510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K162737

B. Purpose for Submission:

To obtain a substantial equivalence determination for Ciprofloxacin 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 Ciprofloxacin: 0.06, 0.12, 0.5, and 1µg/mL (equivalent standard method concentration by efficacy in µg/mL). The MIC result reporting range for the card is ≤0.06-≥4 µg/mL

D. Type of Test:

Automated quantitative or qualitative antimicrobial susceptibility test for Ciprofloxacin

E. Applicant:

bioMérieux, Inc.

F. Proprietary and Established Names:

VITEK 2 AST- GN Ciprofloxacin (≤0.06 - ≥4 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:

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

4. Panel:

   83 Microbiology

H. Intended 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. Indication(s) for use:

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

   Active *in vitro* and in clinical infections:
   - *Citrobacter freundii*
   - *Citrobacter koseri* (*diversus*)
   - *Enterobacter cloacae*
   - *Escherichia coli*
   - *Klebsiella pneumoniae*
   - *Morganella morganii*
   - *Proteus mirabilis*
   - *Proteus vulgaris*
   - *Providencia rettgeri*
   - *Providencia stuartii*
   - *Pseudomonas aeruginosa*
   - *Salmonella typhi*
   - *Serratia marcescens*
   - *Shigella sonnei*

   In vitro data available but clinical significance is unknown:
   - *Enterobacter aerogenes*

Klebsiella oxytoca
Salmonella enteritidis

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):

   Prescription use only

   Limitation:

   "The ability of the AST card to detect resistance with the following combination (s) is unknown because resitant strains were not available at the time of comparative testing:
   - *Ciprofloxacin: Enterobacter cloacae, Providencia rettgeri, Salmonella enteritidis, Salmonella typhi and Shigella sonnei".*

4. Special instrument requirements:

   VITEK 2 and VITEK 2 Compact Systems

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 test card contains 64 microwells. A control well containing only culture medium is included on all cards, with the remaining wells containing premeasured amounts of a specific antimicrobial agent in a culture medium base. A suspension of organism from a pure culture is prepared in a tube containing 0.45-0.5% sterile saline and standardized to a McFarland 0.5 using the DensiCHEK Plus. The VITEK 2 System automatically fills, seals and places the card into the incubator/reader; manual methods can also be used for the inoculation of test cards for use in the VITEK 2 System. The VITEK 2 Compact has a manual filling and sealing operation. The VITEK 2 Systems monitor the growth of each well in the card over a defined period of time (up to 18 hours). 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 Ciprofloxacin has the following concentrations in the card: 0.06, 0.12, 0.5, and 1µg/mL (equivalent standard method concentration by efficacy in µg/mL). The MIC result reporting range for the card is ≤0.06-≥4 µg/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 the Predicate Device

Similarities		
Item	Device VITEK 2 AST- GN Ciprofloxacin	Predicate 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	Saline suspension of organisms	Same
Test Card	VITEK2 Gram Negative Susceptibility Test Card	Same
Instrument	VITEK2 and VITEK2 Compact Systems	Same

Differences		
Item	Device	Predicate
Antimicrobial	Ciprofloxacin	Doxycycline
Antimicrobial Concentration	0.06, 0.12, 0.5, and 1	1, 4, and 16
Reading Algorithm	Growth pattern analysis-Unique to Ciprofloxacin	Discriminate analysis-Unique to Doxycycline
Reporting Range	≤0.06-≥4 µg/mL	≤0.5-≥16

K. Standard/Guidance Document Referenced (if applicable):

- CLSI M100-S24: Performance Standards for Antimicrobial Susceptibility Testing;

Twenty-fourth Informational Supplement, Vol. 34 No. 1 (January 2014)

- CLSI M07-A9: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Ninth Edition" Vol. 32 No, 2 (January 2012)
- Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems; Guidance for Industry and FDA (Issued August 28, 2009)

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 used in the systems use visible light to directly measure organism growth. Transmittance optics are based on an initial light reading of a well before significant growth has begun. Periodic light transmittance samplings of the same well measure organism growth by how much light is prevented from going through the well. The VITEK 2 System monitors the growth of each well in the card over a defined period of time. An interpretive call is made between 4 and 16 hours for a "rapid" read but may be extended to 18 hours in some instances. At the completion of the incubation cycle, a report is generated that contains the MIC value along with the interpretive category result for each antibiotic on the card.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A reproducibility study was conducted at three sites using ten isolates of gram negative bacilli that were consistent with the intended use. Isolates were tested in triplicates over three days for a total of 270 data points. The isolates tested in the reproducibility study included *Klebsiella pneumoniae* (one isolate), *Pseudomonas aeruginosa* (three isolates), *Citrobacter freundii* (one isolate), *Enterobacter aerogenes* (one isolate), *Providencia rettgeri* (one isolate), *Serratia marcescens* (two isolates), and *E. coli* (one isolate). Inocula were prepared using both manual and automatic dilution methods for testing in the VITEK 2. Inocula were prepared manually for testing in the VITEK 2 Compact. The mode MIC value was determined and the reproducibility was calculated based on MIC values falling within ± 1 dilution of the mode MIC value.

