April 2, 2024

Affinity Biosensors
% Peter Trabold, Ph.D., MBA
Regulatory Affairs Specialist
MDC Associates, Inc.
180 Cabot Street
Beverly, Massachusetts 01915

Re: K211815
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: October 13, 2023
Received: October 16, 2023

Dear Peter Trabold:

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.

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)*

K211815

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/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 for the antimicrobial agents and specific target organisms identified below:

- Ampicillin: *Escherichia coli*
- Aztreonam: *Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca*
- Cefazolin: *Klebsiella pneumoniae, Klebsiella variicola*
- Ceftazidime: *Acinetobacter baumannii, Acinetobacter baumannii/nosocomialis group, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella variicola, Pseudomonas aeruginosa*
- Ertapenem: *Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca*
- 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) Summary

The summary of the 510(k) Substantial Equivalence Determination Performance is being submitted in compliance with section 807.92(c).

1. Contact Details

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(805) 960-5100

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Peter Trabold, Regulatory Affairs Specialist
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Beverly, MA 01915
Phone: (978) 927-3808
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2. Device

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

Regulation Name: A cellular analysis system for multiplexed antimicrobial susceptibility testing

Regulation Number: 21 CRF 866.1650

Product Code: SAN
Additional Product Code: LON

Classification: Class II

Predicate Device: Accelerate Pheno system, Accelerate PhenoTest BC Kit (K192665)

3. Device Description Summary

The LifeScale AST system is an *in vitro* diagnostic test designed to quantitatively assess antimicrobial susceptibility using a microfluidic sensor and resonant frequency technology. Specifically engineered for use with positive blood culture samples confirmed positive by Gram stain for Gram-negative rods, the LifeScale LSGN Panel ensures compatibility and accuracy while excluding Gram-positive or polymicrobial samples, thus maintaining specificity and reliability. During the incubation phase, the LifeScale LSGN Panel offers a standard incubation time of 3 hours, extendable up to 6 hours to accommodate varying microbial growth rates. Panels must be read within 8 hours of setup, with automatic cancellation for panels exceeding this timeframe. Panels with delayed readings can be

safely stored in the offline incubator until analysis. Upon reaching sufficient growth in the positive control wells, the LifeScale AST system transitions to data acquisition and readout. Advanced sensors capture essential metrics including microbe count, mass, and fluid volume, processed through sophisticated software algorithms to generate precise AST results for each antibiotic. To maintain hygiene standards, the LifeScale AST system incorporates automated washing and disinfection protocols for the sipper and sensor, minimizing the risk of cross-contamination and organic buildup. The culmination of the testing process involves calculating and reporting AST results (MIC and interpretive results), providing clinicians with actionable insights into antibiotic efficacy. Species-level organism identification is essential for results reporting. AST results are generated based on FDA or CLSI breakpoints validated for laboratory use.

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/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.

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 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*

5. Substantial Equivalency

Feature	New Device Affinity Biosensors LifeScale Gram Negative Kit (LSGN) with the LifeScale AST system (K211815)	Predicate Device Accelerate Pheno™ System and Accelerate PhenoTest BC Kit (K192665)
Intended Use	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/ 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.	The Accelerate PhenoTest BC kit is a multiplexed in vitro diagnostic test utilizing both qualitative nucleic acid fluorescence in situ hybridization (FISH) identification and quantitative, antimicrobial susceptibility testing (AST) methods and is intended for use with the Accelerate Pheno system. The Accelerate PhenoTest BC kit is capable of simultaneous detection and identification of multiple microbial targets followed by susceptibility testing of the appropriate detected bacterial organisms. The Accelerate PhenoTest BC kit is performed directly on blood culture samples identified as positive by a continuous monitoring blood culture system. Results are intended to be interpreted in conjunction with Gram stain results. The Accelerate PhenoTest BC kit identifies the following Gram-positive and Gram-negative bacteria and yeasts utilizing FISH probes targeting organism-specific ribosomal RNA sequences:
Similarities		
Results	Minimum Inhibitory Concentration (MIC) based Antimicrobial Susceptibility Testing direct from Positive Blood Cultures signaled as positive by a continuous monitoring blood culture system.	Minimum Inhibitory Concentration (MIC) based Antimicrobial Susceptibility Testing direct from Positive Blood Cultures signaled as positive by a continuous monitoring blood culture system.
Inoculation Method	Automated	Automated
Read Method	Automated	Automated

Differences		
Organisms Tested	Gram-negative bacilli bacterial pathogens	Gram-positive and Gram-negative bacterial pathogens
Instrument	LifeScale AST system	Accelerate Pheno System
Antimicrobial Agents	Ampicillin Aztreonam Cefazolin Ceftazidime Ertapenem Trimethoprim-Sulfamethoxazole	Amikacin Ampicillin Ampicillin/Sulbactam Aztreonam Ceftazidime Ceftaroline Cefepime Ceftriaxone Ciprofloxacin Daptomycin Ertapenem Gentamicin Linezolid Meropenem Piperacillin/Tazobactam Tobramycin Vancomycin
Sample Prep	Centrifugation and pipetting of sample. Direct from sample. No manual McFarland prep.	Automated. Direct from sample. No manual McFarland prep.
Indicated Organisms	*Acinetobacter baumannii* *Acinetobacter baumannii/nosocomialis* group *Escherichia coli* *Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Klebsiella variicola* *Pseudomonas aeruginosa*	Gram-negative species: *Acinetobacter baumannii* *Citrobacter* spp. *Enterobacter* spp. *Escherichia coli* *Klebsiella* spp. *Proteus* spp. *Pseudomonas aeruginosa* *Serratia marcescens* Additional Gram-positive bacteria and yeast are also included on the Accelerate PhenoTest BC kit.
Time Required for Analysis of Specimen	4 hours, on average	7 hours
Technology Principles	Microfluidic Sensor, Mass Measurement	Morphokinetic Cellular Analysis
IVD Functions	AST only. ID results based on an alternative procedure.	ID and AST together in same system

The differences noted above do not impact the safety and effectiveness of the device.

6. Performance Characteristics

Comparison Study

A comparison study was conducted to evaluate the performance of the LifeScale™ Gram Negative Kit (LSGN) 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 Method (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-R) AST results generated by the LifeScale LSGN kit 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 manufacturer's instructions 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.

Indicated CDC Challenge and Challenge isolates from other reference laboratories were prepared from frozen isolates supplied by Affinity Biosensors to one trial site. 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 new sample was prepared and tested.

