



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT**

I Background Information:

A 510(k) Number

K211815

B Applicant

Affinity Biosensors

C Proprietary and Established Names

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

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
SAN	Class II	21 CFR 866.1650 - A Cellular Analysis System For Multiplexed Antimicrobial Susceptibility Testing	MI - Microbiology
LON	Class II	21 CFR 866.1645 - Fully automated short-term incubation cycle antimicrobial susceptibility system	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for use of the LifeScale Gram Negative Kit (LSGN) with the LifeScale AST system for testing positive blood culture samples containing gram-negative bacilli.

B Measurand:

Antimicrobial

	Reportable Range
Ampicillin	≤ 2 - > 64 $\mu\text{g/mL}$
Aztreonam	≤ 1 - > 64 $\mu\text{g/mL}$
Cefazolin	≤ 0.25 - > 16 $\mu\text{g/mL}$
Ceftazidime	≤ 1 - > 64 $\mu\text{g/mL}$
Ertapenem	≤ 0.125 - > 8 $\mu\text{g/mL}$
Trimethoprim/Sulfamethoxazole	≤ 0.25 - > 8 $\mu\text{g/mL}$

C Type of Test:

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.

III Intended Use/Indications for Use:

A Intended Use(s):

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.

B Indication(s) for Use:

The LifeScale Gram Negative Kit (LSGN) is indicated 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*

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

Limitations:

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*.

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

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

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

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

Testing with the LifeScale AST system should not be performed on polymicrobial samples.

Only the following drug/organism combinations have been shown to provide acceptable results with the LifeScale LSGN kit.

- Ampicillin: *E. coli*
- Aztreonam: *E. coli*, *K. aerogenes*, *K. oxytoca*
- Cefazolin: *K. pneumoniae*, *K. variicola*
- Ceftazidime: *A. baumannii*, *A. baumannii/nosocomiales* group, *Escherichia coli*, *K. aerogenes*, *K. oxytoca*, *K. variicola*, *P. aeruginosa*.
- Ertapenem: *E. coli*, *K. aerogenes*, *K. oxytoca*
- Trimethoprim/Sulfamethoxazole: *E. coli*, *K. aerogenes*, *K. oxytoca*, *K. variicola*

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

- Aztreonam: *K. pneumoniae*, *P. aeruginosa*
- Cefazolin: *E. coli*
- Ceftazidime: *Acinetobacter* spp. (other than *A. baumannii* and *A. baumannii/nosocomialis* group), *K. pneumoniae*
- Ertapenem: *K. pneumoniae*
- Trimethoprim-Sulfamethoxazole: *K. pneumoniae*

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

- Ceftazidime: *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 lack of an intermediate breakpoint).

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

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 performance of the LifeScale LSGN panel has been evaluated using the following blood culture bottles:

- **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

The use of the LifeScale AST system does not eliminate the need for subculture of the positive blood culture.

If the subculture (purity) plate indicates the sample is polymicrobial, the AST results should be voided, and susceptibility testing on each isolate using an alternative method with standard inoculum preparation should be performed.

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.

D Special Instrument Requirements:

The LifeScale Gram Negative Kit (LSGN) assay is performed on the LifeScale instrument with Software version 2.3.58.

IV Device/System Characteristics:

A 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.

Positive Blood Culture Sample Preparation. The assay workflow is as follows: At bottle positivity, the operator performs a Gram stain to confirm the presence of gram-negative rods and, at no longer than 12 hours from bottle positivity, adds a 1.5 mL aliquot of the positive blood culture to a centrifuge vial containing a proprietary Mueller Hinton growth medium. The sample is centrifuged to pellet cellular debris, and then diluted twice in growth medium by the user. Platelets are not removed during the centrifugation process. The operator loads the suspension into the LifeScale instrument. The instrument automatically determines the concentration of cells in the suspension (including bacteria and platelets) and calculates the dilution needed to inoculate the LifeScale LSGN plate at a target concentration of 4.5×10^5 CFU/mL. Platelets are compensated for in the dilution process to increase accuracy when organism concentration is low. Dilutions are automatically prepared by the instrument from the organism suspension loaded into the instrument by the user.

QC sample preparation. Quality control is performed using appropriately subcultured and incubated isolated colonies. A suspension approximating a 0.5 McFarland standard is prepared in saline by the operator and density is confirmed using a densitometer (not a component of the LifeScale AST system). The standardized suspension is diluted in Mueller Hinton broth and loaded into the LifeScale instrument. The instrument automatically determines the concentration of cells in the suspension and calculates the dilution needed to inoculate the LifeScale LSGN plate at the appropriate organism concentration. Dilutions are automatically prepared by the instrument from the organism suspension loaded into the instrument by the user.

Panel inoculation and analysis. The standardized suspension is then inoculated into a proprietary, customized 96-well plate containing lyophilized antimicrobial agents and positive control wells. Sample preparation must be completed, and incubation started, within 30 minutes of transferring the sample to the LifeScale sample vial. Following inoculation, the operator seals the plate and incubates the plate at $35 \pm 2^\circ\text{C}$ either on-board the instrument (on-line) or off-line in an auxiliary laboratory incubator. The off-line incubator must be non-CO₂ and maintain a temperature of $35 \pm 2^\circ\text{C}$. For plates incubated on-line, the system monitors growth through measurements of cell concentration in the positive control well after three hours of incubation. For plates incubated in an off-line incubator, measurement of the control well takes place at baseline (time 0) and again when replaced in the instrument after 3 hours of incubation. The system tracks the inoculated plates and alerts the user when a plate must be retrieved from the off-line incubator and placed on the instrument stage for measurement. Additional incubation time may be required for slowly growing organisms; the maximum incubation time is six hours. However, a plate can remain in an off-line incubator for an additional two hours before being measured. The maximum measurement window for any sample is eight hours from inoculation. Any plates not measured within the 8-hour window are automatically aborted.

Once an AST is started, every user step is guided by the touchscreen on the LifeScale instrument; only appropriate functions are allowed and instructions for each step are provided by touchscreen text.

During measurement, the plate is presented to a sipper (sampler) in sequence. The stage includes a wash station that cleans and disinfects the mass sensor and sipper after sampling. In addition, an integrated bar code reader verifies that the correct plate has been placed on the stage.

When sufficient growth is detected in the growth control wells, the instrument automatically reads all antibiotic-containing wells on the entire plate. The organism identification must be generated by a platform FDA-cleared for use with positive blood culture samples and the identification must be entered into LifeScale instrument before MICs and interpretations are calculated and a final report generated. Interpretations (S, I, R) are determined based on FDA-determined or recognized breakpoints.

Per the intended use of the device, 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 following limitation is included in the device labeling:

The use of the LifeScale AST system does not eliminate the need for subculture of the positive blood culture.