Using VITEK 2 and automatic dilution, all results were on scale.The reproducibility was 100%.

Using VITEK 2 and manual dilution, there was one off-scale result. The best case reproducibility was 98.88% and the worst case reproducibilily was 98.52%.

Using VITEK 2 Compact and manual dilution, there was one off-scale result. The best case reproducibility was 99.62% and the worst case reproducibility was 99.26%

The reproducibility results were acceptable.

b. Linearity/assay reportable range:

Not Applicable

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

Inoculum Density Check:
The inoculum density was monitored using the DensiCHEK Plus™ instrument. The DensiCHEK Plus™ was standardized daily with all results recorded and within expected range.

Purity Check:
A purity check of all organisms was performed at the time of VITEK2 card inoculation. Only results obtained with pure cultures were evaluated.

Quality Control Testing:
QC organisms recommended by both FDA and CLSI, namely *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were tested using both the VITEK 2 AST-GN Ciprofloxacin 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. A summary of QC results is presented in Table 2.

A second set of 20 quality control results was obtained during the additional testing required by the FDA. Testing was conducted at one internal site. Results are presented in Table 2a.

The expected range for *E. coli* ATCC 25922 with Ciprofloxacin is 0.004 – 0.015 µg/mL. Even though the Ciprofloxacin concentrations included in the VITEK 2 AST-Gram Negative card are 0.06, 0.12, 0.5, and 1 µg/mL, the reporting range is ≤0.06 - ≥4 µg/mL. Therefore, all results for the QC strain *E. coli* ATCC 25922 were off scale for the VITEK 2 and VITEK 2 Compact Systems as both VITEK systems report the lowest end of the scale as ≤0.06 µg/mL. The MIC range of the reference test panel was 0.008 to 8 µg/mL and also did not include the low end of the concentration range for this strain (0.004 µg/mL). However, *Pseudomonas aeruginosa* ATCC 27853 was also tested to verify the performance of the device and all results were on-scale since the reporting range on the card and on the concenrations on the reference panel cover the expected range for this organism.

The quality control results are acceptable.

Table 2: Quality Control Results for VITEK 2 (Automatic and Manual Dilution Methods) and for VITEK 2 Compact (Manual Dilution Method) (First Set)

		VITEK 2 Automatic-Dilution		VITEK 2 Manual Dilution		VITEK 2 Compact Manual Dilution	
Organism	**Conc. (µg/mL)**	**Test**	**Ref.**	**Test**	**Ref.**	**Test**	**Ref.**
*E. coli (*ATCC 25922) Expected Range: 0.004-0.015 µg/mL							
	≤0.008		200		102		102
	0.015		21		7		7
	0.03						
	≤0.06	222		109		109	
	0.125						
	0.25		1				
	0.5						
	1						
	2						
	4						
	≥8						
P. aeruginosa (ATCC 27853) Expected Range: 0.25-1 µg/mL	≤0.008						
	0.015						
	0.03						
	≤0.06	1					
	0.125	1	1		1		1
	0.25	18	98	9	52	13	52
	0.5	200	119	98	52	93	52
	1		2		2	1	2
	2						
	4						
	≥8						

Table 2a. Quality Control Data for Ciprofloxacin VITEK 2 Automatic-dilution (Second Set)

		VITEK 2 Automatic-Dilution	
Organism	**Conc. (µg/mL)**	**Test**	**Ref.**
*E. coli (*ATCC 25922) Expected Range: 0.004-0.015 µg/mL			
	≤0.008		8
	0.015		12
	0.03		
	≤0.06	20	
	0.125		
	0.25		
	0.5		
	1		
	2		
	4		
	≥8		
P. aeruginosa (ATCC 27853)	≤0.008		
	0.015		

		VITEK 2 Automatic-Dilution	
Expected Range: 0.25-1 µg/mL	0.03		
	≤0.06		
	0.125		
	0.25	16	14
	0.5	4	6
	1		
	2		
	4		
	≥8		