All LifeScale LSGN testing was performed within 12 hours of the blood culture bottle being flagged as positive. LifeScale LSGN 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 was 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/R).

Reference testing was performed on all enrolled samples in triplicate. Testing was done in accordance with the reference protocol and was performed at one clinical trial site. 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 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. Assessment of categorical agreement (Susceptible/Intermediate/Resistant, S/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 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 5885 tests initiated:

Plate Failures (0.19%): 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 (0.54%): These failures occurred due to issues related to growth during testing.
LifeScale Failures (1.19%): This category involves failures directly attributable to the LifeScale system, including software and hardware failures.
Other Reasons (1.31%): 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 3.23%, with clinical tests accounting for 3.23%, analytical tests for 4.43%, and QC tests for 0.86%.

Upon initiation of any test on the LifeScale system, results were available 96.77% (5695/5885) of the time.

Reason for Exclusion/Incomplete Test	Clinical	Analytical	QC	Overall
Plate Failures*	[3/682] 0.44%	[8/3452] 0.23%	[0/1751] 0.00%	[11/5885] 0.19%
Growth Failures	[6/682] 0.88%	[26/3452] 0.75%	[0/1751] 0.00%	[32/5885] 0.54%
LifeScale Failures**	[4/682] 0.59%	[51/3452] 1.48%	[15/1751] 0.86%	[70/5885] 1.19%
Other Reasons***	[9/682] 1.32%	[68/3452] 1.97%	[0/1751] 0.00%	[77/5885] 1.31%
Total Excluded/Incomplete Tests	[22/682] 3.23%	[153/3452] 4.43%	[15/1751] 0.86%	[190/5885] 3.23%

Table 1. Summary of LifeScale 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 was evaluated with 6 antimicrobials, and an overview of the overall performance is presented in Table 2, below. All antimicrobial/organism combinations met the overall FDA acceptance criteria of ≥90% Essential (EA) and Categorical agreement (CA) rates except for the following: Aztreonam/*K. pneumoniae, P. aeruginosa*, Cefazolin/*E. coli*, Ceftazidime/*Acinetobacter spp.* (other than *A. baumannii*), *K. pneumoniae*, Ertapenem/*K. pneumoniae,* and Trimethoprim-Sulfamethoxazole/*K. pneumoniae.* For Ceftazidime, major errors occurred in *P. aeruginosa* isolates with a MIC value of 16 µg/mL, affecting 5 out of 61 susceptible isolates (8.2%). Adjusted for a lack of an intermediate breakpoint, this equated to 2 major errors (3.3%). Regarding Ertapenem, very major errors were observed in *Klebsiella oxytoca* isolates with MIC values of 0.5 µg/mL, where 2 out of 10 resistant isolates (20%) were affected. Limitations are included in the product labeling.

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%)
Ampicillin - *E. coli* [Breakpoints (µg/mL): 8.0 (S), 32.0 (R)]												
137	137	100.0%	23	23	100.0%	137	100.0%	87	50	0 (0.00%)	0 (0.00%)	0 (0.00%)
Aztreonam - *E. coli, K. aerogenes, K. oxytoca* [Breakpoints (µg/mL): 4.0 (S), 16.0 (R)]												
301	295	98.0%	45	39	86.7%	295	98.0%	91	207	5 (1.66%)	1 (0.48%)	0 (0.00%)
Cefazolin - *K. pneumoniae, K. variicola* [Breakpoints (µg/mL): 2.0 (S), 8.0 (R)]												
143	140	97.9%	69	66	95.7%	132	92.3%	77	62	11 (7.69%)	0 (0.00%)	0 (0.00%)
Ceftazidime - *E. coli, K. aerogenes, K. oxytoca, K. variicola* [Breakpoints (µg/mL): 4.0 (S), 16.0 (R)]												
340	332	97.6%	34	26	76.5%	334	98.2%	101	238	6 (1.76%)	0 (0.00%)	0 (0.00%)
Ceftazidime - *P. aeruginosa* [Breakpoints (µg/mL): 8.0 (S), 16.0 (R)]												
116	107	92.2%	85	76	89.4%	109	94.0%	55	61	0 (0.00%)	5 (8.20%)	2 (3.64%)
Ceftazidime - *A. baumannii, A. baumannii/nosocomialis group* [Breakpoints (µg/mL): 8.0 (S), 32.0 (R)]												
73	72	98.6%	25	24	96.0%	73	100.0%	56	15	0 (0.00%)	0 (0.00%)	0 (0.00%)
Ertapenem - *E. coli, K. aerogenes, K. oxytoca* [Breakpoints (µg/mL): 0.5 (S), 2.0 (R)]												
226	212	93.8%	28	14	50.0%	216	95.6%	43	182	7 (3.1%)	1 (0.55%)	2 (4.65%)
Trimethoprim-Sulfamethoxazole - *E. coli, K. aerogenes, K. oxytoca, K. variicola* [Breakpoints (µg/mL): 2.0 (S), 4.0 (R)]												
340	337	99.1%	16	13	81.3%	337	99.1%	91	249	0 (0.00%)	3 (1.20%)	0 (0.00%)

Table 2. LifeScale LSGN kit performance: Interpretation of MIC results are based on FDA Susceptibility Test Interpretative Criteria (STIC) and the 33rd edition of the CLSI M100, as recognized by FDA.

Ampicillin (AMP)

A total of 137 samples were evaluated with Ampicillin including 91 clinical (prospective and seeded) (66.4%) and 46 challenge (33.6%) samples. 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%)

Species-level performance

A total of 137 *E. coli* samples were included in the performance analysis including 91 clinical (66.4%) and 46 challenge (33.6%) samples. 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%).

Aztreonam (AZT)

A total of 301 samples were evaluated with Aztreonam including 235 clinical (prospective and seeded) (78.1%) and 66 challenge (21.9%) samples. The combined results from clinical and challenge testing from all claimed species demonstrated an EA of 98.0% and a CA of 98.0% with no VMJs (0%) and one MAJs (0.48%)

Species-level performance

A total of 137 *E. coli* samples were evaluated with Aztreonam. The combined results from clinical and challenge testing demonstrated an EA of 98.5% and a CA of 97.8% with no VMJs (0%) and one MAJ (1.23%).

A total of 109 *K. oxytoca* samples were evaluated with Aztreonam. 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 no MAJs (0%).

A total of 55 *K. aerogenes* samples were evaluated with Aztreonam. The combined results from clinical and challenge testing demonstrated an EA of 94.5% and a CA of 98.2% with no VMJs (0%) and no MAJs (0%).