The LifeScale AST system is comprised of:

- **LifeScale Instrument**
Includes the mass sensor, a thermal stage for presentation of the plate for analysis, sample acquisition mechanics, a dedicated computer and electronics, USB ports for communication with the autoinoculator and the manual bar code reader, compartment containing wash solutions
- **Touchscreen monitor**
Interface between the LifeScale Instrument and operator
- **Auto-inoculator**
Connected to the LifeScale Instrument via USB and controlled by LifeScale Acquisition software. Dispenses 100 µL of the organism suspension into each plate well. It also dilutes the initial inoculum to the target microbe concentration by adding growth media to the starting inoculum.
- **Dedicated centrifuge**
Used to remove red blood cells from the blood culture
- **Compressor/waste station**
Floor-sitting unit provides head pressure to drive instrument pneumatics. The unit also includes the wash solution waste container and a solution level scale.
- **Manual bar code reader**
Used to scan well plate and accession number bar codes for unique sample identification
- **Acquisition Software and the MIC Analysis Software version 2.3.58.**
- **Incubator for on-line incubation of the plate**
- **Pager**
This is an optional accessory available from Affinity Biosensors. The pager is carried by operators as desired. Pagers receive alerts when instrument attention is needed, such as when a well plate is to be moved to the instrument stage, or when AST is complete.
- **Sensor**
Consumable that detects organisms in the sample and measures the individual mass of each cell. The sensor is replaced by the user when directed by the instrument.

Each test requires an LSGN Kit which includes:

- LSGN plate containing dried antibiotics.
- Three 20 mL bottles containing cation-adjusted Mueller Hinton growth media (12.25 mL).
- One 5 mL red cap centrifuge vial containing 1.5 mL cation-adjusted Mueller Hinton growth media
- Two empty 0.6 mL vials
- One adhesive plate film cover
- Two sample reservoirs

Table 1. Reportable MIC Ranges and Species-Specific Breakpoints for Antimicrobials Included in the LifeScale System.

Antimicrobial	Indicated Species	LifeScale Reportable Range (µg/mL)	FDA-Recognized Breakpoints		
			S	I	R
Ampicillin	<i>E. coli</i>	≤2 - >64	≤8	16	≥32
Aztreonam	<i>E. coli</i> , <i>K. aerogenes</i> , <i>K. oxytoca</i>	≤1 - >64	≤4	8	≥16
Cefazolin	<i>K. pneumoniae</i> , <i>K. variicola</i>	≤0.25 - >16	≤2	4	≥8
Ceftazidime	<i>A. baumannii</i> , <i>A. baumannii/nosocomialis</i> group	≤1 - >64	≤8	16	≥32
	<i>E. coli</i> , <i>K. aerogenes</i> , <i>K. oxytoca</i> , <i>K. variicola</i>		≤4	8	≥16
	<i>P. aeruginosa</i>		≤8	-*	≥16
Ertapenem	<i>E. coli</i> , <i>K. aerogenes</i> , <i>K. oxytoca</i>	≤0.125 - > 8	≤0.5	1	≥2
Trimethoprim/sulfamethoxazole	<i>E. coli</i> , <i>K. aerogenes</i> , <i>K. oxytoca</i> , <i>K. variicola</i>	≤0.25 - >8	≤2/38	-*	≥4/76

*No intermediate category is defined for these drug/organism combinations.

B Principle of Operation:

The LifeScale AST system employs a microfluidic sensor that detects organisms present in the sample and measures their individual masses. An automated fluidics system loads a small volume of culture from an AST test well onto the sensor. During measurement, differential pressure directs the flow of the sample through the active portion of the sensor at a controlled rate. The sensor's active portion comprises a mechanically vibrating microchannel that transduces mass to frequency. Each dip in the frequency trace is caused by a single microbe passing through the active part of the sensor and the extent of the dip is a measure of its mass. For each well, the LifeScale AST system records the number of microbes, the mass of each microbe, and the fluid volume passing through the sensor. The MIC Analysis software deploys the appropriate MIC Module to analyze these data and determine the MIC for each antibiotic on the plate.

The same measurement method is also used when preparing the sample inoculum. The result is used to dilute the organism suspension to reach the target concentration.

C Instrument Description Information:

1. Instrument Name:
LifeScale AST system, Software version 2.3.58

2. Specimen (Positive Blood Culture) Identification:

Patient and well plate data are entered using a handheld bar code reader.

3. Specimen Sampling and Handling:

The operator is guided through the necessary setup and sample preparation via a series of instructions displayed on the LifeScale AST system touch screen. The sample is prepared from a positive blood culture using a sterile collection device capable of measuring at least 1.5 mL. The 1.5 mL sample is mixed with 1.5 mL growth media and placed in the LifeScale AST system centrifuge. The sample is spun at a pre-programmed time and speed to remove red blood cells. The supernatant is examined to ensure that lysed red blood cells are not present. One mL of the supernatant is added to one vial of Mueller Hinton growth media (12.25 mL); an aliquot of the suspension is added to a dilution vial. The LifeScale AST system will measure the concentration of the organism in the dilution vial and calculate the amount of diluent required to prepare the appropriate organism concentration for testing (4.5×10^5 CFU/mL). If the concentration is appropriate, the LifeScale AST system will prepare an additional dilution for inoculation into the LSGN well plate. If the organism concentration is too high, the LifeScale AST system will guide the operator through an additional dilution step.

The standardized inoculum is added to the sample reservoir by the user and the reservoir is placed on the inoculator. The required volume is automatically aspirated from the reservoir and inoculated into a second reservoir containing growth media. The LSGN panel is placed on the inoculator and automatically inoculated with the appropriate organism suspension.

4. Calibration:

The system automatically checks critical system functions including thermal stage temperature, hatch locks, stage encoders, sensor function, communication with satellite equipment and available quantity of wash solutions. Initiation of the AST can proceed only if all system functions pass the function check.

5. Quality Control:

Quality control (QC) is performed by the operator using manufacturer-specified organisms appropriate for each antimicrobial agent. The QC AST reports “out of range” for any MIC results that are not consistent with expected MIC QC ranges. See more details about Quality Control in section II.A.8 below.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Accelerate Pheno System, Accelerate PhenoTest BC Kit

B Predicate 510(k) Number(s):

K192665

C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>K211815</u> <u>LifeScale Gram Negative Kit (LSGN) with the LifeScale AST system</u>	<u>K192665</u> <u>Accelerate PhenoTest BC Kit</u>
Device Trade Name	LifeScale Gram Negative Kit (LSGN) with the LifeScale AST system	Accelerate PhenoTest BC Kit
General Device Characteristic Similarities		
Intended Use/Indications For Use	The LifeScale AST system is a multiplexed <i>in vitro</i> 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 indicated for use with the LifeScale AST system for <i>in vitro</i> testing of positive blood culture samples confirmed by Gram stain as containing gram-negative bacilli.	The Accelerate PhenoTest BC kit is a multiplexed <i>in vitro</i> diagnostic test utilizing both qualitative nucleic acid fluorescence <i>in situ</i> 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.
Sample	Blood cultures signaled as positive by a continuous	Same