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 Ciprofloxacin were compared to results obtained with the CLSI broth microdilution reference panel. The VITEK 2 AST-Gram Negative card with Ciprofloxacin contains the following concentrations of Ciprofloxacin: 0.06, 0.12, 0.5, and 1 µg/mL (equivalent standard method concentration by efficacy in µg/mL). The MIC results range for the VITEK 2 card is the reporting range is ≤0.06 - ≥4 µg/mL (seven dilutions). The reference panel contained two-fold serial dilutions with a range of 0.0078 to 16 µg/mL (12 dilutions). The testing conditions for the reference method were:

- Medium: Mueller Hinton broth (Cation-Adjusted) with the appropriate dilutions of antimicrobial solution added
- Inoculum: Direct colony suspension
- Incubation: 35^0C; 16- 20 hours

Test inocula were standardized using the DensiCHEK Plus instrument. VITEK 2 AST – Gram Negative cards were inoculated using automatic dilution (for reading on the VITEK 2 instrument) or using a manual dilution method (for reading on the VITEK 2 instrument or on the VITEK 2 COMPACT instrument). Reference panels were inoculated as outlined in the CLSI document M07-A9.

Clinical

A total of 982 clinical isolates were evaluated at three external sites with VITEK 2 AST – Gram Negative cards inoculated by automatic dilution and interpreted using the VITEK 2 instrument. There were four isolates that failed to grow (99.6% growth rate). The results for 978 clinical isolates are provided in Tables 3-5.

The majority of isolates were fresh (808 isolates, 82.3%); 174 isolates (17.7%) were stock isolates. There were 832 *Enterobacteriaceae* including 11 *Salmonella* spp, and 146 *Pseudomonas aeruginosa* isolates tested in the clinical studies.

Challenge

A total of 82 challenge isolates were evaluated at one external site. Per FDA's request an additional set of 88 challenge isolates was tested at an internal site.The challenge sets were tested with both automatic and manual dilution methods on the VITEK 2 System and with the manual dilution method on the VITEK2 Compact System. One isolate failed to grow (99.4% growth rate) during the additional testing. The results of 169 challenge isolates (79 *Enterobacteriaceae* including 10 *Salmonella* spp, and 90 *Pseudomonas aeruginosa*) are provided in Tables 3-5.

Table 3:Overall Performance, All Organisms, VITEK2 Automatic Dilution Method

	Tot	No. EA	EA %	Eval Tot	No. Eval EA	Eval EA %	No. CA	CA %	No. R	min	maj	vmj
Clinical	978	946	96.7	197	165	83.8	948	96.9	137	27	2	1
Challenge	169	168	99.4	62	62	100.0	166	98.2	85	3	0	0
Combined	1147*	1114	97.0	259	227	87.6	1114	97.1	222	30	2	1

EA – Essential Agreement (+/- 2 dilutions)
CA – Category Agreement
EVAL – Evaluable isolates
R or NS – Resistant or non-susceptible 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.

*911 *Enterobacteriaceae* (inclusive of 21 *Salmonella* species) and 236 *Pseudomonas aeruginosa*

Table 3A: Performance of Clinical and Challenge Isolates, VITEK 2 Auto-Dilution

	EA Tot	EA N	EA %	Eval EA Tot	Eval EA N	Eval EA %	CA N	CA %	#R	min	maj	vmj
***Enterobacteriaceae* without *Salmonella* spp**												
Clinical	821	803	97.8	98	80	81.6	803	97.8	110	17	1	0
Challenge	69	69	100	37	37	100	68	98.5	19	1	0	0
Combined	890	872	97.9	135	117	86.6	871	97.8	129	18	1	0
Salmonella spp. (S. typhi and S. enteritidis) [a]												
Clinical	11	11	100	4	4	100	11	100	3	0	0	0
Challenge	10	10	100	2	2	100	10	100	0	0	0	0
Combined	21	21	100	6	6	100	21	100	3	0	0	0
Pseudomonas aeruginosa												
Clinical	146	132	90.4	95	81	85.3	134	91.8	24	10	1	1
Challenge	90	89	98.9	23	23	100	88	97.8	66	2	0	0
Combined	236	221	93.6	118	104	88.1	222	94.1	90	12	1	1

[a] For this analysis the *Salmonella typhi* FDA breakpoints were applied for the analysis of 12 *Salmonella enteritidis*.

Enterobacteriaceae without Salmonella spp

The performance of *Enterobacteriaceae* (Table 3A) was acceptable at 97.9% EA and 97.9% CA, minor discrepancy rate of 2% (18/890), major discrepancy rate of 0.1% (1/759), and no very major discrepancy.

Salmonella spp.

The performance of 21 *Salmonella* spp. (12 *S. enteretidis* and 9 *S. typhi*) was analyzed separately because *Salmonella typhi* breakpoints are different from those for *Enterobacteriaceae*. The performance was acceptable at 100% EA and CA. There were no discrepancies.