Cefazolin (FAZ)

A total of 143 samples were evaluated with Cefazolin including 114 clinical (prospective and seeded) (79.7%) and 29 challenge (20.3%) samples. The combined results from clinical and challenge testing from all claimed species demonstrated an EA of 97.9% and a CA of 92.3% with no VMJs (0%) and no MAJ (0%).

Species-level performance

A total of 105 *K. pneumoniae* samples were evaluated with Cefazolin. The combined results from clinical and challenge testing demonstrated an EA of 98.1% and a CA of 93.3% with no VMJs (0%) and no MAJs (0%).

A total of 38 *K. variicola* samples were evaluated with Cefazolin. The combined results from clinical and challenge testing demonstrated an EA of 97.4% and a CA of 89.5% with no VMJs (0%) and no MAJs (0%). EA evaluable is 96.3%.

Ceftazidime (TAZ)

A total of 529 samples were evaluated with Ceftazidime including 378 clinical (prospective and seeded) (71.5%) and 151 challenge (28.5%) samples. The combined results from clinical and challenge testing from all claimed species demonstrated an EA of 96.2% and a CA of 97.2% with two VMJs (0.94%) and five MAJs (1.59%).

Species-level performance

A total of 73 *A. baumannii* (includes *baumannii/nosocomialis* group) samples were evaluated with Ceftazidime. The combined results from clinical and challenge testing demonstrated an EA of 98.6% and a CA of 100% with no VMJs (0%) and no MAJs (0%).

A total of 137 *E. coli* samples were evaluated with Ceftazidime. The combined results from clinical and challenge testing demonstrated an EA of 97.8% and a CA of 97.1% with no VMJs (0%) and no MAJs (0%).

A total of 55 *K. aerogenes* samples were evaluated with Ceftazidime. The combined results from clinical and challenge testing demonstrated an EA of 92.7% and a CA of 100% with no VMJs (0%) and no MAJs (0%).

A total of 109 *K. oxytoca* samples were evaluated with Ceftazidime. 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 no MAJs (0%).

A total of 39 *K. variicola* samples were evaluated with Ceftazidime. 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 116 *P. aeruginosa* samples were evaluated with Ceftazidime. The combined results from clinical and challenge testing demonstrated an EA of 92.2% and a CA of 94.0% with two VMJs (3.64%) and five MAJ (8.20%). Due to lack of an intermediate breakpoint, one VMJ was in Essential agreement bringing adjusted VMJ rate to 1.82%.

Perform an alternative method of testing prior to reporting results for:
P. aeruginosa at MIC value of 16 µg/mL due to the occurrence of major errors (5 / 61 susceptible isolates, (8.2%) adjusted to 2 major errors (3.3%) due to a lack of an intermediate breakpoint).

Ertapenem (ETP)

A total of 224 samples were evaluated with Ertapenem including 176 clinical (prospective and seeded) (78.6%) and 48 challenge (21.4%) samples. The combined results from clinical and challenge testing from all claimed species demonstrated an EA of 93.8% and a CA of 95.5% with two VMJs (4.76%) and two MAJs (0.55%).

Species-level performance

A total of 66*E. coli* samples were evaluated with Ertapenem. The combined results from clinical and challenge testing demonstrated an EA of 95.5% and a CA of 93.9% with no VMJs (0%) and one MAJs (2.33%).

A total of 51 *K. aerogenes* samples were evaluated with Ertapenem. The combined results from clinical and challenge testing demonstrated an EA of 94.1% and a CA of 96.1% with no VMJs (0%) and no MAJs (0%).

A total of 137 *K. oxytoca* samples were evaluated with Ertapenem. The combined results from clinical and challenge testing demonstrated an EA of 92.5% and a CA of 96.3% with two VMJs (20.0%) and no MAJs (0%). Both VMJs occurred at MIC values of 0.5 µg/mL.

Perform an alternative method of testing prior to reporting results for:

Ertapenem: *K. oxytoca* at MIC values of 0.5 µg/mL due to the occurrence of very major errors (2 /10 resistant isolates, 20%).

Trimethoprim-sulfamethoxazole (SXT)

A total of 340 samples were evaluated with Trimethoprim-Sulfamethoxazole including 264 clinical (prospective and seeded) (77.6%) and 76 challenge (22.4%) samples. The combined results from clinical and challenge testing from all claimed species demonstrated an EA of 99.1% and a CA of 99.1% with no VMJs (0%) and three MAJs (1.20%).

Species-level performance

A total of 138 *E. coli* samples were evaluated with Trimethoprim-Sulfamethoxazole. The combined results from clinical and challenge testing demonstrated an EA of 99.3% and a CA of 99.3% with no VMJs (0%) and one MAJ (1.32%).

A total of 55 *K. aerogenes* samples were evaluated with Trimethoprim-Sulfamethoxazole. The combined results from clinical and challenge testing demonstrated an EA of 98.2% and a CA of 96.4% with no VMJs (0%) and two MAJs (3.92%). Due to a lack of an intermediate breakpoint, the MAJ rate was adjusted to 1.96% (1 MAJ).

A total of 108 *K. oxytoca* samples were evaluated with Trimethoprim-Sulfamethoxazole. 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 39 *K. variicola* samples were evaluated with Trimethoprim-Sulfamethoxazole. The combined results from clinical and challenge testing demonstrated an EA of 97.4% and a CA of 100% with no VMJs (0%) and no MAJs (0%).

Trending

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 than the reference method. Use caution when reporting drug resistance for any antimicrobial. The following drug/organism combinations showed high trending:

- *Ampicillin- E. coli*
- *Aztreonam – E. coli, K. aerogenes, K. oxytoca*
- *Cefazolin – K. pneumoniae*
- *Ceftazidime – E. coli, K. aerogenes, K. variicola, A. baumannii, P. aeruginosa*
- *Ertapenem – E. coli, K. aerogenes, K. oxytoca*
- *Trimethoprim/sulfamethoxazole – E. coli, K. aerogenes, K. variicola*

Quality Control Performance Data

Strains recommended by the FDA and CLSI were tested for each antimicrobial agent evaluated using the LifeScale LSGN kit and the CLSI Reference Broth Microdilution Method. The quality control (QC) strains tested were *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *K. pneumoniae* ATCC 700603. The QC testing results indicate that this device can reliably yield acceptable QC results for >95% of tests.

The measured MIC range for QC data from the six sites are shown in Table 3 through Table 8, along with data from BMD data, QC ranges are shaded grey.

