

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

K170670

B. Purpose for Submission:

To obtain clearance for the addition of Ceftolozane/Tazobactam at concentrations 0.016-256µg/mL µg/mL to the Etest strip for susceptibility testing of Gram negative aerobic microorganisms.

C. Measurand:

Ceftolozane/Tazobactam 0.016 – 256µg/mL. Tazobactam 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[®] Ceftolozane/Tazobactam (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.

Etest Ceftolozane / Tazobactam has been shown to be active against the Gram negative aerobic microorganisms listed below, according to the FDA label for this antimicrobial agent:

Active both in vitro and in clinical infections:

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,
Morganella morganii
Proteus vulgaris
Providencia rettgeri
Providencia stuartii
Serratia liquefacians
Serratia marcescens

2. Indication(s) for use:

Same as the Intended Use

3. Special conditions for use statement(s):

For prescription use only

Limitation(s):

“The ability of the Etest to detect resistance with the following combination (s) 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

- *Ceftolozane/Tazobactam: Citrobacter koseri, Proteus vulgaris, and Serratia liquefacians”*

4. Special instrument requirements:

Manual readings only

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 $\mu\text{g/mL}$. On the other side, 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 Ceftaroline

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

K151873

3. Comparison with predicate:

Table 1: Comparison with the Predicate Device

Similarities		
Item	Device K170670 Etest Ceftolozane /Tazobactam (0.016-256 µg/mL)	Predicate K151873 Etest Ceftaroline (0.002- 32µg/mL)
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 <i>Enterobacteriaceae</i> , <i>Pseudomonas</i> , <i>Staphylococcus</i> , and <i>Enterococcus</i> species and fastidious bacteria, such as anaerobes, <i>N. gonorrhoeae</i> , <i>S. pneumoniae</i> , <i>Streptococcus</i> and <i>Haemophilus</i> 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.	Same
Incubation	35°± 2°C for 16-20 hours	35°; 5% CO ₂ ; 20-24 hours
Inoculation	Isolated colonies from culture	Same
Result	MIC	Same

Differences		
Item	Device	Predicate
Claimed Organisms	<i>Enterobacteriaceae</i> and <i>Pseudomonas aeruginosa</i>	<i>Community-Acquired Bacterial Pneumonia (CABP):</i> <i>Streptococcus pneumoniae</i> <i>Haemophilus influenzae</i> <i>Skin infections:</i> <i>Streptococcus agalactiae</i>
Antimicrobial	Ceftolozane/Tazobactam	Ceftaroline
Antimicrobial Concentration Range	Ceftolozane MIC range: 0.016 – 256 µg/mL. (Tazobactam: 4 µg/mL)	0.002-32 µg/mL
Reading time	Read at 16-20 hours incubation	Etest compared to Broth Microdilution reference method read at 18 and at 23hours

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 “Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically”; Approved Standard, January 2015

CLSI M100-S25 “Performance Standards for Antimicrobial Susceptibility Testing”; Twenty-five Informational Supplement, Volume 35 No. 3, Jan 2015

L. Test Principle:

The Etest gradient technology is based on a combination of the concepts of dilution and diffusion test methods for susceptibility testing. Etest directly quantifies antimicrobial susceptibility in terms of discrete MIC values. When the Etest strip is applied to an inoculated agar plate, the antibiotic is immediately released from the plastic surface into the agar. A predefined, continuous gradient of antibiotic concentrations is created and maintained directly underneath the strip. After incubation whereby bacterial growth becomes visible, a symmetrical inhibition ellipse centered along the strip will be seen. The MIC value in µg/mL is read where the ellipse edge 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

A reproducibility study was conducted at three external sites using 25 organisms of gram negative bacilli that were consistent with the intended use. The isolates tested in the reproducibility study included *E. cloacae* (one isolate), *E. coli* (seven isolates), *K. oxytoca* (three isolates), *K. pneumoniae* (five isolates), *P. aeruginosa* (nine isolates).

Results were within +/- one doubling dilution agreement as compared to the mode MIC value of Ceftolozane/Tazobactam for all organisms at all sites. All results were on scale. The reproducibility was 97.33%.

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 was prepared to match a 0.5 McFarland. Colony counts were performed periodically at each site to demonstrate that the inoculum procedure results were in the expected CFU/mL.

Inoculum density checks were performed on *E. coli* ATCC 25922, quality control strain, reproducibility and 10% of the fresh clinical isolates. The mean inoculum density of *E. coli* ATCC 25922 was 2.6×10^5 CFU/mL.

Quality Control (QC) Testing:

Organisms recommended by both FDA and CLSI, namely *E. coli* ATCC 25922, *E. coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853 were tested against Ceftolozane/Tazobactam at four sites. These recommended QC organisms were tested a minimum of 20 times/site by both the Etest Ceftolozane/Tazobactam and reference method at each site. The Etest results demonstrate that the system can produce QC results in the recommended range > 95% of the time. Table 2 provides a summary of these QC results.

A second set of quality control results were obtained. This testing included one additional quality control organism, *Klebsiella pneumoniae* ATCC 700603. This organism was also tested against Ceftazidime alone to check for integrity of the plasmid-mediated resistance. Testing was conducted at four external sites plus one internal site. Results are presented in Table 3a/3b below.