Device & Predicate Device(s):	<u>K211815</u> <u>LifeScale Gram Negative Kit (LSGN) with the LifeScale AST system</u>	<u>K192665</u> <u>Accelerate PhenoTest BC Kit</u>
	monitoring blood culture system.	
Inoculation Method	Automated	Same
Read Method	Automated	Same
Result Report	Report results as a minimum inhibitory concentration (MIC) and categorical interpretation (S, I, R)	Same
General Device Characteristic Differences		
Antimicrobial Agents	ampicillin, aztreonam, cefazolin, ceftazidime, ertapenem, trimethoprim/sulfamethoxazole	Amikacin, ampicillin, ampicillin/sulbactam, aztreonam, ceftazidime, ceftaroline, cefepime, ceftriaxone, ciprofloxacin, daptomycin, erythromycin, ertapenem, gentamicin, linezolid, meropenem, piperacillin/tazobactam, tobramycin, vancomycin
Organisms tested	Gram-negative bacilli	Gram-positive and gram-negative organisms
Sample Preparation	Centrifugation and pipetting of sample	Automated
IVD Functions	AST	ID and AST
Instrument	LifeScale AST system	Accelerate Pheno System
Technology	Microfluidic and resonant frequency to calculate organism concentration and/or mass distribution	Morphokinetic cellular analysis

VI Standards/Guidance Documents Referenced:

- FDA Class II Special Controls Guidance Document: Antimicrobial Susceptibility (AST) Systems; Guidance for Industry and FDA (Issued August 28, 2009).
- CLSI M100-Ed 33, *Performance Standards for Antimicrobial Susceptibility Testing*; 2023.
- CLSI M100-Ed 32, *Performance Standards for Antimicrobial Susceptibility Testing*; 2022.
- CLSI M07. 11th ed. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 2018.

- FDA STIC Website FDA-Recognized Antimicrobial Susceptibility Test Interpretive Criteria
- IEC 60601-1-2 Edition 4.0 2014-02, Medical electrical equipment - Part 1-2: General requirements for basic safety and essential performance
- IEC 61010-1 Edition 3.1 2017-01, Safety requirements for electrical equipment for measurement, control, and laboratory use
- IEC 62304 Edition 1.1 2015-06 Consolidated Version, Medical device software - Software life cycle processes

VII Performance Characteristics:

A Analytical Performance:

1. Precision/Reproducibility:

Reproducibility testing for the LifeScale LSGN kit was initially conducted at three sites, with an additional site contriving and distributing the samples to the testing sites. Standard Aerobic Blood Culture medium (BACTEC) bottles were inoculated with appropriate isolates, incubated until positivity and tested on the LifeScale system. Isolates were tested in triplicate, each for three days at three sites. When necessary, supplemental reproducibility testing was performed internally on three different systems to simulate three sites.

Panel members generally consisted of species indicated for use with each antimicrobial agent. Reproducibility was determined from the total number (and percent) of results that fell within one dilution ($\pm$ one doubling dilution) of the modal MIC result divided by the total number of results. Reproducibility was evaluated between sites (inter-site) and within sites (intra-site). Both best-case (assumes that off-scale results are less than one dilution from the mode) and worst-case (assumes that off-scale results are more than one dilution from the mode) performance was determined for each antimicrobial agent, as outlined in the AST Special Controls Guidance. Quality control was performed on all instruments each day of replicate testing.

Inter-site and intra-site reproducibility data was found to be acceptable for all antimicrobial agents. Reproducibility results are summarized in Table 2.

Table 2. Reproducibility Results for LifeScale LSGN Kit^a

Antimicrobial	Best Case Reproducibility (%)	Worst Case Reproducibility (%)
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)

Antimicrobial	Best Case Reproducibility (%)	Worst Case Reproducibility (%)
Trimethoprim/ sulfamethoxazole	255/264 (96.6)	243/264 (92.0)

^a Includes data from original and supplemental testing

2. **Inoculum Density Study**

An inoculum density study was performed to determine the effect of organism concentration in a positive blood culture on the performance of the LifeScale LSGN kit and the ability of the LifeScale AST instrument to prepare an appropriate inoculum regardless of the starting concentration. By design, the LifeScale AST instrument automatically determines the concentration of cells in the suspension and will calculate the dilution needed to inoculate the LSGN plate at a target concentration of 4.5×10^5 CFU/mL.

A minimum of ten contrived samples containing high (10^9 CFU/mL) and low (10^6 CFU/mL) concentrations of representative species were prepared for each claimed drug/organism combination, with the exception of ceftazidime. For ceftazidime, six contrived samples containing *A. baumannii* at both concentrations and five contrived samples containing *P. aeruginosa* at 10^6 CFU/mL were prepared. In addition, contrived samples containing very low (10^4 CFU/mL) concentrations of *P. aeruginosa* were tested to confirm that the LifeScale AST system will terminate testing and generate an invalid result if sufficient growth is not detected. All samples were tested in triplicate with the LifeScale LSGN kit and the LifeScale system.

The organism concentration in each contrived sample was confirmed by colony count; all colony counts corresponded to the target organism concentration ranges of 10^4 (for a subset of *P. aeruginosa* samples), 10^6 and 10^9 CFU/mL. AST results were compared to the mode MIC result determined by the reference method.

MIC results for samples containing 10^6 and 10^9 CFU/mL showed good agreement (EA $\geq$ 90%) for claimed drug/organism combinations for the LifeScale LSGN kit as compared to the reference method results regardless of the starting organism concentration.

No results were obtained for samples containing 10^4 CFU/mL of *P. aeruginosa*; all tests were terminated due to insufficient growth, confirming that low organism concentrations in the sample will result in an invalid test.

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 organism concentrations of the positive blood culture samples:

- 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

To address the overall trending observed in the clinical and analytical studies, the following general statement was added to the device labeling;

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*

3. Linearity:

Not applicable

4. Analytical Specificity/Interference:

An interfering substances study was performed to determine the effect of potential interferents in blood cultures on results obtained with the LifeScale LSGN kit. Two different bottle types (BD PLUS Aerobic and BD Standard) were seeded with strains of *E. coli*, *K. pneumoniae*, *A. baumannii* and *P. aeruginosa*; samples included: 1) bottles seeded with organism and the potential interferent and 2) control bottles seeded with organism and no interferent. Endogenous substances were evaluated as possible interferents in concentrations equivalent to concentrations found in human blood (Table 3). Testing of seeded bottles with and without interferent was performed in triplicate. AST performance in the presence of potential interferents was determined by comparison of the EA of MIC results obtained from samples with and without interferents. Comparisons were performed by analysis of MIC mode of the control tests compared with the MIC of each replicate containing interferent.

Table 3. Endogenous Interferents, Concentration Tested

Interfering substances	Concentration Tested	Reference Range in Human Blood
Conjugated bilirubin	0.003 mg/mL	0-0.002 mg/mL
Unconjugated bilirubin	0.003 – 0.012 mg/mL	0.002 mg/mL
Gamma globulin	6 - 13 mg/mL	6-13 mg/mL
Hemoglobin	100 mg/mL	100-200 mg/mL
Triglycerides	5 mg/mL	1.5-5 mg/ml
White blood cells	4.5 x10 ⁶ -1.0 x10 ⁷ cells/mL	4.5 x10 ⁶ -1.0 x10 ⁷ cells/mL
Platelets	≥450,000 platelets/μL	150,000 -400, 000/μl
Heparin	330 units/dL	0.35 – 1 U/mL

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. The following limitation is included in the device labeling:

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

- *All interferents: Cefazidime/A. baumannii*
- *Platelets and heparin: Cefazolin/K. pneumoniae*

For the remaining drug/organism combinations, no interference was noted for any potential interferent as determined by MIC comparison with reference results.

