 4, 8, and ≥16). The reference panel contained two-fold serial dilutions with a range of ≤ 0.0625 to ≥128 µg/mL. The testing conditions for the reference method consisted of the following:

- Medium – Mueller Hinton broth with appropriate dilutions of antimicrobial solution added
- Inoculum – Direct colony suspension
- Incubation – 35°C ambient air incubator; 16-20 hours

All test inocula used for the evaluation of VITEK 2 AST-GN Gentamicin and the reference method were standardized using the DensiCHEK Plus instrument. The cards were inoculated with each test organism by auto-dilution for reading by the VITEK 2 System and by manual dilution for reading on the VITEK 2 and VITEK 2 Compact Systems. Reference broth microdilution panels were inoculated in adherence with CLSI document, M07-A9.

A total of 872 clinical isolates were evaluated at three sites with VITEK 2 AST – Gram Negative cards inoculated by automatic dilution and interpreted using the VITEK 2 instrument. The majority of isolates were fresh (760 isolates, 87.1%); 113 isolates (13%) were stock isolates.

A total of 75 challenge organisms (51 *Enterobacteriaceae* and 26 *P. aeruginosa*) were evaluated at one site. The challenge set was tested with both the auto-dilution and manual dilution options of the VITEK 2 System and with the manual dilution method on the VITEK 2 Compact System. Overall performance of the challenge organisms is shown in Table 2.

Table 2: Performance of Clinical and Challenge Isolates, VITEK 2 Auto-Dilution Method‡

Organism Group	EA Total	EA N	EA %	Eval EA Total	Eval EA N	Eval EA %	CA N	CA %	#R	Min	Maj	Vmj
***Enterobacteriaceae* ≤4 (Susceptible), 8 (Intermediate), ≥16 (Resistant)**												
Clinical	725	720	99.3	16	11	68.8	719	99.2	44	5	0	1
Challenge	51	51	100	4	4	100	50	98.0	10	1	0	0
Combined	776	771	99.4	20	15	75.0	769	99.1	54	6	0	1
***Pseudomonas aeruginosa* ≤4 (Susceptible), 8 (Intermediate), ≥16 (Resistant)**												
Clinical	147	146	99.3	46	45	97.8	141	95.9	12	6	0	0
Challenge	24	24	100	6	6	100	24	100	9	0	0	0
Combined	171	170	99.4	52	51	98.1	165	96.5	21	6	0	0
Enterobacteriaceae* + *Pseudomonas aeruginosa												
Clinical	872	866	99.3	62	56	90.3	860	98.6	56	11	0	1
Challenge	75	75	100	10	10	100	74	98.7	19	1	0	0
Combined	947	941	99.4	72	66	91.7	934	98.6	75	12	0	1

‡**EA** – Essential Agreement (+/- 1 doubling dilution)
CA – Category Agreement
EVAL – Evaluable isolates
R – Resistant isolates
Min – Minor discrepancies
Maj – Major discrepancies
Vmj – Very major discrepancies

Essential Agreement (EA) occurs when there is agreement between the result of the reference method and that of 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. 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 using the auto-dilution method of the VITEK 2 System demonstrated an essential agreement of 99.4% and an overall category agreement of 98.6%. There was one very major (1.3%), 12 minor (1.3%) and no major discrepancies. The Vmj was caused by *K. pneumoniae*.

The performance based on combined clinical and challenge data was acceptable.

There were no resistant *Proteus vulgaris* isolates tested in the studies. A limitation was included in the package insert:
"The ability of the VITEK 2 AST card to detect resistance with the following combination(s) is unknown because resistant strains were not available at the time of comparative testing:
Gentamicin: Proteus vulgaris"

In addition, an insufficient number of resistant isolates were tested during the comparative study for the following organisms: *Citrobacter koseri* (1), *Enterobacter aerogenes* (1)*, Enterobacter cloacae* (2)*,* and *Serratia marcescens* (1). This was addressed by adding the following limitation in the package insert:
"The ability of the VITEK 2 AST card to detect resistance with the following combination(s) is unknown because an insufficient number of resistant strains were

available at the time of comparative testing:
Gentamicin: Citrobacter koseri, Enterobacter aerogenes, Enterobacter cloacae, and *Serratia marcescens"*

Challenge Data – Auto and Manual Dilution:
The challenge set of 75 isolates (51 *Enterobacteriaceae* and 24 *Pseudomonas aeruginosa*) was evaluated on the VITEK 2 System and the VITEK 2 Compact System with the manual dilution method only.

Overall performance is shown in Table 3.

