510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K172150

B. Purpose for Submission:

To obtain a substantial equivalence determination for ceftazidime/avibactam at concentrations of 0.016 – 256 µg/mL for susceptibility testing of gram negative aerobic microorganisms with Etest.

C. Measurand:

Ceftazidime/avibactam 0.016 – 256 µg/mL. The avibactam concentration is fixed at 4 µg/mL in this combination.

D. Type of Test:

Quantitative AST growth-based detection

E. Applicant:

bioMérieux, Inc.

F. Proprietary and Established Names:

Etest Ceftazidime/Avibactam (CZA) (0.016 – 256 µg/mL)

G. Regulatory Information:

1. Regulation section:

   866.1640 Antimicrobial Susceptibility Test Powder

2. Classification:

   II

3. Product code:

   JWY – Manual Antimicrobial Test Systems

4. Panel:

83 – Microbiology

H. Intended Use:

1. Intended use(s):

Etest is a quantitative technique for determination of antimicrobial susceptibility of both non-fastidious Gram-negative and Gram-positive aerobic bacteria such as *Enterobacteriaceae, Pseudomonas, Staphylococcus*, and *Enterococcus* species and fastidious bacteria, such as anaerobes, *N. gonorrhoeae, S. pneumoniae, Streptococcus* and *Haemophilus* species. 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 as tested on agar media using overnight incubation.

Ceftazidime/avibactam has been shown to be active against the Gram negative aerobic microorganisms listed below, according to the FDA label for this antimicrobial agent. Etest CZA can be used to determine the MIC of ceftazidime/avibactam against the microorganisms listed below:

Active both in vitro and in clinical infections:
Citrobacter freundii
Enterobacter cloacae
Escherichia coli
Klebsiella oxytoca
Klebsiella pneumoniae
Proteus mirabilis
Pseudomonas aeruginosa

The following in vitro data are available, but clinical significance is unknown:
Citrobacter koseri
Enterobacter aerogenes
Morganella morganii
Providencia rettgeri
Providencia stuartii
Serratia marcescens

2. Indication(s) for use:

Same as the Intended Use

3. Special conditions for use statement(s):

For prescription use only

Limitations:

Due to the lack of an intermediate interpretive category for ceftazidime/avibactam, testing of Morganella morganii with this drug has resulted in very major discrepancies for isolates that are otherwise within essential agreement of the reference method. Testing should be repeated using an alternative testing/reference method prior to reporting results for M. morganii when the Etest MIC is 8 µg/mL.

The ability of Etest to detect resistance with the following microorganisms is unknown because resistant strains were not available at the time of comparative testing. If such a strain is observed, it should be submitted to a reference laboratory for further testing Ceftazidime/Avibactam: Citrobacter koseri

4. Special instrument requirements:

   N/A

I. Device Description:

Etest consists of a thin, inert and non-porous plastic strip 5mm wide and 60 mm long. One side of the strip carries a two letter code designating the identity of the antibiotic and is calibrated with MIC values in terms of µg/mL. On the reverse, a predefined exponential gradient of the dried and stabilized antibiotic covers a continuous concentration range across 15 two-fold dilutions of a conventional MIC method.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Etest Ceftolozane/Tazobactam

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

   K170670

3. Comparison with predicate:

Table 1. Comparison with the Predicate Device

Similarities		
Item	**Device**	**Predicate**
	K172150 **Etest** **Ceftazidime/Avibactam**	**K170670** **Etest** **Ceftolozane/Tazobactam**
Intended Use	Etest is a quantitative technique for determination of antimicrobial susceptibility of both non-fastidious Gram-negative and Gram-positive aerobic bacteria such as *Enterobacteriaceae*, *Pseudomonas*, *Staphylococcus*, and *Enterococcus* species and fastidious bacteria, such as anaerobes, *N. gonorrhoeae*, *S. pneumoniae*, *Streptococcus* and *Haemophilus* species. 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 microorganism as tested on agar media using overnight incubation.	Same
Antimicrobial Concentration Range	0.016 – 256 µg/mL (Avibactam: fixed at 4 µg/mL)	Same (Tazobactam: fixed at 4 µg/mL)
Test Design	A 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
Inoculation	Isolated colonies from culture	Same
Incubation	35°± 2° C for 16–20 hours	Same
Result	MIC	Same

Differences		
Item	**Device**	**Predicate**
Claimed organisms	*Citrobacter freundii* *Citrobacter koseri* *Enterobacter aerogenes* *Enterobacter cloacae* *Escherichia coli* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Morganella morganii* *Proteus mirabilis* *Providencia rettgeri* *Providencia stuartii* *Pseudomonas aeruginosa* *Serratia marcescens*	*Citrobacter koseri* *Enterobacter cloacae* *Escherichia coli* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Morganella morganii* *Proteus mirabilis* *Proteus vulgaris* *Providencia rettgeri* *Providencia stuartii* *Pseudomonas aeruginosa* *Serratia liquifaciens* *Serratia marcescens*
Antimicrobial Agent	Ceftazidime/Avibactam	Ceftolozane/Tazobactam

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

Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems; Guidance for Industry and FDA

CLSI M07-A10, Method for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard, January 2015.