The effect of antimicrobial agents as interfering substances was not evaluated. The following limitation is included in the device labeling:

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

5. Media Equivalency Study

The purpose of this study was to assess compatibility of the LifeScale LSGN kit with various commonly used blood culture bottle types. Eight blood culture bottle types from three different blood culture manufacturers [bioMérieux, Inc. (BacT/Alert: Standard Aerobic, Standard Anaerobic), Becton Dickinson (BACTEC: Standard Aerobic, Standard Anaerobic, Lytic Anaerobic, Plus Aerobic), and ThermoFisher (VersaTREK: REDOX 1 Aerobic, REDOX 2 Anaerobic)] were evaluated analytically with the LifeScale LSGN kit. A total of 12 well-characterized strains of *E. coli*, *K. pneumoniae*, *A. baumannii* and *P. aeruginosa* were inoculated into each blood culture bottle type; ten replicates of each resulting positive blood culture were tested using the LifeScale LSGN kit at the time of positivity. BACTEC bottles were incubated in a continuous monitoring blood culture instrument; other bottle types were incubated in a conventional incubator with or without mixing per the bottle instructions for use. The time to positivity was estimated for the BacT/Alert and VersaTREK blood culture bottles based on time to positivity for BACTEC media and confirmed by LifeScale AST system cell counts.

MIC results of each bottle type with each antimicrobial/organism combination were compared to the mode MIC value obtained with the broth microdilution reference method; results compared with the LifeScale mode showed similar results.

Results for the majority of drug/organism combinations for claimed species showed good performance with all blood culture bottles with the following exceptions:

- Results obtained with the combination of ertapenem/*E.coli* with BacT/Alert Standard Anaerobic Media (97 total results) and VersaTREK REDOX 1 Aerobic Media (80 total results) showed low CA when compared to the reference method modes. In addition, testing with this drug/organism combination using BACTEC Standard Aerobic media (80 total results) showed low EA when compared to the reference method mode.

To address the results obtained with ertapenem/*E. coli* the following limitation was included in the device labeling:

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 Aerobic and VersaTREK REDOX 1 Aerobic. Use alternative bottle types for determining ertapenem MICs for E. coli.

- Results obtained with the combination of aztreonam/*E. coli* with BACTEC Lytic Anaerobic Media (46 total results) showed a slightly lower than acceptable EA and CA (89.9%) when compared to the reference method mode. Poor results were attributed to a single strain; results were acceptable for the remaining *E. coli* strains tested.

To address the low EA and CA, the following footnote was added to the Media Equivalency Study summary in the device labeling:

Low EA and CA for aztreonam/E. coli with BACTEC Lytic Anaerobic media is attributed to a single E. coli isolate.

- Testing of trimethoprim/sulfamethoxazole with *E. coli* was not performed due to a lack of *E. coli* isolates with on-scale MICs; the following limitation is included in the device labeling:

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

6. Assay Reportable Range:

Not applicable

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

Quality control testing was performed each day that testing was conducted during the clinical and analytical studies. Quality control samples were prepared from isolated colonies as described in Section IVA above (Device Description). Gram-negative CLSI-recommended quality control strains were tested a minimum of 20 times using both the LifeScale LSGN kit and the reference broth microdilution method at three sites during the clinical studies. At least one QC organism was tested each day, rotating through 3 strains (*E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, and *P. aeruginosa* ATCC 27853). For ampicillin, the LifeScale LSGN panel did not include the full CLSI expected range for *E. coli* ATCC 25922. To address the use of this strain in QC testing the following footnote was added to the QC table:

Ampicillin: Does not include the full CLSI expected range

For all antimicrobials, greater than 95% of results obtained during the clinical study were within the expected range; quality control results were acceptable (Table 4 below).

Table 4. QC Results for the LifeScale LSGN Kit, Clinical Study

Antimicrobial	QC Strain	Expected Range µg/mL	No. in Range/No. Tested (%)	
			Reference	LifeScale
Ampicillin ^a	<i>E. coli</i> ATCC 25922	≤2 – 8	112/112 (100)	299/299 (100)
Aztreonam	<i>P. aeruginosa</i> ATCC 27853	2 - 8	110/111 (99.1)	266/266 (100)
Cefazolin	<i>E. coli</i> ATCC 25922	1 - 4	112/112 (100)	299/299 (100)
Ceftazidime	<i>K. pneumoniae</i> ATCC 700603	16 - 64	105/106 (99.1)	283/285 (99.3)
Ertapenem	<i>P. aeruginosa</i> ATCC 27853	2 – 8	107/112 (95.5)	264/267 (98.9)
Trimethoprim/ sulfamethoxazole	<i>E. coli</i> ATCC 25922	≤0.5	112/112 (100)	299/299 (100)

^a Does not include full CLSI expected range. LifeScale MIC results of ≤2 µg/mL were considered acceptable.

Inoculum Density Check: Organism concentration determined automatically by the instrument. Refer the Inoculum Density study above.

LifeScale LSGN Tests Initiated and Failed to Report a Result. Overall, 3.23% of tests initiated during the analytical and clinical studies failed to provide a result (Table 5).

Table 5. LifeScale LSGN Tests Initiated and Failed to Report a Result

Reason for Exclusion/Incomplete Test	No. Exclusion or Incomplete Test/Total No of Tests (%)			
	Clinical Study	Analytical Studies	QC	Overall
Plate Failures^a	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^b	4/682 (0.59)	51/3452 (1.48)	15/1751 (0.86)	70/5885 (1.19)
Other Reasons^c	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)

^a Plate failures include: unable to verify positive controls, sensor clog detected, system unable to calculate MIC.

^b LifeScale failures include: LifeScale system software and hardware failures

^c Other Reasons include: operator error, incubation time greater than 8 hours, user canceled, protocol error

Purity Check: Purity checks were performed for all clinical and analytical tests. Testing was not performed on samples with mixed growth or contamination.

8. Sample Stability Study:

The purpose of this study was to demonstrate that positive blood cultures tested at 12 hours post positivity (T₁₂) and held at incubation temperature (35 °C) are equivalent to results obtained at the time of positivity (T₀).

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

(T₀) and at 13 ± 0.5 hours post bottle ring (T₁₃). 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 timepoint. Resulting MICs were compared to the modal LifeScale MIC determined at T₀. The stability acceptance criterion was ≥ 95% agreement for EA. QC was performed each day of testing.

A total of 21 gram-negative isolates with known MICs as determined by broth microdilution testing were evaluated. Species tested included *Acinetobacter baumannii* (2), *E. coli* (14), *K. pneumoniae* (3) and *P. aeruginosa* (2). Results for ampicillin, aztreonam, cefazolin, ceftazidime and trimethoprim/sulfamethoxazole were acceptable, indicating that results obtained at T₁₂ were equivalent to MIC results obtained at T₀ (Table 6). Additional testing was performed for ertapenem/*E. coli* and showed acceptable results (Table 6).