All Trial Site data, 1(LS) to 6(LS), refer to QC data obtained on the LifeScale LSGN kit (LS).

Ampicillin

			Trial Site						
QC Organism	QC Range	MIC	BMD	1 (LS)	2 (LS)	3 (LS)	4 (LS)	5 (LS)	6 (LS)
E. coli ATCC 25922	2 µg/mL to 8 µg/mL	≤ 2 µg/mL	9	38	15	24	35	13	22
		= 4 µg/mL	102	80	14	20	13	17	4
		= 8 µg/mL	1	3	0	0	1	0	0
Total			112/112 (100%)	121/121 (100%)	29/29 (100%)	44/44 (100%)	49/49 (100%)	30/30 (100%)	26/26 (100%)

Table 3. LifeScale LSGN MIC QC distribution for Ampicillin.

N.B. Ampicillin: Does not include the full CLSI expected range.

Aztreonam

			Trial Site						
QC Organism	QC Range	MIC	BMD	1 (LS)	2 (LS)	3 (LS)	4 (LS)	5 (LS)	6 (LS)
P. aeruginosa ATCC 27853	2 µg/mL to 8 µg/mL	= 2 µg/mL	2	80	24	51	57	24	20
		= 4 µg/mL	85	0	0	1	3	1	4
		= 8 µg/mL	23	0	0	0	1	0	0
		= 16 µg/mL	1	0	0	0	0	0	0
Total			110/111(99.1%)	81/81 (100%)	26/26 (100%)	55/55 (100%)	65/65 (100%)	30/30 (100%)	30/30 (100%)

Table 4. LifeScale LSGN MIC QC distribution for Aztreonam.

Cefazolin

			Trial Site						
QC Organism	QC Range	MIC	BMD	1 (LS)	2 (LS)	3 (LS)	4 (LS)	5 (LS)	6 (LS)
E. coli ATCC 25922	1 µg/mL to 4 µg/mL	= 1 µg/mL	0	1	0	0	1	0	0
		= 2 µg/mL	87	75	21	42	39	26	19
		= 4 µg/mL	25	45	8	2	9	4	7
Total			112/112(100%)	121/121(100%)	29/29 (100%)	44/44 (100%)	49/49 (100%)	30/30 (100%)	36/36 (100%)

Table 5. LifeScale LSGN MIC QC distribution for Cefazolin.

Ceftazidime

			Trial Site						
QC Organism	QC Range	MIC	BMD	1 (LS)	2 (LS)	3 (LS)	4 (LS)	5 (LS)	6 (LS)
K. pneumoniae ATCC 700603	16 µg/mL to 64 µg/mL	= 4 µg/mL	0	0	0	0	2	0	0
		= 16 µg/mL	1	109	25	47	44	25	25
		= 32 µg/mL	56	3	0	2	2	1	0
		= 64 µg/mL	47	0	0	0	0	0	0
		> 64 µg/mL	1	0	0	0	0	0	0
Total			104/105(99.1%)	112/112(100%)	25/25 (100%)	49/49 (100%)	48/48 (100%)	26/26 (100%)	25/25 (100%)

Table 6. LifeScale AST LSGN MIC QC distribution for Ceftazidime.

Ertapenem

			Trial Site						
QC Organism	QC Range	MIC	BMD	1 (LS)	2 (LS)	3 (LS)	4 (LS)	5 (LS)	6 (LS)
P. aeruginosa ATCC 27853	2 µg/mL to 8 µg/mL	≤ 0.125 µg/mL	0	2	0	0	0	0	0
		= 0.25 µg/mL	0	1	0	0	0	0	0
		= 1 µg/mL	5	0	0	0	0	0	0
		= 2 µg/mL	56	76	22	51	57	23	23
		= 4 µg/mL	44	2	2	1	4	2	1
		= 8 µg/mL	7	0	0	0	0	0	0
Total			107/112 (95.5%)	78/81 (96.3%)	23/23 (100%)	52/52 (100%)	61/61 (100%)	25/25 (100%)	24/24 (100%)

Table 7. LifeScale LSGN MIC QC distribution for Ertapenem.

Trimethoprim-Sulfamethoxazole

			Trial Site						
QC Organism	QC Range	MIC	BMD	1 (LS)	2 (LS)	3 (LS)	4 (LS)	5 (LS)	6 (LS)
E. coli ATCC 25922	≤0.5 µg/mL	≤ 0.25 µg/mL	112	121	29	44	49	30	25
		= 0.5 µg/mL	0	0	0	0	0	0	1
Total			112/112(100%)	121/121 (100%)	29/29 (100%)	44/44 (100%)	49/49 (100%)	30/30 (100%)	25/25 (100%)

Table 8. LifeScale LSGN MIC QC distribution for Trimethoprim-Sulfamethoxazole.

Analytical Performance Data

LifeScale LSGN Kit Reproducibility

Organisms Tested:

- *Acinetobacter baumannii* (1 Strains)
- *Escherichia coli* (15 Strains)
- *Klebsiella oxytoca* (1 Strain)
- *Klebsiella pneumoniae* (10 Strains)
- *Pseudomonas aeruginosa* (3 Strains)

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 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 instructions for use. Purity verification for each LifeScale LSGN panel was performed, and panels were incubated on or off-line as dictated by the LifeScale AST system workflow. LifeScale LSGN panels were read at three hours, or when the LifeScale AST system determined the growth threshold in the positive control well was acceptable. A target of nine valid data points per day at each site was generated on three consecutive days for a total of 27 data points per test organism.

LifeScale LSGN kit reproducibility was determined from the total number of evaluable results that fell within 1 dilution (+/- one doubling dilution) of the mode results divided by the total number of results. On-scale LifeScale LSGN kit performance was determined for each antimicrobial agent and was evaluated between and within each site. Both Best Case, which assumes that off-scale results are within one doubling dilution of the mode, and Worst Case, which assumes that off-scale results are more than one doubling dilution of the mode, performance was determined for each drug.

Results

The LifeScale LSGN kit reproducibility acceptance criteria were defined as >95% (Best Case) for each evaluable antimicrobial/organism combination tested. The worst-case scenario acceptance criteria were >89% for each evaluable antimicrobial/organism combination tested.