K. pneumoniae ATCC 700603 is recommended to test against ceftazidime alone to ensure the presence of the plasmid encoding β -lactamase when testing Ceftolozane/Tazobactam. The footnote below is included in the labeling.

“K. pneumoniae ATCC 700603 should be used for routine QC of Ceftolozane/Tazobactam. 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 Ceftolozane/Tazobactam (First Set)

Organism	Conc (µg/mL)	Reference Frequency	Etest Frequency
<i>E. coli</i> ATCC 25922 Expected Range (0.12- 0.5 µg/mL)	≤0.125		
	0.125	34	87
	0.25	59	7
	0.5	1	
	>0.5		
<i>E. coli</i> ATCC 35218 Expected Range (0.06- 0.25 µg/mL)	≤0.064		
	0.064	2	21
	0.125	86	72
	0.25	3	1
	>0.25		
<i>K. pneumoniae</i> ATCC 700603 Expected Range (0.5- 2 µg/mL)	≤0.5		
	0.5	1	
	1	72	62
	2	20	32
	>2		
<i>P. aeruginosa</i> ATCC 27853 Expected Range 0.25- 1 µg/mL	≤0.25		
	0.25		3
	0.5	90	85
	1	4	1
	>1		

Table 3a: Quality Control Data for Ceftolozane/Tazobactam (Second Set)

Organism	Conc (µg/mL)	Reference Frequency	Etest Frequency
<i>E. coli</i> ATCC 25922 Expected Range (0.12- 0.5 µg/mL)	≤0.125		
	0.125	11	93
	0.25	90	7
	0.5		
	>0.5		
<i>E. coli</i> ATCC 35218 Expected Range (0.06- 0.25 µg/mL)	≤0.064		
	0.064		14
	0.125	90	87
	0.25	11	
	>0.25		
<i>K. pneumoniae</i> ATCC 700603 Expected Range (0.5- 2 µg/mL)	≤0.5		
	0.5		
	1	71	48
	2	29	51
	>2		1
<i>P. aeruginosa</i> ATCC 27853 Expected Range 0.25- 1 µg/mL	≤0.25		
	0.25		1
	0.5	99	94
	1	2	6
	>1		

Table 3b: Quality Control Data for Ceftazidime

Ceftazidime	Conc (µg/mL)	Reference Frequency	Etest Frequency
<i>K. pneumoniae</i> * ATCC 700603 Expected Range 16-64 µg/mL (ancillary testing)	≤16	-	NA
	16	4	NA
	32	83	NA
	64	14	NA
	>64	-	NA

*Testing in accordance with:

- CLSI M100-S25 Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fifth Informational Supplement (version recognized at the beginning of clinical trial) (January 2015)-Table 5A
- CLSI M100-S26 Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Sixth Informational Supplement (version recognized at the completion of clinical trial) (January 2016)-Table 5A

The quality control results are acceptable.

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 Etest Ceftolozane/Tazobactam were compared to results obtained with the CLSI broth microdilution reference panel. The CLSI recommended broth microdilution reference panel was prepared and interpreted according to CLSI recommendations. Reference testing was in accordance with the CLSI Standard: CLSI Document M7-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 – Mueller Hinton broth with appropriate dilutions of antimicrobial solution added
- Inoculum – Direct colony suspension
- Incubation – 35°C ambient air incubator; 16-20 hours for all organisms

Clinical testing was performed at five sites (four external and one internal). There were 966 (793 *Enterobacteriaceae* and 173 *Pseudomonas aeruginosa*) clinical isolates, of which 435 were stock (45%), and 531 were fresh (55%). All isolates grew in the Etest Ceftolozane/Tazobactam test.

A challenge set consisting of 90 isolates (51 *Enterobacteriaceae*, and 39 *P. aeruginosa*) was also evaluated at one external site. Each challenge isolate was tested by the Etest Ceftolozane/Tazobactam and the CLSI reference method. All of the challenge organisms grew in the Etest Ceftolozane/Tazobactam test.

The Etest Ceftolozane/Tazobactam results were compared to the CLSI broth microdilution reference method tested at each study site. Results from the 1056 clinical and challenge isolates are summarized in Table 4. The overall performance using the Etest Ceftolozane/Tazobactam was acceptable, with an EA of 97.2%, CA of 98.6%, a minor error (discrepancy) rate of 1.1% (12/1056), and a major error rate of 0.3% (3/928). There were no very major errors.

