510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K190154

B. Purpose for Submission:

To obtain a substantial equivalence determination for Piperacillin/Tazobactam at concentrations of 0.016/4 - 256/4 µg/mL for susceptibility testing of Gram-negative aerobic organisms with ETEST.

C. Measurand:

Piperacillin/Tazobactam 0.016/4 - 256/4 µg/mL

D. Type of Test:

Quantitative Antimicrobial Susceptibility Test growth-based detection

E. Applicant:

bioMérieux, Inc.

F. Proprietary and Established Names:

ETEST Piperacillin/Tazobactam (P/T) (0.016/4 - 256/4 µg/mL)

G. Regulatory Information:

1. Regulation section:

   866.1640 Antimicrobial Susceptibility Test Powder

2. Classification:

   II

3. Product code:

   JWY - Manual Antimicrobial Susceptibility Test Systems

4. Panel:

   83 – Microbiology (83)

H. Intended Use:

1. Intended use(s):

   ETEST is a manual, quantitative technique for determination of antimicrobial susceptibility of non-fastidious Gram-negative and Gram-positive aerobic bacteria and fastidious bacteria. 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 tested on agar media after overnight incubation.

   Piperacillin/Tazobactam has been shown to be active against the Gram-negative aerobic microorganisms listed below according to the FDA label for this antimicrobial agent.

   ETEST P/T can be used to determine the MIC of Piperacillin/Tazobactam against the following microorganisms:

   Active both *in vitro* and in clinical infections:
   Acinetobacter baumannii
   Escherichia coli
   Klebsiella pneumoniae
   Pseudomonas aeruginosa

   In vitro data are available for the following microorganisms, but clinical significance is unknown:
   Citrobacter koseri
   Morganella morganii
   Proteus mirabilis
   Proteus vulgaris
   Serratia marcescens
   Providencia stuartii
   Providencia rettgeri
   Salmonella enterica

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use

   The following limitations are included in the labeling:

   - The ability of ETEST Piperacillin/Tazobactam to detect the following resistant *Enterobacteriaceae* isolates is unknown because resistant isolates were either not

available or an insufficient number was encountered at the time of comparative testing: *Proteus mirabilis, Proteus vulgaris* and *Salmonella enterica*.

4. Special instrument requirements:

   Manual reading only

I. Device Description:

The ETEST gradient technology is based on a combination of the concepts of dilution and diffusion principles for susceptibility testing.

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 has the minimum inhibitory concentration (MIC) reading scale expressed in µg/mL. The other side of the strip contains a predefined continuous exponential 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. Bacterial growth becomes visible during incubation, and a symmetrical inhibition ellipse centered along the strip appears. 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.

ETEST Piperacillin/Tazobactam contains a range of piperacillin from 0.016 to 256 µg/mL, overlaid with a fixed concentration of 4 µg/mL of tazobactam.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   ETEST Ceftazidime/Avibactam 0.016-256 µg/mL

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

   K172150

3. Comparison with predicate:

Table 1: Comparison with the Predicate Device

Item	Device ETEST, **Piperacillin/Tazobactam** (**K190154**)	Predicate ETEST, **Ceftazidime/Avibactam** (**K172150**)
Similarities		
Intended Use	ETEST is a manual, quantitative technique for determination of antimicrobial susceptibility of non-fastidious Gram-negative and Gram-positive aerobic bacteria and fastidious bacteria. 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 tested on agar media after overnight incubation.	Same
Test Design	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
Antimicrobial Concentration Range	0.016/4 – 256/4 µg/mL	Same
Inoculation	Isolated colonies from culture	Same
Incubation	35º ± 2º C for 16 – 20 hours	Same
Result	MIC in µg/mL	Same
Differences		
Claimed Organisms	*Acinetobacter baumannii* *Citrobacter koseri* *Escherichia coli* *Klebsiella pneumoniae* *Morganella morganii* *Proteus mirabilis* *Proteus vulgaris* *Providencia stuartii* *Providencia rettgeri* *Pseudomonas aeruginosa* *Salmonella enterica* *Serratia marcescens*	*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*
Antibiotic	Piperacillin/Tazobactam	Ceftazidime/Avibactam

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

- Guidance for Industry and FDA - Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems – August 28, 2009.
- CLSI M02-A12, Performance Standards for Antimicrobial Disk Susceptibility Test; Approved Standard, January 2015.
- CLSI M07-A11 "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard, Eleventh Edition January 2018".
- CLSI M100-Ed28 "Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Eighth Informational Supplement, January 2018".

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 Piperacillin/Tazobactam ranges from 0.016/4 to 256/4 μg/mL.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Reproducibility testing was conducted at three sites over three days using a Gram-negative panel that included two *A. baumannii*, three *E. coli*, two *K. pneumoniae*, one *M. morganii* and two *P. aeruginosa* isolates. 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 ±1 doubling dilution of the mode.