Antibiotic	Best-case (%)	Worst-case (%)
Ampicillin	268/269 = 99.6%	250/269 = 92.9%
Aztreonam	290/294 = 98.6%	263/294 = 89.5%
Cefazolin	321/324 = 99.1%	321/324 = 99.1%
Ceftazidime	264/265 = 99.6%	244/265 = 92.1%
Ertapenem	291/296 = 98.3%	271/296 = 91.6%
Trimethoprim-Sulfamethoxazole	255/264 = 96.6%	243/264 = 92%

Table 9. Reproducibility data for the LifeScale LSGN panel

LifeScale LSGN Kit Blood Bottle Compatibility

Organisms tested:

- *Escherichia coli* (8 Strains)
- *Klebsiella pneumoniae* (4 Strains)
- *Pseudomonas aeruginosa* (2 Strains)
- *Acinetobacter baumannii* (3 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

For the original study, 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 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 LSGN kit testing, cell counts were performed on LifeScale AST system to determine whether these bottles had reached bottle ring concentration (10^8 CFU/ml). BacT/ALERT and VersaTREK bottles for which LifeScale AST system cell counts indicate concentrations less than bottle ring concentrations, (10^8 CFU/ml), were returned to the offline incubator within 20 minutes.

All susceptibility testing was performed using the LifeScale AST system direct from spiked positive blood cultures, per the LifeScale LSGN kit Instructions for Use. A single LifeScale LSGN panel was prepared from each positive blood culture bottle. LifeScale LSGN panels that were incubated on the LifeScale AST system were read automatically approximately 3 hours from incubation or when sufficient growth had taken place. Panels incubated off-line were read when the LifeScale AST system called for them in accordance with the LifeScale LSGN Kit Instructions for Use.

Results from all MICs from each media/organism were compared to modal MICs from the BMD database. Essential Agreement (EA) was calculated and compared to the mode of MIC results from a database of reference BMD results, for each strain and all blood culture media. EA was also calculated to the LifeScale LSGN MIC mode result. The LifeScale LSGN mode is determined for each strain and antibiotic from the mode of LifeScale LSGN MIC results, combined for all blood culture media. This analysis provided a direct comparison of AST results between media types. An EA agreement of ≥90% for the various blood culture media compared to LifeScale LSGN and Reference BMD modes confirms that the different blood culture media do not affect the MIC results.

Results and Discussion

Results for ampicillin, cefazolin, and ceftazidime (*Enterobacterales* and *P. aeruginosa*) with all blood culture media demonstrated >90% EA compared to the reference method mode and to the LifeScale LSGN mode. Results confirm that blood culture media tested can be used with the LifeScale system and confirms that blood culture media tested with these drugs are substantially equivalent.

Supplemental testing was performed with several drug/media combinations to address low performance. Results obtained in original and supplemental testing with the combination of ertapenem/*E.coli* with bioMérieux Standard Anaerobic Media (97 total results) and REDOX 1 Aerobic Media (80 total results) showed low CA when compared to the reference method mode, and low EA and CA when compared to the mode of LifeScale LSGN kit results. In addition, testing with this drug/organism combination using BD Standard Aerobic media (80 total results) showed low EA compared to the reference method mode.

The following limitation addresses the results obtained with ertapenem/*E. coli*:
The LifeScale LSGN kit showed unacceptable performance for E. coli with ertapenem when tested using the following blood culture bottle types: bioMérieux Standard Anaerobic, BACTEC Standard Anaerobic, and VersaTREK REDOX 1 Aerobic. Use alternate bottle types for determining ertapenem MICs for E. coli.

Results obtained with the combination of aztreonam/*E. coli* with Lytic Anaerobic Media (46 total results) showed slightly lower than acceptable EA and CA compared to both the reference method mode and the mode of the LifeScale LSGN kit results. ***Note:*** *Low EA and CA for aztreonam/E. coli with BACTEC Lytic Anaerobic media is attributed to a single E. coli isolate.* Results were acceptable for the remaining *E. coli* strains tested.

For cefazolin/*K. pneumoniae*, ceftazidime/*A*. *baumannii,* and Trimethoprim-Sulfamethoxazole/*E. coli,* the original data included no isolates with evaluable MICs. Supplemental testing with replicates of a *K. pneumoniae* isolate with on-scale MICs provided acceptable results with cefazolin for all bottle types. Supplemental testing with replicates of an *A. baumannii* isolate with on-scale MICs provided acceptable results with ceftazidime for all aerobic bottle types. Due to a lack of *E. coli* isolates with on-scale MICs for Trimethoprim-Sulfamethoxazole, supplemental testing was not performed and is addressed in the following limitation:

Media equivalency was not determined for Trimethoprim-Sulfamethoxazole/E. coli due to a lack of available isolates with on-scale MICs.

LifeScale LSGN Kit Sample Stability

Organisms tested:

- *Escherichia coli* (9 Strains)
- *Klebsiella pneumoniae* (3 Strains)
- *Pseudomonas aeruginosa* (2 Strains)
- *Acinetobacter baumannii* (2 Strains)

Blood Bottles Tested:

- BD BACTEC™: Standard Aerobic

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 bottle flagged as positive, $T_{pos.}$
- 13 +/- 0.5 hour after bottle flagged as positive, $T_{13.}$

Testing was conducted using BD BACTEC standard aerobic blood culture bottles. A single blood culture bottle was spiked for each experiment. Sixteen organisms were chosen with susceptible and resistant on-scale MICs for each relevant antibiotic on the LifeScale LSGN test panel. The spiked blood culture was then incubated on the BD BACTEC automated blood culture system until flagged as positive (Tpos). The testing was conducted in triplicate; each organism was tested on three LifeScale AST systems at both Tpos and T13. To generate a larger number of evaluable results, some organisms were included in multiple experiments. Positive bottles were removed from the blood culture system and aliquots obtained for LifeScale LSGN kit testing. At the initial time point, T_{pos}, bottles were held at room temperature for less than 20 minutes and then returned to the blood culture system to incubate further until T_{13}. Aliquots were tested with LifeScale LSGN kit within 30 minutes of sampling from the blood culture bottle and within one hour of the bottle being flagged as positive. At the final time point, T_{13}, bottles were removed, and aliquots obtained for LifeScale LSGN tests. This resulted in a total of 126 LifeScale LSGN samples evaluated for performance; 3 at T_{pos}, 3 at T_{13} for each experiment tested.

To confirm organism concentration and purity, colony counts were performed on BAPs direct from the positive control well on the LifeScale LSGN panel, the spiked inoculum, and the positive blood culture at both T_{pos} and T_{13}. The BAPs were prepared immediately after inoculating the LifeScale LSGN well panels.