CLSI M100-S26, Performance Standards for Antimicrobial Susceptibility Testing; Volume 36, No. 1, January 2016.

L. Test Principle:

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 gradient of antibiotic concentrations.

When the 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.

The MIC gradient on Etest Ceftazidime/Avibactam ranges from 0.016 to 256 µg/mL. The

avibactam concentration is fixed at 4 µg/mL in this combination.

M. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

A reproducibility study was conducted at three external sites using 25 isolates of gram negative bacilli that were consistent with the intended use. The isolates tested included *Citrobacter freundii* (two isolates), *Citrobacter koseri* (two isolates), *Enterobacter aerogenes* (two isolates), *Enterobacter cloacae* (two isolates), *Escherichia coli* (five isolates), *Klebsiella oxytoca* (three isolates), *Klebsiella pneumoniae* (three isolates) and *Pseudomonas aeruginosa* (six isolates).

All results were within ± 1 doubling dilution of the mode MIC value for ceftazidime/avibactam. Eleven results were on scale. The reproducibility was acceptable at 100.0%.

b. Linearity/assay reportable range:

Not applicable

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

Inoculum Density Check. Inoculum density checks were performed for all quality control and reproducibility organism suspensions and for 10% of the suspensions prepared for susceptibility testing of the fresh clinical isolates. The mean inoculum density was acceptable at 4.40 X 10^5 CFU/mL.

Purity Check. All clinical, challenge and reproducibility test suspensions were subcultured to assure purity.

Growth or Device Failure. There were no growth or device failures during the course of the study.

Quality Control Testing. Organisms recommended by both FDA and the CLSI were tested with ceftazidime/avibactam at three sites. According to the CLSI M100 S27 document, *K. pneumoniae* ATCC 700603 must be used for routine QC testing of ceftazidime/avibactam. The QC organisms tested included: *E. coli* ATCC 25922, *E. coli* ATCC 35218, *K. pneumoniae* ATCC 700603 and *P. aeruginosa* ATCC 27853. These QC strains were tested a minimum of 20 times per site by both the Etest and the reference method. One quality control strain (*Staphylococcus aureus* ATCC 29213) was tested with the reference method only to provide additional control for this method; 100% of the results obtained with *S. aureus* ATCC 29213 were in the acceptable range. The results demonstrate that the ceftazidime/avibactam Etest can

produce quality control results in the recommended range > 95% of the time. See Table 2 below for a summary of the QC results.

Per the recommendations in the CLSI document M100, additional testing was performed using the reference method with *K. pneumoniae* 700603 with ceftazidime alone and *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 35218 with ampicillin to assure the integrity of the β-lactamase encoding plasmid. The testing demonstrated that the QC strains maintained their resistance profiles throughout the testing as evidenced by obtaining MIC values of 16-64 µg/mL with ceftazidime for *K. pneumoniae* 700603 and obtaining MIC values of >128 µg/mL with ampicillin for the same organism. MIC values of >32 µg/mL were obtained with ampicillin for *E. coli* ATCC 35218.

The sponsor included the following comment in the device labeling: *K. pneumoniae ATCC 700603 should be used for routine QC of ceftazidime/avibactam. This QC strain should also be tested against ceftazidime alone to ensure that the plasmid encoding the beta-lactamase has not been lost in this strain.*

Table 2. Quality Control Data for Ceftazidime/Avibactam

QC organism	Ceftazidime/ Avibactam expected range (µg/mL)	Concentration (µg/mL)	Reference	Etest
E. coli ATCC 25922	0.06 – 0.5	<0.06	-	-
		0.06	1	-
		0.125	74	71
		0.25	10	15
		0.5	1	-
		>0.5	-	-
P. aeruginosa ATCC 27853	0.5 - 4	<0.5	-	-
		0.5	-	-
		1	14	46
		2	71	40
		4	1	-
		>4	-	-
E. coli ATCC 35218	0.03 – 0.12	<0.03	-	-
		0.03	1	-
		0.06	77	76
		0.125	8	10
		>0.125	-	-
K. pneumoniae ATCC 700603	0.25 - 2	<0.25	-	-
		0.25	-	-
		0.5	50	1
		1	35	79

