October 23, 2024

Affinity Biosensors, LLC
Nicole Holliday
Director of Clinical Studies
222 East Canon Perdido Street
Santa Barbara, California 93101

Re: K241324
Trade/Device Name: LifeScale Gram Negative Kit (LSGN) with the LifeScale AST system
Regulation Number: 21 CFR 866.1650
Regulation Name: A Cellular Analysis System For Multiplexed Antimicrobial Susceptibility Testing
Regulatory Class: Class II
Product Code: SAN, LON
Dated: September 23, 2024
Received: September 23, 2024

Dear Nicole Holliday:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

FDA's substantial equivalence determination also included the review and clearance of your Predetermined Change Control Plan (PCCP). Under section 515C(b)(1) of the Act, a new premarket notification is not required for a change to a device cleared under section 510(k) of the Act, if such change is consistent with an established PCCP granted pursuant to section 515C(b)(2) of the Act. Under 21 CFR 807.81(a)(3), a new premarket notification is required if there is a major change or modification in the intended use of a device,

or if there is a change or modification in a device that could significantly affect the safety or effectiveness of the device, e.g., a significant change or modification in design, material, chemical composition, energy source, or manufacturing process. Accordingly, if deviations from the established PCCP result in a major change or modification in the intended use of the device, or result in a change or modification in the device that could significantly affect the safety or effectiveness of the device, then a new premarket notification would be required consistent with section 515C(b)(1) of the Act and 21 CFR 807.81(a)(3). Failure to submit such a premarket submission would constitute adulteration and misbranding under sections 501(f)(1)(B) and 502(o) of the Act, respectively.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part

803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Ribhi Shawar -S

Ribhi Shawar, Ph.D. (ABMM)
Branch Chief, General Bacteriology and Antimicrobial Susceptibility Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K241324

Device Name
LifeScale Gram Negative Kit (LSGN) with the LifeScale AST system

Indications for Use *(Describe)*
The LifeScale AST system is a multiplexed in vitro diagnostic test that uses a microfluidic sensor and resonant frequency to calculate organism concentration and/or mass distribution for quantitative antimicrobial susceptibility testing (AST). Testing is performed directly on blood cultures signaled as positive by a continuous monitoring blood culture system and confirmed by Gram stain. The LifeScale AST system does not provide organism identification and is not indicated for use with polymicrobial samples. Interpretive results (Susceptible/Intermediate/Susceptible-dose dependent/Resistant) are provided for specific drug/organism combinations. Results are intended to be used in conjunction with other clinical and laboratory findings. Standard laboratory protocols for processing positive blood cultures should be followed to ensure availability of isolates for supplemental testing as needed. Additionally, subculture of positive blood culture is necessary for the susceptibility testing of organisms present in polymicrobial samples, for testing antimicrobial agents and species not indicated for testing with the device and for epidemiologic testing and for recovery of organisms present in microbial samples.

The LifeScale AST System and the LifeScale Gram-negative Panel (LSGN) are indicated for testing of positive blood culture samples confirmed by Gram stain as containing Gram-negative organisms for the following antimicrobial agents and specific target organisms identified below:

- Amikacin: Acinetobacter spp., Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella variicola, Pseudomonas aeruginosa
- Ampicillin: Escherichia coli
- Aztreonam: Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca
- Cefazolin: Escherichia coli, Klebsiella pneumoniae, Klebsiella variicola
- Cefepime: Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca, Pseudomonas aeruginosa
- Ceftazidime: Acinetobacter spp. (other than Acinetobacter ursingii), Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella variicola, Klebsiella pneumoniae, Pseudomonas aeruginosa
- Ceftazidime-avibactam: Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca
- Ertapenem: Escherichia coli, Klebsiella aerogenes, Klebsiella pneumoniae, Klebsiella oxytoca
- Gentamicin: Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella variicola, Pseudomonas aeruginosa
- Levofloxacin: Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca, Pseudomonas aeruginosa
- Meropenem: Acinetobacter spp., Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Pseudomonas aeruginosa
- Meropenem-vaborbactam: Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca
- Piperacillin-tazobactam: Acinetobacter spp., Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa
- Trimethoprim-sulfamethoxazole: Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella variicola

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Substantial Equivalence Determination Performance Summary In Compliance with Section 807.92(c)

1. Contact Details

Submitter: Affinity Biosensors
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Correspondent: Nicole Holliday
Director of Clinical Studies
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Santa Barbara, California 93101
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2. Device

Name of Device: LifeScale™ Gram Negative Kit (LSGN) with the LifeScale AST system
Common or Usual Name: LifeScale AST system
Regulation Name:
A cellular analysis system for multiplexed antimicrobial susceptibility testing

Regulation Number:
21 CFR 866.1650
Regulatory Class:
Class II
Product Code:
SAN, LON
Predicate Device:
K211815 LifeScale™ Gram Negative Kit (LSGN) with the LifeScale AST system

Purpose for Submission:

i. To obtain a substantial equivalence determination for the addition of the following antimicrobial agents to the LifeScale Gram Negative Kit (LSGN) Kit: Amikacin, Cefepime, Ceftazidime-avibactam, Gentamicin, Levofloxacin, Meropenem, Meropenem-vaborbactam and Piperacillin-tazobactam. The LSGN Kit is used with the LifeScale AST system for testing positive blood culture samples containing gram-negative bacilli.

Cleared Antimicrobial/Organism Combinations (K211815):

1. **Ampicillin:** *Escherichia coli*
2. **Aztreonam:** *Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca*
3. **Cefazolin:** *Klebsiella pneumoniae, Klebsiella variicola*

4. **Ceftazidime:** *Acinetobacter baumannii, Acinetobacter baumannii/nosocomialis group, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella variicola, Pseudomonas aeruginosa*
5. **Ertapenem:** *Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca*
6. **Trimethoprim-Sulfamethoxazole**: *Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella variicola*

Additional Claimed Antimicrobial/Organism Combinations (Current Submission):

7. **Amikacin:** *Acinetobacter* spp.*, Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella variicola, Pseudomonas aeruginosa*
8. **Cefepime:** *Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca, Pseudomonas aeruginosa*
9. **Ceftazidime-avibactam:** *Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca*
10. **Gentamicin:** *Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella variicola, Pseudomonas aeruginosa*
11. **Levofloxacin:** *Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca, Pseudomonas aeruginosa*
12. **Meropenem:** *Acinetobacter* spp.*, Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Pseudomonas aeruginosa*
13. **Meropenem-vaborbactam:** *Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca*
14. **Piperacillin-tazobactam:** *Acinetobacter* spp.*, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*

ii. Removal of limitations included in in the cleared LifeScale LSGN Kit with the LifeScale AST system (K211815) for Aztreonam, Ceftazidime, Ertapenem, and Cefazolin.

	Antimicrobial/Organism	Limitation	Action
1.	**Ertapenem/*E. coli***	*Due to unacceptable performance of ertapenem/E. coli with incubation in an off-line incubator, perform an alternative method of testing prior to reporting results for ertapenem/E. coli when panels are incubated in an off-line incubator.*	Accept Removal. Additional clinical testing and revised IFU provided for review
2.	**Aztreonam/ *K. pneumoniae***	*Perform an alternative method of testing prior to reporting results for the following antimicrobial/organism combination(s): Aztreonam/K. pneumoniae*	Accept Removal. Additional clinical testing and revised IFU provided for review
3.	**Ceftazidime/ *K. pneumoniae***	*Perform an alternative method of testing prior to reporting results for the following antimicrobial/organism combination(s): Ceftazidime/K. pneumoniae*	Accept Removal. Additional clinical testing and revised IFU provided for review

4.	**Ceftazidime/ *Acinetobacter* spp.**	*Perform an alternative method of testing prior to reporting results for the following antimicrobial/organism combination(s): Ceftazidime/Acinetobacter spp. (other than A. baumannii and A. baumannii/nosocomialis group)*	***Revise current limitation specifically to Acinetobacter ursingii*** ***Revised Limitation:*** *Perform an alternative method of testing prior to reporting results for the following antimicrobial/organism combination(s): Ceftazidime: Acinetobacter ursingii* (revised IFU provided for review.)
5.	**Cefazolin/*E. coli***	*Perform an alternative method of testing prior to reporting results for the following antimicrobial/organism combination(s): Cefazolin/E. coli*	Accept Removal. Additional clinical testing and revised IFU provided for review
6.	**Ertapenem/ *K. pneumoniae***	*Perform an alternative method of testing prior to reporting results for the following antimicrobial/organism combination(s): Ertapenem/K. pneumoniae*	Accept Removal. Additional clinical testing and revised IFU provided for review

Antimicrobials:

Table 1. LifeScale LSGN Antimicrobials and Reportable Ranges

Antimicrobial		Range µg/mL	
		Min (≤)	Max (>)
Amikacin	AMI	4	256
*Ampicillin	AMP	2	64
*Aztreonam	AZT	1	64
*Cefazolin	FAZ	0.25	16
Cefepime	FEP	0.5	64
*Ceftazidime	TAZ	1	64
Ceftazidime-avibactam	CZA	2/4	32/4
*Ertapenem	ETP	0.12	8
Gentamicin	GEN	1	32
Levofloxacin	LEVO	0.25	16
Meropenem	MERO	0.12	16
Meropenem-vaborbactam	MEV	0.5/8	16/8
Piperacillin-tazobactam	P/T	4/4	256/4
*Trimethoprim-sulfamethoxazole	SXT	0.25	8

***Antimicrobics cleared in K211815**

Test Type:
The LifeScale Gram Negative Kit (LSGN) with the LifeScale AST system is a quantitative antimicrobial susceptibility test system that determines the minimum inhibitory concentration of specific organisms from positive blood culture samples.

3. Device Description

The Affinity Biosensors LifeScale Gram Negative Kit (LSGN) is a semi-automated instrument system for antimicrobial susceptibility testing (AST) directly from positive blood cultures for which the Gram stain shows gram-negative bacilli. The system uses a microfluidic sensor that detects organisms in suspension and measures differences in cell mass between bacterial suspensions incubated in the presence and absence of antibiotic. Minimum inhibitory concentrations (MICs) are determined from data obtained during sample measurement including organism concentration and/or cell mass distributions of individual organisms. The system automatically interprets the measurements to determine MIC values and interpretive results (susceptible, intermediate, or resistant) based on FDA-defined or recognized breakpoints. The organism identification determined using a platform FDA-cleared for use with positive blood culture samples is entered by the user. If the organism identification has not been entered or if the sample has not been confirmed as monomicrobial, the system provides a preliminary report that indicates that organism identification or monomicrobial status is pending. The device Instructions for Use indicates that the preliminary laboratory report should not be reported to the healthcare provider. The final report is provided to the healthcare provider when the organism identification is entered into the system and the culture is confirmed to be monomicrobial. Polymicrobial samples should not be tested with the LifeScale LSGN Kit. Preliminary results are available in most cases within four hours from initiation of the assay.

4. Intended Use/Indications for Use

Intended Use:
The LifeScale AST system is a multiplexed *in vit*ro diagnostic test that uses a microfluidic sensor and resonant frequency to calculate organism concentration and/or mass distribution for quantitative antimicrobial susceptibility testing (AST). Testing is performed directly on blood cultures signaled as positive by a continuous monitoring blood culture system and confirmed by Gram stain. The LifeScale AST system does not provide organism identification and is not indicated for use with polymicrobial samples. Interpretive results (Susceptible/Intermediate/Susceptible-dose dependent/Resistant) are provided for specific drug/organism combinations. Results are intended to be used in conjunction with other clinical and laboratory findings. Standard laboratory protocols for processing positive blood cultures should be followed to ensure availability of isolates for supplemental testing as needed. Additionally, subculture of positive blood culture is necessary for the susceptibility testing of organisms present in polymicrobial samples, for testing antimicrobial agents and species not indicated for testing with the device and for epidemiologic testing and for recovery of organisms present in microbial samples.