LifeScale LSGN panels incubated directly on the LifeScale AST system were read automatically at approximately 3 hours from inoculation. Panels incubated off-line were read as soon as the LifeScale AST system requested the LifeScale LSGN panel for reading. AST (MIC) results generated at time T_{pos} and T_{13} were compared to the reference method modal MIC and the LifeScale LSGN MIC mode for each reportable antimicrobial/organism combination.

Results and Discussion

The acceptance criteria were defined as ≥90% agreement with the reference method modal MIC and the LifeScale LSGN MIC mode within +/- one two-fold dilution at T_{pos} and T_{13} for each reportable antimicrobial/organism combination. Table 10 shows essential agreement for T_{pos} and T_{13} data, for all strains that are reportable for each antibiotic.

When evaluated *vs.* the LifeScale LSGN Mode, essential agreement (EA) for 6 of 6 antibiotics met the 90% acceptance criterion at both time points. When evaluated *vs.* Reference BMD, data for 6 of 6 antibiotics meet the 90% acceptance criterion for both time points.

			EA to Ref BMD			**EA to LifeScale LSGN Mode**	
			T=0	**T=13**		**T=0**	**T=13**
Antibiotic	**Organism/s**	**# Organisms Tested**	**EA (EA%)**	**EA (EA%)**		**EA (EA%)**	**EA (EA%)**
Ampicillin	*Escherichia coli*	6	17/18 (94.44%)	18/18 (100.00%)		17/18 (94.44%)	18/18 (100.00%)
Aztreonam	*Escherichia coli*	6	18/18 (100.00%)	18/18 (100.00%)		18/18 (100.00%)	18/18 (100.00%)
Cefazolin	*Klebsiella pneumoniae*	3	9/9 (100.00%)	9/9 (100.00%)		9/9 (100.00%)	9/9 (100.00%)
Ceftazidime	*Acinetobacter baumannii* *Escherichia coli* *Pseudomonas aeruginosa*	2 6 2	29/30 (96.67%)	30/30 (100.00%)		29/30 (96.67%)	30/30 (100.00%)
Ertapenem	*Escherichia coli*	9	38/42 (90.48%)	39/42 (92.86%)		42/42 (100.00%)	39/42 (92.86%)
Trimethoprim/Sulfamethoxazole	*Escherichia coli*	6	18/18 (100.00%)	18/18 (100.00%)		18/18 (100.00%)	18/18 (100.00%)

Table 10. Stability results for reportable antimicrobial/organism combinations at T_{pos} and T_{13}.

LifeScale LSGN Kit Interfering Substances

Organisms tested:

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

Blood Bottles 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
Panellets	≥450,000 panellets/µL

Table 11. Interfering substances and concentrations used in the study.

This study assesses the impact of potentially interfering substances, Table 11, on the LifeScale LSGN Kit 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 five (5) 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 with the LifeScale LSGN kit. 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 LSGN kit 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 ≥ 90% 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.

The combination of ceftazidime/*A. baumannii* with any potential interferent and the combination of cefazolin/*K. pneumoniae* with platelets and heparin were not tested with on-scale isolates; therefore, the effect of these combinations cannot be evaluated. The following limitation applies:

Interference has not been established for the following drug/organism combinations:

- All interferents: ceftazidime/*A. baumannii*
- Platelets and heparin: cefazolin/*K. pneumoniae*

EA to the mode of control MICs exceeds 90% for all interfering substances for all antibiotics considered in this study. This confirms the LifeScale LSGN kit results for samples containing interfering substances are all substantially equivalent to the negative control samples.

For 5 of 6 antimicrobials considered in this study, EA exceeded 90% for all substances. Discrepancies reduced EA to <90% for multiple substances occurred for Ertapenem. These discrepancies were attributed to a single strain which exhibited the same poor EA for the substance and for the control samples, indicating that in all such cases discrepancies were due to behavior of an individual strain and not a result of the interfering substance being tested.

<u>LifeScale LSGN On-Line/Off-Line Incubation</u>

Organisms tested:

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

Study Outline

To improve workload and workflow, the LifeScale AST system utilizes incubation in an off-line non-CO_2 incubator at 35 °C ± 2 °C. For the plates incubated off-line, the LifeScale AST system keeps track of the plate and will alert the operator at 3 hours for the plate to be placed on the system for initial growth assessment. If the growth is sufficient, the plate will be read, and AST data generated. If growth is determined not to be sufficient, the system will estimate the additional incubation time required. Depending on the status of other incubating plates, the operator will either leave the plate on the LifeScale AST system or will be instructed to place the plate back into the incubator until the estimated additional incubation time has occurred.

BACTEC standard aerobic blood culture bottles were inoculated at a target concentration of 1,000 to 10,000 colonies per mL. Spiked blood cultures were incubated in the BACTEC blood culture instrument until flagged positive. LifeScale LSGN kit testing was performed directly from spiked positive blood cultures within 12 hours of the culture being flagged as positive.

One on-line and three off-line incubation test sets were set up and tested at controlled incubation times.

- **On-line testing, placed into the LifeScale AST system after inoculation and incubated on-line.**
- **Off-line Set 1 was read on the LifeScale AST system when the system called for the plate at approximately 3 hours; designated as "at the same time" as the on-line testing plates.**
- **Off-line Set 2 was read after 6 hours of off-line incubation.**
- **Off-line Set 3 was read after 8 hours of off-line incubation.**

LifeScale LSGN kit testing was performed for both incubation scenarios from the same blood culture bottle. Aliquots were taken from each positive blood culture, and all testing performed in triplicate. Plates were inoculated in triplicate: 3 plates for on-line and 3 plates for each off-line incubation time. All plates will be incubated at 35 °C ± 2 °C.

Plates incubated on the LifeScale AST system were read automatically at approximately 3 hours after inoculation. If at the initial read, insufficient growth was detected in the positive control wells, plates were read at a LifeScale AST system determined interval.

The first set of off-line plates, Set 1, was removed from the off-line incubator and placed onto the LifeScale AST system when the LifeScale AST system called for the plates to be read at approximately 3 hours. If the plates had not reached the growth threshold, the plate was incubated as required by the LifeScale AST system. The plate was then read after the extended incubation period. Plates in Sets 2 and 3, were taken from the off-line incubator and placed onto the LifeScale AST system for reading and MIC data generation at the appropriate time (6 or 8 hours).

Results and Discussion

The acceptance criteria were determined as ≥95% agreement within +/- one well for each LifeScale LSGN kit MIC mode, under all incubation conditions, compared to BMD results for each drug/organism combination tested. No claims are made regarding the equivalence of on-line/off-line incubation

times. In addition, LifeScale LSGN kit results, as compared to the reference method, from the clinical study (which included both on-line and off-line incubation) were evaluated for cefazolin, ceftazidime and ertapenem.