   The reproducibility results were acceptable at 100%.

   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 (contemporary) clinical isolates.

The overall mean inoculum densities (CFU/mL) for isolates tested with the reference method ranged from 3.84×10^5 to 5.84×10^5. The overall mean inoculum densities for isolates tested with the ETEST ranged from 5.4×10^7 to 1.71×10^8.

The inoculum densities were acceptable.

Purity Check. Verification of isolate purity was conducted on all clinical, challenge and reproducibility organism suspensions for each ETEST and from each growth control well of the broth microdilution (BMD) reference panel.

Growth or Device Failure. No device failures occurred in the ETEST Piperacillin/Tazobactam clinical trial.

Quality Control (QC) Testing. The FDA and CLSI recommended QC strains (*E. coli* ATCC 25922, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, and *K. pneumoniae* ATCC 700603) were tested at least 20 times per site at four sites using both ETEST and BMD reference methods. The results are summarized in **Table 2**.

Table 2: ETEST Piperacillin/Tazobactam QC Results

QC Organism	Piperacillin/Tazobactam Expected Range	Piperacillin Concentration (µg/mL)[a]	Reference (BMD)	ETEST
E. coli ATCC 25922	1/4 – 4/4 µg/mL	<1	0	0
		1	2	0
		2	79	79
		4	4	6
		>4	0	0
E. coli ATCC 35218	0.5/4 – 2/4 µg/mL	<0.5	0	0
		0.5	1	0
		1	78	20
		2	6	65
		>2	0	0

QC Organism	Piperacillin/Tazobactam Expected Range	Piperacillin Concentration (µg/mL)[a]	Reference (BMD)	ETEST
P. aeruginosa ATCC 27853	1/4 – 8/4 µg/mL	<1	0	0
		1	0	0
		2	13	6
		4	63	79
		8	9	0
		>8	0	0
K. pneumoniae ATCC 700603	8/4 – 32/4 µg/mL	<8	0	0
		8	36	0
		16	49	85
		32	0	0
		>32	0	0

[a]Tazobactam concentration was fixed at 4 µg/mL.

The Quality Control results were within the recommended range 100% of the time and thus acceptable.

Supplemental Quality Control Testing. To ensure the integrity of the plasmids encoding antimicrobial resistance (AR) in the quality control strains *Escherichia coli* ATCC 35218 and *Klebsiella pneumoniae* ATCC 700603, supplemental testing was conducted at four sites. The β-lactamase negative *E. coli* ATCC 25922 was also tested. Per the CLSI M100 standard, QC strains were evaluated by disk diffusion method by testing ampicillin (10 µg) and ceftazidime (30 µg) disks. Results are shown below in **Tables 3 and 4**.

Table 3: Supplemental QC Results - Ampicillin

QC Organism (AR mechanism)	Ampicillin (10 µg) Expected Range	Zone Size (mm)	Disk Results
E. coli ATCC 25922 (β-lactamase negative)	15 – 22 mm	<15	0
		15	9
		16	16
		17	14
		18	17
		19	23
		20	6
		21	0
		22	0
		>22	0
E. coli ATCC 35218 (TEM-1)	6 mm	<5	0
		6	84
		>7	0

Table 4: Supplemental QC Results - Ceftazidime

QC Organism (AR mechanism)	Ceftazidime (30 µg) Expected Range	Zone Size (mm)	Disk Results
E. coli ATCC 25922 (β-lactamase negative)	25 – 32 mm	<25	0
		25	0
		26	2
		27	7
		28	5
		29	6
		30	23
		31	23
		32	19
		>32	0
K. pneumoniae ATCC 700603 (SHV-18, OXA-2, mutations in OmpK35 and OmpK37, TEM-1)	10 – 18 mm	<10	0
		10	0
		11	0
		12	0
		13	4
		14	25
		15	39
		16	16
		17	0
		18	0
		>18	0
P. aeruginosa ATCC 27853 (Inducible AmpC)	22 – 29 mm	<22	0
		22	0
		23	0
		24	0
		25	0
		26	6
		27	12
		28	27
		29	40
		>29	0

Test results demonstrated AR plasmid integrity of the QC isolates with 100% (85/85) of the results within expected range.

