510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K250856

B Applicant

bioMérieux

C Proprietary and Established Names

ETEST Aztreonam/Avibactam (AZA) (0.016/4-256/4 µg/mL)

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
JWY	Class II	21 CFR 866.1640 - Antimicrobial Susceptibility Test Powder	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for aztreonam/avibactam at concentrations of 0.016/4-256/4 µg/mL for susceptibility testing of non-fastidious gram-negative organisms.

B Measurand:

Aztreonam/Avibactam 0.016/4-256/4 µg/mL

C Type of Test:

Quantitative Antimicrobial Susceptibility Test growth-based detection

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
ETEST is a manual, quantitative technique for the 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.

Testing with ETEST Aztreonam/Avibactam (AZA) (0.016/4-256/4 µg/mL) is indicated for Enterobacterales, as recognized by the FDA Susceptibility Test Interpretive Criteria (STIC).

The ETEST Aztreonam/Avibactam (AZA) (0.016/4-256/4 µg/mL) demonstrated acceptable performance with the following microorganisms:

- Enterobacterales:
  - *Escherichia coli*
  - *Klebsiella pneumoniae*
  - *Klebsiella aerogenes*
  - *Citrobacter freundii* complex
  - *Citrobacter koseri*
  - *Enterobacter cloacae* complex
  - *Proteus mirabilis*
  - *Proteus vulgaris*
  - *Morganella morganii*
  - *Providencia stuartii*
  - *Serratia marcescens*

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

Due to unacceptable essential agreement, *Klebsiella oxytoca* should not be tested with the ETEST Aztreonam/Avibactam (AZA) (0.016/4-256/4 µg/mL) and should be tested by an alternative method.

The ability of ETEST Aztreonam/Avibactam to detect the following resistant isolates is unknown because an insufficient number of resistant isolates were available at the time of comparative testing: *Citrobacter freundii complex, Citrobacter koseri, Enterobacter cloacae*

complex, Klebsiella aerogenes, Morganella morganii, Proteus mirabilis, Proteus vulgaris, Providencia stuartii and *Serratia marcescens.*

D Special Instrument Requirements:

Manual reading only

IV Device/System Characteristics:

A Device Description:

ETEST is a thin, inert and non-porous plastic strip carrying the MIC reading scale in µg/mL on one side and a predefined antibiotic gradient on the other side.

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 carries 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.

ETEST Aztreonam/Avibactam contains a range of aztreonam from 0.016 to 256 µg/mL and avibactam at a fixed concentration of 4 µg/mL.

B Principle of Operation:

When the ETEST 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.

V Substantial Equivalence Information:

A Predicate Device Name(s):
ETEST Ceftazidime/Avibactam (CZA) (0.016 - 256 µg/mL)

B Predicate 510(k) Number(s):
K172150

C Comparison with Predicate(s):

Device & Predicate Device(s):	Device: K250856	Predicate: K172150
Device Trade Name	ETEST Aztreonam/Avibactam (AZA) (0.016/4-256/4 µg/mL)	ETEST Ceftazidime/Avibactam (CZA) (0.016-256 µg/mL)
General Device Characteristic Similarities		
Intended Use	ETEST is a manual, quantitative technique for the 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	0.016-256 µg/mL (Avibactam: fixed at 4 µg/mL)	Same
Inoculation	Isolated colonies from culture in a suspension equivalent to 0.5 McFarland. Inoculum is applied to agar with swab manually or with	Same

Device & Predicate Device(s):	Device: K250856	Predicate: K172150
	rotation plate for even distribution of inoculum.	
Incubation	35±2°C for 16-20 hours	Same
Reading	Manual; the point where the edge of inhibition ellipse intersects the MIC Test Strip	Same
Results	MIC (µg/mL)	Same
General Device Characteristic Differences		
Antimicrobial Agent	Aztreonam/Avibactam	Ceftazidime/Avibactam
Indications for Use Organisms	*Escherichia coli* *Klebsiella pneumoniae* *Enterobacter cloacae complex* *Citrobacter freundii complex* *Klebsiella aerogenes* *Proteus mirabilis* *Proteus vulgaris* *Citrobacter koseri* *Morganella morganii* *Serratia marcescens* *Providencia stuartii*	*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*

VI Standards/Guidance Documents Referenced:

- Guidance for Industry and FDA - Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems – August 28, 2009.
- CLSI M07 11th Edition, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically* (09/17/2018)
- CLSI M100 35th ed. *Performance Standards for Antimicrobial Susceptibility Testing* (January 2025)

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

   Reproducibility testing was conducted at three sites using 10 on-scale Enterobacterales isolates (one *C. freundii*, two *C. koseri*, two *E. cloacae*, one *E. coli*, one *K. oxytoca*, two *K. pneumoniae*, and one *S. marcescens*). Each isolate was tested in triplicate over three days for a total of 270 data points. The mode MIC value 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. Four isolates provided off-scale results. The testing resulted in overall reproducibility of >95% for both the best and worst cases. The results were acceptable.