MIC results for ampicillin, aztreonam, cefazolin, ceftazidime and trimethoprim-sulfamethoxazole obtained with both on-line and off-line incubation were equivalent to results obtained with the reference method.

For ertapenem/*E. coli* results obtained with off-line and on-line incubation at three hours incubation were unacceptable for both on-line and off-line incubation, with EAs of 83.3% for both incubation environments. Results from the clinical study showed acceptable performance for samples incubated on-line for 3 hours, however, results for samples incubated off-line for 3 hours showed unacceptable performance with an EA of 87.0%; results at 4 hours for samples incubated off-line were also unacceptable with an EA of 84.6%.

The following limitation applies:
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.

LifeScale LSGN Kit Inoculum Density Study
Organisms tested:

- **Escherichia coli (8 Strains)**
- **Klebsiella pneumoniae (7 Strains)**
- **Pseudomonas aeruginosa (7 Strains)**
- **Acinetobacter baumannii (2 Strains)**

Study Outline
This study demonstrates that the concentration of organisms in a positive blood culture does not impact LifeScale LSGN kit 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 LSGN panel.

Results and Discussion

MIC and final AST results were generated by the LifeScale 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. Acceptable results meet ≥90% essential agreement (within +/- 1 antibiotic dilution) compared to the LifeScale LSGN mode.

At the 10^9 CFU/ml concentration, all tested samples completed, i.e., there was sufficient growth in the positive controls after incubation, plates were read, and results generated. At the 10^6 CFU/ml concentration, 33 of 41 samples (80.5%) were completed, while 8 of 41 samples (19.5%) were terminated due to insufficient growth in the positive control wells. At 10^4 CFU/ml organism concentration, no results were obtained. All samples of *Pseudomonas* species tested at the 10^4 CFU/mL concentration were terminated by LifeScale AST system software due to insufficient growth. These tests confirmed that when organism concentration is at a very low value of 10^4 CFU/ml, the system performed in accordance with specifications, and no results were reported.

Analysis of trending at each tested concentration showed high trending as compared to the reference method for the following antimicrobial/organism combinations at the specified concentrations:

- Aztreonam/*E. coli* at 10^9 CFU/mL
- Cefazolin/*K. pneumoniae* at 10^9 CFU/mL
- Ceftazidime/ *E. coli* at 10^6 CFU/mL and 10^9 CFU/mL
- Ceftazidime/*A. baumannii* at 10^9 CFU/mL
- Ceftazidime/*P. aeruginosa* at 10^6 CFU/mL and 10^9 CFU/mL
- Ertapenem/*E. coli* at 10^6 CFU/mL and 10^9 CFU/mL

			Performance Data					
Antibiotic	**Genus/Species**	**Incubation Category**	**EA%**	**EA% Evaluable**	**CA%**	**#VMJ (VMJ%)**	**#MAJ (MAJ%)**	**#MIN (MIN%)**
Ampicillin	***E. coli***	**10e6 CFU/mL**	**100.0%**	**100.0%**	**100.0%**	**0 (0.00%)**	**0 (0.00%)**	**0 (0.00%)**
Ampicillin	***E. coli***	**10e9 CFU/mL**	**100.0%**	**100.0%**	**100.0%**	**0 (0.00%)**	**0 (0.00%)**	**0 (0.00%)**

Table 12. Inoculum Density performance data, Ampicillin.

			Performance Data					
Antibiotic	**Genus/Species**	**Incubation Category**	**EA%**	**EA% Evaluable**	**CA%**	**#VMJ (VMJ%)**	**#MAJ (MAJ%)**	**#MIN (MIN%)**
Aztreonam	***E. coli***	**10e6 CFU/mL**	**100.0%**	**100.0%**	**100.0%**	**0 (0.00%)**	**0 (0.00%)**	**0 (0.00%)**
Aztreonam	***E. coli***	**10e9 CFU/mL***	**100.0%**	**100.0%**	**100.0%**	**0 (0.00%)**	**0 (0.00%)**	**0 (0.00%)**

Table 13. Inoculum Density performance data, Aztreonam.

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

		Performance Data

Antibiotic	Genus/Species	Incubation Category	EA%	EA% Evaluable	CA%	#VMJ (VMJ%)	#MAJ (MAJ%)	#MIN (MIN%)
Cefazolin	*K. pneumoniae*	10e6 CFU/mL	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Cefazolin	*K. pneumoniae*	10e9 CFU/mL*	100.0%	100.0%	92.9%	0 (0.00%)	0 (0.00%)	2 (7.14%)

Table 14. Inoculum Density performance data, Cefazolin.

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

			Performance Data					
Antibiotic	Genus/Species	Incubation Category	EA%	EA% Evaluable	CA%	#VMJ (VMJ%)	#MAJ (MAJ%)	#MIN (MIN%)
Ceftazidime	*E. coli*	10e6 CFU/mL*	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Ceftazidime	*E. coli*	10e9 CFU/mL*	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Ceftazidime	*A. baumannii*	10e6 CFU/mL	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Ceftazidime	*A. baumannii*	10e9 CFU/mL*	100.0%	100.0%	66.7%	0 (0.00%)	0 (0.00%)	2 (33.33%)
Ceftazidime	*P. aeruginosa*	10e6 CFU/mL**	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Ceftazidime	*P. aeruginosa*	10e9 CFU/mL*	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)

Table 15. Inoculum Density performance data, Ceftazidime.

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

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

			Performance Data					
Antibiotic	Genus/Species	Incubation Category	EA%	EA% Evaluable	CA%	#VMJ (VMJ%)	#MAJ (MAJ%)	#MIN (MIN%)
Ertapenem	*E. coli*	10e6 CFU/mL*	100.0%	100.0%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Ertapenem	*E. coli*	10e9 CFU/mL*	92.6%	86.7%	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)

Table 16. Inoculum Density performance data, Ertapenem

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

			Performance Data					
Antibiotic	Genus/Species	Incubation Category	EA%	EA% Evaluable	CA%	#VMJ (VMJ%)	#MAJ (MAJ%)	#MIN (MIN%)
Trimethoprim-Sulfamethoxazole	*E. coli*	10e6 CFU/mL	100.0%	N/A	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)
Trimethoprim-Sulfamethoxazole	*E. coli*	10e9 CFU/mL	100.0%	N/A	100.0%	0 (0.00%)	0 (0.00%)	0 (0.00%)

Table 17. Inoculum Density performance data, Trimethoprim-Sulfamethoxazole

LifeScale LSGN Contamination and Carry-Over Testing

Organisms Used

- **Escherichia coli (1 Strain)**
- **Klebsiella pneumoniae (1 Strain)**

Study Outline

The LifeScale AST system is designed to prevent contamination by automated cleaning and disinfection of the microfluidics system during and between LifeScale LSGN measurements. Additionally, a film cover, placed on the inoculated LifeScale LSGN panel, protects the sample in each well from cross-contamination during incubation and measurement. Verification of the LifeScale AST system cleaning procedures was performed as described below.