Table 6. Summary of Sample Stability AST Results at T₀ and T₁₃ as Compared to LifeScale LSGN Mode

Antibiotic	No. Samples Tested	Data Points		LifeScale Mode T ₁₃ to LifeScale Mode T ₀
		T ₀	T ₁₃	No. EA/Total Tested (EA%)
Ampicillin	6	18	18	18/18 (100)
Aztreonam	6	18	18	18/18 (100)
Cefazolin	3	9	9	9/9 (100)
Ceftazidime	10	30	30	30/30 (100%)
Ertapenem	14	42	42	42/42 (100%)
Trimethoprim/ Sulfamethoxazole	6	18	18	18/18 (100)
Total	45	135	135	132/135 (97.8)

Colony Counts. To determine organism concentration at the time of test performance, colony counts were performed from the LifeScale LSGN panel positive control well at the time of set-up and from the positive blood cultures at T₀ and T₁₃. The colony counts obtained from positive blood culture bottles at T₀ ranged from 1.0 x10⁷ to 7.1 x10⁸ CFU/mL. The colony counts obtained from positive blood culture bottles at T₁₃ ranged from 1.07 x10⁸ to 1.02 x10¹⁰ CFU/mL. The range for all LifeScale LSGN plate inocula was 1.2 x10⁵ to 1.0 x10⁶ CFU/mL, which is within the target range of 1.0 x10⁵ to 1.0 x10⁶ CFU/ml and is acceptable. See Inoculum Density Study above.

Quality Control. QC results obtained during the sample stability study were acceptable.

Sample Stability Results. The study demonstrates that results from samples tested within one hour after bottle ring provide equivalent results to those tested at 13 hours after bottle ring when incubated in the blood culture monitoring system at 35 °C.

To address the timing of positive blood culture processing, the following limitation is included 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.

9. Off-line and On-line Incubation Study:

The purpose of this study was to demonstrate that results obtained for LSGN plates incubated in an off-line incubator (a non-CO₂ laboratory incubator with a temperature of 35°C ± 2° C) are accurate (as determined by comparison to the reference method) to results obtained for LSGN plates incubated on the integrated thermal stage in the LifeScale AST instrument (on-line). In addition, the study evaluated the effect of an 8-hour incubation on the final MIC (for plates held in an off-line incubator for an additional 2 hours after the maximum incubation time prior to measurement). The LifeScale instrument has the capacity for incubating a single LSGN plate on-line; additional plates processed within that plate's incubation period must be incubated in an off-line incubator. For plates incubated on-line, the system monitors growth in the positive control well through measurements of cell concentration after a minimum of three hours of incubation. For plates incubated in an off-line incubator, measurement of the control well takes place at baseline (time 0) and again when replaced in the instrument after 3 hours of incubation. The system tracks the inoculated plates and alerts the user when a plate must be retrieved from the offline incubator and placed on the instrument stage for measurement.

For this study a set of challenge strains (including *E. coli*, *K. pneumoniae*, *A. baumannii* and *P. aeruginosa*) were inoculated into a BD BACTEC blood culture bottle and incubated until positivity. At positivity, the blood culture was processed in accordance with the LifeScale AST system's instructions for use, inoculated to LSGN plates and tested in triplicate under the following incubation conditions:

- Three hours incubation on-line
- Three hours incubation off-line
- Six hours incubation off-line
- Eight hours incubation off-line

LifeScale LSGN kit results were compared to results obtained with the reference method. LifeScale LSGN kit results as compared to the reference method from samples evaluated in the clinical study (which included samples incubated both on- and off-line) were also evaluated for cefazolin, ceftazidime and ertapenem.

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

Ertapenem/*E. coli* results obtained after three hours incubation in the analytical study 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 for 3 hours on-line, 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%. Results for two samples incubated off-line for 6 hours of incubation were equivalent to results obtained with the reference method.

To address the unacceptable performance with off-line incubation of ertapenem/*E. coli*, the following limitation was added to 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.

Results for all samples incubated off-line for 8 hours were acceptable for all drug/organism combinations. These results demonstrate that plates incubated past the maximum incubation time still provide acceptable results.

Results of the study indicate that on-line and off-line incubation provide acceptable results for ampicillin, aztreonam, cefazolin, ceftazidime and trimethoprim/sulfamethoxazole.

10. Detection Limit:

Not Applicable

11. Assay Cut-Off:

Not Applicable

12. Accuracy (Instrument):

Not Applicable

13. Cross Contamination/Carry-Over:

A two-part study was performed to verify the effectiveness of the LifeScale AST system automated cleaning and disinfection procedures in preventing carry over of microorganisms between processing of successive samples. The first study included paired AST testing of a positive blood culture containing *E. coli* followed by testing of an AST panel containing only growth media. Particles detected in the growth media only plate was below the expected background particle count and was determined to be acceptable. The second study included testing a panel with a resistant strain followed by testing a panel with a susceptible strain and results were evaluated to detect increased MICs in the panel inoculated with the susceptible strain. Testing was performed in triplicate on three LifeScale systems. No transfer of resistant strains was detected, and results were determined to be acceptable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Clinical performance testing with the LifeScale AST system with the LSGN kit was initially performed at six geographically diverse U.S. clinical sites. Additional testing at a single in-house site was performed to supplement the original data to support specific claims. Testing was performed with clinical samples, including fresh, prospective positive blood cultures (201 samples) as well as positive blood cultures contrived with clinical isolates (seeded clinical, 1112 samples). Isolates used for contrived blood cultures were sourced from the clinical testing sites and included: 1) fresh isolates (tested within seven days of isolation, minimally subcultured and not frozen), 2) contemporary isolates (tested within one year of isolation), and 3) stock isolates (recovered from patient specimens and tested within 3 years of isolation). The composition of samples related to time from isolation (fresh/contemporary/stock) was considered acceptable as the original data set included the appropriate percentages of fresh/contemporary/stock isolates. In the final analysis, the percentage of fresh isolates was reduced due to removal of certain drug/organism combinations due to performance issues.

Positive blood cultures contrived with challenge organisms (433 samples), including resistant isolates and isolates with on-scale MIC results, were also tested at the clinical sites.

LifeScale LSGN kit testing was performed per instructions in the device labeling. Plates were incubated on-line (in the LifeScale instrument) or off-line (in an auxiliary incubator). Incubation times for all drug/organism combinations averaged 3.5 hours.

Pure bacterial isolates used for contriving blood cultures as well as those isolates subcultured from prospective positive blood cultures were sent to a central reference laboratory in the U.S. for identification verification using an FDA-cleared MALDI-TOF system. AST testing was performed using the reference broth microdilution (BMD) method, as described in the CLSI document *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, M07, 29th edition. Reference BMD panels were run in triplicate for each isolate and an MIC mode was determined for comparison with the LifeScale LSGN MIC result. If an MIC mode could not be established from the first three BMD results, a second set of BMD assays was run in triplicate and the MIC mode across all six tests was determined. If a mode still could not be established, the median MIC was used for comparison with the LifeScale LSGN MIC result.