Indications for Use:

The LifeScale Gram Negative Kit (LSGN) is intended for use with the LifeScale AST system for *in vitro* testing of positive blood culture samples confirmed by Gram stain as containing gram-negative bacilli for the antimicrobial agents and specific target organisms identified below:

1. **Ampicillin:** *Escherichia coli*
2. **Aztreonam:** *Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca*
3. **Cefazolin:** *Escherichia coli, Klebsiella pneumoniae, Klebsiella variicola*
4. **Ceftazidime:** *Acinetobacter* spp. (other than *Acinetobacter ursingii*), *Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella variicola, Klebsiella pneumoniae, Pseudomonas aeruginosa*
5. **Ertapenem:** *Escherichia coli, Klebsiella aerogenes, Klebsiella pneumoniae, Klebsiella oxytoca*
6. **Trimethoprim-Sulfamethoxazole**: *Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella variicola*
7. **Amikacin:** *Acinetobacter* spp.*, Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella variicola, Pseudomonas aeruginosa*
8. **Cefepime:** *Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca, Pseudomonas aeruginosa*
9. **Ceftazidime-avibactam:** *Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca*
10. **Gentamicin:** *Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella variicola, Pseudomonas aeruginosa*
11. **Levofloxacin:** *Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca, Pseudomonas aeruginosa*
12. **Meropenem:** *Acinetobacter* spp.*, Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Pseudomonas aeruginosa*
13. **Meropenem-vaborbactam:** *Escherichia coli, Klebsiella pneumoniae, Klebsiella aerogenes, Klebsiella oxytoca*
14. **Piperacillin-tazobactam:** *Acinetobacter* spp.*, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*

5. Substantial Equivalence

This submission is an addition of claims to the K211815 and does not impact the safety or effectiveness of the LifeScale AST system.

Device & Predicate Device(s):	**New Device** **Affinity Biosensors Lifescale Gram Negative Kit (LSGN) with the LifeScale AST system**	**K211815** **Predicate Device** **Affinity Biosensors Lifescale Gram Negative Kit (LSGN) with the LifeScale AST system**
Device Trade Name	Lifescale Gram Negative Kit (LSGN) with the LifeScale AST system	Lifescale Gram Negative Kit (LSGN) with the LifeScale AST system
General Device Similarities		
Intended Use/Indications for Use	The LifeScale AST system is a multiplexed *in vitro* diagnostic test that uses a microfluidic sensor and resonant frequency to calculate organism	Same

	concentration and/or mass distribution for quantitative antimicrobial susceptibility testing (AST). Testing is performed directly on blood cultures signaled as positive by a continuous monitoring blood culture system and confirmed by Gram stain. The LifeScale AST system does not provide organism identification and is not indicated for use with polymicrobial samples. Interpretive results (Susceptible/Intermediate/Susceptible-dose dependent/Resistant) are provided for specific drug/organism combinations. Results are intended to be used in conjunction with other clinical and laboratory findings. Standard laboratory protocols for processing positive blood cultures should be followed to ensure availability of isolates for supplemental testing as needed. Additionally, subculture of positive blood culture is necessary for the susceptibility testing of organisms present in polymicrobial samples, for testing antimicrobial agents and species not indicated for testing with the device, for epidemiologic testing and for recovery of organisms present in microbial samples. The LifeScale Gram Negative Kit (LSGN) is intended for use with the LifeScale AST system for *in vitro* testing of positive blood culture samples confirmed by Gram stain as containing gram-negative bacilli.	
Sample	Blood cultures are signaled as positive by a continuous monitoring blood culture system.	Same
Inoculation Method	Automated	Same
Read Method	Automated	Same
Results	Report results as a minimum inhibitory concentration (MIC) and categorical interpretation (S, I/SDD, R)	Same
Sample Prep	Centrifugation and pipetting of sample.	Same
Inoculation Method	Automated	Same
IVD Functions	AST	Same
Technology	Microfluidic and resonant frequency to calculate organism concentration and/or mass distribution	Same
Organisms Tested	Gram-negative bacilli	Same
General Device Characteristic Differences		

Antimicrobial Agents	Amikacin Cefepime Ceftazidime-avibactam Gentamicin Levofloxacin Meropenem Meropenem-vaborbactam Piperacillin-tazobactam	Ampicillin Aztreonam Cefazolin Ceftazidime Ertapenem Trimethoprim-sulfamethoxazole

6. Performance Characteristics

Comparison Study

A comparison study was conducted to evaluate the performance of the LifeScale Gram Negative Kit (LSGN) with the LifeScale AST system in testing prospective clinical blood cultures confirmed positive by Gram Stain for Gram-negative bacilli. This study encompassed testing both prospective positive blood cultures (PBCs) and blood cultures contrived using isolates chosen to generate data required to fulfill intended use claims. All LifeScale AST sample results were compared to the reference Broth Microdilution (BMD). Each sample submitted for BMD testing was assigned a unique Trial ID, and LifeScale results were kept blinded to prevent bias. Performance was evaluated by comparing quantitative (MIC) and qualitative (S/I/SDD/R) AST results generated by the LifeScale AST System with those of the reference BMD.

Prospective PBCs consistent with the inclusion criteria were enrolled and tested at 6 US Clinical sites. For testing of prospective samples, organism ID was performed using an FDA-cleared direct from positive blood culture ID system. Testing was performed on the LifeScale AST System in accordance with the indications for use. PBC bottles were sub-cultured onto Tryptic Soy Agar supplemented with 5% Sheep Blood panels (BAP) and MacConkey Agar panels (MAC) incubated for 18-24 hours and examined for purity and colony morphology. If more than one colony type was observed, each organism was isolated for purity. All organisms isolated were identified using matrix-assisted laser desorption/ionization (MALDI). Polymicrobial samples were withdrawn from the study. ID results generated using a direct from Blood Culture ID system and/or MALDI were entered into the LifeScale AST System, and a final MIC/SIR result was generated using the final LifeScale AST System software. If there was a discordant organism identification between MALDI and a Direct from Blood Culture system, MALDI ID was considered the organism's final identification.

Contrived samples were prepared from frozen isolates supplied by Affinity Biosensors, or they were prepared from contemporary isolates collected by the laboratory and agreed upon by Affinity for study inclusion. Blood cultures with the required amount of blood were spiked with isolated organisms and incubated in the blood culture system. When flagged as positive, the positive blood culture was tested on the LifeScale AST system in accordance with manufacturer's instructions for use. The PBC was sub-cultured to confirm purity. If a mixed (contaminated) culture was observed, a fresh contrived sample was prepared and tested.

All LifeScale system testing was performed within 12 hours of the blood culture bottle being flagged as positive. LifeScale panels were read upon system confirmation of growth. Positive growth was determined automatically by the LifeScale AST system as part of the reading process. If the panel was incubated offline, it would be placed on the LifeScale system to be read. To generate the final AST report, the organism ID was entered into LifeScale AST System. The LifeScale AST System software generated the final AST results (MIC and S/I/SDD/R).

Reference testing was performed on all enrolled samples in triplicate. Testing was done in accordance with the reference protocol and was performed at two trial sites. Clinical sites shipped isolates on transport media from the PBC purity panel following verification of pure culture. Samples contrived from laboratory stock underwent organism identification using MALDI prior to shipping to the reference site for testing. Reference testing was performed in triplicate. The procedure for Broth Microdilution reference testing follows CLSI guidance (CLSI M07).

The performance of the LifeScale AST system with the LSGN Kit was compared to the FDA-recognized reference BMD method for determining quantitative (MIC) AST results direct from Gram-negative positive blood cultures. Acceptable clinical performance was assessed across the following parameters for each antimicrobial agent on the LSGN panel; Essential Agreement (EA), Category Agreement (CA), Essential Agreement of evaluable results (Evaluable EA), Very Major Discrepancy (VMJ), Major Discrepancy (MAJ), Minor Discrepancy (MIN), Growth Failure Rate. For drug/organism group combinations where the susceptible dose-dependent category is recognized in place of the intermediate category, any errors that were observed with this category were designated as minor errors. Assessment of categorical agreement (Susceptible/ Susceptible-Dose Dependent/Intermediate/Resistant, S/SDD/I/R) was conducted utilizing FDA breakpoints (Antimicrobial Susceptibility Test Interpretive Criteria/STIC) and CLSI M100 guidelines, if applicable.

Exclusion Data

Summary of LifeScale LSGN AST Tests Initiated and Failed to Report a Result

The provided table summarizes tests initiated on the LifeScale AST system and the reasons for exclusion or incomplete results during clinical, analytical, and quality control (QC) phases. Out of 6164 tests initiated:

Plate Failures (0.32%): This category includes issues such as being unable to verify positive controls, sensor clogs detected, and the system being unable to calculate MIC.
Growth Failures (0.37%): These failures occurred due to issues related to growth during testing.
LifeScale Failures (1.12%): This category involves failures directly attributable to the LifeScale system, including software and hardware failures.
Other Reasons (0.57%): This includes a variety of reasons such as operator error, incubation time exceeding 8 hours, user cancellation, and protocol errors.

The total percentage of tests excluded or incomplete is 2.38%, with clinical tests accounting for 0.31%, analytical tests for 1.83%, and QC tests for 0.24%.

Upon initiation of any test on the LifeScale system, results were available 97.62% (6017/6164) of the time.

Reason for Exclusion/Incomplete Test	Clinical	Analytical	QC	Overall
Plate Failures*	[5/986] 0.51%	[15/3307] 0.45%	[0/1871] 0.00%	[20/6164] 0.32%
Growth Failures	[6/986] 0.61%	[17/3307] 0.51%	[0/1871] 0.00%	[23/6164] 0.37%
LifeScale Failures**	[4/986] 0.41%	[50/3307] 1.51%	[15/1871] 0.80%	[69/6164] 1.12%
Other Reasons***	[4/986] 0.41%	[31/3307] 0.94%	[0/1871] 0.00%	[35/6164] 0.57%
Total Excluded/Incomplete Tests	**[19/986] 1.93%**	**[113/3307] 3.42%**	**[15/1871] 0.80%**	**[147/6164] 2.38%**

Table 2. Summary of LifeScale LSGN AST tests initiated and failed to report a result

**Plate Failures include: unable to verify positive controls, sensor clog detected, system unable to calculate MIC*
***LifeScale Failures include: LifeScale system software and hardware failures*
****Other Reasons include: operator error, incubation time greater than 8 hours, user canceled, protocol error*

Clinical Performance Data

Overall AST performance for the LifeScale LSGN Kit with the LifeScale AST System was evaluated with 8 additional antimicrobials, and an overview of the overall performance is presented in Table 4, below.