Pseudomonas aeruginosa

The performance of *Pseudomonas aeruginosa* (Table 3A) was acceptable at 93.6% EA and, 94.1% CA, minor discrepancy rate of 5.1% (12/236), one major discrepancy rate of 0.7% (1/137) and a very major discrepancy rate of 1.1% (1/90).

Table 4: Performance of Challenge Isolates, All Organisms, VITEK 2 Manual Dilution Method

	Tot	No. EA	EA %	Eval Tot	No. Eval EA	Eval EA %	No. CA	CA %	No. R	min	maj	vmj
Challenge	169	169	100	63	63	100	168	99.4	85	1	0	0

Table 5 : Performance of Challenge Isolates, All Organisms, VITEK 2 Compact, Manual Dilution Method

	Tot	No. EA	EA %	Eval Tot	No. Eval EA	Eval EA %	No. CA	CA %	No. R	min	maj	vmj
Challenge	169	166	98.2	62	60	96.8	166	98.2	85	3	0	0

Challenge-VITEK 2 Compact
The overall performance of the VITEK 2 Compact (manual dilution) was considered acceptable based on the reproducibility (99.26%), QC (Table 2), and challenge (Table 5) studies.

Resistant Organisms:
A total of 222 resistant isolates were identified out of 1147 organisms tested (19.3%) were found to be resitant to Ciprofloxacin by the reference method. However, the following indicated organisms had no resistant isolates and/or had an insufficient number of isolates around the breakpoint available during comparative testing : *Enterobacter cloacae, Providencia rettgeri, Salmonella enteridis,Salmonella typhi and Shigella sonnei*. This was addressed by adding the following limitation in the labeling:

"The ability of the AST card to detect resistance with the following combination (s) is unknown because resitant strains were not available at the time of comparative testing:

- *Ciprofloxacin: Enterobacter cloacae, Providencia rettgeri, Salmonella enteridis,Salmonella typhi and Shigella sonnei".*

MIC Trends:
Using the combined clinical and challenge data for *Enterobacteriaceae* and *P. aeruginosa* an analysis of trending was conducted. This trending calculation takes into account MIC values that are determined to be one or more doubling dilution lower or higher compared to the reference method irrespective whether the device MIC values are on-scale or not. The combined data of149 for *P. aeruginosa* and 178 for the *Enterobacteriaceae* group constitute the evaluable data for trend analysis which is presented in Table 6.

Table 6. Trending of Clinical and Challenge Isolate Results for the VITEK 2, Automatic Dilution

	No. Eval Isolates for Trending	Difference in MIC as Compared to the CLSI Reference Method				
		≥2 dil. lower	1 dil. Lower	Exact	1 dil. higher	≥ 2 dil. higher
P. aeruginosa [a]	149	4	26	45 (30.2%)	63	11
		30 (20.13%)			74 **(49.66%)**	
Enterobacteriaceae [b]	178	4	42	54 (30.33%)	64	14
		46 (25.84%)			78 **(43.82%)**	

[a] Difference between the higher dilutions and the lower dilutions for *P. aeruginosa* is: 29.53%; 95% CI (18.86% 39.27%)
[b] Difference between the higher dilutions and the lower dilutions for Enterobacteriaceae is : 17.98%; 95% CI (8.09% 27. 38%)
Note: A positive percent difference value indicates higher MIC when compared to the reference method; A negative percent difference value indicates lower MIC when compared to the reference method.

A higher reading trend was observed for both *Enterobacteriaceae and Pseudomonas aeruginosa* compared to the CLSI broth microdilution method, and was found to be significant which raises concerns for potential major errors as summarized in Table 6.

The high trending and the potential for occurrence of major error(s) for Ciprofloxacin when testing clinical and challenge isolate results with VITEK 2 system, was addressed in the labeling by adding the following footnote:

"*VITEK 2 system Ciprofloxacin MIC values for all organisms tended to be one or more doubling dilution higher compared to reference broth microdilution*".

b. Matrix comparison:

Not Applicable

3. Clinical studies:

a. Clinical Sensitivity:

Not Applicable

b. Clinical specificity:

Not Applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not Applicable

4. Clinical cut-off:

Not Applicable

5. Expected values/Reference range:

Table 6: Breakpoints for Ciprofloxacin (FDA Approved labeling)

Organism	Ciprofloxacin FDA MIC Breakpoints (µg/mL)		
	S	I	R
Enterobacteriaceae	≤1	2	4≥
Pseudomonas aeruginosa			
Salmonella typhi[a]	≤0.06	0.12-0.5	≥1

[a] *Salmonella typhi* FDA breakpoints were applied to analyze *Salmonella enteritidis*

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.