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 Piperacillin/Tazobactam were compared to results obtained with the CLSI broth microdilution (BMD) reference panel. The reference panel, prepared and interpreted according to recommendations outlined in the CLSI M07-A10, contained two-fold serial dilutions of piperacillin/tazobactam with a piperacillin concentration range of 0.016 – 256 µg/mL (tazobactam concentration fixed at 4 µg/mL). At the end of incubation, the MIC value obtained from the ETEST (where the complete inhibition of growth intersects the strip) was compared to MIC results obtained with the reference method. The testing conditions for ETEST consisted of the following:

- Inoculum: Direct colony suspension to achieve a suspension equivalent to a 0.5 McFarland standard suspension
- Medium: Cation-adjusted Mueller Hinton agar
- Incubation: 35° C ± 2 for 16-20 hours (except *A. baumannii*, incubate at 35° C for 20-24 hours)

Clinical testing for ETEST Piperacillin/Tazobactam was evaluated at three external sites within the United States and one internal site located outside the United States. Each clinical isolate was tested one time by ETEST and BMD using the same initial standardized suspension. A total of 772 clinical isolates were tested which included 592 *Enterobacteriaceae* [*C. koseri* (41), *E. coli* (140), *K. pneumoniae* (167), *M. morganii* (39), *P. mirabilis* (38), *P. vulgaris* (31), *P. rettgeri* (28), *P. stuartii* (36), *S. enterica* (31) and *S. marcescens* (41)], 74 *A. baumannii* and 106 *P. aeruginosa* isolates. Of all of the tested clinical isolates, 49.2% were considered contemporary (i.e., tested within six months of the organism's original isolation from clinical culture) and 50.8% were considered stock (i.e., no time limit on time from isolation prior to testing).

Challenge testing was performed at one internal site using ETEST and BMD. A total of 89 challenge isolates were tested which included 67 *Enterobacteriaceae* [*C. koseri* (5), *E. coli* (28), *K. pneumoniae* (23), *M. morganii* (2), *P. mirabilis* (3), *S. marcescens* (6)], 9 *A. baumannii* and 13 *P. aeruginosa* isolates.

The comparative study (both clinical and challenge) included 659 *Enterobacteriaceae* isolates [*C. koseri* (46), *E. coli* (168), *K. pneumoniae* (190), *M. morganii* (41), *P. mirabilis* (41), *P. vulgaris* (31), *P. rettgeri* (28), *P. stuartii* (36), *S. enterica* (31) and *S. marcescens* (47)]. Information on the numbers of each *Enterobacteriaceae* species is included as a footnote to the performance table in the labeling.

ETEST Piperacillin/Tazobactam performance observed for clinical, challenge and combined isolates is provided in **Table 5**.

Table 5: Performance of Clinical and Challenge Isolates

Piperacillin/ Tazobactam	Total	EA N	EA %	Eval. Total	Eval. EA N	Eval. EA %	CA N	CA %	#R	min	maj	vmj
***Enterobacteriaceae* (all)**												
Clinical	592	571	96.5	477	456	95.6	566	95.6	131	24	0	2
Challenge	67	60	89.6	57	50	87.7	49	73.1	16	18	0	0
Combined	659	631	95.8	534	506	94.8	615	93.3	147	42	0	2
A. baumannii												
Clinical	74	68	91.9	49	43	87.8	66	89.2	26	8	0	0
Challenge	9	8	88.9	3	2	66.7	8	88.9	6	1	0	0
Combined	83	76	91.6	52	45	86.5	74	89.2	32	9	0	0
P. aeruginosa												
Clinical	106	104	98.1	91	89	97.8	101	95.3	16	5	0	0
Challenge	13	13	100	12	12	100	10	76.9	1	3	0	0
Combined	119	117	98.3	103	101	98.1	111	93.3	17	8	0	0

EA – Essential Agreement
CA – Category Agreement
EVAL – Evaluable isolates
R – Resistant isolates
min – minor errors
maj – major errors
vmj – very major errors

Essential Agreement (EA) is when the ETEST result agrees exactly or within one doubling dilution of the reference broth microdilution result. Category Agreement (CA) is when the ETEST result interpretation agrees exactly with the reference broth microdilution result interpretation.

Overall Performance

ETEST Piperacillin/Tazobactam performance for all *Enterobacteriaceae* isolates is acceptable with 95.8% EA and 93.3% CA. There were 42 minor errors, no major errors, and two very major errors. When evaluating individual species, however, performance of *K. pneumoniae* was unacceptable with 88.9% CA (94.2% EA). Since this species also had a very major error rate of 2% (2 VMJ / 99 resistant isolates), the following footnote is included in the performance section of the device labeling:

> *Categorical errors when testing Klebsiella pneumoniae isolates were mostly due to minor errors (19/190, 10%). Two of 99 resistant isolates gave a very major error. Upon repeat testing, a reference MIC value could not be confidently established for one of the isolates due to extensive variability with the reference method.*

ETEST Piperacillin/Tazobactam performance for all *A. baumannii* isolates is acceptable with 91.6% EA and 89.2% CA. There were nine minor errors and no major or very major errors.