Total Evaluated	No. EA	EA%	Eval Tot	No. Eval EA	Eval EA%	No. CA	CA%	No. R	No. S	#MIN (MIN%)	#MAJ (MAJ%)	#VMJ (VMJ%)
Amikacin-*Acinetobacter spp.* [Breakpoints (µg/mL): ≤16 (S), 32 (I), ≥64 (R)]												
77	77	100.0%	11	11	100.0%	75	97.4%	17	58	2 (2.60%)	0 (0.00%)	0 (0.00%)
[a]Amikacin- *E. coli, K. aerogenes, K. oxytoca, K. pneumoniae, K. variicola* [Breakpoints (µg/mL): ≤16 (S), 32 (I), ≥64 (R)]												
480	467	97.3%	54	41	75.9%	460	95.8%	62	400	17 (3.54%)	3 (0.75%)	0 (0.00%)
[a]Amikacin-*Pseudomonas aeruginosa* [Breakpoints (µg/mL): ≤16 (S), 32 (I), ≥64 (R)]												
59	58	98.3%	15	14	93.3%	56	94.9%	7	50	3 (5.08%)	0 (0.00%)	0 (0.00%)
[a]Cefepime- *E. coli, K. aerogenes, K. oxytoca, K. pneumoniae* [Breakpoints (µg/mL): ≤2 (S), 4,8 (SDD[b]), ≥16 (R)]												
445	412	92.6%	64	31	48.4%	426	95.7%	154	281	16 (3.60%)	3 (1.07%)	0 (0.00%)
Cefepime-*Pseudomonas aeruginosa* [Breakpoints (µg/mL): <8 (S), ≥16 (R)]												
101	94	93.1%	78	71	91.0%	85	84.2%	29	72	0 (0.00%)	13 (18.06%)	3 (10.34%)
[a]Ceftazidime-avibactam- *E. coli, K. aerogenes, K. oxytoca* [Breakpoints (µg/mL): ≤8/4 (S), ≥16/4 (R)]												
303	300	99.0%	7	4	57.1%	300	99.0%	47	256	0 (0.00%)	3 (1.17%)	0 (0.00%)
[a]Gentamicin- *E. coli, K. aerogenes, K. oxytoca, K. pneumoniae, K. variicola* [Breakpoints (µg/mL): ≤4 (S), 8 (I), ≥16 (R)]												

480	474	98.8%	55	49	89.1%	469	97.7%	126	352	8 (1.67%)	2 (0.57%)	1 (0.79%)	
[a]Gentamicin-*Pseudomonas aeruginosa* [Breakpoints (µg/mL): ≤4 (S), 8 (I), ≥16 (R)]													
59	56	94.9%	18	15	83.3%	55	93.2%	10	47	4 (6.78%)	0 (0.00%)	0 (0.00%)	
[a]Levofloxacin- *E. coli, K. aerogenes, K. oxytoca, K. pneumoniae* [Breakpoints (µg/mL): ≤0.5 (S), 1 (I), ≥2 (R)]													
437	429	98.2%	58	50	86.2%	422	96.6%	154	267	15 (3.43%)	0 (0.00%)	0 (0.00%)	
Levofloxacin-*Pseudomonas aeruginosa* [Breakpoints (µg/mL): ≤1 (S), 2 (I), ≥4 (R)]													
101	97	96.0%	40	36	90.0%	89	88.1%	26	69	12 (11.88%)	0 (0.00%)	0 (0.00%)	
Meropenem-*Acinetobacter spp.* [Breakpoints (µg/mL): ≤2 (S), 4 (I), ≥8 (R)]													
78	75	96.2%	42	39	92.9%	76	97.4%	37	41	2 (2.56%)	0 (0.00%)	0 (0.00%)	
[a]Meropenem- *E. coli, K. oxytoca, K. pneumoniae* [Breakpoints (µg/mL): ≤1 (S), 2 (I), ≥4 (R)]													
392	359	91.6%	62	29	46.8%	379	96.7%	123	264	8 (2.04%)	1 (0.38%)	4 (3.25%)	
[a]Meropenem-*Pseudomonas aeruginosa* [Breakpoints (µg/mL): ≤2 (S), 4 (I), ≥8 (R)]													
59	57	96.6%	39	37	94.9%	53	89.8%	25	32	6 (10.17%)	0 (0.00%)	0 (0.00%)	
[a]Meropenem-vaborbactam- *E. coli, K. aerogenes, K. oxytoca, K. pneumoniae* [Breakpoints (µg/mL): ≤4/8 (S), 8/8 (I), ≥16/8 (R)]													
442	420	95.0%	39	17	43.6%	418	94.6%	77	362	22 (4.98%)	2 (0.55%)	0 (0.00%)	
[a]Piperacillin-tazobactam-*Acinetobacter spp.* [Breakpoints (µg/mL): ≤16/4 (S), 32/4-64/4 (I), ≥128/4 (R)]													
89	82	92.1%	23	16	69.6%	84	94.4%	66	22	5 (5.62%)	0 (0.00%)	0 (0.00%)	
[a]Piperacillin-tazobactam- *E.coli, K. pneumoniae* [Breakpoints (µg/mL): ≤8/4 (S), 16/4 (I), ≥32/4 (R)]													
391	355	90.8%	67	31	46.3%	360	92.1%	198	189	25 (6.38%)	3 (1.58%)	3 (1.52%)	
Piperacillin-tazobactam-*Pseudomonas aeruginosa* [Breakpoints (µg/mL): ≤8/4 (S), 16/4 (SDD[b]), ≥32/4 (R)]													
185	174	94.1%	59	48	81.4%	173	93.5%	67	112	10 (5.41%)	1 (0.89%)	1 (1.49%)	

Table 3. LifeScale LSGN performance: Interpretation of MIC results are based on FDA Susceptibility Test Interpretative Criteria (STIC) and the 34th edition of the CLSI M100

[a]In the clinical study or in the Inoculum Density analytical study, the majority of drug/organism combinations tested with the LifeScale LSGN Kit showed MIC values equal to or at least one doubling dilution higher/lower than the reference method. Use caution when reporting drug resistance for any antimicrobial. The following drug/organism combinations showed high trending:

- *Amikacin - Acinetobacter spp., E. coli, K. aerogenes, K. oxytoca, K. pneumoniae, K. variicola, P. aeruginosa*
- *Cefepime - E. coli, K. aerogenes, K. oxytoca, K. pneumoniae*
- *Ceftazidime-avibactam - K. aerogenes, K. oxytoca*
- *Gentamicin - E. coli, K. pneumoniae, K. oxytoca, K. aerogenes, P. aeruginosa*
- *Levofloxacin - K. pneumoniae, K. oxytoca, K. aerogenes*

- *Meropenem - Acinetobacter calcoaceticus species, Acinetobacter lwoffii, K. oxytoca, P. aeruginosa*
- *Meropenem-vaborbactam - K. pneumoniae, K. oxytoca, K. aerogenes*
- *Piperacillin-tazobactam - E. coli, K. pneumoniae*

The following drug/organism combinations showed low trending

- Ceftazidime-avibactam - *E. coli*
- Piperacillin-tazobactam - *A. baumannii*
- Meropenem-vaborbactam - *E. coli*

[b]*SDD- Susceptible dose-dependent.*

Amikacin (AMI)

A total of 616 samples were evaluated with Amikacin including 465 clinical (75.5%) and 151 challenge (24.5%) samples. The combined results from clinical and challenge testing from all claimed species demonstrated an EA of 97.7% and a CA of 95.9% with no VMJs (0%) and 3 MAJs (0.59%).

Species-level performance

- A total of 77 *Acinetobacter spp.* samples were evaluated with Amikacin. The combined results from clinical and challenge testing demonstrated an EA of 100% and a CA of 97.4% with no VMJs (0%) and no MAJs (0%).
- A total of 140 *E. coli* samples were evaluated with Amikacin. The combined results from clinical and challenge testing demonstrated an EA of 96.4% and a CA of 95.7% with no VMJs (0%) and 2 MAJs (1.79%).
- A total of 52 *K. aerogenes* samples were evaluated with Amikacin. The combined results from clinical and challenge testing demonstrated an EA of 100% and a CA of 98.1% with no VMJs (0%) and no MAJs (0%).
- A total of 111 *K. oxytoca* samples were evaluated with Amikacin. The combined results from clinical and challenge testing demonstrated an EA of 99.1% and a CA of 99.1% with no VMJs (0%) and 1 MAJs (0.95%).
- A total of 142 *K. pneumoniae* samples were evaluated with Amikacin. The combined results from clinical and challenge testing demonstrated an EA of 95.8% and a CA of 91.5% with no VMJs (0%) and no MAJs (0%).
- A total of 35 *K. variicola* samples were evaluated with Amikacin. The combined results from clinical and challenge testing demonstrated an EA of 97.1% and a CA of 100% with no VMJs (0%) and no MAJs (0%).
- A total of 59 *P. aeruginosa* samples were evaluated with Amikacin. The combined results from clinical and challenge testing demonstrated an EA of 98.3% and a CA of 94.9% with 0 VMJs (0%) and 0 MAJ (0%).

Cefepime (FEP)

A total of 546 samples were evaluated with Cefepime including 388 clinical (71.1%) and 158 challenge (28.9%) samples. The combined results from clinical and challenge testing from all claimed species demonstrated an EA of 92.7% and a CA of 93.3% with 3 VMJs (1.64%) and 5 MAJs (1.42%).

Species-level performance

- A total of 139 *E. coli* samples were evaluated with Cefepime. The combined results from clinical and challenge testing demonstrated an EA of 94.2% and a CA of 97.1% with no VMJs (0%) and 1 MAJs (1.37%).
- A total of 53 *K. aerogenes* samples were evaluated with Cefepime. The combined results from clinical and challenge testing demonstrated an EA of 90.6% and a CA of 96.2% with no VMJs (0%) and no MAJs (0%).
- A total of 111 *K. oxytoca* samples were evaluated with Cefepime. The combined results from clinical and challenge testing demonstrated an EA of 92.8% and a CA of 92.8% with no VMJs (0%) and 1 MAJs (1.08%).
- A total of 142 *K. pneumoniae* samples were evaluated with Cefepime. The combined results from clinical and challenge testing demonstrated an EA of 91.5% and a CA of 96.5% with no VMJs (0%) and 1 MAJs (1.39%).
- A total of 101 *P. aeruginosa* samples were evaluated with Cefepime. The combined results from clinical and challenge testing demonstrated an EA of 93.1% and a CA of 84.2% with 3 VMJs (10.34%) and 13 MAJ (18.06%). Due to the lack of an intermediate breakpoint the MAJ rate can be adjusted to 2.78% since 11/13 MAJ discrepancies were in essential agreement to the reference method. Due to the high MAJ rate at 4 µg/mL the following limitation is proposed:
    - *For Cefepime, perform an alternative method of testing prior to reporting of results for P. aeruginosa when the MIC is 4 µg/mL due to the occurrence of very major errors (3/29 resistant isolates, 10.34%).*

Ceftazidime-avibactam (AVI)

A total of 303 samples were evaluated with Ceftazidime-avibactam including 232 clinical (76.6%) and 71 challenge (23.4%) samples. The combined results from clinical and challenge testing from all claimed species demonstrated an EA of 99.0% and a CA of 99.0% with no VMJs (0. 0%) and 3 MAJs (1.17%).

Species-level performance

- A total of 53 *K. aerogenes* samples were evaluated with Ceftazidime-avibactam. The combined results from clinical and challenge testing demonstrated an EA of 96.2% and a CA of 94.3% with no VMJs (0%) and 3 MAJs (5.77%). Due to the lack of an intermediate breakpoint, the MAJ rate can be adjusted to 1.92% since 2/3 MAJ discrepancies were within essential agreement to the reference method.
- A total of 111 *K. oxytoca* samples were evaluated with Ceftazidime-avibactam. The combined results from clinical and challenge testing demonstrated an EA of 99.1% and a CA of 100% with no VMJs (0%) and 0 MAJs (0%).