QC organism	Ceftazidime/ Avibactam expected range (µg/mL)	Concentration (µg/mL)	Reference	Etest
		2	1	6
		>2	-	-

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 Etest Ceftazidime/Avibactam were compared to results obtained with the CLSI broth microdilution reference panel. The CLSI panel was prepared and interpreted according to CLSI recommendations outlined in the CLSI Standard: CLSI Document M07-A10, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard – Tenth Edition, Vol. 35, No. 2; January 2015. The testing conditions for the reference method consisted of the following:

- Medium – Cation-adjusted Mueller Hinton Broth with appropriate dilutions of antimicrobial solution added
- Inoculum – Direct colony suspension to achieve a suspension equivalent to a 0.5 McFarland standard suspension
- Incubation 35 °C in ambient air; 16-20 hours for all organisms

Clinical testing was performed at three sites using a total of 1153 clinical isolates (1033 isolates of *Enterobacteriaceae* and 120 isolates of *P. aeruginosa*). Of the clinical isolates, 699 were fresh isolates (60.6 %) and 454 were stock isolates (39.4%). Clinical isolates were tested using both Etest and the reference method.

A total of 86 challenge isolates were tested at a single site using Etest and the reference method (54 isolates of *Enterobacteriaceae* and 32 isolates of *P. aeruginosa*).

Combined clinical and challenge isolates included: *C. freundii* (93 isolates), *C. koseri* (91 isolates), *E. aerogenes* (96 isolates), *E. cloacae* (99 isolates), *E. coli* (109 isolates), *K. oxytoca* (102 isolates), *K. pneumoniae* (107 isolates), *M. morganii* (65 isolates), *P. mirabilis* (95 isolates), *P. vulgaris* (45 isolates), *P. rettgeri* (57 isolates), *P. stuartii* (59 isolates), *S. marcescens* (69 isolates) and *P. aeruginosa* (152 isolates).

For *P. aeruginosa,* the combined results from clinical and challenge testing demonstrated a combined EA of 99.3% and CA of 99.3% (Table 3). A total of 145 isolates were determined to have evaluable results; the EA of evaluable results was 99.3%.

For the *Enterobacteriaceae*, the combined results from clinical and challenge testing demonstrated a combined EA of 99.1% and CA of 99.6% (Table 3). A total of 1060 isolates were determined to have evaluable results; the EA of evaluable results was 99.2%.

Table 3 . Performance of Clinical and Challenge Isolates, Ceftazidime/Avibactam Etest.

	Tot	No. EA	EA %	Eval Tot	Eval EA	EA Eval %	No. CA	CA %	No. R	min	maj	vmj
P. aeruginosa												
Clinical	120	119	99.2	114	113	99.1	119	99.2	16	NA[a]	1	0
Challenge	32	32	100.0	31	31	100.0	32	100.0	18	NA[a]	0	0
Combined	152	151	99.3	145	144	99.3	151	99.3	34	NA[a]	1	0
***Enterobacteriaceae*[b]**												
Clinical	1033	1023	99.0	1015	1006	99.1	1029	99.6	33	NA[a]	2	2
Challenge	54	54	100.0	45	45	100.0	54	100.0	12	NA[a]	0	0
Combined	1087	1077	99.1	1060	1051	99.2	1083	99.6	45	NA[a]	2[c]	2[c,d]

[a] NA, Not Applicable due to a lack of an intermediate breakpoint for ceftazidime/avibactam
[b] *Enterobacteriaceae* included: *Citrobacter freundii, Enterobacter cloacae, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Citrobacter koseri, Enterobacter aerogenes, Morganella morganii Providencia rettgeri, Providencia stuartii,* and *Serratia marcescens*
[c] Etest results are within essential agreement of the reference method
[d] Due to the lack of an intermediate interpretive category for ceftazidime/avibactam, overall very major error rate for *Enterobacteriaceae* of 4.4% (2/45) was adjusted to 0% when considering that all error results were within EA of the reference method

EA – Essential Agreement (+/- 1 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 the result of the reference method and that of Etest Ceftazidime/Avibactam are within plus or minus one serial two-fold dilution of the antibiotic. Evaluable results are those that are on scale for both Etest Ceftazidime/Avibactam and the reference method. Category Agreement (CA) occurs when the interpretation of the result of the reference method agrees exactly with the interpretation of Etest Ceftazidime/Avibactam.