Table 3: Performance of Challenge Isolates, VITEK 2 and VITEK 2 Compact-Manual Dilution

Organism Group	EA Total	EA N	EA %	Eval EA Total	Eval EA N	Eval EA %	CA N	CA %	#R	Min	Maj	Vmj
VITEK 2 System												
Auto-dilution	75	75	100	10	10	100	74	98.7	19	1	0	0
Manual dilution	75	75	100	10	10	100	74	98.7	19	1	0	0
VITEK 2 Compact												
Manual dilution	75	75	100	10	10	100	74	98.7	19	1	0	0

The performance of the challenge isolates using the VITEK 2 Gentamicin test on both the VITEK 2 and VITEK 2 Compact Systems was acceptable.

MIC Trends:
The claimed *Enterobacteriaceae* organisms were also evaluated for trending. This trending calculation takes into account MIC values that are determined to be ≤1 and ≥1 doubling dilutions compared to the reference method irrespective whether the device MIC values are on-scale or not. The analysis showed trending was observed for *Proteus* spp. and *K. pneumoniae.* The trending analysis is shown in Tables 4.1 and 4.2:

Table 4.1: Trending Analysis of Evaluable Clinical and Challenge Results for *Proteus* spp.

Gentamicin ≤1 - ≥16µg/mL	Total[a] (Total # Tested)	Difference in MIC as Compared to the CLSI Reference Method				
		≤2 dil. lower	1 dil. lower	Exact	1 dil. higher	≥2 dil. higher
Proteus spp.	13 (90)	1	9	1 (7.69%)	2	0
		10 (76.92%)[b] 95% CI (49.74 to 91.82%)			2 (15.38%)[b] 95% CI (4.32% to 42.23%)	

[a] Total number of evaluable results for trending analysis
[b] Difference between isolates trending lower and isolates trending higher: 61.54%; 95% CI (23.33% to 80.10%)

A lower MIC result trend was observed in with *Proteus* spp. compared to the CLSI broth microdilution reference method and there are concerns for potential very major

discrepancies. This trending and the potential for occurrence of very major discrepancies for Gentamicin when testing clinical and challenge isolate results with the VITEK 2 GN Gentamicin was addressed by adding the following footnote to the performance table for Gentamicin in the device labeling (Table 127: Performance Characteristics for Gram-Negative Antimicrobial Susceptibility Testing, VITEK 2 Product Information Manual).

Footnote:
"*Of 90 Proteus spp. isolates tested, 13 were evaluable for trending analysis. Based on this analysis, some VITEK 2 Gentamicin MIC values tended to be at least one doubling dilution lower when compared to the reference broth microdilution.*"

Table 4.2: Trending Analysis of Evaluable Clinical and Challenge Results for *K. pneumoniae*

Gentamicin ≤1 - ≥16µg/mL	**Total[a] (Total # Tested)**	**Difference in MIC as Compared to the CLSI Reference Method**				
		≤2 dil. lower	**1 dil. lower**	**Exact**	**1 dil. higher**	**≥2 dil. higher**
K. pneumoniae	13 (163)	1	2	1 (7.69%)	7	2
		3 (23.08%)[b] 95% CI (8.18% to 50.26%)			9 (69.23%)[b] 95% CI (42.37% to 87.32%)	

[a] Total number of evaluable results for trending analysis
[b] Difference between isolates trending lower and isolates trending higher: -46.15%; 95% CI (-69.59% to -7.94%)

A higher MIC result trend was observed with *K. pneumoniae* compared to the CLSI broth microdilution reference method and there are concerns for potential major discrepancies. This trending and the potential for occurrence of major discrepancies for Gentamicin when testing clinical and challenge isolate results with the VITEK 2 GN Gentamicin was addressed by adding the following footnote to the performance table for Gentamicin in the device labeling (Table 127: Performance Characteristics for Gram-Negative Antimicrobial Susceptibility Testing, VITEK 2 Product Information Manual).

Footnote:
"*Of 163 K. pneumoniae isolates tested, 13 were evaluable for trending analysis. Based on this analysis, some VITEK 2 Gentamicin MIC values tended to be at least one doubling dilution higher when compared to the reference broth microdilution.*"

The analysis of the *Pseudomonas aeruginosa* MIC data did not demonstrate notable trending.

b. Matrix comparison:

Not applicable

3. Clinical studies:

   Not applicable

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:

Table 5: Interpretive Criteria for Gentamicin (FDA Drug Label)

Organism	FDA Interpretive Criteria for Gentamicin MIC (µg/mL)		
	S	**I**	**R**
Enterobacteriaceae	≤4	8	≥16
Pseudomonas aeruginosa	≤4	8	≥16

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.