A total of 139 *E. coli* samples were evaluated with Ceftazidime-avibactam. The combined results from clinical and challenge testing demonstrated an EA of 100% and a CA of 100% with no VMJs (0%) and no MAJs (0%).

Gentamicin (GEN)

A total of 539 samples were evaluated with Gentamicin including 407 clinical (75.5%) and 132 challenge (24.5%) samples. The combined results from clinical and challenge testing from all claimed species demonstrated an EA of 98.3% and a CA of 97.2% with 1 VMJs (0.74%) and 2 MAJs (0.50%).

Species-level performance

- A total of 139 *E. coli* samples were evaluated with Gentamicin. The combined results from clinical and challenge testing demonstrated an EA of 99.3% and a CA of 98.6% with no VMJs (0%) and no MAJs (0%).
- A total of 53 *K. aerogenes* samples were evaluated with Gentamicin. The combined results from clinical and challenge testing demonstrated an EA of 100% and a CA of 100% with no VMJs (0%) and no MAJs (0%).
- A total of 111 *K. oxytoca* samples were evaluated with Gentamicin. The combined results from clinical and challenge testing demonstrated an EA of 99.1% and a CA of 98.2% with no VMJs (0%) and 1 MAJs (1.01%).
- A total of 142 *K. pneumoniae* samples were evaluated with Gentamicin. The combined results from clinical and challenge testing demonstrated an EA of 97.2% and a CA of 95.8% with 1 VMJs (1.96%) and 1 MAJs (1.10%).
- A total of 35 *K. variicola* samples were evaluated with Gentamicin. The combined results from clinical and challenge testing demonstrated an EA of 100% and a CA of 97.1% with no VMJs (0%) and no MAJs (0%).
- A total of 59 *P. aeruginosa* samples were evaluated with Gentamicin. The combined results from clinical and challenge testing demonstrated an EA of 94.9% and a CA of 93.2% with 0 VMJs (0%) and 0 MAJ (0%).

Levofloxacin (LEVO)

A total of 496 samples were evaluated with Levofloxacin including 370 clinical (74.6%) and 126 challenge (25.4%) samples. The combined results from clinical and challenge testing from all claimed species demonstrated an EA of 97.6% and a CA of 94.6% with 0 VMJs (0%) and 0 MAJs (0%).

Species-level performance

- A total of 137 *E. coli* samples were evaluated with Levofloxacin. The combined results from clinical and challenge testing demonstrated an EA of 99.3% and a CA of 94.1% with no VMJs (0%) and no MAJs (0%).
- A total of 53 *K. aerogenes* samples were evaluated with Levofloxacin. The combined results from clinical and challenge testing demonstrated an EA of 96.2% and a CA of 92.5% with no VMJs (0%) and no MAJs (0%).
- A total of 108 *K. oxytoca* samples were evaluated with Levofloxacin. The combined results from clinical and challenge testing demonstrated an EA of 98.1% and a CA of 96.3% with no VMJs (0%) and no MAJs (0%).
- A total of 139 *K. pneumoniae* samples were evaluated with Levofloxacin. The combined results from clinical and challenge testing demonstrated an EA of 97.8% and a CA of 95.7% with no VMJs (0%) and no MAJs 0%).
- A total of 101 *P. aeruginosa* samples were evaluated with Levofloxacin. The combined results from clinical and challenge testing demonstrated an EA of 96.0% and a CA of 88.1% with 0 VMJs

(0%) and 0 MAJ (0%). Most of the categorical errors were minor and the EA of evaluable results was 90.0%.

Meropenem (MERO)

A total of 529 samples were evaluated with Meropenem including 393 clinical (74.3%) and 136 challenge (25.7%) samples. The combined results from clinical and challenge testing from all claimed species demonstrated an EA of 92.8 and a CA of 96.0% with 4 VMJs (2.16%) and 1 MAJs (0.30%).

Species-level performance

- A total of 78 *Acinetobacter spp.* samples were evaluated with Meropenem. The combined results from clinical and challenge testing demonstrated an EA of 96.2% and a CA of 97.4% with no VMJs (0%) and no MAJs (0%).
- A total of 139 *E. coli* samples were evaluated with Meropenem. The combined results from clinical and challenge testing demonstrated an EA of 89.9% and a CA of 97.1% with 1 VMJs (2%) and no MAJs (0%).
- A total of 111 *K. oxytoca* samples were evaluated with Meropenem. The combined results from clinical and challenge testing demonstrated an EA of 93.7% and a CA of 97.3% with 2 VMJs (16.67%) and no MAJs (0%). Due to the high VMJ rate the following limitation is proposed:
    - Perform an alternative method of testing prior to reporting results for the following antimicrobial/organism combination:
      Meropenem: *K. oxytoca* when the LifeScale MIC is 0.5 µg/mL due to two very major discrepancies.
- A total of 142 *K. pneumoniae* samples were evaluated with Meropenem. The combined results from clinical and challenge testing demonstrated an EA of 91.5% and a CA of 95.8% with 1 VMJs (1.64%) and 1 MAJs (1.28%).
- A total of 59 *P. aeruginosa* samples were evaluated with Meropenem. The combined results from clinical and challenge testing demonstrated an EA of 96.6% and a CA of 89.8% with 0 VMJs (0%) and 0 MAJ (0%). The EA of evaluable results was 94.9%.

Meropenem-vaborbactam (MEV)

A total of 442 samples were evaluated with Meropenem-vaborbactam including 331 clinical (74.9%) and 111 challenge (25.1%) samples. The combined results from clinical and challenge testing from all claimed species demonstrated an EA of 95.0% and a CA of 94.6% with no VMJs (0%) and 2 MAJs (0.55%).

Species-level performance

- A total of 139 *E. coli* samples were evaluated with Meropenem-vaborbactam. The combined results from clinical and challenge testing demonstrated an EA of 92.8% and a CA of 91.4% with no VMJs (0%) and no MAJs (0%).

- A total of 53 *K. aerogenes* samples were evaluated with Meropenem-vaborbactam. The combined results from clinical and challenge testing demonstrated an EA of 100% and a CA of 98.1% with no VMJs (0%) and no MAJs (0%).
- A total of 110 *K. oxytoca* samples were evaluated with Meropenem-vaborbactam. The combined results from clinical and challenge testing demonstrated an EA of 95.5% and a CA of 94.5% with no VMJs (0%) and no MAJs (0%).
- A total of 140 *K. pneumoniae* samples were evaluated with Meropenem-vaborbactam. The combined results from clinical and challenge testing demonstrated an EA of 95.0% and a CA of 96.4% with no VMJs (0%) and 2 MAJs (1.83%).

Piperacillin-tazobactam

A total of 665 samples were evaluated with Piperacillin-tazobactam including 354 clinical (53.2%) and 311 challenge (46.8%) samples. The combined results from clinical and challenge testing from all claimed species demonstrated an EA of 91.9% and a CA of 92.8% with 4 VMJs (1.21%) and MAJs (1.24%).

Species-level performance

- A total of 89 *A. baumannii, A. baumannii/nosocomialis group* samples were evaluated with Piperacillin-tazobactam. The combined results from clinical and challenge testing demonstrated an EA of 92.1% and a CA of 94.4% with no VMJs (0%) and no MAJs (0%).
- A total of 210 E. coli samples were evaluated with Piperacillin-tazobactam. The combined results from clinical and challenge testing demonstrated an EA of 90.5% and a CA of 94.8% with 2 VMJs (2.0%) and one MAJs (0.92%).
- A total of 181 *K. pneumoniae* samples were evaluated with Piperacillin-tazobactam. The combined results from clinical and challenge testing demonstrated an EA of 91.2% and a CA of 89% with 1 VMJs (1.02%) and 2 MAJs (2.5%). The EA of evaluable results was 52.9%, which is not acceptable. To address this unacceptable CA, the following limitation is included in the device labeling:

    - Perform an alternative method of testing prior to reporting results for: Piperacillin/tazobactam: *K. pneumoniae* when the MIC is 16 µg/mL due to the occurrence of minor errors, that were in essential agreement, resulting in a category agreement below 90%.

- A total of 185 *P. aeruginosa* samples were evaluated with Piperacillin-tazobactam. The combined results from clinical and challenge testing demonstrated an EA of 94.1% and a CA of 93.5% with 1 VMJ (1.49%) and 1 MAJ (0.89%).

Trending

In the clinical study or the Inoculum Density analytical study, the majority of drug/organism combinations tested with the LifeScale LSGN with the LifeScale AST system showed MIC values equal to or at least one doubling dilution higher than the reference method. Use caution when reporting drug resistance for any antimicrobial. The following drug/organism combinations showed high trending:

- Amikacin-*Acinetobacter* spp., *E. coli, K. aerogenes, K. oxytoca, K. pneumoniae, K. variicola, P. aeruginosa*
- Cefepime- *E. coli, K. aerogenes, K. oxytoca, K. pneumoniae*
- Ceftazidime-avibactam- *K. aerogenes, K. oxytoca*
- Gentamicin- *E. coli, K. aerogenes, K. oxytoca, K. pneumoniae*
- Levofloxacin- *K. aerogenes, K. oxytoca, K. pneumoniae*
- Meropenem- *K. oxytoca, P. aeruginosa*
- Meropenem-vaborbactam- *K. aerogenes, K. oxytoca, K. pneumoniae*
- Piperacillin-tazobactam- *K. pneumoniae*

The following drug/organism combinations showed low trending:

- Ceftazidime-avibactam - *E. coli*
- Piperacillin-tazobactam - *A. baumannii*
- Meropenem-vaborbactam - *E. coli*

Quality Control

Strains recommended by the FDA and CLSI and the new LifeScale QC strain (*Enterobacter cloacae* ABGNQC1) were tested for each antimicrobial agent evaluated using the LifeScale AST System LSGN Panel and the CLSI Reference Broth Microdilution Method. The quality control (QC) strains tested were *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, and *Enterobacter cloacae* ABGNQC1. The QC testing results for Amikacin, Cefepime, Ceftazidime-avibactam, Gentamicin, Levofloxacin, Meropenem, Meropenem-vaborbactam, and Piperacillin-tazobactam are detailed in Tables 4-11, indicating that this device can reliably yield acceptable QC results for >95% of tests. Cefepime, Levofloxacin, Meropenem, and Piperacillin-tazobactam incorporate data amalgamated from the initial study K211815 and the supplemental study, referred to as GAP 2. The clinical results for Amikacin, Ceftazidime-avibactam, Gentamicin, and Meropenem-vaborbactam with the new LifeScale LSGN Kit QC organism *Enterobacter cloacae* ABGNQC1, incorporate data from the GAP 2 study.

To validate the use of *E. cloacae* ABGNQC1 as a QC organism with the LifeScale LSGN, a total of 40 replicates were tested by at least two operators per media lot, spanning over a period of at least three days. A minimum of two media lots were tested, resulting in at least 80 data points using the LifeScale AST and the CLSI Reference Broth Microdilution Method. This validation study was done to establish expected QC MIC ranges for Amikacin, Ceftazidime-avibactam, Gentamicin, and Meropenem-vaborbactam. In the clinical study, an additional 92 data points were tested by BMD for a total of 467 data points with >95% in-range, which supported the expected QC ranges of *E. cloacae* ABGNQC1 for Amikacin, Ceftazidime-avibactam, Gentamicin, and Meropenem-vaborbactam in the LifeScale LSGN clinical study.