LifeScale LSGN kit performance was based on criteria outlined in the *Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems*. Performance criteria included essential agreement (EA) and categorical agreement (CA) and the number and percent of categorical errors (minor, major and very major errors). EA was calculated as the percentage of MIC results that fell within ± 1 doubling dilution of the reference result; CA was calculated as the percentage of LifeScale LSGN interpretive results (S/I/R) that were identical to the interpretive categories of the reference result. Acceptable results were obtained with the drug/organism combinations outlined below. Results for drug/organism combinations not listed were not acceptable. To address the use of the device for drug/organism combinations that provided acceptable results, the following limitation was included in the device labeling:

Only the following drug/organism combinations have been shown to provide acceptable results with the LifeScale LSGN kit:

- Ampicillin: *Escherichia coli*
- Aztreonam: *Escherichia coli*, *K. aerogenes*, *K. oxytoca*
- Cefazolin: *K. pneumoniae*, *K. variicola*
- Ceftazidime: *Acinetobacter baumannii*, *A. baumannii/nosocomialis* group, *E. coli*, *K. aerogenes*, *K. oxytoca*, *K. variicola*, *P. aeruginosa*.
- Ertapenem: *Escherichia coli*, *K. aerogenes*, *K. oxytoca*
- Trimethoprim/Sulfamethoxazole: *Escherichia coli*, *K. aerogenes*, *K. oxytoca*, *K. variicola*

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

- Aztreonam: *K. pneumoniae*, *P. aeruginosa*
- Cefazolin: *E. coli*

- *Ceftazidime: Acinetobacter spp. (other than A. baumannii and A. baumannii/nosocomialis group), K. pneumoniae*
- *Ertapenem: K. pneumoniae*
- *Trimethoprim-Sulfamethoxazole: K. pneumoniae*

Positive blood culture samples containing more than one organism should not be tested with the LifeScale AST system. The following limitations are included in the device labeling:

Testing with the LifeScale AST system should not be performed on polymicrobial samples.

If the subculture (purity) plate indicates the sample is polymicrobial, the AST results should be voided, and susceptibility testing on each isolate using an alternative method with standard inoculum preparation should be performed.

A summary of the performance of LifeScale LSGN kit is described below for each antimicrobial agent with indicated species. Complete details including EA, CA and error rate analysis per organism group are summarized in Table 7.

Ampicillin. A total of 137 *E. coli* samples were evaluated with ampicillin. The combined results from clinical and challenge sample testing demonstrated an EA of 100% and CA of 100%. There were no major or very major errors. Performance with other species of Enterobacterales was not evaluated.

Aztreonam. A total of 301 Enterobacterales samples were evaluated with aztreonam [*E. coli* (137), *K. aerogenes* (55) and *K. oxytoca* (109)]. The combined results from clinical and challenge sample testing demonstrated an EA of 98.0% and CA of 98.0%. There was one major error (0.5%) and no very major errors. Performance with *K. pneumoniae* was not acceptable due to a low essential agreement (87.7%), and the occurrence of major errors (4.1%). Although the EA of *P. aeruginosa* with aztreonam was acceptable at 94.0%, CA (87.9%) and the very major error rate (3.7%, 2 very major errors/54 resistant strains) were not acceptable. Limitations describing major errors for *K. pneumoniae* and very major errors for *P. aeruginosa* at particular drug concentrations were not considered appropriate because such a limitation would impact >10% of LifeScale LSGN kit results. The [general limitation](#) listed above addresses the species that have shown good performance with LifeScale LSGN kit testing with aztreonam and for which testing with LifeScale LSGN kit may be performed.

Cefazolin. A total of 143 Enterobacterales samples were evaluated with cefazolin [*K. pneumoniae* (105) and *K. variicola* (38)]. The combined results from clinical and challenge sample testing demonstrated an EA of 97.9% and CA of 92.3%. There were no major or very major errors. Although the EA of *E. coli* with cefazolin was acceptable at 95.6%, CA (85.4%) and the very major error rate (2.7%, 2 very major errors/74 resistant strains) were not acceptable. A limitation describing the very major errors for *E. coli* at a particular drug concentration was not considered appropriate because such a limitation would impact >10% of LifeScale LSGN results. The [general limitation](#) listed above addresses the species that have shown good performance with LifeScale LSGN kit testing with cefazolin and for which testing with LifeScale LSGN kit may be performed.

Ceftazidime/*A. baumannii*. A total of 73 *Acinetobacter* spp. samples were evaluated with ceftazidime [*A. baumannii* (35) and *A. baumannii/nosocomialis* group (38)]. The combined results from clinical and challenge sample testing demonstrated an EA of 98.6% and CA of 100%. There were no categorical errors. Performance of ceftazidime with *A. baumannii* and *A. baumannii/nosocomialis* group was acceptable. Performance with *Acinetobacter* spp. other than *A. baumannii* or *A. baumannii/nosocomialis* group was not acceptable due to a low EA (73.9%), low CA (86.9%) and unacceptable very major error rate (100%). The [general limitation](#) listed above addresses the species that have shown good performance with LifeScale LSGN kit testing with ceftazidime.

Ceftazidime/*P. aeruginosa*. 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 CA of 94.0%. There were 5 major errors (8.2%) and 2 very major errors (3.6%). Due to a lack of an intermediate breakpoint, further analysis of the errors was performed, and adjustments were made by considering the MIC values of the errors compared to the reference MIC value. One of the very major errors were in essential agreement with the reference MIC value. Therefore, the very major error rate was adjusted to 1.8% which is acceptable. Two of the major errors were in essential agreement with the reference MIC value. Therefore, the major error rate was adjusted to 3.3% which was still unacceptable. To address the unacceptable major error rate, the following limitation is included in the device labeling:

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

- *Ceftazidime: 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 lack of an intermediate breakpoint.*

Ceftazidime/Enterobacterales. A total of 340 Enterobacterales samples were evaluated with ceftazidime [*E. coli* (137), *K. aerogenes* (55), *K. oxytoca* (109) and *K. variicola* (39)]. The combined results from clinical and challenge sample testing demonstrated an EA of 97.6% and CA of 98.2%. There were no major or very major errors. Performance with *K. pneumoniae* was not acceptable due to the unacceptable major error rate (4.4%). A limitation describing the very major errors for *K. pneumoniae* at a particular drug concentration was not considered appropriate because such a limitation would impact >10% of LifeScale LSGN results. The [general limitation](#) listed above addresses the species that have shown good performance with LifeScale LSGN kit testing with ceftazidime and for which testing with LifeScale LSGN kit may be performed.