Test 1, Plate-to-Plate Carry-Over with Growth Media

Testing was performed using 5 pairs of plates. The first plate of the pair was inoculated with a microorganism from a processed positive blood culture sample. The second plate of the pair was inoculated with uninoculated growth media except for the wells designated positive controls. After the plates were inoculated, they were incubated on-line or off-line in a conventional incubator at 35 °C ± 2 °C for 3 hours or until the LifeScale AST system called for the plate. The LifeScale AST system read the plates for growth/no growth compared to the positive control wells. Five test pairs of plates were tested, and each pair was read on a different LifeScale AST system.

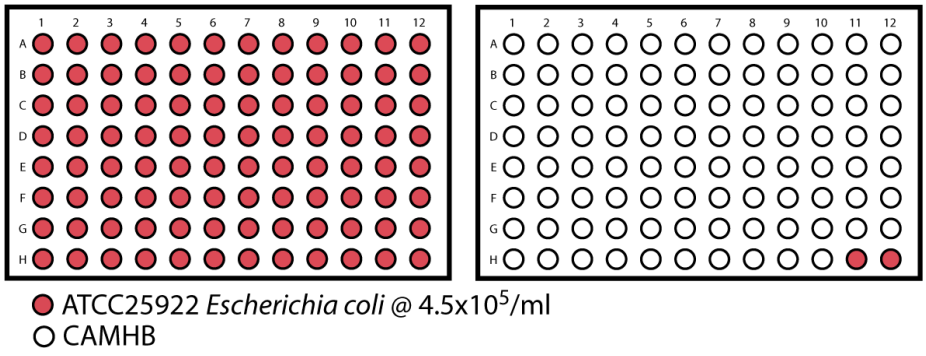

Figure 1. Plate-to-Plate contamination testing (inoculated plate/sterile blank plate).

Test 2, Plate-to-plate Carry-Over with Resistant and Susceptible Organisms

Two organisms were selected for this test. One strain was resistant to many of the antibiotics on the LifeScale LSGN AST plate, *K. pneumoniae* AR0107, the other strain was susceptible, *E. coli* ATCC25922. Both organisms were processed from positive blood cultures per standard procedures. *K. pneumoniae* was inoculated and placed onto the LifeScale AST system and incubated on-line. *E. coli* was inoculated and placed in an off-line incubator until the LifeScale AST system called for the plate to be read. The paired plates were tested in triplicate and read on different LifeScale AST systems.

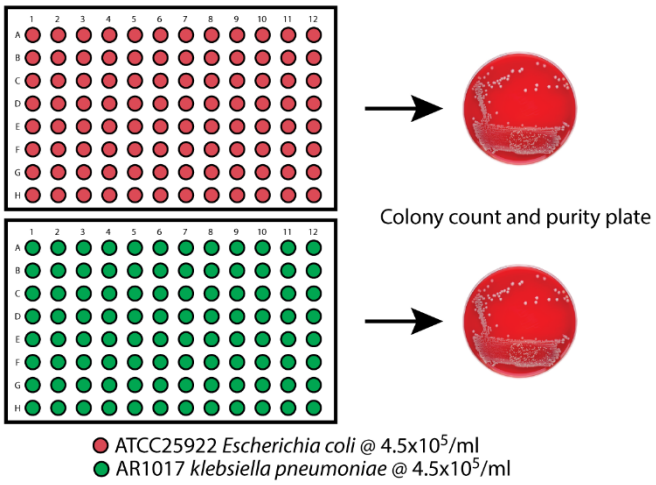

Figure 2. Plate-to-Plate contamination testing (resistant/susceptible strain paired testing).

Test 3, Well-to-Well Cross Carry-Over

Testing was performed by inoculating a blank 96-well plate in a checkerboard pattern. The plate inoculation was performed by manually inoculating alternate wells with bacteria from a processed positive blood culture and un-inoculated growth media. Plates were inoculated and incubated on-line or off-line in a conventional incubator at 35 °C ± 2 °C, for 3 hours, or until the LifeScale AST system called for the test plate. The LifeScale AST system was used to read the plate for "growth/no growth". Well-to-well testing was performed on 5 plates, each plate read on a different LifeScale AST system.

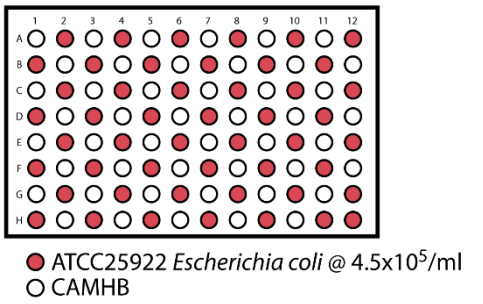

Figure 3. Well to well contamination testing.

Results and Discussion

In Test 1, plate to plate carry-over the second plate of each pair, with growth media, except for the positive control wells, showed no growth. Test 2, plate to plate carry-over with resistant and susceptible organisms, showed that the paired resistant/susceptible test plates, where the resistant strain was followed by a susceptible strain, had expected MIC results for all plates evaluated. In test 3 well-to-well carry-over, all results were as expected. Wells inoculated with uninoculated growth media had no growth, while wells inoculated with processed positive blood culture isolates showed growth for well-to-well and run-to-run testing.

The absence of growth in wells with uninoculated growth media and the expected MIC results of the susceptible strain, which was evaluated after a resistance strain, shows that there was no carry-over from well-to-well or from plate-to-plate that affects LifeScale LSGN results. Testing verifies that the LifeScale AST system cleaning and disinfection procedures result in no contamination or carry-over, ensuring accurate results.

Conclusions

The conclusions derived from both clinical and analytical studies indicate that the LifeScale AST system exhibits similar safety and effectiveness, and its performance is comparable to that of the legally marketed predicate device (as specified in CFR 807.92(3)).