Amikacin

QC Organism	**Expected Range (µg/mL)**	Conc. µg/mL	Reference Frequency	New Device Test Frequency

		Sites →	Ref Lab (ABIO)	ABIO	LACNY	MCW	TRI
Enterobacter cloacae ABGNQC1	8 – 32	<=4					
		8		15	8	7	2
		16	15	34	13	13	22
		32	8			3	
		64					
		128					
		256					
		>256					
Total			**23/23 (100%)**	**49/49 (100%)**	**21/21 (100%)**	**23/23 (100%)**	**24/24 (100%)**

Table 4. LifeScale AST System QC MIC Distribution for Amikacin

Ceftazidime-avibactam

QC Organism	**Expected Range (µg/mL)**	Conc. µg/mL	Reference Frequency	New Device Test Frequency			
		Sites →	**Ref Lab (ABIO)**	**ABIO**	**LACNY**	**MCW**	**TRI**
Enterobacter cloacae ABGNQC1	<=2-8	<=2	9	1	1	2	
		4	14	16	19	18	10
		8		32	1	3	14
		16					
		32					
		>32					
Total			**23/23 (100%)**	**49/49 (100%)**	**21/21 (100%)**	**23/23 (100%)**	**24/24 (100%)**

Table 5. LifeScale AST System QC MIC Distribution for Ceftazidime-avibactam

Cefepime

QC Organism	**Expected Range (µg/mL)**	Conc. µg/mL	Reference Frequency		New Device Test Frequency					
		Sites →	**Ref Lab (TRI)**	**Ref Lab (ABIO)**	**ABIO**	**IND**	**JHM**	**LACNY**	**MCW**	**TRI**
Pseudomonas aeruginosa ATCC 27853	0.5-4	<=0.5					1	1		
		1	47	15	36	13	34	28	14	15
		2	36	6	12	9	18	31	12	11

		4	6	1		2		3	1	
		8								
		16								
		32								
		64								
		>64								
Total			**89/89 (100%)**	**22/22 (100%)**	**48/48 (100%)**	**24/24 (100%)**	**53/53 (100%)**	**63/63 (100%)**	**27/27 (100%)**	**26/26 (100%)**

Table 6. LifeScale AST System QC MIC Distribution for Cefepime

****Does not include full CLSI expected range. LifeScale MIC results of ≤0.5 µg/mL were considered acceptable.***

Gentamicin

QC Organism	**Expected Range (µg/mL)**	Conc. µg/mL	Reference Frequency	New Device Test Frequency			
		Sites →	**Ref Lab (ABIO)**	**ABIO**	**LACNY**	**MCW**	**TRI**
Enterobacter cloacae ABGNQC1	8-32	<=1					
		2					
		4					
		8		3		2	
		16	18	46	19	16	24
		32	5		2	4	
		>32					
Total			**23/23 (100%)**	**49/49 (100%)**	**21/21 (100%)**	**22/22 (100%)**	**24/24 (100%)**

Table 7. LifeScale AST System QC MIC Distribution for Gentamicin

Levofloxacin

QC Organism	**Expected Range (µg/mL)**	Conc. µg/mL	Reference Frequency		New Device Test Frequency					
		Sites →	**Ref Lab (TRI)**	**Ref Lab (ABIO)**	**ABIO**	**IND**	**JHM**	**LACNY**	**MCW**	**TRI**
Pseudomonas aeruginosa ATCC 27853	0.5-4	<=0.25	4							
		0.5	55	17	48	20	48	62	13	23
		1	26	5	1	4	4	1	11	3
		2	4						2	

		4					1			
		8								
		16							1	
		>16								
Total			**85/89 (95.5%)**	**22/22 (100%)**	**49/49 (100%)**	**24/24 (100%)**	**53/53 (100%)**	**63/63 (100%)**	**26/27 (96.3%)**	**26/26 (100%)**

Table 8. LifeScale AST System QC MIC Distribution for Levofloxacin

Meropenem

QC Organism	**Expected Range (µg/mL)**	Conc. µg/mL	Reference Frequency		New Device Test Frequency					
		Sites →	**Ref Lab (TRI)**	**Ref Lab (ABIO)**	**ABIO**	**IND**	**JHM**	**LACNY**	**MCW**	**TRI**
**Pseudomonas aeruginosa* ATCC 27853	0.12-1	<=0.12					1			
		0.25	65	18	48	24	52	58	22	26
		0.5	18	4	1			1	4	
		1	3					1		
		2								
		4						2	1	
		8						1		
		16								
		>16								
Total			**86/86 (100%)**	**22/22 (100%)**	**49/49 (100%)**	**24/24 (100%)**	**53/53 (100%)**	**60/63 (95.2%)**	**26/27 (96.3%)**	**26/26 (100%)**

Table 9. LifeScale AST System QC MIC Distribution for Meropenem

****Does not include full CLSI expected range. LifeScale MIC results of ≤0.12 µg/mL were considered acceptable.***

Meropenem-vaborbactam

QC Organism	**Expected Range (µg/mL)**	Conc. µg/mL	Reference Frequency	New Device Test Frequency

		Sites →	Ref Lab (ABIO)	ABIO	LACNY	MCW	TRI
Enterobacter cloacae ABGNQC1	4-16	<=0.5				2	
		1					
		2					
		4	7				
		8	16	40	14	11	23
		16		9	7	9	1
		>16					
Total			**23/23 (100%)**	**49/49 (100%)**	**21/21 (100%)**	**21/23 (91.3%)**	**24/24 (100%)**

Table 10. LifeScale AST System QC MIC Distribution for Meropenem-vaborbactam

Piperacillin-tazobactam

QC Organism	Expected Range (µg/mL)	Conc. µg/mL	Reference Frequency		New Device Test Frequency					
		Sites →	Ref Lab (TRI)	Ref Lab (ABIO)	ABIO	IND	JHM	LACNY	MCW	TRI
Klebsiella pneumoniae ATCC 700603	8 - 32	<=4								
		8	3		182	24	47	67	44	47
		16	70	48		1	2	2	2	
		32	10	4						
		64								
		128								
		256								
		>256								
Total			**83/83 (100%)**	**52/52 (100%)**	**182/182 (100%)**	**25/25 (100%**	**49/49 (100%)**	**69/69 (100%)**	**46/46 (100%)**	**47/47 (100%)**

Table 11. LifeScale AST System QC MIC Distribution for Piperacillin-tazobactam

Removal of limitations included in K211815

Additional clinical evaluation testing was performed to support the removal of limitations included in in the cleared LifeScale LSGN Kit with the LifeScale AST system (K211815) for Aztreonam, Ceftazidime, Ertapenem, and Cefazolin.

	Total Evaluated	No. EA	EA%	Eval Tot	No. Eval EA	Eval EA%	No. CA	CA%	No. R	No. S	#MIN (MIN%)	#MAJ (MAJ%)	#VMJ (VMJ%)

Ertapenem - *E. coli* [Breakpoints (µg/mL): 0.5 (S), 2.0 (R)]													
Off-line Combined	94	85	90.4%	12	3	25%	85	90.4%	20	74	8 (8.51%)	1 (1.35%)	0 (0.00%)
On-line Combined	108	105	97.2%	5	2	40%	104	96.3%	60	48	3 (2.78%)	1 (2.08%)	0 (0.00%)
[a] Aztreonam - *K. pneumoniae* [Breakpoints (µg/mL): 4.0 (S), 16.0 (R)]													
Combined	160	144	90	21	5	23.8	151	94.4	76	83	6 (3.75%)	2 (2.41%)	1 (1.32%)
[a, b] Ceftazidime - *K. pneumoniae* [Breakpoints (µg/mL): 4.0 (S), 16.0 (R)]													
Combined	160	154	96.3	22	16	72.7	155	96.9	79	79	2 (1.25%)	2 (2.53%)	1 (1.27%)
Ceftazidime - *A. baumannii, A. baumannii/A. nosocomialis group, A. calcoaceticus, A. lwoffii, A. pitti, A. radioresistens, and Acinetobacter spp.* [Breakpoints (µg/mL): 8 (S), 32 (R)]													
Combined	118	112	94.9	58	52	89.7	116	98.3	63	52	2 (1.69%)	0 (0.00%)	0 (0.00%)
[b] Cefazolin - *E. coli* [Breakpoints (µg/mL): 2.0 (S), 8.0 (R)]													
Combined	203	197	97.0	102	96	94.1	178	87.7	117	73	22 (10.83%)	1 (1.37%)	2 (1.71%)
[a, b] Ertapenem - *K. pneumoniae* [Breakpoints (µg/mL): 0.5 (S), 2.0 (R)]													
Combined	158	154	97.5	13	9	69.2	154	97.5	71	85	3 (1.90%)	0 (0.00%)	1 (1.41%)

Table 12. LifeScale LSGN performance for the removal of limitations included in K211815: Interpretation of MIC results are based on FDA Susceptibility Test Interpretative Criteria (STIC) and the 34th edition of the CLSI M100

[a] K. pneumoniae tested with aztreonam, ceftazidime and ertapenem and incubated on-line and K. pneumoniae tested with ertapenem and incubated off-line showed MIC values equal to or at least one doubling dilution higher than the reference method.

[b] The very major error rate was increased for the following:

- *Ceftazidime/K. pneumoniae incubated on-line (2.6%)*
- *Cefazolin/E. coli incubated off-line (2.3%)*
- *Ertapenem/K. pneumoniae incubated off-line (2.9%)*

[c] K. pneumoniae tested with ertapenem showed MIC values equal to or at least one doubling dilution higher than the reference method.

Ertapenem/*E. coli* – Removal of Off-line Limitation

In K211815, evaluation of the performance of on-line and off-line incubation of panels testing Ertapenem/*E. coli* showed unacceptable performance for this drug/organism combination when incubated offline. The following limitation was included in the device labeling:

> *Due to unacceptable performance of Ertapenem/E. coli with incubation in an off-line incubator, perform an alternative method of testing prior to reporting results for Ertapenem/E. coli when panels are incubated in an off-line incubator.*

The current submission provided additional data for both incubation options. The performance for the initial (K211815) and additional data combined for both on-line and off-line incubation is acceptable, and the above limitation was removed from the device labeling.

Aztreonam/*K. pneumoniae*

In K211815, the data for testing Aztreonam/*K. pneumoniae* which showed unacceptable performance (low EA and increased major errors). The following limitation was included in the device labeling:

> *Perform an alternative method of testing prior to reporting results for the following antimicrobial/organism combination(s): Aztreonam/K. pneumoniae*

The current submission included data from testing 54 additional *K. pneumoniae* isolates (seeded clinical and seeded challenge). The performance for the initial (K211815) and additional data combined for Aztreonam/*K. pneumoniae* is acceptable, and the above limitation was removed from the device labeling.

Ceftazidime/*K. pneumoniae*

In K211815, the data for testing Ceftazidime/*K. pneumoniae* which showed unacceptable performance (increased major errors). The following limitation was included in the device labeling:

> *Perform an alternative method of testing prior to reporting results for the following antimicrobial/organism combination(s): Ceftazidime/K. pneumoniae*

The current submission included data from testing 54 additional *K. pneumoniae* isolates (seeded clinical and seeded challenge). The performance for the initial (K211815) and additional data combined for Ceftazidime/*K. pneumoniae* is acceptable, and the above limitation was removed from the device labeling.