2. Linearity:

   Not applicable.

3. Analytical Specificity/Interference:

   Not applicable.

4. Assay Reportable Range:

   Not applicable.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Inoculum Density Check:
   The inoculum was prepared to achieve turbidity equivalent to a 0.5 McFarland standard. Colony counts were performed periodically at each site for all QC replicates, from at least one replicate of each reproducibility isolate on each of the three days of testing, and from a minimum of 10% of the contemporary clinical tested. The inoculum densities were acceptable.

   Purity Check:
   Verification of isolate purity was conducted on all clinical, challenge, QC and reproducibility organism suspensions for each ETEST and from each growth control well of the broth microdilution reference panel. All organism suspensions for both the broth microdilution reference panels and ETEST were pure.

   Growth or Device Failure:
   No device failures occurred in the ETEST Aztreonam/Avibactam (AZA) (0.016/4-256/4 µg/mL) clinical trial.

   Quality Control Testing:

The CLSI-recommended quality control (QC) strain *K. pneumoniae* ATCC 700603 and CLSI QC strains *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were tested at a minimum of 20 times per site at four sites. The QC strains *E. coli* ATCC 25922, and *K. pneumoniae* ATCC 700603 were tested using both ETEST and broth microdilution (BMD) reference methods. The QC strain *P. aeruginosa* ATCC 27853 was tested for BMD only. The results are summarized in **Table 1**.

Table 1. Quality Control Test Results for ETEST Aztreonam/Avibactam

QC Organism	Expected Range (µg/mL)	Concentration (µg/mL)	Reference BMD (All sites)	ETEST (All sites)
E. coli ATCC 25922	0.032-0.125	<0.032		
		0.032	26	14
		0.064	47	69
		0.125	10	1
		>0.125	1	
K. pneumoniae ATCC 700603	0.064-0.5	<0.064		
		0.064	18	
		0.125	49	76
		0.25	17	8
		0.5		
		>0.5		
P. aeruginosa ATCC 27853	2-8	<2		NA
		2	3	
		4	75	
		8	6	
		>8	1	

The Quality Control results were within the recommended range >95% of the time which is acceptable.

6. Detection Limit:

Not applicable.

7. Assay Cut-Off:

Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Results obtained with ETEST Aztreonam/Avibactam (AZA) (0.016/4-256/4 µg/mL) were compared to results obtained with the CLSI broth microdilution reference panel. The reference panel, prepared and interpreted according to recommendations outlined in the CLSI M07 *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically* 11th ed., contained two-fold serial dilutions of aztreonam/avibactam with a concentration range of 0.016/4-256/4 µg/mL. At the end of incubation, the MIC value

obtained from the ETEST (determined based on 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±2ºC for 16-20 hours in aerobic conditions

Clinical testing was performed at three external sites (two US sites and one OUS site) and one internal site with both aztreonam/avibactam (AZA) and the reference method. A total of 602 Enterobacterales isolates (528 clinical isolates and 74 challenge isolates) were tested (including 50 *Citrobacter freundii,* 30 *Citrobacter koseri,* 67 *Enterobacter cloacae,* 150 *Escherichia coli,* 32 *Klebsiella aerogenes*, 118 *Klebsiella pneumoniae*, 30 *Morganella morganii,* 32 *Proteus mirabilis*, 30 *Proteus vulgaris*, 30 *Providencia stuartii*, and 33 *Serratia marcescens*). The clinical testing included 47% contemporary (283/602; isolated no longer than 6 months prior to testing) and 40.7% stock (245/602; no time limit on time from isolation prior to testing) clinical isolates. A total of 74 challenge isolates were also evaluated at one internal site using ETEST Aztreonam/Avibactam (AZA) and the reference method.