Ertapenem. A total of 296 Enterobacterales samples were evaluated with ertapenem [*E. coli* (136), *K. aerogenes* (52) and *K. oxytoca* (108)]. Results for 70 isolates of *E. coli* included in plates incubated in an off-line incubator were removed due to unacceptable performance for plates incubated in this environment (See On-Line and Off-Line Incubation Study above). Results for the remaining 66 isolates of *E. coli* were included in the performance calculation. In the clinical study, the combined results from clinical and challenge isolate testing demonstrated an EA of 93.8% and CA of 95.6% for all Enterobacterales species combined. For *K. aerogenes* and *K. oxytoca*, the EA results were acceptable (94.2% and 92.6% for *K. aerogenes* and *K. oxytoca*, respectively) as were the CA results (96.2% and 96.3% for *K. aerogenes* and *K. oxytoca*, respectively). The major and very major error rate for *K.*

aerogenes was acceptable, however the very major error rate for *K. oxytoca* was unacceptable (20%) due to very major errors at an MIC of 0.5 µg/mL; there were no major errors for *K. oxytoca*. To address the performance of ertapenem with *K. oxytoca*, the following limitation is included in the device labeling:

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

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

For *E. coli*, the overall EA and CA results were acceptable. However, when stratified by on-line and off-line incubation, results obtained for samples incubated off-line were unacceptable with an EA of 87.0% for samples incubated for 3 hours and 84.6% for samples incubated for 4 hours. Samples containing *E. coli* and incubated for 3 hours on-line showed an EA of 95.4% which is acceptable.

To address the unacceptable EA for samples containing *E. coli* and incubated off-line, the following limitation was added to 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.

Performance with *K. pneumoniae* was not acceptable due to an unacceptable major error rate (4.4%). The [general limitation](#) listed above addresses the species that have shown good performance with LifeScale LSGN kit testing with ertapenem and for which testing with LifeScale LSGN kit may be performed.

Trimethoprim/sulfamethoxazole. A total of 340 Enterobacterales samples were evaluated with trimethoprim/sulfamethoxazole [*E. coli* (138), *K. aerogenes* (55), *K. oxytoca* (108) and *K. variicola* (39)]. The combined results from clinical and challenge sample testing demonstrated an EA of 99.1% and CA of 99.1%. The major and very major error rates were acceptable. Performance with *K. pneumoniae* was not acceptable due to an unacceptable major error rate (5.7%). A limitation describing the major errors for *K. pneumoniae* at a particular drug concentration is not appropriate because such a limitation would impact >10% of LifeScale LSGN results. The [general limitation](#) listed above addresses the species that have shown good performance with LifeScale LSGN kit testing with trimethoprim-sulfamethoxazole and for which testing with LifeScale LSGN kit may be performed.

Table 7. Performance for All Antimicrobial Agents with All Organisms Groups

	Tot	No. EA	EA %	Eval EA Tot	No. Eval EA	Eval EA %	No. CA	CA %	No. R	No. S	min	maj	vmj
Ampicillin <i>E. coli</i> [Breakpoints (µg/mL): 8 (S), 16 (I), 32 (R)]													
Seeded Challenge	46	46	100	2	2	100	46	100	39	7	0	0	0
Prospective	23	23	100	6	6	100	23	100	9	14	0	0	0
Seeded Clinical	68	68	100	15	15	100	68	100	39	29	0	0	0

	Tot	No. EA	EA %	Eval EA Tot	No. Eval EA	Eval EA %	No. CA	CA %	No. R	No. S	min	maj	vmj
Combined	137	137	100	23	23	100	137	100	87	50	0	0	0
Aztreonam Enterobacterales: <i>E. coli</i>, <i>K. aerogenes</i>, <i>K. oxytoca</i> [Breakpoints (µg/mL): 4 (S), 8 (I), 16 (R)]													
Seeded Challenge	66	64	97.0	20	18	90.0	66	100	52	14	0	0	0
Prospective	30	29	96.7	3	2	66.7	28	93.3	2	28	1	1	0
Seeded Clinical	205	202	98.5	22	19	86.4	201	98.0	37	165	4	0	0
Combined	301	295	98.0	45	39	86.7	295	98.0	91	207	5	1	0
Cefazolin Enterobacterales: <i>K. pneumoniae</i>, <i>K. variicola</i> [Breakpoints (µg/mL): 2 (S), 4 (I), 8 (R)]													
Seeded Challenge	29	29	100	1	1	100	28	96.6	29	0	1	0	0
Prospective	38	35	92.1	33	30	90.9	32	84.2	6	29	6	0	0
Seeded Clinical	76	76	100	35	35	100	72	94.7	42	33	4	0	0
Combined	143	140	97.9	69	66	95.7	132	92.3	77	62	11	0	0
Ceftazidime Enterobacterales: <i>E. coli</i>, <i>K. aerogenes</i>, <i>K. oxytoca</i>, <i>K. variicola</i> [Breakpoints (µg/mL): 4 (S), 8 (I), 16 (R)]													
Seeded Challenge	75	74	98.7	9	8	88.9	75	100	63	12	0	0	0
Prospective	33	32	97.0	3	2	66.7	32	97.0	2	31	1	0	0
Seeded Clinical	232	226	97.4	22	16	72.7	227	97.8	36	195	5	0	0
Combined	340	332	97.6	34	26	76.5	334	98.2	101	238	6	0	0
Ceftazidime <i>P. aeruginosa</i> [Breakpoints (µg/mL): 8 (S), 16 (R)]													
Seeded Challenge	55	54	98.2	30	29	96.7	55	100	37	18	0	0	0
Prospective	15	15	100	12	12	100	14	93.3	2	13	0	0	1
Seeded Clinical	46	38	82.6	43	35	81.4	40	87.0	16	30	0	5	1
Combined	116	107	92.2	85	76	89.4	109	94.0	55	61	0	5	2
Ceftazidime <i>A. baumannii</i>/<i>A. baumannii</i> nosocomialis group [Breakpoints (µg/mL): 8 (S), 16 (I), 32 (R)]													
Seeded Challenge	21	21	100	5	5	100	21	100	21	0	0	0	0
Seeded Clinical	52	51	98.1	20	19	95.0	52	100	35	15	0	0	0
Combined	73	72	98.6	25	24	96.0	73	100	56	15	0	0	0
Ertapenem Enterobacterales: <i>E. coli</i> (on-line incubation only), <i>K. aerogenes</i>, <i>K. oxytoca</i> [Breakpoints (µg/mL): 0.5 (S), 1 (I), 2 (R)]													
Seeded Challenge	50	45	90.0	9	4	44.4	48	96.0	31	18	0	1	1
Prospective	12	12	100	0	0	NA	12	100	0	12	0	0	0

	Tot	No. EA	EA %	Eval EA Tot	No. Eval EA	Eval EA %	No. CA	CA %	No. R	No. S	min	maj	vmj
Seeded Clinical	164	155	94.5	19	10	52.6	156	95.1	12	152	7	0	1
Combined	226	212	93.8	28	14	50	216	95.6	43	182	7	1	2
Trimethoprim/Sulfamethoxazole – Enterobacterales: <i>E. coli</i>, <i>K. aerogenes</i>, <i>K. oxytoca</i>, <i>K. variicola</i> [Breakpoints (µg/mL): 2 (S), 4 (R)]													
Seeded Challenge	76	75	98.7	4	3	75.0	75	98.7	50	26	0	1	0
Prospective	33	33	100	2	2	100	33	100	7	26	0	0	0
Seeded Clinical	231	229	99.1	10	8	80.0	229	99.1	34	197	0	2	0
Combined	340	337	99.1	16	13	81.3	337	99.1	91	249	0	3	0

EA - Essential Agreement

R - Resistant isolates

min – minor errors

CA - Category Agreement

S – Susceptible isolates

maj – major errors

Eval – Evaluable isolates

vmj - very major errors

Trending

A trending analysis using combined clinical and challenge sample results was conducted to evaluate antimicrobial-organism combinations for which LifeScale LSGN MIC results were determined to be one or more doubling dilutions lower or higher than the reference result (Table 8). MIC results that were off-scale for both the reference and LifeScale LSGN kit were not considered in the trending analysis. Antimicrobial-organism combinations for which the difference between the percentage of isolates with higher or lower MIC values was $\geq 30\%$ with a statistically significant confidence interval were considered to have evidence of trending and is addressed in the labeling.