Ceftazidime/*Acinetobacter* spp.

In K211815, the data for testing Ceftazidime with a variety of *Acinetobacter* spp. Performance for *A. baumannii* and *A. baumannii/A. nosocomialis* group was acceptable but combined testing of other *Acinetobacter* spp. showed unacceptable performance (increased very major errors). The following limitation was included in the device labeling:

> *Perform an alternative method of testing prior to reporting results for the following antimicrobial/organism combination(s): Ceftazidime/Acinetobacter spp. (other than A. baumannii and A. baumannii/nosocomialis group).*

The current submission indicates that the unacceptable percentage of very major errors was due to very major errors observed only with *Acinetobacter ursignii*. The limitation could be revised to limit reporting to *Acinetobacter* spp. other than *A. ursignii*. The performance data for all *Acinetobacter* spp. including *A. baumannii, A. baumannii/A. nosocomialis group, A. calcoaceticus, A. lwoffii, A. pitti, A. radioresistens*,

and *Acinetobacter* spp. showed acceptable performance. The following revised limitation was included in the device labeling:

> *Perform an alternative method of testing prior to reporting results for the following antimicrobial/organism combination(s): Ceftazidime/Acinetobacter ursingii*

Cefazolin/*E. coli*

In K211815, the data for testing for testing Cefazolin/*E. coli* which showed unacceptable performance. The following limitation was included in the device labeling:

> *Perform an alternative method of testing prior to reporting results for the following antimicrobial/organism combination(s): Cefazolin/E. coli*

The current submission included data from testing 66 additional *E. coli* isolates (seeded clinical and seeded challenge). The performance for the initial (K211815) and additional data combined for Cefazolin/*E. coli* is acceptable, and the above limitation was removed from the device labeling.

Ertapenem/*K. pneumoniae*

In K211815, the data for testing for testing Ertapenem/*E. coli* which showed unacceptable performance (due to increased very major errors). The following limitation was included in the device labeling:

> *Perform an alternative method of testing prior to reporting results for the following antimicrobial/organism combination(s): Ertapenem/K. pneumoniae*

The current submission included data from testing 54 additional *K. pneumoniae* isolates (seeded clinical and seeded challenge). The performance for the initial (K211815) and additional data combined for Ertapenem/*E. coli* is acceptable, and the above limitation was removed from the device labeling.

Analytical Performance Data

Reproducibility

Initial Study Overview

Reproducibility was performed with well-characterized on-scale strains. Each strain was spiked from pure culture to a BACTEC Standard Aerobic bottle. Once the blood culture bottles flagged positive, 12 aliquots were generated from each positive blood culture bottle. Aliquots were label-coded and shipped to three testing sites. Test organisms were blind-coded, and the identification of the organism was unknown to the three test sites. Each day for three days, three replicates of each test organism from a single positive blood culture were aliquoted within a few hours of the BACTEC instrument positive indication and tested at the three sites within 12 hours of the positive flag.
Upon sample receipt of the positive blood samples at each test site, the samples were processed for testing according to the LifeScale LSGN protocol. Purity verification for each LifeScale LSGN plate was

performed, and plates were incubated on or off-line as dictated by the LifeScale AST system workflow. LifeScale LSGN plates were read at three hours, or when the LifeScale AST system determined the growth threshold in the positive control well was acceptable.

Supplemental Study Overview

The purpose of the supplemental study was to verify that the LifeScale LSGN Kit with the LifeScale AST System provides acceptable reproducibility of on-scale MIC results for certain antimicrobials. There was an insufficient number of isolates tested for certain antibiotics. This report includes the additional testing performed for Cefepime, Ceftazidime-avibactam, and Meropenem.

All additional testing was performed at Affinity at three distinct sites within the facility. Each strain was tested in triplicate at the three internal sites, on three consecutive days. For each antibiotic, reproducibility was evaluated specific to the essential agreement between MIC results and the mode of MIC value for each strain. Data from the supplemental testing was combined with the data from the initial study.

Results and Discussion

Best-case (≥95%) and worst-case (>89%) reproducibility was acceptable for all antibiotics tested (Amikacin, Cefepime, Ceftazidime-avibactam, Gentamicin, Levofloxacin, Meropenem, Meropenem-vaborbactam, and Piperacillin-tazobactam). Inter-site and intra-site reproducibility data was found to be acceptable for all antimicrobial agents. Combined Site reproducibility results for the initial and supplemental studies are summarized in Table 13.

Table 13. Reproducibility Data for the LifeScale LSGN Panel*

Antimicrobial	Best Case No. Within 1 +/- Dil/ Total Tests (%)	Worst Case No. Within 1 +/- Dil/ Total Tests (%)
Amikacin	Best case: 468/481 = 97.3%	Worst case: 450/481 = 93.6%
Cefepime	Best case: 504/509 = 99%	Worst case: 459/509 = 90.2%
Ceftazidime-avibactam	Best case: 345/350 = 98.6%	Worst case: 339/350= 96.9%
Gentamicin	Best case: 503/509 = 98.8%	Worst case: 466/509 = 91.6%
Levofloxacin	Best case: 373/373 = 100%	Worst case: 361/373 = 96.8%
Meropenem	Best case: 508/534 = 95.1%	Worst case: 477/534 = 89.3%
Meropenem-vaborbactam	Best case: 274/287 = 95.5%	Worst case: 261/287 = 90.9%
Piperacillin-tazobactam	Best case: 555/566 = 98.1%	Worst case: 521/566 = 92.0%

Sample Stability

Organisms tested:

- *Escherichia coli* (6 Strains)
- *Klebsiella pneumoniae* (3 Strains)
- *Pseudomonas aeruginosa* (2 Strains)
- *Acinetobacter baumannii* (2 Strains)

Blood Bottles Tested:

- BD BACTEC™: Standard Aerobic

Study Outline

To demonstrate sample stability at 12 hours, post positivity, spiked positive blood cultures were tested with the LifeScale LSGN panel at the following time intervals:

- Within one hour of the bottle being flagged as positive, T_0.
- 13 +/- 0.5 hour after bottle flagged as positive, T_{13}, when stored at 35 °C

The purpose of this study was to demonstrate that positive blood cultures tested at 12 hours post positivity (T_{12}) and held at incubation temperature (35 °C) are equivalent to results obtained at the time of positivity (T_0). Contrived positive blood culture specimens (prepared in BD Standard Aerobic blood culture bottles) containing the recommended blood volume were tested within one hour of bottle ring (T_0) and at T_{13} ± 0.5 hours post bottle ring (T_{13}). Testing at 13 hours was performed to support the stability at 12 hours post bottle ring as listed in the device labeling. Testing was performed in triplicate for each sample at each time point. Resulting MICs were compared to the modal LifeScale MIC determined at T_0. The stability acceptance criterion was ≥ 95% agreement for EA. QC was performed on each day of testing.

Table 14. Summary of Sample Stability AST Results at T_{13} as Compared to LifeScale LSGN Mode at T_0

Antibiotic	No. Samples Tested	Data Points		LifeScale T_{13} to LifeScale Mode T_0
		T_0	T_{13}	No. EA/Total Tested (EA%)
Amikacin	13	39	39	36/39* (92.31%)
Cefepime	11	33	33	31/32 (96.88%)
Ceftazidime-avibactam	11	33	33	33/33 (100.00%)
Gentamicin	11	33	33	33/33 (100.00%)
Levofloxacin	11	33	33	33/33 (100.00%)
Meropenem	13	39	39	36/39* (92.31%)
Meropenem-vaborbactam	9	27	27	24/27* (88.89%)
Piperacillin-tazobactam	13	39	39	38/39 (97.44%)

TOTAL	**92**	**276**	**276**	**264/275 (96.00%)**

* Comparison to the BMD reference mode MIC resulted in an EA of 100%.

Results and Discussion

Results for Cefepime, Ceftazidime-avibactam, Gentamicin, Levofloxacin, and Piperacillin-tazobactam at T_{13} meet acceptance criteria essential agreement to the mode of the MIC results obtained at T_0, supporting the 12-hour stability claim. The essential agreement for Amikacin, Meropenem, Meropenem/vaborbactam were not acceptable (EA <95%). When comparing the LifeScale MICs at T0 and T13 to the BMD reference mode MIC, the overall EA for these antimicrobials resulted was 100%. This is noted in a footnote in the device labeling.

To address the timing of positive blood culture processing, the following limitation included in the original 510(k) pre-market submission (K211815), should remain in the device labeling:

> *Positive blood cultures must be processed immediately on the LifeScale AST system or within 12 hours of blood culture bottle positivity should delays be unavoidable.*

Inoculum Density

Inoculum Verification

Organisms tested:

- *Escherichia coli* (6)
- *Klebsiella pneumoniae* (3)
- *Pseudomonas aeruginosa* (2)
- *Acinetobacter baumannii* (2)

Study Outline

This study demonstrates that the concentration of organisms in a positive blood culture does not impact LifeScale AST system performance and that the dilution function of the LifeScale AST system provides the appropriate inoculum for the assay. In blood cultures, at the time of positivity, organism concentration is estimated to be near 10^8 CFU/mL, with a range of approximately one Log greater or less. At the time a blood culture is flagged positive, concentrations below 10^7 CFU/ml are not likely. Higher concentrations may be observed if the blood culture incubates for an extended time after being flagged as positive. A secondary objective is to confirm that the LifeScale System terminates the test in cases of extremely low inoculum concentrations for which growth does not meet the required threshold after incubation. Contrived positive blood culture samples from thirteen strains were tested at target organism concentrations of 10^6 CFU/mL and 10^9 CFU/mL to challenge the system beyond the range of expected blood culture organism concentrations. Blood culture was also seeded with slow-growing organisms at target concentrations of 10^4 CFU/mL to verify that the LifeScale AST system, tests are terminated according to system specification when sufficient organism growth is not detected.
The slow growth rate of *Pseudomonas aeruginosa* combined with extremely low organism concentration provided a worst-case test of organism growth in the LifeScale AST system.

Results and Discussion

MIC and final AST results were generated by the LifeScale LSGN Kit with the LifeScale AST System for each strain and antibiotic and were compared to Broth Microdilution (BMD) Reference mode MIC data for the tested strains. Data from each inoculum level were compared to the Reference mode.
This study demonstrates that the concentration of organisms in a positive blood culture does not impact LifeScale LSGN performance and that the dilution function of the LifeScale AST system provides the appropriate inoculum for the assay. In blood cultures, at the time of positivity, organism concentration is estimated to be near 10^8 CFU/ml, with a range of approximately one Log greater or less. At the time a blood culture is flagged positive, concentrations below 10^7 CFU/ml are not likely. Higher concentrations may be observed if the blood culture incubates for an extended time after being flagged as positive. A secondary objective is to confirm that the LifeScale System terminates the test in cases of extremely low inoculum concentrations for which growth does not meet the required threshold after incubation.
Contrived positive blood culture samples from thirteen strains were tested at target organism concentrations of 10^6 CFU/ml and 10^9 CFU/ml to challenge the system beyond the range of expected blood culture organism concentrations. Blood culture was also seeded with slow-growing organisms at target concentrations of 10^4 CFU/ml to verify that the LifeScale AST system, tests are terminated according to system specification when sufficient organism growth is not detected.
The slow growth rate of *Pseudomonas aeruginosa* combined with extremely low organism concentration provided a worst-case test of organism growth in the LifeScale AST system.