Table 4: Performance of Clinical and Challenge Isolates

	Tot	No. EA	EA %	Eval Tot	No. Eval EA	Eval EA %	No. CA	CA %	No. R	min	maj	vmj
<i>Enterobacteriaceae</i>												
Clinical	793	768	96.85	780	755	96.79	781	98.49	56	10	2	0
Challenge	51	49	96.08	38	36	94.74	49	96.08	23	1	1	0
Combined	844	817	96.8	818	791	96.7	830	98.34	79	11	3	0
<i>Pseudomonas aeruginosa</i>												
Clinical	173	171	98.8	163	162	99.4	172	99.4	17	1	0	0
Challenge	39	38	97.4	24	24	100	39	100	18	0	0	0
Combined	212	209	98.6	187	186	99.5	211	99.5	35	1	0	0
All Organisms												
Clinical	966	939	97.2	943	917	97.2	953	98.6	73	11	2	0
Challenge	90	87	96.7	62	60	96.8	88	97.8	41	1	1	0
Combined	1056	1026	97.2	1005	977	97.2	1041	98.6	114	12	3	0

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) is when the Etest results agree with the reference test panel results exactly or within one doubling dilution of the reference method. Category agreement (CA) is when the Etest result interpretation agrees exactly with the reference panel result interpretation.

Enzyme Groups Molecular Characterization:

Molecular characterization was conducted for *Pseudomonas aeruginosa* and for *Enterobacteriaceae* to determine the beta lactamase enzyme groups and

other resistance markers noted in the FDA drug label. The performance of the Etest with Ceftolozane/Tazobactam included 38 *Pseudomonas aeruginosa* and 45 *Enterobacteriaceae* isolates that were characterized as detailed below.

Pseudomonas aeruginosa:

Molecular characterization was conducted to determine beta lactamase enzyme resistance marker status for *Pseudomonas aeruginosa* isolates for the following: chromosomal AmpC, and loss of OprD. Enzyme characterization was also performed for *Pseudomonas aeruginosa* isolates that produce serine carbapenemases [*K. pneumoniae* carbapenemase (KPC)] and metallo- β -lactamases.

Pseudomonas isolates with MexXY and MexAB up-regulation enzymes were not evaluated as this information was not available at the time of testing. This was addressed by adding the following footnote in the labeling:

“Enzyme group characterization was not available for the following organisms at the time of comparative testing, and therefore the performance of the Etest with Ceftolozane/Tazobactam is unknown for: Pseudomonas aeruginosa (up-regulation of MexXY and MexAB) and Enterobacteriaceae (OXA)”.

Enterobacteriaceae (ESBLs):

Molecular characterization was conducted to determine beta lactamase enzyme resistance marker status for claimed *Enterobacteriaceae* isolates for the following ESBLs: TEM, SHV and CTX-M. However, information on *Enterobacteriaceae* isolates with OXA enzymes was not available at the time of testing and was not provided. This was addressed by adding the footnote listed above in the labeling.

Enterobacteriaceae (KPC and β -lactamases):

Enzyme characterization was also conducted for to determine carbapenemase enzyme resistance marker status for claimed *Enterobacteriaceae* isolates that produce serine carbapenemases [*K. pneumoniae* carbapenemase (KPC)]. However, MIC testing did not include *Enterobacteriaceae* isolates with known metallo-beta lactamase status with the exception of one *Proteus mirabilis* isolate tested that contain VEB metallo beta-lactamase. Tazobactam is not an inhibitor of carbapenemases such as KPC and metallo- β -lactamases such as IMP and VIM. In conformance with the Ceftolozane/Tazobactam Pharmaceutical Drug label, the following footnote was added in the labeling

“Ceftolozane/Tazobactam is not active against Enterobacteriaceae that produce KPC or metallo-beta lactamases”.

Resistant Organisms:

A total of 114 out of 1056 organisms tested (10.8%) were found to be resistant to Ceftolozane/Tazobactam by the reference method. However, the following indicated organisms had no resistant isolates available during comparative testing: *Citrobacter koseri*, *Proteus vulgaris*, and *Serratia liquefacians*. This was addressed by adding the following limitation in the labeling:

“The ability of the Etest to detect resistance with the following combination (s) 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

- *Ceftolozane/Tazobactam: Citrobacter koseri, Proteus vulgaris, and Serratia liquefacians”*

MIC Trends:

Using the combined clinical and challenge data for *Enterobacteriaceae* 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 for 821 results constitute the evaluable data for trend analysis which is presented in Table 5.

Table 5. Trending in Combined Clinical and Challenge *Enterobacteriaceae*

Organism	Difference in MIC as Compared to the CLSI Reference Method			
	# Isolates	≤1 dil lower	Exact	≥1 dil. higher
<i>Enterobacteriaceae</i>	821	282 (34.35%)*	441 (53.71%)	98 (11.94%)*

*Difference 22.41% 95%CI: 18.44% 26.30%

A trend towards lower MIC reading was observed in the overall performance of *Enterobacteriaceae* compared to the CLSI broth micro-dilution method, which raises concerns for potential occurrence of very major errors. This trending and the potential for occurrence of very major error(s) for Ceftolozane/Tazobactam was addressed in the labeling by adding the following footnote:

“Etest Ceftolozane/Tazobactam MIC values tended to be in exact agreement or at least one doubling dilution lower when testing Enterobacteriaceae compared to the reference broth micro-dilution”.

- 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: FDA Interpretive Criteria for Ceftolozane/Tazobactam

Organism	MIC (µg/mL)		
	S	I	R
<i>Enterobacteriaceae</i>	≤2/4	4/4	≥8/4
<i>Pseudomonas aeruginosa</i>	≤4/4	8/4	≥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.