ETEST Piperacillin/Tazobactam performance for all *P. aeruginosa* is acceptable with 98.3% EA and 93.3% CA. There were eight minor errors and no major or very major errors.

At the time of comparative testing, resistant isolates were not available for several bacterial species. Thus, the following limitation is included in the device labeling:

> *The ability of ETEST Piperacillin/Tazobactam to detect the following resistant Enterobacteriaceae isolates is unknown because resistant isolates were either not available or an insufficient number was encountered at the time of comparative testing: Proteus mirabilis, Proteus vulgaris and Salmonella enterica.*

To address testing of non-indicated species, the sponsor included the following statement in the Warnings and Precautions section of the device labeling:

> *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.*

Inoculator and ETEST Strip Applicator Options

Culture media plates for ETEST can be inoculated and streaked by swabs manually or with the RETRO C80 inoculator. ETEST strips can be applied onto inoculated media using forceps or the NEMA C88 vacuum pen.

The ETEST Piperacillin/Tazobactam studies used manual inoculation with swabs and applied ETEST strips with forceps at all test sites. The following footnote is included in the performance section of the device labeling:

> *Optional inoculator and ETEST strip applicator can be used for plate inoculation and applying ETEST strips onto agar media. In the ETEST Piperacillin/ Tazobactam clinical studies, swabs were used for plate inoculation/streaking and forceps were used for ETEST strip application.*

Trending

A trending analysis was conducted using the combined data (clinical and challenge) for each organism species and group. 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.

Trending results were stratified by species to determine if species-related trends were observed (**Table 6**). Species for which the difference between the percentage of isolates with higher versus lower MIC values was ≥30% and for which the confidence interval was determined to be statistically significant were considered to have evidence of significant trending and is addressed in labeling.

A trend toward lower MIC values was observed for *K. pneumoniae* while a trend toward higher MIC values was observed for *A. baumannii, M. morganii, P. mirabilis, P. rettgeri, P. stuartii and S. marcescens*. The following footnotes are included in the performance section of the device labeling to address the trending:

ETEST Piperacillin/Tazobactam MIC values tended to be in exact agreement or at least one doubling dilution lower when testing Klebsiella pneumoniae compared to the CLSI reference broth microdilution.

ETEST Piperacillin/Tazobactam MIC values tended to be in exact agreement or at least one doubling dilution higher when testing A. baumannii, M. morganii, P. mirabilis, P. rettgeri, P. stuartii and S. marcescens compared to the CLSI reference broth microdilution.

Table 6. Trending by Species (clinical and challenge isolates combined)

Organism	Total Evaluable for Trending	≥1 dil. Lower # (%)	Exact # (%)	≥1 dil. Higher # (%)	Percent Difference (95% CI)	Trending Noted
C. koseri	37	8	26	3	-13.51%	No
E. coli	163	28	88	47	11.66%	No
K. pneumoniae	109	52	42	15	-33.94% (-44.62 to -21.99)	Yes
M. morganii	40	1	20	19	45.00% (27.11 to 60.14)	Yes
P. mirabilis	41	4	15	22	43.90% (24.26 to 59.36)	Yes
P. vulgaris	31	7	17	7	0.00%	No
P. rettgeri	22	2	6	14	54.55% (26.64 to 72.42)	Yes
P. stuartii	33	3	13	17	42.42% (20.62 to 59.48)	Yes
S. enterica	31	3	19	9	19.35%	No
S. marcescens	43	5	16	22	39.53% (20.23 to 55.19)	Yes
Enterobacteriaceae (all)	550	113	262	175	11.27%	No
A. baumannii	53	2	20	31	54.72% (38.59 to 67.27)	Yes
P. aeruginosa	104	17	60	27	9.62%	No

Resistance Markers

Resistance markers for indicated isolates were provided in the submission. They consisted of β-lactamses including AmpC (CIT, CMY, DHA, FOX), ESBL (CTX-M, SHV, TEM) and carbapenemases (IMP, KPC, NDM, OXA, VIM).

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:

The FDA-recognized susceptibility interpretive criteria for piperacillin/tazobactam are listed in **Table 7**.

Table 7: FDA Recognized Interpretive Criteria for Piperacillin/Tazobactam (µg/mL)[a]

Organism	S	I	R
Enterobacteriaceae	≤16/4	32/4 - 64/4	≥128/4
Pseudomonas aeruginosa	≤16/4	32/4 - 64/4	≥128/4
Acinetobacter spp.	≤16/4	32/4 - 64/4	≥128/4

[a]According to CLSI M100-Ed28 and FDA STIC Website https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm410971.htm

N. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a finding of substantial equivalence.