Summary of Results at 10^6

E. coli and *P. aeruginosa* showed MICs exceeding the essential agreement acceptance criteria when compared to the reference method for most antimicrobials.

K. pneumoniae strains exhibited variable results (specifically strain JMI1083162) for some of the drugs, showing discrepancies in essential agreement (EA) compared to the reference mode.

Gentamicin, levofloxacin, and meropenem generally showed good agreement with the reference mode across all tested strains, though there were occasional discrepancies.

Piperacillin-tazobactam exhibited variable results for 10^6 but performed well at 10^9.

Summary of Results at 10^9

Overall, all tested strains demonstrated MICs within EA to the reference mode across the various drugs, indicating improved agreement compared to the lower inoculum density.

These findings suggest that the higher inoculum density at (10^9) result in better essential agreement between the LifeScale method and the reference mode for determining MICs of antimicrobial agents against certain strains.

Inoculum Density Performance Data for LifeScale when compared to the Reference BMD

AMIKACIN (AMI)		Performance Data									
Antibiotic	**Genus/Species**	**Incubation Category**	**#Samples**	**#Organisms**	**#Evaluable**	**EA%**	**EA% Evaluable**	**CA%**	**#VMJ (VMJ%)**	**#MAJ (MAJ%)**	**#MIN (MIN%)**
Amikacin	*Escherichia coli*	10e6 CFU/mL	17	6	6	94.1%	83.3%	88.2%	0 (0.00%)	0 (0.00%)	2 (11.76%)
Amikacin	*Escherichia coli*	10e9 CFU/mL	18	6	6	100.0%	100.0%	66.7%	0 (0.00%)	0 (0.00%)	6 (33.33%)
Amikacin	*Acinetobacter baumannii*	10e6 CFU/mL	6	2	6	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Amikacin	*Acinetobacter baumannii*	10e9 CFU/mL	6	2	6	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Amikacin	*Klebsiella pneumoniae*	10e6 CFU/mL	4	2	3	75.0%	66.7%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Amikacin	*Klebsiella pneumoniae*	10e9 CFU/mL	7	3	1	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Amikacin	*Pseudomonas aeruginosa*	10e6 CFU/mL	5	2	2	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Amikacin	*Pseudomonas aeruginosa*	10e9 CFU/mL	6	2	3	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)

Table 15. Inoculum Density Performance Data, Amikacin. The following drug/organism combinations showed high tending:
Amikacin/E. coli at 10^9 CFU/mL

CEFEPIME (FEP)		Performance Data									
Antibiotic	**Genus/Species**	**Incubation Category**	**#Samples**	**#Organisms**	**#Evaluable**	**EA%**	**EA% Evaluable**	**CA%**	**#VMJ (VMJ%)**	**#MAJ (MAJ%)**	**#MIN (MIN%)**
Cefepime	*Escherichia coli*	10e6 CFU/mL*	17	6	3	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Cefepime	*Escherichia coli*	10e9 CFU/mL*	18	6	3	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Cefepime	*Klebsiella pneumoniae*	10e6 CFU/mL	4	2	4	75.0%	75.0%	75.0%	1 (25.00%)	0 (0.00%)	0 (0.00%)
Cefepime	*Klebsiella pneumoniae*	10e9 CFU/mL	7	3	4	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Cefepime	*Pseudomonas aeruginosa*	10e6 CFU/mL	5	2	5	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Cefepime	*Pseudomonas aeruginosa*	10e9 CFU/mL	6	2	6	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)

Table 16. Inoculum Density Performance Data, Cefepime.

** The LifeScale MIC values tended to be equal to or at least one doubling dilution higher than the reference broth microdilution method*

Ceftazidime-avibactam (CAZ)		Performance Data									
Antibiotic	**Genus/Species**	**Incubation Category**	**#Samples**	**#Organisms**	**#Evaluable**	**EA%**	**EA% Evaluable**	**CA%**	**#VMJ (VMJ%)**	**#MAJ (MAJ%)**	**#MIN (MIN%)**
Ceftazidime-avibactam	*Escherichia coli*	10e6 CFU/mL*	17	6	3	94.1%	66.7%	94.1%	0 (0.00%)	0 (0.00%)	0 (0.00%)

Ceftazidime-avibactam	*Escherichia coli*	10e9 CFU/mL	18	6	3	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Ceftazidime-avibactam	*Klebsiella pneumoniae*	10e6 CFU/mL	4	2	0	100.0%	N/A	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Ceftazidime-avibactam	*Klebsiella pneumoniae*	10e9 CFU/mL	7	3	0	100.0%	N/A	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Ceftazidime-avibactam	*Pseudomonas aeruginosa*	10e6 CFU/mL	5	2	5	60.0%	60.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Ceftazidime-avibactam	*Pseudomonas aeruginosa*	10e9 CFU/mL	6	2	6	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)

Table 17. Inoculum Density Performance Data, Ceftazidime-avibactam.

**The LifeScale MIC values tended to be equal to or at least one doubling dilution lower than the reference broth microdilution method.*

GENTAMICIN (GEN)		Performance Data									
Antibiotic	**Genus/Species**	**Incubation Category**	**#Samples**	**#Organisms**	**#Evaluable**	**EA%**	**EA% Evaluable**	**CA%**	**#VMJ (VMJ%)**	**#MAJ (MAJ%)**	**#MIN (MIN%)**
Gentamicin	*Escherichia coli*	10e6 CFU/mL	17	6	9	100.0%	100.0%	94.1%	0 (0.00%)	0 (0.00%)	1 (5.88%)
Gentamicin	*Escherichia coli*	10e9 CFU/mL*	18	6	9	100.0%	100.0%	72.2%	0 (0.00%)	0 (0.00%)	5 (27.78%)
Gentamicin	*Klebsiella pneumoniae*	10e6 CFU/mL	4	2	3	75.0%	66.7%	75.0%	0 (0.00%)	0 (0.00%)	1 (25.00%)
Gentamicin	*Klebsiella pneumoniae*	10e9 CFU/mL	7	3	1	100.0%	100.0%	85.7%	0 (0.00%)	0 (0.00%)	1 (14.29%)
Gentamicin	*Pseudomonas aeruginosa*	10e6 CFU/mL	5	2	5	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Gentamicin	*Pseudomonas aeruginosa*	10e9 CFU/mL	6	2	6	100.0%	100.0%	66.7%	0 (0.00%)	0 (0.00%)	2 (33.33%)

Table 18. Inoculum Density Performance Data, Gentamicin.

** The LifeScale MIC values tended to be equal to or at least one doubling dilution higher than the reference broth microdilution method*

LEVOFLOXACIN (LEVO)		Performance Data									
Antibiotic	**Genus/Species**	**Incubation Category**	**#Samples**	**#Organisms**	**#Evaluable**	**EA%**	**EA% Evaluable**	**CA%**	**#VMJ (VMJ%)**	**#MAJ (MAJ%)**	**#MIN (MIN%)**
Levofloxacin	*Escherichia coli*	10e6 CFU/mL	17	6	6	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Levofloxacin	*Escherichia coli*	10e9 CFU/mL	18	6	6	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Levofloxacin	*Klebsiella pneumoniae*	10e6 CFU/mL	4	2	1	100.0%	100.0%	75.0%	0 (0.00%)	0 (0.00%)	1 (25.00%)
Levofloxacin	*Klebsiella pneumoniae*	10e9 CFU/mL	7	3	6	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Levofloxacin	*Pseudomonas aeruginosa*	10e6 CFU/mL	5	2	5	100.0%	100.0%	40.0%	0 (0.00%)	0 (0.00%)	3 (60.00%)
Levofloxacin	*Pseudomonas aeruginosa*	10e9 CFU/mL	6	2	6	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)

Table 19. Inoculum Density Performance Data, Levofloxacin

MEROPENEM (MERO)		Performance Data									
Antibiotic	**Genus/Species**	**Incubation Category**	**#Samples**	**#Organisms**	**#Evaluable**	**EA%**	**EA% Evaluable**	**CA%**	**#VMJ (VMJ%)**	**#MAJ (MAJ%)**	**#MIN (MIN%)**
Meropenem	*Escherichia coli*	10e6 CFU/mL	217	6	0	100.0%	N/A	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Meropenem	*Escherichia coli*	10e9 CFU/mL	18	6	0	100.0%	N/A	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Meropenem	*Acinetobacter baumannii*	10e6 CFU/mL	5	2	3	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Meropenem	*Acinetobacter baumannii*	10e9 CFU/mL	6	2	3	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Meropenem	*Klebsiella pneumoniae*	10e6 CFU/mL	4	2	4	75.0%	75.0%	25.0%	0 (0.00%)	0 (0.00%)	3 (75.00%)
Meropenem	*Klebsiella pneumoniae*	10e9 CFU/mL	7	3	4	85.7%	75.0%	71.4%	0 (0.00%)	0 (0.00%)	2 (28.57%)
Meropenem	*Pseudomonas aeruginosa*	10e6 CFU/mL	5	2	5	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Meropenem	*Pseudomonas aeruginosa*	10e9 CFU/mL	6	2	6	100.0%	100.0%	50.0%	0 (0.00%)	0 (0.00%)	3 (50.00%)

Table 20. Inoculum Density Performance Data, Meropenem.

MEROPENEM-VABORBACTAM (MEV)		Performance Data									
Antibiotic	**Genus/Species**	**Incubation Category**	**#Samples**	**#Organisms**	**#Evaluable**	**EA%**	**EA% Evaluable**	**CA%**	**#VMJ (VMJ%)**	**#MAJ (MAJ%)**	**#MIN (MIN%)**
Meropenem-vaborbactam	*Escherichia coli*	10e6 CFU/mL	17	6	0	100.0%	N/A	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Meropenem-vaborbactam	*Escherichia coli*	10e9 CFU/mL	18	6	0	100.0%	N/A	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Meropenem-vaborbactam	*Klebsiella pneumoniae*	10e6 CFU/mL	4	2	4	50.0%	50.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Meropenem-vaborbactam	*Klebsiella pneumoniae*	10e9 CFU/mL	7	3	4	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)

Table 21. Inoculum Density Performance Data, Meropenem-vaborbactam.

PIPERACILLIN-TAZOBACTAM (P/T)	Performance Data

Antibiotic	Genus/Species	Incubation Category	#Samples	#Organisms	#Evaluable	EA%	EA% Evaluable	CA%	#VMJ (VMJ%)	#MAJ (MAJ%)	#MIN (MIN%)
Piperacillin-tazobactam	*Escherichia coli*	10e6 CFU/mL	29	8	17	86.2%	76.5%	96.6%	0 (0.00%)	1 (5.88%)	0 (0.00%)
Piperacillin-tazobactam	*Escherichia coli*	10e9 CFU/mL*	18	6	7	94.4%	85.7%	88.9%	0 (0.00%)	0 (0.00%)	2 (11.11%)
Piperacillin-tazobactam	*Acinetobacter baumannii*	10e6 CFU/mL	5	2	2	100.0%	100.0%	60.0%	0 (0.00%)	0 (0.00%)	2 (40.00%)
Piperacillin-tazobactam	*Acinetobacter baumannii*	10e9 CFU/mL	6	2	6	66.7%	66.7%	50.0%	0 (0.00%)	0 (0.00%)	3 (50.00%)
Piperacillin-tazobactam	*Klebsiella pneumoniae*	10e6 CFU/mL	16	4	13	93.8%	92.3%	62.8%	0 (0.00%)	0 (0.00%)	3 (31.25%)
Piperacillin-tazobactam	*Klebsiella pneumoniae*	10e9 CFU/mL	7	3	3	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Piperacillin-tazobactam	*Pseudomonas aeruginosa*	10e6 CFU/mL	25	5	25	92.0%	92.0%	92.0%	2 (8.00%)	0 (0.00%)	0 (0.00%)
Piperacillin-tazobactam	*Pseudomonas aeruginosa*	10e9 CFU/mL	6	2	6	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)

Table 22. Inoculum Density Performance Data, Piperacillin-tazobactam.