The performance of 602 clinical and challenge isolates is summarized in **Table 2.**

Table 2. Performance of ETEST Aztreonam/Avibactam with Enterobacterales

	Tot*	No. EA	EA %	Eval Tot	No. Eval EA	Eval EA %	CA Tot	CA %	No. R	No. S	min	maj	vmj
Enterobacterales* [Breakpoints (µg/mL): ≤ 4/4(S), 8/4 (I), ≥16/4 (R)]													
Clinical	528	504	95.5	528	504	95.5	521	98.7	5	516	6	1	0
Challenge	74	72	97.3	74	72	97.3	69	93.2	7	58	5	0	0
Combined	602	576	95.7	602	576	95.7	590	98.0	12	574	11	1	0

* Includes isolates of *C. freundii, C. koseri, E. cloacae, E. coli, K. aerogenes, K. pneumoniae, M. morganii, P. mirabilis, P. vulgaris, P. stuartii,* and *S. marcescens.*

EA – Essential Agreement
CA – Categorical Agreement
S – Susceptible
maj – Major Errors
Eval – Evaluable MICs
R – Resistant
min – Minor Errors
vmj – Very Major Errors

Essential agreement (EA) occurs when the MIC result of the reference method and that of the ETEST Aztreonam/Avibactam (AZA) are within plus or minus one serial two-fold dilution of the antibiotic. Evaluable results are those that are on scale for both the reference method and the ETEST Aztreonam/Avibactam (AZA) or those in which an off-scale result is at least two doubling dilutions from the on-scale result. Category agreement (CA) occurs when the interpretation of the result of the reference method agrees exactly with the interpretation of the ETEST Aztreonam/Avibactam (AZA).

ETEST Aztreonam/Avibactam (AZA) (0.016/4-256/4 µg/mL) performance for all Enterobacterales isolates (clinical and challenge) is acceptable with an EA of 95.7% and CA of 98.0%. There was one (1) major error among 574 susceptible isolates (1/574 = 0.2%) which is acceptable. There were no very major errors.

An insufficient number of resistant strains were evaluated for the following species: *Citrobacter freundii* complex*, Citrobacter koseri, Enterobacter cloacae* complex, *Klebsiella aerogenes, Morganella morganii, Proteus mirabilis, Proteus vulgaris, Providencia stuartii* and *Serratia marcescens.* The sponsor included the following limitation in the device labeling:

> *The ability of ETEST Aztreonam/Avibactam to detect the following resistant isolates is unknown because an insufficient number of resistant isolates were available at the time of comparative testing: Citrobacter freundii complex, Citrobacter koseri, Enterobacter cloacae complex, Klebsiella aerogenes, Morganella morganii, Proteus mirabilis, Proteus vulgaris, Providencia stuartii and Serratia marcescens*

During the clinical study, 48 clinical and challenge isolates of *K. oxytoca* were tested with ETEST Aztreonam/Avibactam. However, the EA obtained for this species was not acceptable (77.1%). The sponsor removed this organism from the Indications of Use and included the following limitation in the device labeling to address testing of this species:

> *Due to unacceptable essential agreement, Klebsiella oxytoca should not be tested with the ETEST Aztreonam/Avibactam (AZA) (0.016/4-256/4 µg/mL) and should be tested by an alternative method*.

Testing/Reporting Non-Indicated Species

For this review, the interpretative criteria are applied to the organisms/organism groups according to the FDA STIC website. As required under 511A(2)(2)(B) of the Federal Food, Drug and Cosmetic Act, the following statement is added in the precautions section of 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 labelling 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, the NEMA C88 vacuum pen or the automatic Applicator SIMPLEX C76.

The ETEST studies for aztreonam/avibactam used manual inoculation with swabs and applied ETEST strips with forceps at all test sites. The following statement is included as a footnote to the performance table in the device labeling:

> *In the ETEST Aztreonam/Avibactam clinical studies, swabs were used for plate inoculation/streaking and forceps were used for ETEST strip application. Testing with the optional Inoculator RETRO C80, Vacuum Pen NEMA C88, and Applicator SIMPLEX C76 was not evaluated during the clinical studies.*

MIC Trending Analysis

Using the combined clinical and challenge data, an analysis of trending was conducted for Enterobacterales isolates. Results are to determine if species-related trends were observed (**Table 3**). This trending calculation considers MIC values that are determined to be one or more doubling dilutions lower or higher compared to the reference method irrespective of whether the device MIC values are on-scale or not. Results that are not clearly at least one dilution lower, at least one dilution higher or in exact agreement with the CLSI reference method are not considered in the trending analysis.

Species for which the difference between the percentage of isolates with higher vs. lower readings was ≥ 30% and for which the confidence interval was determined to be statistically significant were considered to show evidence of trending. Trending that provides higher or lower MIC values compared to the reference is addressed in labeling.