A total of 79 resistant isolates were evaluated; however, no resistant strains of *Citrobacter koseri* were evaluated. The sponsor added the following limitation to the device labeling:

> *The ability of Etest to detect resistance with the following microorganisms is unknown because resistant strains were not available at the time of comparative testing. If such a strain is observed, it should be submitted to a reference laboratory for further testing.*
> *Ceftazidime/Avibactam: Citrobacter koseri*

Three clinical isolates (one isolate each of *P. stuartii, E. aerogenes* and *P. aeruginosa*) were determined to be susceptible by the reference method but resistant with ceftazidime/avibactam Etest. Both the *P. stuartii* and *E. aerogenes* MIC results were within essential agreement of the results obtained with the reference method. For the single isolate of *P. aeruginosa* Etest results were not within essential agreement with the reference method. However, the major error rates for *Enterobacteriaceae* and for *P. aeruginosa* were determined to be acceptable at 0.2% and 0.8%, respectively.

Two isolates of *M. morganii* were determined to be resistant by the reference method but susceptible with Etest Ceftazidime/Avibactam resulting in an overall very major error rate for *Enterobacteriaceae* of 4.4%. Because there is no intermediate breakpoint for ceftazidime/avibactam and because the Etest MIC values obtained with these two isolates were within essential agreement of the reference method, the overall very major error rate for *Enterobacteriaceae* was adjusted to 0.0% and considered to be acceptable. The following footnote was added to the performance table in the device labeling:

> *The overall very major error rate for Enterobacteriaceae was 4.4%. The two very major errors were one dilution apart from the reference method and as such fall within essential agreement. Based on the essential agreement and the lack of an intermediate breakpoint, the adjusted very major error rate is 0.0%*

In addition, because both of the very major errors occurred with *M. morganii* (for a very major error rate of 100% for this species) the following limitation was added to the device labeling to address the possibility of very major errors when testing *M. morganii*:

Due to the lack of an intermediate interpretive category for ceftazidime/avibactam, testing of Morganella morganii with this drug has resulted in very major discrepancies for isolates that are otherwise within essential agreement of the reference method. Testing should be repeated using an alternative testing/reference method prior to reporting results for M. morganii when the Etest MIC is 8 µg/mL.

Enzyme Group Molecular Characterization:
Isolates of *Enterobacteriaceae* and *P. aeruginosa* harboring various molecular mechanisms of resistance noted in the FDA drug label were tested with ceftazidime/avibactam. The following resistance mechanisms were evaluated:

- *Enterobacteriaceae* – ESBL, not specified, CTX-M, TEM, SHV, KPC
- *P. aeruginosa* – chromosomal AmpC, OprD loss, KPC, IMP, VIM, other metallo-β-lactamase

Enterobacteriaceae isolates harboring AmpC and OXA were not evaluated. The sponsor included the following footnote to the performance table in the device labeling:

> *Enzyme characterization was not available for the following organisms at the time of comparative testing, and therefore the performance of Etest Ceftazidime/Avibactam is unknown for Pseudomonas aeruginosa (up-regulation of MexXY and MexAB) and Enterobacteriaceae (OXA and AmpC).*

The sponsor also included the following footnote regarding additional resistance mechanisms:

> *Ceftazidime/avibactam is not active against bacteria that produce metallo-beta-lactamase enzymes and may not have activity against gram-negative bacteria that overexpress efflux pumps or have porin mutations.*

MIC Trending
An analysis of trending was conducted using the combined clinical and challenge data for *P. aeruginosa* and for *Enterobacteriaceae.* 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 of whether the device MIC values are on-scale or not.

The data for 152 *P. aeruginosa* results determined to be evaluable for trending analysis showed no evidence of trending.

The data for 1061 *Enterobacteriaceae* results determined to be evaluable for trending analysis is presented in Table 4.

Table 4. Trending in *Enterobacteriaceae*, Clinical and Challenge Isolates

Organism	Difference in MIC as Compared to the CLSI Reference Method			
	No. results evaluable for trending analysis	**No. results ≥1 dilution lower**	**No. results Exact**	**No. results ≥1 dilution higher**
Enterobacteriaceae	1061	80 (7.54%)*	628 (59.19%)	353 (33.27%)*

*Difference: 25.73%, 95% CI (22.46 – 28.96%)

A trend towards higher MIC readings was observed in the overall performance of *Enterobacteriaceae* compared to the CLSI broth microdilution method which raises concerns for the potential occurrence of major errors. The sponsor included the following footnote to the performance table to address the trending observed for ceftazidime/avibactam:

Etest Ceftazidime/Avibactam MIC values tended to be in exact agreement or at least one doubling dilution higher when testing Enterobacteriaceae compared to the reference broth microdilution method.

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):

No applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Table 5. Breakpoints and Interpretive Categories for Ceftazidime/Avibactam (FDA Drug Label)

Organism	FDA Interpretive Criteria for Ceftazidime/Avibactam MIC (µg/mL)		
	S	I	R
Enterobacteriaceae and *P. aeruginosa*	≤ 8/4	NA	≥ 16/4

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.