** The LifeScale MIC values tended to be equal to or at least one doubling dilution higher than the reference broth microdilution method*

Media Equivalence

LifeScale LSGN Blood Bottle Compatibility
Organisms tested:

- *Escherichia coli* (5 Strains)
- *Klebsiella pneumoniae* (3 Strains)
- *Pseudomonas aeruginosa* (2 Strains)
- *Acinetobacter baumannii* (2 Strains)

Blood Bottles Tested:

- BD BACTEC™: Standard Aerobic, Standard Anaerobic, Plus Aerobic, Lytic Anaerobic
- BacT/ALERT™: Standard Aerobic, Standard Anaerobic
- VersaTREK™: REDOX 1 Aerobic media, REDOX 2 Anaerobic media

Study Outline

A minimum of 12 strains were tested using each blood culture media type. Testing was performed on replicates of ten (10) per organism/media type. Each organism tested was spiked into the blood culture media to be tested, at a target concentration of 1,000-10,000 CFU per mL. Testing was conducted with the eight media types listed above.

BACTEC bottles were placed in the automated blood culture instrument. The remaining blood bottle types were incubated off-line at 35 °C +/- 2 °C. The VersaTREK Redox2 bottle was not mixed in accordance with the VersaTREK IFU. A VersaTREK connector was attached to the VersaTREK Redox1 bottle, which is required for incubation on the VersaTREK system to detect pressure changes and prevent gas build up. It was used in this study to prevent dangerous gas build up within the mixing VersaTREK REDOX bottles. The connectors were inserted in the bottle top prior to incubation and removed after incubation.

The BACTEC incubation time was used as a guide to incubation time for the equivalent BacT/ALERT and VersaTREK bottles. Prior to LifeScale AST testing, cell counts were performed on LifeScale AST to determine whether these bottles had reached bottle ring concentration (10^8 CFU/ml). BacT/ALERT and VersaTREK bottles for which LifeScale AST cell counts indicate concentrations less than bottle ring concentrations, (10^8 CFU/ml), were returned to the off-line incubator within 20 minutes.

All susceptibility testing was performed using the LifeScale LSGN Kit with the LifeScale AST System direct from spiked positive blood cultures, per the LifeScale LSGN Instructions for Use.

A single LifeScale LSGN panel was prepared from each positive blood culture bottle. LifeScale LSGN plates that were incubated on the LifeScale AST system were read automatically approximately 3 hours from incubation or when sufficient growth had taken place. Plates incubated off-line were read when the LifeScale AST system called for them in accordance with the LifeScale LSGN Instructions for Use.

Since all other analytical studies used a single bottle type (i.e., BD BACTEC Standard Aerobic Media), and all bottle types were tested in the clinical study, results from all MICs from each media/antimicrobial were directly compared to LifeScale AST modal MICs from the BD BACTEC Standard Aerobic Media (i.e., the "comparator").

Essential Agreement (EA) was calculated and compared to the LifeScale AST mode MIC results from the BD BACTEC Standard Aerobic Media, for each antimicrobial and blood culture media.

An EA agreement of ≥95% for the various blood culture media compared to LifeScale AST mode MICs from the BD BACTEC Standard Aerobic Media for each antimicrobial confirms that the different blood culture media do not affect the MIC results.

Results and Discussion

The performance of various blood bottle types was evaluated for Amikacin, Cefepime, Ceftazidime-avibactam, Gentamicin, Levofloxacin, Meropenem, Meropenem-vaborbactam and Piperacillin-tazobactam.

A total of 888 samples were tested on the LifeScale AST system. There were 10 "Failed" samples whose measurement failed to complete. There were 4 "Unreported" occurrences when the measurement of a plate was completed but the system could not give an MIC for a specific antibiotic due to a failed measurement of individual wells, an empty well, or an anomalous growth pattern. There were a total of 90 withdrawn samples due to *Acinetobacter* and *Pseudomonas* data collected with Anaerobic blood bottles.

All blood culture media demonstrated ≥95% EA for each antimicrobial when compared to the LifeScale AST modal MICs from the BD BACTEC Standard Aerobic Media, except Amikacin, Gentamicin and Meropenem.

Amikacin
All blood bottle types showed excellent performance with EA ≥95%, except Standard Aerobic (BMX) and REDOX 1 aerobic media.

Acinetobacter baumannii varied across the blood bottle types. The Standard Aerobic (BD) and Plus Aerobic (BD) bottles demonstrated 100% EA, indicating reliable detection. However, the Standard Aerobic (BMX) bottle type showed a decrease in performance with an EA of 84.2%, and the REDOX 1 aerobic bottle at 77.8% EA, suggesting potential limitations in these bottle types for detecting *A. baumannii*.

Gentamicin
All blood bottle types showed excellent performance with EA ≥95%, except Plus Aerobic (BD), Standard Aerobic (BMX) and Standard Anaerobic (BMX).

Klebsiella pneumoniae varied across blood bottle types and conditions. The Standard Aerobic (BD) bottle type showed high EA (96.7%). REDOX 1 Aerobic media demonstrated 100% EA, indicating reliable agreement of *Klebsiella pneumoniae* in aerobic conditions. However, some bottle types, such as Plus Aerobic (BD), Standard Aerobic (BMX) and Standard Anaerobic (BMX), exhibited lower EA. The low EA for these combinations is due to the results of a single strain, *K. pneumoniae* IHMA1427927 with a bimodal distribution across multiple bottle types lower the performance:

Gentamicin/Plus Aerobic (BD): *K. pneumoniae* IHMA1427927 (EA: 1/10)
Gentamicin/Standard Aerobic (BMX): *K. pneumoniae* IHMA1427927 (EA: 0/10)
Gentamicin/Standard Anaerobic (BMX): *K. pneumoniae* IHMA1427927 (EA: 1/10)

Meropenem
All blood bottle types showed excellent performance with EA ≥95%, except REDOX 1 Aerobic Media and the Standard Anaerobic (BMX) media.

Escherichia coli performance showed variability across bottle types. The Standard Aerobic (BMX) bottle type demonstrated 100% EA, indicating robust performance, while REDOX 1 Aerobic media showed slightly lower EA. Bottle types including the REDOX 1 Aerobic Media and the Standard Anaerobic (BMX) media exhibited lower EA. The low EA for these combinations is due to the results of a single strain, *E. coli* JMI1054207 with various MIC results across multiple bottle types lower the performance:

Meropenem/Standard Anaerobic (BMX): *E. coli* JMI1054207 (EA: 0/10)
Meropenem/REDOX 1 Aerobic: *E. coli* JMI1054207 (EA: 0/10)

Conclusion

Amikacin, cefepime, ceftazidime-avibactam, levofloxacin, gentamicin, meropenem, meropenem-vaborbactam, and piperacillin-tazobactam were assessed for their susceptibility testing against various

bacterial strains across different blood bottle types and aerobic/anaerobic conditions. Results for most drug/organism combinations for claimed species showed good performance with all blood culture bottles with the following exceptions:

- Amikacin/*A. baumannii* with Standard Aerobic (BMX) and REDOX 1 aerobic bottle

Overall, the study suggests that these antibiotics can effectively assess the susceptibility of bacterial strains in blood cultures, but there may be variations in performance depending on the specific antibiotic, bacterial strain, and blood bottle type used resulting in potential limitations.

Interfering Substances

Organisms tested:

1. *Escherichia coli* (7 Strains)
2. *Pseudomonas aeruginosa* (3 Strain)
3. *Acinetobacter baumannii* (2 Strain)
4. *Klebsiella pneumoniae* (3 Strains)

Blood Bottle Tested:

BD BACTEC™: Standard Aerobic

Interfering Substances

Interfering substances	Testing Concentrations
Conjugated bilirubin	0.003 mg/mL
Unconjugated bilirubin	0.003 - 0.012 mg/mL
Gamma-globulin	6 - 13 mg/mL
Hemoglobin	100 mg/mL
Triglycerides	5 mg/mL
White cells	$4.5X10^6$ - $1.0X\ 10^7$ cells/mL
Heparin	330 units/dL
Platelets	≥450,000 platelets/µL

Table 23. Interferring substances and concentrations used in the study.

Study Outline

This study assesses the impact of potentially interfering substances on the LifeScale LSGN Kit with ifeScale AST system performance by adding a potentially interfering substance to blood culture media containing a test organism and blood. The test blood culture bottle is incubated in a blood culture system until the blood culture is flagged positive. The positive blood culture is then tested on the LifeScale

system and the results for the six antibiotics on the LifeScale LSGN panel are compared to those for positive blood cultures that do not contain interfering substances.
BACTEC Standard Aerobic media was used for testing. Testing for each interferent was carried out with a minimum of fifteen (15) test organisms. Each interfering substance was tested in triplicate for each test organism/media combination.
Only bottles that flagged positive within 48 hours of inoculation were tested in the LifeScale AST system. A bottle containing no interfering substance was tested in triplicate each time testing was performed with interfering substances. When the blood culture was flagged positive, a LifeScale LSGN AST was performed directly from the positive blood culture using the LifeScale AST system as described in the LifeScale manual.

Interfering Substances

The interfering substance and 10 mL of whole blood, the volume recommended by the manufacturer, were added to the blood culture bottle. The blood culture was inoculated with the test organism and placed onto the BACTEC Blood culture instrument until it flagged positive.

The effect of antimicrobial agents as interfering substances was not evaluated. The following limitation applies:

> *Potential interference by antimicrobial agents that may be present in a patient blood specimen has not been established with the Affinity LifeScale LSGN Kit. Use caution when interpreting results if information is available about the patient treatment with antimicrobial agents.*

Essential Agreement (EA) was determined by comparing MICs for bottles containing interfering substances to the mode of MICs for bottles not containing interfering substances for each organism under test. EA ≥ 95% was acceptable and indicated that the interfering substance had no substantial impact on results. Analysis was performed for each interfering substance and both media types.

Results and Discussion

Essential Agreement for Interfering Substances.

For a majority of the antimicrobials in this study, EA exceeded 95% for most substances. Discrepancies reduced EA to <95% for multiple substances occurred for Meropenem/*A.baumannii, E.coli, K.pneumoniae and P.aeruginosa*, and Amikacin/*A.baumannii*.

For most of the discrepancies (<95%), the essential agreement (EA) for samples with interferents and no interferents compared to the No Substance Mode remaining unchanged suggests that the presence of interfering substances didn't introduce additional variability into the testing process. The unchanged performance indicates that the testing conditions remained stable regardless of the presence of the interfering substance.

It can be concluded that while the presence of interfering substances didn't significantly affect the performance of the system, the variability in MICs emphasizes the inherent complexity of antimicrobial testing, where multiple factors can influence the outcome.