Table 8. LifeScale LSGN Kit – Analysis of Trending in the Clinical Study

Drug	Organism	Total Evaluable for Trending	≥ 1 Dilution lower No. (%)	Exact No. (%)	≥ 1 Dilution Higher No. (%)	Percent Difference (CI)	Trending Noted in the Clinical Study
Ampicillin	<i>E. coli</i>	48	6 (12.5)	18 (37.5)	24 (50)	38% (19.0% to 53%)	Yes high
Aztreonam	<i>E. coli</i> ^a	63	7 (11.1)	13 (20.6)	43, (68.3)	57% (41% to 69%)	Yes high
	<i>K. aerogenes</i>	18	3 (16.7)	4 (22.2)	11, (61.1)	44% (13% to 66%)	Yes high
	<i>K. oxytoca</i>	51	8 (15.7)	2 (3.9)	41, (80.4)	65% (47% to 76%)	Yes high
Cefazolin	<i>K. pneumoniae</i> ^a	78	16 (20.5)	38 (48.7)	24, (30.8)	10% (-3% to 24%)	No
	<i>K. variicola</i>	18	4 (22.2)	11	3 (16.7)	-6% (-31% to 21%)	No

Drug	Organism	Total Evaluable for Trending	≥ 1 Dilution lower No. (%)	Exact No. (%)	≥ 1 Dilution Higher No. (%)	Percent Difference (CI)	Trending Noted in the Clinical Study
Ceftazidime	<i>A. baumannii</i> ^a	56	15 (26.8)	21 (37.5)	20, (35.7)	9% (-8% to 25%)	No
	<i>A. baumannii</i> / <i>nosocomialis</i> group	18	6 (33.3)	6 (33.3)	6 (33.3)	0.0 -28.6 to 28.6	No
	<i>P. aeruginosa</i> ^a	89	15 (16.9)	38 (42.7)	36, (40.5)	24% (10% to 36%)	No
	<i>E. coli</i> ^b	21	10 (47.6)	4 (19.1)	7 (33.3)	-14% (-40% to 14%)	No
	<i>K. aerogenes</i>	12	3 (25)	0 (0)	9 (75)	50% (10% to 73%)	Yes high
	<i>K. oxytoca</i>	19	6 (31.6)	2 (10.5)	11 (57.9)	26% (-5% to 51%)	No
	<i>K. variicola</i>	9	0 (0)	1 (11.1)	8 (88.9)	89% (45% to 98%)	Yes high
Ertapenem	<i>E. coli</i> ^b	21	2 (9.5)	1 (4.8)	18 (85.7)	76% (48% to 88%)	Yes high
	<i>K. aerogenes</i>	12	1 (8.3)	2 (16.6)	9 (75)	67% (28% to 84%)	Yes high
	<i>K. oxytoca</i>	68	10 (14.7)	1 (1.5)	57 (83.8)	69% (54% to 79%)	Yes high
Trimethoprim/ sulfamethoxazole	<i>E. coli</i>	5	0 (0)	1 (20.0)	4, (80)	80% (19% to 96%)	Yes high
	<i>K. aerogenes</i>	17	0 (0)	4 (23.5)	13 (76.5)	76% (46% to 90%)	Yes high
	<i>K. oxytoca</i>	3	1 (33.3)	0 (0)	2 (66.7)	33% (-32% to 72%)	No
	<i>K. variicola</i>	18	1 (5.6)	4 (22.2)	13 (72.2)	67% (36% to 83%)	Yes high

^a High trending noted in the Inoculum Density study for positive blood culture samples with an organism concentration of 10⁹ CFU/mL.

^b High trending noted in the Inoculum Density study for positive blood culture samples with an organism concentration of 10⁶ and 10⁹ CFU/mL.

Analysis of trending in the clinical study indicated that LifeScale LSGN MIC values for the following antimicrobial/organism combinations tended to be at least one doubling dilution higher than the reference MIC value:

- Ampicillin- *E. coli*
- Aztreonam – *E. coli*, *K. aerogenes*, *K. oxytoca*
- Ceftazidime – *K. aerogenes*, *K. variicola*
- Ertapenem – *E. coli*, *K. aerogenes*, *K. oxytoca*
- Trimethoprim/sulfamethoxazole – *E. coli*, *K. aerogenes*, *K. variicola*

To address the overall observed trending with the LifeScale LSGN kit in both the clinical study and inoculum density study, the following statement was added to the device labeling:

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*

2. Matrix Comparison:
NA

C Clinical Studies:

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

D Clinical Cut-Off:

NA

E Expected Values/Reference Range:

Table 9. FDA-Recognized Interpretive Criteria for the Antimicrobials Indicated With the LifeScale LSGN Kit

Antimicrobial	Organism Group	Interpretive Criteria ^a		
		S	I	R
Ampicillin	<i>E. coli</i>	≤8	16	≥32
Aztreonam	Enterobacterales	≤4	8	≥16
Cefazolin	Enterobacterales	≤2	4	≥8
Ceftazidime	Enterobacterales	≤4	8	≥16
	<i>A. baumannii</i>	≤8	16	≥32
	<i>P. aeruginosa</i>	≤8	-	≥16
Ertapenem	Enterobacterales	≤0.5	1	≥2
Trimethoprim/sulfamethoxazole	Enterobacterales	≤2/38	-	≥4/76

S = Susceptible; I = Intermediate; R = Resistant

^a FDA-Recognized Antimicrobial Susceptibility Test Interpretive Criteria Website

<https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm410971.htm>

F Other Supportive Instrument Performance Characteristics Data:

NA

VIII Proposed Labeling:

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

IX Conclusion:

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

To support the implementation of changes to FDA-recognized susceptibility test interpretive criteria (i.e., breakpoints), this submission included a predetermined change control plan (PCCP) that was reviewed and accepted by FDA as described in the [Antimicrobial Susceptibility Test \(AST\) System Devices – Updating Breakpoints in Device Labeling guidance](#). This PCCP addresses future revisions to device labeling in response to breakpoint changes that are recognized on the FDA STIC webpage (<https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm410971.htm>). The protocol outlined the specific procedures and acceptance criteria that Affinity Biosensors intends to use to evaluate the LifeScale AST system when revised breakpoints for indicated drugs are published on the FDA STIC webpage. The PCCP included with the submission indicated that if specific criteria are met, Affinity Biosensors will update the LifeScale AST system label to include (1) the new breakpoints, (2) an updated performance section after re-evaluation of data in this premarket notification with the new breakpoints, and (3) any new limitations as determined by their evaluation.