Table 3. Trending Observed with ETEST Aztreonam/Avibactam

Organism Name	Total Evaluable for Trending	≥ 1 Dilution lower No. (%)	Exact No.	≥ 1 Dilution Higher No. (%)	Percent Difference (CI)*	Trending Noted
Citrobacter freundii	49	17, (34.6)	24	8, (16.3)	-18% , (-34%, -1%)	No
Citrobacter koseri	27	6, (22.2)	14	7, (25.9)	4% , (-19%, 26%)	No
Enterobacter cloacae	66	25, (37.9)	31	10, (15.2)	-23% , (-37%, -8%)	No
Escherichia coli	143	41, (28.7)	81	21, (14.7)	-14% , (-23%, -4%)	No
Klebsiella aerogenes	31	6, (19.4)	20	5, (16.1)	-3% , (-22%, 16%)	No
Klebsiella pneumoniae	116	48, (41.4)	61	7, (6.0)	-35% , (-45%, -25%)	Yes
Morganella morganii	20	9, (45.0)	9	2, (10.0)	-35% , (-57%, -7%)	Yes
Proteus mirabilis	7	3, (42.9)	2	2, (28.6)	-14% , (-52%, 30%)	No
Proteus vulgaris	4	4, (100)	0	0, (0)	-100% , (-100%, -31%)	Yes
Providencia stuartii	12	6, (50.0)	5	1, (8.3)	-42% , (-67%, -5%)	Yes
Serratia marcescens	33	10, (30.3)	22	1, (3.0)	-27% , (-44%, -9%)	No

Analysis of trending indicated that MIC values for *K. pneumoniae, Morganella morganii, Proteus vulgaris,* and *Providencia stuartii* tended to be at least one doubling dilution lower than the reference MIC values. The following statement is added as footnote to the AST performance table:

ETEST Aztreonam/Avibactam MIC values tended to be in exact agreement or at least one doubling dilution lower when testing Klebsiella pneumoniae, Morganella morganii, Proteus vulgaris and Providencia stuartii compared to the CLSI reference broth microdilution method.

Resistant Isolates:
A total of 12 Enterobacterales resistant isolates were available in the ETEST Aztreonam/Avibactam performance evaluation studies: *Klebsiella pneumoniae* (2), *Escherichia coli* (8), *Serratia marcescens* (1) and *Enterobacter cloacae* complex (1).

Resistance Mechanism Characterization
Challenge isolates of Enterobacterales harboring various molecular mechanisms of resistance were evaluated with ETEST Aztreonam/Avibactam (AZA) (0.016/4-256/4 µg/mL). The following antimicrobial resistance mechanisms were evaluated: acquired cephalosporinase, carbapenemases (MBL, NDM, OXA-48 like, KPC), overproduction of natural cephalosporinase and reduced permeability, ESBL, etc. For identifying the resistance mechanisms, a number of antimicrobial resistance marker genes (e.g., DHA, TEM, KPC, VEB, OXA, SHV, ACT, NDM, OXA-like, ampH, Ompk, GES, CTX-M, CMY, FOX, etc.) were tested.

2. Matrix Comparison:

   Not applicable.

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable.

2. Clinical Specificity:

   Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   Not applicable.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

The FDA recognized susceptibility interpretive criteria for aztreonam/avibactam are listed in **Table 4**.

Table 4. FDA Identified Interpretive Criteria for Aztreonam/Avibactam

	Minimum Inhibitory Concentration (µg/mL)[a]		
Organisms	**S**	**I**	**R**
Enterobacterales	≤4/4	8/4	≥16/4

[a]According to the FDA STIC Webpage

VIII Proposed Labeling:

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

IX Conclusion:

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

To support the implementation of changes to FDA-recognized susceptibility test interpretive criteria (i.e., breakpoints), this submission included a breakpoint change protocol that was reviewed and accepted by FDA. This protocol addresses future revisions to device labeling in response to breakpoint changes that are recognized on the FDA STIC webpage (https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm410971.htm). The protocol outlined the specific procedures and acceptance criteria that bioMérieux intends to use to evaluate the bioMérieux ETEST Aztreonam/Avibactam (AZA) (0.016/4-256/4 µg/mL) when revised breakpoints for Aztreonam/Avibactam are published on the FDA STIC webpage. The breakpoint change protocol included with the submission indicated that if specific criteria are met, bioMérieux will update the ETEST Aztreonam/Avibactam device label to include (1) the new breakpoints, (2) an updated performance section after re-evaluation of data in this premarket notification with the new breakpoints, and (3) any new limitations as determined by their evaluation.